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Langmuir and Langmuir–Blodgett films of a novel tryptophan peptide lipid†

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A novel tryptophan peptide lipid, $C_{18}H_{35}O$ (SA)-Gly-Trp-Gly-OH, was synthesized and studied for its surface chemistry and spectroscopic properties.

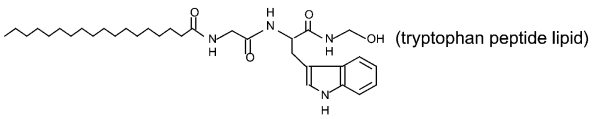
Fluorescence spectroscopy or microscopy is an indispensable analytical technique for chemistry and biochemistry research. In order to observe fluorescence, a molecule should either contain an intrinsic fluorescent unit such as amino acid tryptophan in protein or be covalently or non-covalently labeled with a fluorescent probe. In the area of surface chemistry, fluorescence is among the most sensitive and convenient techniques to investigate molecular structures and properties in organized monomolecular thin films.[1–3] Very recently, a novel research direction of engineering functional lipid molecules into artificial receptors or protein-like supramolecular structures through Langmuir and Langmuir–Blodgett thin film techniques has arisen.[4–9] The most unique feature of this approach is that lipid molecules are aggregated or assembled together into artificial receptors during the monolayer formation process with their polar moieties interacting with each other through non-covalent bonding.

5-Octadecanoylamino fluorescein (ODFL) is a common fluorescence probe in protein monolayer study. Unfortunately, our recent study showed that this fluorescein amphiphile significantly aggregates at the air–water interface. Fluorescence quenching was observed from both the pure ODFL monolayer and a monolayer of ODFL mixed with stearic acid. However, a mixed monolayer of ODFL and three synthetic peptide lipids partially reduced the fluorescence quenching.[10] We pointed out clearly that the immiscibility between the fluorescent probe lipids with other lipids would lead to the failure of the fluorescent lipid as a probe and therefore must be carefully used. Since tryptophan itself is a natural amino acid and is miscible with other amino acids, it would be interesting to investigate its potential as an intrinsic fluorescence probe for peptide lipid monolayer research. As a first-stage investigation, the two purposes of this study include the monolayer surface properties and UV absorption and fluorescence emission spectroscopic studies of a synthesized tryptophan-containing peptide lipid.

The peptide lipid is a tripeptide Gly-Trp-Gly connected with a stearic acid long aliphatic chain through the amino terminal of the peptide (SA-Gly-Trp-Gly-OH). The synthesis of this lipid is accomplished by solid phase synthesis as reported previously and described in the ESI.†

Fig. 1 shows the isotherms of the surface pressure (π) and the perpendicular dipole moment ($\mu_\perp$) as a function of the surface area (A) per molecule of the amphiphilic peptide lipid. A couple of features can be found from this isotherm. First of all, the surface pressure starts to rise at a molecular area of 75 Å^2 molecule^{-1} and three kink points appear on the isotherm at different compression stages. From surface pressure nil to the first kink point (molecular area 50 Å^2 molecule^{-1}), the monolayer is in a liquid expanded phase. From the second (area 38 Å^2 molecule^{-1}) to the third kink point (area 30 Å^2 molecule^{-1}), the monolayer appears as a liquid condensed phase and above the third kink point, the monolayer is compressed to a solid phase until collapse. When extrapolating the solid phase to surface pressure nil, a limiting molecular area of 42 Å^2 molecule^{-1} is obtained for the tryptophan peptide lipid. From the CPK model, the maximum molecular area of the peptide lipid is around 108 Å^2 molecule^{-1}. After energy minimization using molecular mechanics calculations in a vacuum (HyperChem), the tryptophan peptide was found to adopt an 'L' geometry, with the vertical part representing the aliphatic long tail and the bottom horizontal part representing the Gly-Trp-Gly peptide moiety. The molecular area from this configuration is around 54 Å^2 molecule^{-1}. Compared to the limiting molecular area obtained from the surface pressure-area isotherm extrapolated from the solid phase, we believe that at the air-water interface, the peptide lipid changes from the 'L' conformation to a 'ℓ' conformation due to the intermolecular interactions of peptide with water, leading to a decreased experimental molecular area compared to that obtained from a vacuum environment.

The surface dipole moment–area isotherm further reveals some important molecular orientational change during the monolayer formation. Based on the surface potential measurements, the effective dipole moment expressed in mD ($\mu_\perp$) perpendicular to the surface has been calculated using the Helmholtz equation,[11] $\mu_\perp = A\Delta V/12\pi$. The dipole moment $\mu_\perp$ varies under compression of the monolayer, as can be seen in Fig. 1. From a molecular area of 160 to 80 Å^2 molecule^{-1}, the dipole moment fluctuates significantly, indicating the existence of domains of monolayer in the gaseous phase. Then the dipole moment increases steadily as the area decreases from 75 to 50 Å^2 molecule^{-1} and passes through a maximum (+187 mD), indicating the gradual formation of a closely packed monolayer.



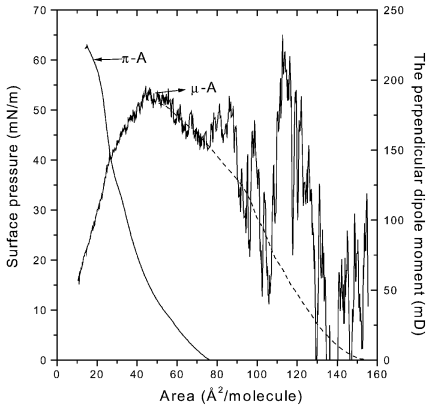


Fig. 1 Surface pressure–molecular area isotherm (π–A) and the perpendicular dipole moment (average value, ---)–molecular area isotherm ($\mu_\perp$–A) of the amphiphilic peptide lipid on the pure water subphase (pH = 5.8, T = 20.0 ± 0.5 °C).

† Electronic supplementary information (ESI) available: synthesis of tryptophan peptide lipid. See http://www.rsc.org/suppdata/cc/b1/b106597c/

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Upon further compression, the effective dipole moment began to decrease.

There is a good correspondence between the π–A and the $\mu_{\perp}$–A curves. At the lifting point (75 Å molecule^{-1}) of the surface pressure–area isotherm, the dipole moment values do not fluctuate any more. The dipole moment reaches a maximum value at 50 Å molecule^{-1}, which corresponds to the first kink point of the surface pressure–area isotherm. From the first to the second kink point, the dipole moment decreases from 50 to 38 Å^2 molecule^{-1} and beyond the second kink point, the dipole moment continues to decrease, but with a different slope. From this analysis, it is clear that the kink points from the surface pressure–area isotherm represent the molecular orientational change of peptide lipid at the air–water interface.

In situ UV spectra of the peptide lipid on the pure water subphase at different surface pressures are shown in Fig. 2. There are two bands centered at 222 and 278 nm, respectively. The spectra show that there is no shift of the peak position during the compression of the peptide lipid at the interface and the absorbance intensity increases linearly with the increase of surface pressure (see inset of Fig. 2). This is a good indication that no static aggregates such as dimer, trimer or oligomer are formed during the compression of the monolayer, although domains were observed in the gaseous phase in 2D by surface potential measurements at the large surface area.

The tryptophan peptide lipid shows a native fluorescence emission at 360 nm when excited with a 290 nm light beam in a methanol solution (10^{-5} M), but no fluorescence was observed in chloroform solution at the same concentration. At the surface pressures of 10, 20 and 30 mN m^{-1}, the Langmuir films were transferred to the quartz substrate. No fluorescence can be observed if we directly measure the fluorescence with the dry L–B films. Then we kept the L–B film in different organic vapors and it was found that the fluorescence of the tryptophan peptide lipid on the L–B film can be observed in the vapor of methanol, ethanol and 1,4-dioxane at rt. For example, a droplet of methanol was placed in the cuvet where the L–B film slide was inserted into, which resulted in a vapor pressure of 108 Torr at rt. In this paper, all the LB film fluorescence emission spectra were observed in the environment of methanol vapor.

The fluorescence emission spectra of the L–B monolayer films deposited at different surface pressures reveal that the highest fluorescence intensity was observed at a surface pressure of 10 mN m^{-1}. At higher surface pressures such as 20 or 30 mN m^{-1}, the fluorescence intensity decreases because of the self-quenching. The multilayer depositions were tested at a surface pressure of 10 mN m^{-1}. From the results shown in Fig. 3, we can see that the highest fluorescence intensity was observed for a single dipping. Fluorescence quenching between neighbor layers might be responsible for the decrease in fluorescence in the case of a multilayer deposition. A second possibility to explain a decrease in fluorescence intensity is related to the fact that the transfer ratios dropped dramatically for the 2 and 3 dippings which reduce the number of fluorescent monomers.

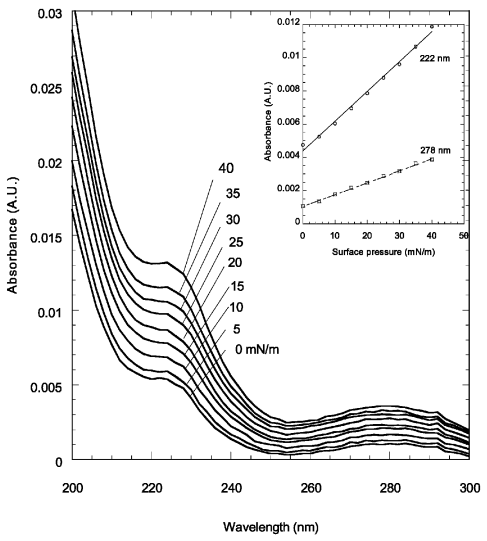

Fig. 2 UV absorption spectra of the tryptophan peptide lipid at the air–water interface at different surface pressures (pH = 5.8, T = 20.0 ± 0.5 °C).

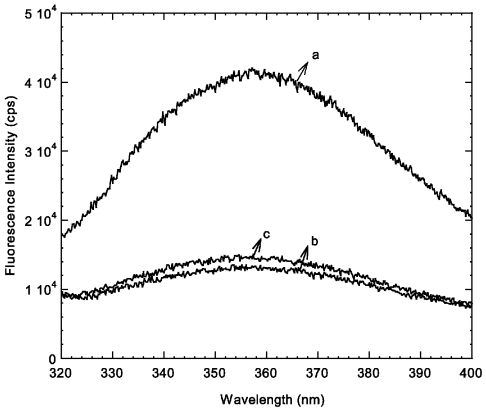

Fig. 3 Fluorescence emission spectra of the L–B film of the tryptophan peptide lipid deposited at surface pressure of 10 mN m^{-1} with different numbers of layers, a: 1 monolayer, b: 2 monolayers and c: 3 monolayers.

We also attempted to reduce the self-quenching of the tryptophan fluorescence by mixing the tryptophan peptide lipid either with stearic acid (SA) or a synthesized peptide lipid SA-Ala-Gly-His-Gly-OH. For both mixtures in L–B films (1 monolayer) with two different molar ratios for each mixture (tryptophan peptide lipid/SA or SA-Aln-Gly-His-Gly, 1 : 10 and 1 : 100), we were not able to detect any fluorescence from the tryptophan moiety.

From the interfacial and spectroscopic studies described above, it reveals that the tryptophan peptide lipid can form a homogeneous monolayer at the air–water interface. The Langmuir film can be transferred to a quartz slide with deposition ratios of about 1. The fluorescence of the tryptophan can be readily observed with 1 monolayer when the L–B film is exposed to methanol, ethanol or 1,4-dioxane vapor at rt. This study shows that the tryptophan lipid may be used as an intrinsic fluorescence probe for the study of Langmuir and L–B films of peptides or peptide lipid libraries.

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