Small Angle X-ray Scattering (SAXS) and X-ray Reflectivity (XRR) Studies on Structure and Phase Behavior of Bicelles

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Over the past decade, there were lots of biophysical and biochemical studies on the mimic of biological membranes. In order to understand the interactions and to characterize the membrane associated biomolecules, the idea of bilayered micelles, or so-called bicelle, could be very useful model system. This kind of binary mixture usually refers to a disc-shaped aggregation which composed of both long-chain lecithin in the planar core area and short-chain lecithin in the rim region in order to protect the hydrophobic long-chain lipid tail from contacting with water.

Figures 1 and 2 show the the SAXS data recorded for samples of DPPC/DiC7PC and DPPC/DiC6PC mixture suspended in water. We found that phase transition occured when more short chain lipids are added in the long chain lipid system as shown in Fig. 1. By observing the intensities and full width at half maximum (FWHM) of these Bragg peaks caused by multilamellar structure of DPPC, we found that the bilayer stacking is getting disorder and the number of lipid bilayers is decreasing as indicated by the intensity decrease and the broadening FWHM. The multilamellar diffraction peaks do not disappear until DPPC/DiC7PC ratio is at 3/1. During the transition of multilamellar vesicle to unilamellar vesicle, the Bragg peak slightly shift from q=0.100 Å^{-1} to a lower q=0.094 Å^{-1}, for pure DPPC multibilayers and mixture of DPPC/DiC7PC=3/1 with repeat distance of 6.28 and 6.68 nm, respectively. It indicates that it is not only the bilayer structure is destroyed by adding DiC7PC but also enlarged the repeat distance of DPPC bilayer as increasing the short chain to long chain lipid ratio.

Other than the DPPC associated with DiC7PC, we also attempt to determine the mixture of DPPC and DiC6PC. From the SAXS profile in Fig. 2, actually, we found the presence of short chain lipid of DiC6PC did not alter the DPPC multibilayer structure too much, even at the ratio of DPPC/DiC6PC=3/1. DPPC bilayer stacking still dominates the main structure. To compare with the DPPC/DiC7PC mixture, there is only one phase in DPPC/DiC6PC system. However, the addition of DiC6PC still slightly affect the multilamellar structure.

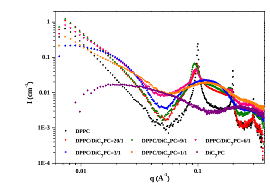
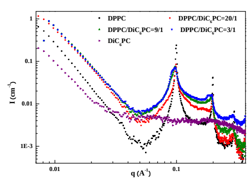

Fig. 1 (left) and 2 (right): X-ray small angle scattering results of DPPC mixed with DiC7PC and DiC6PC, respectively, in ratio of 1/0, 20/1, 9/1, 6/1, 3/1, 1/1, and 0/1 at temperature of 30^{0}C.

Besides the SAXS scattering technique, we make an effort to understand the formation of such mixture in the form of solid supported oriented multilayer film. The X-ray reflectivity method was applied for determining the in-depth profile of such oriented multilayer film. From Fig. 3 and Fig. 4, we found that there is not so much difference between DPPC/DiC7PC and DPPC/DiC6PC systems for the short-chain to long chain ratio below 1/3. The results show that the DPPC multilamellar structure incorporated with the short-chain lipids would induce a new set of Bragg peaks that occurr at around times of q=0.125 Å^{-1}, corresponding to a repeat diatance of 5.03 nm, and the intensity of these diffraction peaks increases with increasing amounts of short-chain lipid. When the mixing ratio of long-chain lipid to short-chain lipid is 3/1, or even to 1/1, its reflectivity profiles exhibit the totally different formation form the ratio above 9/1. We can find another new thinner phase has take place of DPPC multibilayers and short-chain induced phase. At DPPC/DiC7PC=3/1, there is only one phase left with a repeat distance of 4.36 nm, q=0.144 Å^{-1}. For the case of DPPC/DiC7PC=1/1, however, there two separate phases again, which indicates the mismatch of these two lipids in forming the plannar bilayer structure.

To compare with the results obtained at lower relative humidity conditions, around 40% relative humidity (results not shown), we observed that only one phase exist in the mixture of DPPC with DiC6PC and the repeat distance of that phase seems to have only little change at different ratios of short (diC6PC) to long chain ratios. These results show indicate that the short-chain lipid, DiC6PC, does not affect the DPPC multibilayers too much at the environment condition of lacking water molecules. However, the reflectivity results of DPPC/DiC7PC at 40% RH are consist with that at high relative humidity, 98% RH with two phases and the repeat spacing could be swelled by the presence of water from 5.71 nm to 6.28 nm for DPPC phase and from 3.14 nm to 4.83 nm for DiC7PC-induced phase. On the other word, water content plays an important role on the short-chain-induced phase. From the above discussions, we can conclude that the effects of water content on the structure of the mixed DiC7PC and DiC6PC with DPPC multibilayers are extremely different.

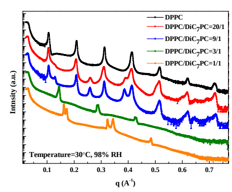
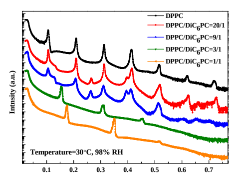

Fig. 3 (left) and 4 (right): Multilayer x-ray reflectivity results of DPPC mixed with DiC7PC and DiC6PC, respectively, in ratio of 20/1, 9/1, 3/1, and 1/1 at temperature of 30 0C and 98% RH