Facilitated Formation of Helical Polypeptide Assemblies in a Lipid Monolayer by an Interfacial Polyion Complexation

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Considerable efforts have been directed[1] toward the design and synthesis of small model proteins to elucidate interactions involved in protein folding. Several groups[2] have devised a strategy in which a rigid molecule serves as a template to assemble secondary structures in simple model proteins. We now report the formation of α-helix polypeptide assemblies in lipid monolayers on water by using a polyion complexation technique.[3]

Surface monolayers are a useful tool for ordering molecules two-dimensionally. By the use of it, water-insoluble poly(α-amino acid)s have been spread on water and revealed their conformational properties on the basis of surface pressure (π)–area (A) isotherms.[4] Recently, water-soluble biopolymers such as proteins[5] and synthetic polymers[6] were organized through their specific binding to the monolayers from the bulk aqueous phase. Amphiphiles of polyelectrolytes or hydrophilic polymers connected covalently with long alkyl chains could also be aligned at the air–water interface.[7,8] These polymer assemblies have shown characteristics uniquely different from those in homogeneous media. As part of our research into the polymer assemblies, we have prepared amphiphilic poly(γ-benzyl L-glutamate) (PBLG-N^+), whose one terminus is modified with a quaternary ammonium group so as to form a monolayer on water and to interact with a polyanion such as sodium poly(styrenesulfonate) (PSS^-) in the subphase, and describe the mixing behavior and second-order structure of PBLG-N^+ with L-α-dipalmitoylphosphatidylcholine (DPPC) as a matrix lipid monolayer on both pure water and aqueous PSS^-.

PBLG-N^+ was prepared via the following three steps. First, polymerization of γ-benzyl L-glutamate-*N*-carboxylic anhydride[8] initiated with the primary amino group of propylamine was carried out, then the residual amino group of the chain end of the polymer obtained was reacted with ω-bromoundecanoyl chloride, and finally, the polymer was treated with a large excess of trimethylamine to the terminal bromine of the polymer. The resulting amphiphilic polypeptide (PBLG-N^+) consisted of a quaternary ammonium group as the hydrophilic part and the PBLG segment as the hydrophobic part whose degree of polymerization (n) was 41. DPPC and PSS^- were purchased from commercial sources (Wako and Aldrich, respectively).

The monolayers were obtained by spreading a benzene–chloroform (8:2) solution containing PBLG-N^+ and DPPC on purified water (Milli-Q system, Millipore Ltd.) or on aqueous PSS^- ([styrenesulfonate unit] = 2.4×10^{-4} M). Twenty minutes after spreading, the monolayer was compressed continuously at a rate of 1.2 $cm^2\ s^{-1}$.[9] Wilhelmy's plate method and a Teflon-coated trough with a microprocessor-controlled film balance, FSD-50 (San-Esu Keisoku, Ltd.) with a precision of 0.01 mN m^{-1}, were used for surface pressure measurements.

Chart 1

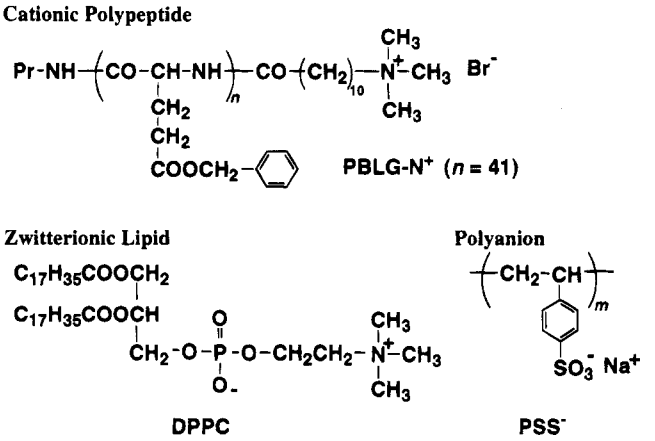

Figure 1 shows surface pressure (π)–area (A) isotherms of PBLC-N^+ and DPPC on pure water and on aqueous PSS^- at 20 °C. PBLG-N^+ is found to give an extremely expanded monolayer compared with DPPC. When PSS^- was added into the subphase, the PBLG-N^+ monolayer further expanded while the zwitterionic DPPC monolayer showed no change in area, as was expected. This suggests that an ionic complexation between PBLG-N^+ and PSS^- through electrostatic force occurs at the air–water interface. To obtain more quantitative information for complexation, the composition of an LB (Langmuir–Blodgett) film[10] transferred from a monolayer of pure PBLG-N^+ on aqueous PSS^- was determined by X-ray photoelectron spectroscopy (XPS). As a result, XPS data clearly showed that the LB film was composed of a 1:1 polyion complex of PBLG-N^+ and PSS^-.[11]

The mixing behavior of PBLG-N^+ and DPPC has been investigated in monolayers on pure water and on aqueous PSS^-. π–A isotherms for different compositions of a two-component monolayer have been recorded (not shown here). The mixing behavior of lipids of monolayers can be studied by using the phase rule of Crisp.[12,13] The area of the mixed monolayers at defined surface pressure is plotted against the mole fraction of the mixture. For an ideal mixing behavior (complete miscibility or complete phase separation) these areas show a linear dependence on the mole fraction. Any deviation from that linearity is caused by interactions of the different amphiphiles which demonstrates at least partial miscibility. Figure 2 shows a typical mean area–composition diagram (at a constant pressure of 20 mN m^{-1}) for PBLG-N^+ and DPPC. On pure water, apparent deviation from the linearity is observed, meaning partial miscibility of both components. In contrast, on aqueous PSS^- the area is linearly dependent on the composition of the monolayer. This demonstrates the ideal behavior of these amphiphiles. On the basis of these measurements alone one cannot distinguish between an ideal miscibility and a phase-separated system for the mixture of PBLG-N^+ and DPPC. Thus, an additional method is necessary to distinguish between such ideal systems. We have employed circular dichroism (CD) spectroscopy to characterize the interaction and secondary structure of PBLG-N^+ in the mixed monolayers.

Figure 3 displays a CD spectrum (JASCO J-720) of an LB film[14] transferred from the mixed monolayer of

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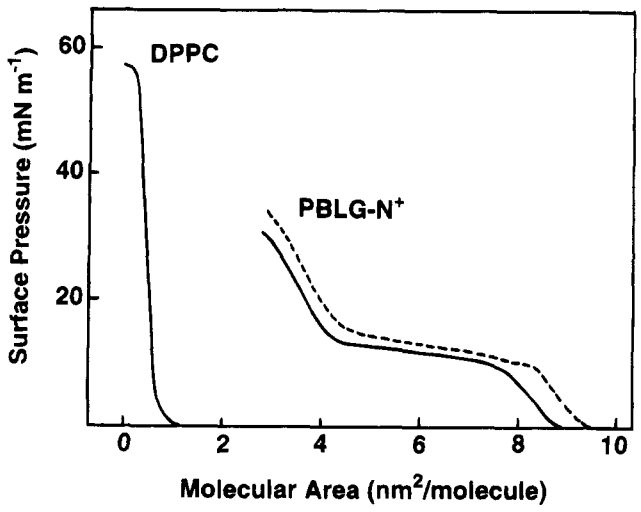

Figure 1. Surface pressure (π)–molecular area (A) isotherms of PBLG-N$^+$ and DPPC on pure water (solid line) and on aqueous PSS$^-$ ([styrene sulfonate] = 2.4 × 10^{-4} unit M) (broken line) at 20 °C. Spreading conditions are described in the text.

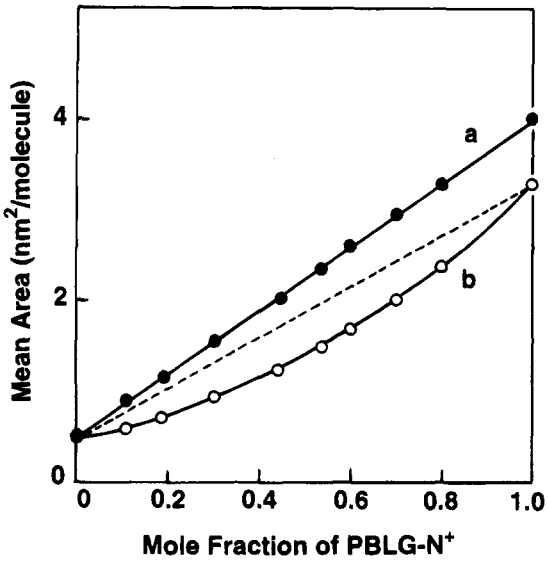

Figure 2. Mean molecular area as a function of the composition of monolayers for the system PBLG-N$^+$/DPPC on aqueous PSS$^-$ (a) and on pure water (b) at a constant surface pressure of 20 mN m^{-1}, 20 °C.

PBLG-N$^+$ and DPPC (mole fraction of PBLG-N$^+$, 0.20) on aqueous PSS$^-$ at a constant surface pressure of 25 mN m^{-1}, at which the monolayer is in a condensed phase. We have tried to transfer the same monolyer without polyanion PSS$^-$ in the subphase (i.e., on pure water) under several conditions but have failed.

In Figure 3, a CD spectrum of PBLG-N$^+$ in 1,1,1,3,3,3-hexafluuoro-2-propanol (HFP) solution (1.0 × 10^{-4} unit M) is also shown for comparison. The spectrum of this solution exhibits the two negative bands at 222 and 208 nm, indicating a typical α-helix conformation in a manner similar to that of α-helical polypeptides.[15] On the other hand, the LB film gives a different CD spectrum from that encountered in the homogeneous HFP solution. It has been reported that CD spectra show red shifting of the 222-nm band toward 225–230 nm and progressive flattening of the 208-nm band when aggregation of α-helices progresses.[16] The observed spectral feature for our LB film is very similar to that derived from aggregation of α-helices, suggesting formation of PBLG-N$^+$ domains in the mixed monolayer with

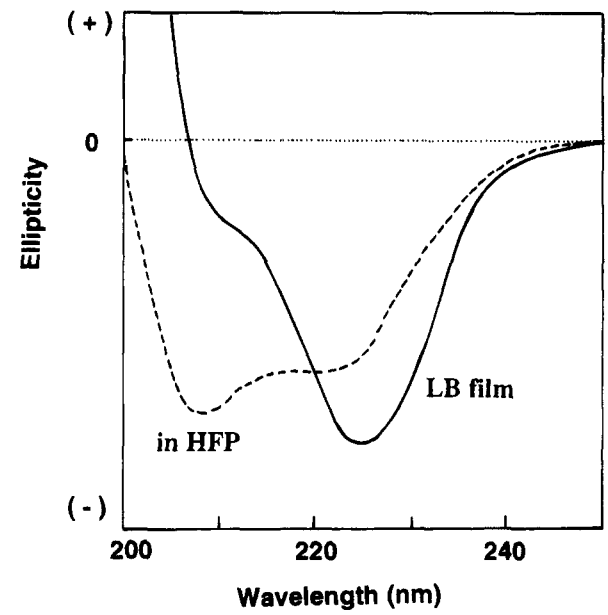

Figure 3. CD spectra for an LB film transferred from the mixed monolayer of PBLG-N$^+$ and DPPC (mole fraction of PBLG-N$^+$, 0.2) on aqueous PSS$^-$ at 25 mN m^{-1} and for an HFP solution of PBLG-N$^+$ (1.0 × 10^{-4} unit M).

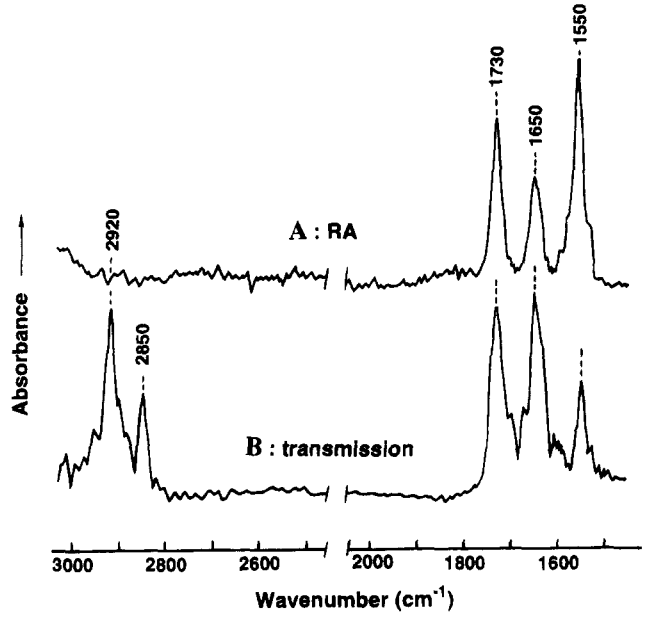

Figure 4. FTIR spectra for the same LB film as described in Figure 3: (A) reflection–absorption spectrum for the LB film deposited on a Au-evaporated glass plate; (B) transmission spectrum for the LB film deposited on a CaF_2 plate.

PSS$^-$. Therefore, the polyanion, PSS$^-$, must have interacted selectively with the cationic PBLG-N$^+$ but not with DPPC and then caused an aggregation of PBLG-N$^+$ helices, yielding a phase-separated monolayer at the air–water interface.

Subsequently, FTIR spectra (Nicholet System 800) were measured for the same LB film. Molecular orientation in an LB film can be estimated by comparison of band intensities in IR transmission and reflection–absorption (RA) spectra.[17] Figure 4 shows transmission and RA[18] spectra of one-complexed monolayer (mole fraction of PBLG-N$^+$, 0.2) LB films deposited on a CaF_2 plate and an Au-evaporated glass plate, respectively.

In the amide I and amide II regions (1700–1500 cm^{-1}), both spectra exhibit two bands at 1650 and 1550

cm^{-1}, which are assigned to the α-helix conformation[19] of PBLG-N^+, comparable to the above CD spectroscopic data. In addition, the C=O stretching band due to ester groups of DPPC and the side chain of PBLG-N^+ is observed at 1730 cm^{-1}. Upon going from the RA spectrum to the transmission one, an obvious difference in band intensities appears in the CH_2 stretching region, and in the amide I and amide II regions. In the CH_2 stretching region (2920 and 2850 cm^{-1}), the RA spectrum shows no peak due to CH_2 symmetric and antisymmetric stretching modes while the transmission spectrum does. This observation clearly demonstrates that alkyl chains of DPPC are oriented perpendicularly to the surface.[17] On the other hand, in the amide I and amide II regions the relative band intensity of amide I (1650 cm^{-1}) to amide II (1550 cm^{-1}) is found to be different between the transmission and RA spectra. Since the amide I and amide II bands are derived from the C=O stretching and the C—N stretching, including the N—H in-plane bending, respectively, on the basis of peptide bonds of the PBLG-N^+ main chain, it might not be easy to evaluate the orientation of PBLG-N^+ chains by these spectra alone due to their helical conformation even if they would align perpendicularly to the surface normal. Recently, Samulski et al.[20] proposed a method to estimate the helix orientation in self-assembled PBLG films on a gold substrate using the IR dichroic properties. Estimation of such an orientation of our α-helix is now in progress.

In conclusion, the present study has demonstrated that the α-helix polypeptide assemblies in the lipid monolayers can be produced by addition of the polyion if the end of the polypeptide chains is appropriately modified with an oppositely charged group. We believe that this concept is extended to other polypeptide—lipid combinations and is useful to model the structure of biomembranes.

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(9) Below this value (1.20 cm^2 s^{-1}), the effect of compression rate on the monolayer area was within experimental error.

(10) The LB film was transferred in the vertical mode at a transfer rate of 2 mm min^{-1} onto a quartz plate. The transfer ratio (±10%) was 1 both in the down-stroke and in the up-stroke mode.

(11) XPS (instrument, Shimadzu ESCA-1000; X-ray source, Mg Kα; takeoff angle of the photoelectron is 50°) spectra for the LB film (10 layers) transferred from a monolayer of pure PBLG-N^+ on aqueous PSS^- at a surface pressure of 20 mN m^{-1} gave peaks including those of C_{1s}, N_{1s}, O_{1s}, and S_{2p}. The S_{2p} peak is derived from PSS^-. Two peaks at 404 and 402 eV due to N_{1s} appeared, which can be assigned to the cationic ammonium nitrogen (N^+) and nitrogen in the peptide bonds, respectively. Peaks due to Na and Br were not detected, meaning that the ion-exchanging (complexation) reaction between PBLG-N^+ and PSS^- occurred at the air—water interface. The elemental ratio, $N(N^+)/S$, was determined to be 1:0.95(±0.2), in close agreement with that expected from complete pairing of the sulfonate anion in PSS^- and the ammonium cation in PBLG-N^+, from the area ratio of the $N_{1s}(N^+)$ and S_{2p} peaks after correction for relative sensitivity factors [*Practical Surface Analysis by Auger and X-Ray Photoelectron Spectroscopy*; Briggs, D., Seah, M. P., Eds.; Wiley: New York, 1983].

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