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Carrier-Mediated Transport Across Phospholipid-Composite Membranes Containing Valinomycin

Artificial membranes containing a phospholipid and valinomycin were prepared. Valinomycin in these membranes acts as an ionophore (a mobile carrier for potassium ions) at a temperature above the phase transition point of the phospholipid. Experimental data were explained by the theory of carrier-mediated transport accompanying formation of a carrier-ion pair complex.

EIZO SADA, SHIGEO KATOH, MASAAKI TERASHIMA and YOSHIHIRO TAKADA

Chemical Engineering Department
Kyoto University
Kyoto 606, Japan

SCOPE

The objects of the present work are to prepare phospholipid-composite membranes containing a carrier for potassium ions and to clarify the mechanism of carrier-mediated transport for potassium ions in these membranes.

Among the unique functions of biomembrane, the carrier-mediated transport of solutes is the most interesting one. Although the mechanism of carrier-mediated transport of various ions has been widely studied with liquid membranes (Ward III, 1970; Reusch and Cussler, 1973), bilayer membranes, and liposomes (Szabo et al., 1969), on account of their mechanical unstability it is desirable to prepare more stable membranes containing carriers.

In previous work (Sada et al., 1983), we have reported that the phospholipid-composite membrane prepared from a hydrophobic polymer and a phospholipid shows similar permeability characteristics to those reported with biomembrane models such as liposomes and bilayer membranes. In the present work we intended to incorporate valinomycin, a carrier for potassium ions in biomembranes into those phospholipid-composite membranes, which are far more stable than liposomes and liquid membranes. At the temperature above the phase transition point of the phospholipid, valinomycin is expected to act as a carrier for potassium ions in a similar manner as in biomembranes.

These membranes are useful for studying transport phenomena in living systems and for the selective separation of solutes on an industrial scale by selecting suitable carriers. They further may be suitable for reconstitution or immobilization of hydrophobic enzymes which are active only in the phospholipid bilayer of biomembranes.

CONCLUSION AND SIGNIFICANCE

Valinomycin was incorporated into the stable phospholipid-composite membranes. The fluxes of potassium ions through these membranes increased steeply with potassium concentration and reached a plateau at high salt concentration above the phase transition temperature of the phospholipid. They also increased in proportion to carrier concentration. So it was presumed that valinomycin acted as a mobile carrier. The behavior of valinomycin was analyzed by the theory of mobile-carrier mechanism, in which valinomycin makes a carrier-ion pair complex at one side of the membrane, diffuses across the membrane, and releases ions at the other side.

From these results we concluded that valinomycin in the phospholipid-composite membranes acted as the mobile carrier for potassium ions at a temperature above the phase transition point of the phospholipid. The experimental data agreed well with the predictions based on the theory of carrier-mediated transport accompanying formation of a carrier-ion pair complex. Further, these membranes could be used stably at least for four weeks.

By selecting and designing suitable carriers, these types of composite membranes may make important contributions to industrial processes for selective separation of solutes.

INTRODUCTION

Bilayer lipid membranes and liposomes have been widely used as experimental models to reconstitute several functions of biomembranes such as the permeability change due to the phase-transition and the selective transport of several kinds of solutes (Szabo et al., 1964; Inoue, 1974). Among them, the selective and facilitated transport of solutes is the most important one from the viewpoint of industrial applications. It is well known that some molecular species having a high affinity to a particular solute can dissolve into the hydrophobic region of biomembranes on account of their hydrophobicity and act as a mobile carrier of the solute.

Although liquid membranes containing mobile carriers have also been used for the selective transport of cations (Ward III, 1970; Reusch and Cussler, 1973), the mechanical stability of these systems becomes a serious problem in practical use. Therefore, new attempts to prepare stable artificial membranes containing carriers for the facilitated transport of cations have been reported (Oda et al., 1981; Sugiura, 1981; Shimizu et al., 1981).

In a previous paper (Sada et al., 1983), we reported that the phospholipid-composite membrane prepared from a hydrophobic polymer and a phospholipid showed an abrupt change of permeability at the phase transition temperature of the phospholipid from gel state to liquid-crystal state. The object of the present work was to prepare phospholipid-composite membranes containing a carrier for potassium ions and to clarify the mechanism of carrier-mediated transport for potassium ions. These membranes are expected to be more stable than liquid membranes and to be suitable for reconstitution of biomembrane functions, in comparison with known artificial membranes of the carrier type. By use of these membranes the fluxes of potassium and lithium ions were measured above and below the phase transition temperature of the phospholipid. The experimental data were compared with the predictions based on the theory of the carrier-mediated transport mechanism with formation of a carrier-ion pair complex.

THEORY

Reusch and Cussler (Cussler, 1971; Reusch and Cussler, 1973) proposed the mobile-carrier mechanism for the facilitated transport of cations in liquid membranes containing crownethers. According to this mechanism, the mobile carrier is assumed to react rapidly at one interface of the membrane with a cation and an anion to form an uncharged carrier-ion pair complex. The complex diffuses slowly across the membrane and then both cation and anion are released from the carrier at the other interface of the membrane.

In the present case, potassium ions coexist with lithium ions in the compartment I shown in Figure 1. In this figure S represents the mobile carrier. At the membrane interface, potassium and lithium ions react competitively with valinomycin molecules, which have a higher affinity to potassium ions:

$$K^+ + Cl^- + S \rightleftharpoons KCl - S \tag{1}$$

$$Li^+ + Cl^- + S \rightleftharpoons LiCl - S \tag{2}$$

The reaction producing these uncharged complexes may occur in several ways. For example, the cation could first dissolve and form a complex with the carrier in the membrane. Then the anion could dissolve and react with the complex to form the ion pair. Alternatively, the cation and anion could dissolve simultaneously and form an ion pair, and then the ion pair could react with the carrier. These reactions, however, are fast in comparison with the diffusion process and an equilibrium in formation of the carrier-ion pair complex is assumed to be established. The exact sequence of the reactions does not affect the total flux.

In this case, the total flux J^T of potassium and lithium ions can be represented by the following equations (Reusch and Cussler, 1973).

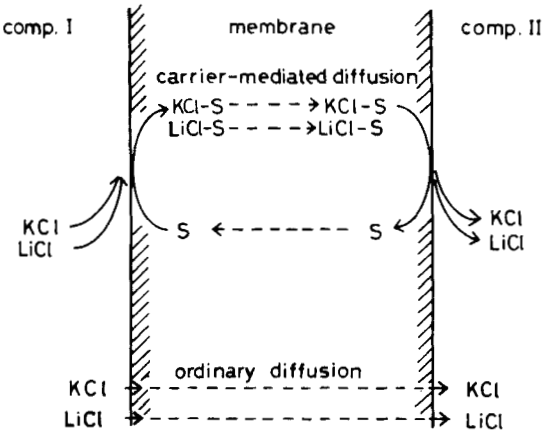


Figure 1. Schematic drawing of mobile carrier mechanism.

$$J_K^T = \frac{Dk_1}{L}(C_K^I C_{Cl}^I - C_K^{II} C_{Cl}^{II}) + \frac{D_s k_1 K_1 C_s}{L}\left(\frac{C_K^I C_{Cl}^I}{1 + k_1 K_1 C_K^I C_{Cl}^I + k_2 K_2 C_{Li}^I C_{Cl}^I} - \frac{C_K^{II} C_{Cl}^{II}}{1 + k_1 K_1 C_K^{II} C_{Cl}^{II} + k_2 K_2 C_{Li}^{II} C_{Cl}^{II}}\right) \tag{3}$$

$$J_{Li}^T = \frac{Dk_2}{L}(C_{Li}^I C_{Cl}^I - C_{Li}^{II} C_{Cl}^{II}) + \frac{D_s k_2 K_2 C_s}{L}\left(\frac{C_{Li}^I C_{Cl}^I}{1 + k_1 K_1 C_K^I C_{Cl}^I + k_2 K_2 C_{Li}^I C_{Cl}^I} - \frac{C_{Li}^{II} C_{Cl}^{II}}{1 + k_1 K_1 C_K^{II} C_{Cl}^{II} + k_2 K_2 C_{Li}^{II} C_{Cl}^{II}}\right) \tag{4}$$

The first terms on the right-hand side of Eqs. 3 and 4 represent the ordinary diffusion of ion pairs and the second terms represent the carrier mediated diffusion. K_1 and K_2 are association constants shown by Eqs. 1 and 2, and k_1 and k_2 are partition coefficients of KCl and LiCl ion pairs, respectively. If the ordinary diffusion terms of Eqs. 3 and 4 can be experimentally subtracted, and if both the carrier-mediated back diffusion from compartment II to I and the concentration of ions in the compartment II are very low, i.e., $k_1 K_1 C_K^{II} C_{Cl}^{II} + k_2 K_2 C_{Li}^{II} C_{Cl}^{II} \ll 1$, $C_K^I C_{Cl}^I \gg C_K^{II} C_{Cl}^{II}$, and $C_{Li}^I C_{Cl}^I \gg C_{Li}^{II} C_{Cl}^{II}$, Eqs. 3 and 4 become

$$J_K = \frac{D_s k_1 K_1 C_s}{L} \frac{C_K^I C_{Cl}^I}{1 + k_1 K_1 C_K^I C_{Cl}^I + k_2 K_2 C_{Li}^I C_{Cl}^I} \tag{5}$$

$$J_{Li} = \frac{D_s k_2 K_2 C_s}{L} \frac{C_{Li}^I C_{Cl}^I}{1 + k_1 K_1 C_K^I C_{Cl}^I + k_2 K_2 C_{Li}^I C_{Cl}^I} \tag{6}$$

J_K and J_{Li} are the fluxes of potassium and lithium ions mediated by the carrier, respectively. According to these equations, the fluxes of potassium and lithium ions should increase in proportion to the carrier concentration and depend on both the cation and the anion concentrations. The ion selectivity $S_{K/Li}$ is defined as the ratio of Eq. 5 to Eq. 6:

$$S_{K/Li} = J_K / J_{Li} = k_1 K_1 / k_2 K_2 \tag{7}$$

Under the condition of the equal concentration of cations ($C_K^I = C_{Li}^I = C_o$, $C_{Cl}^I = 2C_o$), Eqs. 5 and 6 become

$$\frac{C_s C_o^2}{J_K} = \frac{(k_1 K_1 + k_2 K_2)L}{D_s k_1 K_1} C_o^2 + \frac{L}{2 D_s k_1 K_1} \tag{8}$$

$$\frac{C_s C_o^2}{J_{Li}} = \frac{(k_1 K_1 + k_2 K_2)L}{D_s k_2 K_2} C_o^2 + \frac{L}{2 D_s k_2 K_2} \tag{9}$$

Then, the values of $k_1 K_1$, $k_2 K_2$ and D_s could be evaluated using Eqs. 8 and 9.

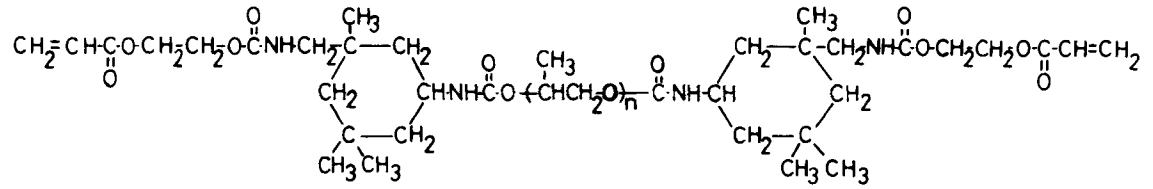


Figure 2. Structure of prepolymer ENTP.

EXPERIMENTAL

Materials

DL-α-dipalmitoyl phosphatidylcholine (DPPC; Sigma Chemical Co.) and valinomycin (Boehringer Mannheim GmbH. Mw 1,111) were used. Potassium chloride and lithium chloride were of reagent grade. Photo-cross-linkable resin prepolymers (ENTP 2000 and 4000; Figure 2) were used for preparation of hydrophobic membranes containing DPPC and valinomycin. The prepolymer was synthesized from hydroxyethyl-acrylate isophorone diisocyanate and poly (propyrene glycol)-2000 (av. Mw = 2,000) or poly (propyrene glycol)-4000 (av. Mw = 4,000) from Kansai Paint Co. Ltd., Japan, (Sonomoto et al., 1979). The physical properties are as follows: compressive strength, 5×10^6 Pa; tensile strength, 1×10^6 Pa; glass transition temperature, below 273 K.

Preparation of Membranes

840 mg of ENTP 4000, 360 mg of ENTP 2000, 27 mg of benzoin ethyl-ether, 800 mg of DPPC, and 0–80 mg of valinomycin were dissolved in 4 cm^3 of chloroform. Then the solution was spread on a rectangular plate (7 cm $\times$ 7 cm) and photo-cross-linked by ultraviolet illumination for 40 min. The solvent was removed overnight in water. The average thickness of the membranes was 300 μm and these membranes were stable for at least four weeks. According to scanning electron microscope observation, the phospholipid constituted a continuous phase in the framework of crosslinked polymer. It was verified by thin-layer chromatography that valinomycin was still contained in the phospholipid-composite membrane after a series of the experiments lasted for four weeks. The chromatograms were developed on silica gel plate (Art. 5721, Merck) with methanol as the developing solvent for 60 min.

Measurement of Cation Flux

Figure 3 shows a schematic diagram of the experimental apparatus used for measurement of the cation flux. The test cell consisted of two acrylic resin compartments, 4 cm I.D. and 3 cm long, separated by a membrane. The volume of each compartment was 46.0 cm^3 and the membrane area was 7.1 cm^2. Each compartment was equipped with a water jacket and a six-blade paddle stirrer 3.2 cm in diameter. In the beginning, both compartments were continuously washed with distilled water for a few days to reduce the concentrations of potassium and lithium ions in the effluent from both compartments below 10^{-6} M. Then, distilled water containing KCl and LiCl [0.025—2.0 M (= mol/dm^3)] was charged in compartment I while compartment II was continuously washed with distilled water. After confirming that the concentration of the cations in the effluent had reached a very low and constant value, the experiment was begun by stopping the infusion of distilled water and clamping the tube connected to compartment I. A small amount of the solution in compartment II was taken at predetermined time intervals to measure the concentrations of the cations by atomic absorption and flame emission spectrophotometer (Nippon Jarrel-Ash AA8200). In the present work, most of the experiments were carried out with equimolar solutions of KCl and LiCl at 323 K, at which temperature DPPC layers were in the liquid-crystal state (phase transition temperature, 315 K). The stirring speed in both compartments was 50 rpm in all the experiments and the liquid film resistances for mass transfer on both sides of the membrane were neglected in this condition.

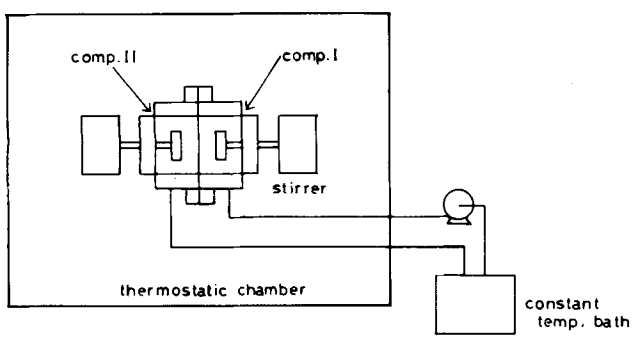


Figure 3. Schematic diagram of experimental apparatus.

RESULTS AND DISCUSSION

Figure 4 shows the representative change of the cation concentration in compartment II with time. Since they increase linearly with time, total fluxes of the cations can be determined from their slopes. In Figure 5, the total fluxes (J_K^T, J_{Li}^T) of the cations are plotted against the concentration of each salt in compartment I. The fluxes were corrected for the variation in the membrane thickness from 300 μm on assumption of inverse proportionality of the flux to the membrane thickness. In the case of the phospholipid-composite membranes with valinomycin, the total fluxes were larger than those through the membranes without valinomycin and the increase of the potassium fluxes was remarkable. They increased steeply and reached a plateau at high salt concentration. In addition, the previous study (Sada et al., 1983) showed DPPC bilayers within the membrane are the liquid-crystal state at 323 K. It is suggested that valinomycin within the phospholipid bilayers in the membrane may act as the mobile carrier for potassium ions. In the case of the phospholipid-composite membranes without valinomycin, the total flux might be approximated by the straight lines shown in Figure 5 because it was much smaller than those through the membrane with valinomycin, although it should increase as the square of the salt concentration according to Eqs. 3 and 4. It can be estimated that the ordinary diffusion flux across the membranes containing valinomycin is approximately the same as that across the membrane without valinomycin. Therefore, the fluxes due to the carrier-mediated diffusion (J_K, J_{Li}) may be evaluated by subtracting the ordinary diffusion flux from the total flux; they are plotted in Figure 6 against the each salt concentration. The fluxes mediated by the

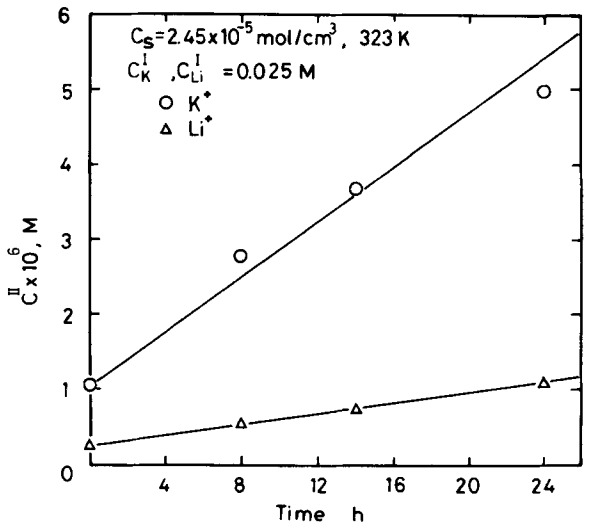


Figure 4. Time-course of cation concentration in compartment II.

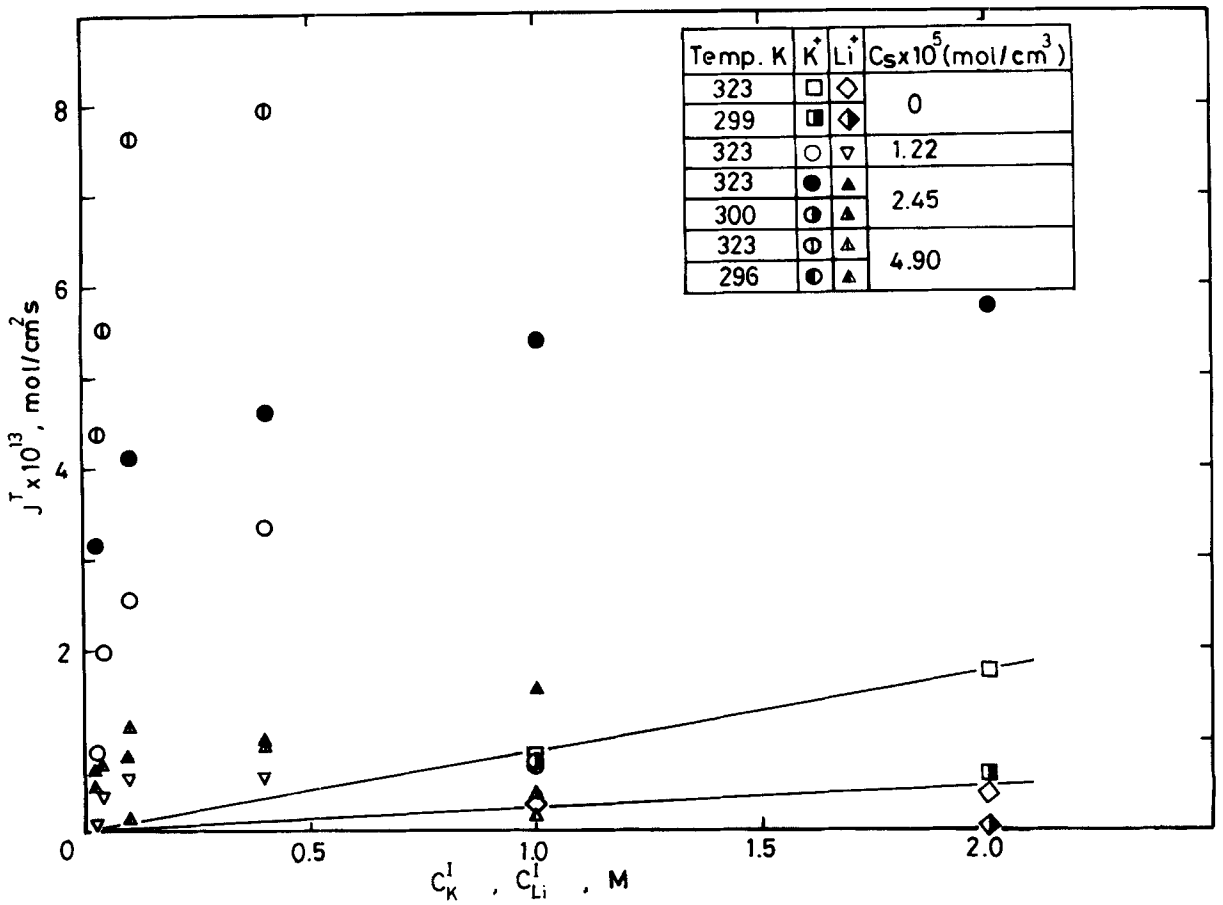


Figure 5. Total flux of cations.

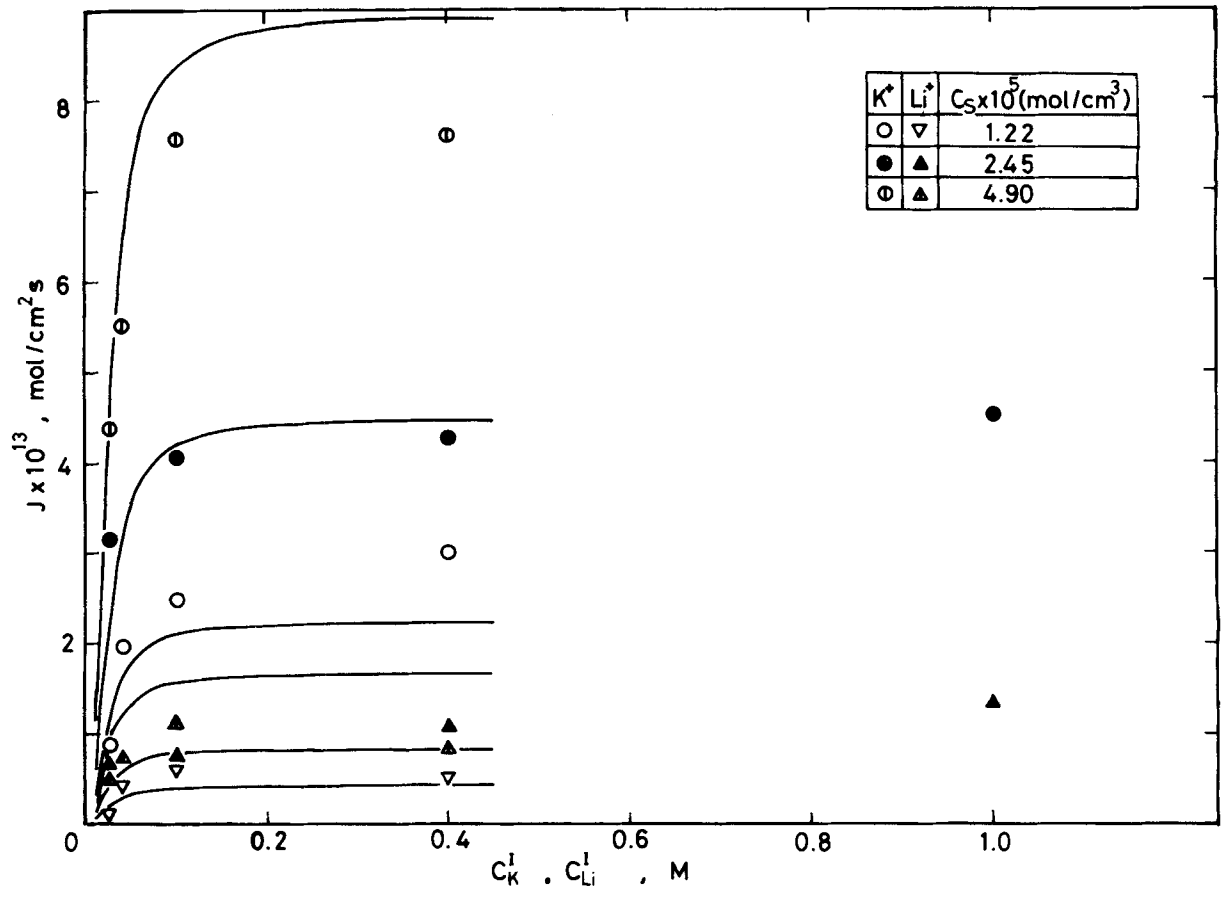


Figure 6. Carrier-mediated flux of cations.

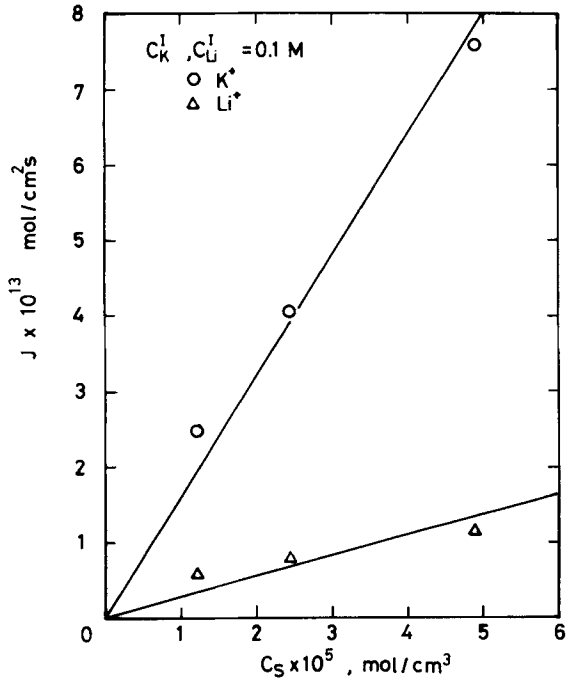
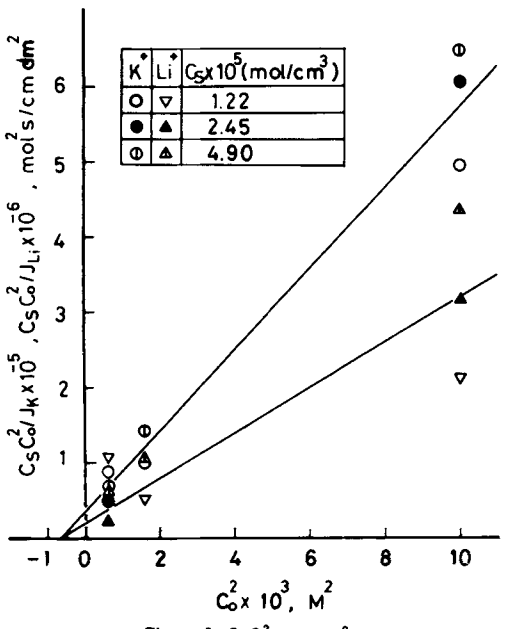


Figure 7. Carrier-mediated flux vs. carrier concentration.

carrier are plotted in Figure 7 against the carrier concentration in the case of $C_K^I = C_{Li}^I = 0.1$M. As expected from Eqs. 5 and 6, they increased in proportion to the carrier concentration. The inequality $k_1K_1C_K^{II}C_{Cl}^{II} + k_2K_2C_{Li}^{II}C_{Cl}^{II} \ll 1$ is satisfied, because the salt concentrations in compartment II were less than 10^{-5}M and the concentrations at the half saturation of the flux were larger than 10^{-2}M, as shown in Figures 5 and 6. Therefore, Eqs. 8 and 9 can be applied under the present experimental conditions and suggest that the plots of $C_SC_o^2/J_K$, $C_SC_o^2/J_{Li}$ against C_o^2 give straight lines. These relations are shown in Figure 8. From the slopes and the intercepts of these lines, values of k_1K_1, k_2K_2, and D_s were evaluated by using the least-squares method and the ion selectivity $S_{K/Li}$ was calculated. They are summarized in Table 1. Using the values in Table 1, the carrier-mediated fluxes were calculated from Eqs. 5 and 6. Solid curves in Figure 6 show these calculated values. They show good agreement with the experimental data. Although the value of the ion selectivity was low compared with those reported in bilayer systems, the microenvironment of the carrier at or near the membrane interface may be somewhat different from that in the black lipid membrane. The ion selectivity might be improved by selecting or designing other suitable carriers.



Figuer 8. $C_sC_o^2/J$ vs. C_o^2

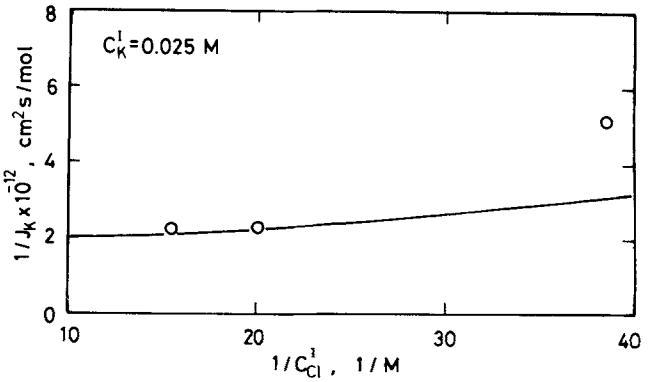


Figure 9. Variation of potassium ion flux with chlorine concentration.

TABLE 1. VALUES OF EXPERIMENTAL FACTORS

D_s (cm²/s)	6.52×10^{-10}
k_1K_2 (dm⁶/mol²)	673
k_2K_2 (dm⁶/mol²)	126
$S_{K/Li}$	5.3

Equations 8 and 9 also predict the dependence of the carrier-mediated flux of potassium ions on both chlorine and lithium ion concentrations. Figure 9 shows the variation of J_K with the concentration of chlorine ions. In these experiments, the KCl concentration in compartment I was fixed at 0.025 M and the LiCl concentration in the same compartment was varied from 1×10^{-3} M to 4×10^{-2}M. The solid curve in Figure 9 shows the theoretical prediction from Eq. 5 by use of the values in Table 1. The prediction shows an increase of the flux with increasing LiCl concentration because the contribution of the increase of chlorine ions to the flux is larger than that of the competition between potassium and lithium ions and it shows general agreement with the experimental data. With a model assuming formation of a carrier-cation complex, for example, the flux should decrease with the LiCl concentration because of the competition between potassium and lithium ions.

In this work, it is shown that the fluxes of potassium ions across phospholipid-composite membranes containing valinomycin were higher than those across the membranes without it, and that the facilitated transport across the phospholipid-composite membranes, which are far more stable than liquid membranes and bilayer membranes, was well explained by the carrier-mediated mechanism. It is expected that with use of these type of composite membranes, the selective separation of various solutes may be achieved by selecting suitable carriers and experimental conditions. The phospholipid-composite membranes may be suitable also for reconstitution or immobilization of hydrophobic enzymes, which are active only in the phospholipid bilayer of biomembranes.

NOTATION

C = ion concentration, mol/dm³
C_o = initial ion concentration in compartment I, mol/dm³
C_s = carrier concentration in membrane, mol/cm³
D = diffusion coefficient of ion pair in membrane, cm²/s
D_s = diffusion coefficient of carrier and carrier-ion pair complex in membrane, cm²/s
J = carrier-mediated flux of cation, mol/cm²·s
J^T = total flux of cation, mol/cm²·s
k_1 = partition coefficient of KCl, dm⁶/mol·cm³
k_2 = partition coefficient of LiCl, dm⁶/mol·cm³
K_1 = association constant shown by Eq. 1, cm³/mol

K_2 = association constant shown by Eq. 2, cm^3/mol
L = membrane thickness, cm
$S_{K/Li}$ = ion selectivity

Superscripts

I = compartment I
II = compartment II

Subscripts

K = potassium ion
Li = lithium ion

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R & D NOTES

Computation of Phase Equilibrium: Optimization with Thermodynamic Inconsistency

TANMOY CHAKRAVARTY
C. W. WHITE, III, and
W. D. SEIDER

Department of Chemical Engineering
University of Pennsylvania
Philadelphia, PA 19104

In 1979, Gautam and Seider used the Rand method and a new algorithm for phase-splitting to compute the compositions at equilibrium for a mixture of 40 mol % ethylene glycol, 30% lauryl alcohol, and 30% nitromethane at 295 K and 1.013 bar (1 atm). The extended van Laar equation was used with the interaction coefficients for the binary pairs determined by Null (1970), who fit the experimental data of Francis (1956) with three liquid phases at equilibrium. The compositions computed by Gautam and Seider agreed to four significant figures with those of Null and the chemical potentials of the species in the three liquid phases agreed to nearly four significant figures.

However, they obtained the iteration history of the dimensionless Gibbs free energy shown in Figure 1. A single liquid phase is assumed at equilibrium. This is split into two liquid phases with compositions and Gibbs free energies shown in Table 1. As the Rand method adjusts the compositions from iteration 1–6, G/RT *increases*, and the chemical potentials agree to within two significant figures. Phase 2 is split into two liquid phases, with a significant decrease in G/RT. Then, the Rand method reduces G/RT to a minimum after iteration 8, but follows with an *increase* until the convergence criteria (fractional change in $n_{jl} \leq 10^{-3}$) are satisfied.

This spurious behavior can be traced to the thermodynamic inconsistency of the extended van Laar equation. Although the Gibbs-Duhem equation is not satisfied, the Rand method sets the chemical potentials equal (i.e., $\mu_{j1} = \mu_{j2} = \mu_{j3}$, $j = 1, \ldots, C$), but does not locate a stationary point in the Gibbs free energy surface.

This note is intended to explain the impact of thermodynamic inconsistency upon the performance of optimization procedures that are similar to the Rand method. First, the extended van Laar equation and the UNIQUAC equation are reviewed briefly and additional results presented before the spurious behavior of the Rand method is explained.

T. Chakravarty is presently at Clarkson University; C. W. White, III, at West Virginia University.