Membrane Fluidity of Egg Yolk Phosphatidylcholine/Detergent Mixed Aggregates Studied by Spin Label Method

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Anisotropic electron spin resonance (ESR) spectra of 5-doxylstearic acid and small membrane fluidity of hydrocarbon chains of egg yolk phosphatidylcholine (EggPC)/sodium taurocholate mixed micelles suggested considerably ordered arrangement of the hydrocarbon chains, while isotropic ESR spectra and relatively large fluidity of EggPC/octylglucoside mixed micelles indicated rather disordered arrangement of hydrocarbon chains of EggPC.

The spin label method has been frequently employed for obtaining membrane properties, such as fluidity, diffusion, flip-flop, partitioning of a spin probe between aqueous and lipid phase, and so on. On the other hand, the mechanism of transition from micelles to vesicles has been widely studied in close relationship to the functional reconstitution of membrane proteins after having been purified in detergent solution. The vesicle formation have been known to be symmetrical opposite to those of the vesicle destruction by detergent.[1] We have studied the micelle–vesicle transition mechanism in order to find out the size-determining factors of liposomes with the aid of electron micrography, particle sizes, and turbidities of detergent-containing mixed aggregates, partition coefficients of detergents between water and lipid, and so on.[2–5] Consequently, the destruction of egg yolk phosphatidylcholine (EggPC) large unilamellar vesicles (LUV) by a detergent, such as octylglucoside (OG) or sodium cholate, proceeded via three transition points with an increase in detergent concentrations: stage 1 is that of distribution of detergents into vesicles, resulting in size growth of the vesicles, stage 2 is of transition to small vesicles containing large amount of detergent (SUV*), stage 3 is of transformation to intermediate structures, and stage 4 is of formation of mixed micelles.[3–5] The present report deals with membrane fluidity of EggPC/sodium taurocholate (TC) and EggPC/OG mixed aggregates obtained by two spin probes, 5- and 16-doxylstearic acid (5- and 16-DS), which monitors the behavior of membranes near polar head groups and at the end of the hydrocarbon chains of EggPC, respectively. Especially, anisotropic electron spin resonance (ESR) spectra and small membrane fluidity of EggPC/TC micelles will be discussed in comparison with isotropic ESR spectra and large fluidity of EggPC/OG micelles.

Suspensions of EggPC LUV, prepared by extrusion (diameter: 200 nm) and then added by a known amount of a detergent, was mixed with a thin film of 5- or 16-DS at the probe to EggPC molar ratio of 1:100, and left for 1 d. Then, ESR measurement was performed with a JEOL JES-TE100 spectrometer (X band, 100 kHz modulation) equipped with a temperature control accessory at 25 °C. The microwave power, modulation amplitude, scan field, and scanning time were set at 12 mW, 0.4 mT, ±5 mT, and 4 min, respectively. The EggPC concentration was kept at 5 mM. The peaks of external manganese dioxide were used for determination of peak positions and intensities of the ESR signals. Rotational correlation time, τ_c, for isotropic ESR spectrum (16-DS) on the nanosecond time scale was calculated according to Cannon et al.[6]

$$\tau_c = 0.65 \times W_0[(h_0/h_{-1})^{1/2} - 1], \quad (1)$$

where W_0 is the maximum-to-minimum width of I (nuclear spin angular momentum) $= 0$ hyperfine splitting band, h_0 and h_{-1} are maximum-to-minimum heights of $I = 0$ and -1 hyperfine splitting bands, respectively. On the other hand, the τ_c value for anisotropic spectrum (5-DS) on the nanosecond time scale was calculated according to Freed et al.[7]

$$\tau_c = a \times (1 - A'_z/A_z)^b, \quad (2)$$

where A'_z is one-half the separation of the outer hyperfine extrema, and A_z is the rigid limit value of the same quantity (3.36 mT for DS). Constants $a = 0.54$ and $b = -1.36$ for the Brownian diffusion model[7] was employed for the calculation.

With increase in detergent concentrations, the phase transition from EggPC vesicles (stage 1) to SUV* (stage 2), then to intermediate structures (stage 3), and thereafter to mixed micelles (stage 4) proceeded. ESR spectra of 5-DS in (A) EggPC LUV in the absence of a detergent, EggPC/TC aggregates, and (B) EggPC/OG aggregates are illustrated in Figure 1. As can be seen from Figure 1, ESR spectra of detergent-free LUV and mixed vesicles containing small amount of detergent (stages 1 and 2) are anisotropic, irrespective of the kinds of detergents. At further increased detergent concentrations (stages 3 and 4), the spectra of EggPC/TC micelles still remain to be anisotropic, although outer hyperfine splitting constants decrease with increase in TC concentrations, whereas those of EggPC/OG micelles are isotropic. In other words, lowly fluid anisotropic bands change

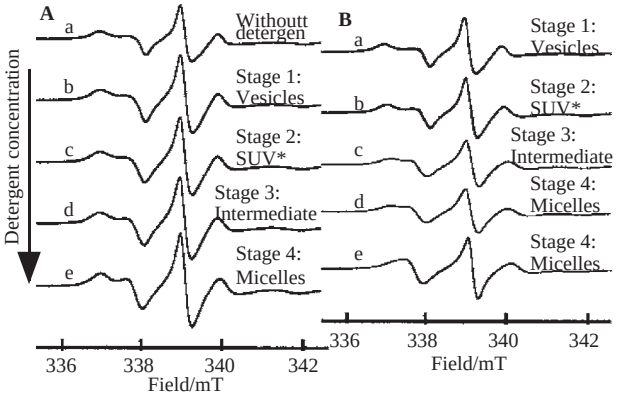

Figure 1. (A) ESR spectra of 5-DS in (a) EggPC LUV, EggPC aggregates containing TC of (b) 1.5, (c) 2.75, (d) 4.75, and (e) 7.0 mM. B) ESR spectra of 5-DS in EggPC aggregates containing OG of (a) 10, (b) 16, (c) 26, (d) 32, and (e) 36 mM at 25 °C.

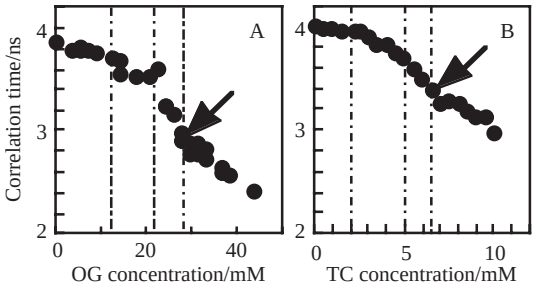

Figure 2. Dependence of rotational correlation times of 5-DS incorporated in (A) EggPC/OG and in (B) EggPC/TC mixed aggregates on detergent concentrations at 25 °C. Three vertical dashed lines represent three phase transition points during vesicle solubilization. Arrows indicate micelles containing minimal amount of detergents.

to more isotropic bands as OG concentrations in the micellar region increase. Although data are not shown, ESR spectra of 5-DS in EggPC/sodium cholate or EggPC/3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) micelles were anisotropic, while those of 5-DS in EggPC/octaethyleneglycol mono-*n*-dodecyl ether ($C_{12}C_8$) micelles were isotropic.

Figure 2 depicts the dependence of the τ_c values of 5-DS in (A) EggPC/OG and (B) EggPC/TC mixed aggregates on detergent concentrations. Three phase transition points, represented by dashed lines, were actually determined by size and optical density (turbidity) measurements, and electron micrographs of the detergent-containing aggregates. It can be seen from Figure 2 that the τ_c values decrease slowly up to OG concentration of 12 mM, become almost unchanged at $\tau_c = 3.5$ ns up to OG concentration of 22 mM, suggesting that a lamellar state is still maintained. At a concentration over 22 mM, the τ_c value drastically decreases, indicating the beginning of the destruction of the lamellar structures, i.e., vesicle solubilization. The destruction of the lamellar structure is completed at an OG concentration of 28 mM. In the case of EggPC/TC aggregates (Figure 2B), the τ_c values are nearly constant in the first stage, decrease slowly in the second stage. In the thirds stage (TC concentration from 5.0 to 6.5 mM), the τ_c value abruptly decreases, indicating the destruction of the lamellar structure. The destruction of lamellar structure is completed at a TC concentration of 6.5 mM. In both cases, the τ_c values inflect around phase transition points. Next, a larger τ_c value of 5-DS in EggPC micelles containing minimal amount of TC ($\tau_c = 3.4$ ns, the arrow in Figure 2B) than that in EggPC micelles containing minimal amount of OG ($\tau_c = 2.9$ ns, the arrow in Figure 2A) also suggests significantly smaller membrane fluidity of EggPC/TC micelles than that of EggPC/OG micelles. The τ_c values of 5-DS in TC- and OG-containing EggPC micelles further decrease with further increase in the concentration of micellar region.

ESR spectra of 16-DS dissolved in EggPC aggregates containing any amount of OG, $C_{12}E_8$, sodium cholate, CHAPS, or TC were characterized by isotropic triplet bands, having various h_0 to h_{-1} ratios. Plots of the τ_c values of 16-DS in (A) OG- and

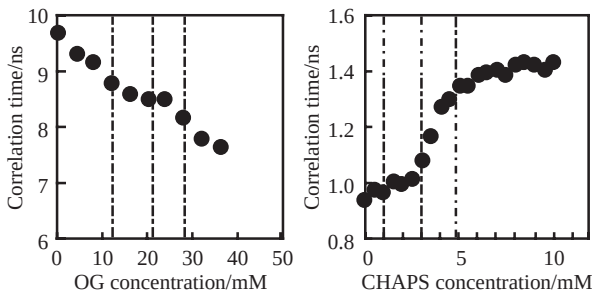

Figure 3. Plots of rotational correlation times of 16-DS incorporated in (A) EggPC/OG and (B) EggPC/CHAPS mixed aggregates against detergent concentrations at 25 °C.

(B) CHAPS-containing EggPC aggregates against detergent concentrations are shown in Figure 3. The τ_c values of EggPC/OG aggregates decrease, while those of EggPC/CHAPS aggregates increase with increase in detergent concentrations. Also in this case, the τ_c values inflect around phase transition points. Similarly, the τ_c values of $C_{12}E_8$-containing aggregates decreased, whereas those of sodium cholate- or TC-containing mixed aggregates increased with increase in detergent concentrations.

As can be seen from Figures 1 and 2, membrane fluidity of hydrocarbon chains of EggPC near the polar head group is much smaller than that at the end of the chains. The presence of three inflection points may suggest that the vesicle destruction mechanism (presence of three phase transition points) is common to all detergents examined. Anisotropic ESR spectra and small membrane fluidity of EggPC/TC micelles indicate more ordered arrangement of hydrocarbon chains compared to that of hydrocarbon chains in EggPC/OG micelles. Especially, the large τ_c values of 5-DS in EggPC/TC micelles and increase in τ_c values of 16-DS with an increase in CHAPS concentration may be caused by solidification of membranes due to the presence of steroidal skeleton in TC or CHAPS. Probably, difference between fluidity of EggPC/OG and that of EggPC/TC micelles containing minimal amount of detergent (arrows in Figure 2) may affect the properties of vesicles, metastable states, prepared on removal of the detergents or orientation of proteins reconstituted in the vesicles.

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