A Spontaneous Aggregate Formed by Polypeptide-based Amphiphile in Water

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In order to construct a molecular aggregate with well-defined structure, a novel amphiphile consisting of a phosphate head group and hydrophobic polypeptide tail was prepared. This amphiphilic polypeptide was soluble in water and spontaneously assembled to form a ribbon-like molecular aggregate. FT-IR and circular dichroism (CD) spectra showed that the aggregated polypeptide adopts an anti-parallel β-form conformation.

In *vivo*, biolipids are arranged as the double layer structures (lipid bilayer) and act mainly as barriers to the transport of water-soluble molecules.[1] Since the discovery that bilayer membranes can be made by dialkylammonium salts,[2] a large variety of artificial amphiphiles displaying membrane organization comparable to that of biolipids have been successfully prepared. These amphiphiles can form several types of molecular aggregates based on the hydrophobic association between long hydrocarbon chains, such as spherical particles, bilayer membranes and hexagonal cylinders. These are well-defined structures,[3-9] however, their interior consisting of the liquid hydrocarbons may be rather statistical. Furthermore, there are few studies of an amphiphile consisting of non-hydrocarbon tails. [10]

In this study, we prepared and estimated its molecular organization of amphiphilic polypeptides in water, instead of employing hydrocarbon-containing amphiphiles. As a result, the amphiphile can self-assembled into a molecular aggregate with a highly ordered interior.

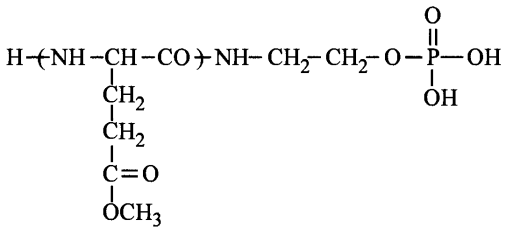

Figure 1. Chemical structure of PMG_{10}-P.

A novel polypeptide-based amphiphile, poly(γ-methyl L-glutamate) with a phosphate moiety at the terminal (PMGn-P; Figure 1), was obtained by the polymerization of the N-carboxy anhydride of L-glutamic acid γ-methyl ester (MG-NCA; Ajinomoto Co., Ltd. Japan) with *O*-phosphorylethanolamine as an initiator. The MG-NCA (1.0 g) was solved in ethyl acetate (50 ml; Katayama Chemical Co., Ltd., Japan). The obtained solution was added dropwise to the aqueous solution (pH 9.0) containing the initiator and the mixture was vigorously stirred for two hours at room temperature. The method gave an amphiphilic polypeptide with the average degree of polymerization, n, of 10 (PMG_{10}-P; Mw=1571), which was estimated from ^{1}H-NMR analysis of trifluoroacetic acid solution of the polypeptide. A distribution of molecular weight ($\overline{Mw}/\overline{Mn}$) of PMG_{10}-P, 1.10, was obtained by gel permeation chromatographic analysis using a G4000HXL column (Tosoh Ltd., Japan).

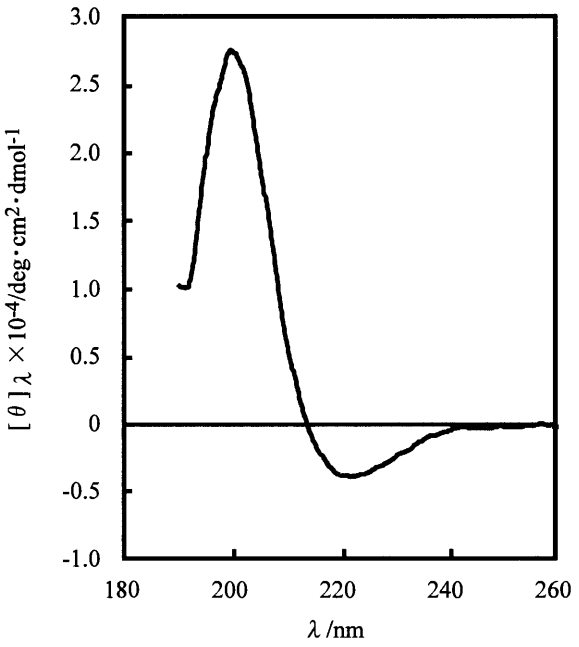

Figure 2. CD spectrum of PMG_{10}-P in aqueous solution.

PMG_{10}-P was soluble in water by sonication (Biomic model 7250B; SEIKO I&E Co. Ltd., Japan). The conformation of PMG_{10}-P in aqueous solution was characterised by circular dichroism (CD). The CD spectrum of PMG_{10}-P exhibited positive band at 198 nm and negative band at 218 nm, respectively (Figure 2). The spectrum showed the hydrophobic polypeptide was in a β-form conformation in water.[11] Furthermore, the FT-IR spectrum of PMG_{10}-P cast film from aqueous solution also showed the typical spectrum for anti-parallel β-sheet conformation (Amide I :1625 cm^{-1}; Amide II: 1550 cm^{-1}).[12] In this system, intermolecular interactions must be necessary to drive the β-form conformation. Therefore, it was expected that this interaction may yield molecular aggregates in this aqueous system.

The morphology of the PMG_{10}-P aggregate in water was studied by transmission electron microscopy (TEM). After the sonication PMG_{10}-P was dispersed in water forming globular aggregate whose diameter was 50-150 nm (Figure 3(a)). One hour later, globular aggregates joined each other to form fibrous aggregates (Figure 3(b)). Twenty-four hours later, the fibrils assembled to form twisted ribbon-like aggregate (Figure 3(c)). The thickness of the ribbon-like sheet was ca. 4.0 nm and this value is compatible with the chain length of PMG_{10}-P when it

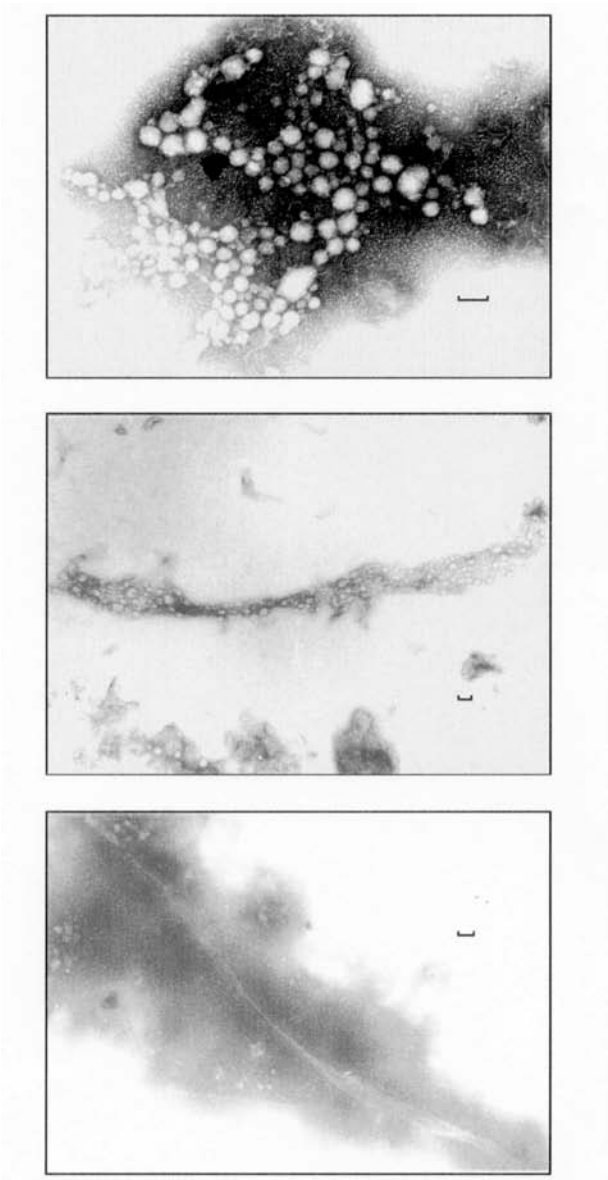

Figure 3. Transmission electron microscopy of PMG10-P assembly in water after sonication (a), aging 1 h (b), aging 24 h (c). —— ; 100nm

is in the β-form conformation.

In conclusion, poly(γ-methyl L-glutamate) with an phosphate moiety at the terminal can associate with each other *via* the intermolecular hydrogen bonds between the peptide backbones, in addition to the hydrophobic interactions between the side-chains, to form spontaneously the ribbon-like sheet structure (Figure 4). A highly ordered β-form polypeptide layer interior of the aggregate is the remarkable and unique structural feature that is not observed with the aggregates composed of amphiphiles having hydrocarbon tail.

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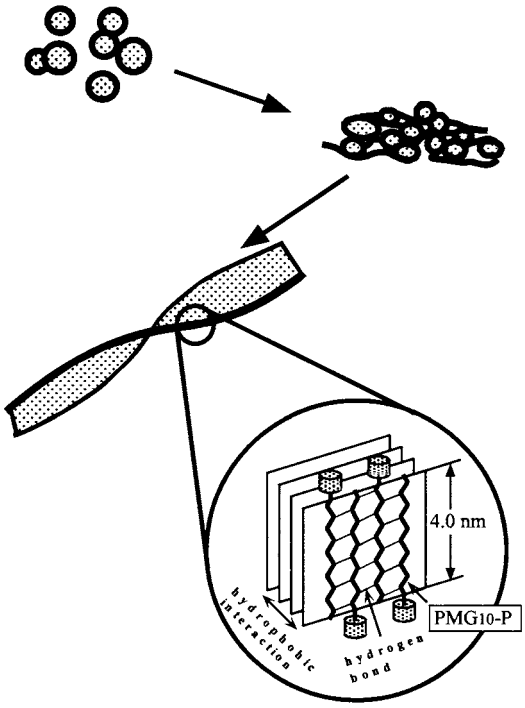

Figure 4. Schematic illustration of the structure of PMG10-P assembly in the aqueous.

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