Partition Behavior of a Nonionic Detergent, Octyl Glucoside, between Membrane and Water Phases, and Its Effect on Membrane Permeability†

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ABSTRACT: The partition equilibrium of an nonionic detergent, octyl glucoside, between the membrane phase and water and the effect of the detergent on the barrier efficiency of the vesicle membrane were studied. When the detergent concentration was lower than 4 mM in the water phase, or a mole fraction of 0.3 in the membrane phase, the partition coefficient of the detergent was independent of the detergent concentration and was 75 M^{-1}. This value was about twice the value predicted from the critical micelle concentration. In this concentration region, the permeability of Cl^- was relatively low [$(2–5) \times 10^{-10}$ cm/s]. When the detergent in the membrane phase exceeded a mole fraction of 0.3, the apparent partition coefficient decreased, and the permeability of Cl^- abruptly increased. These observations are explained by the following model: If the effective cross-sectional areas of phospholipid molecules and detergent molecules are similar to each other, a detergent molecule in the membrane phase will be surrounded only by phospholipid molecules as long as the mole fraction of the detergent in the membrane phase is below 0.3, and in this condition, the membrane barrier efficiency is high. At a mole fraction higher than 0.3, the detergent molecules come into contact with each other, and the membrane barrier efficiency decreases.

Since relatively homogeneous unilamellar vesicles can be prepared by the removal of detergent from phospholipid–detergent mixed micelles, the detergent removal method has been widely used for liposome preparation [e.g., see Brunner et al. (1976), Rhoden and Godin (1979), Allen et al. (1980), Zumbuehl and Weder (1981), Mimms et al. (1981), Nozaki et al. (1982), Schurtenberger et al. (1984, 1985), Almog et al. (1986), and Ueno et al. (1984, 1986, 1987, 1988)]. This method has been especially favorably applied to solubilization and reconstitution of functional membrane protein in phospholipid vesicles [e.g., see Mimms et al. (1981), Martin and Ueno (1985), and Kramer et al. (1986)]. In this case, it is essentially important to clarify the influence of detergent incorporated in the membrane phase on membrane properties.

It is well-known that there is a special region where the barrier efficiency of the vesicle membrane is abruptly reduced, which occurs at a far lower concentration of the detergent than that at which the vesicle is destroyed (Castellino et al., 1979; O'Conner et al., 1985; Ueno, 1987; Ruiz et al., 1988). In those studies, the overall concentration of detergent has been the only object of study with no reference to the behavior of the detergent incorporated into the membrane. It is more important to know the concentration of the detergent partitioned in the membrane phase than to know the overall concentration in order to clarify the action of detergent on membrane barrier efficiency. For this purpose, the study on the partition equilibrium of detergent between the membrane phase and the water phase should be most essential. In some cases, the partition coefficient of detergent between the membrane phase and the water phase has been indirectly estimated, with some asumptions, from the variation of vesicle size, depending on detergent concentration (Schurtenberger et al., 1984, 1985; Ollivon et al., 1988), or from the critical micelle concentration (cmc)[1] of the detergent in the water phase (Ueno et al., 1984; Ollivon et al., 1988). However, the apparent partition coefficient as a ratio of detergent concentration (not activity) in both phases is generally dependent on the concentration except in a very low concentration region. The indirect methods, in which the concentration dependence of the apparent partition coefficient has been neglected, should lead to the inaccurate results. There are few reports based on direct measurement of the partition equilibrium in a restricted concentration region (Ueno et al., 1984; Schurtenberger, 1985). In this report, we will clarify the partition behavior of a detergent, octyl glucoside, between membrane and water phases, extending over a wide concentration range, by the direct measurement of the detergent concentration in both phases. This way is far more simple and accurate than the indirect methods. On the basis of the results, we will discuss the relation between state of detergent in the vesicle membrane and its effect on membrane properties.

Experimental Procedures

Materials. L-α-Phosphatidylcholine from egg yolk was purchased from Green Cross Co. (no less than 99% purity). Octyl β-D-glucoside (OG) used as a detergent was purchased from Wako Chemicals. [^{14}C]Octyl glucoside and [^{3}H]dipalmitoylphosphatidylcholine were from New England Nuclear. All other reagents were of reagent grade from Wako Chemicals.

Methods. Detergent-free vesicles were prepared according to Mimms et al. (1981). Vesicle size was determined by quasi-elastic light scattering using Otsuka Electronics LPA 3000/3100. Cl ion permeability was determined by electrometric measurement of Cl^- efflux into $NaNO_3$ solution using an Ionalyzer (Orion Research, Model 701A) with a Cl ion selective solid membrane electrode. The apparent partition coefficient (concentration ratio) of detergent between the

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[1] Abbreviations: cmc, critical micelle concentration; OG, octyl glucoside; PC, phosphatidylcholine; TES, *N*-[tris(hydroxymethyl)-methyl]-2-aminoethanesulfonic acid.

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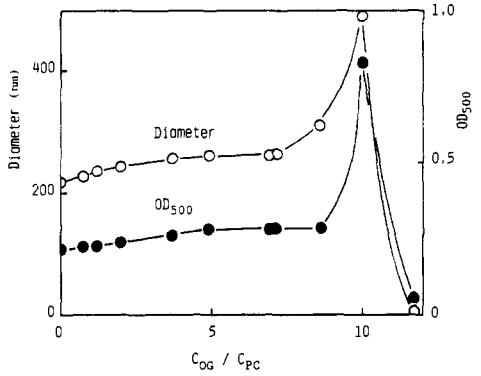

FIGURE 1: Diameter of vesicles or mixed micelles and optical density at 500 nm of the suspension as a function of molar ratio of octyl glucoside to phospholipid. Phospholipid concentration is 2 mM.

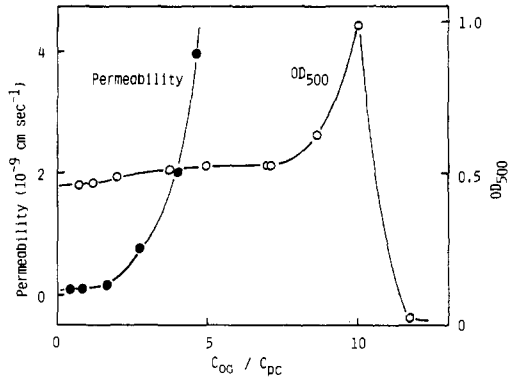

FIGURE 2: Permeability of Cl^- ion through the vesicle membrane and optical density at 500 nm of the vesicle suspension at 25 °C.

membrane phase and the water phase was directly measured by equilibrium dyalisis as follows: the vesicle suspension was incubated with an appropriate amount of detergent for 1 day using a dialysis cell composed of two compartments, separated by a dialysis membrane. Detergent concentration in both compartments and phospholipid concentration were measured by the radiotracer technique. The optical density at 500 nm (OD_{500}) of the vesicle suspension was measured as a index of the turbidity.

Detergent and phospholipid concentrations were determined by monitoring their radioactivities using an Aloka LSC-903 liquid scintillation system. The micro method of Bartlett (1959) was also sometimes adopted to determine phospholipid concentration. The phospholipid concentrations measured by both methods agreed well with each other in experimental accuracy (data not shown). The buffer solution used was usually composed of 250 mM NaCl/1 mM EDTA/20 mM TES (pH 7.0).

Results and Discussion

Vesicle Size and Turbidity. When a detergent is added to a phospholipid vesicle suspension, the detergent partitions into the membrane phase of the vesicle. With high concentrations of detergent, the vesicle is solubilized and forms mixed micelles with the detergent.

Figure 1 shows the relationship between detergent concentration and vesicle diameter or turbidity (optical density at 500 nm). As the detergent concentration increased, the OD and vesicle diameter slightly increased until C_{OG}/C_{PC} (molar ratio) reached about 8. At that point, apparent vesicle size and OD abruptly increased, which suggests the formation of aggregates of phospholipid other than vesicle or ordinary shaped mixed micelles. Above this concentration, apparent particle size and turbidity were abruptly reduced, showing the formation of mixed micelles.

Similar behavior has been observed in the process of detergent removal from phospholipid–detergent mixed micelles. As the detergent was removed from the mixed micelle, the apparent particle size and turbidity passed through a maximum and then decreased (Schurtenberger et al., 1984; Ueno et al., 1988).

Membrane Barrier Efficiency. Next, Cl^- permeability was measured to investigate the effect of detergent on the barrier efficiency of the vesicle membrane.

If ion permeation obeys first-order kinetics:

$$\ln (C_\infty - C) = \ln (C_\infty - C_0) - kt \qquad (1)$$

where k is the rate constant of Cl^- permeation, C is the measured concentration at time t, C_0 is the initial concentration, and C is the concentration at t infinity, that is, the concentration inside and outside at equilibrium. The permeability coefficient is obtained from the rate constant by use of

$$P(\text{membrane area/vesicle}) = k(\text{internal volume/vesicle}) \qquad (2)$$

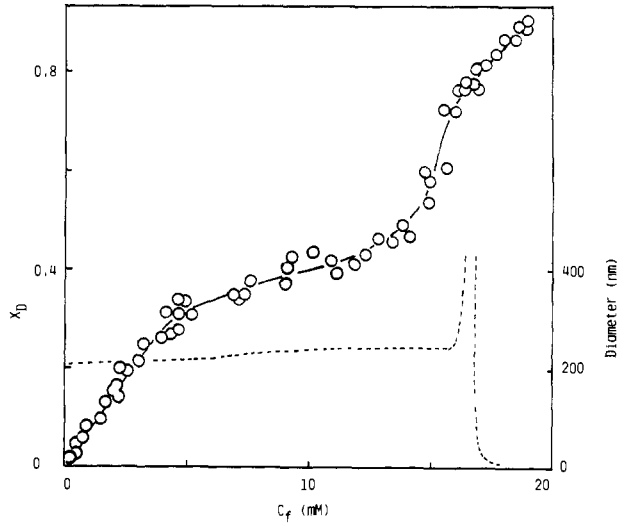

FIGURE 3: Partition equilibrium of octyl glucoside between the membrane phase and the water phase at 25 °C. The concentration of the detergent in the water phase (C_f) is represented in the molar concentration scale (mM) and that in the membrane phase (X_D) in the mole fraction scale. The phospholipid concentration is 2–4 mM. The dashed line represents vesicle or mixed micelle diameter.

Permeability coefficients and the turbidity at 500 nm of the suspension at 25 °C were plotted against the molar ratio of detergent to phospholipid (Figure 2). At a molar ratio of 10, the apparent turbidity became maximum. Following that, the turbidity was abruptly reduced, indicating that vesicle destruction or mixed micelle formation had occurred. At a far lower detergent concentration, there was a region in which Cl ion permeability increased abruptly, or the barrier efficiency of the vesicle membrane diminished.

Partition Equilibrium of Detergent between the Membrane Phase and the Water Phase. Since it is more important to know the detergent concentration in the membrane phase than the overall detergent concentration, we studied the partition equibrium of the detergent between membrane and water phases as shown in Figure 3. The ordinate represents the detergent concentration in mole fraction in the membrane phase in partition equilibrium, and the abscissa represents the detergent concentration in the water phase. The insert shows the diameter of the vesicle or the mixed micelle. In the low

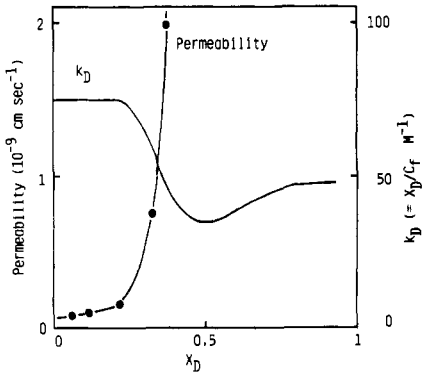

FIGURE 4: Permeability of Cl^- ion and apparent partition coefficient of the detergent (k_D) as a function of mole fraction of the detergent in the membrane phase.

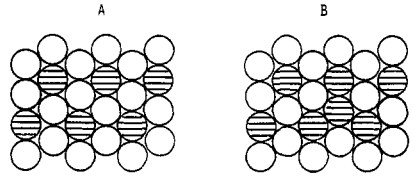

FIGURE 5: Model for molecular arrangement of the detergent and phospholipid in the membrane phase. (A) Detergent concentration in the membrane phase is below than 0.3 in the mole fraction scale. (B) Detergent concentration is greater than 0.3 in the mole fraction scale. (Hatched circles) Detergent molecule; (open circles) phospholipid molecule.

concentration region, this plot gives a linear relation. This slope corresponds to the inverse of the cmc if ideal mixing within the bilayer occurs and the detergent concentration in the water phase is below the cmc (Ueno et al., 1984; Tanford, 1980). The experimental result shows that the slope is 75 M^{-1} and about twice the predicted value (40 M^{-1}) from the cmc (25 mM at 25 °C) (Shinoda et al., 1959). This suggests that detergents are thermodynamically more stable when they are surrounded by phospholipid molecules than by detergent alone. The former state corresponds to the membrane phase composed of phospholipid containing a small amount of detergent and the latter state to the micellar phase in water composed of detergent alone. Above 0.3 mole fraction, the slope becomes lower, or the apparent partition coefficient decreases, while the only change observed in the vesicle is a slight increase in diameter, due to the incorporation of detergent into the membrane phase. As shown in Figure 4, the partition coefficient and permeability coefficient were plotted against the mole fraction of the detergent in the membrane phase. Abrupt lowering of the partition coefficient was found to be accompanied by an abrupt rise in permeability, or a lowering of barrier efficiency of the vesicle membrane.

Model of Molecular Arrangement of Detergent and Phospholipid in the Membrane Phase. Figure 5 shows a simple model of the closest packing of the molecules having the same cross-sectional area as each other. If the cross-sectional areas of phospholipid and detergent molecules are tentatively assumed to be similar to each other, then the detergent molecule partitioned in the membrane phase will be surrounded only by phospholipid molecules, as long as the mole fraction of the detergent in the membrane phase is below 0.3 (Figure 5A). At concentrations of detergent higher than 0.3, detergent molecules may come in contact with each other, according to the geometric viewpoint (Figure 5B). In other words, at a detergent concentration below 0.3, the partition coefficient would be independent of the detergent concentration, and the effect of detergent on the barrier efficiency of

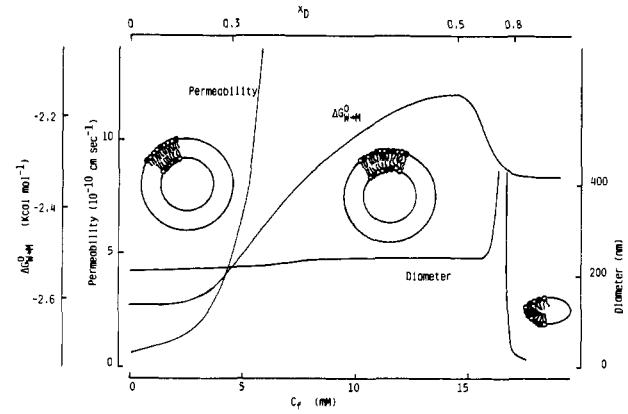

FIGURE 6: Transfer free energy of the detergent, Cl^- ion permeability through the vesicle membrane, and vesicle diameter as functions of detergent concentration in the water phase and the membrane phase, and corresponding schematic representations of the vesicles and mixed micelles.

the membrane would be relatively small, although the effect is dependent on detergent concentration; that is, the permeability coefficient ranged from 2×10^{-10} to 5×10^{-10} cm/s, which can be compared with that of unperturbed egg PC bilayer, 0.76×10^{-10} cm/s (Mimms et al., 1981) or 0.8×10^{-10} cm/s (Ueno et al., 1984). The values cited here are 5–10 times higher than the published value (Hauser et al., 1973). It should be noted that the permeability measurement of Hauser et al. (1973) was made at 4 °C, where permeability is severalfold lower than at room temperature. The cited values are comparable with the permeability at 20 °C obtained by Toyoshima and Thompson (1975). At a concentration of greater than 0.3, the partition coefficient could be expected to depend on the detergent concentration, because detergent molecules come into contact with each other by simple geometric requirement, and the barrier efficiency is reduced as the result. Experimental results support the propriety of this model.

Thermodynamic Parameters of Transfer of Detergent from the Water Phase to the Membrane Phase. Thermodynamic parameters of transfer of detergent from the water phase to the membrane phase were obtained from the temperature dependence of the partition coefficient in the low concentration region where the $X_D - C_f$ plot could be regarded as a linear relation (see Figure 3) and activity coefficients of detergent in both phases could be assumed to be unity. The results at 25 °C are as follows: transfer standard free energy $\Delta G°$ = −3 kcal mol^{-1}, enthalpy $\Delta H°$ = 3 kcal mol^{-1}, and entropy $\Delta S°$ = 20 cal deg^{-1} mol^{-1}. Positive transfer enthalpy ensures that as the temperature goes up, the detergent transfers from the water phase to the membrane phase.

Conclusion

The conclusion is summarized in Figure 6. When the detergent concentration is lower than 4 mM in water, or 0.3 of mole fraction in the membrane phase, the detergent molecules partitioned in the membrane phase are surrounded by phospholipid molecules and are not in contact with other detergent molecules. In this concentration region, the partition coefficient of detergent between the membrane phase and the water phase is independent of the detergent concentration; that is, the transfer free energy is constant, and the membrane barrier efficiency is rather high. When the detergent concentration in the membrane phase exceeds 0.3 in mole fraction scale, the transfer free energy becomes high even though the form and size of the vesicle do not change significantly, and the membrane barrier efficiency abruptly decreases due to the

contact of detegent molecules with each other. As the detergent concentration continues to increase, the transfer free energy increases until the vesicle is eventually destroyed.

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Rotational Motion of Yeast Cytochrome Oxidase in Phosphatidylcholine Complexes Studied by Saturation-Transfer Electron Spin Resonance

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ABSTRACT: Cytochrome oxidase from yeast has been covalently labeled with a nitroxide derivative of maleimide and reconstituted in lipid-substituted complexes with dimyristoyl-, dioleoyl-, or dielaidoylphosphatidylcholine. The rotational mobility of the enzyme in the complexes has been studied as a function of temperature and time, and of lipid/protein ratio, using saturation-transfer electron spin resonance spectroscopy. For complexes with dimyristoylphosphatidylcholine, the rotational mobility of the protein decreases abruptly below the gel-to-fluid-phase transition. This change is accompanied by a lateral segregation of the protein, as seen by freeze-fracture electron microscopy, and by an increase in the activation energy for the enzymatic activity. A time-dependent decrease in the rotational motion of the protein is observed on incubating at temperatures in the fluid phase of the lipid. This corresponds with a time-dependent loss of enzyme activity observed on incubation at temperatures in the fluid phase, but not at temperatures in the gel phase, over a period of 3 h. The rotational mobility decreases with increasing protein concentration in the complexes, both in the fluid and in the gel phases. The dependence of the protein mobility on lipid/protein ratio can be interpreted quantitatively in terms of the effect of increased random protein-protein contacts in the fluid phase. The maximum limiting rotational correlation time for the protein diffusion at high lipid/protein ratios in the fluid phase is $\tau_{R_l} \approx 25$ μs, suggesting that the protein is present as either a monomer or more probably a dimer in the reconstituted membrane.

Cytochrome *c* oxidase is the terminal member (complex IV) of the mitochondrial respiratory chain, being responsible for the transfer of electrons from cytochrome *c* to molecular oxygen, with concomitant proton pumping. The enzyme is a multisubunit integral protein situated in the inner mitochondrial membrane. The relative orientation and proximity of this enzyme complex to that of cytochrome *c* reductase, the preceding member (complex III) of the electron-transport chain, is a factor of considerable relevance to the function of

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