Size Growth Factor in the Process of Vesicle Formation from Phospholipid-Detergent Mixed Mecelles

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Small unilamellar vesicles (SUV*) appeared in the transition region were reproduced by adding proper amount of detergent to SUV prepared by ultrasonication. It was found that SUV* are fusible to grow their size time-dependently resulting in formation of large unilamellar vesicles (LUV) if octylglucoside was used as a detergent, but are not if sodium cholate was used. On the basis above results it was discussed that post-vesiculation size growth is a crucial factor to regulate vesicle size in the process of vesicle formation from detergent-phospholipid mixed micelles.

The mechanism of micelle-vesicle transition has been widely studied in close relationship to the functional reconstitution of membrane proteins after they have been purified in detergent solution. It is, however, still unclear why the size of the vesicles prepared by removing detergent by dialysis from phospholipid-detergent mixed micelles becomes larger than that of the vesicles prepared by rapid detergent removal using hydrophobic porous beads or gel filtration, and why the size of the vesicles prepared using octylglucoside as a detergent is large, while that using cholate, SDS or polyoxiethylene alkylether small.[1] The purpose of this study is to find out the size determining factor and to show how to control the vesicle size in the process of vesicle formation.

In the process of vesicle formation by detergent removal from phospholipid-detergent mixed micelles it has been observed that with detergent depletion first the turbidity of the solution (or suspension) and apparent particle size grow and take maximum at certain detergent concentration, followed by decrease and then vesicles form[2,3]. The similar behavior was observed in the process of vesicle destruction by adding detergent[4]. The phenomena observed here have been known to be opposite symmetry[5]. We have found the appearance of small vesicles before vesicle destruction by adding detergent to initially prepared vesicles as a common phenomenon in spite of the variety of detergents, even in solubilization process of LUV (large unilamellar vesicle)[6]. We tentatively named these small vesicles (containing high amount of detergent) SUV* to distinguish the vesicles from SUV (small unilamellar vesicle) prepared by sonicaion (which does not contain any detergent). In this paper we show large unilamellar vesicles are formed by fusion of the SUV* and from the results discuss that the post vesiculation size growth[5,7] is a critical factor for size regulation of the vesicles prepared by detergent removal method.

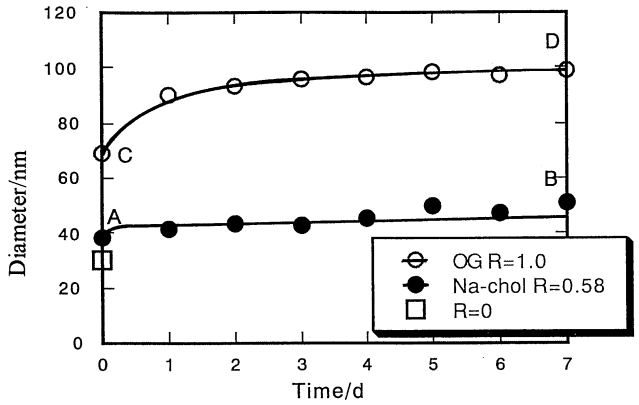

Figure 1. Size change of vesicles after addtion of detergents.

SUV* was produced by adding detergent to SUV prepared by ultrasonicaion (for 40 min using a probe type sonicator) with the concentration of 1.0 or 1.1 of "effective" molar ratio, that is, detergent / phospholipid (egg yolk phosphatidylcholine) molar ratio in membrane phase for octylglucoside or 0.6 for sodiumcholate(Na-chol). The phospholipid concentration was 5 mM in a buffer solution (20 mM Tes, 250 mM NaCl, 1 mM EDTA; pH 7.0). Dtergent concentration was measured by the radiotracer technique and phospholipid concentration was determined as phosphorus by the method of Ames[8]. All experiments were performed at 25°C. In the detergent concentration region SUV* or aggregate of SUV* has been observed in both processes of vesicle formation by detergent removal from mixed micelles and vesicle destruction by adding detergent to vesicles prepared before hand[6,9]. And also in this concentration region the turbidity and apparent vesicle size were known to be time-dependent. Figure 1 shows the size change of SUV after addition of detergent. The size a little increased just after addition of sodium cholate at 0.6 of molar ratio but the size increase was rather small (solid circle). On the other hand, as shown in open circle, the size growth of the vesicles containing octylglucoside of 1.0 in molar ratio was very large and time dependent. The size reached maximum about 2 days after addition of octylglucoside. Figure 2 shows the freeze fracture electron micrographs of the vesicles after addition of sodim cholate. Significant size change was not observed after addition of sodium cholate.

On the other hand, in the case of addition of octylglucoside as a detergent, a notable time-dependent size growth was observed as

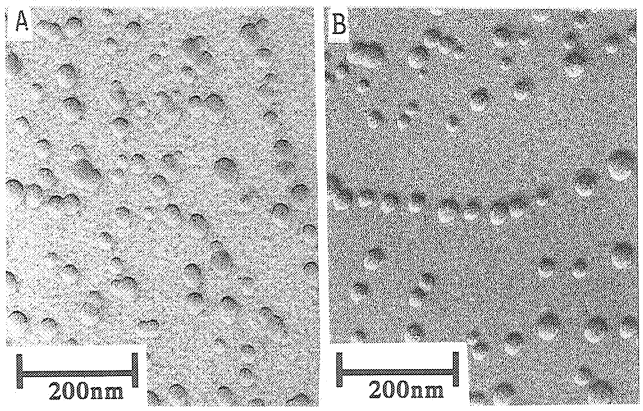

Figure 2. Freeze fracture electron micrograph of vesicles after addition of sodium cholate. A; immediately, B; 7 days after addition of detergent.

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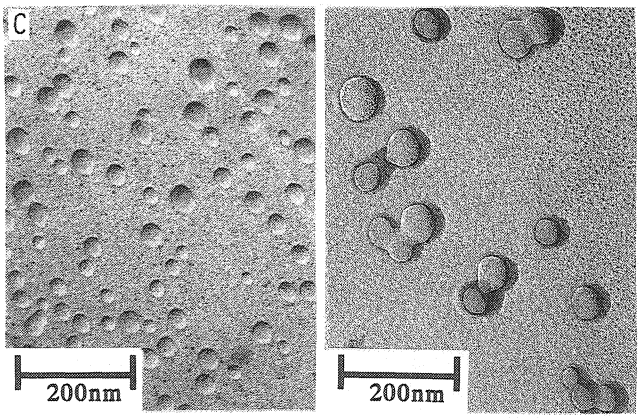

Figure 3. Freeze fracture electron micrograph of vesicles after addition of octylglucoside. C; immediately, D; 7days after addition of detergent.

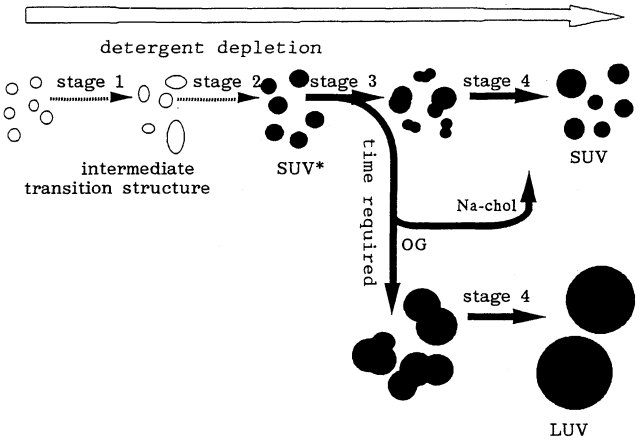

Figure 5. Schematic representation of micelle-vesicle transition and size regulation.

shown in Figure 3 in accordance with the results of Figure 1. Even 7 days after addition of octylglucoside an image of fusion was observed.

Next, the following cycle was devised. Octylglucoside was added to initially detergent free SUV with 35 nm of diameter at the concentration of 1.1 in effective molar ratio and the size of the vesicles increased from point E to F in the Figure 4. Then the vesicles were incubated for 6 hours and the size increased from F to G. Finally the detergent was removed by dialysis and the size a little decreased from G to H. In this cycle the vesicle size grew about 3 times the original size to be about 120 nm.

Figure 5 shows the schematic representation of micelle-vesicle transition and size regulation. Sage 1 is that of micellar growth with detergent depretion; stage 2 is of micelle-to-vesicle transition; stage 3 of size growth of small vesicles (SUV*) by fusion; stage 4 of detergent removal from fused vesicles. In this report we cleared stages 3 and 4, where the states of size growth of vesicles were depicted in black figure. From the results the following conclusion can be reached regarding the size regulation of the vesicles prepared by detergent removing method: Initially formed vesicles are small, but the size increases slowly thereafter by fusion, if detergent is not removed too quickly. This will answer the question, why the vesicles prepared by removing detergent by dialysis are larger than those prepared by rapid detergent removal. Some time is required for fusion to be completed. The results also answer the question why the size of the vesicles prepared using octylglucoside is large and that using cholate, SDS or polyoxyethylene alkylether small. The small vesicles containing octylglucoside are fusible in the process of detergent removal due to their geometrical distortion related to their curvature and high surface energy caused by depletion of detergent. The vesicles containing cholate or SDS can not easily aggregate and fuse with each other because of its electrostatic repulsion. The vesicles containing polyoxyethylene alkylether also can hardly be fusible because of large hydration of its ethyleneoxide. Very recently, the vesicle growth containing surfactant at subsolublizing concentration was reported in another experimental system[10]. Thus, post vesiculation size growth has explained the time and detergent-dependent size variation of the vesicles prepared by detergent removal from phospholipid-detergent mixed micelles. The results obtained here can make it possible to control vesicle size in the stage of vesicle preparation and to choose the most suitable detergent for a work, such as , protein reconstitution.

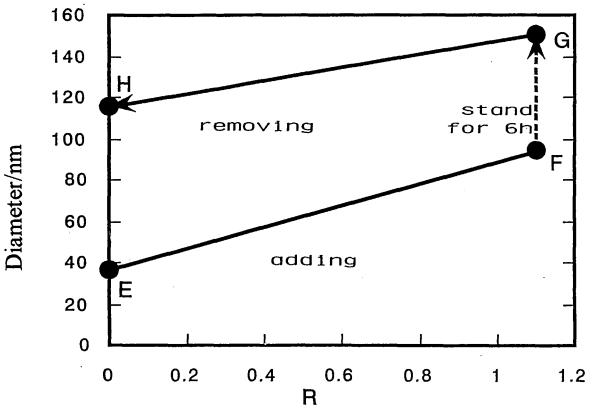

Figure 4. Size change of vesicles by adding and then removing octylglucoside.

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