Highly Selective Membrane Transport of Zn^{2+} Ion by a Cooperative Carrier Composed of 1,10-Diaza-18-crown-6 and Palmitic Acid

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(Received March 23, 1992)

A mixture of 1,10-diaza-18-crown-6 and palmitic acid was found to be an excellent cooperative carrier for the uphill transport of Zn^{2+} ions through a chloroform liquid membrane. In the presence of histidine, as a suitable metal ion acceptor in the receiving phase, it can selectively and efficiently transport Zn^{2+} ion from aqueous solutions containing other cations such as Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Hg^{2+}, Pb^{2+}, and Cd^{2+}. Amount of Zn^{2+} transport across the liquid membrane after 2 h is about 90%, while that of other cations is <4%.

Transport of metal ions across a membrane plays an essential role in many biochemical processes[1] and may have useful practical applications in separation science. As artificial ligand models, several types of macrocyclic molecules have been prepared and demonstrated specifically to transport alkali, alkaline earth, and organic ammonium ions with high selectivities.[2-6] In marked constract, little attention has been directed toward the transport of transition and heavy metal ions.[7-11] Despite the important role of Zn^{2+} ion in the environmental,[12] medical,[13] and biological processes,[14,15] information about the transport of this cation across liquid membrane is quite sparse.[16,17] Hence, the development of a new membrane carrier for selective transport of Zn^{2+} ion is still a challenging problem.

In this paper we describe a new highly selective liquid membrane system containing 1,10-diaza-18-crown-6 (DA18C6) and palmitic acid CH_3-$(CH_2)_{14}$-COOH, known as one of the major building blocks of lipids,[13] for uphill transport of Zn^{2+} ion, and present an example which mimicked well the characteristics of biological transport. The receiving phase contains an amino acid which found to play an important role in transport process. We believed that the present case is the first successful example of ligand membrane transport in which zinc ion is transported with surprisingly high efficiency and excellent selectivity.

Experimental

Reagents. DA18C6 and other crown others used were purchased from Merck company and were used as received. Reagent grade chloroform (Merck) was used as the membrane organic solvent. All other chemicals used in this study were of the highest purity available and were used without further purification except for vacuum drying over P_2O_5 for 72 h. Triply distilled water was used throughout.

Apparatus. A bulk type liquid membrane cell[7,18] was used in all experiments. pH measurements of the aqueous phases were performed with a Corning 125 pH meter using a combined glass electrode. A Philips Pye Unicam SP9 atomic absorption spectrophotometer was used for monitoring the metal content of the aqueous phases.

Procedure. All transport experiments were carried out at ambient temperature. A cylindrical glass cell (inside diameter 4.0 cm) holding a glass tube (inside diameter 2.0 cm), thus separating the two aqueous phases, was used. The inner aqueous phase (I) contained zinc perchlorate (5 cm^3). The outer aqueous phase (II) included histidine as metal ion receptor (10 cm^3). The pH of solution was adjusted to 7.0 with either sodium hydroxide or hydrochloric acid. The chloroform solution (30 cm^3) containing 1.2×10^{-3} M DA18C6 (1 M=1 mol dm^{-3}) and 1.5×10^{-3} M palmitic acid lay below these aqueous phases, and bridged the two aqueous phases (I) and (II). The organic layer was magnetically stirred by a Teflon®-coated magnetic bar (3 cm×5 mm diameter). Samples of both aqueous phases were analyzed for metal content by AAS. Reproducibility was confirmed as ± 5% or better.

A similar transport experiment was carried out in the absence of the cooperative carriers for reference. The detailed conditions are included in the tables of the text. It should be mentioned that the final pH of both aqueous phases (I) and (II) were determined after each transport experiment, and it was found that, in all cases studied, the difference between initial and final pH values is negligible.

Results and Discussion

The liquid membrane operated here is shown schematically in Fig. 1. After complexation of the cation with carrier on the left side of the membrane, the complex diffuses down its concentration gradient. On the right side of the membrane, Zn^{2+} ion would be extracted into the receiving aqueous phase via forma-

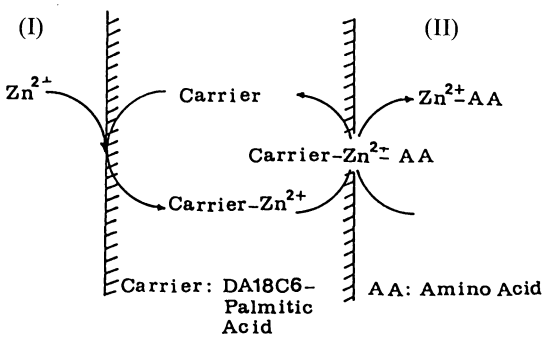

Fig. 1. Liquid membrane system for transport of Zn^{2+} ions.

tion of a ternary complex (carrier–metal ion–amino acid). Then, the free carrier diffuses back across the membrane. The net result is the transport of Zn^{2+} ion from the aqueous source phase to the aqueous receiving phase across the bulk of the organic phase (the membrane).

It was primarily observed that neither DA18C6 nor palmitic acid alone does transport the cation, but a given mixture of them (at a mole ratio of about 1 : 1) mediates the transport with surprisingly high efficiency (Table 1), in the presence of histidine in the receiving phase. This confirms the cooperative action of the two components as carrier. A possible explanation for this cooperative behavior would be the existence of some proton-donor proton-acceptor interactions between palmitic acid (as donor) and nitrogen atoms of DA18C6 (as acceptor) which can impart a greater degree of lipophilicity to the crown ether–cation complex, in order to facilitate the cation transport through liquid membrane.

It is noteworthy that, the preliminary extraction experiments showed a substantial bleeding of DA18C6 of about 60% its initial concentration from the chloroform phase into the aqueous phase, as determined by spectrophotometric titration of the organic phase with iodine.[19] However, the addition of palmitic acid to the organic phase was found to dramatically reduce the degree of DA18C6 loss by a factor of about 65%.

Permeability of the membrane system for Zn^{2+} ion also depends largely on the nature of amino acid in the receiving phase (Table 2), which is believed to play an essential role in the metal ion releasing process via formation of a ternary metal ion–carrier–amino acid complex. Such a ternary complex has already been recognized as being an important transient species in biological transport.[20] As it is seen from Table 2, 92% of Zn^{2+} ion transports through the liquid membrane during 2 h in the presence of histidine in the receiving phase, while the use of other amino acids results in no promising Zn^{2+} transport even if the experiment was carried out overnight. However, amino acids are hardly soluble in the membrane phase and hence they found to scarcely move into the aqueous source phase.

Moreover, the transport efficiency was found to be

Table 1. Effect of DA18C6 and Palmitic Acid Concentration on Zn^{2+} Transport[a]

Palmitic acid M×10³	DA18C6 M×10³	%Transported into receiving phase
1.5	0	1
1.5	0.43	85
1.5	0.87	89
1.5	0.98	96
1.5	1.2	96
1.5	1.7	94
1.5	2.6	50
1.5	4.0	36
0	1.2	2

a) Initial transport condition: Source phase, 5 cm³ of 4.0×10^{-4} M $Zn(ClO_4)_2$; receiving phase, 10 cm³ of 2.0×10^{-3} M histidine; liquid membrane, 30 cm³ of varying concentrations of carriers in chloroform; time of transport, 2 h.

Table 2. Effect of Amino Acid Present in Receiving Phase on Zn^{2+} Transport[a]

Amino acid	%Transported into receiving phase	Time of transport /h
His	92	2
Ala, Arg or Asn	<2	5
Gln	6	5
Asp	40	16
Gly	22	15
Thr	16	16
Ser	11	14
Phe	7	15
Tyr, Val, Try, Pro, Met, Lys, Lev, Glu, Cys or *iso*Lev	<5	15

a) Initial transport condition: Source phase, 5 cm³ of 4.0×10^{-4} M $Zn(ClO_4)_2$; receiving phase, 10 cm³ of 2.0×10^{-3} M amino acid; liquid membrane, 30 cm³ of 1.2×10^{-3} M DA18C6 1.5×10^{-3} M palmitic acid in chloroform.

Table 3. Effect of Carrier Structure on Zn^{2+} Transport[a]

Crown ether	Fatty acid	%Transport into receiving phase	%Remaining in source phase
18C6	Palmitic acid	0.5	96
DB18C6	Palmitic acid	0.4	99
DC18C6	Palmitic acid	0.6	99
B15C5	Palmitic acid	Trace	100
DB24C8	Palmitic acid	0.5	95
DB30C10	Palmitic acid	1.0	98
DA15C5	Palmitic acid	78	0.4
DA18C6	Palmitic acid	94	0.1
DA18C6	Oleic acid	92	0.1
DA18C6	Stearic acid	71	1.0

a) Initial transport condition: Source phase, 5 cm³ of 4.0×10^{-4} M $Zn(ClO_4)_2$; receiving phase 10 cm³ of 1.2×10^{-3} M histidine; liquid membrane, 30 cm³ of 1.2×10^{-3} M crown ether and 1.5×10^{-3} M fatty acid in chloroform; time of transport, 2 h.

critically depend on the nature of macrocycle used in the binary carrier and, in lower extent, on the structure of fatty acid used. In other experiments (and under the same experimental conditions) we tried several macrocyclic crown ethers other than DA18C6 as well as three different fatty acids as carrier for the transport of Zn^{2+} ion and the results are given in Table 3. As it is seen, with the exception of DA15C5 with 78% Zn(II) transport, in all other cases the results show just a negligible amount of ion transport. Thereby, it seems reasonable to assume that only those crown ethers containing nitrogen atoms as proton acceptor in their ring can act cooperatively in the presence of fatty acids, as proton donor centers, for the efficient transport of Zn^{2+} ions. Another words, the data seem to support the necessity of some proton donor-proton acceptor interactions between the two compounds of the binary carrier for its cooperative action in metal ion transport process. However, the difference in the complexing ability of DA18C6 and DA15C5 with metal ion[21] could be responsible for their different transport efficiency in the membrane system.

In addition, the nature of fatty acid used would also influence the flux of zinc transport. As it is seen from Table 3, the rate of Zn^{2+} transport varies in the order palmitic acid>oleic acid>stearic acid. Based on the above discussions, both the acidity and lipophilicity of fatty acids are expected to influence the transport efficiency.

Figure 2 shows the time dependence of Zn(II) transport througth liquid membrane under the optimum experimental condition. It is quite obvious that the extraction of Zn^{2+} ion from the aqueous source phase into the organic membrane occurs very rapidly, so that the extraction seems to be completed after approxi-

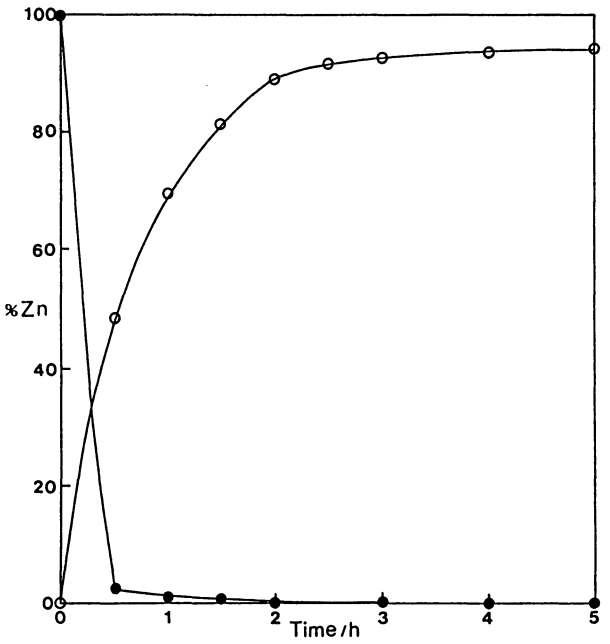

Fig. 2. Time dependence of Zn^{2+} transport. Initial transport condition: Source phase, 5 cm^3 of 4.0×10^{-4} M $Zn(ClO_4)_2$; receiving phase, 10 cm^3 of 2.0×10^{-3} M histidine; liquid membrane, 30 cm^3 of 1.2×10^{-3} M DA18C6 and 1.5×10^{-3} M palmitic acid in chloroform. (○) % Transported; (●) % remaining.

Table 4. Amount of Cation Transported from Various Cation Mixtures through Membrane[a]

Cation	Log K_f(water)[b] of M^{n+}-DA18C6	%Transported into receiving phase	%Remaining in source phase	%Existing in membrane
Mixture 1				
Zn^{2+}	4.31	90.0	4.8	5.2
Cu^{2+}	7.59	2.7	2.0	95.3
Ni^{2+}	3.43	1.9	46.0	52.1
Co^{2+}	3.25	0.3	74.0	25.7
Mixture 2				
Zn^{2+}	4.31	98.0	0.1	1.9
Hg^{2+}	18.75	<2	<2	>96
Pb^{2+}	6.90	<2	2.1	>96
Cd^{2+}	5.31	2.5	0.1	97.4
Mixture 3				
Zn^{2+}	4.31	88.0	1.2	10.8
Ca^{2+}	1.80	2.9	82.0	5.1
Mg^{2+}	—	3.8	—	—
K^+	—	<0.1	—	—
Na^+	—	<0.1	—	—
Mixture 4				
Zn^{2+}	4.31	95.0	0.7	4.3
Mn^{2+}	—	2.7	—	—
Mixture 5				
Zn^{2+}	4.31	91.0	1.0	8.0
Fe^{2+}	—	4.5	93.0	2.5

a) Initial transport condition: Source phase, 5 cm^3 of 4.0×10^{-4} M of each cation in the mixture; receiving phase, 10 cm^3 of 2.0×10^{-3} M histidine; liquid membrane, 30 cm^3 of 1.2×10^{-3} M DA18C6 and 1.5×10^{-3} M palmitic acid in chloroform, time of transport, 2 h. b) Ref. 18.

mately half an hour. However, the release of zinc ion into the aqueous receiving phase occurs at a slower rate. It was confirmed that about 96% of Zn^{2+} ion was transported form aqueous phase (I) into the aqueous phase (II) after 2 h, under the optimal conditions. The reproducibility of zinc transport was investigated and the percent of metal ion transported after 2 h obtained from ten replicate measurements was found to be 96.0±2.1.

In Table 4 are listed the percent of transported Zn^{2+} and M^{n+} cations, which were present with zinc in equimolar concentrations, into the receiving phase. The stability constants of the metal ion complexes with DA18C6 in aqueous solution are also included for comparison. The surprisingly high selectivity of zinc transport over the other cations used can be related to the previously proposed hypothesis[18,23] that predicts the existence of an optimum value for the stability constant K_f above or below which the rate of ion transport decreases. The data given in Table 4 indicate that cations Hg^{2+}, Pb^{2+}, Cd^{2+}, and Cu^{2+} are extracted almost completely in the organic membrane phase, but the resulting complexes seem too stable to be extracted into the aqueous receiving phase. On the other hand, Ni^{2+}, Co^{2+}, and Ca^{2+} ions form much weaker complexes with DA18C6 and, therefore, would remain largely in the source phase. The maximum observed transport occurs for Zn^{2+} ion with an intermediate complex stability of log K_f=4.31, which is probably within the optimum stability range necessary for a successful ion transport.

However, the high affinity of histidine for Zn^{2+} ion over other cations used[22] is another important factor in determining the efficiency of ion transport. For example, in the case of Ni^{2+} and Co^{2+} ions despite the low stability constants of their corresponding complexes with DA18C6, it is seen that a relatively large fraction of the source phase cations has been extracted into the organic membrane (Ni^{2+}: 52.1% and Co^{2+}: 25.7%). This large degree of extraction into the membrane implies that it is not for lack of extraction that these cations are transported poorly, but rather for lack of their release into the aqueous receiving phase. Thus, it seems reasonable to assume that poor transport of Ni^{2+} and Co^{2+} ions must more probably be due to the relatively low affinity of histidine for these two cations as compared to Zn^{2+} ion.

Furthermore, to investigate the contribution of kinetic factor on the transport selectivity, the transport experiments on two different cation mixtures were performed overnight and the results are given in Table 5. From the results it is evident that in the case of Cu^{2+}, Pb^{2+}, and Cd^{2+} ions which form stable DA18C6 complexes of much higher stabilities than the optimum range, the selectivity of Zn^{2+} over these cations seems to be mainly controlled by the thermodynamic factor. It is interesting to note that despite the higher stability of Cu^{2+} -DA18C6 complex than Pb^{2+} -DA18C6 and Cd^{2+} -DA18C6, the amount of copper transport overnight is much higher than that of Pb^{2+} and Cd^{2+} ions. This unexpected behavior can be due to higher affinity of histidine for copper is comparison with lead and cadmium.[22] On the other hand, in the case of Co^{2+}, Ni^{2+}, and Ca^{2+} ions with weaker DA18C6 complexes than Zn^{2+} ion, the kinetic factor strongly influences the selectivity behavior of the membrane system; the interferring effect of these cations increases in the order $Ca^{2+}<Co^{2+}<Ni^{2+}$, which is in fact the order of the increase in the stability of the corresponding DA18C6 complexes.

Table 5. Amount of Cation Transported from Various Cation Mixtures through Membrane after 15 h[a]

Cation	%Transported into receiving phase	%Remaining in source phase
Mixture 1		
Zn^{2+}	96.0	0.4
Cu^{2+}	11.4	0.9
Ni^{2+}	93.0	2.5
Co^{2+}	83.0	6.7
Mixture 2		
Zn^{2+}	100	0
Ca^{2+}	10.2	80
Pb^{2+}	0.18	0
Cd^{2+}	1.2	0

a) Initial transport condition: Source phase, 5 cm^3 of 4.0×10^{-4} M of each cation in the mixture; receiving phase, 10 cm^3 of 2.0×10^{-3} M histidine; liquid membrane, 30 cm^3 of 1.2×10^{-3} M DA18C6 and 1.5×10^{-3} M palmitic acid in chloroform.

In conclusion, the excellent efficiency and high degree of selectivity for Zn^{2+} ion transport demonstrated by the membrane system investigated has significant implications. Beside the biological importance of the membrane system studied here, our experiments demonstrate the potential application to selective removal, concentration or purification of zinc ion from mixtures.

The authors gratefully acknowledge the support of this work by the Shiraz University Research Council. The assistance of H. Parham from this research group in running some of the experiments is also acknowledged.

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