The Molecular Orientation of a Peptide-based Amphiphile at Hexane/Water Interface

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A rod-like amphiphile, hydrophobic poly(γ-methyl L-glutamate) containing β-cyclodextrin as a hydrophilic terminal group (PMG-CyD), lay down in its monolayer membrane at air/water interface, however, it has a possibility for the perpendicular orientation at hexane/water interface above specific surface pressure.

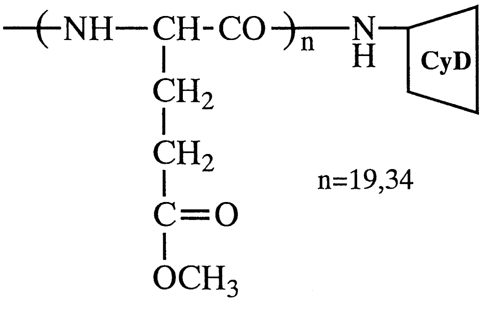
Molecular orientation at an interface is one of the most interesting topics in interfacial phenomena in biological systems and their related science and technology. For example, it has been recognized that the orientation of a key segment in membrane proteins decides the functions such as molecular recognition and energy and/or information transfer on and through the biological membranes.

Simple model systems give a basic insight into this problem. Among them, rod-like molecules, such as α-helical polypeptides, may be convenient, since the small changes in their orientation are drastically reflected to the interfacial properties. It has been reasonably considered that α-helices lie down in their membrane and at every possible interfaces,[1] however, recently a highly ordered layer with α-helices oriented nearly perpendicular to the surface of substrates was pruduced.[2]

In this study, we prepared a rod-like molecule with a molecular recognition site, α-helical poly(γ-methyl L-glutamate) containing β-cyclodextrin at the terminal (PMG-CyD; Figure 1), and formed its monolayer at oil/water interface in order to elucidate a possibility for control of the monolayer order, i.e., molecular orientation of this rod-like molecule with its functional end group.

The PMG-CyD (Figure 1) was obtained by polymerization of N-carboxy L-glutamic acid anhydride γ-methylester with 6-amino-cyclodextrin[3] as an initiator. The average degree of polymerization of PMG moiety in the PMG-CyD was determined to be 19 and 34, PMG_{19}-CyD and PMG_{34}-CyD, respectively, by ^1H-NMR.

PMG-CyD forms a stable monolayer at air/water(A/W) interface by spreading a DMF solution onto a pure water subphase. The monolayers were accumulated five times on a quartz plate and analyzed with CD spectroscopy. The typical α-helix CD pattern showed that the helix conformation was found to be kept on the quartz plates[4]. At A/W interface the pressure-area (π-A) isotherms of PMG_{19}-CyD and PMG_{34}-CyD showed a steep increase. Extrapolation of the each steep part of the isotherm to $\pi=0$ gives the extrapolated area, 312 Å^2/molecule (PMG_{19}-CyD) and 600 Å^2/molecule (PMG_{34}-CyD), respectively. X-ray analysis of the PMG solid film showed that the diameter of PMG rod including side chain region is 12.2 Å. Based on this diameter we can calculate the difference in the area per molecule owing to the difference in the degree of polymerization between the two PMG-CyDs to be ca. 275 Å^2/molecule when the polypeptides lie down on the water surface. This difference is almost equal to that obtained from the extrapolated area, thus confirming the parallel orientation of the α-helices to the A/W interface.



$$-\!\!\left(NH-\underset{\underset{\underset{\underset{OCH_3}{|}}{C=O}}{|\;CH_2}}{\overset{|}{CH}}{}_{\,\text{}}\!\!-CO\right)_n\!-\underset{H}{N}-\text{CyD}$$

Figure 1. Chemical structure of PMG-CyD.

On the other hand, at hexane/water (H/W) interface the PMG-CyD also forms a stable monolayer[5]. The π-A isotherms for both systems (Figure 2) in this case showed two steep increase

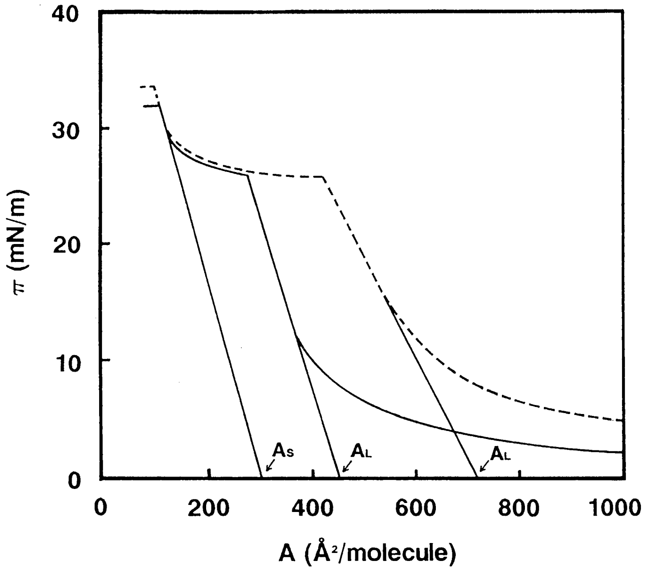


Figure 2. Pressure-area (π-A) isotherms of PMG_{19}-CyD(——) and PMG_{34}-CyD(- - - -).

Table 1. Limiting area estimated from π-A isotherms

	A_L(Å^2/molecule)	A_S(Å^2/molecule)
PMG_{19}-CyD	455	305
PMG_{34}-CyD	720	305

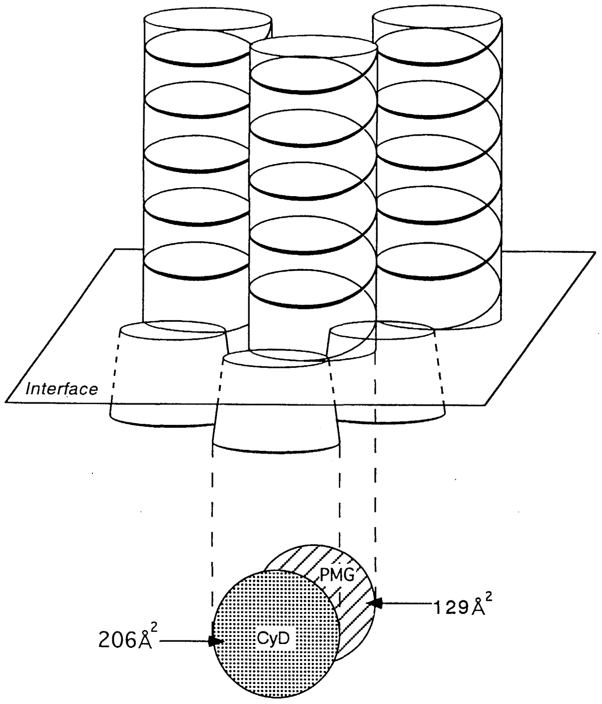


Figure 3. Schematic picture of PMG$_{19}$-CyD and PMG$_{34}$-CyD at hexane/water interface.

parts, respectively, suggesting that the monolayers can take two possible orders in the solid state monolayer by introducing the hexane phase. Extrapolations of the each steep parts of the isotherm to π=0 gave two extrapolated values of area per PMG-CyD molecule (A_L and A_S in Table 1). As for the larger area, A_L, there was a difference, 265 Å^2/molecule, between PMG$_{19}$-CyD and PMG$_{34}$-CyD, which is roughly consistent with the difference calculated above. However, the absolute values, 455 Å^2 and 720 Å^2, are higher than those at A/W interface, respectively. This means that hexane molecules penetrate in the alkyl side chain region of the PMG segments and weakened the interaction among them. On the other hand, the values of A_S, the smaller area, were interestingly found to be consistent with each other. One of the possible structure based on this fact is that, under the smaller area than A_L, α-helical segments of PMG-CyD may be oriented perpendicular to H/W interface (Figure 3). However, the area, 305 Å^2/molecule is much larger than those of the cross sectional area, 206 Å^2, of CyD and that, 129 Å^2, for the rod of PMG. This difference may be explained in terms of the deviation of the center axis between PMG helix and CyD (Figure 3).

In this study, we showed a possibility for the control of molecular orientation of a rod-like amphiphile, PMG-CyD, in the monolayer membrane system, i.e., the possibility for a perpendicular orientation of α-helix rod at hexane/water interface. The spectroscopic study of the helix rod orientation in the monolayer is in progress and will be reported elsewhere.

References and Notes

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3 L.D.Melton and K.N.Slessor, *Carbohydrate Research*, **18**,29 (1971).
4 The α-helix conformation of the samples was also confirmed by Amide I (1548 cm^{-1}) and Amide II (1653 cm^{-1}) in the FT-IR spectra of their cast thin films on KBr plate.
5 For the preparation of the monolayer at oil/water interface, see: B.B. Sauer, M Kawaguchi, and H. Yu, *Macromolecules*, **20**, 2732 (1987).