Binding Behavior of Metal Cations and Hydrophobic Ions from a Mixed Solution

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Critical experimental data are presented on the competitive adsorption of metal cations with different ionic valences to phospholipid vesicles, and on the binding of hydrophobic ions from mixed solutions with multivalent metal cations produced under different conditions by means of the Inductively Coupled Argon Plasma. It is apparent that the preferential effect in binding between the metal cations depends not only on their ionic valences but also on the concentrations of each ion at which the corresponding binding is carried out; i.e., the binding strength of the metal cations on the membrane surface increases in the following order: $K^+ < Mg^{2+} < Ca^{2+} < La^{3+} < Th^{4+}$, and this order comes to be more pronounced in the high-concentration region of each component. Furthermore, it is realized that, in binding from mixed solutions of hydrophobic ions and multivalent metal cations (Ca^{2+} or Th^{4+}), a synergistic interaction between these ions occurs; i.e., the binding of hydrophobic anions is promoted by the presence of metal cations, while the binding of hydrophobic cations is hindered by the metal cations.

From a structural point of view, phospholipids are perhaps the most important of all constituents of living systems, for without them a living cell could not exist. Among the various functions of a biological membrane, its permeability property is one of the most interesting points with relation to its interfacial behavior. Many studies[1] of metal-cation binding to phospholipid vesicles in terms of the permeability have indicated that the metal cations, especially the multivalent cations, are bound to the vesicles with a high affinity and that the presence of even small amounts of the cation exerts a profound effect on the vesicle dispersion.[2,3]

On the other hand, some organic ions, such as detergents, antibiotics, and some other lipidsoluble ions, can be bound to the phospholipid vesicles by hydrophobic interaction with the lipid molecules. It is expected, therefore, that the adsorption of these organic ions into the membrane will bring about not only a change in the membrane-surface potential, but also a change in the dipole potential in the membrane arising from the oriented dipoles of the polar-head groups of phospholipid molecules.[4,5]

Biological membranes sometimes operate in a medium containing simultaneously, several kinds of metal cations with different ionic valences and a few hydrophobic ions. For example, some sea plants grow quickly in sea water in which various ionic materials are included. It is assumed that in their growing stage, the permeability of their ionic materials through the cell membrane plays an important role. In such a case, it is important to know the relation between the permeability of the membrane and the composition of the ionic materials included.

In this work, some critical data on the binding character of these metal cations with different ionic valences on the phospholipid vesicles from their mixed systems have been obtained. Further, the binding behavior of hydrophobic ions from the mixed solution with multivalent metal cations has been examined under various component conditions. We believe that these critical data on the binding will provide a crucial illustration of the functionality of phospholipid bilayers.

Experimental

Materials. Bovine brain-phosphatidylethanolamine (PE) and -phosphatidylserine (PS) were used as the membrane-forming lipids; they were purchased from the Sigma Chemical Co., Ltd. (U.S.A.). Sodium tetraphenylborate (TPB) and tetraphenylphosphonium chloride (TPP), which has been obtained from Dojindo Laboratories, were used as a hydrophobic anion and a hydrophobic cation respectively. The other inorganic reagents (metal chlorides) were of an analytical grade. All the solutions of these materials were prepared by dissolving them in deionized (Barnstead, NANO pure system) and doubly distilled water.

Vesicle Preparation. The PE and PS vesicles were prepared by the usual sonication method in a nitrogen atmosphere at 4 °C, without using any buffer to reduce the spurious effects of the buffer ions included.[6] After the removal of the undispersed materials by centrifugation (26000 G, 1 h), the dispersions were filtered through a membrane filter (0.1 μm pore size).

Measurements of Amounts of Binding. The binding strength of each metal cation from a single-component system on the PE (or PS) vesicles has been measured as follows. Ten-ml portions of the vesicle dispersion (0.3 g dm^{-1}) were poured into 10-ml portions of various salt solutions containing different ionic concentrations, after which the mixture was left standing for one night at 25 °C. Only the vesicle particles were then separated from the medium by ultrafiltration (Millipore PTGC), and the concentrations of the metal cations and hydrophobic ions were measured analytically by means of the Inductively Coupled Argon Plasma (ICP) method (Jarrell-Ash 975), which counts the amounts of boronium for TPB and of phosphonium for TPP respectively. By observing the

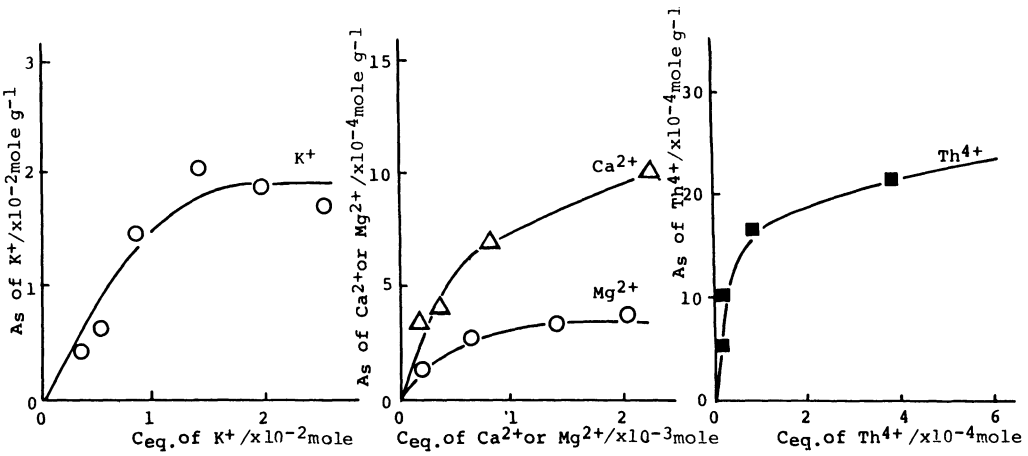

Fig. 1. Binding isotherms of K^+, Ca^{2+}, Mg^{2+}, and Th^{4+} to PE vesicles.

different concentrations in the solutions before and after (C_{eq}) the adsorption process, the amounts of adsorption per unit of vesicle weight (As) were calculated using a calibration curve.

The amounts of binding of each component ion from these mixed solutions of the different ionic species were determined by the same procedure as that used for the singlecomponent system. After a binding period of 24 h, the concentrations of the individual ions remaining in the filtrate as well as in the initial solution were determined simultaneously using the ICP method. By this analysis, the concentrations of many atomic species can be analysed simultaneously from a single liquid solution.

In the simultaneous binding of two ionic species, the effects of the order of addition on their binding are studied as follows. A 5-ml portion of one salt solution is poured into a cylinder with the same volume of the vesicle dispersion, and then the mixture is stored in a thermostat kept at 25 °C for 24 h. After that, a 5-ml portion of another salt solution including the second component ion was added, and the new mixture stored again for 24 h. After the completion of the second binding process, the binding amounts of each component ion were determined in the same analytical way.

Results and Discussion

1) The Binding Behavior of Metal Cations from Single- and Two-Component Systems. Figure 1 presents some typical examples of binding isotherms for metal cations. As may be seen, the affinity of the binding of each cation is much dependent on the ionic valence of the ions, and the affinity increases with an increase in the ionic valence. Especially, the multivalent cations (La^{3+} and Th^{4+}) are bound to the vesicles with very high affinities; even a trace of these ions exerts an important effect on the stability of vesicle dispersion.[2] The present work demonstrated that the binding strength of metal cations to PE and PS vesicles increases in the following order: $K^+ < Mg^{2+} < Ca^{2+} < Mn^{2+} < La^{3+} < Th^{4+}$. Considering their binding order, the competitive (or preferential) binding between the different metal cations has been examined simultaneously in systems including two such species. Figures 2, 3, and 4 show the binding isotherms of K^+ and Ca^{2+}, Ca^{2+} and Mg^{2+}, and Ca^{2+} and Th^{4+}, respectively, as obtained from mixed solutions. It is apparent from Fig. 2 that the preference of Ca^{2+} relative to K^+ can be observed over a wide region except for the lower concentration region of less than 1×10^{-4} mol dm^{-3} $CaCl_2$. It is interesting to notice that the preferential effect of a special ion depends not only on the valence of the ion, but also on the concentration of each ion at which the corresponding binding is carried out. Figure 3 shows the results obtained from the simultaneous binding of Mg^{2+} and Ca^{2+} on the PE vesicles. In the same figure, the isotherms obtained from single-component system are also illustrated by the dotted lines. It follows that

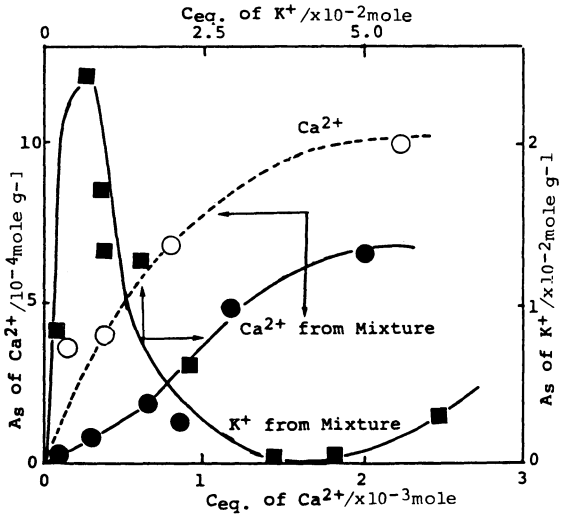

Fig. 2. Binding isotherms of K^+(■) and Ca^{2+}(●) to PE vesicles obtained from their mixed solutions, where the concentration ratio of both the ions is fixed at 30 (K^+) : 1(Ca^{2+}). The dotted line shows the isotherm of Ca^{2+}(○) from the single component system.

the results show a preferential binding of Ca^{2+} in comparison to the binding of Mg^{2+} over the whole concentration region and that the preference of Ca^{2+} depends on the concentration and becomes apparent in concentrations of each ion higher than 1×10^{-3} mol dm^{-3}. As may be seen from Fig. 4, similar results may be found in the simultaneous binding of Ca^{2+} and Th^{4+}, where the concentration of Th^{4+} is fixed at 1×10^{-4} mol dm^{-3}. It is found that the amount of the binding of Th^{4+} is kept constant over the whole concentration region and that only the amount of the adsorption of Ca^{2+} varies with its bulk concentration. All these findings regarding the simultaneous binding between the different metal cations suggest that the binding of metal cations on the membrane surface is influenced by various factors; the ionic species, the ionic valence, the concentration, their ratio in a two-component system and other enviromental factors, and that it is hard to predict the final binding layer from the composition of the equilibrated bulk solution.

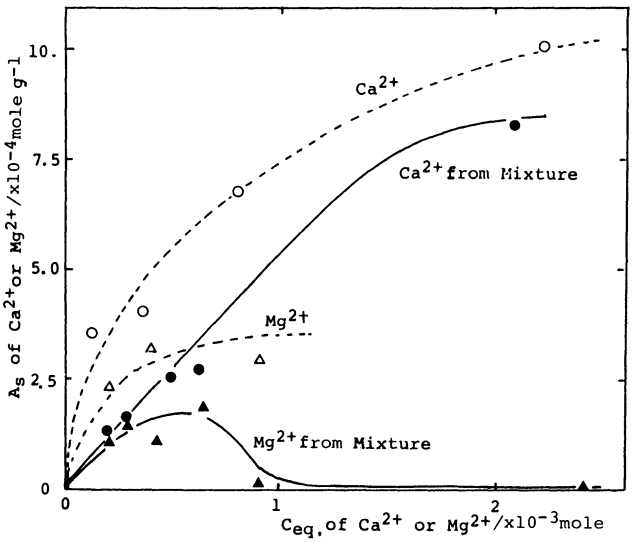

Fig. 3. Binding isotherms of Ca^{2+}(●) and Mg^{2+}(▲) to PE vesicles obtained from their mixed solutions, where the concentration ratio of both the ions is fixed at 1 (Ca^{2+}) : 1(Mg^{2+}). The dotted lines show the isotherms of Ca^{2+}(○) and Mg^{2+}(△) from the single component systems.

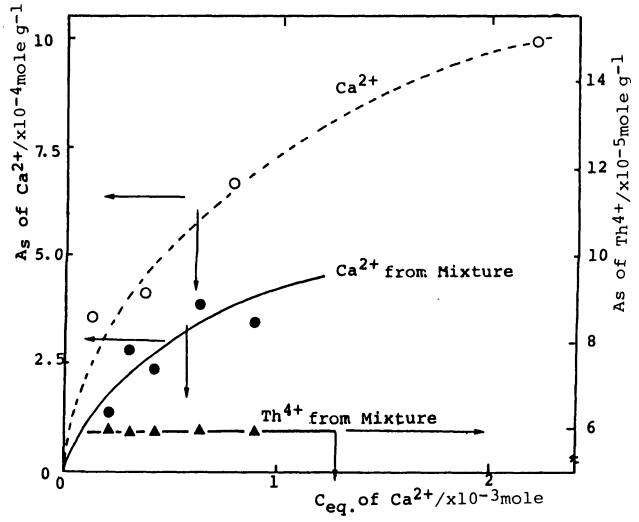

Fig. 4. Binding isotherms of Ca^{2+}(●) and Th^{4+}(▲) to PE vesicles obtained from their mixed solutions, where the concentration of Th^{4+} is kept constant at 1×10^{-4} mol dm^{-3}. The dotted line shows the isotherm of Ca^{2+}(○) obtained from the single component system.

This complexity of the situation in the metal-cation binding from a mixed solution indicates that the binding of their ions will be influenced by the order of the addition of the species. Figure 5 shows some typical results obtained from the two different ways of addition for Ca^{2+} and Th^{4+}, where the concentration of Th^{4+} is fixed at 1×10^{-4} mol dm^{-3}; only the comparison in the binding amounts for Ca^{2+} is indicated for the two mixing methods. As may be seen from the figure, the amounts of bound Ca^{2+} are greatly influenced by the order of addition; the initial addition of Th^{4+} results in decreased amounts of Ca^{2+}. This result indicates that a multivalent cation such as Th^{4+} binds on the membrane surface with an irreversible character and that it is hard to exchange occupied binding-site for other metal cations.

2) Binding Behavior of Hydrophobic Ions from Mixed Solutions with Metal Cations. It has already been reported that there is a great difference between the adsorption behavior of TPB and TPP toward the lipid vesicles from their single-component systems; i.e., the amounts of the binding of TPB are greater than those of TPP, especially at high bulk concentrations.[7] Here, the difference between the binding behavior of the two hydrophobic ions and that of their coexistent systems with the metal cations has been

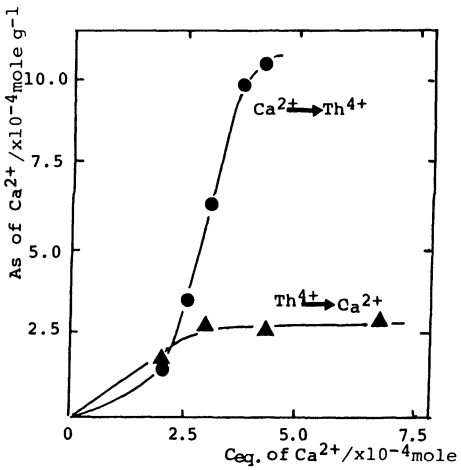

Fig. 5. Binding isotherms of Ca^{2+} to PE vesicles obtained from the two different methods of addition for Ca^{2+}, where the concentration of Th^{4+} is fixed at 1×10^{-4} mol dm^{-3}.

examined using the ICP method. Figures 6 and 7 show the binding isotherms of TPB and Ca^{2+} to the PE and PS vesicles obtained from their mixed solutions with Ca^{2+}. The dotted lines in these figures show the isotherms obtained from the single-component systems of each ion. It is found that the amounts of the binding of TPB are enhanced extensively by the coexistence of Ca^{2+}; a similarly enhanced value is observed in the binding to the PE and PS vesicles. Furthermore, it is apparent that the binding of Ca^{2+} to the PE and PS vesicles is also promoted by the coexistence of TPB. These results indicate that, when the TPB anion and the metal cation are present in the same solution, a synergistic effect by their ions occurs and each ion acts as a cooperator for its binding to the membrane surface.[8]

On the other hand, when the TPP and the metal cations are bound to the vesicles from a mixed solution, very different behavior has been observed. Figures 8 and 9 display the binding isotherms obtained from the mixed solutions of Ca^{2+} plus TPP and of Th^{4+} plus TPP respectively. In Fig. 9, the concentration of Th^{4+} was fixed at 1×10^{-4} mol dm^{-3} and only the binding of TPP is compared. It may be

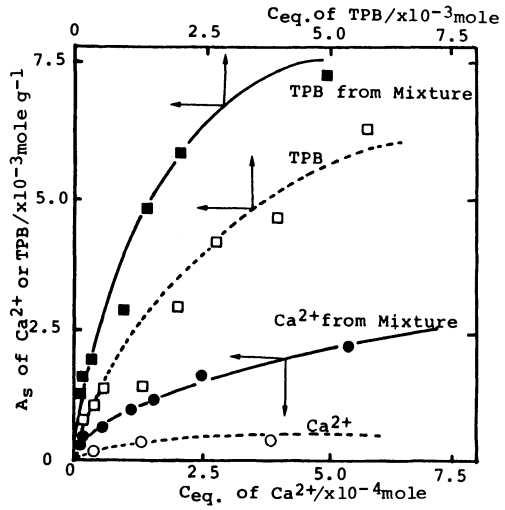

Fig. 6. Binding isotherms of Ca^{2+}(●) and TPB(■) to PE vesicles obtained from their mixed solutions, where the concentration ratio of both the ions is fixed at 1(Ca^{2+}) : 6(TPB). The dotted lines show the isotherms of Ca^{2+}(○) and TPB(□) obtained from the single component systems.

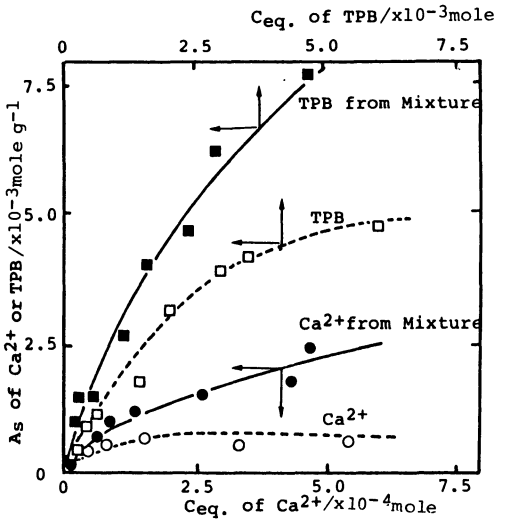

Fig. 7. Binding isotherms of Ca^{2+}(●) and TPB(■) to PS vesicles obtained from their mixed solutions, where the concentration ratio of both the ions is fixed at 1 (Ca^{2+}) : 6(TPB). The dotted lines show the isotherms of Ca^{2+}(○) and TPB(□) obtained from the single components ystems.

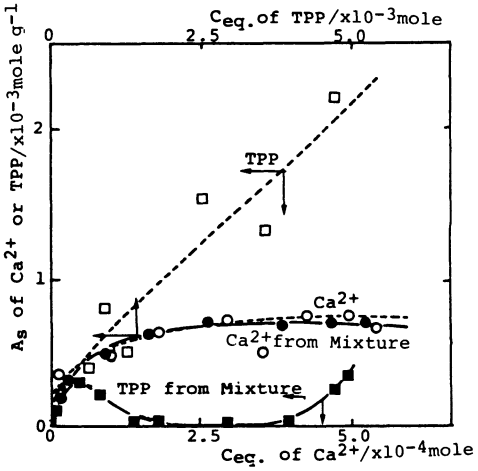

Fig. 8. Binding isotherms of Ca^{2+}(●) and TPP(■) to PE vesicles obtained from their mixed solutions, where the concentration ratio of both the ions is fixed at 1 (Ca^{2+}) : 6(TPP). The dotted lines show the isotherms of Ca^{2+}(○) and TPP(□) obtained from the single component systems.

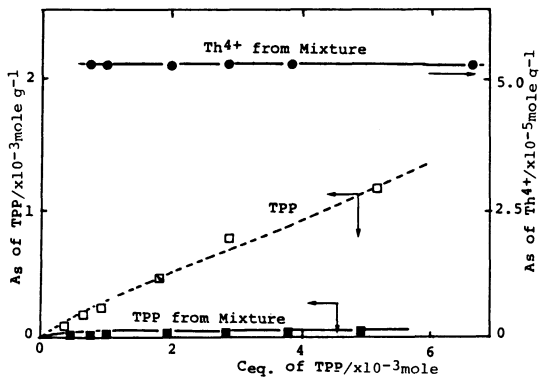

Fig. 9. Binding isotherms of Th^{4+}(●) and TPP(■) to PE vesicles obtained from their mixed solutions, where the concentration of Th^{4+} is fixed at 1×10^{-4} mol dm^{-3}. The dotted line shows the isotherm of TPP (□) obtained from the single component system.

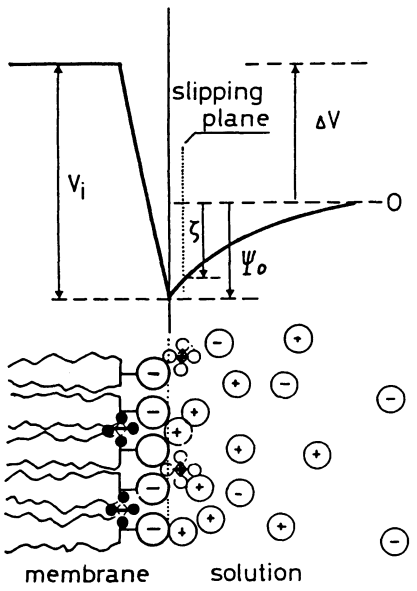

Fig. 10. Schematic picture showing the electrostatic potential through membrane-solution interface and the binding sites on the layer of the lipid membrane (●◇●; TPB, ○◈○; TPP).

seen from Fig. 8 that the binding of TPP is hindered by Ca^{2+} except in some special concentration regions of TPP. It may be observed by comparison with Fig. 2 that the relations between Ca^{2+} and TPP in their preferential binding resemble those between Ca^{2+} and K^+, suggesting that the binding affinity of TPP results from the same cause as the binding of the metal cation. On the other hand, as may be seen from Fig. 9, the binding of TPP to the vesicles is completely hindered by the coexistence of Th^{4+} because of the strong binding character of Th^{4+} to the membrane surface.

Such a difference in the binding of TPB and TPP to the vesicles from that in mixed solutions with the metal cations may be explained by their different contributions to the electrostatic potential through the membrane-solution interface. Figure 10 shows a schematic picture to elucidate the potential profile near the membrane surface. The membrane potential is described as a combination of potentials arising from the oriented dipoles of polar-head groups of phospholipid molecules and the ionic double-layer potential on their surface.[9] This potential profile can be applied to the PE vesicles under negatively charged conditions.[2] It is usually known that the metal cations bind to the membrane surface and cause the ionic double-layer potential to change. This consideration can be confirmed by measurements of the zeta-potentials of the vesicles (ζ in Fig. 10) and of the surface potentials of the monolayers (ΔV in Fig. 10) in various ionic solutions. On the other hand, the binding of TPB causes a change in both the dipole potential and the double-layer potential as a result of their hydrophobic interaction.[8] According to this concept, if the metal cations and the TPB anions are both present in a solution including PS or PE vesicles, some synergistic interaction in their binding to the vesicles may be expected; some metal cations will initially bind to the negative surface of the vesicles and decrease the negative double-layer potential. This will induce an increase in the binding of some TPB anions by means of their electrostatic forces and again result in a negative surface potential. By repeating the process, a positive cooperative binding between the metal cation and the TPB anion can be observed. On the other hand, when the lipid soluble cations (TPP) are present in a solution with the metal cation, the binding of TPP will be hindered by the positive potential effect induced by the binding of the metal cation to the negative membrane surface. Furthermore, because of the restricted number of binding sites on the outer layer of the lipid membrane which are occupied preferentially by the metal cations, the binding of TPP is extensively hindered by the coexistence of metal cations.

It is clear from the present binding work that the binding sites on the membrane surface for TPP are very different from those for TPB, although they have the same hydrophobic character.

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