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Partially induced transition from horizontal to vertical orientation of helical peptides at the air–water interface and the structure of their monolayers transferred on the solid substrates

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ABSTRACT

To apply the Langmuir–Blodgett (LB) technique as a platform for investigating the fundamental properties of amphiphilic peptides (APs), we have investigated the structure of LB films using the APs. To vertically orient the helical APs like transmembrane proteins in the membrane, the primary structure of the APs was designed to have two domains: a hydrophilic domain (three amino acids) and a hydrophobic domain (ca. 20 amino acids). However, we are still far from having full control of their orientation. This study reports the contribution of the subphase temperature to the change in the orientation of helical APs. When the surface pressure–area isotherm of AP was observed at the subphase temperature at 41.5 °C, the isotherm exhibited a plateau, implying that a phase transition of the monolayer at the air–water interface occurred. Circular dichroism (CD) spectra of the monolayers transferred on the solid substrates revealed that the orientation of the helices changed at the pressure, where the plateau of the isotherm was observed. This change was not observed at 21.5 °C, i.e., the horizontal alignment of helixes was maintained. Atomic force microscopy (AFM) was used to systematically investigate the surface structure of the monolayers transferred at different surface pressures. A structural model of the monolayer that did not contradict with the results obtained by the three different techniques (the isotherm, CD spectroscopy, and AFM) was derived, and it was concluded that the horizontally oriented helices partially changed their orientation to vertical upon compression in the plateau region of the isotherm.

1. Introduction

Because of their amphiphilic nature, membrane proteins are more difficult to investigate than water-soluble proteins [1]. To isolate them from cells, a treatment using detergents is required. To investigate their biological functionality, they have to be placed in similar conditions as in the cell, e.g., reconstitution of membrane proteins into lipid vesicles. Because of these obstacles, the progress in the structure determination of membrane proteins is far behind water-soluble proteins [2], even though one-third of proteins encoded in the human genome are membrane proteins [3,4] and more than half of pharmaceutical targets correspond to membrane proteins [5,6]. Therefore, tremendous efforts are being made to understand the chemical and biological functionalities and the structure of membrane proteins, as well as to develop techniques for their characterization [7,8]. Besides the direct investigation of the proteins themselves, there is a "divide and conquer" approach to membrane proteins [9–11]. In this approach, fragments of the membrane proteins (peptides) are the targets investigated to understand the membrane proteins. When the protein is disassembled into peptides, the above-mentioned difficulties in the membrane proteins are reduced and various experimental techniques can be applied. Specifically, in the case of α-helical peptides, which correspond to one of the transmembrane (TM) domains of the α-helical TM proteins, the experimental techniques and the TM domains investigated have been summarized in Ref. [11]. However, the application of the Langmuir–Blodgett (LB) technique to TM peptides was hardly mentioned.

The LB technique, which involves studying the monolayer at the gas–liquid interface (Langmuir monolayer), has been applied to various peptides to understand the fundamental properties of polypeptides [12] and develop molecular architectures [13–15]. However, in the case of helical peptides, it is difficult to align the helical axis perpendicular to the interface [13,14,16–24], and vertically oriented helical peptides often contain non-gene encoded amino acids (AAs) [14,16,17,19,21–23]. This is one of the reasons why the LB technique is rarely applied to investigate the TM peptides in biological aspects. To use the LB technique as a platform to investigate the fundamental properties of TM peptides, how the helical axis of peptides consisting of the gene-

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encoded AAs can be oriented perpendicular by the LB technique needs to be studied, because the TM domains of the TM proteins vertically orient to the cell membrane.

Gas–liquid and gas–solid interfaces are unfavorable situations for mimicking the membrane environment of TM peptides. Conversely, because of the advantage of the LB technique, we could apply a variety of spectroscopic and microscopic methods that are surface- and interface-sensitive to analyze and determine the structure and dynamics of materials at the interface [20,25–35]. This will help to understand the fundamental properties of TM peptides.

Thus, we restricted ourselves to peptides whose AAs are gene-encoded and whose sequences consist of hydrophobic and hydrophilic domains [36,37]. Because of the two domains, when the peptides form the α-helical conformation, amphiphilic helices are formed, and they are expected to orient their helical axes perpendicular to the surface of the aqueous subphase. Recently, we had indicated that amphiphilic peptides (APs) whose AA sequences of the hydrophobic domains are the same as TM peptides proposed for the lipid bilayer [38,39] are able to form an α-helix at the air–water interface and these helices partially orient perpendicular in peptide/lipid mixed LB films [37].

The present work aims to further investigate the important parameters that determine the orientation of the amphiphilic helices at the air–water interface, and we have investigated how the temperature at the air–water interface and the hydrophobic moment (HM) of the hydrophobic domain of the peptide affect the controllability of the orientation of the amphiphilic helices by the LB technique. For simplicity, the Langmuir monolayers of the APs were formed without lipids, and the AA sequences of the APs for the monolayers are shown in Table 1. K3-19W is one of the peptides we have used previously [37]. To investigate the influence of the HM value on the orientation of the helical AP in the monolayers, the W residue of K3-19W was changed to the A residue to reduce the HM value of the hydrophobic domain. This AP was called K3-19A. The helical wheel projections of their hydrophobic domains are shown in Fig. 1 together with the values and the directions of the HMs. The HM values were calculated using the equation in Ref. [40] and the hydrophobicity values used for the calculation were the whole-residue hydrophobicity scale for the lipid bilayer interface (ΔG_{wif}) [41]. The temperature dependence of the surface pressure–area (π–A) isotherm of the Langmuir monolayer of AP was investigated. The circular dichroism (CD) spectra of the Langmuir monolayers transferred on the solid substrates by the LB technique (1-layer LB films) were recorded to analyze the orientation of the helices. The topography images of the 1-layer LB films of the APs were obtained by atomic force microscope (AFM). A structure model of the 1-layer LB film that does not contradict with the results obtained by three different techniques was deduced.

2. Experimental

The APs consisting of 22 AAs (Table 1) were synthesized by Operon Biotechnologies, K.K. (Tokyo, Japan) and their purities were >90%. Pure water (>18 MΩ cm) was prepared in a Milli-Q system (Elix Advantage 3, Merck Millipore, Darmstadt, Germany). All of the organic solvents, citric acid, and trisodium citrate were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

Table 1
Primary structure and average hydrophobicity scale [41] of the amphiphilic peptides.

Abbreviation	Primary structure	Average hydrophobicity scale[a]
K3-19W	NH_2–KKKALALAAAALWLAAAALALA–$CONH_2$	−0.009 kcal/mol
K3-19A	NH_2–KKKALALAAAALALAAAALALA–$CONH_2$	0.083 kcal/mol

The underlined parts correspond to the hydrophobic domains.
[a] Because the hydrophobic/hydrophilic residues are scaled to be negative/positive, the average scale indicates that K3-19A has lower hydrophobicity than K3-19W.

The solvent for the spreading solution of the APs was dichloromethane/methanol (5:2 v/v), and the concentrations of K3-19W and K3-19A in the spreading solutions were 47.2 and 49.9 μM, respectively. A citric acid–sodium citrate buffer (5 mM, pH 6.0) was used for the aqueous subphase of the Langmuir monolayer, and the temperature of the subphase was controlled at 21.5 ± 0.5, 31.5 ± 0.5, or 41.5 ± 0.5 °C. At pH 6.0, the three K residues are expected to be protonated.

The π–A isotherms of the Langmuir monolayer were measured by the trough with two movable barriers (MiniMicro LB system, KSV NIMA, Biolin Scientific Holding AB, Stockholm, Sweden). The inner width (effective barrier width) and the inner length of the trough were 50 and 170 mm, respectively. The spreading solution was spread drop by drop on the subphase by a micro syringe. A period of 10 min was allowed for the solvent evaporation before compression of the Langmuir monolayer, and the compression speed was 500 mm^2/min. The number of molecules spread on the subphase for a single measurement of the isotherm was 1.14×10^{15} and 0.90×10^{15} for K3-19W and K3-19A, respectively, corresponding to 40 and 30 μL of the spreading solution of K3-19W and K3-19A, respectively.

For the CD measurements, the Langmuir monolayer on the subphase was transferred at specific surface pressures (π_{dep}'s) to the fused silica substrate (10 mm × 40 mm) by the vertical deposition method [42]. The CD spectra were recorded using a J-820 CD spectropolarimeter (JASCO Corp., Tokyo, Japan), and the temperature of the 1-layer LB films was kept at 20 °C during the measurements by the sample holder equipped with the thermoelectric (Peltier) devices. The 1-layer LB film was set perpendicular to the optical axis of the spectropolarimeter. An elementary analysis of the helix orientation was performed on the CD spectra under the assumption that all of the APs in the 1-layer LB film formed an α-helix [37]. The shape of the CD spectrum depends on the angle between the incident axis and the helical axis of the α-helix. The spectral shapes as a function of wavelength when the incident axis is parallel to the helical axis ($G_1(\lambda)$) and when the incident axis is perpendicular to the helical axis ($G_2(\lambda)$) are given in Ref. [43]. When the helices are uniformly oriented at a tilt angle (θ) of the helical axis from the film normal, the CD spectrum of the 1-layer LB film, whose plane is perpendicular to the optical axis of the spectropolarimeter, is given by

$$\mathrm{CD}(\lambda, \theta) = C\left\{G_1(\lambda)\cos^2\theta + G_2(\lambda)\sin^2\theta\right\}, \quad (1)$$

where C is the amplitude coefficient.

The surface morphology of the 1-layer LB film was observed by AFM (Dimension Icon, Bruker AXS GmbH, Karlsruhe, Germany) in tapping mode. The Langmuir monolayer of the AP was deposited on a fleshly cleaved mica substrate (8 mm × 51 mm) by the vertical deposition method [42] at a given π_{dep}. The resonant frequency and the spring constant of the cantilever (NCHV-10 V) were 361–400 kHz and 20–80 N/m, respectively. The topography image was collected at 512 × 512 pixel resolution. The flattening of the obtained images and the analyses of the height histogram were carried out using the software WSxM [44].

For the vertical deposition of the Langmuir monolayer, the trough with two movable barriers was used except for the deposition of the monolayer of K3-19W at π_{dep} = 29 mN/m from the subphase at 41.5 °C. In this condition, the trough with a single movable barrier (Filgen, Inc., Nagoya, Japan) had to be used because the barrier can move in a wider range and this trough can compress the Langmuir monolayer into a smaller area than the trough with two barriers. The inner width and length of this trough were 80 and 525 mm, respectively, and the compression speed was 880 mm^2/min. The Langmuir monolayer was compressed up to π_{dep} and was stabilized for 5–15 min before the deposition. During the deposition, the substrate was vertically lifted from the subphase to air at 6 mm/min for both troughs, and the surface pressure was kept constant at π_{dep}. The transfer ratio, which is defined as the decrease in area of the Langmuir monolayer during the deposition divided