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A DYNAMICAL PROFILE OF METAL INCORPORATION TO A LIPOSOMAL MEMBRANE AS STUDIED BY ELECTRON SPIN RESONANCE

Yuji ISHIKAWA, Kunihiko TAJIMA, Kazuo MUKAI, Osamu OCHI, Kazuhiko ISHIZU,* and Hiromu SAKURAI†

Department of chemistry, Faculty of Science, Ehime University, Mastuyama, Ehime 790
†Faculty of Pharmaceutical Science, University of Tokushima, Tokushima 770

An Egg-Yolk lecithin liposome containing the surfacant cyclic polyamine was prepared, and a dynamical behavior of its Cu(II) ion uptake was studied by monitoring a time dependent decay of the isotropic copper nucleus hyperfine splitting in the ESR upon metal incorporation into a liposomal membrane. The reaction kinetics studied by ESR justified the fact that there are the two kinds of metal binding processes, in which the distinctive time course and the activation energy can be clearly demonstrated.

A liposomal membrane obtained from egg-phosphatidylcholine has often been utilized as a model of the biogenic membrane and enzyme. Several surface active metallo-porphyrin were recently synthesized, further, the role of photosensitized electron transport[1] and oxygen-binding affinity[2] were investigated in the vesicles incorporating these surfacant metal complexes as a model active site. On the other hand, the kinetics for some transition metal ion interacting with phospholipid vesicles have been studied by application of a spin-label assay for metal ion chelation and complex formation.[3] To our present knowledgement, however, a little information has been reported on the dynamical behavior of metal ion chelating to the ligand, which is included in the liposomal system as the metal coordination site. In the view of a spin probe technique, a divalent copper complex can be utilized to distinguish the paramagnetic species capsulated, from these existing in the out side of the membrane, since a slower molecular motion of the encapsulated molecule results an enhanced anisotropy in the observed ESR hyperfine structures. In the present paper, a kinetic ESR for copper ion incorporation was investigated by using a liposomal membrane including the surfacant cyclic polyamine.

The surface-active cyclic polyamine such as 3-undecyl-2,4-dioxo-1,5,8,12-tetraazacyclotetradecane (DD14N4), (Fig. 1), was obtained by condensation reaction between the alkylated malonate and 1,3-N,N'-bis-(2-aminoethyl)-1,3-propanediamine.[4] The crude products were purified on alumina column chromatography, and the purity was checked by elemental analysis; Found: C, 66.34; H, 11.44; N, 13.89%. Calcd for $C_{22}H_{48}N_4O_2$: C, 66.62; H, 11.18; N, 14.18%.

The liposome was prepared in the following processes; the chloroform solution of the ligand (5 mM/1 ml) and of egg-yolk lecithin[5] (0.5 mM/2 ml) were evapolated in vacuo. The tris-buffer solution (0.2 M/2 ml, pH 8.0) was added to these and the mixture was ultrasonicated for 30 min in ice bath. The resultant was examined by gel permeation on Sephadex column (Sephadex -G75 superfine). The single compartment liposome was fractionated from the multilayered one by monitoring with

the wavelength of 660 nm. The particle size was determined by an electronmicroscope distributed in the diameter from 200-250 nm. ESR spectra were measured by a JEOL FE2XG X-band spectrometer with 100 kHz magnetic field modulation at room temperature and liquid nitrogen temperature (77 K). The magnetic field was calibrated by the splitting of Mn(II) in MgO (Δ_{3-4} = 8.69 mT) and g-values were standardised using Li-TCNQ (g = 2.00252) as a reference.

The total concentration of the ligand in the liposome (10^{-4} M) was determined by a photometric titration with reference to the characteristic d-d band absorption of the monomeric Cu(II)-DD14N4 1:1 complex (λ_{max} = 507 nm, ε = 100). A time resolved ESR measurement for the metal incorporation was carried out at the room temperature with mixing the copper solution and the liposome suspension as to adjust in the equimolar ligand/metal ion ratio. At an early stage of mixing (1 min), the ESR mainly observed is isotropic copper hyperfine structure with the average g-factor and the copper nucleus hyperfine splitting; (g_0 = 2.100, $|A_0|$ = 101 x 10^{-4} cm^{-1}). At the same time, a part of the anisotropic copper nucleus hyperfine splitting (B) overlapped together with the isotropic ESR absorption can be also detected at the higher magnetic field as indicated in Fig. 2. The g_0 and A_0 values of (A) are identical with those of the reference prepared by mixing the copper ion and the liposome in the same buffer without addition of the surfacant ligand. The ESR hyperfine component (A) in Fig. 2 thus can be safely assigned to the copper complex, which exists in the outside sphare of the membrane, and undergoes a free tumbling molecular motion. With standing for 1 min, a magnitude of the isotropic hyperfine componet (A) begins to decrease, on the other hand, that for the anisotropic hyperfine component (B) starts to increase. After 30 min, the well defined anisotropic hyperfine component (B) can be seen in place of the isotropic hyperfine component (A). The ESR parameters determined are $g_{//}$ = 2.143, $g_{\perp}$ = 2.051 and $|A_{//}|$ = 199 x $10^{-4}cm^{-1}$, respectively. These values are nearly identical with those of the Cu(II)-DD14N4 complex measured in a frozen matrix at 77 K; $g_{//}$ = 2.163, $g_{\perp}$ = 2.057 and $|A_{//}|$ = 214 x $10^{-4}cm^{-1}$. Based on the similarity seen in both cases, the anisotropic

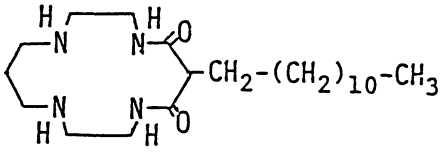

Fig. 1. Chemical structure of DD14N4.

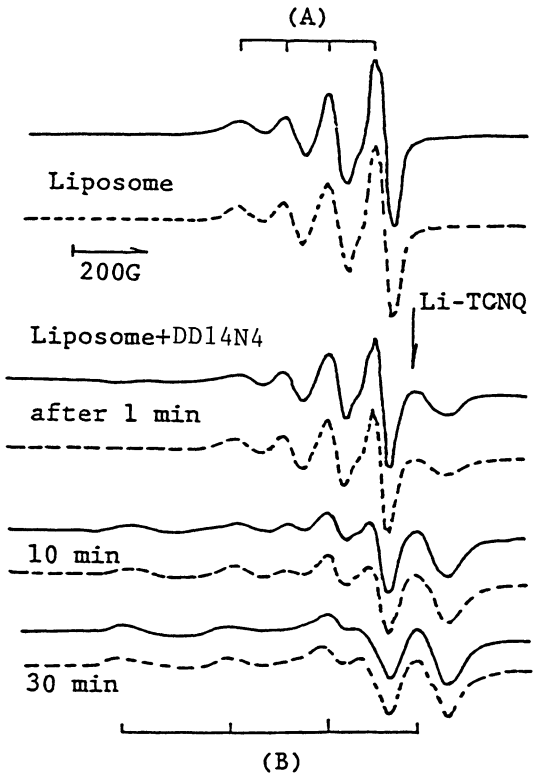

Fig.2. The time dependent ESR of the metal incorporation in the liposome suspension. The $CuSO_4$ (0.01 M/10 µl) was mixed with the liposome (0.5 mM) suspended in the tris-buffer solution (0.2 M/1 ml) at pH=8.0. After mixing, the ESR spectra were immediately recorded at room temperature (22°C). The dotted line is a computer superposition of (A) and (B).

hyperfine component is assigned to the Cu(II) complex encapsulated in the liposome matrix, where a molecular motion of the copper complex is largely restricted even at the room temperature. A small discrepancy of the ESR parameters seen in both cases would be attributed to a partial fluctuating movement of the encapsulated molecule in the liposome matrix.

A decay of the hyperfine component (A) is taken as the parameter to measure the metal incorporation and a time course for the decay was studied by assuming the pseudo-first order reaction kinetics, where the line intensity (I) of the isotropic hyperfine component (A) at any observation time (t) was estimated by a computer superposition of both the isotropic hyperfine component (A) and the anisotropic hyperfine component (B). As shown in Fig. 3-a the logarithmic decay of the ESR intensity (I) can be treated as a summation of the two independent linear relations in terms of the distinctive reaction constants k_1 and k_2. The observed relationship between the ESR intensity (I) and time course, (t) thus can be expressed written as;

$$I = \alpha \cdot e^{-k_1 t} + \beta \cdot e^{-k_2 t}$$

The computer simulation obtained after a trial and error calculation gives the best fit with the observed using the following parameters determined at 15 °C; $\alpha/\beta = 2$, $k_1 = 4.06 \times 10^{-2}\ s^{-1}$ and $k_2 = 4.87 \times 10^{-3}\ s^{-1}$ (Fig. 3-b). The temperature dependence on k_1 and k_2 was studied in the range from 273 to 303 K and the activation energy (E_α, E_β) for the metal incorporation in each α and β site was estimated to be 14.6 and 69 kJ/mol, respectively (Fig. 4).

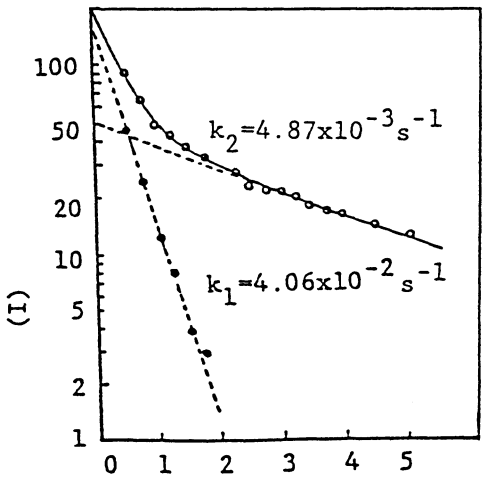

Fig.3-a. Time/ min

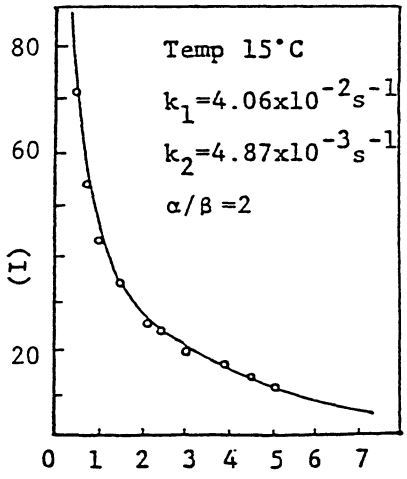

Fig.3-b. Time/min

Fig.3-a. The logarithmic plots of the ESR intensity (I) as a function of the observating time course.
Fig.3-b. The relation between the observed ESR intensity (I) and the reaction time interval (t). The open circle denotes the observed plots and the solid line is the computer simulated.

The α site as characterized by the lower activation energy could be assigned to those distributed on the surface of the outer phase of the liposome. We cannot uniquely specify the structure of the β site, which would be capsulated in the lipid layer of the liposome. Nevertheless, it is interesting to note that the difference of the activation energy $E_\alpha - E_\beta$ is fairly close to the energy barrier reported for a transverse diffusion (flip-flop) of the phospholipid[6] in a bilayer membrane. A datailed investigation is now in progress.

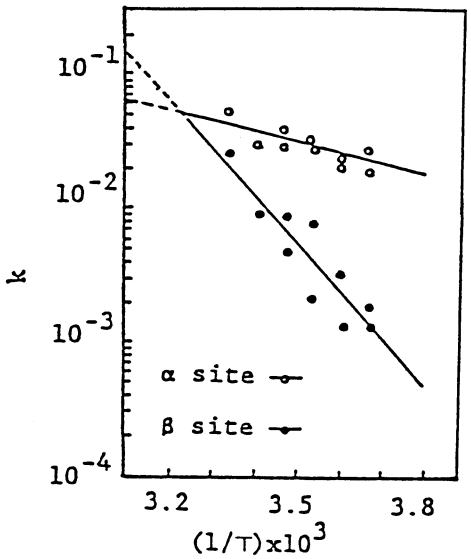

Fig.4. Arrhenius plots for the metal incorporation.
E_α=14.6kJ/mol, E_β=69.0kJ/mol.

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