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Interaction between Liposomal Membrane and Sodium Dodecyl Sulfate as Studied by an ESR Spin Probe Method

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Interactions between phospholipid liposomal membranes and sodiumdodecyl sulfate in a phosphate buffer solution (50 mM, pH 7) were studied by the ESR spin probe, 2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-1,3-oxazolidin-3-oxyl. Sodium dodecyl sulfate molecules (2 mM) incorporated into phospholipid bilayers (13.6 mM) appeared to form clusters by themselves, causing phase separation of the bilayers. The possibility of two distinct liposomes, one phospholipid-rich and the other, SDS-rich, was excluded on the basis of results obtained by a centrifugal experiment under the above conditions.

Introduction

Liposomes are now being developed as drug carriers¹⁾. The interaction of surfactant molecules with liposomes has been extensively investigated with regard to the leakage of the contents encapsulated in the interior of liposomes. Also, liposomes are widely used as biomembrane models²⁾. One of the actions of anaesthetics on the biomembrane can be correlated with a change in the fluidity of the biomembrane³⁾. In order to clarify the action of anaesthetics, it is very important to investigate the fundamental interaction of anaesthetics with biomembranes and the influences of fluidity on them. It seems advantageous to use surfactants as anaesthetic models because their use enables the control of the amphiphilicity by changing their alkyl chain lengths. Hence, understanding of the interaction between model biomembranes and surfactants is important⁴⁾.

We will report, in the first phase of our experiments, that the influence of sodium dodecyl sulfate (SDS) on the fluidity of the phospholipid bilayers was investigated using a spin labelled stearic acid.

Experimental

Materials : L- β , γ -Dipalmitoyl- α -phosphatidylcholine (DPPC) was purchased from Fluka

AG. L- α -phosphatidylcholine from frozen egg yolk (type VII-E), 2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-1,3-oxazolidin-3-oxyl (5NS), 2-(10-carboxydecyl)-2-hexyl-4,4-dimethyl-1,3-oxazolidin-3-oxyl (12NS), and 2-(14-carboxytetradecyl)-2-ethyl-4,4-dimethyl-1,3-oxazolidin-3-oxyl (16NS) were obtained from the Sigma Chemical Company and used without further purification.

Preparation of Liposomes : Multilayered liposomes were prepared according to the method of Bangham *et al.*⁵⁾. Evaporating the mixture of 100 μ L of chloroform solution containing the phospholipid (13.6 μ mol) and 0.7 mL of ethanol solution containing a spin probe (0.14 μ mol) so that the mixture of the phospholipid and spin probe molecules dried as a thin film on the surface of the flask. One milliliter of the surfactant solution containing 50 mM phosphate buffer (pH 7) was poured into the flask. The final concentration of the phospholipid was 13.6 mM. The dispersion of phospholipid was carried out by shaking on a Vortex mixer at a temperature of about 30°C higher than the phase transition temperature of the phospholipid being used.

ESR Measurement : The molar ratio of spin probes to phospholipids was about 1 : 100. The liposome suspension was drawn up in a 1 mm o.d. capillary tube in the presence of air. ESR spectra were recorded at 50°C on

a Japan Electron Optics Laboratory Model JES-1 X spectrometer with 100 kHz-0.63 gauss modulation. The order parameter, designated as S , was estimated from the spectra. The quantity of S was defined by

$$S = 3/2 \times (A_{\parallel} - a_N) / (A_{zz} - (A_{xx} + A_{yy})/2) \quad (1)$$

where $A_{\parallel}$ is the parallel components of the hyperfine splitting. A_{xx} , A_{yy} , A_{zz} are assumed to be 6.3, 5.8, and 33.6 gauss, respectively⁶⁾. The value of a_N is defined by

$$a_N = (A_{xx} + A_{yy} + A_{zz})/3 \quad (2)$$

Therefore, the experimental value of $A_{\parallel}$ yields the S value.

Centrifugation: Aliquots of the dispersions were spun at 430 g for 10 min in a Hitachi 05 PR-22 rotor. The clear supernatant was removed by decantation and used in the ESR experiments.

Results and Discussion

Suspensions of DPPC liposomes and egg yolk lecithin liposomes containing 5 NS and various amounts of SDS were prepared. When the ESR spectra for DPPC liposomes were measured at room temperature, the obtained spectra were composed of superposed spectra provided by partitioning of 5 NS between the aqueous phase and the lipid phase of the liposomes. The former gave rise to three sharp resonance lines and the latter gave magnetically anisotropic and broad lines. In order to make the spectral analysis easier, these measurements were performed at 50°C, a temperature that is higher than that of the phase transition of DPPC and egg yolk lecithin liposomes. The spectra for those liposomes without SDS were the well known patterns as illustrated in **Figs.-1 (a)** and **2 (a)**. The S values for DPPC and egg yolk lecithin were 0.51 and 0.52, respectively. Since the examined temperature was well above the phase transition temperature, both calculated order parameters are very close to each other.

Under such a condition that the molar ratio of SDS to phospholipid is well below zero point five, the spectra obtained in the presence of SDS can be interpreted as a superposition of two different configurations of the 5 NS molecules. Namely, as shown in **Fig.-1 (c)**,

signal **A**, indicated by arrow **A**, comes from the rather extended form of 5 NS and signal **B** is due to a folded configuration⁶⁾. A folding of the 5 NS hydrocarbon chain means a fast rotational isomerization between the trans and gauche conformations indicating the circumstances around this probe are more fluid than those around the other probes with a signal **A**.

The interaction between Triton X-100 and phosphatidylcholine liposomes was studied by Anzai *et al.*,⁷⁾ they postulated from the analysis of the spectral change based on a spin-spin exchange broadening effect than, in some cases, heterogeneous mixing may occur on the liposomal membranes and that Triton-rich regions and phospholipid-rich regions are separated to some extent.

The spectra observed in this study directly show that the 5 NS molecules are incorporated at the two different levels; one is phospholipid-rich regions and the other is SDS-rich regions. The component with the smaller S value will correspond to a signal for 5 NS localized in the SDS-rich regions. The intensity for the component with the smaller S value became larger with increasing amounts of SDS as illustrated in **Fig.-1**. On the other hand, when the concentration of the liposomes was changed and the concentration of SDS was kept constant, the intensity of the component with the smaller S value increased with decreasing amounts of liposomes. (Data are not shown.) Such an observation is a similar relation to that described above. Under these conditions, the degree of the outer hyperfine splitting with the larger S value did not change regardless of the concentration of SDS added. Hence, SDS molecules incorporated into the phospholipid bilayers are supposed to form clusters made up of large portions of the SDS molecules.

Alternatively, one could imagine that there exists two types of liposomes; one is phospholipid-rich and the other SDS-rich. In order to exclude this possibility, we examined the additional ESR measurements. DPPC liposomes treated with 2 mM of SDS solution were fractionated by centrifugation at 430 × g for 10 min. The lower layer after centrifuging had a milky turbidity and almost all of the

Fig.-1 ESR spectra of 5 NS in the presence of DPPC liposomes in 50 mM phosphate buffer (pH 7) measured at 50°C. The concentration of DPPC was 13.6 μ mol/mL and that of SDS is shown in the figure. Arrow **A** indicates a signal originated from the component with the larger S value while arrow **B** indicates a signal from the component with the smaller S value. The order parameter, S , was calculated using the equation described in the text.

Fig.-2 ESR spectra of 5NS in the presence of egg yolk lecithin liposomes measured under the same conditions as described in **Fig.-1**.

liposomes were concentrated in this layer. The spectrum for this layer was similar to that before centrifuging as shown in **Fig.-3**. On the other hand, the appearance of the upper layer was almost transparent. This layer might contain rather small liposomes and/or SDS-phospholipid mixed micelles. However, the spectrum for the upper layer was the same as that before centrifuging. If such SDS-phospholipid mixed micelles are formed, the resonance lines observed should have been more isotropic. Therefore, SDS is presumed

Fig.-3 ESR spectra of fractionated liposome suspensions. Spectrum (a) was obtained with 2 mM SDS. Spectra (b) and (c) were obtained from the upper supernatant and the lower milky layer, respectively. The conditions for ESR measurements were the same as described in **Fig.-1**.

to act in a manner so that the intrasegregation may take place on the liposomal membranes. Increasing the concentration of SDS above 6 mM, the intensity of the outer peak A decreased and the isotropic resonance lines were observed at 20 mM of SDS, where the mixed micelles of SDS and phospholipid may be formed.

Furthermore, the ESR measurements were done using the other spin probes. A spin probe of 12 NS or 16 NS gave rise to three symmetric resonance lines and the rotational correlation time, τ_c , was calculated from the spectrum according to an equation described in the literature⁸). For egg yolk lecithin liposomes, the value of τ_c was 1×10^{-9} s for 12 NS and 4×10^{-10} s for 16 NS. In spite of adding 2 mM of SDS, the value of τ_c was unchanged. This means that close to the polar regions of the bilayer the motion of the spin probe is highly anisotropic and influenced by added SDS molecules, but becomes more isotropic toward the bilayer interior and independent in the presence of SDS.

The last question to be answered was whether a phase separation caused by SDS might result in the presence of 5 NS molecules themselves. SDS is an anionic surfactant and 5 NS is also anionic. In order to investigate the repulsion effect generated between the same charges of 5 NS and SDS, a fatty acid was used in place of SDS. The spectrum obtained is the well known pattern as illustrated in **Fig.-4**. No superposition was observed. Incorporated fatty acid mol=

Fig.-4 ESR spectrum of 5NS in the presence of DPPC liposomes with 2 mM sodium dodecanoate. The conditions for ESR measurements were the same as described in **Fig.-1**.

ecules may have a tendency to be evenly distributed in the bilayer. Thus, we speculated that the repulsion effect between 5 NS molecules and the anionic surfactant molecules is not responsible for the intrasegregation in the bilayer.

It can be concluded at this time that the interaction between phospholipid liposomes and SDS may cause the intrasegregation in the bilayer under such a condition that the molar ratio of SDS to phospholipid is well below zero point five and that the phase separation can be observed using a labelled stearic acid, 5 NS, which has a doxyl radical moiety close to the polar head group.

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ESR スピンプローブ法で調べられた リポソーム膜と SDS の相互作用

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50 mM リン酸緩衝液中におけるリン脂質リポソーム膜と SDS の相互作用が ESR スピンプローブ, 2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-1,3-oxylを用いて調べられた。リン脂質二分子膜内 (13.6 mM) に侵入した SDS 分子 (2 mM) はそれ自身でクラスターを形成し、二分子膜の相分離を引き起こすと思われる。このような条件下で、二種類のリポソーム、すなわちリン脂質に富むものと、SDS に富むものが別々に存在するという可能性は遠心分離による実験で除外された。

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