Biological Science

9C, 15A, 2007G656

On the hydration of metastable subgel and highly crystalline phases in dimyristoylphosphatidylglycerol bilayers

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Introduction

Dimyristoylphosphatidylglycerol (DMPG) bilayers have been extensively studied as a model for the negatively charged biomembrane. DMPG bilayers show a unique low-temperature phase behavior. In the presence of more than about 100 mM NaCl, DMPG bilayers assume vesicular structures and the stable phase at low temperature is the highly crystalline (HC) phase, which is more ordered than two other metasatable subgel phases (L_1 and L_2 phases [1]).

Using differential scanning calorimetry, we have studied on the process of the HC phase formation under high Na^+ concentrations and revealed that the time course of the HC formation consists of three stages, i.e., initial delay, growth and plateau stages [2]. In addition, our results indicated that the transition from the L_2 phase to the HC phase gradually proceeded without intermediate states during incubation at 1°C over 4 h. For this transition, we also inferred that at least three possible processes are involved in the L_2-to-HC phase transition: (1) dehydration, (2) formation of hydrogen bond networks among PG headgroups and (3) Na^+ ion binding to negatively charged PG headgroups. Here, we report the results of small angle X-ray diffraction (SAXD) experiments performed to confirm the involvement of extensive dehydration in the L_2-to-HC phase transition [2].

Materials and methods

The sodium salt of DMPG was purchased from Avanti Polar Lipids Inc. and used without further purification. DMPG dissolved in chloroform was dried under a flow of nitrogen and then under reduced pressure overnight. The resulting lipid film was dispersed into a buffer containing 10mM HEPES (pH 7.4) and an appropriate concentration of NaCl. The L_2 phase was formed by incubating for 4h at 1°C in the presence of 175mM NaCl. When the sufficiently hydrated DMPG bilayers are incubated at 1°C, the HC phase formation reaches a plateau in an NaCl concentration-dependent manner [2]. Thus, the HC phase coexists with the metastable subgel phase at the plateau. On the basis of density difference, we separated the HC phase fraction by centrifugation using a H_2O/D_2O mixture. Imaging plate was used as an X-ray detector.

Results and Discussion

Figure 1 shows SAXD profiles of the L_2 and HC phases of DMPG bilayers. A broad peak centered at 0.23 nm^{-1} (4.35 nm) indicates that DMPG vesicles in the L_2 phase consist of mutually independent single bilayers. The peak position reflects the thickness of a single bilayer [3]. In contrast, the SAXD profile of the HC phase (Fig. 1b) shows a sharp reflection from a multilamellar system with the lamellar spacing of 4.76 nm (0.21 nm^{-1}). Thus, the notable interbilayer water loss, dehydration, may take place in the L_2-to-HC phase transition.

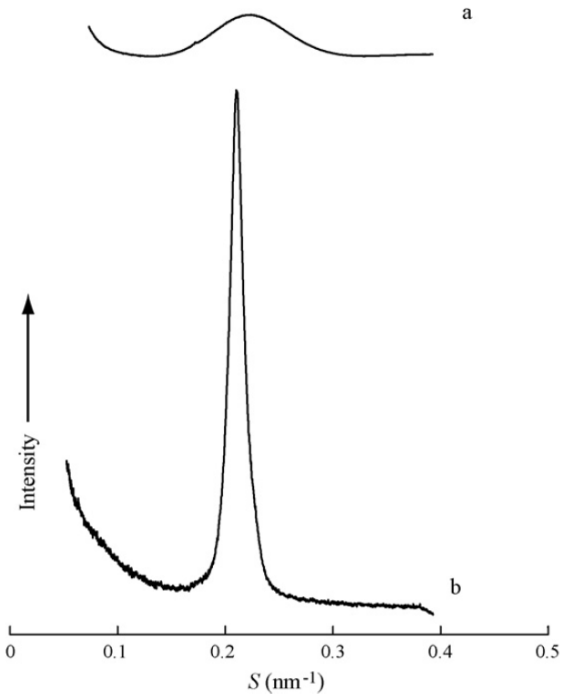

Figure 1 SAXD profiles of (a) the L_2 phase and (b) the HC phase of DMPG bilayers.

References

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