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Effect of ultra-fine bubble water on the structure of protein and lipid membrane

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1. Introduction

Ultra-fine bubble (UFB) is usually defined as small bubbles with the diameter less than 1 μm, which is different from microbubbles generally having approximately 1 ~ 100 μm in diameter. UFB is colorless and transparent to the naked eye, and is stable for several weeks or so in solutions. UFB enables to entrap various kinds of gases for a long time. When oxygen is as entrapped gas, the amount of dissolved oxygen can be increased efficiently. Unlike chemicals, it can contribute to promote the growth promote of organisms. It is said that UFB acts on various physiological functions in living bodies to improve the function. Even the approach to use of UFBs as DDS is now progressing.

Therefore, recently, the industrial applications of UFB have been focused and highlighted in the various fields such as agricultural and fishery industries, and medical therapy. However, the detailed mechanism underlying the performance of UFB, especially the effect of UFB on biological materials, is not known.

Then, we investigated the effect of UFB on protein and lipid membrane structures by using small-and-wide angle X-ray scattering (SWAXS). The present experiment would be the first study on the effect of UFB on biological materials in the world as far as we know.

2. Experimental

In the present study, we have investigated the effect of two different types of UFB on the proteins and the liposomes. UFB used was the ultra-high-density UFB occluding O_2 or N_2 gas (10 billion particles / mL or more) offered by NANOX Co. The proteins were myoglobin from equine skeletal muscle, bovine serum albumin, and lysozyme from chicken egg-white, which were purchased from SIGMA co. The liposome was the large uni-lamellar vesicle (LUV) or small uni-lamellar vesicle (SUV) composed of lipid mixture (ganglioside (G_{M1}), cholesterol, and phospholipid (DMPC, DPPC, DOPC, POPC). where the phospholipid concentration was fixed at 1 %w/v. The time-resolved SWAXS measurements were carried out by using the X-ray scattering spectrometer installed at BL10C beam port of PF at KEK, Tsukuba, Japan. The X-ray wavelengths were 1.0 Å and 1.5 Å. The sample-to-detector distances were 50 cm and 300 cm. X-ray scattering intensity was recorded by a hybrid pixel detector, PILATUS3 2M (Dectris). The exposure time was 60 seconds. Sample cells composed of a pair of thin-quartz windows with 1 mm path length were used.

3. Results and Discussion

After the mixing of the protein or liposome solution with the UFB solution at the volume ratio of 1/5, the time-resolved SWAXS measurement was started under an appropriate time-interval. Fig. 1 shows the time evolution of the scattering curves of LUV ([G_{M1}] / [cholesterol] / [DMPC] = 0.1/0.1/1). The broad hump at q = ~0.1 Å^{-1} and the sharp peak at q = ~1.46 Å^{-1} reflect the lipid bilayer structure and the alkychain packing and its fluctuation [1, 2]. As shown clearly, we did not recognize any evident change of the lipid bilayer structure. However, we found significant changes of the inter-alkychain distnace and its fluctuation. As shown in Fig. 2, both alky chain packing and chain fluctuation of lipids at gel phase in liposome tend to be suppressed in O_2-UFB with in N_2 bubble and pure buffer. The detailed results will be seen elsewhere.

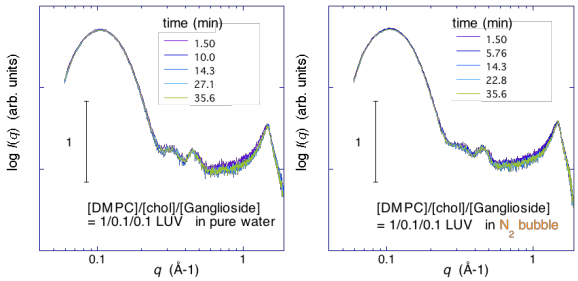

Fig. 1. Time evolution of the scattering curve of LUV ([G_{M1}]/[cholesterol]/[DMPC]=0.1/0.1/1) after dispersed into pure water (on the left) and N_2-UFB solution (on the right).

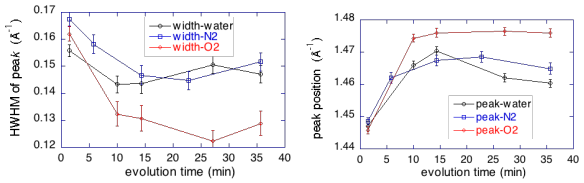

Fig. 2. Time evolution of the position (on the right) and HWHM (on the left) of the peak at ~ 1.4-1.5 Å^{-1} in Fig. 1.

References

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