Controllable Orientation of the Peptide-Based Surfactant at Air-Water Interface

Akiko Toyotama,* Shin-ichi Kugimiya, Masakatsu Yonese, Takatoshi Kinoshita,† and Yoshiharu Tsujita†
Faculty of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya 467
†*Department of Materials Science and Engineering, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya 466*

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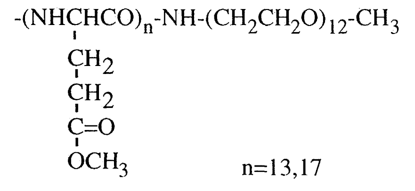
A polypeptide-based amphiphile, poly(γ-methyl L-glutamate)-polyethyleneglycol block copolymer (PMG-PEG), formed a stable monolayer membrane at the air-water interface. The orientation of the α-helical amphiphile in the membrane was shown to be strongly dependent on the surface pressure.

It has been recognized that the molecular orientation at interfaces is directly related to the cell membrane function and activity. Then there is a lot of cases, such as bacteriorhodopsin, that they are beared by closely packed α-helices,which extend nearely perpendicular to the plane of the membrane. Therefore, it is well known that much effort has been made to clarify and regulate the structure of monolayer of variety of surfactants.[1] For example, Yamamoto *et al.*[2] showed that the structure of monolayer composed of amphiphilic diblock copolymer is quite different between low and high pressures. Furthermore the structure and properties of monolayers of polypeptides at air-water interface were systematically investigated by Malcolm.[3] It was found that the α-helical polypeptide monolayers are consistent with a structure consisting of condensed ordered arrays of α-helices keeping its axis parallel to the interface (Figure 1(a)). The collapse pressure of such the monolayer was also shown to be around 20 mN/m. However it seems necessary to have the ordered structure, virtical to the membrane, of α-helical segments spanning the stable membrane for getting the biological function and activity. Therefore, we aim for it, prepared a polypeptide-based amphiphile, PMG-PEG, and characterized the molecular orientation of helix rod in the monolayer as a function of the surface pressure.



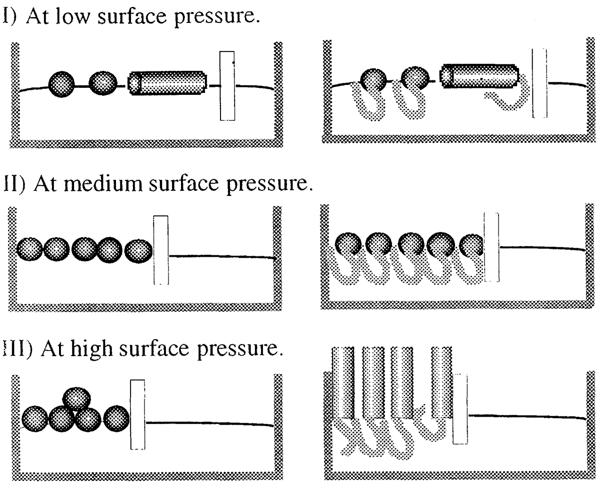
$$\begin{array}{l} -(\mathrm{NH\underset{|}{C}HCO})_n\text{-NH-}(\mathrm{CH_2CH_2O})_{12}\text{-CH}_3 \\ \;\;\;\;\;\;\;\;\;\mathrm{\underset{|}{C}H_2} \\ \;\;\;\;\;\;\;\;\;\mathrm{\underset{|}{C}H_2} \\ \;\;\;\;\;\;\;\;\;\mathrm{\underset{|}{C}{=}O} \\ \;\;\;\;\;\;\;\;\;\mathrm{OCH_3} \end{array}$$

n=13,17

Figure 2. Chemical structure of PMG-PEG.

The block copolymer , PMG-PEG (Figure 2), was obtained by polymerization of N-carboxy L-glutamic acid anhydride γ-methylester with ω-aminopolyethyleneglycol methyl ether[4] as an initiator. The average degree of polymerization of PMG moiety in the PMG-PEG was determined to be 13 and 17, PMG13-PEG and PMG17-PEG, respectively, by ^{1}H-NMR.[5] We also confirmed from circular dichroism (CD) spectroscopy and X-ray analysis that it formed a novel micelle, in aqueous phase,having ordered core structure with hexagonal packing of α-helix molecules.[6]

In order to prepare PMG-PEG monolayer, the sample was spread from a solution of TFE on the clean distilled water surface in a trough. And surface pressure (π) - area (A) isotherms were obtained by Wilhelmy plate method. The both amphiphiles were found to form stable monolayer (Figure 3). The monolayers were accumulated five times, respectively, on a quartz plate and



(a) α-helical polypeptide (b) PMG-PEG

Figure 1. Schematic illustration of monolayers at the air-water interface.

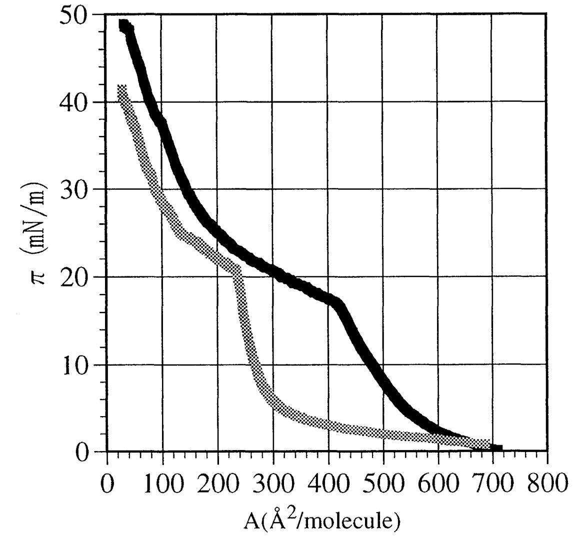


Figure 3. Pressure-area(π-A) isotherms of PMG13-PEG(▒) and PMG17-PEG(▬).

Table 1. Limiting area estimated from π -A isotherms

	A_L (Å^2/molecule)	A_S (Å^2/molecule)
PMG13-PEG	290	240
PMG17-PEG	560	240

analyzed with CD spectroscopy. The α-helical conformation was found to be kept on the quartz plate. The π-A curves, for both systems, showed two steep increase parts in π, suggesting a structure change taking place on the water surface above specific pressures. Extrapolations of the each steep parts of the isotherm to π=0 give the two limiting areas (A_L, A_S) per PMG-PEG molecules (Table 1). At A_L, the large area, there was much difference between PMG17-PEG and PMG13-PEG. This difference is due to the difference of moleculer weights of the two surfactants. On the other hand, the value of A_S, the small area, was found to be consistent with each other. Thus, at the smaller area than A_L, PMG moiety of PMG-PEG may be oriented perpendicular to air-water interface. This means that PMG-PEG lie flat on the water surface at low surface pressure (<35 mN/m), at higher pressure (>35 mN/m), it formed a highly oriented structure vertical to the air-water interface (Figure 1(b)). The hydrophilic segment, PEG, which is movable in aqueous phase, can come into gap of helices, resulting in the decrease in the interaction between the PMG helices. So that PMG helices rise up against the interface by degrees. These estimated areas are above twice the value, 120 Å^2, expected for a close packed monolayers estimated from X-ray analysis.[6] This also supports the penetration of PEG segment among PMG helices. Furthermore this monolayer have high collapse pressure, above 40 mN/m, than that of hydrophobic polypeptide monolayer.[3] This may arise from the unique structure proposed above (Figure 1(b)).

In this study, we revealed the following (I) PEG attached peptide weaken the interaction between the peptide helices and assist in aggregation high ordered and stable structure at surface above specific pressure, (II) the fine orientation of this peptide-based surfactant at air-water interface depended on surface pressure (*i.e.* at low surface pressure the molecule exists flat on the air-water interface, at high surface pressure this exists vertical to the interface).

References and Notes

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4 It was prepared from the corresponding poly(ethyleneglycol) monomethyl ether.

5 It obtained by comparing the peak area of β-, γ-methylene protons to the PMG (δ=2.0～2.8 ppm) and methylene protons to the PEG (δ=3.9～4.1 ppm).

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