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Ligand-induced association of helical polypeptides at the air–water interface. Orientation of helix rods on water

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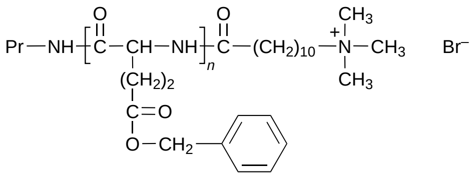
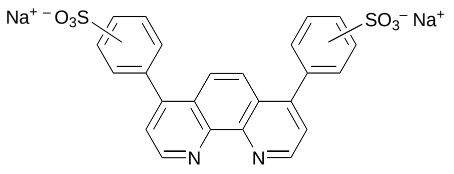
Helix bundle structure has been successfully constructed at the air–water interface using ion complexation. A quaternary ammonium-terminated poly(γ-benzyl-L-glutamate) (PBLG-N^+) was prepared and used as the helical component. Bathophenanthroline disulfonate (BPS) was employed as a template to associate α-helices. The ion complexations of the ammonium group of PBLG-N^+ with the sulfonate groups of BPS or the iron BPS complex (PBLG-N^+/BPS or PBLG-N^+/Fe-BPS) were carried out in solution. The surface pressure(π)–area(A) curve of PBLG-N^+/Fe-BPS gave a more expanded monolayer compared with those for PBLG-N^+ and PBLG-N^+/BPS. The PBLG helical rod in the PBLG-N^+/Fe-BPS monolayer was calculated to tilt at an angle $\langle\theta\rangle = 41°$ from the surface normal by means of FTIR spectroscopy. Subsequently, the complexations were performed at the air–water interface. When the iron BPS complex was added into the subphase, the monolayer of PBLG-N^+ gave a condensed π–A isotherm compared with that on pure water, suggesting ion complexation between the monolayer and the iron BPS complex. The average tilt, $\langle\theta\rangle$, of the helical axis in this monolayer was estimated to be 25°. To improve the orientation of the helical rods, the PBLG-N^+/BPS monolayer was spread on an aqueous solution containing BPS and Fe^{2+}. As a result, the helix rod was found to align with almost vertical orientation.

Considerable efforts have been directed toward the molecular design and synthesis of model proteins to elucidate interactions related to protein folding.[1–3] Protein tertiary structures can be looked upon as assemblies of secondary structures connected by flexible loops. This model has been the basis for the design of artificial proteins. The design process is simple; one first creates secondary structure modules and then connects them together with appropriate loops. A recent strategy for protein design is to replace flexible loop structures with a rigid template molecule. Many artificial proteins have been prepared by covalently attaching α-helices to templates and these have been characterized in aqueous solutions.[4–8]

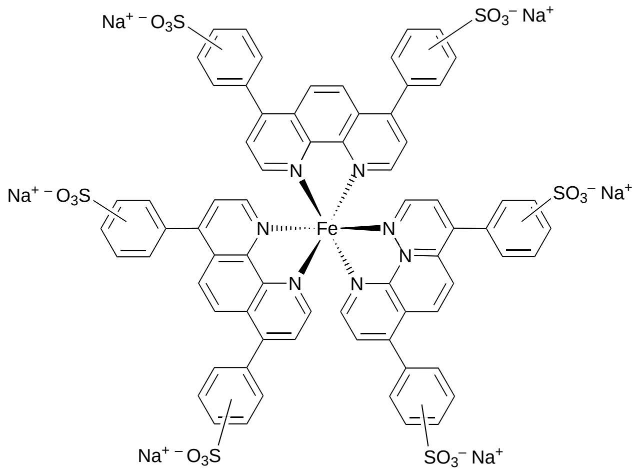
We have devised a strategy in which an interfacial polyion complexation is employed for fabricating an α-helix bundle structure.[9] As a secondary structure module, we have chosen poly(γ-benzyl-L-glutamate), one of whose termini is modified with a quaternary ammonium group (PBLG-N^+) so as to form monolayers on water and interact with a polyanion such as poly(styrene sulfonate) added in the subphase. Consequently, it has been found that α-helix polypeptide (PBLG-N^+) assemblies in a zwitterionic lipid monolayer can be produced by addition of the polyanion *via* polyion complexation-induced phase separation of the monolayer. In the present paper, we describe facilitated formation of a helix bundle structure of PBLG-N^+ at the air–water interface using a bathophenanthroline disulfonate (BPS) iron complex.



PBLG-N+
(n = 35)



BPS



Fe-BPS

Results and Discussion

Preparation of ion complexes of PBLG-N^+ with BPS or Fe-BPS and their monolayer characteristics

Since PBLG-N^+ as the helix component bears a quaternary ammonium salt moiety, ion complexes would be formed by ion-exchange reaction with sodium sulfonate groups of BPS or Fe-BPS. Ion complexes (PBLG-N^+/BPS and PBLG-N^+/Fe-BPS) were prepared by mixing PBLG-N^+ and 1/2 equiv. of BPS or 1/6 equiv. Fe-BPS in a DMF–water mixed solvent. The stoichiometric complexations were confirmed by ^1H NMR spectroscopy. It has been reported that the N-CH_3 proton resonance in the NMR spectrum of the sodium polystyrene sulfonate–dodecyltrimethylammonium bromide polyion complex exhibits an upfield shift compared with that

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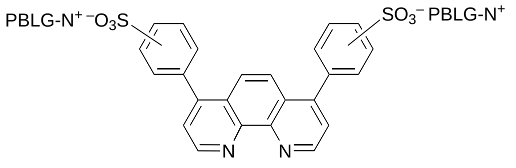
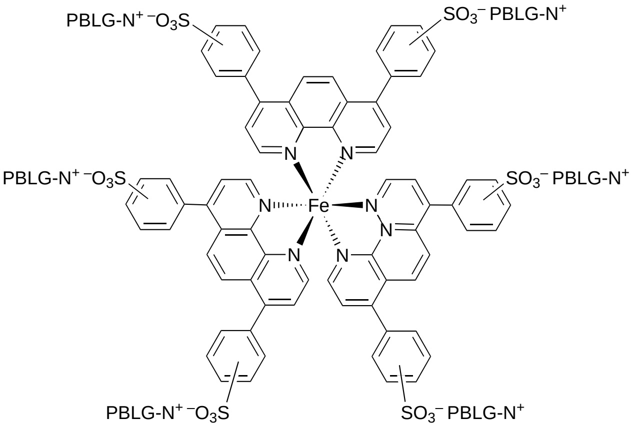
in solution containing dodecyltrimethylammonium bromide due to the ring-current effect of the aromatic groups.[10] For our ion complexes, such ring-current shifts were also observed. The N-CH_3 proton resonance (3.2 ppm) in a $CDCl_3$ solution of PBLG-N^+ disappeared completely upon complexation with BPS or Fe-BPS due to an upfield shift. This result demonstrates that no unreacted, free PBLG-N^+ exists and thus the structure of the complexes are as shown.



PBLG-N+/BPS



PBLG-N+/Fe-BPS

Subsequently, π–A isotherms of these complexes were measured on pure water at 20 °C and these are shown in Fig. 1, in which the data for PBLG-N^+ measured under the same conditions are also included for comparison. The π–A curves are found to expand in the order PBLG-N^+/Fe-BPS≫PBLG-N^+/BPS>PBLG-N^+. In particular, PBLG-N^+/Fe-BPS having six PBLG chains gives an extremely expanded monolayer. It is reasonable that the limiting molecular area of the PBLG-N^+/BPS monolayer having two PBLG chains is about twice as great as that of the PBLG-N^+ monolayer. However, the limiting molecular area of the PBLG-N^+/Fe-BPS monolayer is seven times as large as that of the PBLG-N^+ monolayer, suggesting that the orientation of the PBLG chains of this complex monolayer is not as simple as predicted by the molecular structure of the complex. To elucidate the structural properties of this complex monolayer, the visible spectrum and the reflection–absorption (RA)-FTIR spectrum were measured for the surface monolayer and the LB film deposited on a gold substrate, respectively. Fig. 2 shows the visible spectrum of the PBLG-N^+/Fe-PBS complex monolayer on water at a surface pressure of 28 mN m^{-1}. An absorption maximum due to the d–d transition of the iron complex of BPS is clearly observed, which is consistent with that for the Fe-BPS complex in aqueous solution, indicating that the structure of the iron complex of BPS is maintained even at the air–water interface.

Recently, Samulski *et al.* reported that the nature of the order and average orientation of the polypeptide α-helix in the self-assembled films can be inferred from RA-FTIR data.[11] Fig. 3(*a*) displays the RA-FTIR spectrum of the C=O stretching region for the one-layer LB film transferred from the monolayer of PBLG-N^+/Fe-BPS on water at 28 mN m^{-1}. In the amide I and amide II regions (1700–1500 cm^{-1}), the spectrum exhibits two bands at 1656 and 1546 cm^{-1}, which are assigned to the α-helix conformation of PBLG.[12,13] In addition, the C=O stretching band due to the ester groups of the side chains of PBLG is observed at 1734 cm^{-1}. By using the observed ratio of the amide I and amide II integrated intensities: $D = A_I/A_{II}$,[11] the average tilt of the helical axis from the surface normal, $\langle\theta\rangle$, was estimated to be 41°. Taking the structural features of this complex into account, it is impossible that six α-helical rods align in the same direction at the two-dimensional interface. Therefore, the $\langle\theta\rangle$ value obtained is an average angle of the helices in various directions.

PBLG-Helix association by complexation at the air–water interface

To improve the orientation of PBLG-helices, an ion complexation of the PBLG-N^+ monolayer and Fe-BPS complex added in the subphase was performed at the air–water interface. Fig. 4 shows the effect of addition of the Fe-BPS complex into the subphase on the π–A isotherm of PBLG-N^+. Curves (*a*) and (*b*) indicate the π–A isotherms on pure water and on an aqueous solution containing the Fe-BPS complex (2.2×10^{-7} mol dm^{-3}), respectively. In the presence of Fe-BPS, the PBLG-N^+ monolayer has a tendency to shrink slightly, suggesting formation of the ion complex of ammonium groups of PBLG-N^+ with sulfonate groups of Fe-BPS. Actually, the RA spectrum of this monolayer deposited on a gold plate at 28 mN m^{-1} gave an absorption peak at 539 nm due to the d–d transition of Fe-BPS complex (data not shown here). The

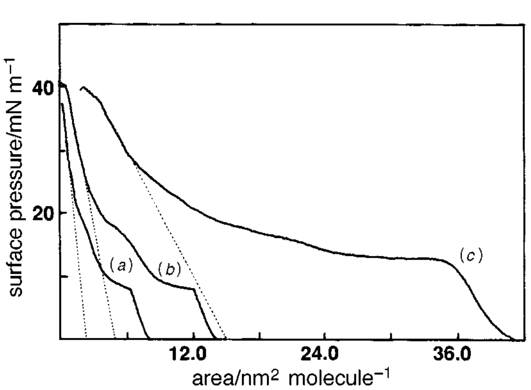


Fig. 1 Surface pressure–area isotherms of (*a*) PBLG-N^+, (*b*) PBLG-N^+/PBS and (*c*) PBLG-N^+/Fe-BPS on pure water at 20 °C

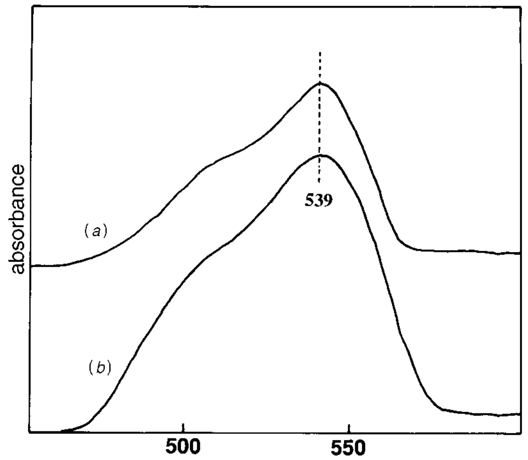


Fig. 2 Absorption spectra of Fe-BPS (*a*) in aqueous solution and (*b*) the surface monolayer of the PBLG-N^+/Fe-BPS complex on pure water at a surface pressure of 28 mN m^{-1}

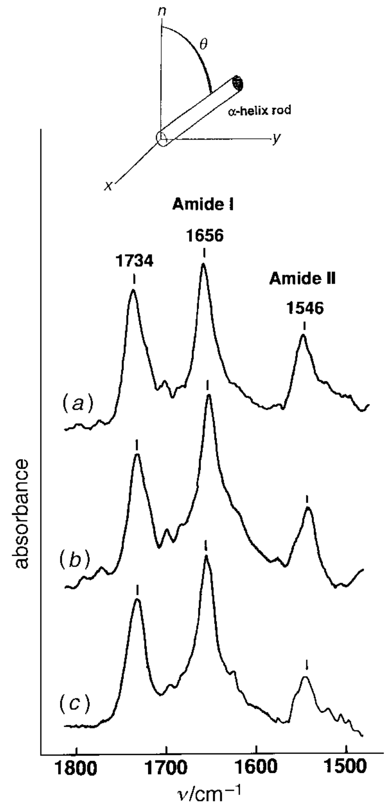


Fig. 3 RA-FTIR spectra of (*a*) a one-layer LB film transferred onto a gold plate from a monolayer of PBLG-N$^+$/Fe-BPS, (*b*) a one-layer LB film transferred onto a gold plate from an interfacially complexed monolayer of PBLG-N$^+$ with Fe-BPS added in the subphase, and (*c*) a one-layer LB film transferred onto a gold plate from an interfacially complexed monolayer of PBLG-N$^+$/BPS with Fe^{2+} and BPS added in the subphase, at a surface pressure of 28 mN m^{-1}. The inset shows the average tilt of the helical axis from the surface normal.

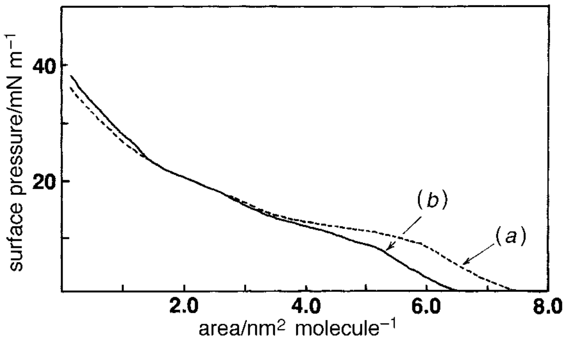


Fig. 4 Surface pressure–area isotherms of PBLG-N$^+$ (*a*) on pure water and (*b*) on an aqueous solution containing Fe-BPS complex (2.2×10^{-7} mol dm^{-3}) at 20 °C

RA-FTIR spectrum was measured for the same deposited monolayer and is displayed in Fig. 3(*b*). PBLG chains are found to take the α-helical conformation on the basis of the amide I and amide II bands. From the value of the ratio *D*, the average tilt of the helical axis, $\langle\theta\rangle$, was evaluated in the same manner as stated above and found to be 25°. Such a tilting angle implies that the orientation of helix rods was considerably improved by using the interfacial ion complex method and became much closer to a vertical alignment of helix rods.

Finally, the following two approaches to the association of helix rods were studied at the air–water interface: (i) interfacial complexation of the PBLG-N$^+$/BPS monolayer with Fe^{2+} in the subphase and (ii) interfacial complexation of PBLG-N$^+$/BPS monolayer with Fe^{2+} and BPS in the subphase.

Fig. 5 shows a comparison of π–*A* isotherms obtained by methods (i) and (ii) at 20 °C. The curve (*a*) [method (ii)] was recorded as follows. The monolayer of PBLG-N$^+$/BPS spread on pure water was first compressed to a surface pressure of 28 mN m^{-1} and then, at that surface pressure, Fe^{2+} was added carefully into the subphase ([Fe^{2+}] = 6.5×10^{-7} mol dm^{-3}). Ten minutes after the addition, BPS was added into the subphase ([BPS] = 6.5×10^{-8} mol dm^{-3}). A further 10 min after BPS addition, the monolayer was compressed continuously and the surface pressure was simultaneously measured. The curve (*b*) [method (i)] was recorded in the same way, except for the absence of BPS in the subphase. It can be seen from Fig. 5 that there is no difference between π–*A* curves (*a*) and (*b*), suggesting that it is difficult to obtain information about interfacial complexation from these π–*A* isotherms even if some interaction between the monolayer and substrates in the subphase takes place. Thus, visible spectra were measured for these monolayers. Fig. 6 shows the reflection–absorption spectra for the one-layer LB films transferred from the monolayers onto gold plates, and then gently washed several times to remove physisorbed iron complexes. In the case of addition of Fe^{2+} into the subphase, no peak appears in the range 400–600 nm. However, by adding BPS together with Fe^{2+} into the subphase, an absorption peak appears at 539 nm due to the d–d transition of the Fe-BPS complex. These results strongly suggest that the PBLG-N$^+$/BPS monolayer cannot form an iron complex in the presence of Fe^{2+} only, but can form a stable iron complex in the presence of BPS as well as Fe^{2+} as schematically illustrated in Fig. 7. If the illustrated structure of the Fe-complex at the air–water interface is correct, complexes having free sulfonate groups should exist. In order to confirm this postulate, an adsorption experiment using

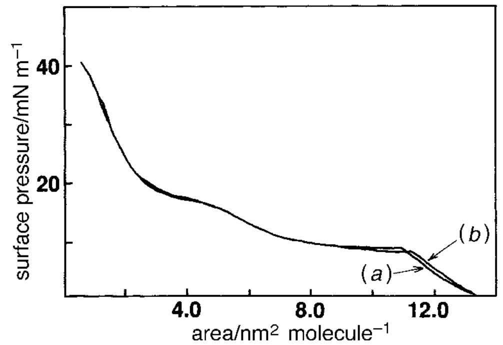


Fig. 5 A comparison of surface pressure–area isotherms in (*a*) method (i) and (*b*) method (ii) (see text) at 20 °C

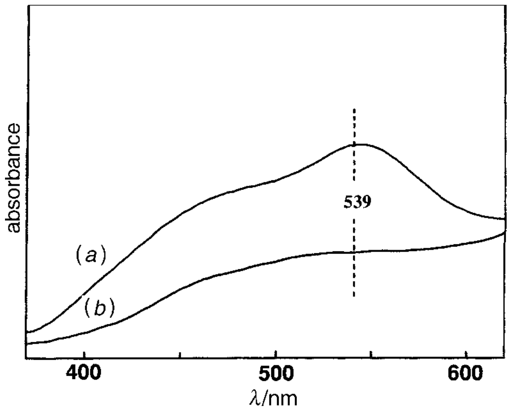


Fig. 6 Reflection–absorption spectra of one-layer LB films transferred onto gold plates from the monolayers of PBLG-N$^+$/BPS on aqueous solutions containing (*a*) Fe^{2+} (6.5×10^{-7} mol dm^{-3}) and BPS (6.5×10^{-8} mol dm^{-3}) and (*b*) Fe^{2+} (6.5×10^{-7} mol dm^{-3}) only, at a surface pressure of 28 mN m^{-1}, 20 °C

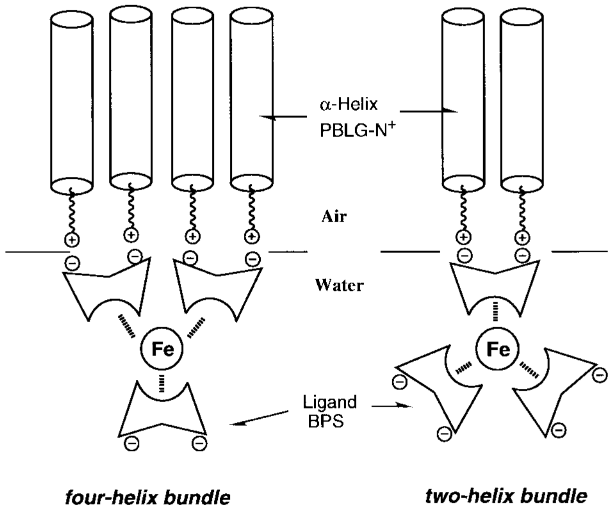


Fig. 7 Possible illustration for the association of helical polypeptide induced with an ion complexation at the air–water interface

Methylene Blue, a typical cationic dye, was carried out. The two-layer LB films, in which the Fe-BPS complex moiety faces the outside, prepared on a gold plate by method (ii) were immersed in the Methylene Blue solution (1×10^{-4} mol dm^{-3}) for 10 min. The LB film thus treated was rinsed with water several times to remove free Methylene Blue and then the reflection–absorption spectrum of this film was measured. The absorption bands of Methylene Blue were clearly observed at 614 and 664 nm, implying that free sulfonate groups from BPS are certainly present and that these can bind the cationic dye electrostatically. From the RA-FTIR spectral data of the one-layer LB film prepared by method (ii) and shown in Fig. 3(*c*), the average tilt, $\langle\theta\rangle$, of α-helical PBLG rods was evaluated to be 7°, meaning an almost vertical orientation of the helices at the two dimensional interface. Formation of such a highly oriented assembly of α-helices is also supported by the result of π–A isotherms in Fig. 5, in which complexation did not affect the π–A curve.

In summary, we have demonstrated the ligand-induced association of helical PBLGs which can be achieved at the air–water interface by the complexation of Fe^{2+} and BPS. In these assemblies, it has been found that PBLG helix rods align vertically and that the structural unit is a two-helix or four-helix bundle.

Experimental

Materials

The preparation of PBLG-N$^+$ has been described elsewhere.[9] BPS (Aldrich) was used as received. The iron complex of BPS(Fe-BPS) was prepared by reacting BPS with 1/3 equiv. of iron(II) [as freshly prepared $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ solution]. The stoichiometry of Fe-BPS was determined to be Fe : BPS = 1 : 3 by UV–VIS titration of BPS with iron(II). Other chemicals used were of analytical grade.

Ion complexation

Ion complexes were prepared by an ion-exchanging reaction between the ammonium group of PBLG-N$^+$ and the sulfonate groups of BPS or the Fe-BPS complex. PBLG-N$^+$ was reacted with 1/2 equiv. of BPS or 1/6 equiv. of Fe-BPS complex in DMF–water (5:1, v/v). After removal of the solvent, the residue was washed with water several times and then dried *in vacuo*. The stoichiometric composition of these ion complexes was determined by ^{1}H NMR spectroscopy (JEOL, JNM-GX400 FTNMR spectrometer).

Measurement

The monolayers were obtained by spreading a solution of PBLG-N$^+$ or ion complexes on purified water (Milli-Q system, Millipore Ltd.). Ten minutes after spreading, the monolayer was compressed continuously at a rate of 1.2 cm^2 s^{-1}. Wilhelmy's plate method and a Teflon-coated trough with a microprocessor-controlled film balance (USI system Ltd., Japan) with a precision of 0.01 mN m^{-1}, were used for surface pressure measurements.

One-layer LB films were transferred in the vertical mode with the upward stroke at a transfer rate of 2 mm min^{-1} onto gold plates.

UV–VIS spectra of the surface monolayers on water and Langmuir–Blodgett (LB) films deposited on gold substrates were measured by a multichannel photodetector (Otsuka Electronics, MCPD-100).

The reflection–absorption (RA) FTIR spectra of these LB films were measured and recorded on a Nicolet System 800 by using a reflection attachment at the incident angle of 80 Å, together with a polarizer.

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