Vertical Standing of Amphiphilic Helical Peptides at the Hexane-Water Interface

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Amphiphilic poly(*L*-leucine) (n=20) helixes having a trimethylammonium head group at the terminal end were stood vertical with a tilt at the hexane-water interface, although they usually laid down to the air-water interface. Structures of the transferred monolayer on a substrate were confirmed by FT-IR spectra and atomic force microscopy (AFM).

Helical structures of oligopeptides are one of key motifs of three dimensional protein structures. Supramolecular assemblies based on α-helical peptides have been widely studied.[1-3] For example, when hydrophobic helical peptides having a SH-groups at the terminal end are designed, they form a self-assembled monolayer, in which helix axes aligned perpendicular to an Au surface.[4] When hydrophobic helical peptides having a hydrophilic terminal group are spread at the air-water interface, their helix axes have been reported to align parallel to the interface, and it has been difficult to align perpendicular to the interface.[5-8] If helical peptides could be aligned perpendicular on a substrate, it is interesting as a molecular material in which macro dipoles oriented in one direction.

In this communication, we first report that amphiphilic poly(*L*-leucine) (n=20) helixes having a trimethylammonium head group at the N-terminal, $^{+}$N(*L*-Leu)$_{20}$OBn, can be stood vertical at the hexane-water interface and transferred on a substrate retaining their orientations. The orientation of helical peptides was confirmed by π-A isotherms, FT-IR spectra, and AFM measurements.

An amphiphilic oligopeptide, $^{+}$N(*L*-Leu)$_{20}$OBn, was prepared according to the conventional solid-phase peptide synthesis.[9] Purification of the polypeptides was performed by reversed phase HPLC equipped with UV and RI detectors (column: ODS-80Ts, φ4.6 x 20 cm, elution solvent: CH_3CN/H_2O = 1:1). Identification was made by MALDI-TOF MS and elemental analysis.

Figure 1 shows surface pressure (π) - area (A) isotherms of amphiphilic $^{+}$N(*L*-Leu)$_{20}$OBn monolayers at the hexane-water[10] and air-water interfaces at 20 °C. At the air-water interface, the surface pressure showed a simple and steep rise near 3 nm^2 due to the formation of the solid monolayer [a curve (a)]. This value is corresponding to the area per molecule where $^{+}$N(*L*-Leu)$_{20}$OBn helix peptides aligned parallel to the interface. In contrast, at the hexane-water interface, $^{+}$N(*L*-Leu)$_{20}$OBn monolayers showed a surface pressure of 10-20 mNm^{-1} at the low molecular area that means an expanded liquid phase of monolayer solubilized in the hexane layer [a curve (b)]. The steep increase of surface pressure near 1.1 nm^2 indicates the formation of the solid monolayer, whose area corresponds to the bottom area of the helical peptide. When the organic solution of hydrophobic peptide helixes was spread on the air-water interface, peptide helixes immediately crystallize and align parallel to the interface as a stable form after evaporation of organic

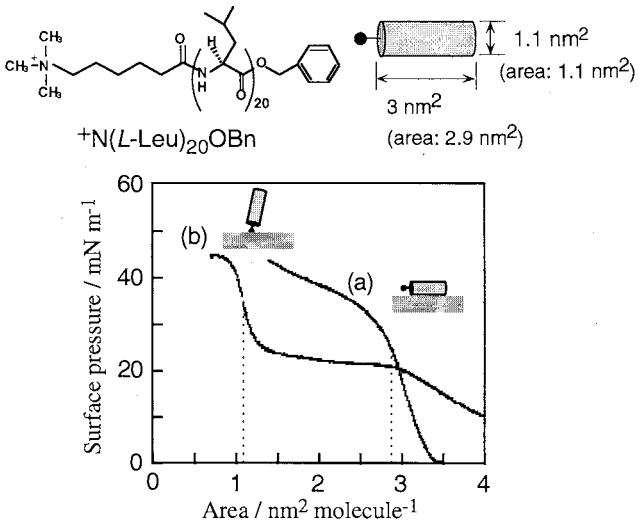

Figure 1. π–A isotherms of $^{+}$N(L-Leu)$_{20}$OBn, (a) at the air-water interface and (b) at the hexane-water interface at 20 °C.

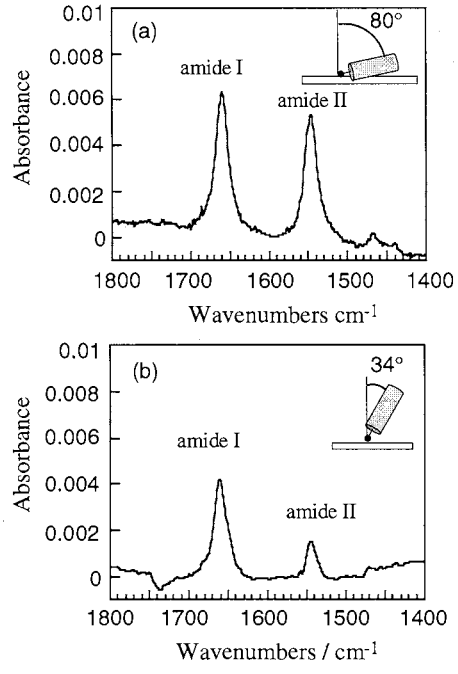

Figure 2. FT-IR reflection absorption spectra (RAS) of the monolayers transferred onto the Au-deposited glass plate from (a) the air-water interface, and (b) the hexane-water interface.

solvents. At the hexane-water interface, however, they can align perpendicular to the interface solubilizing in the hexane layer to form expanded monolayer and condensed to the solid layer keeping the perpendicular orientation.

A monolayer of $^{+}$N(*L*-Leu)$_{20}$OBn was transferred vertically on an Au-deposited glass plate from the subphase to the air phase both at the air-water and hexane-water interfaces.[11] The structure of the monolayers was analyzed by FT-IR reflection absorption

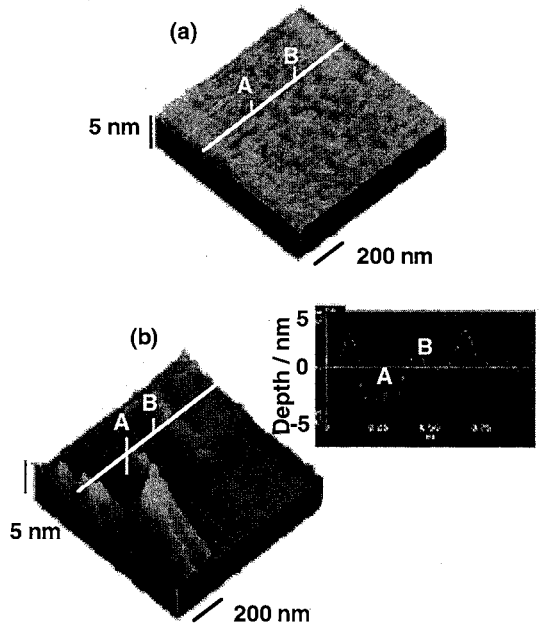

Figure 3. AFM Images of (a) the $^{+}$N-(L-Leu)$_{20}$OBn monolayer transferred on a mica plate from the hexane–water interface, and (b) the monolayer treated with scratch by a cantilever tip and its depth profile.

spectra (RAS) (Figure 2).[12] Both peptide monolayers showed typical amide I ($\nu_{C=O}$) and amide II (δ_{N-H}) absorptions near 1660 and 1546 cm^{-1}, respectively. It is indicated from peak decomborution that $^{+}$N(*L*-Leu)$_{20}$OBn peptides exist as an α-helical structure more than 80% in the monolayer.[13]

The tilt angle of the helix axis from the right angle to the surface could be evaluated from the ratio of the individual intensities of amide I to amide II absorptions. The monolayer transferred from the air-water interface showed the similar intensities of amide I and II absorptions, in which helix peptides are calculated to align on the surface with the tilt angle of 80-85° from the right axis. On the contrary, the monolayer transferred from the hexane-water interface, the amide I absorption was larger than the amide II, in which the tilt angle of helix axis could be calculated to be 34°. Thus, monolayers could be transferred on a substrate as their orientations both from the air-water and hexane-water interfaces.

The monolayer structures were also confirmed by an atomic force microscopy (AFM) in air.[14] The AFM image of the monolayer transferred onto a flat mica surface was shown in Figure 3a. The surface of the monolayer onto a mica was rather flat with the variation of 0.3 nm. Figure 3b shows the AFM image and the depth profile when the monolayer was treated with scratching by the cantilever at a force stronger than 1 nN. After scratching, the sample had a cavity of 3.3 ± 0.5 nm in depth (A in Figure 3b), similar to the long axis of peptide helixes (thickness of the monolayer). In the case of the monolayer transferred from the air-water interface, the membrane surface was also flat and thickness of the monolayer was determined to be 1.1 ± 0.5 nm by a scratching method, which is corresponding to the diameter of helix peptides (data are not shown).

In conclusion, although it is difficult to align amphiphilic helical peptides perpendicular to the interface, the organic-water interface is useful to prepare a peptide monolayer in which helix axes stood vertical to the interface. In other words, we can control the orientation of amphiphilic helical peptides by changing the interface.

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