Chapter IV

CHEMISTRY OF AQUATIC ORGANISMS AND THEIR UTILIZATION

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Aquatic environment is largely different from terrestrial environment with highly diversified habitats including coral reefs, deep sea, tropical mangrove estuary, and polar sea. Aquatic organisms are adaptively radiated to all these various aquatic environments, and are different from terrestrial organisms in their chemical and biochemical features. Thus, they have many interesting and unique characteristics. Recently, the relationship between their adaptation to environment and biological compounds or their metabolism is being gradually understood. In this chapter, the chemical and biochemical features of aquatic organisms will be described and the present status and future prospect of their utilization will also be considered.

(Hiroki Abe)

1. CHEMISTRY AND BIOCHEMISTRY OF AQUATIC ORGANISMS

1.1 Proximate composition

As food materials, fish and shellfish are different from terrestrial food resource in various aspects. At first, fish and shellfish include variety of species even if we consider only those used for food materials. Including the jelly fish in Coelenterata, sea cucumber and sea urchin in Echinoderm and whales in Mammalia, almost all phyla are utilized as aquatic food resource. Secondly, seasonal variations are prominent. For example, salmon were originally marketed only in autumn and winter, their season of migration to mother rivers. This character is similar to vegetables, but nowadays the seasonality of many fish and vegetables is becoming unclear due to the development of storage and culture technologies. The contents of lipids and carbohydrates change widely with season even in the same species and the chemical compositions differ with the size and part of the body.

Postmortem changes in muscles of fish and shellfish are generally rapid

Table 4.1. Proximal composition of major fish and shelfish.

Species	in 100g of edible part																	Remarks
	Moisture	Protein	Lipids	Carbohydrate	Ash	Inorganic substances					Vitamins							
						Sodium	Potassium	Calcium	Phosphorus	Iron	A			B_1	B_2	Naiacin	C	
											Retinol	α-carotene	β-carotene					
			(g)					(mg)				(μg)				(mg)		
Jack mackerel	74.4	20.7	3.5	0.1	1.3	120	370	27	230	0.7	10	Tr	Tr	0.10	0.20	5.4	Tr	*Trachurs japonicus*
Ayu (wild)	77.7	18.3	2.4	0.1	1.5	70	370	270	310	0.9	35	(0)	(0)	0.13	0.15	3.1	2	
Ayu (cultured)	72.0	17.8	7.9	0.6	1.7	55	360	250	320	0.8	55	(0)	(0)	0.15	0.14	3.5	2	
Sardine	64.4	19.8	13.9	0.7	1.2	120	310	70	230	1.8	40	Tr	Tr	0.03	0.36	8.2	Tr	*Sardinops melanostictus*
Eel	62.1	17.1	19.3	0.3	1.2	74	230	130	260	0.5	2,400	0	1	0.37	0.48	3.0	2	
Skipjack tuna	72.2	25.8	0.5	0.1	1.4	43	430	11	280	1.9	5	0	0	0.13	0.17	19.0	Tr	Caught in spring
Salmon	72.3	22.3	4.1	0.1	1.2	66	350	14	240	0.5	11	0	0	0.15	0.21	6.7	1	*Oncorhynchus keta*
Mackerel	65.7	20.7	12.1	0.3	1.2	140	320	9	230	1.1	24	0	0	0.15	0.28	10.4	Tr	*Scomber japonicus*
Pacific saury	55.8	18.5	24.6	0.1	1.0	130	200	32	180	1.4	13	0	0	0.01	0.26	7.0	Tr	
Red sea bream	72.2	20.6	5.8	0.1	1.3	55	440	11	220	0.2	8	0	0	0.09	0.05	6.0	1	*Pagrus major*
Cod	80.4	18.1	0.2	0.1	1.2	130	350	41	270	0.4	56	0	0	0.07	0.14	1.1	0	*Theragr chalcogramma*
Herring	66.1	17.4	15.1	0.1	1.3	110	350	27	240	1.0	18	0	0	0.01	0.23	4.0	Tr	
Marbled flounder	76.8	20.0	2.0	Tr	1.2	46	440	22	240	0.1	12	0	0	0.04	0.11	5.0	3	*Paralichthys olivaceus* (wild)
Puffer	78.9	19.3	0.3	0.2	1.3	100	430	6	250	0.2	3	0	0	0.06	0.21	5.9	Tr	*Takifugu rubripes* (wild)
Yellowtail (wild)	59.6	21.4	17.6	0.3	1.1	32	380	5	130	1.3	50	—	—	0.23	0.36	9.5	2	
Yellowtail (cultured)	60.8	19.7	18.2	0.3	1.0	37	310	12	200	0.9	28	0	0	0.16	0.19	9.1	2	
Tuna (lean meat)	70.4	26.4	1.4	0.1	1.7	49	380	5	270	1.1	83	0	0	0.10	0.05	14.2	2	Pacific blue fin tuna
Tuna (fatty meat)	51.4	20.1	27.5	0.1	0.9	71	230	7	180	1.6	270	0	0	0.04	0.07	9.8	4	Pacific blue fin tuna
Japanese littleneck	90.3	6.0	0.3	0.4	3.0	870	140	66	85	3.8	2	1	21	0.02	0.16	1.4	1	*Ruditapes philippinarum*
Common oriental clam	88.8	6.1	0.5	1.8	2.8	780	160	130	96	2.1	7	0	25	0.08	0.16	1.1	1	*Meretrix lusoria*
Scallop	82.3	13.5	0.9	1.5	1.8	320	310	22	210	2.2	10	1	150	0.05	0.29	1.7	3	*Mizuhopecten yessoensis*
Squid	79.0	18.1	1.2	0.2	1.5	300	270	14	250	0.1	13	0	0	0.05	0.04	4.2	1	*Todarodes pacificus*
Shrimp	76.1	21.6	0.6	Tr	1.7	170	430	41	310	0.5	0	0	49	0.11	0.06	3.8	Tr	*mar*
Crab	84.0	13.9	0.4	0.1	1.6	310	310	90	170	0.5	Tr	—	—	0.24	0.60	8.0	Tr	*Chionoecetes opilio*
Octopus	81.1	16.4	0.7	0.1	1.7	280	290	16	160	0.6	5	—	—	0.03	0.09	2.2	Tr	*Octopus vulgarisc*
Sea cucumber	92.2	4.6	0.3	0.5	2.4	680	54	72	25	0.1	0	0	5	0.05	0.02	0.1	0	

Tr: marginally contained, but not reached minimum value for record.
(0): not measured, but it can be estimated not to be contained from literatures.
—: not measured or not measurable.
(From *Standard Table of Food Composition in Japan, Fifth Revised and Enlarged Edition*, 2008.)

compared to those of animals. This is related to the fact that fish and shellfish are ectothermic organisms and their habitats are aquatic environments where temperatures are generally lower than terrestrial environments. Preservation at low temperatures is more important for the treatment of fish and shellfish than for other animal meat.

These features of fish and shellfish are sometimes advantageous. Characteristic tastes and seasonal variations of fish and shellfish provide variety and richness in our dietary life.

Proximate composition, the basic information on the composition of food, represents the contents of moisture, proteins, lipids, carbohydrates and ash in food. "*Standard Tables of Food Composition in Japan*" (fifth edition, published in 2005), provides detailed information on dumping rates, energy, inorganic substances (minerals and ash), vitamins, fatty acids, cholesterol, dietary fiber, salt equivalent amount etc. (refer Table 4.1 for details of common fish and shellfish). These values are important indicators to know the nutritional value of foods. The energy is calculated from composition of protein, lipids and carbohydrates. As described above, proximate compositions of fish and shellfish muscles change with species, season, sex, nutritional conditions, size and part of the body. As can be observed from the table, generally the moisture content is 70 to 80%, protein 15–20%, lipids 1–10%, carbohydrates about 0.5%, ash content 1.0–1.5%. Protein content is predominantly higher except moisture followed by lipids. Generally muscles of migratory fish such as mackerel and sardine contain large amount of lipids, though the seasonal fluctuation is wide, exceeding 20% in some cases (Table 4.1). In tuna, dorsal muscle contains 1–2% lipids, while the ventral muscle has more than 20%. Lipid contents of eel and yellowtail are high. There is no information in the table, but generally the protein content is lower and lipids higher in dark muscles compared to ordinary muscles.

The moisture content is higher and protein content is inversely poorer in invertebrate than in fish meat. The moisture content of the body wall of sea cucumber is higher than 90%. Abalone muscles contains a large amount of collagen as a major component of protein, although the content fluctuates seasonally which influences the texture of raw abalone. Bivalves are rich in carbohydrates because it accumulates glycogen as energy storage similar to lipids in fish (Fig. 4.1).

1.2 Protein

Proteins are major components of organisms. A typical relatively pure protein that we can see in daily life is the egg white. Protein, with a calorie equivalent of 4 kcal/g is one of the three major nutrients including lipids and carbohydrates. Recently in Japan, 40% of animal protein intake is dependent on fish and shellfish. Fish proteins, which are almost equivalent to that found in the meat of terrestrial animals, and poultry are a good source of protein supply for balancing the essential amino acids which cannot be synthesized in human body. In invertebrates, however, the contents of several amino acids such as tryptophan are less than the recommended value and hence their nutritive value is limited.

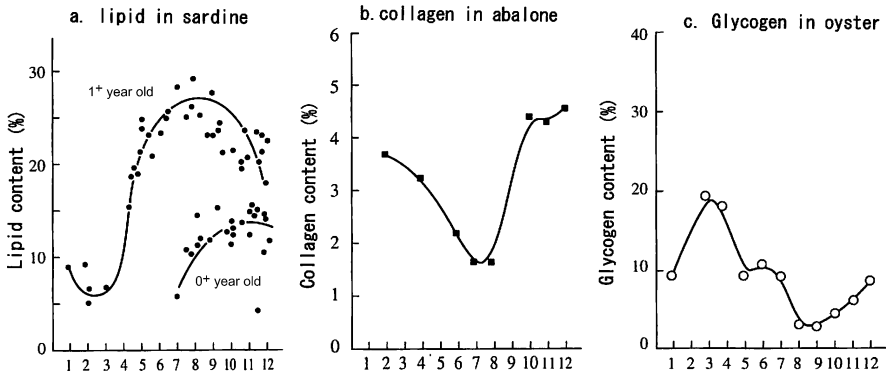


Fig. 4.1. Seasonal changes in chemical composition of aquatic animals (Watabe, 2008).

1) Structures and functions of proteins from aquatic organisms

Proteins are produced by a combination of 20 L-amino acids (standard amino acids). Amino acids have in common an amino group (basic part), a carboxyl group (acidic part), and a carbon bound to a hydrogen atom (α-carbon) in addition to a side chain which characterizes each amino acid. In organisms, amino acids also exist as a free state and are utilized for protein synthesis and osmoregulation in aquatic invertebrates.

Many amino acids bound through a peptide bond formed between the carboxyl group of one amino acid and the amino group of another form a polypeptide. Polypeptides shorter than about 50 amino acids are called peptides and the larger molecules are categorized as proteins. Molecular weight of proteins varies widely from 6,000 (insulin) to 3,700,000 (titin). Proteins have electric charges originated from constitutive amino acids. Molecular weights and isoelectric points of some proteins from aquatic organisms are listed in Table 4.2. Isoelectric points are determined by amino acid composition of proteins. Chemical characteristics of these proteins are not so different from those of higher vertebrates, although differences in stability or enzymatic activity among species are occasionally encountered.

The linear sequence of amino acid residues in protein (amino acid sequence) from the amino terminal side is referred to as a primary structure. If we compare the amino acid sequences of corresponding proteins in fish and higher vertebrates, some muscle proteins such as actin are highly conserved, while some such as myoglobin show much less sequence identity (~70%) as a result of drastic molecular evolution.

Information on the primary structures is encoded as nucleotide sequences in genes contained in the DNA. The sequence information is transcribed from DNA to messenger RNAs (mRNAs) followed by translation to amino acid sequences in ribosome. Just after the translation, major secondary structures are nearly completed and the configurations of amino acid side chains (namely, the tertiary

Table 4.2. Profiles of proteins originated from aquatic organisms.

Protein (species)	Number of amino acid	Molecular weight	Isoelectric point	Function
Balbalbmine (Toadfish)	109	11,757	4.47	Ca transportation?
Myoglobin (Yellowfin tuna)	146	15,529	9.00	Preservation of enzyme
Tripsin (Crayfish)	237	25,022	4.02	Protease
Tropomyosin (Zebra fish)	284	32,723	4.70	Regulation of muscle contraction
Pepsin (Pacific cod)	324	34,014	4.48	Protease
Actin (Tiger puffer)	377	41,945	5.22	Regulation of muscle contraction
Myosin heavy chain (Medaka)	1,937	221,694	5.54	Regulation of muscle contraction
Twitchin (Mediterranean mussel)	4,736	526,838	6.48	Regulation of muscle contraction in bivalve

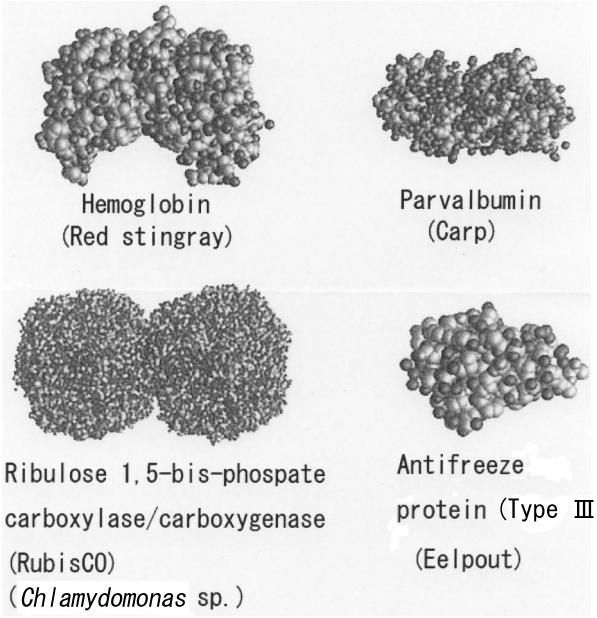


Fig. 4.2. Various shapes of proteins. Each atom is expressed as a ball. Sizes of the models are not proportional to the actual size. Dark balls stand for oxygen atoms. The larger the size of atom, the smaller the protein size. The models are constructed from the data of crystal structures registered in Protein Data Bank. The names in the parantheses are the common name of species from which each protein is from.

structure) are mostly formed through a step called folding. Protein structure is generally determined by the amino acid sequence, and the folded structure has a close relationship with the function of proteins. The folded structure of protein is also thermodynamically advantageous and totally in accordance with negative energy change through complete structural breakdown or denaturation (endothermal reaction). In a few cases, proteins are spliced or modified with sugar chains, lipids, etc., by so-called post-translational modifications. N-termini of proteins from aquatic organisms are frequently subjected to modification such as acetylation. Final functional structure of proteins varies widely: namely, round, elongated, a combination of both etc. Figure 4.2 shows the tertiary structures (ball model) of four proteins from aquatic organisms.

Protein structure is perturbed under strong acidic and basic conditions, or by organic solvents, denaturing agents, heavy metal ions etc. This phenomenon called denaturation is generally accompanied by loss of function such as aggregation, decrease in activity etc. Proteins from aquatic organisms are generally unstable as compared with mammalian proteins and are relatively susceptible to denaturation factors. Proteins become more susceptible to proteases when their native structures are damaged even in live organisms. The duration of proteins

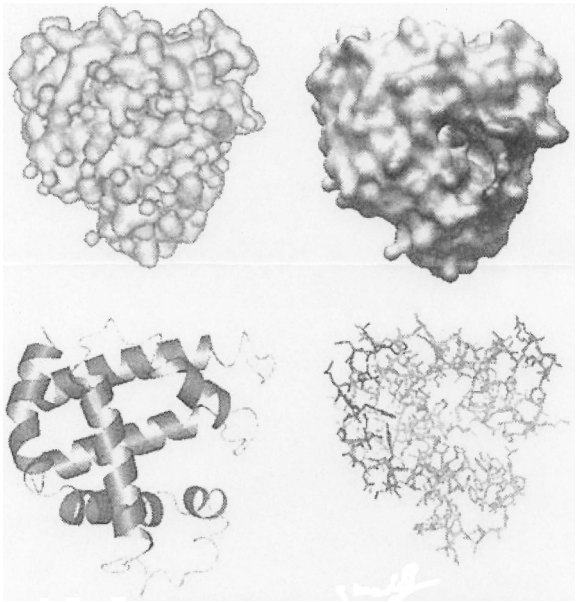


Fig. 4.3. Variety of tertiary structure model of myoglobin from yellowfin tuna. From upper left, accessible surface of solvents, electorostatic potential, ribbon model, and ball and stick model, viewed from the same angle.

from synthesis to decomposition greatly differs. Half life time, the period for replacement of half of a given protein, varies from around 10 minutes for short-life proteins (such as some enzymes) to as long as 6 months for long-life proteins (such as collagen).

Higher structures of proteins are mostly determined by the characterization of protein crystals by X-ray analyses. Unveiling the structures makes it easy to understand the relationship between structure and function of proteins. Figure 4.3 shows the structure of myoglobin from yellowfin tuna. α-Helical content of this protein is less than that of mammalian counterparts. The structural models can be drawn in a variety of ways. By hiding side chains of amino acids in the model, the inner structure becomes clearer, although actual protein molecules have component atoms of amino acid packed in a narrow space of the molecule. Structural changes (fluctuations) might take place in the active state of a given protein, but cannot be determined by the present analytical methods.

Some proteins are composed of more than two polypeptide chains like hemoglobin and RuBisCO (Fig. 4.2). In these protein complexes, each polypeptide chain is called a subunit, and the whole structure of the protein is called a quaternary structure. In some cases, like a proteasome, many different subunits are assembled into a huge complex to achieve a function. In others, proteins are assembled into macromolecular structures with other biogenic substances (nucleic

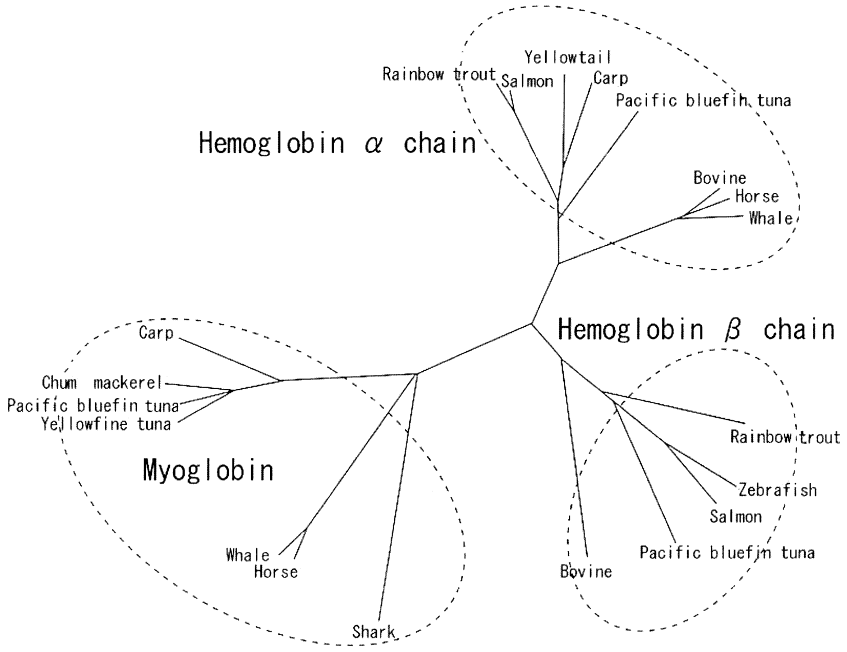


Fig. 4.4. Molecular dendrogram of globin superfamily based on amino acid sequences. Each protein forms clear clusters.

acids, lipids, and so on) for higher biological functions.

In order to perform the expected functions in living organisms, some proteins need to have flexible structures. Stability of the protein generally shows positive relationship with body temperature (or the surrounding water temperature for most aquatic animals). The reason the deterioration of freshness is generally rapid in seafood is that stability of the components such as proteins are reduced as a result of trade-off for their effective functions at lower temperature and under higher pressure environment. As a result, postmortem biochemical changes of the components generally proceed rapidly.

2) Species- and tissue-specificity of protein components

Nucleotide sequences of whole genomes have been revealed in many organisms, and the numbers of genes have been determined to be about 23,000 in sea urchin, 15,800 in sea squirt, 22,400 in pufferfish and 29,000 in human etc. Total numbers of proteins functioning in living organisms are considered to be much larger than the numbers of genes. Many proteins have their isoforms transcribed by different combinations of exons by alternative splicing from a single gene. Clear differences in protein components are observed among tissues even in the same species. For example, the ordinary (white) and dark muscles of fish show different protein compositions. In comparison with the corresponding proteins from different species, the replacement ratio of amino acids is generally

large between remotely related species in taxonomy. The molecular phylogenic tree of the globin superfamily based on their amino acid sequences is shown in Fig. 4.4. Each globin family forms a clear cluster.

3) Changes of function and structure of proteins encountered in aquatic organisms

Proteins are involved in vital activities, although they rarely reveal their presence by working as "background players". It is thus difficult to recognize the changes in function and structure of proteins in our daily life. In this paragraph, several instances of changes related with proteins that are recognizable by our senses (such as color, taste, and texture) will be briefly introduced.

The color of skeletal muscles varies with fish species. For example, the color of muscle is red in tuna but white in flounder. This is due to the differences in the content of a heme protein called myoglobin in the fish muscle. Myoglobin facilitates the sustained swimming of migratory fish. When raw tuna meat is left at room temperature, the color gradually darkens. Such discoloration is caused by oxidation of the heme protein. On the other hand, color of crab and shrimp shells turns red by boiling. This is due to the color of carotenoids that appear on the carapace as a result of structural change in the carotenoid-binding protein. Protein degradation of phycobilin protein due to thermal denaturation is also responsible for the change of color in *nori* (seaweed) caused by roasting. Active blinking of pigment cells is observed on the dermis of live squids. The blinking is caused by instant retraction and expansion of chromatophores driven by the tiny muscles under the control of the nervous system. Bioluminescence of jelly fish and corals is emitted by fluorescence proteins. Green fluorescent protein (GFP) was discovered in jelly fish (*Aequorea victoria*). This protein has outstanding characteristics in that the chromophore is a part of the protein itself and composed of only three amino acids and no substrate is required for fluorescence emission. On the other hand, mollusks have hemocyanin as a respiratory pigment instead of hemoglobin. Hemocyanin has copper in the molecule instead of iron. Therefore, the blood of mollusks looks pale when it binds with oxygen. Brilliance of pearl is produced by refined layered structure of calcium carbonate crystal with the aid of a protein, conchiolin.

Fresh "*sashimi*" or sliced raw fish has a tough texture, which is due to the network of fibrous structure mainly composed of collagen, myosin and actin. As a result of postmortem change (autolysis), the texture gradually softens. Rigor mortis is caused by the strong binding between myosin and actin, being triggered by the breakdown of ATP (more detailed process is described in Section 3). Muscle is then softened by fragmentation of the sarcomeres by the action of endogenous proteolytic enzymes. Increase of taste active components (glutamic acid, inosinic acid, etc.) by the enzymatic breakdown of protein and ATP contributes to improvement of taste, but further decomposition of the protein results in diminishing the taste of muscle with a simultaneous increase in unfavorable taste and odor components.

Collagen, forming tough fibers in organisms, is gelatinized by heat treatment. Due to this, abalone muscle containing collagen in large amount, softens by

boiling. When the broth obtained after boiling fish with skin and bones is left to stand at low temperature, extracted gelatinized collagen tends to form a gel (called "*nikogori*"). When fish muscle is ground with 2–3% of salt, actomyosin dissolves and forms sol which is changed to gel under proper heating conditions (fish meal or "*kamaboko*"). This phenomenon is explained mainly by the formation of network between actomyosin and water. Crosslinking of proteins by transglutaminase is also considered to be responsible for the gel formation.

Proteins also exert important functions in adaptation of organisms to their environments. The reason fish inhabiting the polar areas can survive without being frozen, even though the water temperature is below freezing point, is the inhibition of ice crystal growth by antifreeze proteins present in their blood plasma (Fig. 4.2). On the other hand, in the tide pools, some organisms can survive in the environment even though the water temperature can be extremely high under scorching sunlight during daytime. These are considered to have the ability to express heat shock proteins to overcome such an extreme environment.

Some proteins show toxicity. Sea anemones possess toxic proteins in their nematocytes. Cone snails inject toxic proteins into their enemies and preys. Even non toxic proteins sometimes cause harmful symptoms to human beings. Causative substances of allergy by fish and shellfish are protein components of muscle such as tropomyosin, parvalbumin and collagen. Mechanism of intoxification by puffer toxin (tetrodotoxin) and paralytic shellfish poison (PSP) is the blockage of neurotransmission by binding to membrane proteins in the sodium channel (see Section 4).

As described above, life style and postmortem changes of organisms are closely related to the nature and functions of proteins possessed. These facts have been accumulated by a number of dedicated researchers mainly through biochemical and physiological approaches. The researches may have evolved just out of the curiosity for the uniqueness of aquatic organisms, or may have originated from the needs in fisheries and food industries. Proteins of aquatic organisms, living at low temperature and under high pressure environment, are generally unstable as described above, and thus the storage and processing of fish and shellfish are difficult compared to terrestrial animals. Research targeting the proteins from aquatic organisms seems to require a lot more efforts and devices for further understanding their roles and for effective utilization of aquatic resources. However, in spite of the difficulties in handling, one may feel a stronger motivation to elucidate their secrets of life.

(Yoshihiro Ochiai)

1.3 Lipids

"Lipids" are low molecular substances which do not dissolve in water, and involve a variety of substance groups. Furthermore, various organisms exist in the aquatic environment and have more complex lipid compositions compared to terrestrial organisms. Therefore, this section describes mainly the lipids contained in fish and shellfish consumed as seafood.

1) Distribution and properties of lipids in aquatic organisms

Similar to lipids of terrestrial organisms, lipids in aquatic organisms also play the roles as energy sources, components in cell membranes, essential nutrients, and metabolic regulators. The lipids of aquatic organisms, however, are characterized by high contents of unsaturated fatty acids as a component. It is also another unique feature of aquatic organisms that some of them accumulate large amounts of non-glyceride lipids.

Lipids are classified into depot lipids and tissue lipids by the difference of existing forms in organisms. Depot lipids are accumulated in the adipose tissues under skin, mesenterium and liver when the organisms are in good nutritional states. The contents of depot lipids show wide fluctuation. Fatty fish and oily fish were the words originally used for fish such as sardine and saury having enough lipids. Generally, large individuals, immature fish with undeveloped ovary, and fish inhabiting the low temperature environments have higher lipid contents compared to small individuals, mature fish before spawning, and fish inhabiting the high temperature environments, respectively. Comparing wild and cultured fishes, the latter have higher lipid content due to over feed intake and shortage of exercise. In the season when the fish are tasty (referred to as "*shun*"), their lipid contents are generally high. Lipid contents differ among regions of fish body even in the same individual. Fish species such as saury and yellowtail accumulate lipids in muscle and have relatively low lipid contents in liver. Anterior portion of fish body is abundant in lipids compared with the posterior one. Ventral part contains higher lipid contents compared to the dorsal part. In tuna, for instance, ordinary meat contains only about 1% of lipid, while in the fatty meat of the ventral part ("*toro*"), it reaches near 30%. In contrast, fishes with low lipid contents in muscle, such as pufferfish, anglerfish, and cod, often accumulate lipids in their liver. In crustaceans such as crab and shrimp, and mollusks such as octopus, squid, and shellfish, lipid content is lower in muscle and high in the midgut gland, the organ corresponding to liver and pancreas, and other internal organs.

In contrast to depot lipids, tissue lipids exist as constituents of cell membrane and are important for the maintenance of homeostasis. Thus, tissue lipids do not fluctuate so much even under the individual nutritional and physiological conditions compared with the depot lipids. When comparing tissue and depot lipids, the former contains higher rate of unsaturated fatty acid than the latter. This is because unsaturated fatty acids in the membrane lipids can keep the fluidity of the membrane even under low temperature conditions.

Lipids can also be classified into nonpolar and polar lipids based on the differences in polarity caused by chemical structures. The nonpolar lipids have low affinity to water and involve acylglycerols, waxes, glyceryl ethers, sterol fatty acid esters, carotenoid fatty acid esters. They also include hydrocarbons and the hydrolyzed forms of these compounds such as fatty acids, fatty alcohols, sterols, and carotenoids. Polar lipids contain phospholipids such as glycerophospholipids and sphingomyelin, and glycolipids such as glyceroglycolipids and sphingoglycolipids.

2) Fatty acids

Fatty acids are one of the important components of various lipids. Although existing mainly as acylglycerol, glycerophospholipid, or acylglyceryl ether through ester bonds, fatty acids can also exist as free fatty acids. Some fatty acids combine with hydroxy group of sterols or carotenoids via ester bonds. Fatty acids are synthesized from acetyl CoA through the elongation of carbon chain by the addition of two carbon units on the carboxyl terminus, successively. As a result, most of the natural fatty acids are composed of even numbers of carbon atoms. Carbon numbers of most fatty acids are from 14 to 22 in fish and shellfish. Fatty acids are classified into two main groups: saturated fatty acids which have no double bond and unsaturated fatty acids with double bonds. The unsaturated ones with more than two double bonds are called poly unsaturated fatty acid (PUFA). PUFA with more than 20 carbons and more than three double bonds are called highly unsaturated fatty acids (HUFA). Most of the double bonds in natural unsaturated fatty acids are *cis* form (Fig. 4.5). In the case of fatty acids having more than two double bonds, the double bonds mainly form a divinylmethane structure (–CH=CH–CH_2–CH=CH–), although fatty acids which have conjugated double bonds (–CH=CH–CH=CH–) are also discovered.

According to the nomenclature system by International Union of Pure and Applied Chemistry (IUPAC), the position of double bond is expressed by the number of carbon from carboxyl terminnus. For example, the oleic and lenoleic acids in Fig. 4.5 are expressed as $18{:}1\Delta^{9}$ and $18{:}2\Delta^{9,12}$, respectively. However, this notation system is rather inconvenient considering the biosynthetic process of PUFA. In the carbon-chain elongation reaction of fatty acid, the corresponding enzyme adds new carbon at carboxyl terminus and the number designating the position of double bond is changed along with the elongation reaction. For instance, when the carbon chain of oleic acid is elongated to 20, the IUPAC notation of the fatty acid will be $20{:}1\Delta^{11}$ (Fig. 4.5). If the carbon at methyl terminus is arranged as n-1 and indicated the position of double bonds by the number from the carbon at methyl terminus, oleic acid can be expressed as 18:1n-9. Similarly, linoleic acid, α-linolenic acid, arachidonic acid, icosapentaenoic acid (IPA), and docosahexaenoic acid (DHA) are expressed as 18:2n-6, 18:3n-3, 20:4n-6, 20:5n-3, and 22:6n-3, respectively. It is easily understandable by this notation system that linoleic acid and arachidonic acid have double bonds in the same position of n-6 and α-linolenic acid, IPA, and DHA have them in the same position of n-3. Thus, each HUFA group is easily distinguished to originate from each biosynthetic group. In another form of expression, carbons next to carboxyl terminus in the carbon chain are named as α, β, γ, and that in the methyl terminus as ω indicating the final position. In this system, the position of double bonds can be designated as ω3, ω6, ω9, and so on.

Marine animals contain higher amounts of n-3 HUFA than terrestrial animals. However, in contrast to plants and bacteria, animals cannot insert double bond in the position of n-6 and n-3, although they can introduce double bond in the position closer to the carboxyl terminus than n-9. Therefore, fish and shellfish have to intake n-6 and n-3 fatty acids produced by bacteria and plants through

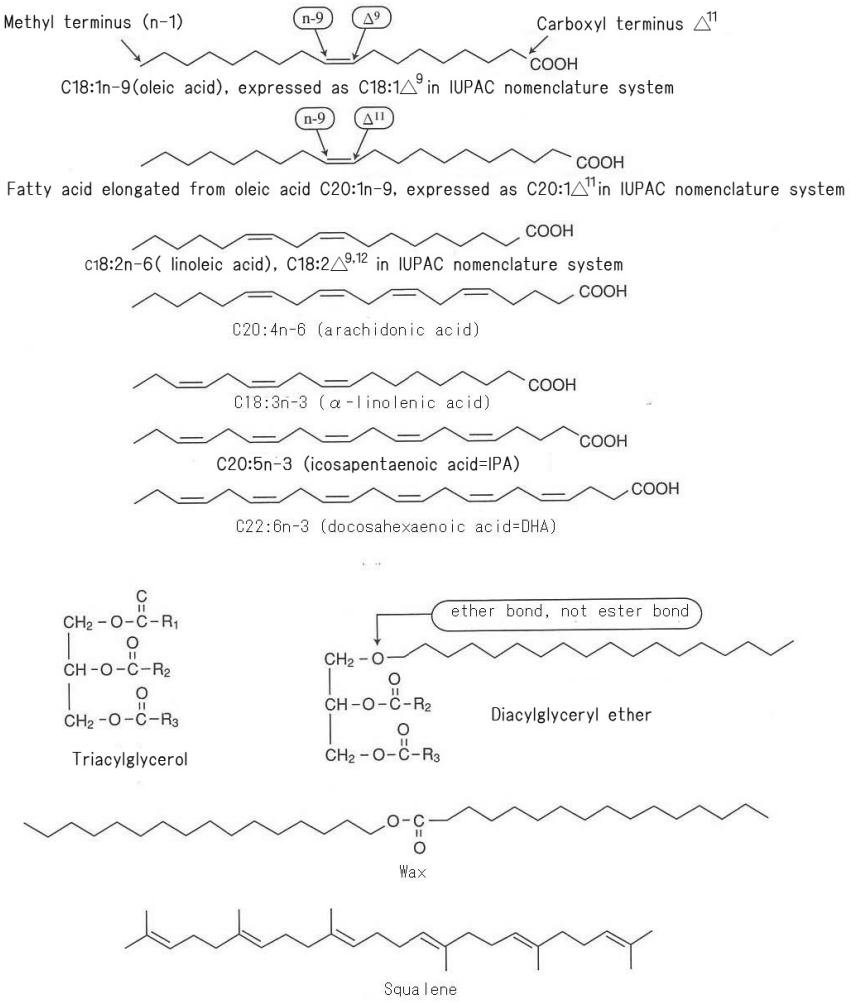


Fig. 4.5. Structure of various lipids.

food chain and utilize them directly or after further elongation of carbon chain or after desaturation. Highly unsaturated fatty acids have low melting point and high fluidity. These characteristics are suitable for living in aquatic environment with low temperature. Fatty acid compositions vary among species, with the differences being due to the ingested food, capacity of elongation, and desaturation of carbon chains in the fatty acids. Marine fishes contain higher amounts of IPA and DHA than freshwater fishes, although marine fishes have low activities of enzymes for carbon-chain elongation and desaturation from 18:2n-6 and 18:3n-3. Thus, they have to intake IPA and DHA directly from food. The contents of n-3 HUFA tend to be higher in tissue lipids such as phospholipids than in depot lipids such as

triacylglycerols. For this reason, the ratio of n-3 HUFA in total fatty acid tends to be high in fish with low lipid contents.

3) Acylglycerols

Acylglycerols are compounds in which glycerol binds with fatty acids by ester bond and classified into mono-, di-, and tri-acylglycerol based on the number of fatty acid bound with glycerol. Generally, mono- and di-acylglycerol are trace components and tri-acylglycerol is a major one. In many fish, triacylglycerols exist as depot lipids mainly in adipose tissues and liver. The contents of triacylglycerol fluctuate widely, because when required, it is utilized as energy source after being broken down to glycerol and fatty acids by lipase. Skilfish (*Erilepis zonifer*) is an exceptional fish which accumulates extremely large amounts of triacylglycerol in muscles. Therefore, overeating of old skilfish flesh is said to cause diarrhea.

As the distribution pattern of fatty acids binding to glycerol through ester bond in a triacylglycerol molecule, saturated fatty acids mostly bind at the first position of glycerol, IPA or DHA as well as short chain fatty acids mainly bind at the second position, and third position is mainly occupied by a long chain fatty acid.

4) Waxes

Waxes are compounds in which fatty acids are esterified with a long-chain primary fatty alcohol in place of glycerol. They are found in large amount in several fishes such as oilfish (*Ruvettus pretiosus*), escolar (*Lepidocybium flavobrunneum*), and several Lanternfish etc., and in crustaceans, arrowworms and squids living in mesopelagic and bathypelagic zone. The other fishes such as carp also have a small amount of waxes in their liver and waxes are known to be distributed widely in the living world. Compositions of fatty alcohols found in wax of marine animals are simple. Chemical species with relatively low unsaturation such as 14:0, 16:0, 18:0, 20:1, and 22:1 are common. The fatty acids found in wax are mainly composed of C_{14}–C_{22}, although waxes of crustaceans sometimes contain 22:6 fatty acid. The composition of fatty acids composing wax is more complex than that of fatty alcohols, although it is much simpler than that of fatty acids found in common triacylglycerol. Unsaturation of fatty acids in wax is also relatively low. Enzyme activities for decomposition of wax at ester bond are high in copepods which accumulate waxes in their body and lantern fish which feed on the copepods. When the copepods which accumulate triacylglycerol and wax are starved, triacylglycerol decreases at first and wax decreases after the disappearance of triacylglycerol, suggesting that both triacylglycerol and wax are utilized as energy sources. Human beings have only low capacity to decompose wax and excessive ingestion of wax leads to diarrhea. For this reason, commercial sale of oilfish and escolar as food is prohibited. Salted and dried mullet roe ("*karasumi*") contains relatively higher contents of wax but there is no problem when people eat a small amount of the product as a delicacy. Specific gravity of wax is lower than that of triacylglycerol and the low specific gravity of wax is considered to contribute in obtaining buoyancy for the vertical migration of copepods living in mesopelagic and bathylpelagic zone.

5) Glycerylether lipids

Glycerylethers are compounds in which a fatty alcohol binds at the first position of glycerol by ether bond. There are two types of glycerylether: one is alkylether in which saturated fatty alcohol binds to glycerol and another is alkenylether in which unsaturated fatty alcohol binds. Diacylglycerylether, in which two fatty acids bind to hydroxyl group of a glycerol moiety by ester bond, exist in large amounts in the liver and muscle of several elasmobranchs such as spiny dogfish (*Squalus acanthias*) and basking shark (*Cetorhinus maximus*). Most teleost fishes have only a trace amount of diacylglycerylether, although silver kingfish (*Rexea prometheoides*) has a relatively large amount of diacylglycerylether. Diacylglycerylether is hardly decomposed by human digestive enzymes, and one must be careful while eating fish with high amount of diacylglycerylether. The physiological function of diacylglycerylether in the above mentioned sharks is still not known, although it is expected to be involved in regulating buoyancy because of its low specific gravity compared to triacylglycerol.

6) Hydrocarbons

Several deep-sea sharks such as spiny sharks (*Centrophorus* sp.) accumulate a large amount of a hydrocarbon, squalene which is a precursor of sterols. It is supposed that the accumulation of a large amount of squalene in deep-sea sharks occur due to the slow progress of lanosterol synthesis by ring-closure of squalene 2,3-epoxide converted oxidatively from squalene by squalene monooxygenease under the low oxygen environment in deep-sea. However, not all fish in deep-sea accumulate squalene and deep-sea sharks also have a sufficient amount of sterols for the maintenance of homeostasis. Thus, there should be some positive reasons for the accumulation of squalene in the deep-sea sharks. Specific gravity of squalene is also lower than triacylglycerol as seen in wax and glycerylether and squalene is also possible to be responsible to regulate buoyancy.

7) Sterols and sterol esters

Similar to sterols in mammals, fish sterols are composed mainly of C_{27} cholesterol synthesized from squalene through lanosterol, of which carbon number is 30, and are an important component of cell membranes. Cholesterol exists as free sterol or ester with fatty acid. Various kinds of sterols with various structures exist in marine invertebrates. Algae also contain different types of sterols from animals. Sterols are isoprenoid compounds. Isopentenyl diphosphate and dimethylallyl diphosphate, which are the common precursors of isoprenoids, are supplied from the mevalonic acid pathway in animals. In plants, however, the mevalonic acid pathway exists in cytoplasm and methylerythritol phosphate pathway is in chloroplasts. These pathways are properly used depending on the kind of targeted isoprenoid (Rodríguez-Concepción and Boronat, 2002). Several invertebrates such as crustaceans lack the capacity of sterol biosynthesis and have to ingest sterols from food.

8) Polar lipids

In phospholipids which are important polar lipids, there exist glycerophospholipids and sphingophospholipids. Glycerophospholipids combine

with ethanolamine, serine, glycerol, inositol etc. on their phosphate group and are major components of cell membranes and other membranes in the cell as tissue lipids. Most phospholipids in fish muscle are phosphatidylcholine and phosphatidylethanolamine. The first position of glycerol moiety of phosphatidylcholine combines with a fatty acid having low unsaturation degree such as 16:0 or 18:1 and the second position combines with a highly unsaturated fatty acids such as IPA and DHA. As a specific phospholipid in aquatic organisms, phosphonolipids are found in shellfish such as oysters and scallops. Phosphonolipids include both glycerophosphonolipids and sphingophosphonolipids. Abundant phosphonolipds in shellfish are sphingophosphonolipids which distribute mainly in adductor muscle.

9) Physiological function to human beings

Lipids in aquatic organisms serve not only as energy source, but also have various important functions beneficial to human beings. An epidemiologic survey revealed that even though the Inuits ingest high fat diets, their mortality by cardiovascular disease is less than Western people (Dyerberg *et al*., 1975). The reason was explained as that they ingest a large amount of lipids from fish and marine mammals which are very rich in HUFA such as IPA and DHA. This report stimulated the researches into the physiological functions of HUFA. As a result, it was found that IPA from fish oil is transformed to some hormone-like substances (icosanoids) in human body according to demand, which works antagonistically with icosanoids from arachidonic acid and exhibit remarkable physiological activities. Therefore, the balance between n-6 and n-3 fatty acid in our food is important for the maintenance of our health. IPA and DHA are scarce in lipid from terrestrial animals and plants, and the demand for lipids from aquatic organisms are globally increasing. However, HUFA is easily oxidized because of the existence of many double bonds. Thus, care is necessary in the processing of seafood. People who live in inland country and who have no habit of eating seafood do not prefer to eat fish oil. Several attempts are implemented to make oils having no fishy odor and containing HUFA from recombinant plants by introduction of genes of fatty acid desaturation enzymes from marine microalgae to terrestrial plants (Robert *et al*., 2005).

(Shigeru Okada)

1.4 Low molecular weight components

Low molecular weight compounds dissolved in the cells of organisms are important metabolites for maintenance of their lives. The major compounds are referred to as extractive components. Extractive components are comprised of organic compounds extracted by water or hot water, but proteins, nucleic acids, lipids, polysaccharides, pigments, and vitamins are excluded. Inorganic ions are also not included as a rule. Extractive components are classified into nitrogenous compounds such as amino acids and nucleotides and non-nitrogenous compounds such as sugars and organic acids.

Table 4.3. Composition of extractive components in several fisheries animals (mg/100 g).

Chemical compoud	Bigeye tuna[1]	Red sea bream[2]	Salmon shark[3]	Bigfin reef squid[4]	Japanese spiny lobster[5]
Major free amino acid					
Taurine	6	138	44	310	201
Glutamic acid	1	5	12	4	9
Proline	1	2	7	1,029	114
Glycine	6	12	21	896	1,191
Alanine	11	13	19	178	92
Valine	6	3	7	11	25
Leucine	6	4	8	5	18
Lysine	4	11	3	6	15
Arginine	1	2	6	689	515
Histidine	231	4	8	1	+
Dipeptide					
Carnosine	2	+	—		
Anserine	919	+	1,060		
Nucleotide					
ATP	4	11	—	3	13
ADP	9	6	7	40	120
AMP	20	10	5	249	92
IMP	363	342	112	+	101
Guanidyl compound					
Creatine	530	718	507		
Creatinine	—	17	33		
Methylamine					
TMAO	130	246	1,100	624	282
TMA	+	+		+	1
Glycinebetaine				732	501
Homarine				6	152
Organic acid					
Lactic acid	920			+	
Succinic acid				4	
Other					
Urea			1,520		

Open space: not analyzed, +: trace, —: not detected.
[1]Koriyama *et al*. (2000), [2]Konosu *et al*. (1974), [3]Suyama and Suzuki (1975), [4]Kani *et al*. (2007), [5]Shirai *et al*. (1996).

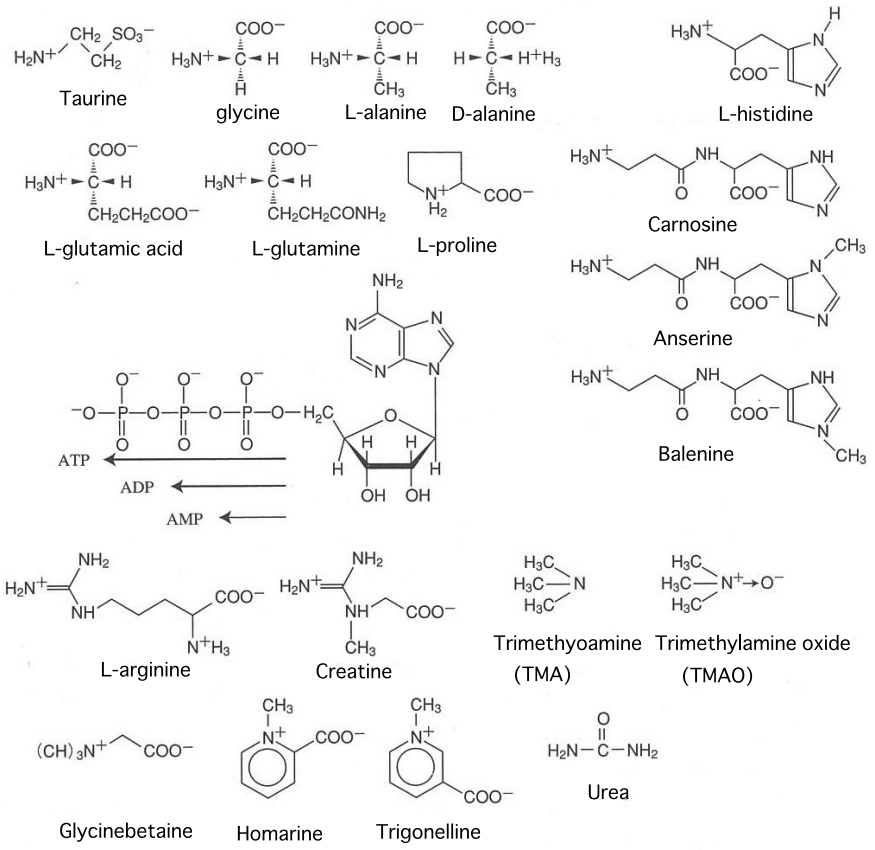


Fig. 4.6. Structures of major extractive components.

Typical compositions and structures of extractive components in aquatic animals are shown in Table 4.3 and Fig. 4.6, respectively. In free amino acids, taurine is abundant in white-fleshed fishes. In red-fleshed fishes, however, dark (red) muscle contains rather large amounts of taurine. Taurine content is much higher in the muscle of invertebrates. Red-fleshed fishes have a large amount of histidine. The histidine contents in ordinary (white) muscle of skipjack tuna and tunas sometimes reach 1,500 mg/100 g. During the deterioration of freshness, histamine is produced from histidine by the action of bacterial histidine decarboxylase and sometimes causes allergy-like food poisoning. Invertebrates contain much larger amounts of free amino acids than fishes, because most invertebrates accumulate non-essential amino acids such as glutamine, glycine, alanine, and proline for osmoregulation (described in Subsection 1.6). Although amino acids in organisms generally consist of L isomer, Japanese spiny lobster (*Panulirus japonicus*) contains 40% of D-alanine in total free alanine pool. This

is a specific feature of many crustaceans and some bivalve mollusks belonging to subclass Heterodonta such as hard clam (*Meretrix lusoria*) and otter shell (*Tresus keenae*). Actively moving invertebrates also contain a large amount of arginine. It exists as phosphoarginine which plays a role as a reservoir of ATP in place of phosphocreatine in vertebrates. These amino acids contribute to the taste of seafood as described in Subsection 2.2.

Large amounts of imidazole dipeptides exist in the muscle of fish depending on the species. Carnosine (β-alanyl-L-histidine) is not abundant in most fishes except for Japanese eel (*Anguilla japonica*) and conger eel (*Astroconger myriaster*) in which carnosine content ranges about 400 mg/100 g, and is a major extractive component of these fishes. Terrestrial animals contain a large amount of carnosine in their muscle. In the ordinary muscle of skipjack tuna and tunas, billfishes, and salmonids contain a large amount of anserine (β-alanyl-π-methyl-L-histidine). From the ordinary muscle of Pacific blue marlin (*Makaira nigricans*), 2,500 mg/100 g of anserine was detected. The content is over 500 mg even in salmonids. The content of anserine in dark muscle is generally low, corresponding to 1/10 to 1/4 of the content in ordinary muscle. As shown in Table 4.3, several shark species have also a large amount of anserine, depending on the species. In the other animals than fish, anserine is abundant in white muscle of avians such as chicken and turkey. As for the other imidazole dipeptide in muscle, balenine (also called as ophidine: β-alanyl-τ-methyl-L-histidine) exists in whales, particularly in large amounts (1,200–1,600 mg/100 g) in the skeletal muscle of baleen whale. Balenine is also abundant in the muscle of snakes such as cobra. These imidazole dipeptides play an important role as proton buffering constituents that capture protons (H^+), which are accumulated in muscle during severe anaerobic exercise and causes muscle fatigue, and thus prolong the period of anaerobic exercise. It has also been known that these imidazole dipeptides exhibit a variety of physiological functions such as scavenging activity of reactive oxygen species and anti-aging activity.

Adenosine triphosphate (ATP) and its related compounds are the most important constituents of the nucleotides, nucleosides, and nucleic acid bases which form the nucleic acids. In living cells, ATP, adenosine diphosphate (ADP), and adenosine monophosphate (AMP) are the major nucleotides in muscle depending on the energetic states. As described in Subsection 3.1, however, inosine monophosphate (IMP) is produced by AMP deaminase during the post-mortem period of fishes and crustaceans, and then decomposed to inosine and hypoxanthine. Because of the weak decomposing activities of IMP, it tends to accumulate in muscle. In other invertebrates such as squids and octopuses, AMP is decomposed to inosine and hypoxanthine via adenosine by 5′-nucleotidase. As the decomposing activities are weak, AMP tends to accumulate in these invertebrates. In the liver of squids, however, IMP also accumulate with AMP. As mentioned in Subsection 2.2, IMP and AMP contribute largely to the umami taste of seafood.

Compounds having guanidyl group in their molecules are referred to as guanidino compounds. By definition, arginine is also a guanidino compound.

Creatine works as a reservoir of ATP in muscle as phosphocreatine which is a sole phosphagen (high energy ATP reservoir) in vertebrates and is similar to phosphoarginine in invertebrates. In the initial phase of muscle exercise, phosphocreatine transfers phosphate group to ADP and is transformed into creatine along with the formation of ATP, and supports initial muscle exercise for several to several tens seconds. In the resting fish muscle, 10–30 μmol/g muscle of phosphocreatine can be detected. Phosphocreatine is generally determined as creatine by the decomposition during the preparation of extracts or preservation of fish muscle (Table 4.3). Creatine content tends to be higher in white-fleshed fishes than red-fleshed ones and higher in ordinary muscle than in dark muscle. Creatine is synthesized from arginine and glycine via guanidinoacetate (glycocyamine). Only a trace amount of creatinine is found in living cells and fresh fishes, but it is produced by dehydration and ring-formation during the process of heating or fermentation of fish.

Methylamines have so far been called as quaternary ammonium base, in which nitrogen is combined with methyl group(s). They are now collectively called as methylamines. Trimethylamine oxide (TMAO) and trimethylamine (TMA) are not found in terrestrial animals and are characteristic compounds in aquatic animals. Sharks and rays (cartilaginous fish), and coelacanths and codfishes have an extremely high amount of TMAO (Table 4.3). Marine fishes contains higher amounts of TMAO than freshwater ones but several freshwater fishes such as tilapia have also substantial amounts of TMAO. In invertebrates, TMAO content is high in the mantle muscle of squids and octopuses (cephalopoda) but low in shellfishes.

As for the TMAO synthetic pathway in fish, it is known to be produced in liver and kidney from TMA, synthesized by intestinal bacteria from choline originating from foods, and accumulated in muscle. TMA is extremely scarce in living cells but produced from TMAO mainly by the bacterial enzyme after death, and causes the fishy smell. Codfishes are specific and have TMAO demethylase (demethylating enzyme) in their muscle and produce dimethylamine and formaldehyde. The latter binds with proteins and causes the degradation of meat quality. TMAO demethylase is an especially unique enzyme and has a basic structure consisting mainly of aspartic acid.

Several kinds of betaines have been isolated and identified from invertebrates. Quantitatively major betaines in aquatic animals are glycinebetaine having chain structure and circular homarine. Trigonelline is an isomer of homarine and the contents are generally one order lower than homarine. In freshwater crustaceans, however, the content of trigonelline is higher than that of homarine and similar to that of glycinebetaine. A large amount of glycinebetaine is found in the muscle and viscera of marine invertebrates and the content is high over 1,500 mg/100 g in Ezo-neptune (*Neptunea polycostata*) and Pacific oyster (*Crassostrea gigas*). In several species, glycinebetaine is known to be an osmolyte contributing to the osmoregulation. Two synthetic pathways are estimated for glycinebetaine: one from choline and another from glycine via sarcosine. Homarine, on the other hand, is known to be synthesized by the methylation of picoline or by the pathway

from glycine and succinyl-CoA. Trigonelline was confirmed to be synthesized by methylation of nicotinic acid. These betaines also exist in plants and are known to work as stress resistance and growth stimulating factors.

Urea is synthesized from urea cycle and is a waste of amino nitrogen in mammals. Aquatic animals, however, generally excrete amino nitrogen as ammonia from gills. Fish also have urea cycle but the activity is low. Therefore, arginine, a component of urea cycle, is an essential amino acid for fish species. Urea is an important osmolyte together with TMAO in cartilaginous fishes (see Subsection 1.6). Sharks and rays synthesize urea and accumulate it in large amounts in every part of the body, from blood to muscle (Table 4.3). In sharks and rays, therefore, ammonia is formed from urea after death and their meats are redolent of ammonia. Lungfishes, which are called "living fossil" and distributed in Africa and South America, excrete amino nitrogen as ammonia from gills in wet season. In dry season, however, they burrow into the mud, secrete a kind of filamentous substances like cocoon, and estivate in it. During the estivation, disposable ammonia enters into urea cycle and is excreted as urea into urine.

As for non-nitrogenous components, trace amounts of monosaccharide such as glucose and ribose, and sugar phosphates which are components of glycolysis are found in aquatic animals and the contents increase after their death. In organic acids, lactate, a final product of glycolysis, is found in large amounts in fish muscle. Lactate is produced by the exhaustive exercise during the catch. Citrate, malate, and succinate which are components of citric acid cycle are detected only in trace amounts. In bivalve mollusks, in particular, succinate content increase when they close their shells tightly (during anaerobiosis). In this situation, propionate is also known to be produced.

(Hiroki Abe)

1.5 Components of algae

The definition of algae is difficult, as it contain two kind of organisms: one having chloroplast evolved by primary symbiosis in which primitive eukaryotes incorporated cyanobacteria, and various other organisms developed as a result of secondary symbiosis in which eukaryote having chloroplast are incorporated in the other protista. Generally, the word "algae" means macro algae, namely green algae, brown algae, and red algae. Among these, green and red algae evolved by primary symbiosis, while brown algae evolved by secondary symbiosis. Each class of algae has specific components. In this section, the characteristics of polysaccharides and algae pigments will be described.

1) Carbohydrates

In contrast to fish and shellfish, the proteins and lipids content are low in algae except for some cases, and carbohydrates are the major components. Carbohydrates in algae can be classified into structural polysaccharides in cell walls, mucilaginous polysaccharides in intercellular cement, and intracellular storage polysaccharides. There are differences in each polysaccharide among the green, red, and brown algae. The composition of polysaccharides also differs

among species in the same class of algae and is very complicated. Algae contain commercially important polysaccharides such as agar and alginic acid.

a) Cell wall structural polysaccharides

Polysaccharides composing the cell wall of green algae is cellulose in which D-glucopyranoses link together by β-1,4-glycosidic linkage. In green algae, some species have cellulose I in which the chains of glucose (glucan molecules) are set in parallel to each other and other species have cellulose II in which a part of glucans are set in the opposite direction. Several species have β-1,3-xylane or β-1,3-mannan (hemicellulose) as structural polysaccharides. Structural polysaccharides in cell walls of brown algae are composed of cellulose II and hemicelluloses. In red algae, species belonging to Florideophycea have cellulose II and hemicelluloses similar to brown algae and species belonging to Bangiophyceae such as *Porphyra* spp. do not have cellulose but have polysaccharides composed of β-1,3-xylane and β-1,4-mannan.

b) Intercellular mucilaginous polysaccharides

Green algae have water-soluble sulfate polysaccharides such as sulfur containing xyloarabinogalactan. Alginic acid and fucoidan are intercellular mucopolysaccharides in brown algae.

Alginic acid is composed of two kinds of uronic acids, namely D-mannuronic acid (M) and L-gluronic acid (G). Alginic acid consists of M block, where M molecules forms a linear chain linked to each other by β-1,4 linkage, G block, where G molecules are linked by α-1,4 linkage, and MG block, a mixture of M and G molecules (Fig. 4.7). The ratio of M and G contained in alginic acid varies among species. It also fluctuates among parts and with seasons even in the same individual. Sodium salts of alginic acid are useful for plasticizing agents in food processing.

Fucoidan is the name of sulfate polysaccharides mainly composed of L-fucose and sulfate ester (Fig. 4.7). Most of fucoidans contain galactose, xylose, glucuronic acid as well as L-fucose as component monosaccharides. Among them, fucoidan which contains less than 5% of glucuronic acid are called true fucoidan and the others are called fucan. Some fucoidans have more glucuronic acid than fucose. The structure of fucoidan is very complicated.

Agar and carrageenan are representatives of mucopolysaccharides in red algae. Both are polysaccharides formed from galactose residues. They have gel forming activities and have demands for moisturizing agent in cosmetics and food processing. Agar is a polysaccharide found in red algae belonging to Gelidiales, Gigartinales, and Ceramiales, and is a mixture of agarose and agaropectin. In agarose, D-galactose and 3,6-anhydro-L-galactose are linked by β-1,4-linkage and form a unit of disaccharide called agarobiose, and the units are linked together by α-1,3-linkage to form a linear chain of polysaccharide (Fig. 4.7). Agaropectin is basically the same as agarose but have some pyruvic acids and sulphate groups bound on some agarobiose unit of agarose. The gel forming capability of agar is high along with the increase of 3,6-anhydro-L-galactose contents and decrease of sulfate group contents. As a result, the capability depends on the contents of agarose in agar and the content varies with season.

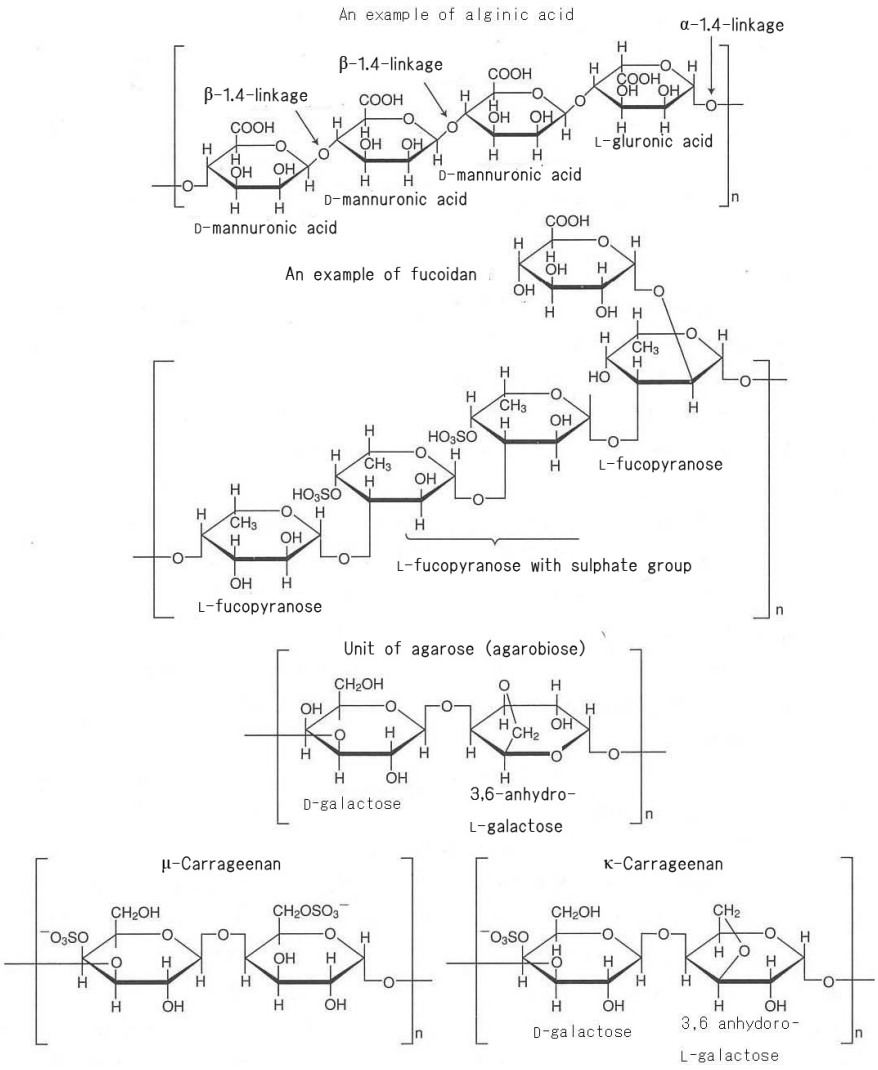


Fig. 4.7. Mucopolysaccharides in algae.

Carrageenan is also a sulfate polysaccharide distributed in red algae belonging to Gigartinaceae, Solieriaceae, and Phyllophoraceae. Based on the number and position of sulfate group on the D-galactosyl residues, carrageenans are classified as ι, κ, λ, μ, ν, ξ. D-galactose having a sulfate group at hydroxyl group of 4th position and D-galactose having a sulfate at hydroxyl group on 6th position form a disaccharide unit. The unit is linked successively by α-1,3-linkage to μ-carrageenan. κ-carrageenan is induced from μ-carrageenan, if the D-galactose

unit with a sulfate of 6th position in μ-carrageenan is replaced with 3,6-anhydro-D-galactose. Thus, μ-carrageenan is considered to be the precursor of κ-carrageenan. Similarly, ν and λ are considered to be the precursors of ι and ξ, respectively. Contents and compositions of carrageenan vary with age, region of algae and seasons. Some other red algae have porphyran and funoran.

c) Storage polysaccharides

As storage polysaccharides, green algae accumulate starch which is composed of amylose and amylopectin. The former is a linear chain of α-glucose by α-1,4-linkage, and the latter has highly branched D-glucose linked by α-1,6-linkage. Storage polysaccharide of brown algae is laminaran, which has a main linear chain of D-glucose linked by β-1,3-linkage and branches by β-1,6-linkage. Some have mannitol at reducing end and others do not. The ratio of glucose residues linked by β-1,6-linkage and existence of mannitol at reducing end differ from species. It is known that laminaran increase in summer and is inversely related with the content of mannitol. Red algae has a storage polysaccharide named as floridean starch in which α-1,4-glucan is the major component.

2) Photosynthetic pigments

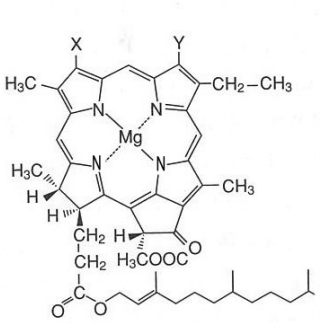
There are three kinds of photosynthetic pigment in algae, namely chlorophylls, carotenoids, and phycobilins. The distribution of these pigments in algae is highly related to the evolutionary lineage. Chlorophyll *a* is a major photosynthetic pigment in all algae and other chlorophylls, carotenoids, and phycobilins work as accessory pigments to transfer excitation energy from absorbed light to chlorophyll *a*.

a) Chlorophylls

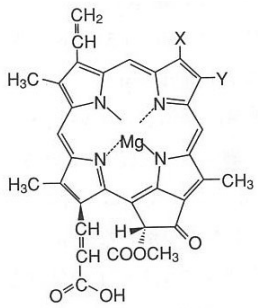
Chlorophyll (Chl) is a pigment having a fat-soluble metal porphyrin coordinated with magnesium (Mg). Chlorophyll is commonly divided into Chl *a*, Chl *b*, Chl *c*, and Chl *d* (Fig. 4.8). Green algae have Chl *a* and Chl *b* similar to that in terrestrial plants. Brown algae have Chl *a* and Chl *c*, which is further classified into Chl c_1, Chl c_2, and Chl c_3 depending on the structure of side chain. Brown algae mostly have Chl c_1 and Chl c_2 but some have Chl c_3. Red algae have only Chl *a*. Chl *d* was found in an extract from red algae but its existence as a natural compound had been an enigma for a long period. In 1996, the existence of Chl *d* was confirmed in a cyanobacterium, *Acaryochloris marina*, isolated from the body of ascidian in Palau. Another cyanobacterium in the same group was found to be attached on the red algae. Thus, Chl *d* was finally concluded to have originated from cyanobacteria (Miyashita *et al.*, 1996).

b) Carotenoids

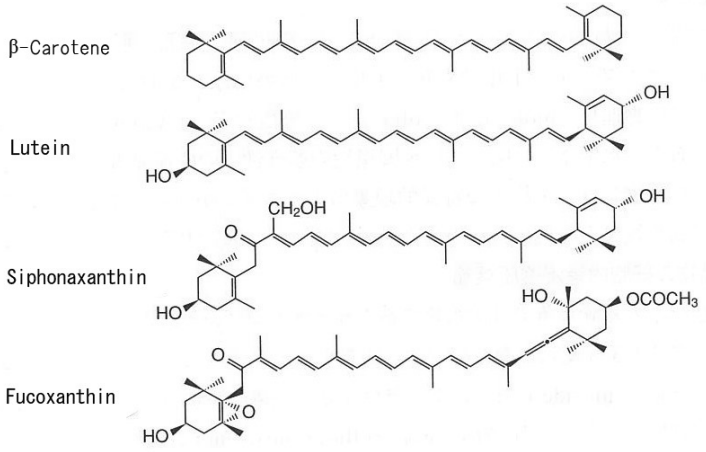
Carotenoids are basically fat-soluble tetraterpenes having 8 isoprenoid units, with a carbon number of 40. They are divided into two groups, i.e., carotenes, which are hydrocarbons, and xanthophylls, which contain oxygen atom in the molecule. Carotenoids work as antenna pigments, which absorb photoenergy at 400–500 nm and transfer it to chlorophyll for photosynthesis, and also have a function to prevent damage by excessive light. Distribution of carotenoids in algae is also related to phylogenetic lineage. Green algae have a similar composition of carotenoids as the terrestrial plants and have β-carotene

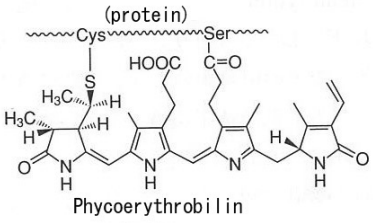


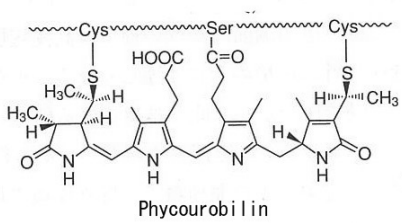
Chlorophyll *a*: X=CH=CH$_2$ Y=CH$_3$
Chlorophyll *b*: X=CH=CH$_2$ Y=CHO
Chlorophyll *d*: X=CH=CHO Y=CH$_3$



Chlorophyll *c*$_1$: X=CH$_3$ Y=CH$_2$CH$_3$
Chlorophyll *c*$_2$: X=CH$_3$ Y=CH=CH$_2$
Chlorophyll *c*$_3$: X=COCH$_3$ Y=CH=CH$_2$







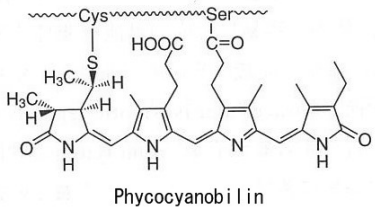


Fig. 4.8. Pigments in algae.

and lutein as their main carotenoids (Fig. 4.8). In addition, they also possess α-carotene, violaxanthin, neoxanthin, antheraxanthine, zeaxanthin etc. other than the above two major carotenoids. Several species inhabiting deeper layer have siphonaxanthin and siphonein, a fatty acid ester of siphonaxanthin. They absorb light of longer wave length (around 540 nm) than ordinary carotenoids. The wave length corresponds to green light which can reach deeper layer, suggesting the adaptation to deeper layer where the light hardly reaches. Major carotenoids in brown algae are β-carotene and fucoxanthin. The content of fucoxanthin is several times higher than that of β-carotene. Total carotenoids content is higher than that of total chlorophyll in brown algae. Thus, the algae look brown because the green color of chlorophylls is masked by the color of carotenoids, especially by the orange color of fucoxanthin. Carotenoid composition of red algae is similar to that of green algae and contains β-carotene, lutein, zeaxanthin, and so on.

c) Phycobilins

Different from the green and brown algae, red algae have phycobilins as antenna pigments for photosynthesis. In phycobilin, bilin which is a linear chain of open-ringed tetrapyrrole binds with protein. Phycobilin is a water-soluble protein and assembles on the thylakoid membrane of the chloroplast and forms granules called phycobilisome. Phycobilins absorb light energy at 550–650 nm and are traditionally classified by their spectroscopic natures into phycoerythrin, phycocyanin, and allophycocyanin. Recently, however, they are classified in detail by the nature and number of chromophore binding with protein. Phycoerythrin contains phycoerythrobilin only or phycourobilin as the prosthetic group. Six units of $\alpha\beta$-monomer composed of an α-subunit and a β-subunit associate into a hexamer. In some phycoerythrin, a γ-subunit combines with a hexamer of $\alpha\beta$-subunit. Phycocyanin and allophycocyanin have only phycocyanobilin as bilin, and basically three $\alpha\beta$-monomers aggregate into a trimer.

(Shigeru Okada)

1.6 Comparative biochemistry

The discipline of comparative physiology and biochemistry is the field to understand the physiology, ecology, morphology, behavior of species and the mechanisms of environmental adaptation using various methods including biochemistry and molecular biology. Therefore, comparative physiology and biochemistry always focuses on the biological evolution and environmental adaptation as the background of the study. Comparative biochemistry of aquatic animals has stimulated interests of scientists for a long time and recent development of analytical methods makes it possible to clarify the mechanisms of various specific phenomena of aquatic animals. In this chapter, the authors will introduce some particular phenomena of aquatic animals and their mechanisms.

1) Intracellular osmotic regulation in invertebrates

Regardless of their being freshwater or marine species, teleost, the dominant group of fishes, maintain the osmotic pressure of blood to around 1/3 of seawater.

This is also true even in terrestrial animals. Teleost fishes regulate the osmotic pressure by controlling excretion and intake of inorganic ions by chloride cells on gills (extracellular aniso-osmotic regulation). Invertebrates, however, have open blood-vascular system in which the blood (hemolymph) comes out from heart and after circulating through the whole body, returns back again to heart. In species having low salt regulatory capability at gills, the osmotic pressure of hemolymph is almost in equilibrium with that of outside medium under high salinity because of the influx of salts into hemolymph. Some species such as Japanese mitten crab (*Eriocheir japonicus*) can maintain considerably lower osmotic pressure than medium even under high salinity environment. In a freshwater environment, however, almost all invertebrate species maintain higher hemolymph osmotic pressure than outside medium owing to the transport of compounds such as nutrients through hemolymph.

Invertebrates inhabiting brackish waters have to tolerate daily fluctuations of salinity. They cannot survive if the intracellular osmotic pressure fluctuates along with the surrounding salinity changes. Thus, in high salinity environment, many invertebrates accumulate some easily biosynthesizable compounds such as non-essential amino acids, betaines, TMAO etc. (refer to Subsection 1.4) in their tissues, and increase the intracellular osmotic pressure to prevent the inflow of inorganic ions from hemolymph to cytoplasm. This is referred to as intracellular iso-osmotic regulation. The compounds adopted for this purpose are called osmolytes. Of many amino acids, basic and acidic amino acids would cause the instability of higher structure of proteins by interacting with them, if accumulated in large amounts in the cell. On the other hand, neutral amino acids have little chance to interact with proteins and avoid such influence. Especially, glycine and alanine have short side chains and no additional functional groups interacting with proteins. For this reason, these amino acids and taurine, betaines, TMAO etc. are named "compatible solute" (see Table 4.3).

Figure 4.9 shows the changes of free amino acid contents in the muscle of red-swamp crayfish (*Procambarus clarkii*) during acclimation from freshwater to full-seawater. Direct transfer from freshwater to half-seawater causes no harmful impact on the physiology of crayfish, although the acclimation to full-seawater requires longer time. The osmolytes which increase under high salinity environment differ from species and organs in a particular species. In the muscle of crayfish, D- and L-alanine, L-glutamine, glycine, and L-proline increased significantly and the content of total free amino acids doubled during acclimation from freshwater to full-seawater. These amino acids are the osmolytes responsible for iso-osmotic regulation in crayfish. Taurine increased only in hepatopancreas and only D- and L-alanine and L-proline are responsible for the increase of total amino acids in nervous tissues. As shown in the metabolic pathway in Fig. 4.10, these amino acids are easily biosynthesized via glycolytic pathway and citric acid (TCA) cycle. Salinity acclimation is a rapid process, in which amino acid increase can reach equilibrium within 24–36 h after the transfer to high salinity environment. Although the physiological functions of free D-amino acids have not so far been understood in invertebrates, the function of D-alanine as osmolyte was known

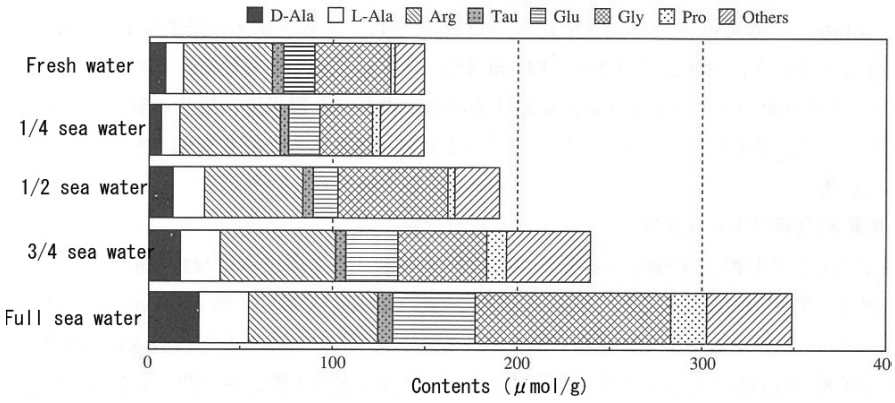


Fig. 4.9. Fluctuation of amino acid during acclimatizing process of from fresh water to seawater in red swamp crawfish.

only recently. D-Alanine contributes to iso-osmotic regulation at least in crustaceans and bivalve mollusks belonging to subclass Heterodonta. Bivalves belonging to Pterimorphia such as Pacific oyster (*Crassostrea gigas*) use taurine almost as a sole osmolyte. It is known that crustacean and Heterodonta bivalves acclimated to high salinity become tastier because of the increase of salts and sweet amino acids such as glycine and alanine.

2) Protein stabilizing function of TMAO in sharks and rays

Unlike teleost fishes, the osmotic pressure in the blood of cartilaginous fishes is almost similar to that in seawater. Cartilaginous fishes accumulate large amounts of urea and TMAO in all tissues including blood and increase the osmotic pressure (see Subsection 1.4). Urea is an agent for denaturing of protein and its accumulation is a fatal blow for the internal environment of the cells. TMAO, however, has a highly stabilizing activity against the protein structure and is considered to be a "counteracting solute" which anuls the protein denaturing action of urea. As shown in Table 4.3, both urea and TMAO contents are considerably high in salmon shark (*Lamna ditropis*) and the molecular ratio of urea:TMAO is 253:147 μmol/g which is close to 2:1. When urea and TMAO plus other methylamines exist in this ratio, the effect of urea on protein structure is considered to be minimal. In addition, it is known that proteins in sharks and rays are rather resistant to the denaturing effect of urea.

3) Hypoxia/anoxia tolerance

Although all animals must respire molecular oxygen to survive, aquatic animals usually have to tolerate the low oxygen concentration, which is only 1/30 of terrestrial environment. Thus, some aquatic animals have evolved high tolerance against hypoxia or anoxia. Tolerance to hypoxia has been well studied in diving marine mammals such as whales and seals. Pacific oyster is a champion at tolerating anoxic conditions, and survives for 3 weeks at 25°C after shell

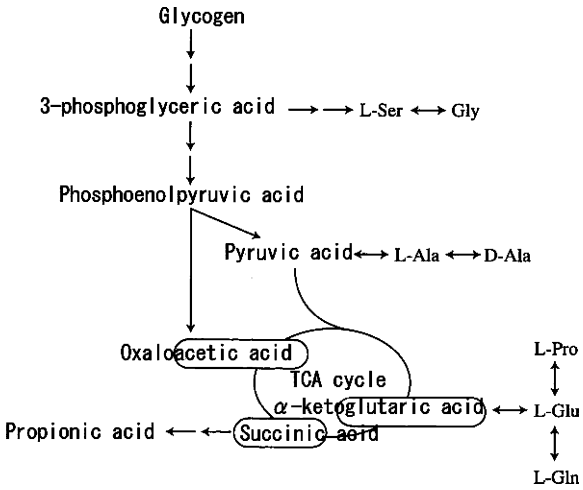


Fig. 4.10. Metabolic pathway of amino acids and organic acids which increase during acclimatization process to hyper osmotic pressure in invertebrates.

closure where the oxygen disappears promptly. Some other bivalves such as hard clam (*Meretrix lusoria*) can also survive for about 10 days. However, if they accumulate lactate as an anaerobic end product under anoxia, they cannot survive for such a long time. The handling of anaerobic end products is a serious problem for animals under anaerobiosis. In invertebrates, pyruvate produced in glycolysis is not transformed to lactate, but reacts with amino acids to form neutral compounds collectively called opines. Several different types of opines are produced in different invertebrate species. When pyruvate reacts with arginine, the product is called octopine. The other known opines are alanopine (reacts with alanine), strombine (glycine), tauropine (taurine), and β-alanopine (β-alanine). All opines are produced by several different dehydrogenases generally termed as opine dehydrogenase. These reactions regenerate NAD^+ with the consumption of NADH, similar to lactate dehydrogenase reaction. By these reactions, therefore, redox balance (recycle of NAD^+) in glycolysis can be maintained.

Other than the production of alanopine from pyruvate under anaerobiosis, bivalve mollusks also produce succinate from phosphoenolpyruvate. In this pathway, oxaloacetate produced from phosphoenolpyruvate by phosphoenolpyruvate carboxykinase, follows the inverse direction of TCA cycle to make succinate via malate and fumarate. Mollusks can also produce propionate from succinate. It has been clarified that bivalves accumulate alanine and succinate from pyruvate in the early phase of anaerobiosis and also accumulate alanopine and propionate during the prolonged anaerobiosis. The carbon flow is regulated at the stage of phosphoenolpyruvate and the stage is called "phosphoenolpyruvate branch point". Under anoxic conditions, lowering the pH and alanine accumulation in muscle inhibit the activity of pyruvate kinase, which

produce pyruvate from phosphoenolpyruvate, and activate the phosphoenolpyruvate carboxykinase reaction. These changes lead the carbon flow to oxaloacetate. When lactate or alanopine is produced from a glucose unit of glycogen, 3 ATP molecules are obtained. When succinate or propionate is produced from a glucose unit, 5 or 7 ATP molecules are recovered, respectively. Thus, the ATP yield is improved by this metabolic shift. Under anoxic stress, bivalves largely suppress their metabolism to reduce the consumption of glycogen. The same strategy is adopted by other invertebrates. It is known that some parasitic helminths such as roundworm (*Ascaris lumbricoides*) also accumulate succinate and propionate in the body of mammals, the final hosts, where oxygen occurs only periodically.

In squid and octopus, phosphoarginine is used as ATP reservoir (see Subsection 1.4) and produces a large amount of arginine in the early phase of severe exercise. Arginine is a basic amino acid and has positive charge in its side chain. It causes harmful impact on the structure of proteins and enzyme activities in cytosol. To prevent the effect, squids and octopuses seldom produce lactate as an anaerobic end product but accumulate octopine produced from pyruvate and arginine. Octopine is a neutral ampholyte with far lesser effect on protein structure than arginine. The formation of octopine from pyruvate and arginine by octopine dehydrogenase needs NADH and NAD^+ is regenerated by the reaction. As a result, the redox balance is maintained as in the case of lactate production. As stated so far, in contrast to the terrestrial animals, several animals in aquatic environments do not produce lactate by anaerobic metabolism under hypoxic stress or during exercise.

Even in fish, some species can tolerate hypoxic stress and do not produce lactate. Cyprinid fish can tolerate high temperature and hypoxic stresses. When goldfish and common carp await spring in a small pond that freezes over, they do not produce lactate but ethanol, and release it to the outside water to prevent the accumulation of anaerobic end product. There are two possible pathways for the production of ethanol: via acetaldehyde in the same way as ethanol production by yeast and via acetyl-CoA and acetaldehyde, the former being considered as the main pathway. Besides, the fish suppress consumption of ATP by remaining dormant, which enables it to survive the long freezing winter.

(Hiroki Abe and Naoko Yoshikawa)

1.7 Genetic engineering

1) Structure and function of genes

The gene is composed of nucleic acids. A nucleotide, the unit of nucleic acids as well as an important compound for energy metabolism, is composed of a base, a phosphate group and sugar. The bases are classified into purines, with a combination of six-member and five-member rings, and pyrimidines, composed of a six-member ring. Ribose or deoxyribose is combined with bases and form nucleosides. Phosphate groups bind with carbon at 5′ position of the sugar to form nucleotides. Nucleic acids are long chains of nucleotides each of which has one

phosphate. Nucleic acids with deoxyribose are DNA (deoxyribonucleic acid) and that with ribose are RNA (ribonucleic acid). The four bases contained in DNA are adenine, guanine, cytosine and thymine. Important functions of genes composed of DNA are transmission of heredity to the next generation by DNA duplication and transcription of genetic information through RNA. In RNA, uracil is contained in place of thymine in DNA. RNA functions as ribosomal RNA and transfer RNA, whereas messenger RNA (mRNA) is used as a template in the synthesis of proteins (translation).

2) Principle genetic techniques

Basic techniques in genetic engineering are cleavage, binding, amplification and determination of base sequence of DNA. Restriction enzymes used for cleavage of DNA are endonucleases which recognize specific nucleotide sequences in DNA and cleave DNA at specific position. There are various restriction enzymes which recognize different nucleotide base sequences. The enzyme for binding DNA is ligase which can combine any two DNA. The reconstructed DNAs by cleavage and rebinding are called recombinant DNAs. There are two methods for amplification of DNA. One is to introduce recombinant DNA to organisms such as *E. coli* and use *in vivo* amplifying system. The other is to amplify specific regions of DNA by polymerase chain reaction (PCR). For RNA, cleavage by restriction enzymes and amplification by PCR are possible after preparing complementary DNA (cDNA) by reverse transcription reaction using RNA as template.

Two methods are available for determination of nucleotide sequences. Automatic DNA sequencers, which employ cycle sequencing method adopted from the method of Sanger have prevailed dominantly.

3) Recombinant proteins

When a recombinant DNA with information of amino acid sequence of protein is introduced to cells after inserting into the vector with proper transcription regulatory sequence, cells transcript mRNA from DNA and subsequently produce the protein. Proteins thus obtained are called recombinant protein. *E. coli* and yeast are commonly used as host to introduce DNAs. *In vitro* expression systems composed of enzymes for transcription and translation are also available. These methods are useful to obtain proteins which are at low levels in living body and difficult for purification in bulk. Trials have been implemented for production of various hormones as recombinant proteins and their application. One of the issues to be solved for the application of recombinant proteins is posttranslational modification such as glycosylation and polymer formation in host organisms. For this, utilization of fish eggs as host to produce recombinant proteins has been tried, resulting in the production of active gonad stimulation hormone (GSH) of goldfish, dimer glycoprotein in rainbow trout egg was reported.

4) Molecuar phylogenics and species identification

Substitution in the nucleotide sequence of DNA result from errors in genetic duplications, damages by ultraviolet, and so on. That is so-called mutation. Disadvantageous mutations for living organism are eliminated during evolution, though advantageous or not disadvantageous mutations are not eliminated. As a

result, mutations are accumulated in DNA with the progress of time during evolution. Pauling compared amino acid sequences of hemoglobin, protein transporting oxygen in blood, among species showing that though hemoglobins from various species show similar functions, they have different amino acid sequences. Hemoglobins from human being and carp show 49% amino acids variations. Further, they have liner correlation between the number of variations in amino acid and year of their diversification. Since 1980s, molecular phylogenetic analysis for estimation to determine evolutionary and genetic relationship among taxa based on the amino acid or DNA nucleotides sequences became common and new concepts of genetic relationship among aquatic organisms have been proposed.

Species identification from species specific nucleotide sequences used in molecular phylogenetic analysis is possible. Species identification by molecular phylogenetic methods enables species identification of larval fish and tissue materials of which species identifications from external morphology are impossible. These methods have also advantage in species identification of rare samples with extremely small quantities, because DNA can be amplified enormously by PCR. Further, DNA is heat stable and PCR can amplify specific DNA sequence from mixed samples. Therefore, the method is also useful to detect materials used for processed food such as canned, filleted and surimi-based sea food. For identification of species, the determination of nucleotides sequence is most accurate. However, we need longer time, and it is laborious to determine nucleotide sequences of all samples for a large number of samples. To settle this problem, other methods are adopted to detect the differences in the nucleotide sequence. Single strand length polymorphism (SSLP) is used to detect the difference in the length of nucleotide sequence using repeated sequences which are commonly formed in microsatellite in the sequence. If there are differences in the number of repeats between any two species, they have different DNA lengths. We can easily detect their differences by electrophoresis. If there is no difference in the length of DNA, they may have different nucleotide sequences recognized by restriction enzymes. In this case, we can detect the differences by electrophoresis as the differences in the length of fragments (restriction fragment length polymorphism: RFLP) after treatment of DNA with restriction enzymes. Another method developed to detect single nucleotide polymorphism (SNP) is the single strand conformation polymorphism (SSCP) using differences in higher structure of single strand DNA and hybridization with complementary DNA.

It is thought that even in the same species, population specific nucleotide sequence will exist in local populations in habitats separated for long period. These differences are useful for identification of local origins. Even in cases where the separation is incomplete and several levels of exchanges of individuals occur between the local populations, the local populations may have different frequency of individuals with altered nucleotide sequences. For detection of local population, we should analyze plural numbers of individuals, and not one single individual, and differences in the frequency of specific genes should be statistically analysed. This method is used in population genetics.

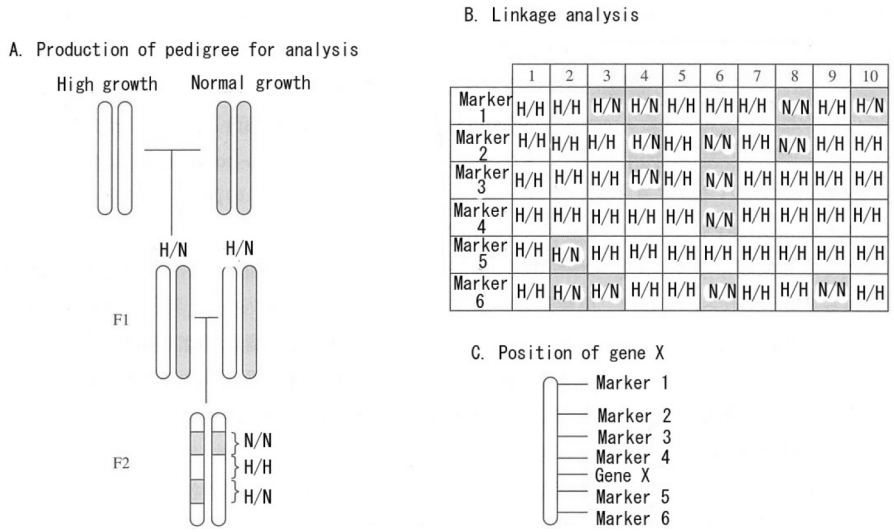
5) Genome project

Genome project is an attempt to analyze all genetic information of a species. For this purpose, determination of whole DNA nucleotide sequences of the genome has been undertaken in various species. In aquatic organisms, puffer fish has gained attentions due to its smallest genome size in vertebrates. The genome size of puffer is 1/8 of human genome. Outlines of the whole genome sequences were reported for tiger puffer (*Takifugu rubripes*) and green puffer (*Tetraodon nigroviridis*) prior to the advance in other vertebrates except human being. The other project now under progress are that of zebra fish (*Ddanio rerio*), medaka (*Oryzias latipes*) and threespine sticleback (*Gasterosteus aculeatus*) in osteichthyes, amphibians, protochodates and algae including porphyra. As a result, the comparison in the whole genome, not the comparison in respective genes, is now possible. These data base use powerful tools to clarify evolutionary process of living organisms and extract functional gene information from huge DNA nucleotide sequences. Nowadays, performance of DNA sequencers is rapidly improving. Next generation sequencers which can read several billions of base pairs in one run are now available. Now we can analyze whole genome sequences of individuals, can detect the individual difference by using SNP, and analyze differences in the phenotype in relation to genomic differences at the individual level.

6) Linkage analysis and identification of quality trait loci

The phenomena in which a set of genetic loci on the same chromosome inherit together to the next generation are called genetic linkage. Loci on different chromosome inherit independent of each other and do not link, although some loci on the same chromosome sometime also happen not to link. The linkage probability between two loci on the same chromosome is high when their physical distance on the chromosome is short and the probability is low when the distance is long. Therefore, by analyzing the linkage probability among various loci on a same chromosome, we can obtain the information of positional relationship of various loci on the chromosome. This is the linkage analysis and the map showing position of genes is called linkage map. The linkage probability between any two genes is expressed by genetic distance. Genetic distance is proportional to the physical distance of the two genes in DNA.

Using linkage map, we can find genes relating to specific phenotypes. As an example, Fig. 4.11 shows the process to identify the genes relating to the phenotype of high growth. This process is gene mapping by using linkage analysis. Actually, many genes are thought to have phenotype of high growth, which are called as quantitative trait. We need to evaluate the degree of linkage between any two genes statistically. Quantitative trait loci (QTL) analysis is a method to identify the position of genes relating to quantitative trait. In aquaculture, strains which have advantageous genetic characters such as fast growth and disease resistances have been established by selective breeding. Linkage maps have been constructed for Atlantic salmon (*Salmo salar*), tiger puffer (*Takifugu rubripes*), yellowtail (*Seriola quinqueradiata*), goldstriped amberjack (*Seriola lalandi*), sea bream (*Pagrus major*), rainbow trout, tilapia, catfish, carp, kuruma



	1	2	3	4	5	6	7	8	9	10
Marker 1	H/H	H/H	H/N	H/N	H/H	H/H	H/H	N/N	H/H	H/N
Marker 2	H/H	H/H	H/H	H/N	H/H	N/N	H/H	N/N	H/H	H/H
Marker 3	H/H	H/H	H/H	H/N	H/H	N/N	H/H	H/H	H/H	H/H
Marker 4	H/H	H/H	H/H	H/H	H/H	N/N	H/H	H/H	H/H	H/H
Marker 5	H/H	H/N	H/H	H/H	H/H	H/H	H/H	H/H	H/H	H/H
Marker 6	H/H	H/N	H/N	H/H	H/H	N/N	H/H	H/H	N/N	H/H

Fig. 4.11. Mapping of high growth gene using linkage map. A: Production of pedigree for analysis, F1 are produced from crossbreeding of individuals which have high growth phenotype and normal growth phenotype. The F2 generation is produced by backcrossing between parent and F1 or intercrossing among F1. This process is for production of pedigree for analysis. Chromosome of F2 are mixture of chromosomes originated from high growth individual and normal growth individual. B: Linkage analysis. The relation between the phenotype (high growth or normal) and genotype (origin of the gene) of which locus on the chromosome are known (marker gene) are analyzed. The genes of which ratio of homozygous on the gene in high growth individuals are larger than 50% link with gene related to growth, and the genetic distance between the loci of marker and the gene related to growth can be calculated. The figures are indicating the result of the analysis using 6 markers on the chromosome using ten F2 individuals representing high growth. Maker 4 and 5 are strongly linked with the genome X (high-growth related gene) with increase of distance between loci of the genome X and marker, linkage became weak. The loci of the gene X is located between the loci of Marker 4 and 5.

shrimp (*Penaeus japonicus*), tiger shrimp (*Penaeus monodon*), and the maps are useful for exploration of genes relating to prominent phenotypes.

7) Screening of mutant fish

Screening of mutants which expresses interesting phenotype is conducted by induction of mutation in DNA in high efficiency using ultraviolet and treatment with chemicals such as alkylating agent. Once responsible genes are identified by mapping described above, we can clarify the function of the gene screened. This analysis requires existence of linkage map, the treatment of a large number of individuals and short generation time. This analytical strategy was first developed for nematodes and drosophila. In 1990s, a large scale screening of mutants was implemented in zebrafish (*Danio rerio*) and, later, similar analysis was performed for medaka (*Oryzias latipes*). Zebrafish and medaka are particular species in

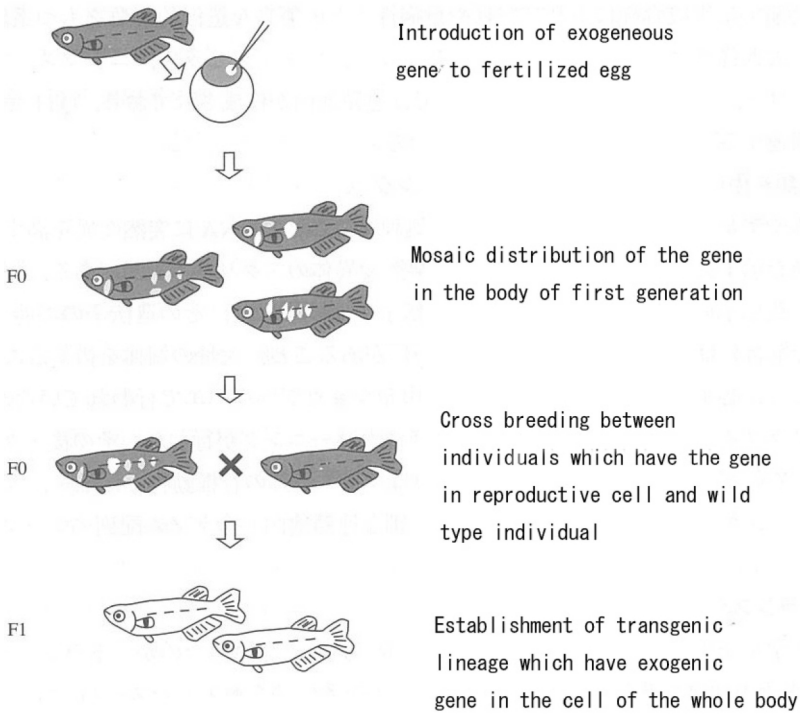


Fig. 4.12. Production of transgenic fish. Firstly, the gene is introduced into fertilized egg. Microinjection is mainly used for the introduction. In the case of introduction of gene to fertilized egg, the gene can be introduced in only a portion of the cells and F0 is the cell mosaic of the gene. Fo are divided from further generation as transient transgenic fish. In the individuals which have the gene in reproductive cells in Fo can produce eggs or sperm which has the gene, the individuals from the eggs or sperm have the gene in the cells in whole body. The transgenic lineage in fish in which the gene is fixed (stable transgenic fish) can be produced by this method.

vertebrates except mouse where large scale mutant screening is possible. Detailed linkage map and whole genome sequence data of the two fish are now available.

8) Transgenic fish

Production of transgenic organisms has been developed by introducing gene engineering technology combined with cell engineering and embryological engineering technologies. The production of transgenic super mouse in which exogenous growth hormone gene was introduced were reported in 1985. However, it is easier to introduce exogenous genes into fish, because fish eggs develop outside the mother's body. Basic protocol for the production of transgenic fish is shown in Fig. 4.12. Since mid 1980s, the transient gene expression of exogenous genes has been reported. In 1988, a stable transgenic line of zebrafish in which exogenous genes were incorporated into chromosomes was reported. Since then, transgenic fish incorporating various exogenous genes have been reported in

various fish species. The production of super coho salmon which was 37 times larger than wild type in weight by expressing excess growth hormone is a well known example. Not only the gene transfer but also elimination of specific gene is possible. Knockout mouse is an example. This technology is based on murine embryonic stem (ES) cell, which has both pluripotency and indefinite proliferative potential. At first, EM cells which lack a gene is produced using homologous recombination. Next, individuals which lack the gene in whole body is produced from ES cells. In fish, aiming for establishment of knockout technology, the establishment of culture cells which has pluriopotency and the production of germ line chimera are on trial not only in model fish such as zebrafish and medaka but also in cultured fish such as sea bream and flounder.

Visualization of molecules in living body, live imaging, by the introduction of fluorescent protein gene expands the possibility for the utilization of transgenic organisms. This method is frequently employed in research for developmental biology and especially useful for fish, where eggs are transparent and their development progresses outside the body. Transgenic fish in which various tissues and cells are visualized have been developed predominantly in zebrafish and medaka. The production of rainbow trout sperm and eggs in the body of seema (*Oncorhynchus masou*: Yamame) were successful. In the trial, primordial germ cells were visualized by green fluorescent protein (GFP) to pick up. Womb borrowing technique to use foreign womb which produce next generation in the body of different species has possibilities for various application such as conservation of endangered species and production of knockout fish. The research is getting attention from various research field.

In relation to the treatment of transgenic organisms, Cartagena Protocol for biosafety was adopted in 2000 for the protection of biodiversity. After the adoption, the treatment of transgenic organisms has been strictly regulated by law.

(Yoshiharu Kinoshita and Shugo Watabe)

2. NUTRITIONAL AND PHYSIOLOGICAL FUNCTION OF SEAFOOD

2.1 Nutritional function

In view of the rising problem of obesity and life style-related diseases in the present world, superiority of Japanese dietary habits or eating fish is now globally recognized and "*sushi*" has gained global popularity. Protein content of fish is about 20% which is almost similar to that of terrestrial meats and poultry. In olden times, fish meat was considered to be low in quality than the meats of terrestrial animals, because of low tryptophan content. After the reduction of tryptophan requirement level in the standard for amino acid score, fish meat is considered to be a good protein source having 100 or nearly ideal amino acid score, the score used for evaluation of protein quality estimated from amino acid composition. On the other hand, there is a large species difference in protein contents among invertebrates and their amino acid scores are generally lower than fish meat.

Lipid content of fish meat varies widely depending on the species and seasons from nearly 0% to 30% as in tuna and eel (see Subsection 1.3). Lipid contents of invertebrates are generally less than a few percent, except for the gonad of sea urchin which is around 10%. Thus, invertebrates are low fat diets. The seafood lipids have high concentrations of IPA and DHA, which is one of the important benefits of eating fish (see Subsection 1.3).

Seafood is generally considered to have high cholesterol. However, if cholesterol contents of the liver of anglerfish (*Lophius litulon*, 560 mg/100 g), and the fully matured *shishamo* (*Spirinchus lanceolatus*) and Japanese smelt (*Hypomesus nipponensis*) with roe (210–230 mg) are excluded, fish cholesterol is as low as that in the meat of terrestrial animals and poultry (50–80 mg). Although the cholesterol content in fish roe is high (350–480 mg), the values are not comparable to that in chicken egg (1,400 mg). The content shows relatively high value in the muscle of eel (230 mg) and conger eel (140 mg). Apart from fish meat, cholesterol contents in the muscle of invertebrates are generally high as seen in squids (210–350 mg) but the contents in bivalves and crabs are as low as those in fish. Whereas many seafood contain rather large amount of cholesterol, they are very rich in taurine and IPA, which have cholesterol reducing activities. Thus, eating seafood is not a problem for those not suffering from hypercholesterolemia.

Among the fish, taurine is rich in white-fleshed fishes and in the dark muscle of red-fleshed fishes. Invertebrates accumulate larger amounts of taurine than white-fleshed fishes (Table 4.3). In particular, mollusks contain a large amount of taurine which reaches around 1.5–2 g/100 g in some species. Taurine can be obtained almost only from seafood. Due to the preventive effects on hypercholesterolemia and arteriosclerosis, it is thought to contribute largely to the prevention of cardiac disease in fish eating people.

Taurine also has an antihypertensive function which is due to the suppression of the excitation of sympathetic nerves. It is known that taurine lowers blood pressure as well as heart rate by suppressing the excretion of adrenaline and noradrenaline. Moreover, it is also known to reduce reactive oxygen species, accelerate the development of retina, and detoxication in liver.

Seafood is also a good source of several vitamins. The contents of several vitamins are shown in Table 4.4. Among the fat soluble vitamins, vitamin A is rich in fish especially in the liver. Aged big fish such as tuna and seabass (*Stereolepis ischinagi*) have a prominently large amount of vitamin A in liver and eating the liver of these fishes sometimes causes vitamin A intoxication. Vitamin A_1 (retinol) and A_2 (3-dehydroretinol) are rich in liver of marine and freshwater fish, respectively. The content of vitamin A in muscle including fish and invertebrates are around several tens μg/100 g. Algae in dried form are also rich in vitamin A. Although brightly colored vegetables contain large amounts of carotene and are rich vitamin A source, fish and algae are also good sources of vitamin A supply. Vitamin A is essential for the growth and differentiation of organs, strengthens skin and mucous membrane, and participates in visual functions.

Table 4.4. Distribution of vitamins and calcium in fisheries products.

	Vitamin A Equivalent amount to retinol (μg/100 g)
Liver of monkfish	8,300
Lampern	8,200
Liver of eel	4,400
Eel	2,400
Firefly squid	1,500
Sablefish	1,100
Conger eel	500
Dried Nori	7,200
Dried Matsumo (*Analipus japonicus*)	5,000
Dried Iwanori (natural *Porphyra*)	4,600
Dried green laver	2,800
Chiken liver	14,000
Pork liver	13,000
Cow liver	1,100
Beefsteak plant leaf	1,800
Carrot	1,510
Chili	1,280
Spinach	1,000

	Vitamin D (μg/100 g)
Liver of monkfish	110
Dried sardine	50
Dried anchovy larva	46
Thread-sail filefish	43
Indo-Pacific blue marlin	38
Sockey salmon	33
Chum salmon	32
Bullet tuna	22
Pacific herring	22
Pacific saury	19
Pacific bluefin tuna (fatty tissue)	18
Duck	32.5
Beaf	0–2.1
Pork	0.1–2.0
Chicken	0–0.4
Dried snow fungus	970
Jew's ear fungus	435
Dried shiitake mushroom	16.8

Table 4.4. (continued).

	Naiacin (mg/100 g)
Salted ovary of Alaska pollock	49.5
Albacore	20.7
Skipjack tuna	19.0
Yellowfin tuna	17.5
Bullet tuna	16.2
Amberstripe scad	15.2
Pacific bluefin tuna	14.2
Indo-Pacific blue marlin	13.5
Bigeye tuna	13.5
Barred marlin	10.4
Chub mackerel	10.4
Yellow tail	9.1
Beaf	0.7–7.6
Cow liver	13.5
Pork	1.4–9.4
Pork liver	14
Chicken	3.3–11.6
Chicken liver	4.5

	Vitamin B_{12} (μg/100 g)
Salted kidney of salmon	327.6
Bowel of natural ayu	60.3
Salmon caviar	47.3
Liver of monkfish	39.3
Japanese fluvial sculpin	28.2
Salted ovary of Alaska pollock	18.1
Pacific saury	17.7
Pacific herring	17.4
Round herring	14.2
Corbicula spp. (shijimi)	62.4
Bloody clam	59.2
Japanese littleneck	52.4
Beaf	0.3–2.6
Cow liver	52.8
Pork	0.2–0.6
Pork liver	25.2
Chicken	0.1–0.7
Chicken liver	44.4

Typical example of vitamin D deficiency is rickets, causing spinal curvature. As the pathogenesis of rickets has become very rare in recent years, the interest in vitamin D has rather declined. However, vitamin D has again attracted interest recently due to the rise in osteoporosis problem. Vitamin D accelerates the

Table 4.4. (continued).

	Calcium (mg/100 g)
Sweatend dried anchovy	2,500
Dried anchovy	2,200
Seasoned dried silver-stripe round herring	1,400
Boiled crucian carp in soy sauce	1,200
Boiled goby in soy sauce	1,200
Oriental wheather loach	1,100
Dried anchovy sheet	970
Boiled smelt in soy sauce	970
Dried Japanese sand lance	740
Dried shrimp	7,400
Dried sakura shrimp	2,000
River snails	1,300
Hijiki	1,400
Dried *Monostoroma*	920
Dried *Laminaria*	900
Dried Arame (Eisenia bicyclis)	790
Dried Wakame (*Undaria*)	780
Bovine milk	110–130

absorption of calcium from intestinal tract and is essential for the maintenance of homeostasis and osteogenesis. Vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecaciferol) are the common forms of vitamin D. Vitamin D_3 content is high in fish. As terrestrial animals and invertebrates only have a small quantity of vitamin D, it is no exaggeration to say that vitamin D is a fish specific vitamin (Table 4.4). Vitamin D is abundant in migratory red-fleshed fishes, eels, and salmons, and its intake is not anticipated without eating those fishes. Although duck meat has exceptionally large amounts of vitamin D, the other terrestrial meats and poultry contain only a trace amount of vitamin D.

Water soluble vitamins are generally abundant in liver and dark muscle of fish. Vitamin B_1 level in the muscle of freshwater fishes is high and almost the same as that in pork. Vitamin B_2 content is high in migratory red-fleshed fishes, loach, mud snail (*Cipangopaludina* sp.), and freshwater clam (*Corbicula* sp.). Niacin is a precursor of NAD^+ and $NADP^+$, which are coenzymes of oxidoreductases, and found to be more in active fishes than in inactive ones (Table 4.4). Niacin is a unique vitamin common in ordinary muscle than in dark muscle. Fish is a good source for niacin supply. Vitamin B_{12} is an animal vitamin and not found in plants. Liver and other visceral organs are rich in vitamin B_{12}, and the content is also high in the dark muscle of fish. Unexpectedly, bivalves such as freshwater clam and ark shell (*Scapharca broughtonii*) contain a large amount of vitamin B_{12}. Cows and pigs have a large amount of B_{12} in liver,

although the content is very low in muscle. Seafood is a good source of vitamin B_{12} supply.

Seafood is also abundant in minerals. It may generally be believed that fish contain a large amount of calcium. The contents, however, are generally not so high except for small fishes eaten with bone or shrimps eaten with shell. As shown in Table 4.4, calcium contents are high in traditionally processed seafood such as *tsukuda-ni* (boil fish with soy sauce). Algae such as Hijiki (*Hizikia fusiforme*) are also good calcium source. Intestinal absorption rate of calcium is 50% for cow's milk, 35% for fish bone, and 20% for algae. However, small fish is a good calcium source after milk.

Iron (Fe) content is high in red-fleshed fishes containing myoglobin in large amounts, but is higher in bivalves such as oyster than in fish. Mud snails (*Cipangopaludina* sp.) commonly inhabited paddy fields exceptionally have large amounts of various minerals such as calcium, iron, and magnesium.

Trace elements such as zinc (Zn), copper (Cu), and selenium (Se) are known to be indispensable and important nutrients. Trace elements are accumulated in organisms through respiration and food chain in the sea, a large pool of trace elements. As a result, seafood have large amounts of trace elements depending on the species. The accumulation of metals sometimes causes problems in food safety such as mercury in tuna and arsenic in Hijiki (see Subsection 4.2).

Epidemiological surveys by WHO and other institutions have reported that the more the fish eaten, the lesser is the risk of cancer, and that Japanese diet is the major reason for health and long life of Japanese people (Konosu *et al.*, 1994). The benefit of fish eating is now globally recognized.

(Hiroki Abe)

2.2 Physiological functions

The various tastes of seafood are probably the most understood physiological functions of the seafood components. Seafood, particularly invertebrates, are fascinating for their specific tastes, which are mainly produced by low molecular weight organic compounds such as free amino acids (see Subsection 1.4). These compounds, named as "taste-active components" characterizing the special taste of seafood, have been identified in a dozen of delicious seafood. Table 4.5 shows the taste-active components of fresh seafood determined so far. Although not listed in the table, sodium, potassium, and chloride ions are taste-active components in all seafood so far analyzed, with phosphate ion also being the taste-active component in many species. The taste effects of these ions are very high. For example, salt is the most effective suppressor of the bitterness of arginine and increases the sweetness of glycine and alanine and the basic taste (umami) of glutamate.

Even though the contents vary among species, glutamate is a common taste-active component in all species. Regardless of the content in food, glutamate shows umami synergistically with nucleotides, increases sweetness, and also

Table 4.5. Taste-active components in fresh fish and shell fish.

Chemical compound	Green sea urchin[1]	Snow crab[1]	Japanese spiny lobster[2]	Fan lobster[2]	Abalone (*Haliotis discus*)[1]	Japanese scallop[1]	Japanese littleneck[1]	Bigfin reef squid[3]	Yellowtail[4]
Free amino acid									
Glutamic acid	103	19	12	15	109	140	90	4	15
Glycine	842	623	1,200	580	174	1,925	180	896	
Alanine	261	187	95	50		256		178	
Arginine		579	520	710		323	53	689	
Proline			120	245				1,029	
Valine	154			60					
Methionine	47			25					
Others				Ile 45			Tau 555		His 800
				Leu 45					*
Nucleotide									
AMP		32	110	190	90	172	28	249	
IMP	2		125						340
GMP	2	4							
Methylamine									
Glycinebetaine		357	550	900	975			1,042	
TMAO			445	1,040				678	
Sarcosine				40					
Organic acid									
Succinic acid							65		
Others		CMP 6							

*α-aminobutanoic acid (2 mg/100 g), γ-aminobutanoic acid (5), α-aminoadipic acid (10), β-isobutyric acid (1).
Ile: Isoleucine, Leu: Leucine, Tau: Taurine, His: Histidine.
[1]Fuke and Konosu (1991), [2]Shirai *et al*. (1996), [3]Kani *et al*. (2008), [4]Kubota *et al*. (2002).

increases flavor characteristics such as taste body. Glycine is a sweet amino acid and contributes to the sweet taste of invertebrates as a common taste-active component in invertebrates, and alanine and proline, also the sweet amino acids, are taste-active components in many species. Arginine is a bitter amino acid but the bitterness is suppressed by glutamate, adenosine monophosphate (AMP), and salt, and represents slight sweet taste and increases flavor characteristics.

The other amino acids are species specific. Valine in green sea urchin (*Hemicentrotus pulcherrimus*) contributes to the specific bitterness of the gonad, and methionine contributes to the specific taste of sea urchin. The amino acids are also taste-active in shovel-nosed lobster (*Ibacus ciliatus*). Although not shown in the table, methionine is also taste-active in fish sauce. We cannot detect any taste for the taurine crystal, but it is a taste-active component only in short-necked clam (*Ruditapes philippinarum*). It is considered that taste panels could detect the weak taste of taurine in the clam because the contents of the other taste-active components are very low in clam. A large amount of histidine is considered to be taste-active in yellowtail and contributes to the sour and umami tastes. In yellowtail, amino acids in minor quantities, which do not constitute proteins, are judged to be taste-active.

Among the nucleotides, AMP occurs in large amount in the invertebrates, and has been judged to contribute to the umami taste in invertebrates by synergistic action with glutamate. In several species, inosine monophosphate (IMP) and a trace quantity of guanosine monophosphate (GMP) contribute as taste-active components. Cytidine monophosphate (CMP) is a taste active component only in snow crab (*Chionoecetes opilio*). In yellowtail, a large amount of IMP is a major taste-active component. Thus, the synergistic action between glutamate and IMP is the base of the taste of fish meat.

In methylamines, a large amount of glycinebetaine which has a bitter taste with weak sweetness and considered to add thickness and complexity to taste is considered to be a taste-active component in various invertebrates. TMAO (see Subsection 1.4) contributes to the weak sweetness in shrimps and oval squid (*Sepioteuthis lessoniana*), while despite the small amount, TMA (not shown in the table) is considered to contribute to the specific seafood-like taste in boreo Pacific gonate squid (*Gonatopsis borealis*).

Among the organic acids, succinate is identified as taste-active only in short-necked clam. As described in Subsection 1.6, succinate is one of the anaerobic end products in bivalves and contributes to their umami taste. In dried skipjack tuna (not included in the table), lactate is known to contribute to the sour taste and enhance the overall taste.

As described above, seafood taste is highly diversified but the taste-active component of each species consists of only a small number of extractive components which are common in many species. Therefore, it is considered that a specific balance among the common taste-active components produces the species specific taste.

(Hiroki Abe)

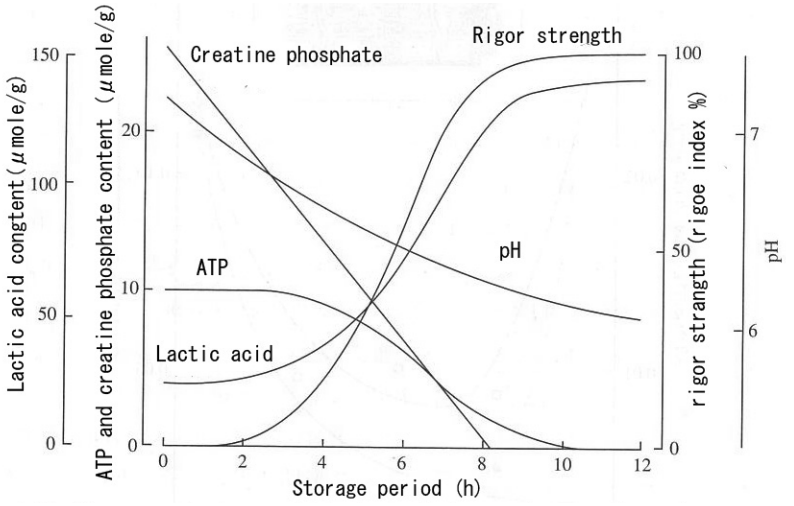


Fig. 4.13. Progress of rigor and biochemical changes in normal muscle in sardine during ice storage after immediate death by beheading following sufficient rest (from Watabe *et al*., 1991).

3. QUALITY CONTROL OF FISH AND SHELLFISH

3.1 Post mortem changes

Most prominent change observed externally in muscles following the death is the rigor mortis. This is a phenomenon in which muscle becomes hard, losing extensibility and elasticity.

Muscle cells are composed of myofibril as the structural unit. Myofibrils contain proteins which enables the muscle to contract. Among these, myosin and actin, called contractile proteins, respectively occupy about 60% and 20% of the whole myofibrillar proteins. In ordinary muscle, which belongs to fast muscle because of fast contractile speed and occupies the most part of edible portion of fish, the two proteins respectively form thick and thin filaments in myofibrils. In the presence of magnesium ion, myosin ATPase is activated by interaction with actin and the chemical energy obtained by the reaction is converted into mechanical energy. As a result, both filaments slide against each other and the muscle constricts. This ATPase reaction is regulated by tropoin, which will be explained in later part. The ATPase combined with functions of other myofibrillar protein is myofibrillar ATPase.

Metabolic reaction for synthesis of ATP is blocked after death, whereas ATP is consumed gradually and exhausted by ATPase activity leading to rigor mortis. Under these conditions, myosin and actin bind irreversibly and the muscle loses elasticity. However, rigor mortis does not occur immediately after death. Decomposition from ATP to ADP occurs even in resting muscle of living fish.

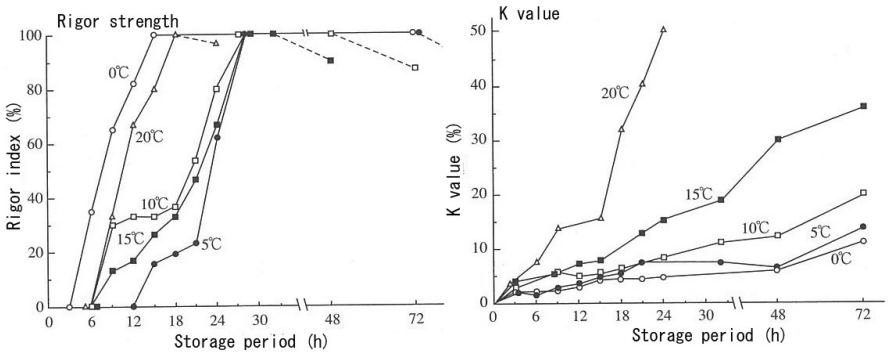


Fig. 4.14. Changes in rigor strength and K value of flounder under different temperatures (modified from Iwamoto *et al*., 1987).

ADP receives the high energy phosphate from creatine phosphate accumulated in resting muscle to regenerate ATP. Thus, creatine phosphate is consumed and creatine accumulates in muscle in early phase after the death (Fig. 4.13). Furthermore, lactic acid is also accumulated and the muscle pH decreases. The decrease of pH after death depends on the glycogen content in living state. In muscles of migratory fish which contain large quantities of glycogen, pH decreases to around 5.5.

3.2 Preservation of freshness

In Japan, the high commercially valuable fish such as sea bream and flounder are often eaten raw and particularly high freshness is required when eaten as sashimi. These fish are often transported to the market in living state. On the other hand, the fish prior to the onset of rigor mortis are sold at a price similar to the living fish. Therefore, technology to postpone the progress of rigor mortis is commercially useful. As compared to fish agonized to death, fish killed instantly while resting by quick disruption of the central nervous system at the medulla prominently delays the rigor mortis. Storage temperatures have strong influence on the progress of rigor mortis. As can be seen in Fig. 4.14, the flounder exposed to instant death shows more rapid progress of rigor mortis at 0°C than at 10°C. This result is different from the general chemical reaction, where higher temperature induce faster reaction, and can be explained by an increased concentration of intracellular calcium ion at 0°C, when we compare the myofibril ATPase activity at different temperatures (Fig. 4.15). The interaction between myosin and actin become possible by binding of calcium with troponin contained in myofibrils in the presence of magnesium ion leading to muscle contraction. Even in the resting fish killed instantly, ATP content gradually decreases by the myofibrillar ATPase. At 0°C, calcium ion content increases in cells and ATPase activity is increased by increased calcium ion concenration. These changes cause

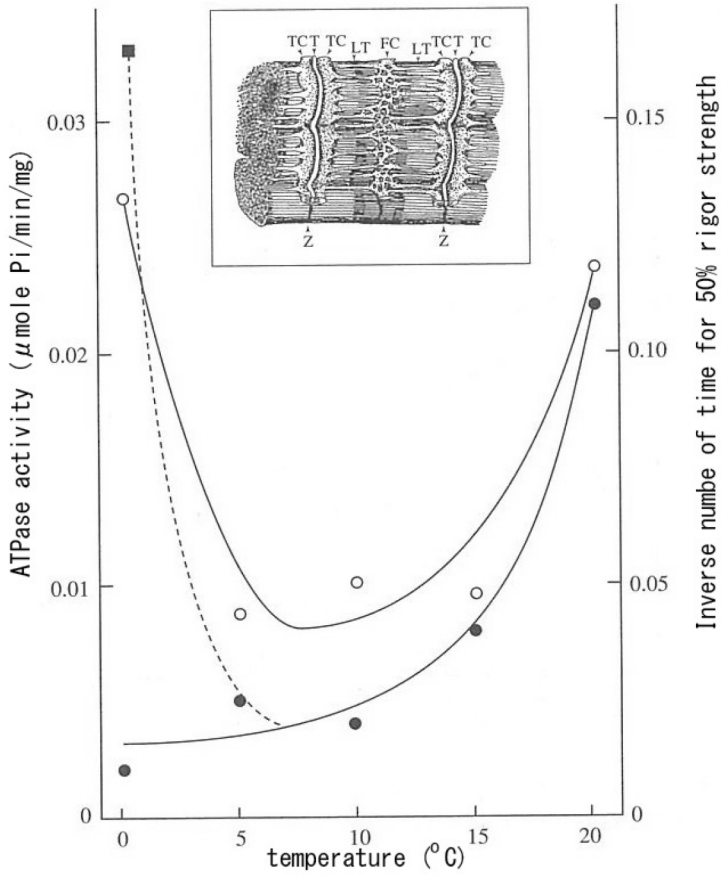


Fig. 4.15. Comparison of rigor mortis progress rate of spiked plaice (○) with its myofibrillar Mg^{2+} ATPase activity in the presence of 0.1 mM Ca^{2+} (■) at 0°C and 1 mM EGTA (●) at various temperatures (Watabe and Hashimoto, 1989). Rigor mortis progress rate is expressed as $T^{50\%}{}_{Ri}(h-1)$ calculated from the data reported in Iwamoto *et al*. (1987). The inset is the network of sarcoplasmic reticulum surrounding a myofibril. TC, terminal cisternae; T, transverse tubule; LT, longitudinal tubule; FC, fenestrated cisternae; Z, Z band.

rapid consumption of ATP and the progress of rigor mortis is accelerated. It is confirmed that uptake of calcium ion by sarcoplasmic reticulum, the organelle for storing calcium, is extremely low at 0°C. This is because, the calcium pump which transports calcium ion positively from cell to sarcoplasmic reticulum does not work at 0°C. This phenomenon is observed only at 0°C in fish (Fig. 4.16). Progress of rigor mortis shows relation to the temperature of the fish habitat. In cods, which live in low temperature environment less than 5°C, rigor mortis is not accelerated at 0°C. Furthermore, within the same species, rigor mortis progressed slowly in fish caught in winter compared to the fish caught in summer. The

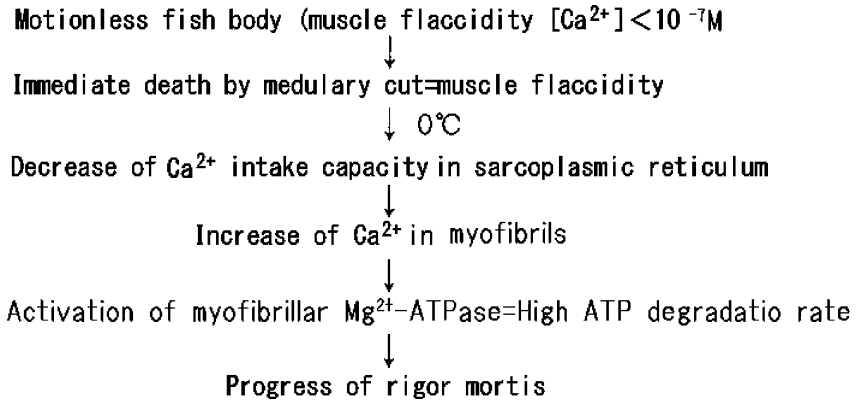


Fig. 4.16. Mechanisms involved in the onset of rigor mortis in fish muscle stored at 0°C (Watabe and Hashimoto, 1989).

relation between temperature of habitat and progress of rigor mortis is prominent in fish living in wide temperature range such as carp, which can live at temperatures from 0°C to 30°C. Fish express various isoforms of proteins which have different optimum temperatures for activities although the functions are similar. Not only myosin, but calcium pump in sarcoplasmic recticulum and ATP synthase in mitochondria are increased by acclimatation at low temperature. All these proteins have deep relation to the progress of rigor mortis. Increased myosin ATPase activity by rearing in low temperature environment rather accelerates the rigor mortis, but the increase of activities in sarcoplasmic reticulum and ATP synthase in mitochondria overcome the increase of the ATPase. Trials are implemented to utilize these temperature-dependent changes of protein in aquaculture and treatment of fisheries products.

3.3 Chemical changes during preservation

Changes in fish and shellfish occurring in the early phase after death are described in the above section in relation to rigor mortis. Briefly, ATP level is kept constant in the early phase before rigor mortis. After that, with progress of rigor mortis and softening, ATP is decomposed sequentially to ADP, AMP, inosinemonophosphate (IMP), inosine (HxR), Hypoxanthine (Hx), and the muscle undergoes autolysis. Reactions from AMP to IMP are weak in fish, and it is understood that the ratio of cumulative amount from ATP to IMP against that of HxR and Hx is strongly correlated with the freshness. The ratio is expressed as K value using following equation and used as good indicator of fressness.

$$\text{K value} = \frac{[\text{HxR}]+[\text{Hx}]}{[\text{ATP}]+[\text{ADP}]+[\text{AMP}]+[\text{IMP}]+[\text{HxR}]+[\text{Hx}]} \times 100.$$

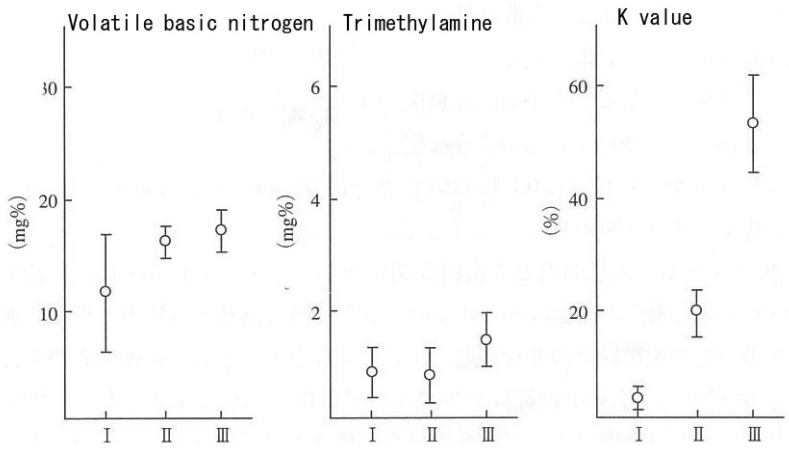


Fig. 4.17. Comparison of freshness estimation methods (from Uchiyama *et al.*, 1970). I. Fish meat immediately after death. II. Fish meat as ingredient for sushi in high class Sushi restaurant. III. Fish meat as ingredient for sushi in cheaper sushi restaurant.

In addition, mollusks have an alternative pathway through which AMP is converted to adenosine and not to IMP—one of the umami compounds. Fish and shellfish are not only healthy food but also tasty food since they quickly accumulate IMP.

K value has the highest reliability among freshness indices, although it is sometimes inconsistent with the results from sensory evaluation. Figure 4.17 shows a comparison of three freshness indices for tuna fillet of different freshness: contents of volatile basic nitrogen (VNB) including ammonia, contents of trimethylamine (TMA), a volatile compound responsible for fishy smell, and K value. K value, but not other indices, apparently distinguishes the two types of tuna fillets, one taken immediately after death and served in a fancy sushi restaurant and the other served in a local sushi restaurant. The changes in K value of spike plaice in Fig. 4.14 indicate that the storage at 0°C is preferable once rigor-mortis is completed, although the storage at 5–10°C is effective to delay the onset of rigor mortis.

As described in Subsection 1.1, post mortem changes are more rapid in fish meat than in livestock meat. This means that fish and shellfish are spoiled more rapidly. After the resolution of rigor mortis, the muscle of fish and shellfish undergoes softening due to the autolysis by endogenous proteases followed by bacterial degradation of muscle proteins (Fig. 4.18). Degradation of muscle proteins by endogenous proteases increase the amount of peptides and free amino acids, which contribute to the taste of fish and shellfish. IMP is also increased in the early phase after resolution of rigor mortis.

After autolysis, fish and shellfish are spoiled by the proliferation of bacteria that utilize low molecular weight component such as peptides and free amino acid as nutrients. Storage at low temperatures including ice storage is necessary to

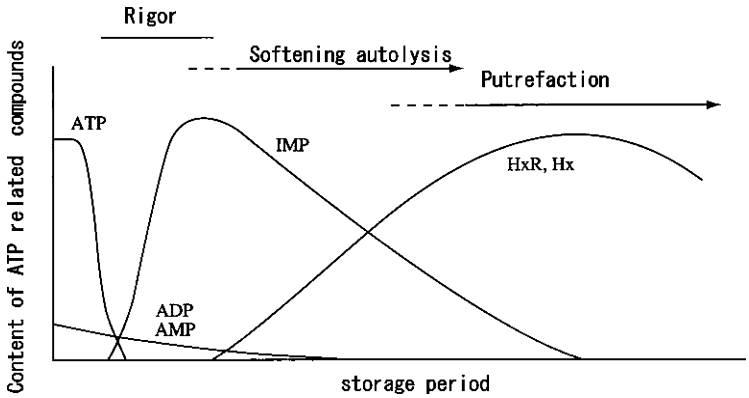


Fig. 4.18. Changes of fish meat during storage (modified from Iwamoto *et al*., 1987; Ohta, 1990; Watabe *et al*., 1991).

prevent these undesirable changes, especially in fish and shellfish that show rapid post mortem changes. In addition to the "spoilage" that is commonly observed, bacterial growth sometimes causes an allergy in lean fish such as tuna and skipjack tuna containing large quantities of free histidine not corporate to proteins. In these fish, decarboxylation of histidine by bacteria leads to the accumulation of histamine, a substance inducing allergy-like disease.

On the other hand, there are various processed food in fish and shell fish such as salted, salted and dried, fermented etc. The autolytic reaction is used to produce these products, because protease of fish and shellfish are active even at low temperature due to their characteristic structure. The high activity of protease as well as other enzymes at low temperatures enables fish and shellfish to live in low temperature environments. Mechanisms involved in the high enzyme activity of fish and shellfish at low temperatures have been extensively studied in fish reared at different temperatures.

(Shugo Watabe and Gen Kaneko)

4. SEAFOOD SAFETY

4.1 Fish and shellfish poisons

Food poisonings of seafood are mainly caused by the microbial contamination. However, several species of aquatic organisms possess deadly poison. Almost all the poisons in fish and shellfish are obtained from their diets. Therefore, it is difficult to forecast poisoning of seafood by methods other than checking the appearance of the primary producers of the toxins.

1) Poison in fish

a) Puffer poison

Puffer poison was documented in ancient China and in Japan from the Heian

period (794–1185). Many satirical *haiku* (short poems) composed in Edo period (1603–1867) deal with puffer. Therefore, it is assumed that puffer eating habit was popularized in Edo period. The major constituent of the puffer poison is tetrodotoxin contained in the fish belonging to family Tetraodontidae. Tetrodotoxin has many hydroxyl groups and a guanidyl group in the molecule. Furthermore, it has a unique functional group called hemilactal. Toxicities of puffer are surveyed in species and tissue levels. Ovary and liver of *Takifugu niphobles*, *T. poecilonotus*, *T. pardalis*, *T. snyderi*, *T. porphyreus*, *T. obscurus* are known to be deadly toxic. None of the species caught along the coast of Japan show toxicity in the muscle. However, the muscle of *Lagocephalus lunaris*, a tropical puffer species, has high toxicity and several species in tropical area, such as Taiwan and Philippines, have toxicity in all tissues including muscle.

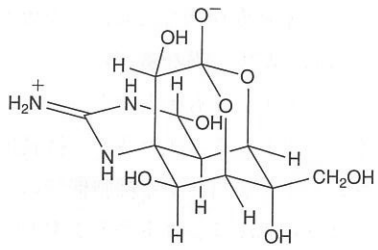
Toxicity of puffer varies widely among fishing ground and individuals. Tetrodotoxin is distributed in a wide variety of organisms such as newts, a frog, goby (*Yongeichthys criniger*), blue-ringed octopus (*Hapalochlaena fasciata*), Japanese ivory-shell (*Babylonia japonica*), Japanese triton (*Charonia sauliae*), starfish (*Astropecten polyacanthus*), crab (*Atergatis floridus*), nemertea, flatworms, etc. It is also distributed in the red alga, *Himemosazuki* (*Jania adhaerens*, Corallinaceae). Bacteria isolated from tetrodotoxin-containing organisms produce tetrodotoxin under cultured conditions. Therefore, tetrodotoxin is believed to be produced by bacteria.

Tetrodotoxin binds to sodium channel in nerves and muscles and interferes with the influx of sodium ion into the cell. Therefore, neurotransmission is blocked and muscles are paralyzed. As a result, breathing becomes difficult, which is the major cause of death by puffer poisoning. Possibility of survival is increased by continuing to breath because sensitivity of the sodium channel of cardiac muscle to tetrodotoxin is lower than that of skeletal muscle, and heart can continue to beat after dysfunction of skeletal muscle. Lethal dosage of tetrodotoxin in puffer is several hundred times higher than in fish species other than puffers. Mutations of amino acid residues are observed in the sodium channel of pufferfish which lower the binding potential toward tetrodotoxin.

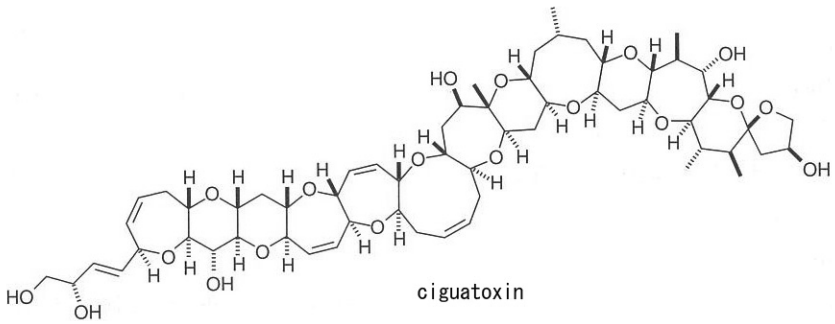
b) Ciguatera poisoning

Low mortality food poisonings occur by eating fish inhabiting the coral reefs of the tropics. Among these, poisoning caused by ciguatoxin is called ciguatera poisoning, annual prevalence of which is estimated to be 20,000 to 50,000 cases in the world. This is the largest poisoning caused by seafood toxins. Major symptoms of ciguatera are digestive disorder and disturbance of perception. The latter symptom persists longer and is the most characteristic symptom of ciguatera. It is called dry ice sensation because patients feel keen pain as if touching dry ice when they touch something cold.

Causative toxin of ciguatera is ciguatoxin, which is a compound collectively called as ladder form polyether. Ciguatoxin is produced by a benthic dinoflagellate *Gambierdiscus toxicus*. The toxin is accumulated in larger carnivorous fish through food chain. Toxicity of the ciguatoxin increases as it undergoes modifications due to the metabolism of each organism through the food chain.



Tetrodotoxin



ciguatoxin

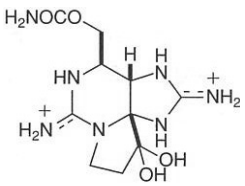
Lethal dose of ciguatera to mouse is about 1/20 of tetrodotoxin. However, mortality by ciguatera is low, because ciguatoxin has high toxicity to fish and fish cannot accumulate large amounts of ciguatoxin. Causative toxins of ciguatera in South Pacific Ocean and Caribbean Sea are the ladder form polyether, even though their chemical constitutions are slightly different from each other.

Ciguatoxin binds to sodium channels same as tetrodotoxin. By binding with ciguatoxin, sodium channel is continuously opened and sodium ion concentrations in cells increase. Abnormal modulation of cell functions related to sodium channel causes the symptoms of ciguatera.

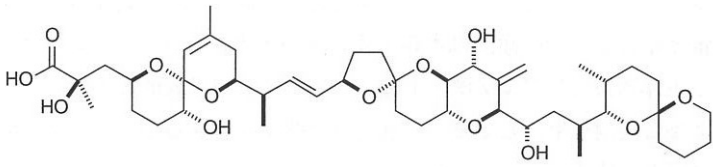
G. toxicus also produces mytotoxin in addition to ciguatoxin. Mytotoxin is a ladder form polyether with molecular size and toxicity higher than that of ciguatoxin. It expresses its toxicity by accelerating influx of calcium ion into the cell. However, it is not observed to be transferred to higher trophic level via food chain. Thus, mytotoxin is not the major causative toxin of ciguatera.

c) Palytoxin

Palytoxin is a highly toxic substance found in a cnidarian (*Palythoa tuberculosa*), which had been used as arrow poison by indigenous Hawaiians. Benthic dinoflagellate *Ostreopsis* also produces palytoxin. It was identified as the causative substance in food poisoning caused by eating knobsnout parrotfish (*Scarus ovifrons*) caught off Tokushima. Many cells of *Ostreopsis* were found in the digestive tract of the fish. *Ostreopsis* was attached on algae in the habitat of knobsnout parrotfish and it was estimated that the derivative of palytoxin



saxitoxin



okadaic acid

produced by *Ostreopsis* was transferred to the fish. Palytoxin was also identified as the causative substance in the food poisoning of scrawled filefish (*Aluterus scriptus*), round scad (*Decapterus lajang*), and black triggerfish (*Melichthys vidua*), and is also suspected to be the causative substance of a high mortality sudden food poisoning called clupeotoxism caused by eating plankton feeding fish such as herrings and anchovies. Palytoxin binds to sodium pump in cells and continuously opens the channel of sodium ion. As a result, toxicity occurs due to the increase of intracellular sodium ion concentration.

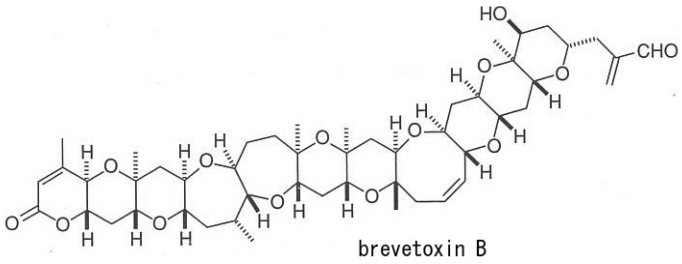
2) Shellfish poisoning

a) Paralytic shellfish poisoning

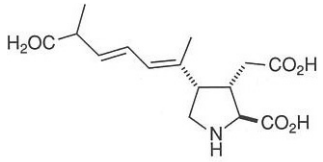
It had been known along the coast of North America that bivalves have paralytic poison caused by accumulation of the toxin produced by dinoflagellates of genus *Alexandrium*. The distribution of toxic dinoflagellates is expanding all over the world. The genera *Alexandrium* and *Gymnodinium* are the producers of paralytic shellfish poisoning in arctic and temperate zones and *Pyrodinium bahamense* var. *compressum* in the tropical zone. Bivalves such as Pacific oyster (*Crassostrea gigas*), short-necked clam (*Ruditapes philippinarum*), blue mussel (*Mytilus* sp.) as well as scallop (*Patinopecten yessoensis*) in Hokkaido and Tohoku area in northern Japan become poisonous occasionally in summer.

The causative toxins are a water soluble basic substance called saxitoxin and its analogs. Saxitoxin has similar toxicity to tetrodotoxin and also similarly binds to the sodium channel on cell membrane. Monitoring of shellfish poisoning is performed to prevent shipping of poisonous shellfish. Regulation value of shellfish toxin is 4 MU/g (1 g of edible part contains toxin which can kill 4 mice of 20 g body weight).

Puffers in tropical zones have paralytic shellfish poisons derived from *Pyrodinium bahamense* var. *compressum* occurring in the habitats of puffers in



brevetoxin B



domoic acid

Florida. Several freshwater cyanobacteria also produce paralytic shellfish poisons which cause shellfish poisonings in freshwater lakes.

b) Diarrhetic shellfish poison

Food poisoning which causes diarrhea by ingesting shellfish is called diarrhetic shellfish poisoning. Diarrhetic shellfish poisoning occurred along the coast of Tohoku area for the first time in Japan, and then in various places not only on the coast of Japan but also on coasts all over the world. Causative substances are a polyether compound called okadaic acid and its analogs. Okadaic acid had been discovered as a cytotoxic substance from a sponge (*Halichondria okadai*) before the discovery as the causative toxin of diarrhetic shellfish poison.

Okadaic acid inhibits the activity of protein phosphatase 2A (see Subsection 5.1) in cell and exhibits cytotoxicity by this inhibition. Diarrhetic shellfish poisons are produced by dinoflagellates of the genus *Dinophysis*. In the monitoring for diarrhetic shellfish poisoning, the regulation value is 0.05 MU/g for edible parts.

c) Other shellfish poisons

Neurotoxic shellfish poisoning

When a red tide bloom by *Gymnodinium breve* occurs, it is followed by mass mortality of fish, occurrence of toxic shellfish, and health disorders caused by breathing the seawater mist. The causative substances of these phenomena are a ladder form polyether called brevetoxin and its analogs. The symptom by eating the toxic bivalves is mild ciguatera like symptom. Brevetoxins also bind to sodium channel and accelerate influx of sodium ion into the cell same as ciguatoxin. Metabolites of brevetoxin are accumulated in bivalves, although the toxicity is maintained in the metabolites. Red tides by *G. breve* occur frequently in Florida and the Gulf of Mexico as well as in New Zealand.

Amnestic shellfish poisoning

Poisoning by cultured blue mussel (*Mytilus edulis*) occurred in Canada. Major symptoms of the poisoning are emesis, diarrhea, and amnesia. The causative substance is domoic acid produced by diatoms *Psuedonitzchia* spp. that multiplied explosively in the area. Domoic acid was discovered from a red alga, *hanayanagi* (*Chondria armata*) that was used for anthelmintic as a folk medicine in Tokunoshima Island in Kagoshima (southern Japan). It binds to glutamate receptor and disturbs the neurotransmission. Because many strains of the diatom produce domoic acid, keeping a watch on the diatom is needed in bivalve cultures.

(Shigeki Matsunaga)

4.2 Other substances

There are about 100 elements on the earth, some of which support the life of organisms. These elements are called minerals or inorganic substances in nutritional science. Minerals are one of 5 major nutrients along with proteins, lipids, carbohydrates and vitamins. Zinc, potassium, calcium, chrome, selenium, ion, copper, sodium, magnesium, iodine, phosphorus etc. are determined as essential trace elements.

On the other hand, many of these essential trace elements are also toxic heavy metals. Copper and zinc are essential trace elements for living organisms, although they have optimum concentrations in the body. Concentrations lower than the optimum level leads to deficiency while higher concentration turns out to be harmful. Heavy metals combine with sulfur in living body, and the trend for combination is as follows: mercury ion > copper ion > cadmium ion > lead ion. Cysteine, one of amino acids, is a typical sulfur-containing substance in living body. This amino acid has important role in protein functions. When cysteine binds with heavy metals, the function of proteins is lost leading to heavy metal toxicity.

Aquatic organisms draw surrounding water into their body through body surface or gills for respiration. They also ingest water simultaneously when they eat food. Algae and zoo- and phyto-planktons accumulate trace element in their body directly from surrounding water. Organisms concentrate the trace elements derived from food through the food chain and finally trace elements are ingested by human beings. The famous accident happened in Japan, in which organic mercury was converted from inorganic mercury in surrounding water, accumulated in fish, and finally damaged the central nervous system in animals and human beings who ate the fish leading to death in worst cases (Minamata Disease). Much mercury is still accumulated in offshore migratory fish which are on the higher position of food chain and fish and shellfish which ingest bottom organic substances as their food. Japan's Ministry of Health, Labour and Welfare issued a guide line of reasonable levels for ingestion of these food.

It is also well known that aquatic organisms contain arsenic at the level or measuring up to 18 and 63 ppm in shrimp and laminaria (Kombu), respectively.

The value reaches 110 ppm in Hijiki (*Sargassum fusiforme*, brown algae). Compared to the regulation value for tap water in Japan (0.05 ppm), the concentration in natural sea water is very low (0.0037). Inorganic aresenicum is more toxic than organic aresenicum. Hijiki has more inorganic aresenicum, while other algae have more organic. In the processing of Hijiki, 60–80% of arsenicum is removed by boiling and rinsing. In Japan, Hijiki is considered a good source of trace elements including iodine. Also it functions as dietary fiber. Well balanced ingestion of Hijiki is therefore necessary.

Recently, man-made toxic chemical substances flow into lakes and sea through rivers. Impacts of these substances on environments and safety hazard to human beings who eat fish and shellfish living in these environment, is a matter of concern. Typically, such substances are dioxins, which are accumulated in the bottom of lake and sea. It is known that dioxins have toxic effects on reproduction, development and immunity of human beings and are also carcinogenic. Dioxins are mainly contained in fatty tissues and internal organs of fish. Japanese ingest 1.3 pg TEQ/kg body weight every day and 85% of it is from fish and shell fish (TEQ: Toxicity equilibrium quantity). However the amount is only 30% of internationally recognized tolerable amount for ingestion (4 pg TEQ/1 kg). When we consider excellent functions of fish and shellfish as food resources, well balanced ingestion is important for prevention of risk of toxic substances. Endocrine disrupting chemicals, so called environmental hormone, are not so much in news as earlier, although these chemicals still exist in water environment. Typical endocrine disrupting chemical in water is organotin, which causes sex reversal of female snail to male. Other than this, it was suspected that 20–30 chemical substances exist in sea water. Noniphenol, octhinophenol etc. show female hormone like function to male fish and shellfish. Impacts of these substances on the ecosystem are of concern.

(Shugo Watabe)

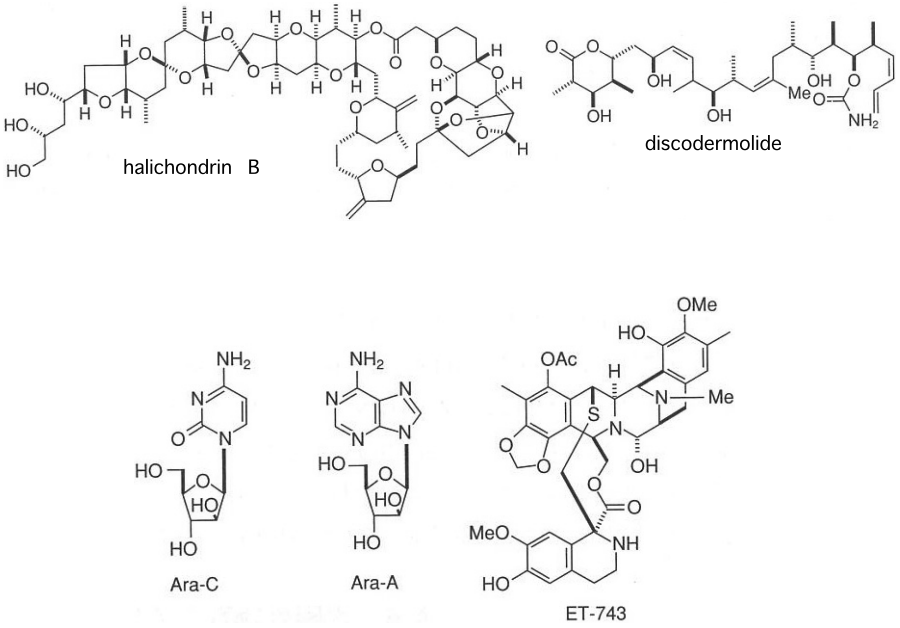
5. BIOCHEMICAL USE OF AQUATIC NATURAL PRODUCTS

5.1 Bioactive substances

Substances commonly existing in all organisms such as proteins, lipids, carbohydrates, and nucleic acids are called primary metabolites. On the other hand, components only contained in particular species are called secondary metabolites or natural products. Various secondary metabolites derived from terrestrial plants and soil microorganisms such as actinomycetes are used as medicines and agrochemicals. Some marine invertebrates and microorganisms produce secondary metabolites with chemical structures and/or biological functions different from those derived from the terrestrial counterparts.

1) Cytotoxic and anticancer substances

Cancer is the first cause of death in many developed countries and explorations of effective anticancer therapeutic agents with less side effects are actively pursued. More than half of commercially available anticancer agents are natural



products or their analogs. Many substances discovered from aquatic organisms show cytotoxicity to cancer cells or anticancer activity in animal experiments.

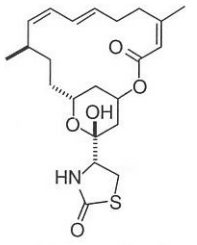
a) Substances which bind to tubulin

Cancer cells proliferate more rapidly than normal cells. Therefore, many anticancer agents block cell division. Tubulin is a protein which composes spindle fiber (microtubule) by polymerization and separates chromosomes in the process of cell division. Vincristine which disturbs the polymerization, and taxol which stabilizes the microtubule are both derived from plants and used as anticancer agents. Among substances derived from aquatic organisms, halichondrin B and discodermolide, both derived from the sponge, have the same mechanism of action as vincristine and taxol, respectively.

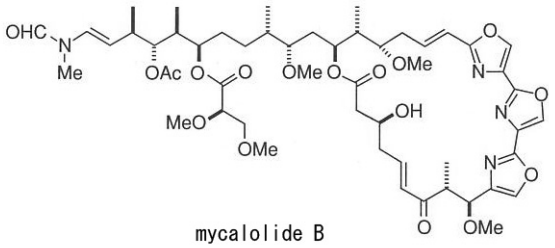
b) Substances affecting the replication of nucleic acid

Normal nucleosides which compose nucleic acids have a pentose called ribose. The substance in which ribose in nucleoside is replaced with arabinose was discovered for the first time in a sponge. By using this scheme of modification, Ara-C and Ara-A were chemically synthesized. In the process of DNA replication, they are mistaken as normal nucleosides that have ribose and bind to the DNA chain. Thus, the DNA replication process is inhibited. Ara-C is used for the treatment of leukemia and Ara-A is used as an antiviral agent.

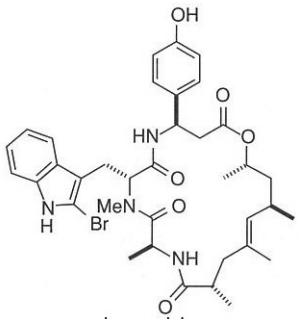
Ecteinascidin 743 (ET-743), which was discovered from a colonial tunicate, exhibits not only cytotoxicity but also antitumor activity in animal experiments and was approved as an anticancer agent after clinical trials. It is impossible to obtain sufficient amount of ET743 directly from the tunicate. A large amount of



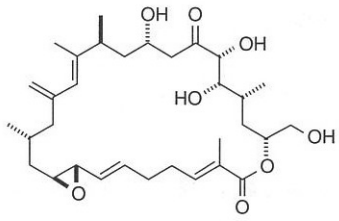
latrunculin A



mycalolide B



jaspamide



amphidinolide H

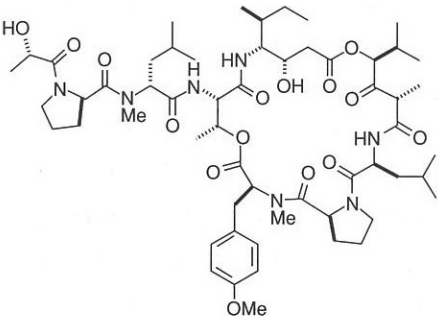
cyanosafracin B was produced by fermentation and ET-743 was produced from cyanosafracin B by chemical transformations. ET-743 exhibits antitumor activity through interference of DNA replication by alkylation of DNA.

c) Substances which interact with actin

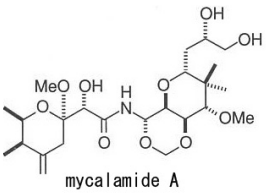
Actin is the most abundant protein in eukaryotic cells. The monomeric form called G-actin is polymerized to form F-actin, which participates in the maintenance of cell morphology and cell movement. Cytochalasin, a metabolite of a fungus, is well-known to interfere with the polymerization by binding to G-actin. Phalloidin, derived from mushrooms, binds to F-actin and stabilize the polymer. Both substances interfere with the equilibrium between G- and F-actin and induce cell death. Latrunculin A and mycalolide B, both derived from the sponge, block the polymerization of G-actin. On the other hand, jaspamide, derived from a sponge and amphidinolide H, from a dinoflagellate, stabilize F-actin. Substances which affect actin show toxicity to cancer cells in low concentrations, although they are also harmful to normal cells because actin also exists in normal cells.

d) Protein synthesis inhibitor

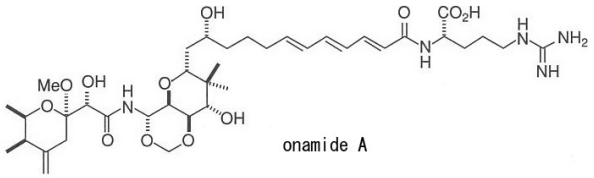
The structure of ribosome, which is a factory for protein synthesis in the cell, is significantly different between prokaryotes and eukaryotes. In fact, many chemical substances used as antibiotics have no impact on ribosome of eukaryote but inhibit ribosome of prokaryote. Several compounds discovered in aquatic organisms inhibit ribosome of eukaryotes selectively. Didemnin B and its analogues, derived from colonial tunicates, exhibit antitumor activity by inhibition



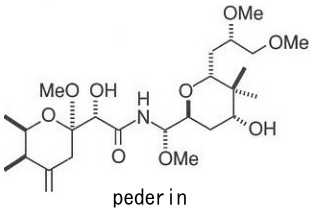
didemnin B



mycalamide A



onamide A

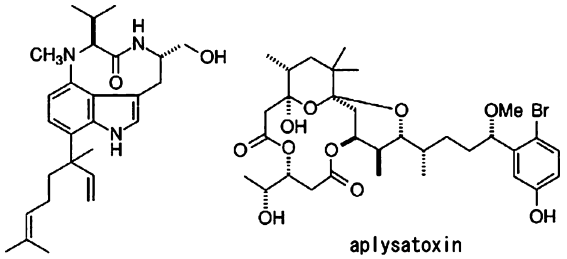


pederin

of protein synthesis, but their application for medicinal use failed because of its strong toxicity to mammals.

Mycalamide A and onnamide A, both derived from the sponges, are cytotoxic substances and also exhibit antiviral activity. The common structure on the left side of these molecules also exists in pederin which is the toxic constituent of rove beetle (*Paederus fuscipes*). These substances interfere with protein synthesis of eukaryotes.

Secondary metabolites contained in sponges and colonial tunicates have chemical structures similar to those produced by microorganisms. It had been suspected that symbiotic bacteria in the sponges and colonial tunicates may be the one producing these substances. Biosynthetic genes of pederin were first cloned from the symbiotic bacteria of the beetle. Based on this genetic information, DNA from the sponge that contains onnamide A was surveyed and genes similar to biosynthetic genes of pederin were cloned. The gene was shown to constitute



bacterial genome. From these facts, it was proven that onnamide A is produced by bacteria in the sponge.

2) Substances that affect enzyme activities

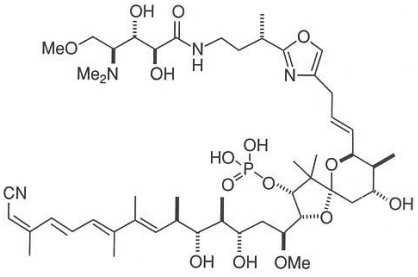
Essentially, life processes are sequences of chemical reactions and each reaction is strictly controlled by an enzyme. Natural products which interfere or accelerate the activity of specific enzymes are used as medicinal drugs and reagents for cell-biology.

a) Enzymes related to phosphorylation and dephosphorylation

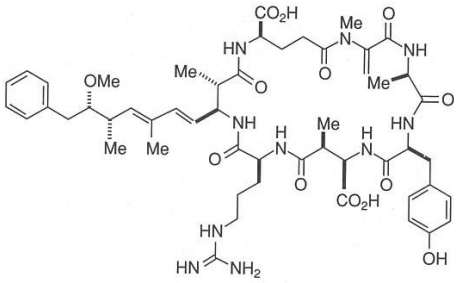
Many proteins in cells have phosphate group(s) in its molecule as phosphate esters. Protein kinase works in phosphorylation and phosphoprotein phosphatase is involved in dephosphorylation. When a phosphate group is introduced in proteins, the changes of enzyme activity of the protein and its distribution (localization) in the cell as well as the changes of conformation of the molecule would occur because of highly hydrophilic and anionic natures of the phosphate group. Therefore, phosphorylation of protein strongly participates in the regulation of functions of the proteins.

One of the enzymes involved in the phosphorylation is protein kinase C (PKC), which is activated in the presence of calcium ion and lipids such as phosphatidylserine and diacylglycerol. TPA, a terpenoid compound derived from plants, and teleocidin, an alkaloid derived from the actinomycete, are tumor promoters that activate PKC, and thus phosphorylating numerous proteins in the cells. They activate PKC in far lower concentrations than lipids mentioned above. Tumor promoter is a substance which activates cancer cells and enhances their amplification. Lyngbyatoxin A and aplysiatoxin, derived from cyanobacteria, also activate PKC and show tumor promoting activity.

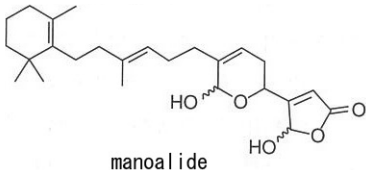
Phosphoprotein phosphatase 1 and 2A (PP1 and PP2A) are the major dephosphorylating enzymes in eukaryotic cells. Substances which selectively inhibit these enzymes were found in aquatic organisms. Okadaic acid (see Subsection 4.1) and calyculin A are such substances derived from sponges, and microcystins are derived from cyanobacteria. Calyculin A is cytotoxic to cancer cells, but also a tumor promoter. Microcystins are hepatotoxins which are produced by Microcystis, a cyanobacterium that form blooms called "*aoko*" in lakes and ponds in summer. They also function as tumor promoters in the liver.



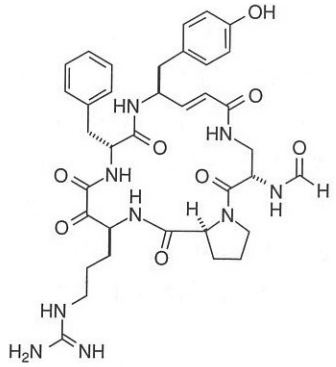
calyculin A



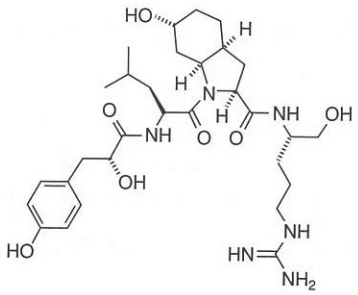
mycrocystin YR



manoalide



cyclotheonamide A



aeruginosin298-A

b) Enzymes related to hydrolysis of lipids

Phospholipase A_2 (PLA_2) is an enzyme which releases arachidonic acid from phospholipids in the cell membrane. Arachidonic acid is converted to prostaglandins which have hormonal function in the organisms. Substances which interfere with the release of arachidonic acid eventually inhibit the production of prostaglandins and exhibit anti-inflammatory effect. Manoalide, a terpenoid derived from a sponge, binds to PLA_2 irreversibly and inhibits its activity.

c) Enzymes related to the hydrolysis of proteins

Hydrolysis of proteins is catalyzed by protease. Abnormality in the regulation of protease activity causes a variety of diseases. Therefore, protease inhibitors are expected to be therapeutic agents for the diseases. Cyclotheonamide A, derived from the sponge, and aeruginosins, derived from the cyanobacteria, strongly inhibit serine proteases such as trypsin and thrombin.

3) Other bioactive substances

These include various excitatory amino acids, lectins, antibacterial and antifungal substances, and prostaglandins. However, on account of the limited space, the author cannot introduce various other bioactive substances found in aquatic organisms. Those interested may refer to Blunt and Munro (2007).

(Shigeki Matsunaga)

5.2 Other substances

From the viewpoints of effective utilization of fisheries resources and environmental concern, studies on the intensive utilization of under-utilized or waste aquatic resources have been on the rise. Various excellent products are developed and used in the area of food and cosmetics.

Chitin, the major component in the shell of crustaceans, is a polysaccharide in which *N*-acetyl-D-glucosamine bind to each other by β-1,4-glycoside linkage (see Subsection 1.5). Chitin is a poorly soluble compound and difficult to be used as it is. Chitosan is obtained after partial deacetylation by heating in concentrated alkali. The product is a mixture of chitin and chitosan called chitin-chitosan, used as it is or as derivatives after several treatments such as carboxymethylation. Chitin-chitosan is used for various purposes such as a coagulant agent for waste-water treatment, antibacterial agents, artificial skins and suture threads for surgical operation, humectants, wound healing drugs, and so on. In food industry, chitin-chitosan used for dietary fibers is also known to have antihypertensive and blood cholesterol-lowering effects, and used as a specified health food for people suffering from high blood cholesterol. It is also reported that chitin-chitosan has calcium absorption facilitating function, immunostimulatory action, and anticancer activity. Thus, the future utilization of chitin-chitosan is highly expected.

Collagen obtained from skin and scale of fish and mantle of scallop is used as marine collagen. Collagens from fish and shellfish living in low temperature environment have advantage in low denaturation temperature, maintaining moisture at room temperature, and water retention at low temperature. Marine collagen is used as the medium for cell culture. For medical use, therapeutic actions for osteoporosis and arthritis are recognized by oral administration. It is also used as supplements for moisture retention of skin and hair and for anti-aging.

Chondroitin sulfate obtained from the cartilage of shark and nasal cartilage (called as *hizu*) of salmon is an acid mucopolysaccharide in which sulfate groups bind to the backbone structure composed of *N*-acetyl-D-galactosamine and D-

glucuronic acid. It is used for relief from arthritis, post-operative care of cataract, and in eye drop for dry eyes. Chondroitin sulfate is also reported to reduce the risk of heart attack. Hyaluronic acid is a mucopolysaccharide composed of *N*-acetyl-D-glucosamine and D-glucuronic acid and has no sulfate group. It is used for the medical cure for arthritis and injury and also for anti-aging and clear skin. *N*-acetyl-D-glucosamine produced by the decomposition of chitin is a precursor for the synthesis of hyaluronic acid, administration of which is reported to be effective for improvement of skin quality. It is also confirmed to have beneficial effect on improvement of gonarthrosis (osteoarthrosis of knee) and capacity for learning and memory.

Many studies have been carried out on utilization of substances from wastes left behind after extraction, bivalve shells, fish scale, etc. Anserine, a dipeptide described in Subsection 1.4, purified from cooking water of skipjack tuna, is utilized as supplements for athletes for the recovery from fatigue. Studies for exploitation of other physiological functions continue to progress.

(Hiroki Abe)

CONCLUSION

As described in this chapter, a variety of biological compounds in aquatic organisms differs greatly from species to species and includes unique components which cannot be found in terrestrial organisms. In this context, it can be said that each species is valuable for human beings. Aquatic organisms provide us various useful materials as well as food. However, fish and shellfish resources caught as food are on the decline, and the catches of major fish and shellfish have sharply decreased recently. Therefore, we have to put together ideas for conservation and recovery of fish stocks, to make the aquatic organisms available for long term future use by human beings. Technologies of human beings are extremely developed in 20th century but we have lost a lot in exchange for the development. To make the loss up is an important challenge in 21st century.

(Hiroki Abe)

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