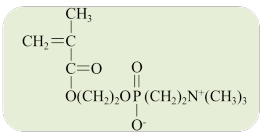
論 文 の 内 容 の 要 旨

論文題目: **Control of molecular aggregation with water-soluble amphiphilic phospholipid polymer biomaterials**
（分子構造を規定した水溶性・両親媒性リン脂質ポリマーバイオマテリアルの会合状態の制御）

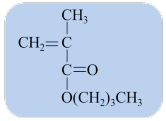
氏　　名: 牟　鳴薇

Motivation and objective: For many bioactive compounds that can be used in medical treatment, a problem remaining is their poor solubility in aqueous media. Thus, enhancing the solubility of hydrophobic biomolecules in aqueous media is highly demanding. The using of amphiphilic polymers bearing both hydrophobic groups and hydrophilic group, as a matrix material is an effective method to solve this problem.

A typical example is the polymers composed of 2-methacryloyloxyethyl phosphorylcholine (MPC) units, which are well-known for their biocompatibility. Indeed, the poly(MPC-*co*-*n*-butyl methacrylate)(PMB), composed of 30 mol% MPC units, is commercially available as a solubilizing test reagent for some pharmaceutical compounds. However, the random type structure of the developed amphiphilic polymers is not fit for deepening the understanding on the polymer aggregation behaviors, which is significant to determine their practical performance. Thanks to the progress in the radical polymerization field, preparation of well-defined polymers become available. In this study, we are mainly focus on elucidating the aggregation behaviors of amphiphilic polymers in aqueous media, thus to provide a profound insight to the applications of amphiphilic polymers in biomedical areas.



2-Methacryloyloxyethyl phosphorylcholine (MPC)



n-butyl methacrylate (BMA)

Background: The preparation of safer and more effective injectable formulations for bioactive compounds is a key objective for pharmaceutical treatments. The bioactive compounds must be dissolved in a suitable medium, most likely an aqueous medium. However, there are many bioactive

compounds with excellent therapeutic properties that are sometimes poorly water-soluble (*e.g.* paclitaxel (PTX)). In general, solubility of these poorly water-soluble bioactive compounds can be enhanced using pharmaceutical solubilizers such as natural phospholipid assemblies, synthetic surface-active molecules, and water-soluble amphiphilic polymers.

As for the use of amphiphilic polymers, they can usually form core-shell type aggregations in aqueous media: the hydrophilic shell makes the entire assembly water-soluble while the hydrophobic core solubilizes hydrophobic molecules as a "microcontainer". However, most of the developed amphiphilic polymers have random type structures, which endow them good water solubility while unclear aggregation process.

With the developments of controlled radical polymerization (CRP), preparation of predesigned polymers with desired specific architecture and low polydispersity becomes possible. The typical CRP methods include atom transfer radical polymerization (ATRP), reversible addition-fragmentation chain transfer polymerization (RAFT) and nitroxide-mediated polymerization (NMP). Additionally, the activators regenerated by electron transfer (ARGET) ATRP method is further developed which is thought capable to be used in biochemistry synthesis because of the much lower quantity of metal and ligand needed during the synthesis. By changing the CRP agent and monomers, well-defined polymers with controlled compositions, chain lengths can be obtained.

MPC-derived amphiphilic polymers prove to have high potential to be used in biomedical areas. For example, random-type PMB (PMBr) composed of 80 mol% MPC units, is used in the pharmaceutical and cosmetic fields as a moisturizing component. In addition, water-soluble PMB composed of 30 mol% MPC units (PMB30r) is commercially available as a solubilizing test reagent for some pharmaceutical compounds. In this work, well-defined PMB with various composition, chain lengths and architectures were synthesized. Comparative study of the aggregation behaviors of diblock-type PMBs and random type PMBs are conducted. A new self-gelation process was discovered based on the triblock-type PMBs in an aqueous media.

Experiments: The PMBs were synthesized as shown in the scheme. For the sample labels, for example, PMB60b, in which, the "60" means the molar percentage of MPC units; while the "b" means the structure type "block-type

copolymer".

Results and discussion: The water-solubility of the PMBs was depended on the composition and architecture of the polymer, however, almost the PMBs obtained in this study showed good water solubility. A comparative study on the aggregation behaviors of both random-type and block-type PMBs can be conducted.

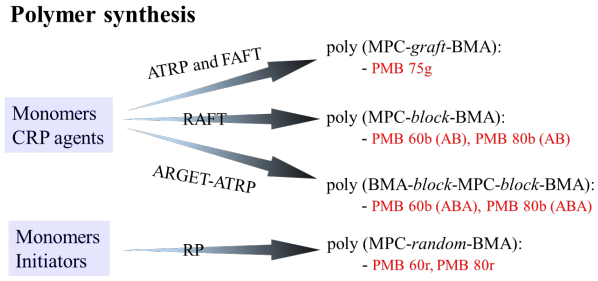
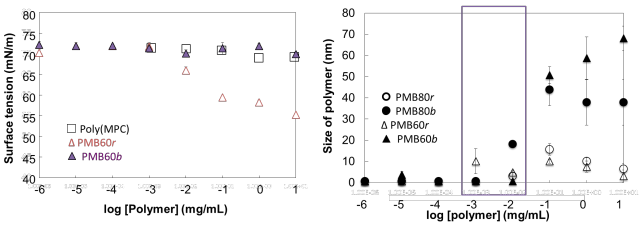
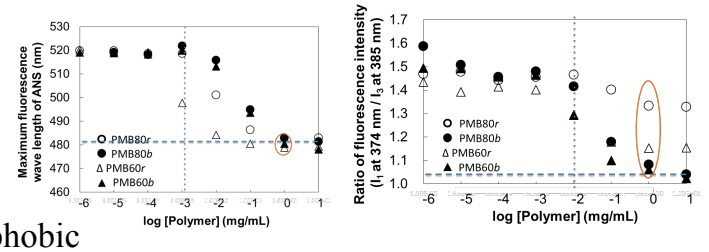


Abb.	MPC unit mole fraction* In feed	in copolymer	Mw** (10^4)	Mw/Mn**	Yield (%)	Solubility H_2O 1 mg/mL
PMB60*r*	0.60	0.61	9.2	3.2	88	++
PMB80*r*	0.80	0.82	8.4	3.4	89	++
PMB60*b*-AB	0.60	0.65	1.3	1.2	71	+
PMB80*b*-AB	0.80	0.80	1.4	1.2	68	+
PMB60*b*-ABA	0.60	0.68	-	-	77	-
PMB80*b*-ABA	0.80	0.86	2.1	1.4	87	+
PMB75*g*	0.75	0.76	2.5	1.4	73	+

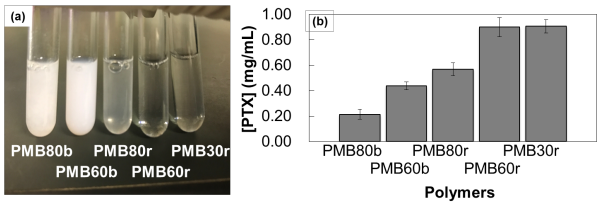
The solubilization of the polymer can be considered from the surface tension measurements. The surface tension of an aqueous solution of both poly(MPC) and PMB60b did not depend on the concentrations, indicating that the hydrophobic main chain is completely covered with hydrophilic side chains. While for the PMB60r, the surface tension decreased obviously. Moreover, according to the DLS tests, remarkable difference between the size of aggregates for PMB60b and PMB60r was observed. The PMB60b showed much larger aggregates size, implying concentrated hydrophobic domains.



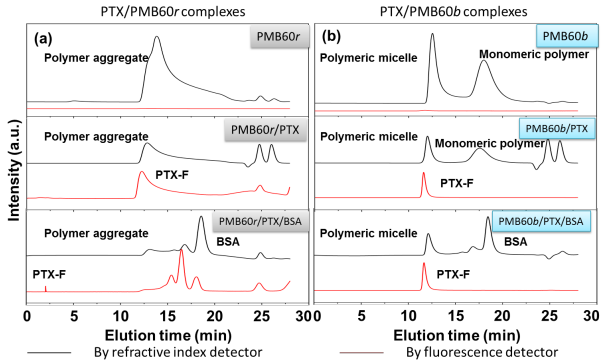
To further clarify the different aggregation behaviors for PMBb and PMBr, two probe molecules with disparate hydrophobic domains were introduced: 8-anilinonaphthalene-1-sulfonic acid (ANS) and much hydrophobic pyrene. As the increased concentration of PMBs, the ANS was gradually localized in the hydrophobic domains, resulting in the wavelength shift. During these changes, the ANS could interact with the PMBr at less concentration than PMBb. When the pyrene was used, although the similar concentration dependence was observed in both PMBr and PMBb system. The PMBb oppositely showed sharper concentration dependence.



These results demonstrate that the PMBb formed a totally core-shell structured aggregates with protected, large hydrophobic domains in the core part; while the PMBr just gave some loose, small and flexible aggregates.



Although giving different aggregation behaviors, all the PMBs could be used to enhance the water-solubility of PTX, a mode drug candidate for treating cancer *in vivo*. Considering the similar hydrophobic size of PTX and ANS, it was reasonable that the PMBr was more effectively in enhancing the solubility. However, when the stability of containing the bioactive compounds is taken into consideration, the PMBb showed advantage over the PMBr.



Another discovery to be noted is that the triblock-type PMBb with a PMPC segment in the middle part and two symmetrical PBMA segments at two ends behaved self-gelation property through designed processes. This unique gel formation behavior, the first of this kind, was due to the rigidity of the PMPC segment. Similar to the above discussed diblock-type PMBb, this triblock-type PMBb based gel system can also be used for a sustainable release of bioactive compounds included proteins and peptides.

Over all, based on this work, the different aggregation behaviors of random and block type PMBs are clarified. With well-defined structures and strong hydrophobic interaction, block type PMBs showed high potential to be used as carriers for hydrophobic biomolecules.

Future perspective: The previous study mainly focuses on the properties of the PMBs themselves. For practical application, it is necessary to further exploring the evolution process of the materials when containing bioactive compounds and proceeded under simulated *in vivo* environments.