Permeation Control of Polymerized Liposome with Various Surfactants

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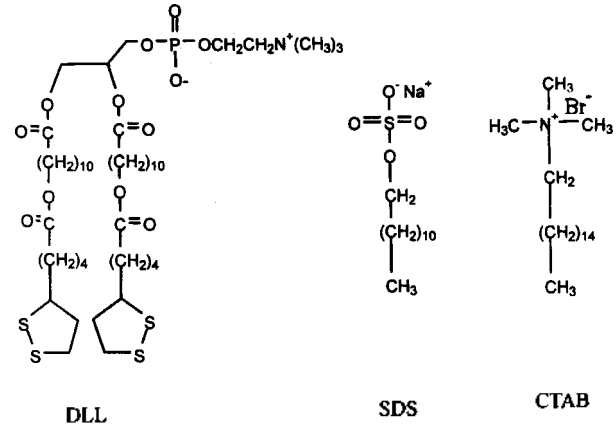
After all the continuous efforts of clarifying the bilayer properties of our polymerized liposome (PL), it was concluded that the PL had encapsulated the relatively big fluorescence markers well, and released them quite slowly enough for drug delivery. It was already reported that the PL was able to encapsulate the small-sized carboxyfluorescein (CF) in the presence of DPPC (Dipalmitoyl phosphatidylcholine) and DMPC (Dimyristoyl phosphatidylcholine) at a critical ratio of DLL : DPPC : DMPC=2 : 1 : 1.[1] All of the results found out, so far, intrigued us to modify the tightness of the membrane by combining either single or double chain surfactants.

This type of strategy reminded us of the Nylon capsule membrane coated with synthetic amphiphile, in which the permeability could be controlled by the phase transition of the corking surfactants.[2] Meanwhile, numerous reports have been published in the field of PL together with its application.[3] In this report, we present quite unexpected results by incorporating surfactants which are generally known to disrupt the bilayer membrane. The monomer of the PL was 1,2-bis[12-(lipoyloxy)dodecanoyl]-sn-glycero-3-phosphorylcholine (DLL),[4,5] and was available from the previous experiment.[1]

Actually, the disruptable surfactants were added to check their effects on the stability and permeability of the PL containing encapsulated fluorescence markers. The PL was stable and the permeation rate was not affected by the presence of surfactants. Instead, these results suggested that the PL could be fortified by some of the surfactants.

Different forms of surfactants in the number of chains and the types of charge were incorporated in the experiment. One of the surfactants such as SDS (Sodium Dodecylsulfate), CTAB (Cetyltrimethylammonium Bromide), PA (Palmitic Acid), Triton X-100, DHAB (Dihexadecylammonium Bromide), DCP (Dicetyl Phosphate), and Cholesterol was covesiclized with the DLL in various mole ratios (5, 10, 20, 30, 50%). From the gel-filtration profiles, it was found out that the encapsulation of small molecules like carboxyfluorescein was possible only in the presence of surfactants at a particular ratio, although CF was freely leaking through the PL of DLL alone. As for the encapsulation and release of CF out of the DLL liposome, look at the stylized view in Figure 1.

Typically, 3 mg of DLL and a designated mol% of surfactant in choloroform were mixed to form a clear solution, and dried to a dry lipid film. 1 mL of 1 mM carboxyfluorescein in pH=6.4 0.02 M phosphate buffer was added, and dispersed to make a turbid mixture by freeze-thawing and vortex mixing (x5). pH of the turbid MLV (Multilamellar Vesicle) solution was adjusted to 8.4 by 0.3 M NaOH, and the solution was then polymerized with cysteine (5 mol% of DLL) as an initiator by shaking overnight



Structure 1.

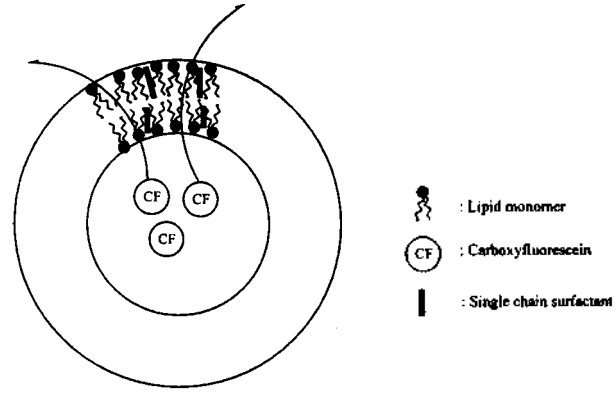


Figure 1. Release of the encapsulated carboxyfluorescein from the liposome.

at room temperature. The pH was reduced back to 6.4 after the polymerization was over. The whole mixture was gel-filtered through Sephadex G-50 column (1.5×40 cm) to remove the unencapsulated CF, and the gel-filtration results from the various surfactant/DLL mixtures were listed in Table 1. The representative gel-filtration profile of SDS/DLL combination is shown in Figure 2. Fractions with encapsulated CF were collected, and dialyzed against pH=6.4 buffer at 45 °C. The release kinetics were followed by measuring the fluorescence intensity of the periodically taken samples as shown in the permeation plot of SDS/DLL combination (see Figure 3). The permeation data are also included in Table 1, and the permeation of CF is not so variant for all the surfactant/DLL combinations.[6]

Mostly, the optimum ratios of surfactant/DLL were in the range of 5-10 mol% except cholesterol which required more than 50%. At the ratios out of this range, the encapsulation was very poor. If the surfactant/DLL ratio was raised over

Table 1. Gel-Filtration and Permeation Results of Surfactants/DLL Mixtures

Surfactant[a]	Mol%	Encapsulation[b]	10^9P[c] (cm/hr)
SDS	10	H	83
Triton X-100	5	M	83
CTAB	10	M	50
PA	A[d]	NE	-
DHAB	10	M	50
DCP	A[d]	NE	-
Cholesterol	50	H	42

[a] SDS: Sodium dodecylsulfate, CTAB: Cetyltrimethylammonium Bromide, PA: Palmitic acid, DHAB: Dihexadecylammonium Bromide, DCP: Dicetylphosphate. [b] H: Highly encapsulated, M: Moderately encapsulated, NE: No encapsulation. [c] Permeation coefficient was measured at 45 °C, and permeation coefficient was an average of three independent experiments. [d] A=5, 10, 20, 30 mol%.

50%, the surfactant micellar solution had dissolved away the DLL monomers, so that the solution became clear immediately. In general, the surfactant/DLL ratio should be less than 10% to get some encapsulation.

At the optimum ratio of surfactant/DLL, the lipid solution with CF was extruded through 0.4, 0.2, 0.1 μm PC membrane 5 times each at 45 °C to form an LUV (Large Unilamellar Vesicle) solution, and then polymerized by the same procedure as above. The polymerized LUV solution

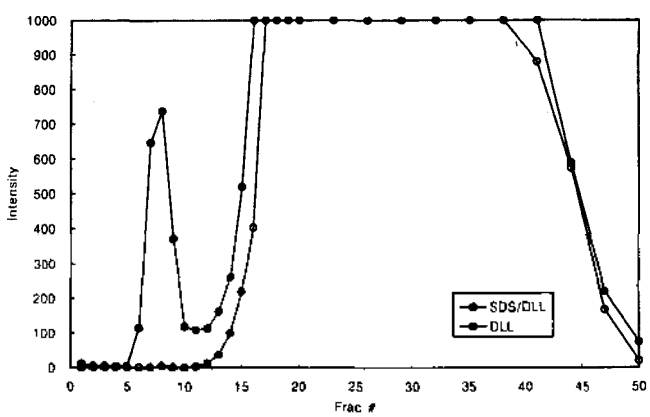


Figure 2. Gel-filtration Profile of CF Encapsuated with SDS/DLL.

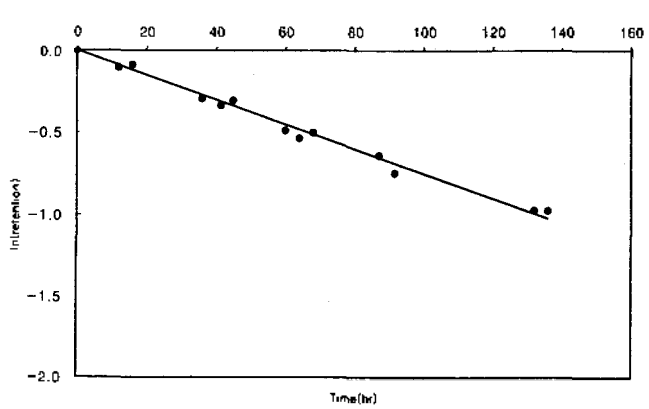


Figure 3. CF Release from SDS/DLL.

was also gel-filtered to determine the percentage of encapsulated CF. Compared with MLV solution, LUV was not so effective in inducing encapsulation. For all of the surfactant/DLL mixtures, the amount of encapsulation was much less than that of MLV. The permeation of CF from the LUV also showed different style of release profile from MLV. For example, the release of CF from the LUV of CTAB/DLL did not show slow and gradual decrease, but the fluctuation of release profile proposed the possibility of the bilayer destabilization in the presence of surfactants. Probably LUV's bilayer was thoroughly affected by the surfactants so that the encapsulated CF leaked out unexpectedly. For cholesterol, some of the undissolved cholesterol had clogged the PC membrane, and accordingly, the extrusion process was not feasible.

The fully polymerized DLL had offered enough stability to liposome structure, but the membrane tightness might be hampered due to the rigid structure. To overcome this shortcoming, the partially polymerized DLL, which could form tighter bilayer due to the flexible unpolymerized part of DLL, was prepared by reducing the duration of polymerization to 1/2 and 1 hr which were much less than 4 hr necessary for complete polymerization. Unpolymerized lipid monomer could be detected by TLC and UV absorbance (330 nm). The partially polymerized liposome also did not show any significant encapsulation of CF, as the fully polymerized one did not.

As the polymerized DLL failed to assemble a tight bilayer membrane presumably because of the bent from the carboxyl group in the middle of the chain and the crosslinking at the chain ends, CF encapsulation was possible only with the aid of DPPC and DMPC. As per the above results, the role of phospholipid could be replaced with simple surfactants irrespective of their potential membrane disruption. The effect of the charge was not significant and, instead, the hydrophobicity of the chain seemed to be more crucial factor for the tighter membrane packing. Especially, SDS and cholesterol were outstanding candidates for filling-up the bilayer of PL, and the negative charge of SDS might have given additional interaction between the choline moiety of DLL and SDS.

Once the leaky DLL was sealed with surfactant, the permeation rate of CF was not much dependant on surfactants. The permeation coefficient was about one hundred times smaller than that of DLL/DPPC/DMPC mixture, and this was resonablely due to the permeation from MLV, not from LUV. There are still more rooms for fine-tunning the permeability of the polymerized liposome.

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First Observation of Metal-Mediated Interligand Tautomerism. Cobalt(III) Complexes Containing Mixed Pyridine-2-thiol and Pyridine-2-thione Ligands

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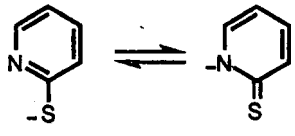
This work stems from our interest on the unprecedented molecular nonrigidity directed toward metal-mediated interligand tautomerism between mixed-tautomeric ligands in metal complexes. Tautomeric equilibria have often been used to deduce general structure-stability relationships,[1,2] and the results have been extended beyond the initial studies to such diverse areas as quantum mechanical calculations,[3] molecular switches,[4] tautomeric catalysts,[5] and theories of genetic mutation.[1] Many important biological molecules have been found to exist as tautomers and their chemistry is of considerable interest. For instance, the tautomeric equilibria of purine and pyrimidine bases have been suggested to affect RNA transcription and DNA replication in biological systems,[1] behaviors of nucleic acids,[6,7] and *etc.* In particular, thio-bases such as thiouracil and thiocytosine that can be tautomerized are normal constituents of some *t*-RNA species.[8,9] Among such tautomeric systems, the simplest example is pyridine-2-thiol and its analogs, which are capable of chelating to metals as either pyridine-2-thiol (A, thiol) or pyridine-2-thione (B, thion).[10,11] The coordination



A B

modes of the ligand are directed by various factors such as the basicity of the central metal, the overall charge of the complex, and medium effects *etc.*[12-20] Some metal complexes of the ligands have been structurally elucidated in the solid state, but their solution-behaviors are not evaluated unambiguously due to surprisingly delicate NMR spectra in solution.[21-24] In this communication, we describe a preliminary investigation of a unique molecular fluxionality in cobalt(III) complexes containing mixed-tautomeric thiol-thion ligands.

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The present synthetic procedure[25] of the title complexes afforded the same *mer*-Co(N-S)$_3$ isomer (N-S=pyridine-2-thio (PyS); pyrimidine-2-thio (PymS)[26]) as the literature procedures.[22,24] Intricate signals of ^{1}H NMR ($CDCl_3$) result from three non-equivalent ligands in Co(PyS)$_3$, indicating that the *mer* isomer is still retained without any geometrical isomerism accompanied by a bond-rupture in the solution. Among the signals, in particular, two broad resonances at 8.28 ppm and 7.09 ppm in a 2 : 1 integral ratio are notable and somewhat illegible. From ^{1}H/^{13}C 2D-heteronuclear spectrum of Co(PyS)$_3$ (Figure 1), the two signals are clearly characterized as the protons of ring carbons adjacent to nitrogen atoms of PyS ligands along with the assignment of

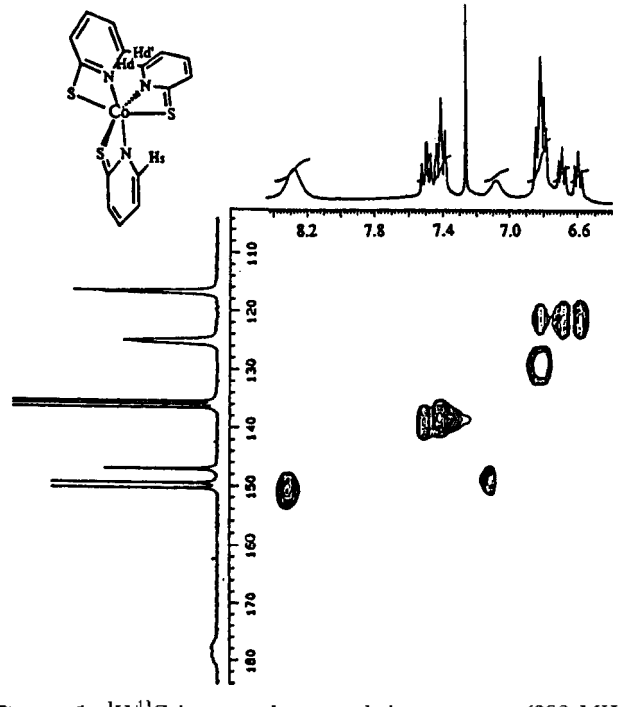

Figure 1. ^{1}H/^{13}C heteronuclear correlation spectrum (300 MHz, $CDCl_3$) of Co(PyS)$_3$.