論文の内容の要旨

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論文題目

Structural and Mechanistic Insight into a Novel Enoate Reductase
in Fatty-acid Metabolism Pathway
（脂肪酸代謝経路における新規エノン還元酵素の構造と機能の解明）

In a living organism, fatty acids exist as the constituents of neutral and polar lipids, the covalent attachments to distinct eukaryotic proteins, and the parts of eucaryotic second-messenger molecules and serve many essential functions. They can be remodeled by elongation, insertion or removal of double bonds. Several metabolic pathways have been discovered and studied to investigate the mechanism of the reactions and the regulation of fatty acids. Recently, a novel saturation pathway of linoleic acid was discovered from *Lactobacillus plantarum* AKU 1009a. Among the enzymes constituting the pathway, CLA-ER was identified as an enoate reductase and saturates the C=C bond of 10-oxo-*trans*-11-octadecenoic acid (KetoB) to generate oxooctadecanoic acid (KetoC). Based on the amino-acid sequence, CLA-ER is concluded to belong to the NADH oxidase/flavin reductase superfamily. The majority of the family members have an NAD(P)H:FMN oxidoreductase activity and catalyze the reduction of flavins and nitroaromatic compounds. Some enzymes in this superfamily have been also reported to take other activities. For example, IYD, an iodotyrosine

deiodinase, salvages iodide from mono- and diiodotyrosine with a reduced FMN. BluB, which is involved in the biosynthesis of vitamin B12, triggers the fragmentation and contraction of flavin mononucleotide and cleavage of ribityl tail to form DMB and D-erythrose 4-phosphate. However, no enzyme has been reported to show an enoate reduction activity. Here, I report both the substrate-free and substrate-bound structures of CLA-ER, which elucidate the novel reaction mechanism and the structural basis of substrate specificity.

The crystal structures of CLA-ER/FMN, CLA-ER/FMN/KetoB, CLA-ER/FMN/KetoC were determined to 2.10-, 2.35- and 2.15-Å resolutions, respectively. CLA-ER forms a homodimeric structure, which is consistent with the result of size exclusion chromatography. A CLA-ER molecule can be divided into two subdomains, stable subdomain and flexible subdomain. The stable subdomain is composed of central β-sheet (β1–β5) and 10 α-helices (α1–4, 7–12). The α7-helices in stable subdomains form extensive hydrophobic interactions in the central part of dimer interface, and the domain swapping of C-terminal tails also contribute to the dimer formation. The flexible subdomains adopt helix-loop-helix structures (residues 97–132) in each protomer. The two flexible subdomains exist at the both sides of the stable subdomains in the dimeric structure. Two FMN molecules locate on the interface of two stable subdomains and a long groove is formed with CLA-ER and FMN together.

The structures of CLA-ER complexed with fatty acids are similar to the structure of fatty acid-free state (RMSD of 0.141 Å). The fatty acid molecules were observed in the binding tunnel, which is formed by both of the long groove and the flexible subdomains in fatty acid-free state. The tunnel for fatty acid binding adopts a long sandglass shape that is narrow in the middle and relatively wide in two ends. Although the tunnel is hydrophobic to interact with the long alkyl chain of fatty acids, an arginine residue is located at one end of the tunnel to form a basic environment suitable for the carboxyl group of fatty acids.

Superposition among the structures of CLA-ER with and without fatty acids reveals

the completely same conformation of the stable subdomain. However, a significant conformational change was observed at the flexible subdomain. Upon the substrate binding, the flexible subdomain rotates about 45 deg towards the stable subdomain of the neighbor protomer in dimer. The helix-loop-helix structure acts as a “cap”, which captures a fatty acid when it binds to the groove in the interface of homodimer.

The conformational change of the “cap” structure makes CLA-ER change to it closed form and results in the formation of the tunnel for fatty-acid recognition and reaction. A cysteine residue is the sole hydrophilic one around the enoate group. Mutations of this cysteine residue showed decreased activities indicating it is important for the reaction. Besides, a tyrosine residue and a phenylalanine residue were also observed in the active site. Mutational analysis of these residues revealed that both the mutants of the tyrosine residue and the phenylalanine residue almost lost their activities. The phenylalanine residue is considered to recognize the C=C bond of substrate; the tyrosine residue located at 3.3 Å to the cysteine residue, and may be involved in both electron transfer and induction of conformational change of the phenyl alanine residue.

I try to explain the reason for the novel activity found in CLA-ER. As mentioned in reaction mechanism, a hydrophobic residue involved in the “cap” structure is critical for the spatial arrangement of C=C group. Therefore, a “cap” structure must be essential for the recognition of substrate in enoate reduction and deletion of this “cap” structure in enzymes may lead to the loss of enoate reduction activities. Among the NADH oxidase/flavin reductases whose structures are available, limited enzymes contain “cap” structures like CLA-ER. Sequence alignments of the enzymes with “cap” structures reveal that none of them conserved both the critical cysteine residue and the tyrosine residue. Mutations of these residues have been demonstrated to decrease activity remarkably. Therefore, all the “cap” structure, the hydrophobic “key” (phenylalanine residue), the cysteine residue and the tyrosine residue are the structural features that make CLA-ER identified as enoate reductase preferentially.