Trapping the Metastable Phase of Cholesterol *en route* to Crystallization of the Thermodynamically Stable Monohydrate Phase

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Abnormally high physiological levels of cholesterol may develop into detrimental precipitants containing cholesterol crystallites that are associated with atherosclerotic plaques\(^2\) and with gallstones in the bile.\(^2\) Evidence had come forth from an X-ray powder diffraction study of early-formed crystallites obtained from bile solution that an unknown metastable cholesterol phase is formed en route to precipitation of the thermodynamically stable monohydrate phase.\(^3\)

In previous grazing-incidence X-ray diffraction (GIXD) studies\(^4\) on the growth of a cholesterol crystalline phase three layers thick at the air-water interface we have demonstrated that cholesterol undergoes a transition from a 10x7.5Å\(^2\) bilayer motif into a 12.4x12.4 Å\(^2\) trilayer motif with a structure akin to the stable monohydrate phase, which we tentatively interpret as water-mediated. The GIXD pattern of the 10x7.5Å\(^2\) bilayer resembled the powder X-ray diffraction pattern of the unknown polymorph of cholesterol.\(^3\) We had also found that stigmasterol forms a crystalline trilayer in the 10x7.5 Å\(^2\) motif in an arrangement similar to that of its 3-D hemihydrate crystal structure.\(^3\)

We adopted the following two procedures to help trap the metastable 10x7.5Å\(^2\) layer motif phase in the form of a multilayer in order to establish the overall molecular packing, the structure of occluded water, and the nature of the above-mentioned phase transition.

1. Various cholesterol-stigmasterol (C:S) mixtures, ranging from 85:15 to 25:75, were studied to induce formation of a multilayer 10x7.5Å\(^2\) motif. For this purpose use was made of surface pressure–molecular area (π-A) isotherms and GIXD. The latter showed that the 85:15 and 75:25 C:S mixtures form crystalline bilayers adopting the 10x7.5 Å\(^2\) motif as pure cholesterol on water. The various mixtures from 63:37 to 25:75 yielded crystalline films about two-three bilayers thick appearing in the 10x7.5 Å\(^2\) motif, with unit cell dimensions (as determined from the positions of the intensity modulations along the various Bragg rods, see Fig.1), \(a = 10.1 Å, b = 7.6 Å, c = 35.8 Å, \alpha = 90°, \beta = 95.2°, \gamma = 90°\) in a monoclinic \(P2_1\) space group. We are in the process of determining the multilayer molecular arrangement from the GIXD data. It is evident at this stage that ordered water is occluded between the bilayers, but whether the phase is a monohydrate or hemihydrate remains to be elucidated.

2. In order to establish the influence of phospholipids on crystal nucleation of cholesterol, 5:1 mixtures of cholesterol with different phospholipids were examined. The 5: 1 cholesterol: L-\(\alpha\)-phosphatidylcholines \(\gamma\)-oleoyl yielded a crystalline phase about three bilayers thick in the 10x7.5Å\(^2\) motif according to the positions of the Bragg peaks and widths FWHM(\(q_z\)) of their corresponding Bragg rod intensity modulations. The unit cell dimensions, as determined from the various Bragg rods are \(a = 10.2 Å, b = 7.5 Å, c = 36.3 Å, \alpha = 96.3°, \beta = 95.3°, \gamma = 90°\) in a triclinic \(P1\) space group. Once again, the GIXD data are being analyzed for determination of the multilayer crystal structure, with a particular focus on the water arrangement.

We have also obtained additional data on the transition from the 10x7.5Å\(^2\) bilayer motif to the 12.4x12.4 Å\(^2\) cholesterol monohydrate phase. The film incorporated about four-five bilayers of the monohydrate structure in coexistence with the metastable bilayer phase in about equal amounts. The experiments were done at 5°C and also at 20°C (in order to better approach biological conditions), unlike the earlier experiments\(^3\) in which a weakly formed trilayer of the monohydrate phase was formed at 5°C. These results will help us to analyze the bilayer to monohydrate phase transition.
Figure 1. GIXD pattern $l(q_{xy},q_z)$, as a function of the X-ray scattering vector components $q_{xy}$ and $q_z$, of the crystalline multilayer on water obtained from a 4:6 cholesterol:stigmasterol mixture.

References


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