

Interaction of cholesterol and 7-dehydrocholesterol with bilayers of sphingomyelin

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Introduction

The membrane surrounding living cells consists of a bilayer of polar lipids that represents the matrix supporting the different membrane proteins. The lipid matrix is now recognised as an active participant in facilitating and transducing transmembrane signalling processes. One such mechanism is by the creation of lipid domains. Domains with restricted motion, referred to as liquid-ordered phase, are rich in sphingomyelin and cholesterol. The signalling system sonic hedgehog, associated with embryonic development, is characterised by a failure to catalyse the biosynthesis of cholesterol from its precursor, 7-dehydrocholesterol. We have examined model membranes comprised of sphingomyelin and cholesterol or 7-dehydrocholesterol to compare their phase behaviour.

Materials and Methods

Mixed aqueous dispersions of egg-sphingomyelin with equimolar amounts of cholesterol or 7-dehydrocholesterol were prepared. The structure of the dispersed lipids was examined by static X-ray diffraction on Beamline 15A. Powder patterns were recorded on image plates at temperatures about the physiological range. The patterns were analysed by circular integration of the scattering intensities and d-spacings calibrated using crystalline cholesterol.

Results and Discussion

The small-angle X-ray scattering intensity from equimolar mixtures of sphingomyelin with cholesterol or 7-dehydrocholesterol was recorded at temperatures between 20° and 55°C. The regions showing the first two orders of diffraction of lamellar phases are shown in Figure 1. The wide-angle region consisted of a broad scattering band at all temperatures suggesting that the sterols prevented formation of a gel phase by the sphingomyelin. The lamellar reflections from the mixture containing cholesterol shows a repeat spacing of 6.42nm and this was unchanged in the temperature range 20° to 50°C. By contrast, a dominant lamellar d-spacing of the mixture containing 7-dehydrocholesterol was 6.47nm seen over the temperature range 20° to 55°C but above 37°C a phase separation takes place evidenced by the appearance of another lamellar phase characterised by a d-spacing of 5.79nm. This suggests that subtle differences in structure between the two sterol molecules

influences the phase behaviour of sphingomyelin-sterol phases. Such effects may underly the defect in signalling associated with the sonic hedgehog pathway.

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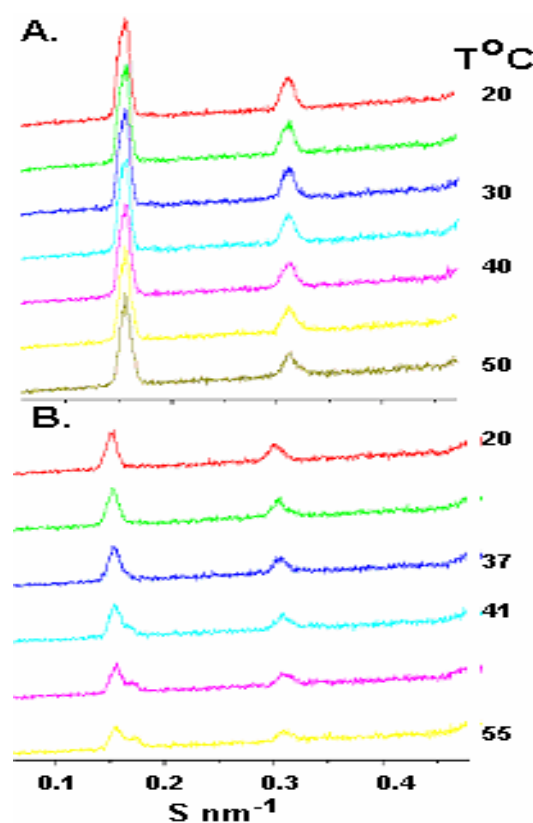


Fig.1. SAXS patterns from dispersions of sphingomyelin with A. cholesterol B. 7-dehydrocholesterol.