CHEMISTRY LETTERS, pp. 1801-1804, 1991.

Morphological Transformation of Solubilization Process of Large Unilamellar Vesicle by a Nonionic Detergent, Octyl Glucoside

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Solubilization process of large unilamellar vesicles was characterized by 5 stages; (1) simple incorporation of detergents into the membrane; (2) appearance of convex and concave on the membrane surface; (3) closing of the convex and concave resulting in formation of small vesicles; (4) dispersion or aggregation of the small vesicles; (5) solubilization of the small vesicles resulting in formation of mixed micelles.

As a small amount of a detergent is added to a phospholipid vesicle, the detergent distributes in the vesicle membrane, and distribution equilibrium is basically attained. On the other hand, a large amount of the detergent solubilizes the vesicle and makes a mixed micelle with phospholipids of the vesicle. The study on the mechanism of solubilization process is very important from a view point of the solubilization and reconstitution of a membrane protein, and many models concerning mixed micelle-vesicle transition have been considered.[1-3] Almost all models describe three stages during vesicle-solubilization process. One typical and the latest was reported by Ollivon et al.;[3] first (A) vesicles swell during equilibration with detergents, and then (B) vesicle-to-sheet transition is followed by (C) sheet-to-micelle transition. These models can roughly illustrate the phenomena of the vesicle to micelle transition, but can not explain the appearance of small particles observed just before vesicle destruction as well as kinetic factors of vesicle size control.[4,5] In this report, we propose a model of vesicle solubilization process which will be consistent with all experimental results obtained.

In this study, we used a large unilamellar vesicle prepared by detergent-removal method. Egg yolk phosphatidylcholine (PC) was used as a phospholipid. Octyl glucoside (OG) was used as a nonionic detergent adopted for vesicle solubilization. The particle size was estimated by quasi elastic light scattering. The diameters of the detergent-free vesicle lay between 180 nm and 250 nm. Experimental temperature was 25 ^{o}C, which exceeds a phase transition temperature of the egg phosphatidylcholine (below 0 ^{o}C).

As the concentration of the detergent increases, the vesicle size grows larger

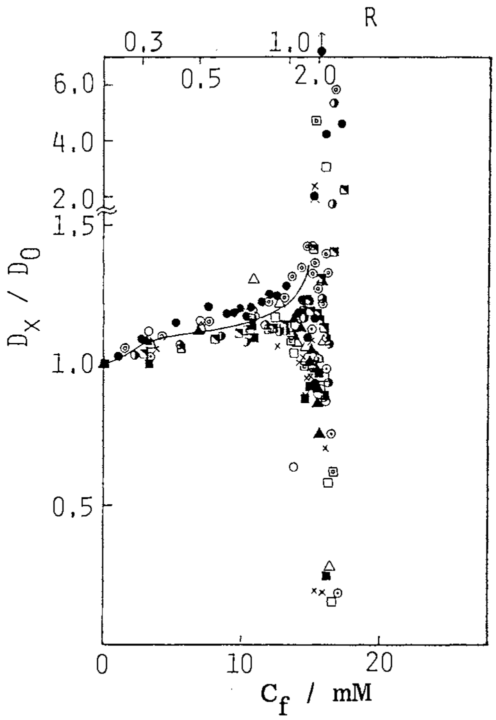

Fig. 1. D_x / D_o vs. C_f (OG concentration in water phase) plots. Solid line represents theoretical curve calculated from Eq. 1 (see context). Symbols represent experimental values.

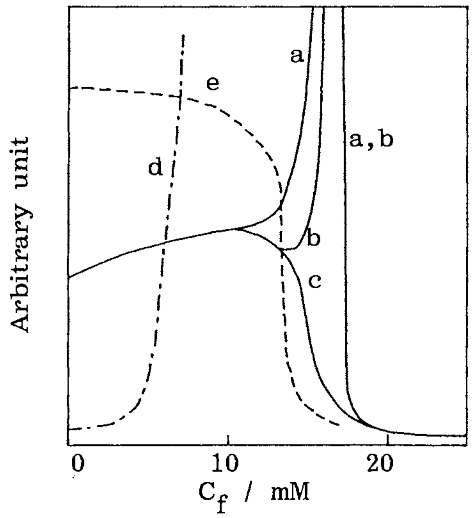

Fig. 2. Schematic representations of typical behavior of vesicle size (a, b, c) and barrier efficiency (d, e) depending on C_f. Line a: high concentration of phospholipid (>7 mM); Line b: medium concentration (2.4-7 mM); Line c: low concentration (< 2.4 mM). Line d: permeability of Cl^- (M. Ueno, Biochim. Biophys. Acta, 904, 140 (1987)); Line e: survival rate of urokinase (this work).

little by little. If the glowth of the vesicle size is only due to the extension of the bilayer by the detergent molecules incorporated into the vesicle membrane, the size of the vesicle containing the detergent (Dx) can be simulated using the "effective" cross-sectional area of the detergent molecules (S_{OG}) incorporated. If the "effective" cross-sectional area of the detergent is assumed half that of phospholipid (Sp), by allowing for single chain of the detergent and double chain of the phospholipid, we get next equation;

$$Dx / Do = \sqrt{1 + R/2} \qquad (1)$$

where Do is the diameter of the detergent-free vesicle, R is molar ratio of detergent /phospholipid in membrane. R is a function of OG concentration in water phase (C_f) in equilibrium and obtained from an experiment of partition equilibrium of OG between water and membrane phases.[6] Thus Dx/Do is expected to be independent of Do and dependent on the detergent concentration in water. Figure 1 shows Dx/Do vs. C_f plots. Below 10 mM of OG in water phase, the theoretical line coincides with the experimental data within experimental errors. This means the vesicle size increases by the corresponding cross-sectional area of the detergent incorporated. Over 10 mM, the experimental data can not be described by a simple theoretical line (Fig. 1, solid line). In Fig. 2, solid lines (a, b, and c) give typical patterns of the experimental plots appeared in

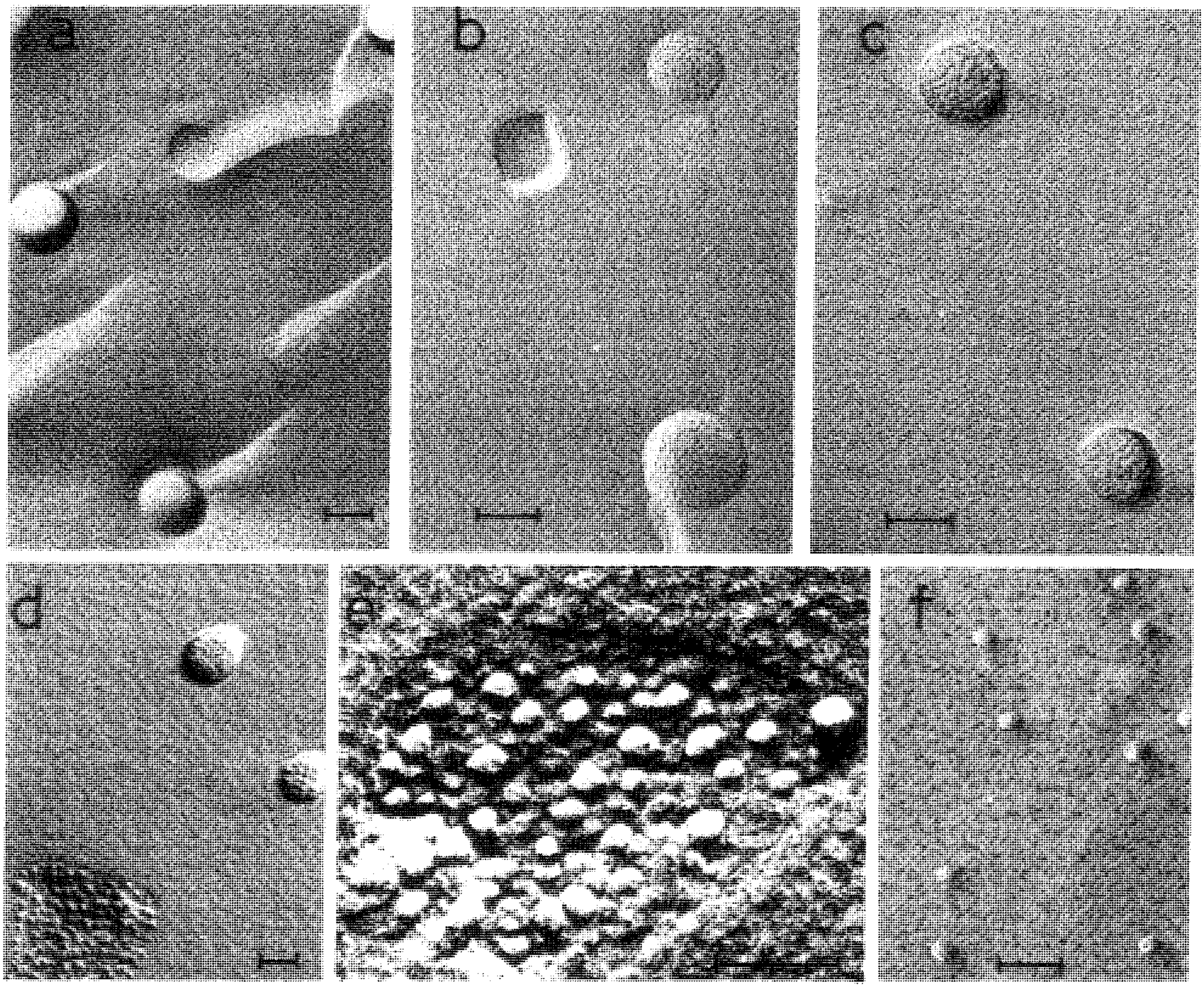

Fig. 3. Freez fracture electron micrograph. a: detergent-free vesicle; b: R = 0.3 (corresponding C_f = 3 mM); c: R = 2.2 (C_f = 14 mM); d,e,f: R = 2.6 (C_f = 16 mM). Each bar represents 100 nm.

Fig. 1. Line (a) shows that after the abrupt increase in diameter, the abrupt decrease takes place, resulting in mixed micelles. Line (b) shows that a temporizing decrease in diameter is followed by the abrupt increase in diameter, and after that the abrupt decrease in diameter comes about, resulting in mixed micelles. Line (c) shows that the vesicle is solubilized into mixed micelles without the abrupt increase in diameter. At the sufficient concentration of the phospholipid (above 7 mM), the behavior as (a) is likely to appear. On the other hand, at the low concentration (below 2.4 mM), the behavior as (c) is observed. These observations suggest that the abrupt increase in the apparent particle size observed during micelle to vesicle transition or vesicle to micelle transition is not essential but incidental in the transition. In Fig. 3, electron microscopic pictures in the range of transition of the vesicle to the mixed micelle are shown. The pictures suggest that the abrupt increase of apparent size observed is due to coagulation of small particles. The sizes of the small particles are 20-30 nm. It is interesting to note that the size is similar to that of the

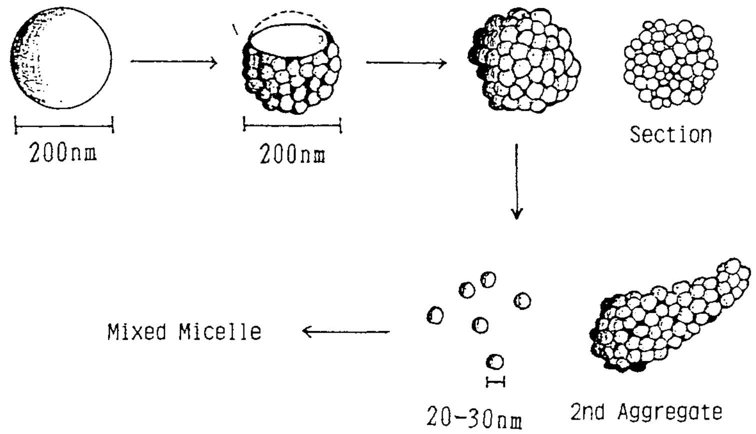

Fig. 4. Illustration of transition process of large unilamellar vesicle-to-mixed micelle by addition of detergents.

small unilamelar vesicle prepared by ultrasonication method. The appearance of small particles (small vesicles) in the transition range was originally predicted by Lichtenberg et al.[2] Before vesicle destruction, the surface of the vesicle becomes "rough" as increasing of the detergent, where the barrier efficincy of the membrane for a low molecular weight compound (d in Fig. 2) disappears but the ability holding a high molecular weight compound has been kept yet (e in Fig. 2) until 13 mM of the detergent in water in equilibrium. Over 15 mM of the detergent in water phase, small particles with 20-30 nm in diameter or their aggregates appear. At the same time, the barrier efficiency for a high molecular compound disappear, which indicates that the original vesicle had been destroyed.

The process of vesicle solubilization by addition of detergents can be described as follows. Stage 1; detergent incorporated in membrane distributes homogeneously through vesicle membrane and the increase of the vesicle size can be simulated by the corresponding cross-sectional area of the detergent incorporated. Stage 2; as increasing of the detergent, the convex and concave appear on the surface, but the vesicle structure holds yet, of which size is approximately simulated by Eq. 1. Stage 3; the convex and concave close to become small particle (suposing to be small vesicle). Stage 4; the small particles disperse (in the low concentration of the phospholipid) or make the 2nd aggregates (in the high concentration), whose forms are unsettled. Stage 5; the small particles (perhaps small vesicles) are solubilized into mixed micelles, most likely according to the mechanism shown by Ollivon et al.[3]

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(Received July 22, 1991)