Contrast variation SAS study in DDABr/D2O system using ASAXS

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Amphiphilic molecules like phospholipids or surfactant self-assemble the fluid membranes in the water. The mechanism of the structural formation of these membranes are interesting to investigate one of the biomembranes. Dipalmitoylphosphatidylcholine(DPPC), one of the most studied phospholipids, usually shows a multi-lamellar vesicle in the water and we have been studying its structure using small angle X-ray scattering (SAXS) .

The synthetic cationic double-chain surfactant didodecyldimethylammonium bromide (DDABr) /water system shows two distinct thermodynamically stable lamellar phases as shown in Fig.1: a dilute Lα phase, and a condensed Lα' phase[1]. As mentioned above DDABr is similar to DPPC in the shape of molecule however the phase behavior of its aqueous solution is different. In particular, it is interesting why Lα' phase coexists with Lα phase, where the inter-bilayer distance is very large and the repulsion between bilayers is effective in long range. The phase transition from Lα to Lα' phase is also interesting to study the fusion of the membranes. In order to investigate the mechanism of the above behavior, we performed anomalous small angle X-ray scattering(ASAXS) . Since DDABr molecule includes a Br atom whose absorption edge is 13.473keV(0.93423Å), the contrast of the scattering amplitude varies with the energy of X-ray because of anormalous dispersion of the Br atom. From the contrast variation experiment, we can obtain more information of the structure of the system.

The ASAXS experiments were performed were performed at BL40B2. The sample-detector distance was 1m and the energy of the incident X-ray changed in the range of 12.6keV-13.47keV. The samples were prepared to be Φ_{DDAB}=5, 10, 15, 20, 25, 30, 35wt.% and temperature was 40ºC.

The obtained ASAXS profiles are shown in Fig.2. The profiles change with the energy. Since it is not easy to analyse these data properly, we have been improving the analysis of the contrast variation method like Nagao et al[2].

Reference

[1] M. Dubois, Th. Zemb, N. Fuller, R.P. Rand and V.A. Persegian, J. Chem. Phys. **108** (1998) 7855.
[2] M. Nagao, H. Seto, M. Shibayama and N.L. Yamada, J. Appl. Cryst. **36** (2003) 602.

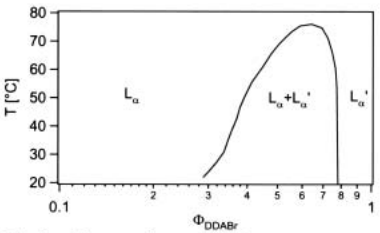

Fig.1: Phase diagram of temperature vs weight fraction. The swollen lamellar Lα phase is a multi-lamellar vesicle. The collapsed Lα' phase is the open bilayer phase.[1]

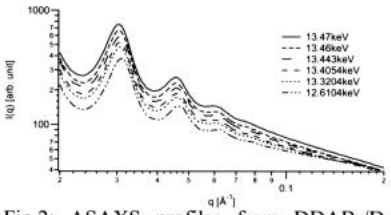

Fig.2: ASAXS profiles from DDABr/D2O system(Φ_{DDAB}=5wt.%) . The profiles change with the energy.

Crystal Structure of the L Intermediate of Bacteriorhodopsin

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Bacteriorhodopsin (bR), a light-driven proton pump found in the cell membrane of *Halobacterium salinarum*, contains retinal as the chromophore. Photoisomerization of the retinal from the *trans* to the 13-*cis* configuration initiates a reaction cycle involving several intermediates (bR_{570} -> K_{590} -> L_{550} -> M_{412} -> N_{560} -> O_{640} -> bR_{570}). For elucidation of the proton pumping mechanism, it is important to obtain structural information of the reaction intermediates. Using a 3D crystal belonging to the space group P622, we have previously determined the structures of the ground state (bR_{570}), the K intermediate and the M intermediate at 2.3 - 2.6Å resolutions. Our structural data show that, in the K intermediate, the retinal chromophore has a largely twisted 13-cis, 15-anti configuration. The distortion in the retinal polyene chain is suggested to derive a vertical movement of helix G upon formation of the M intermediate, which is accompanied by a large rearrangement in the hydrogen-bonding network in the proton release channel.

An increasing number of experimental data have shown that internal water molecules play an important role in regulating the pK_a values of key residues in the pathway of proton transport. The current structural model of the unphotolysed state (bR_{ground}) shows that water molecules existing in the active site participate in stabilizing the protonated Schiff base with a very high pK_a (~ 13) and unprotonated Asp85 with a very low pK_a (~ 2.5). Recent FTIR studies of the L intermediate have suggested that, before the primary proton transfer, rearrangements of the internal water molecules take place in such a manner that they cause a significant reduction of the pK_a of the Schiff base and a concomitant increase of the pK_a of Asp85. But, the structure of a key intermediate, i.e., the L intermediate, has not yet been determined convincingly, making it difficult to quantitatively analyze the detailed movements of the internal water molecules.

In the present study, we carried out a quantitative analysis for x-radiation damages and searched for the best condition for X-ray measurements to minimize undesired effects of X-ray-induced structural changes. After a careful structural analysis, we constructed a structural model of the L intermediate at 2.4 Å resolution. In addition to this structural determination, structural changes taking place in the L-to-M transition were also investigated. Combined with our previous study of the K intermediate [1], the present study leaded us to propose a novel proton-pumping mechanism, in which the vertical translocation of the water molecules in the active site is a key event determining the directionality of proton translocation in the protein [2].

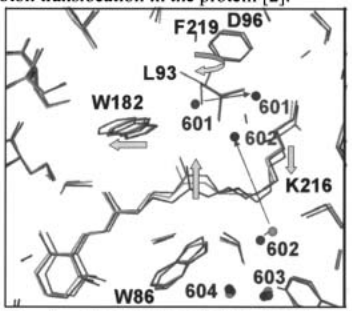

Fig. 1. Movements of key water molecules (indicated by thin arrows) and conformational changes in Leu93, Trp182 and the retinal—Lys216 chain (indicated by thick arrows) during the bR_{ground}→to→K and the K→to→L transitions. Atoms in bR_{ground}, K and L are drawn in different colors.

Reference:

1. Y. Matui et al.: Specific damages induced by X-ray radiation and structural changes in the primary photoreaction of bacteriorhodopsin. *J. Mol. Biol.* **324**, 468-481 (2002)
2. T. Kouyama, et al.: Crystal structure of the L intermediate of bacteriorhodopsin: Evidence for vertical translocation of a water molecule during the proton pumping cycle (Submitted *J. Mol. Biol.*)