Molecular Orientation Control in Amphiphilic α-Helical Copolypeptide Monolayer at Air/Water Interface

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Molecular orientation of amphiphilic α-helical di-block type copolypeptide PLLysZ$_{25}$–P(MLG$_{42}$/LGA$_{18}$) was controlled in a monolayer at an air/water interface by controlling the secondary structure of hydrophilic segment of the peptide. The orientation of the peptide was investigated by surface-area (π-A) measurement, FT-IR reflection-absorption spectroscopy (RAS), and atomic force microscopy (AFM).

To create novel two dimensional nanostructured materials, interest in rod-like α-helical polypeptides has been growing because of their unique properties such as well-defined secondary structure, macrodipole moment, and bio-degradability. It has been reported that the α-helical polypeptides containing a thiol group at the terminus of each molecule formed a self-assembled monolayer, in which their helix axes were oriented perpendicular to the solid surface.[1,2] In these systems, it is difficult to control molecular packing and produce a multi-layered structure. A few studies have been done on orienting α-helical polypeptides perpendicularly in a monolayer at an oil/water interface.[3,4] These methods still have the difficulty in transferring monolayer onto a substrate. It is necessary, therefore, to control molecular orientation at air/water interface.

In this study, a novel method to orient rod-like molecules at an air/water interface is described.

Preparation of amphiphilic α-helical copolypeptide composed of poly(ε-benzyloxycarbonyl L-lysine)$_{25}$–poly[(γ-methyl L-glutamate)$_{42}$/(L-glutamic acid)$_{18}$] (PLLysZ$_{25}$–P(MLG$_{42}$/LGA$_{18}$)) (Figure 1) was previously described.[5]

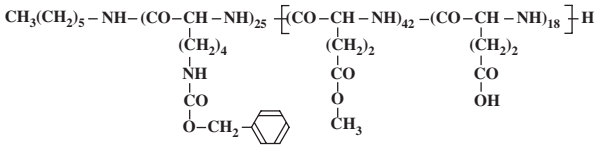

Figure 1. Chemical structure of PLLysZ$_{25}$–P(MLG$_{42}$/LGA$_{18}$).

Secondary structure of PLLysZ$_{25}$–P(MLG$_{42}$/LGA$_{18}$) in aqueous solutions at several pHs was examined by circular dichroism (CD) spectroscopy. When pH of the subphase was 5, the positive band at 195 nm and negative band at 224 nm based on α-helical conformation were observed. With increasing pH, these peak intensities gradually decreased. This suggests that the hydrophilic P(MLG$_{42}$/LGA$_{18}$) segment undergoes a helix-coil transition in aqueous solution depending on pH. Below pH 4, the peptide precipitated.

The molecular orientation of PLLysZ$_{25}$–P(MLG$_{42}$/LGA$_{18}$) at the air/water interface was characterized by π-A isotherms of the monolayers (Figure 2). The monolayer was prepared by spreading a DMF/benzene (1:20 in vol.) solution on water at several subphase pH. The surface pressure was increased by

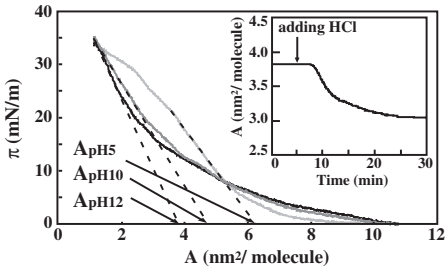

Figure 2. π-A isotherms for monolayer of PLLysZ$_{25}$–P(MLG$_{42}$/LGA$_{18}$) with 0.1 mol/L KCl in aqueous solution at pH 5 (——), pH 10 (——), and pH 12 (——). Inset: Time course of the molecular occupied area while keeping surface pressure constant after adding HCl.

compressing the monolayer. From these isotherms, limiting area A_{pH5}, A_{pH10}, and A_{pH12} were estimated to be 6.26, 4.74, and 3.92 nm^2/molecule, respectively. $A_{//}$ and $A_{\perp}$, the area per molecule when the peptide rods were oriented parallel ($A_{//}$) and normal ($A_{\perp}$) to the surface, were calculated to be 17.0 and 2.39 nm^2/molecule, respectively. The value of A_{pH5} was smaller than that of $A_{//}$. This indicates that α-helical PLLysZ$_{25}$–P(MLG$_{42}$/LGA$_{18}$) is not perfectly parallel to the air/water interface when pH of the subphase is 5. The limiting area gradually decreased as pH increased. This suggests that the hydrophobic PLLysZ$_{25}$ rod is oriented somewhat perpendicular to the air/water interface because the hydrophilic part ionized and formed a random coil structure, which then dissolved into the bu1k.

It has already been confirmed that the α-helical poly(L-leucine) segment of amphiphilic block copolymer, poly(L-leucine)–polyethyleneglycol [PLeu–PEG], stands in the monolayer at the air/water interface by compressing the monolayer.[6,7] The hydrophilic PEG segment is essential for the tilting of the hydrophobic PLeu segment in the copolymer monolayer system. The solubilization of the hydrophilic PEG segment into water may make the PLeu segment stand at the interface.[6,7]

To orient the molecule more perpendicularly, at first, the monolayer was compressed until the limiting area was up to 3.9 nm^2/molecule when the subphase pH was 12. In this case, the hydrophobic PLLysZ$_{25}$ segment was oriented somewhat perpendicularly. Following the orientation, hydrochloric acid was added to the subphase to decrease the pH down to 5 and to make the hydrophilic part form an α-helical conformation, and then the monolayer was compressed again. When the surface pressure was kept constant, the limiting area gradually decreased to 3.0 nm^2/molecule (Figure 2 inserted). This suggests that the α-helical molecule can be oriented more perpendicularly.

Molecular orientation of the α-helical peptide in a Langmuir–Blodgett (LB) film was investigated by FT-IR/RAS using Perkin Elmer Spectrum 2000. The monolayers from the air/wa-

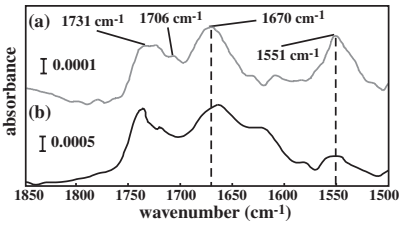

Figure 3. FT-IR reflection absorption spectra of LB film transferred onto gold substrate from PLLysZ$_{25}$–P(MLG$_{42}$/LGA$_{18}$) monolayer on water at pH 5 aqueous solution (a) and after molecular orientation control (b).

ter interface at pH 5 and from controlling the molecular orientation were transferred onto a gold-coated glass substrate by a horizontal drawing-up method while keeping the surface pressure at $\pi = 25\,\mathrm{mN/m}$ using a LB film balance (Nippon Laser & Electronics Lab., NL-LB240-MWA), respectively. The tilt angle of the α-helical axis from the surface normal was calculated by the method proposed by Samulski et al.[8]

The spectra of the LB film with a single layer are shown in Figure 3a without molecular orientation control and 3b with molecular orientation control. Both spectra showed Amide I(α) band at 1670 cm^{-1} and Amide II(α) at 1551 cm^{-1} because of the presence of α-helix, in addition to the bands resulting from the urethane bond of the side chain of LLysZ at 1705 cm^{-1} and the carbonyl bond of the side chain of MLG and LGA at 1731 cm^{-1}. In Figure 4b, the shoulder bands resulting from β-sheet were observed at 1626 cm^{-1} (Amide I(β)) and 1522 cm^{-1} (Amide II(β)), respectively. This may be caused by the strong interaction between LGA residues, previously described by Higashi et al.[9] This could be improved by using non β-sheet forming amino acid sequences as the hydrophilic segment. These spectra show that the PLLysZ$_{25}$–P(MLG$_{42}$/LGA$_{18}$) with molecular orientation control is more perpendicularly oriented than that of without control. Tilt angle without molecular orientation control was estimated to be 72°. In the case of orientation controlled LB film, tilt angle was not calculated because of a small amount of β-sheet and random coil structure. However, it seems that apparent value could be smaller than 72° considering the small amide II band based on α-helix.

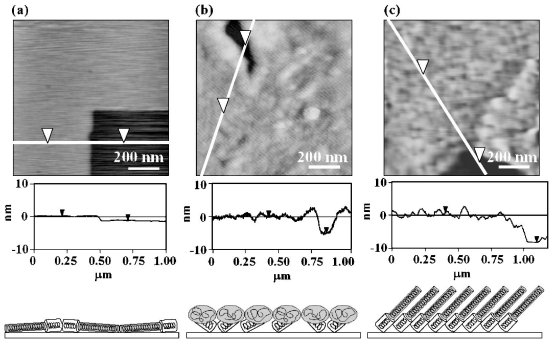

Figure 4. AFM images (1 μm × 1 μm) of PLLysZ$_{25}$–P(MLG$_{42}$/LGA$_{18}$) LB films transferred onto mica substrate at pH 5 (a), at pH 12 (b), and after molecular orientation control (c).

The morphology of the LB films with and without molecular orientation control was observed by AFM (Nanoscope IIIa, Digital Instruments) using contact mode, respectively. In the case of pH 5, without orientation control, the surface was flat (Figure 4a). From the depth of the cavity that was made by scratching with a cantilever, the thickness of the LB film was estimated to be ca. 1.4 nm. This value is equivalent to the average diameter of α-helical PLLysZ$_{25}$–P(MLG$_{42}$/LGA$_{18}$). This means that the α-helical peptide lies on the mica substrate even though the α-helix axes are not completely parallel to the air/water interface. This suggests that molecular orientation is changed while transferring the monolayer onto the substrate.

When pH of the subphase was 12, the surface of the LB film was not smooth (Figure 4b). This might be based on polydispersity of molecular weight of the peptide. The thickness of the LB film was estimated to be ca. 5 nm. This suggests that the hydrophobic part of PLLysZ$_{25}$ is oriented somewhat perpendicular to the mica substrate.

After performing the molecular orientation control, the morphology of the LB film was observed (Figure 4c). The surface was not smooth. The thickness of the LB film was estimated to be ca. 9 nm. This suggests that the α-helical PLLysZ$_{25}$–P(MLG$_{42}$/LGA$_{18}$) is oriented even more perpendicular to the mica surface than in the case at pH 12. From the thickness of the film, tilt angle of the rod was estimated to be 45°. This value is consistent with the result from FT-IR/RAS measurement.

In conclusion, it was possible to control the molecular orientation of α-helical amphiphilic peptides in a monolayer at an air/water interface by controlling the secondary structure of the hydrophilic segment of the peptide. This method to orient rod-like molecules could be useful to create nanostructured functional thin films.

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