Guest Intercalation at Corrugated Surface of Host Monolayer Crystal on Water: Cholesteryl-L-Glutamate and Water-Soluble Amino Acids

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Corrugated surfaces are attracting interest in fields such as catalysis, chromatography, or material science, owing to their ability to stereospecifically intercalate "guest" molecules.[1,2] Such interfacial structures, thus far studied by indirect means, may be elucidated on the molecular level using grazing incidence X-ray diffraction (GIXD)[3]. Here we examine the interaction between a corrugated Langmuir film of cholesteryl-L-glutamate (CLG) and various α-amino acids (H_2N-CHX-CO_2H) at the air−aqueous solution interface. The CLG molecule is composed of a hydrophobic steroid moiety with a cross-sectional area of 38 Å^2 and a hydrophilic glutamate substituant with an area of 24 Å^2 (Figure 1a). This amphiphile was expected to self-assemble as a monolayer at the air−water interface, generating a corrugated surface with hydrophobic chiral grooves exposed to the water, appropriate for stereospecific inclusion of water-soluble hydrophobic amino acids. Furthermore, these architectures might induce thin film crystallization of the water-soluble amino acids at the periphery of the crystalline clusters by line epitaxy.[4]

The CLG molecule was synthesized according to ref 5. The surface pressure−molecular area (π−A) isotherm of CLG on pure water (Figure 1b) displays a sharp increase in the surface pressure at A = 39 Å^2, the value corresponding to close packing of cholesterol moieties, in a monolayer form. The isotherm becomes more expanded than on water (Figure 1b) when CLG is deposited on saturated solutions of enantiomeric L-leu (X = CH_2-CH-$(CH_3)_2$), L-met (X = $(CH_2)_2$-S-CH_3), or L-Phe (X = CH_2-C_6H_5). Scheme 1 shows architectures that can be formed at the air−solution interface. The water-soluble amino acid can (a) avoid any interaction with the CLG monolayer, (b) intercalate between the glutamate moieties, (c) adsorb at the interface, (d) adsorb and dock within the hydrophobic grooves. An increase of the limiting area per CLG molecule would be observed for the two latter cases.

To distinguish between the various models, GIXD measurements using synchrotron radiation have been performed.[6] The GIXD patterns of CLG (Figure 1c) spread on pure water measured at A = 50 Å^2, π = 0 mN/m and A = 38 Å^2, π = 4 mN/m display

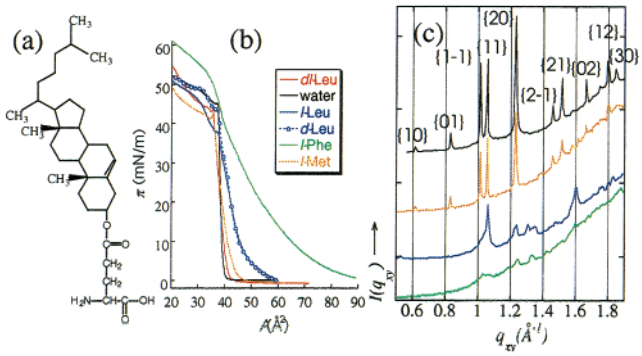
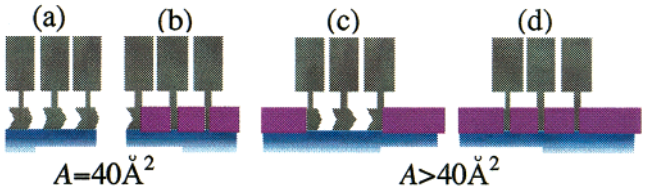


Figure 1. (a) Cholesteryl-L-glutamate (CLG), (b) π−A isotherms at 5 °C for a film of CLG spread on different subphases, water, and saturated solutions of amino acids at 0 °C of *dl*-Leu (8 g/L), *d*-Leu (22 g/L), L-Leu (22 g/L), L-Phe (20 g/L), and L-Met (18 g/L), (c) GIXD patterns obtained in the uncompressed state from a monolayer of CLG spread on water (black), L-Met (yellow), L-Leu (blue), L-Phe (green). Solutions of L-Ser (70 g/L), L-Asn (17 g/L), L-Gln(30 g/L), L-Ala (128 g/L) and Gly (142 g/L) give the same diffraction signal as on water.

Scheme 1. Possible Packing Arrangements of CLG Spread on Solutions of Water-Soluble Amino Acids, Viewed Parallel to the Interface



10 sharp Bragg peaks.[7] These have been indexed in an oblique unit cell with dimensions a = 10.2 Å, b = 7.6 Å, γ = 92.8°, typical values for a close packing motif of the cholesterol moieties.[8] The widths of the Bragg peaks indicate a crystal coherence length of 300 Å. The Bragg rods show only one modulation with a full width at half-maximum (fwhm) of 0.26 Å^{-1}, yielding a film thickness of 2π/fwhm = 24 Å which is 7 Å larger than that of a monolayer of pure cholesterol on water (17 Å).[8] This 7 Å corresponds to the length of the glutamate moiety, showing that they are ordered, despite a cross-sectional area (∼23 Å^2) lower than that of the steroid. The crystal structure was determined by refining a starting molecular model constructed from the 2-D crystal structure of cholesteryl esters on water.[10]

The agreement between the experimental and the calculated Bragg rods (Figure 2a) corresponding to the refined monolayer structure (Figure 2b,c) is satisfactory since the strongest reflections {1−1}, {11}, and {20} are well fitted. The CLG packing is determined by the cholesterol moieties, resulting in a relatively open lattice of the amino acid groups, that would necessitate intercalated water molecules to achieve an ordered hydrogen-bond network. The area of the unit cell is 77 Å^2, of which only ∼48 Å^2 are occupied by the glutamate moieties. This mismatch should allow intercalation of short amino acids between the glutamate groups (Scheme 1b) with a possible rearrangement of the CLG molecules. Figure 1c presents the GIXD patterns of a

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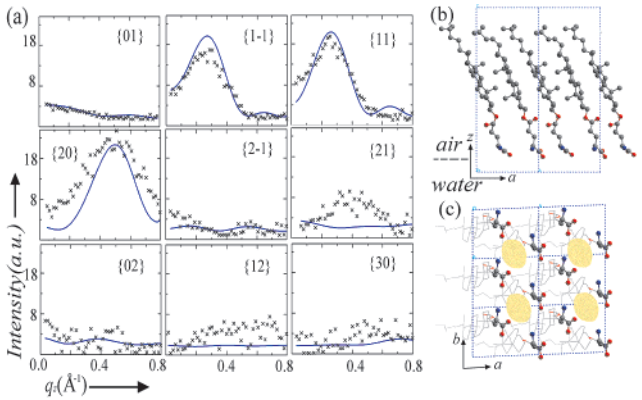

Figure 2. (a) Experimental Bragg rods $I(q_z)$ (crosses) from a monolayer of CLG on water and calculated rods (solid lines) based on a refined molecular structure. X-ray structure factor least-squares refinement using Shelx[11] was initiated, treating the two independent molecules in the unit cell as rigid bodies and then relaxing this constraint, refining the cholesterol and amino acid moieties as separate rigid groups. (b−c) The refined structure as viewed parallel, and normal to, the air−water interface. The yellow patches highlight the "pockets" within which a short amino acid may dock.

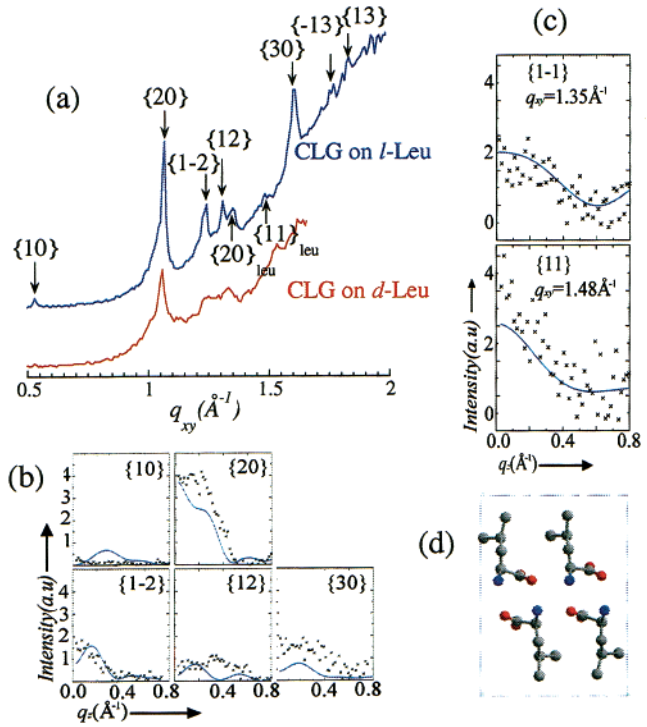

Figure 3. (a) GIXD patterns $I(q_{xy})$ for CLG spread on Leu. The same pattern was obtained for two concentrations of Leu (22 and 11 g/L). The $\{hk\}$ indices of the unit cell arising from the monolayer containing CLG and L-Leu are denoted by arrows from above, whereas the $\{hk\}_{leu}$ indices arising from the Leu bilayer are denoted from below. (b−c) Experimental (dotted line) and calculated (solid line) Bragg rods of CLG and L-Leu monolayer and the bilayer of L-Leu respectively, (d) The molecular model proposed or the bilayer of L-Leu, viewed along the air−water interface, was not refined.

thin film of CLG spread on aqueous solutions of three different amino acids. The patterns on pure water and on L-Met solution are the same, indicating that the solute molecules are not bound to the film. A similar behavior was also deduced for the hydrophilic α-amino acids L-Asp (X = CH_2-$CONH_2$), L-Gln(X = $(CH_2)_2$-$CONH_2$), L-Ser (X = CH_2OH), L-Ala (X = CH_3) and Gly. In contrast, the GIXD pattern is drastically changed by the presence of hydrophobic L-Leu or L-Phe in the subphase.

With L-Leu in solution, the Bragg peaks are weaker and broader than on water, yet the GIXD pattern is sufficiently rich to permit an X-ray structure factor analysis, providing evidence of intercalation of L-Leu within the CLG monolayer.

The GIXD pattern displays 10 distinct Bragg peaks. The fwhm of the Bragg rods indicate the coexistence of two phases. The film thickness of one phase is 27 Å, corresponding to a monolayer, with cell dimensions $a = 11.9$ Å, $b = 10.9$ Å, $\gamma = 94.5°$. The unit cell area of ∼129 Å^2 can accommodate three CLG molecules, with a molecular area of 43 Å^2, of which each glutamate moiety covers an area of ∼22 Å^2. The remaining area of 63 Å^2 can accommodate three L-Leu molecules. Thus, a molecular model containing three CLG and three L-leu molecules was examined, where the positions and orientations of the six molecules were adjusted to yield a reasonable fit between the experimental and calculated Bragg rods (Figure 3b). The Bragg peaks corresponding to the second phase (Figure 3c) were identified by their Bragg rods that give a film thickness of ∼14 Å, which cannot be due to the CLG. Since the film thickness of 14 Å matches that of the bilayer repeat in the L-Leu crystal structure[12] ($a = 9.61$ Å, $b = 5.31$ Å, $c = 14.7$ Å), we attribute these Bragg peaks to a crystalline bilayer of L-Leu with cell dimensions $a = 9.3$ Å, $b = 4.8$ Å (Figure 3d). The calculated Bragg rods fit the experimental profiles and support this hypothesis. Since the glutamate moieties and L-Leu have the same sense of chirality, we assume that the monolayer containing CLG and L-Leu stabilizes the L-Leu bilayer.

Replacing L-Leu by *d*-Leu in the aqueous subphase would destroy such a balance. The $\pi-A$ isotherm of a CLG film on *d*-Leu (Figure 1) is the same as that on L-Leu, indicating the presence of *d*-Leu at the air−liquid interface. The GIXD pattern of CLG on *d*-Leu solution (Figure 3a) exhibits Bragg peaks decidedly broader and weaker than those observed for L-Leu, all indexed in a unit cell $a = 11.9$ Å, $b = 10.5$ Å, $\gamma = 91.3°$. The corresponding Bragg rods show that this phase has a thickness comparable to a CLG monolayer on water. Thus, there is no evidence of the formation of a crystalline bilayer of *d*-Leu. The weak diffraction suggests that *d*-Leu cannot be as easily incorporated as L-Leu between the glutamate moieties of CLG. The effect of chirality of the water-soluble component was also manifest from experiments with *dl*-Leu. The GIXD pattern observed, but only for areas per CLG molecule less than 40 Å^2, is the same as that on water. Thus, spontaneous formation of a crystalline CLG monolayer is inhibited by DL-Leu, but upon compression of the film, pure CLG crystallites are formed.

In conclusion, it is possible to design and to monitor by GIXD the complexation of "guest" chiral molecules at corrugated surfaces of thin films at the air−aqueous solution. The incorporation of the "guest" molecules within the "host" monolayer depends on the hydrophibicity, shape, and chirality of the solute molecules.

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Supporting Information Available: Crystallographic details of the various thin films (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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