Influence of Cholesterol on Polydispersity and Interfacial Properties of Large Unilamellar Vesicles

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Cholesterol-rich phases in lipid mixtures, commonly referred to as lipid "rafts" have garnered a great deal of interest because of their putative roles in biological functions ranging from T-cell activation [1] to intracellular trafficking [2]. One of the central questions in studies of lipid - cholesterol mixtures is, what mechanism or mechanisms drive the formation and modulate stability of cholesterol rich phases? One possible mechanism for domain formation in lipid - cholesterol mixtures is phase separation resulting from cholesterol's strong affinity for and ability to condense one lipid component and not the other, which, in turn is modulated by the degree of lipid chain unsaturation. Here, we use SAXS from unilamellar vesicles to study possible differences in the influence of cholesterol on membranes composed of lipids with either one or two unsaturated chains, stearyloleoyl (SOPC) and dioleoyl phosphatidylcholine (DOPC). Structural differences, either in liposome size and polydispersity or in membrane bilayer structure reflect differences in interactions of cholesterol with these lipids, due to the presence of an additional double bond in the sn-1 chain of DOPC when compared to SOPC. Such differences in interactions may have important implications for the efficacy of these lipids in forming the “non-raft” component of lipid “raft” mixtures.

The primary objectives of this study were to simultaneously measure both the vesicle and bilayer form factors for low polydispersity large unilamellar vesicles (LUVs) prepared by extrusion, and to compare the possible differences in the influence of cholesterol on SOPC and DOPC membranes in LUVs. LUVs prepared by extrusion had a nominal diameter of 50 nm and were prepared in a 20% by weight sucrose solution, in order to enhance the electron density contrast between the bilayer and the medium. Four sample conditions were investigated, SOPC and DOPC vesicles prepared without and with 30 mole % cholesterol. Measurements were performed at two sample to detector distances, 3 m and 70 cm, corresponding to a range of approximately 0.064 nm^{-1} to 4.6 nm^{-1}. The complete SAXS curves are shown in Figure 1. The scattering minima corresponding to the vesicle form factor are indicated by the solid arrows in Figure 1. Using the relation between the average vesicle radius and first scattering minimum, $q\langle R\rangle = \pi$, we find values for the vesicle radii of 20.0, 17.5, 19.8, and 15.2 nm for SOPC, SOPC + cholesterol, DOPC and DOPC + cholesterol, respectively, assuming that these vesicles are spherical. The width of the minima is indicative of the relative polydispersity, and it is clear from the figure that the DOPC + cholesterol vesicles are significantly more polydisperse than the other three samples. We should qualify, however, that we have not considered the influence of vesicle morphology on the position and depth of the vesicle form factor scattering minima, and there are indications that the effects of vesicle morphology may contribute significantly to the vesicle form factor in the vicinity of the scattering minima [3].

The scattering minima corresponding to the bilayer form factor are indicated in Figure 1 with dashed arrows. In this case the characteristic thickness of the bilayer is given by $qt = 2\pi$. The characteristic thicknesses of the SOPC, SOPC + cholesterol, DOPC and DOPC + cholesterol bilayers are 2.4, 2.4, 2.2, and 2.2 nm, respectively. By performing an indirect continuous Fourier transform on the scattering data over the large q range ($q > 0.4$ nm^{-1}), it is possible to resolve the electron density profile of the bilayer (as shown in Figure 2). On examination of these electron density profiles, we find that the characteristic lengths determined from Figure 1 correspond to the width of the deepest region of the electron density profiles. It is in these profiles that we see the most significant differences in the influence of cholesterol on the bilayer structure. The primary influence of cholesterol on the electron density profile of the bilayer is due to the higher electron density of cholesterol's steroid rings than that of adjacent lipids. Thus increases in electron density with addition of cholesterol most likely correspond to the average vertical position of cholesterol within the bilayer. A comparison of the curves showing SOPC + cholesterol and DOPC + cholesterol suggests that the average vertical position of cholesterol is closer to the membrane-water interface in DOPC membranes than SOPC membranes.

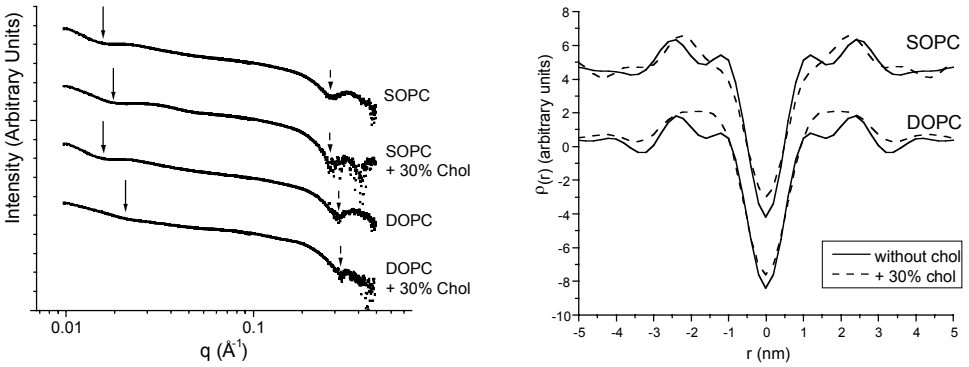

Figure Left: SAXS results for SOPC and DOPC LUVs with and without 30 mole % cholesterol. Solid arrows indicate positions of scattering minima associated with the vesicle form factor, while dashed arrows correspond to scattering minima associated with the bilayer form factor. Curves have been shifted on the vertical axis to facilitate viewing.

Figure Right: Electron density profiles of SOPC and DOPC bilayers without and with 30 mole % cholesterol, determined via an indirect continuous Fourier transform of the high q region data shown in Figure 1. Curves have been shifted on the vertical axis to facilitate viewing.

References

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