Selective Uptake of Copper(II) and Zinc(II) Ions Using Liposomes without Ionophores

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The multilamellar vesicles (MLVs) without ionophores were applied to the separation media of Cu^{2+} and Zn^{2+}. Mn^{2+}, Fe^{2+}, Co^{2+} and Ni^{2+} were impermeable into the MLVs. The uptake rate and efficiency of Cu^{2+} were dependent on the charge-type of the MLVs surface, while the effect of copper salts on the uptake rate and efficiency was little observed.

Liposomes prepared from phospholipids are vesicular lipid bilayers that close a volume of aqueous solution. Liposomes are hardly permeable to metal ions, since phospholipids which have markedly hydrophobic chains prevent the passage of water-soluble materials through membranes. Ionophores such as valinomycin are used for transporting alkali and alkaline earth metal ions across lipid barriers including artificial and biological membranes.[1] On the other hand, Oku *et al.* measured the permeation of Cu^{2+} into lipid bilayer membranes in the absence of ionophores by the fluorometric method.[2] However, there are no reports on the uptake of other transition metal ions by liposomes and the application of liposomes to the separation media of transition metal ions.

In the course of our studies on the uptake of first transition metal ions by multilamellar vesicles (MLVs) without ionophores, we have found that Cu^{2+} and Zn^{2+} permeated selectively into the inner phase of the MLVs, and those metal ions were trapped in the MLVs without leakage through the lipid membranes. The MLVs can thus be applied to the separation media for Cu^{2+} and Zn^{2+}.

Egg yolk phosphatidylcholine (PC), cholesterol (Chol) and stearylamine (SA) were obtained from Wako Pure Chemicals. Dicetylphosphate (DCP) was purchased from Sigma Chemical Co. Calcein (3,3'-bis[N,N-bis(carboxymethyl)aminomethyl] fluorescein) was bought from Dojindo Laboratories. The concentration of PC was calculated by use of a molecular weight of 765.[3] A 1.0×10^{-2} M (1 M = 1 mol dm^{-3}) solution of metal ion was prepared by dissolving metal salt: $MnCl_2$, $Fe(NH_4)_2(SO_4)_2$, $CoCl_2$, $NiCl_2$, $CuCl_2$ and $ZnCl_2$.

Different charge-types of MLVs were prepared using standard methods.[4] The preparation of anionic MLVs is as follows: a mixture (20 μ mol PC, 20 μ mol Chol, 2 μ mol DCP) in chloroform was added to a 100-ml round-bottom flask. Chloroform was removed by rotary evaporation at 30 °C under reduced pressure and a stream of nitrogen gas forming a lipid film on the wall of the flask. After at least 1 h *in vacuo* , a 0.9 ml portion of 1.0×10^{-2} M 3-(N-morpholino)propanesulfonic acid (Mops) buffer solution (pH 7.2) was added into the flask and whole contents were extensively mixed on a Vortex stirrer for 15 min at 25 °C.

Cationic MLVs were prepared according to the procedude for anionic MLVs, except that SA (20 μ mol in chloroform) was employed in place of DCP. Zwitterionic MLVs were prepared by use of lipid mixture of PC (22 μmol) and Chol (20 μ mol) in chloroform.

The uptake procedure of metal ion with MLVs consisted in pipetting a 0.9 ml MLVs suspension and a 0.1 ml of 1.0×10^{-2} M metal ion solution into a glass cuvette. The mixture was shaken for 1 h by a water bath incuvator at 25 °C. Next, the separation of metal ion encapsulated in the MLVs and free metal ion was performed on a Sephadex G-50 column (column size, 10x300 mm). The column was equilibrated with 10 mM Mops buffer (pH 7.2), and the flow rate was 18 ml/h. A 275 μ l portion of the mixture was applied to the column. The amounts of the MLVs and metal ion eluted from the column were determined by measuring phosphorus and metal ion in each fraction tube by an inductively coupled plasma atomic emission spectrometer (ICP-AES) with an ultrasonic neblizer.[5]

The fluorescence (FL) intensity of calcein in the MLVs suspension was measured as follows: different charge-types of the calcein-trapped MLVs were prepared according to the procedure, except that a 1.0 ml portion of 5.0×10^{-8} M calcein solution was added into the flask in place of the buffer solution in the swelling of the lipid film. A 50 μ l portion of the MLVs suspension was diluted with a 2.0 ml portion of the buffer solution in a 1-cm quartz cell. Next, a 5 μl portion of 1.0×10^{-2} M Cu^{2+} or Co^{2+} solution was injected into the cell. Vigorous agitation by a magnetic stirrer was continued during the FL measurement. The exitation and emission wavelength were set at 490 and 513 nm, respectively.

First, we carried out the uptake of the metal ions with the anionic MLVs. A typical elution profile of Cu^{2+} is shown in Figure 1. Two peaks appeared in the elution profile of Cu^{2+}. On the other hand, one peak was observed in the elution profile of phosphorus. By comparison of two elution profiles, first and second peaks in the elution profile of Cu^{2+} are ascribed to the elution of the MLVs containing Cu^{2+} and free Cu^{2+}, respectively.

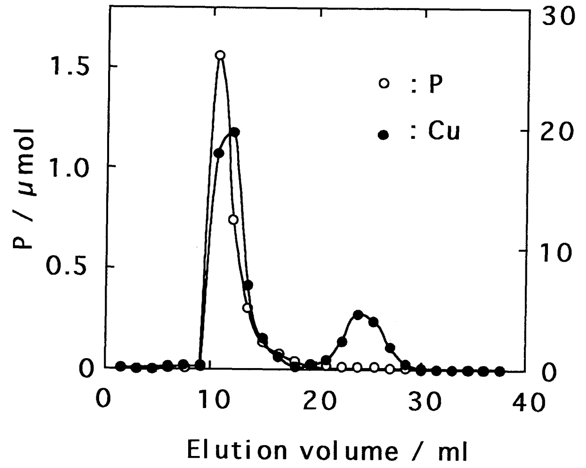

Figure 1. Separation of the MLVs containing Cu^{2+} from free Cu^{2+}.
Mobile phase : 10mM Mops buffer (pH 7.2),
Column : Sephadex G-50 (10×300mm),
MLVs suspensions : 275μl.

As shown in Figure 1, Cu^{2+} was encapsulated in the MLVs without leakage through the lipid membranes during gel-chromatography. The uptake efficiency is referred to as the mole ratio of metal ion trapped in the MLVs to total mole of metal ion in the MLVs suspension applied to the column. We then determined the concentration of Cu^{2+} trapped in the MLVs by mixing the fractions of the MLVs containing Cu^{2+}. The uptake efficiency of Cu^{2+} was 18%. The amount of phosphorus was also determined by ICP-AES. The amount of phosphorus in the MLVs containing Cu^{2+} was almost equal to that in the MLVs suspensions applied to the column. Therefore, the MLVs containing Cu^{2+} was essentially excluded from the column.

A similar elution profile was obtained in the separation of the MLVs containing Zn^{2+} and free Zn^{2+}, though first peak height was smaller than that observed in Cu^{2+} (result not shown). The uptake efficiency of Zn^{2+} was 6%. On the other hand, uptake was little observed in the elution profiles of Mn^{2+}, Co^{2+} and Ni^{2+}. In the case of Fe^{2+}, the MLVs condensed by mixing the Fe^{2+} solution. Therefore, the MLVs was found to be unsuitable for the separation media of Mn^{2+}, Fe^{2+}, Co^{2+} and Ni^{2+}.

Next, the Cu^{2+} uptake was carried out using cationic and zwitterionic MLVs. However, in the separation of the MLVs containing Cu^{2+} and free Cu^{2+} by gel-chromatography, no peak corresponding to the MLVs was observed in the elution profile. Since the FL intensity of calcein decreases by the formation of complexes with Co^{2+} or Cu^{2+},[6] we then examined the effect of different chage-type of MLVs on the Cu^{2+} uptake by measuring the change of the FL intensity of calcein trapped in the MLVs.

In order to confirm the permiation of Cu^{2+} into the inner phase of the MLVs, the FL intensity-time profile was also measured in the presence of Co^{2+}. This is because Co^{2+} reacts with free calcein in the MLVs suspension and calcein existing on the surface of MLVs and is unreactive to calcein exsiting in the inner phase of the MLVs.[2] In addition, the ratio of the FL intensity in the presence of Cu^{2+} to that of Co^{2+} at any reaction time was obtained from the FL intensity-time profiles of Co^{2+} and Cu^{2+}. Figure 2 shows the FL intensity ratio in the presence of different chage-type of MLVs. When Cu^{2+} is unreactive to calcein exsiting in the inner phase of the MLVs, the FL intensity ratio should be 1.0 at any reaction time, since the complex formation rate of calceine with Cu^{2+} was exactly equal to that with Co^{2+}. However, as shown in Figure 2, the decrease of the FL intensity in the presence of Cu^{2+} was greater than that of Co^{2+}. Therefore, the result suggests that Cu^{2+} could penetrate the lipid bilayer membrane and react with calcein exsiting in the inner phase of the MLVs.

On the other hand, the uptake rate and efficiency increased in the following order: cationic < anionic < zwitterionic MLVs. Therefore, these results indicate that the control of surface charge of the MLVs could be important for the improvement of uptake rate and efficiency of Cu^{2+}. Next, the effect of the copper salts was investigated using the anionic MLVs. Differences in the uptake rate and efficiency for Cu^{2+} were little observed between chloride, bromide and sulfate ions.

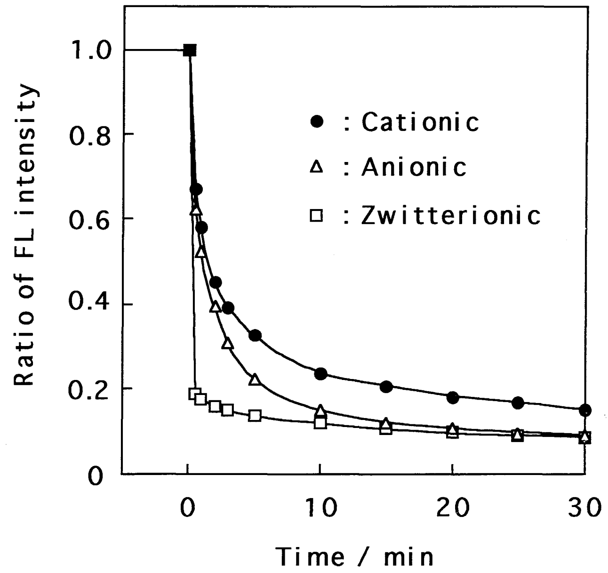

Figure 2. Ratio of fluorescence intensity of calcein with Cu^{2+} to that with Co^{2+} in the presence of different charge-type MLVs.

Liposomes are practically impermeable to metal ions, since hydrophobic phospholipids prevent the passage of metal ions through membranes. Therefore, liposomes generally require the presence of transport carriers such as ionophores. However, no specific transport carriers were added in the lipid membranes of the MLVs. Therefore, the results obtained suggest that anionic ions such as chloride and bromide could react with Cu^{2+} to form a nonionic permeant species. We then determined the concentrations of bromide in the MLVs containing Cu^{2+} by ICP-AES. However, bromide was not detected in the MLVs. Further studies on the uptake mechanism of Cu^{2+} and Zn^{2+} with the MLVs are under way.

References and Notes

1 B.C.Pressman, *Fed. Proc.*, **32**, 1698 (1973).
2 N.Oku, D.A.Kendall, and R.C.Macdonald, *Biochim. Biophys Acta*, **691**, 332(1982).
3 H.Scott and A.A.F.Sayead, *J.Collid Surface Sci.*, **9**, 274 (1986).
4 A.D.Bangham, M.W.Hill, and N.G.A.Miller, *J. Methods Membrane Biol.*, **1**, 1(1974).
5 T.Kamidate, T.Suita, and H.Watanabe, *Anal.Biochem.*, **241**, 264(1966).
6 D.F.H.Wallach and T.L.Steck, *Anal.Chem.*, **35**, 1035(1963).