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Cooperative Effect of Lipophilic Amine and Neutral Crown Ether on Potassium Ion Active Transport through a Chloroform Membrane

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A competitive proton-driven transport of alkali metal ions with co-transported picrate anion was investigated in a chloroform membrane system containing either dicyclohexano-18-crown-6 (DC18C6), octadecylamine ($C_{18}NH_2$), or a DC18C6/$C_{18}NH_2$ mixture as cooperative carrier. The cooperative carrier effectively transported K^+ against its concentration gradient between the two aqueous phases. The DC18C6 alone, though being a proton-unionizable carrier, also mediated the uphill transport of K^+ when the receiving phase was strongly acidic. The mechanisms for these transport behaviors are discussed referring to additional data on the partitioning of species at membrane/aqueous phase interfaces.

The active transport of specific ions in an artificial liquid membrane system has received much attention in view of simulating biological membrane functions and developing the separation science methodology.[1-3] Research is in progress toward the synthesis of ionophores bearing a proton-ionizable moiety such as carboxyl,[4-6] phenolic hydroxyl,[7] and amino groups,[8] or a donor nitrogen atom in the macrocycle,[9,10] which are capable of active-transporting metal ions.

On the other hand, cooperative carrier-mediated uphill transports have been demonstrated, e.g., metal ion/proton counter-transport by a mixture of long-chain fatty acid and neutral crown ether[11] and amino acid anion/proton co-transport or amino acid anion/anion counter-transport by a mixture of lipophilic amine and neutral crown ether.[12] However, as to the proton-driven transport of metal ions, to our knowledge, no report has ever appeared on the cooperative carrier system with a lipophilic amine-crown ether mixture. Recently, a "double carrier" system for the uphill transport of metal ion has been described by Umezawa and co-workers.[13] Their system is different from the cooperative carrier system since they utilize two discrete liquid membranes containing neutral crown ether and lipophilic amine separately.

Most works on such an active transport of ions have focused on the behavior of the ions to be transported, except for a few cases.[13,14] For a better understanding of the overall transport mechanism, it is necessary to investigate in detail the behaviors of co- or counter-transported species as well. We report here a competitive proton-driven uphill transport of potassium ion by a cooperative carrier composed of octadecylamine and dicyclohexano-18-crown-6 through a chloroform liquid membrane. To gain insight into the mechanistic details, the transport and partitioning behaviors of both the metal ions in question and the co- or counter-transported anion were studied. Picrate was employed as a co-anion, which is frequently used in both facilitated and active transport systems because of its lipophilicity. The use of picrate salts permitted us to obtain information on the structure of the ion pair extracted into or carried across the chloroform membrane, by optical spectroscopy.

Experimental

Materials. Reagent grade dicyclohexano-18-crown-6 (DC18C6) was from Merck and was used without further purification. Reagent grade chloroform and dichloromethane from Wako Pure Chemicals were used as membrane solvents. Chloroform employed in solvent extraction experiments was of spectrograde from Dojindo Laboratories. Reagent grade KOH, LiOH, NaOH, KSCN, HCl, H_3PO_4, triethanolamine (TEA), tris(hydroxymethyl)aminomethane (Tris), picric acid (HPic) were from Wako Pure Chemicals, and tetramethylammonium hydroxide (TMAOH), 2-morpholino-1-ethanesulfonic acid (MES), ethanolamine (EA), octadecylamine ($C_{18}NH_2$) were from Tokyo Kasei Kogyo.

Transport Experiments. Membrane transport experiments were performed with a U-shaped glass tube placed in a thermostated incubator (20±0.2 °C) as previously reported.[15] The rates of ion transport were measured for the liquid membranes with either of three carrier systems: $C_{18}NH_2$ (1 mM; 1 M=1 mol dm^{-3}), DC18C6 (1 mM), and a mixture of $C_{18}NH_2$ (1 mM) and DC18C6 (1 mM). An alkali metal chloride (10 mM) and picric acid (5 mM) were dissolved in both the source (basic) aqueous phase and the receiving (acidic) aqueous phase in contact with the liquid membrane. The aqueous solutions were made up from a standardized stock solution (pH≈7) containing NaOH, LiOH, KOH, HCl, and picric acid. The pH of the receiving phase was varied while that of the source phase was kept constant (pH=12; adjusted with TMAOH). The acidity of the receiving phase was adjusted to pH 2 with a H_3PO_4–TEA buffer, pH 3—4 with a H_3PO_4-TMAOH buffer, pH 5 with a MES-TMAOH buffer, pH 7—9 with a Tris-HCl buffer, and pH 10 with an EA-HCl buffer. Aliquots (200 µl) of the aqueous solutions of both phases were withdrawn at appropriate intervals and then diluted 50 times. The alkali metal ions were determined on a Seiko Instruments ICP atomic emission spectrometer Model SPS 1200-VR and the picrate ion (Pic$^-$) concentration was determined spectrophotometrically at 354 nm (ε=14500 cm^{-1} M^{-1})[16] on a JASCO spectrometer Model Ubest-50.

Solvent Extration Experiments. Extraction experiments were carried out with a water/chloroform system by the procedure similar to that described previously.[15] The extracted amount (%E) of K^+ and Pic^- was calculated from the difference in their concentrations before and after the extraction. The pH in the aqueous phase was adjusted with one of the buffer solutions listed above. The absorbance of the picrate salt in the chloroform phase was measured in a wavelength range 300—500 nm.

Results and Discussion

Active Transport. Figure 1 shows a typical profile for the competitive proton-driven transport of three alkali metal ions through the chloroform membrane containing a $C_{18}NH_2$-DC18C6 mixture as a cooperative carrier, together with the initial concentrations of species. The cooperative carrier mediates an uphill transport of K^+ selectively from the basic aqueous phase (Aq. 1) to the acidic one (Aq. 2). This phenomenon is based on the protonation-deprotonation cycle of $C_{18}NH_2$ at the membrane/aqueous phase interface and on the complexation-decomplexation cycle of DC18C6 with K^+ at each interface, as represented in Fig. 2. At the Aq. 2/membrane interface, $C_{18}NH_2$ is protonated and then incorporated into the crown ring forming a lipophilic crown ether-$C_{18}NH_3^+$ complex. The complex is transferred through the membrane to the basic interface with associated Pic^-. At the Aq. 1/membrane interface, $C_{18}NH_3^+$ is deprotonated and proton is released into the basic phase. Immediately the DC18C6 forms selectively a complex with K^+ and carries it through the membrane to the acidic interface with co-transported Pic^-. At the acidic interface, K^+ is

Aq. 1	Membrane	Aq. 2
10 mM NaCl 10 mM KCl 10 mM LiCl 5 mM HPic	1 mM $C_{18}H_{37}NH_2$ 1 mM DC18C6 ($CHCl_3$)	10 mM NaCl 10 mM KCl 10 mM LiCl 5 mM HPic
0.1 M TMAOH pH = 12.1		0.1 M Tris-HCl pH = 7.1

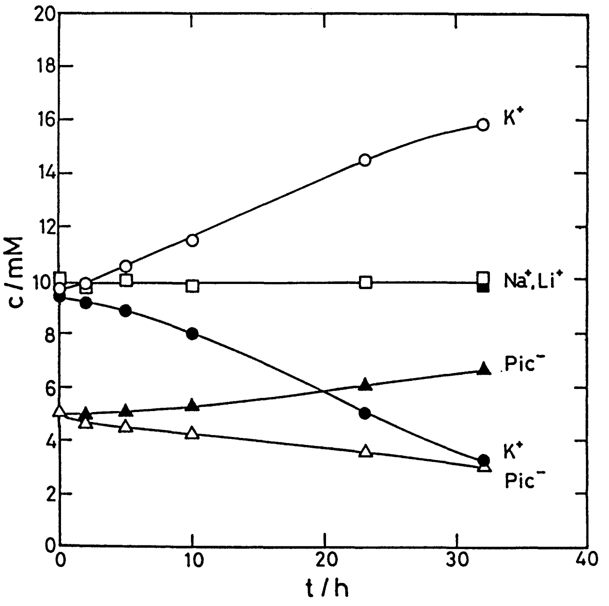

Fig. 1. Proton-driven active transport of K^+ through a chloroform membrane containing DC18C6 and $C_{18}NH_2$ as carrier. Solid and open symbols denote the concentration of ions in source (Aq. 1) and receiving (Aq. 2) aqueous phases, respectively.

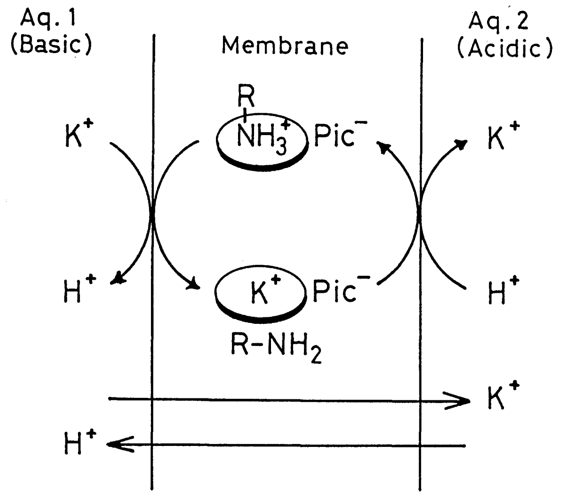

Fig. 2. Uphill transport of K^+.

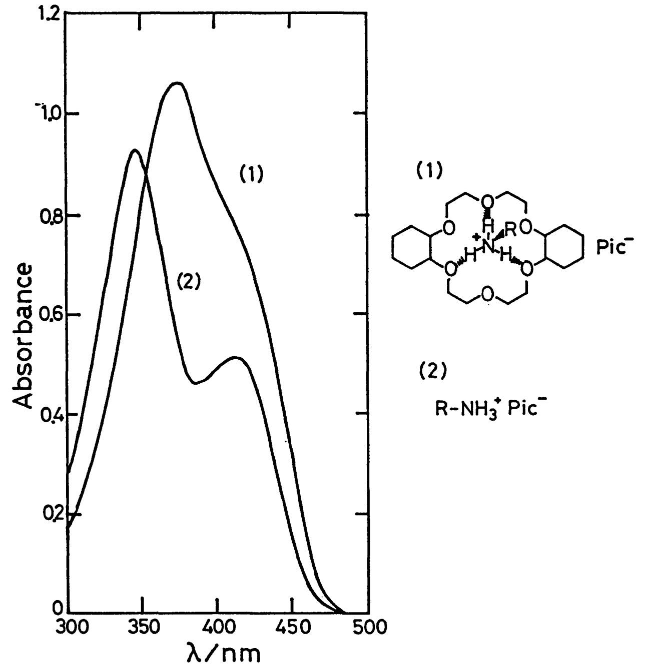

Fig. 3. Optical spectra of picrate salts in $CHCl_3$ extracted from HPic (2 mM) aqueous solution (pH=2.0) into chloroform containing (1) DC18C6 (1 mM)-$C_{18}NH_2$ (1 mM) mixture and (2) $C_{18}NH_2$ (1 mM).

released into the aqueous phase with concomitant complexation of DC18C6 with $C_{18}NH_3^+$. Thus, the pH gradient between the two aqueous phases promotes the transport of a specific metal ion against its concentration gradient.

Aq. 1	Membrane	Aq. 2
10 mM NaCl 10 mM KCl 10 mM LiCl 5 mM HPic 0.1 M TMAOH pH = 12.3	1 mM DC18C6 ($CHCl_3$)	10 mM NaCl 10 mM KCl 10 mM LiCl 5 mM HPic 0.1 M H_3PO_4-TEA pH = 2.0

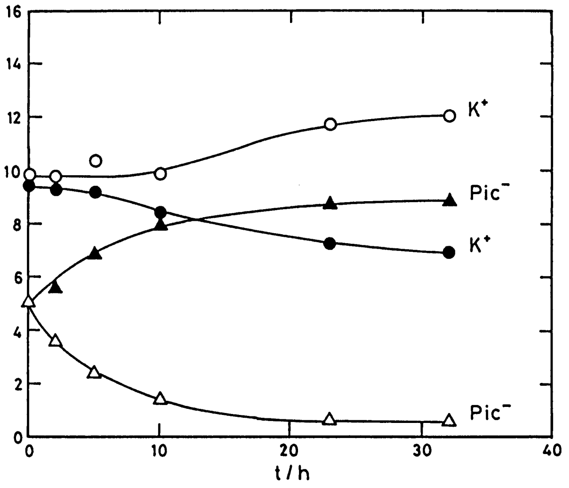

Fig. 4. Time courses for the uphill transport of K^+ and Pic^- through a chloroform membrane containing DC18C6 as carrier. Symbols are the same as in Fig. 1.

This finding suggests that formation of the DC18C6-$C_{18}NH_3^+$ complex enhances the release of K^+ at the acidic interface in the present system, resulting in an effective transport of K^+. Figure 3 shows the optical spectra of the picrate salts extracted from the acidic aqueous solution of picric acid into the chloroform with $C_{18}NH_2$ or a DC18C6-$C_{18}NH_2$ mixture. The ammonium cation picrate ($C_{18}NH_3^+Pic^-$) extracted into the $C_{18}NH_2$-containing chloroform is a tight ion pair with absorption maxima at 346 nm and 413 nm. Addition of DC18C6 to the chloroform phase caused a pronounced red-shift of the main peak from 346 nm to 374 nm. A second transition at 413 nm is little affected by the presence of DC18C6 and becomes a shoulder due to an overlap with the red-shifted main band. This bathochromic shift indicates the formation of an intermolecular complex of lipophilic ammonium cation-crown ether. Such a complexation has been suggested on the basis of a bathochromic shift observed in the optical spectra of homogeneous tetrahydrofuran solutions of a lipophilic amine, crown ether, and picric acid.[8] Our results demonstrate the complexation in the membrane solution in contact with the acidic aqueous solution of picric acid.

The transfer of proton from the acidic to the basic phase results in a simultaneous transfer of K^+ in the opposite direction, as predicted by the scheme in Fig. 2. Concomitantly, and unexpectedly, Pic^- was transferred across the membrane against its concentration gradient in the reverse direction of the K^+ transport (Fig. 1). On the other hand, DC18C6 alone, though being a proton-unionizable compound, was found to mediate the uphill transport of K^+ by rapidly pumping Pic^- from the acidic to the basic phase, as depicted in Fig. 4. To elucidate the background of these trans-

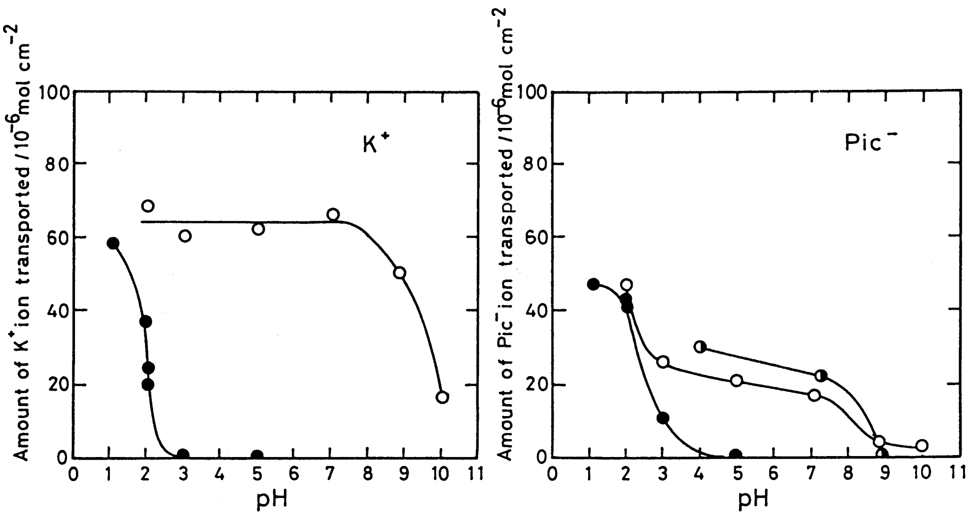

Fig. 5. Amounts of K^+ and Pic^- transported in 32 h as a function of pH in the receiving aqueous phase. The carriers employed are DC18C6 (●), DC18C6-$C_{18}NH_2$ mixture (○), and $C_{18}NH_2$ (◑).

port phenomena, we examined the effect of pH in the aqueous phases on the transport behaviors of K^+ and Pic^- by varying pH in the receiving phase (Aq. 2). The results are given in Fig. 5. Each experiment was repeated at least twice and the results were consistent within ±10%. There is little change in K^+ transportability by the DC18C6-$C_{18}NH_2$ cooperative carrier over the receiving phase pH range 2—7. In contrast, the counter-transport of Pic^- decreases monotonically with an increase in pH. The Pic^- transport should be driven by the pH gradient with co-transport of proton. With the use of DC18C6 alone as carrier, the uphill transport of K^+ occurs at the receiving phase pH values below 3, and the pH increase in the receiving phase leads to abrupt diminution in the transportability of both K^+ and Pic^-. The details of the underlying mechanisms for these phenomena could be understood on the basis of the partitioning of species between the aqueous and chloroform phases.

Extraction in $H_2O/CHCl_3$ Systems. Figures 6 and 7 depict the percent extraction (%E) of ions against the pH in the aqueous phase in a $H_2O/CHCl_3$ extraction system. The %E values obtained in 3—5 independent runs for a given system agreed with each other within ±10% except for the values for K^+ at pH 2 and 5 in the HPic-KCl/DC18C6 system. In the HPic (aqueous phase)/DC18C6 (organic phase) system, the %E values for Pic^- rises below pH 4 in the aqueous phase (Fig. 6). Pic^- would be extracted as an undissociated acid form into chloroform; the pK_a for HPic is 0.38.[17] A large amount of picric acid was actually extracted into neat chloroform from the acidic aqueous solution (pH 2) as in the following observations: 58.7% into reagent grade $CHCl_3$; 58.4% into spectrograde $CHCl_3$ 58.4% into alcohol-free $CHCl_3$ washed three times with deionized water. The amount of Pic^- extracted, therefore, is lowered as HPic is dissociated with increasing pH. Although the increasing pH lowers the extracted amount of Pic^- in HPic/$C_{18}NH_2$ system as well, the %E values remain high even at pH's above 4 where Pic^- is hardly extracted in the HPic/DC18C6 system. The high extractability may be due to the extraction in the form of an ion pair, $C_{18}NH_3{}^+Pic^-$. No picrate, hence, is extracted at pH 10 which is near the pK_a (10.6)[17] of $C_{18}NH_2$. In the HPic/DC18C6-$C_{18}NH_2$ system, formation of a lipophilic DC18C6-$C_{18}NH_3{}^+$ complex enhances the uptake of Pic^- into chloroform, so that the %E values are higher at pH's above 4 than those for the HPic/$C_{18}NH_2$ system. However, a negligible amount of Pic^- is extracted at pH 12 where $C_{18}NH_2$ is no longer protonated.

Addition of KCl to the aqueous phase affects the extraction of Pic^- (Fig. 7). In the case of DC18C6 alone, the extractability of Pic^- is constant at pH's above 5, while K^+ is extracted over a wide pH range.

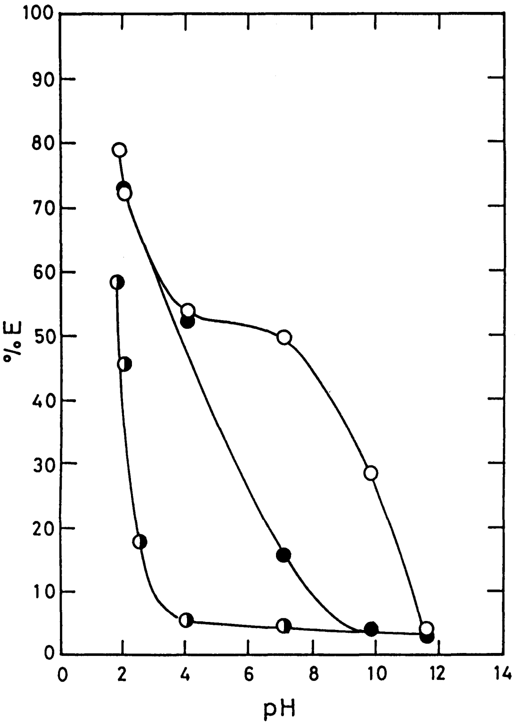

Fig. 6. Effect of pH in the aqueous phase on the extractability (%E) of picric acid in the $H_2O/CHCl_3$ extraction systems: HPic/DC18C6(◑), HPic/$C_{18}NH_2$ (●), and HPic/DC18C6-$C_{18}NH_2$ (○). [HPic] in water is 2 mM; [DC18C6] or [$C_{18}NH_2$] in $CHCl_3$ is 1 mM.

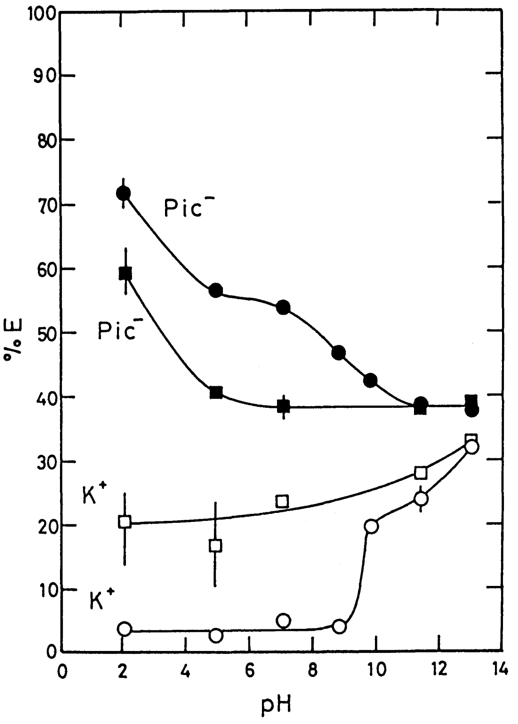

Fig. 7. Effect of pH in the aqueous phase on the %E for K^+ and Pic^-. □, ■: HPic-KCl/DC18C6; ○, ●: HPic-KCl/DC18C6-$C_{18}NH_2$. [HPic] and [KCl] in the aqueous phase are 2 mM and 4 mM, respectively.

The uptake of Pic^- and K^+ indicates the ion pair formation between the K^+-DC18C6 complex cation and Pic^-. The high extractability of Pic^- at pH's below 5 is a result of the overlap with the dissolution in the undissociated picric acid. In the DC18C6-$C_{18}NH_2$ systems, K^+ is hardly extracted at pH's up to 9 and the extraction behavior of Pic^- is similar to that in the absence of K^+ in the aqueous phase. However, the %E values for K^+ increase at pH above 9. This extraction behavior clearly reflects the spectral change for species in the chloroform phase.

In Table 1, the absorption maxima (λ_m) are listed for the picrate salts in the chloroform phase as a function of aqueous phase pH. In the HPic/DC18C6-$C_{18}NH_2$ system, the absorption maximum is virtually independent of pH in the aqueous phase, indicating that the species in question is a $[C_{18}NH_3\text{-DC18C6}]^+Pic^-$ complex. The presence of K^+ in the aqueous phase leads to a blue-shift with pH increasing beyond 7. Furthermore, the absorption maximum at pH 13 (368 nm) agrees closely with that for the $[K\text{-DC18C6}]^+Pic^-$ complex (367 nm) extracted with DC18C6 alone at pH 13. This λ_m is close to that (365 nm) for $[K\text{-18C6}]^+Pic^-$ complex in chloroform reported by Wong et al.[18] Therefore, the hypsochromic shift suggests the conversion of the $C_{18}NH_3^+$-DC18C6 complex into the K^+-DC18C6 complex, caused by deprotonation of $C_{18}NH_3^+$. These results are compatible with the pH dependence of the K^+ extractability in the DC18C6-$C_{18}NH_2$ system shown in Fig. 7.

Table 1. Effect of pH in the Aqueous Phase on the Absorption Maxima (λ_m) of Picrate Salts in Chloroform for Three Salt-Ligand Combinations in $H_2O/CHCl_3$ Extraction Systems[a)]

Salt (Aq phase)	Ligand ($CHCl_3$ phase)	pH	λ_m nm
HPic	DC18C6-$C_{18}NH_2$	2.0	374
		7.0	377
		11.6	377
HPic-KCl	DC18C6-$C_{18}NH_2$	2.0	374
		4.9	376
		7.0	377
		8.8	374
		9.8	372
		11.4	369
		13.0	368
HPic-KCl	DC18C6	13.0	367

a) Initial concentrations of species in the extraction systems are given in Figs. 6 and 7.

Transport Mechanism. The uphill transport of K^+ by the proton-unionizable DC18C6 into a strongly acidic receiving phase could be explained in terms of the scheme illustrated in Fig. 8. As is evident in Fig. 4, Pic^- is rapidly pumped into the basic source phase, resulting in the build-up of Pic^- concentration gradient between the two aqueous phases, and then the K^+ transport begins. This finding suggests that the uphill transport of K^+ in this case is driven by the concentration gradient of Pic^-, so that the K^+ transport is reduced with decreasing counter-transport of Pic^- due to the increasing pH of Aq. 2 (Fig. 5). The picrate probably diffuses in an undissociated form across the chloroform membrane from Aq. 2 to Aq. 1

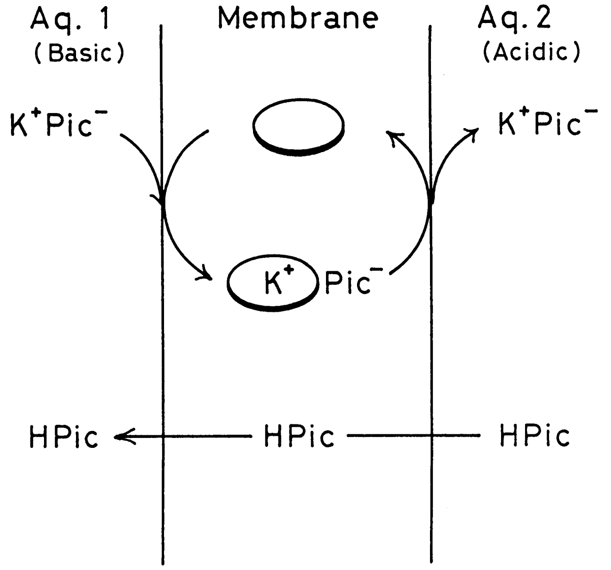

Fig. 8. Active transport of K^+ through a chloroform membrane containing DC18C6.

Table 2. Amounts of K^+ and Pic^- Transported in 32 h through Liquid Membranes

Entry	pH Aq.1	pH Aq.2	Carrier	Membrane solvent	Amount transported 10^{-6} mol cm^{-2} K^+	Amount transported 10^{-6} mol cm^{-2} Pic^-
1[a)]	12.3	2.0	—	$CHCl_3$	—	49
2	12.3	2.0	DC18C6	$CHCl_3$	24	41
3	12.3	2.2	DC18C6	CH_2Cl_2	29	40
4	11.9	2.1	DC18C6	$CHCl_3$	0	—
5	12.1	2.0	DC18C6-$C_{18}NH_2$	$CHCl_3$	68	47
6	12.3	2.2	DC18C6-$C_{18}NH_2$	CH_2Cl_2	64	33

a) No carrier. Aq.1 and Aq.2 of Entry 4: [KSCN]=10 mM; Aq.1 of Entries 3 and 6: [NaOH]=[LiOH]=[KOH]=10 mM, [HPic]=5 mM.

driven by the H^+ gradient without complexation with carrier. This picture is supported by the following three observations: Pic^- is extracted from a strongly acidic aqueous solution not only into chloroform containing DC18C6 but also into ligand-free chloroform (see above). Second, a large amount of Pic^- is transported through the chloroform membrane containing no carrier from Aq. 2 to Aq. 1 against its concentration gradient, as given in Table 2 (Entries 1—3) in comparison with the values for DC18C6. Thirdly, a highly ionizable KSCN was not transported by DC18C6 alone even at pH 2 (Entry 4 in Table 2). Such an uphill transport of metal ion by a neutral crown ether alone would produce an artifact when we evaluate a proton-coupled uphill transport by a proton-ionizable carrier.

On the other hand, K^+ transport by a DC18C6–$C_{18}NH_2$ mixture occurs even at receiving phase pH's above 3, and the amount of K^+ transported is constant in spite of a decrease in the counter-transport of Pic^-. The K^+ transport is apparently driven not only by the Pic^- gradient. If Pic^- is transferred only in the direction from Aq. 2 to Aq. 1, the K^+ transport requires co-transported anion (A^-) other than Pic^-, as depicted in Fig. 9. Beside picrate, OH^- and Cl^- are present in the source aqueous phase as co-transportable anions. The highly hydrated OH^- may not be co-transported by the K^+-DC18C6 complex cation through a low polarity liquid membrane such as chloroform and dichloromethane. Maruyama et al. demonstrated that Cl^- is transported as an antiport anion in the anion/amino acid anion counter-transport through a chloroform membrane containing a lipophilic ammonium cation-crown ether complex as carrier.[19] To see whether the Cl^- is co-transported or not in the present systems, the K^+ transport was studied in the system with a Cl^--free source aqueous phase by using a dichloromethane membrane which would not liberate HCl photochemically. The results are given in Table 2 (Entries 5 and 6) in comparison with the value for $CHCl_3$. The K^+ transportability was not affected by the absence of Cl^- both in the source aqueous and liquid membrane phases. Consequently, the K^+-DC18C6 complex should be transferred with associated Pic^- from the source to the receiving phase, i.e., A^- is Pic^- in Fig. 9.

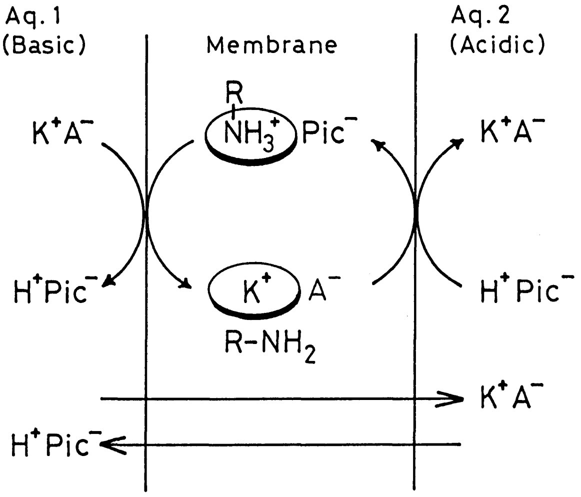

Fig. 9. Active transport of K^+ through a chloroform membrane containing a cooperative carrier, DC18C6-$C_{18}NH_2$.

However, if the ion transport obeys a 1 : 1 stoichiometry between K^+ and H^+ as shown in Fig. 9 or Fig. 2, Pic^- is merely recycled and no Pic^- transport would result. Hence, we have to envisage other processes to account for the Pic^- transport observed here. There are two possibilities; one is the pumping up of Pic^- by $C_{18}NH_2$ using the concentration gradient of H^+, and the other is the diffusion of undissociated HPic driven by the pH gradient. The former was confirmed by the transport experiments where $C_{18}NH_2$ alone was used as carrier, as seen in Fig. 5. The pH dependence of the Pic^- transportability is similar to that for the DC18C6-$C_{18}NH_2$ carrier system. When the receiving solution is strongly acidic, the latter process indeed occurs as mentioned above. These results incite us to conclude that the three processes are occurring simultaneously in the DC18C6-$C_{18}NH_2$ cooperative carrier system, as depicted schematically in Fig. 10.

The dependence of the K^+ and Pic^- transportabilities on the pH in the receiving phase (Fig. 5) could be

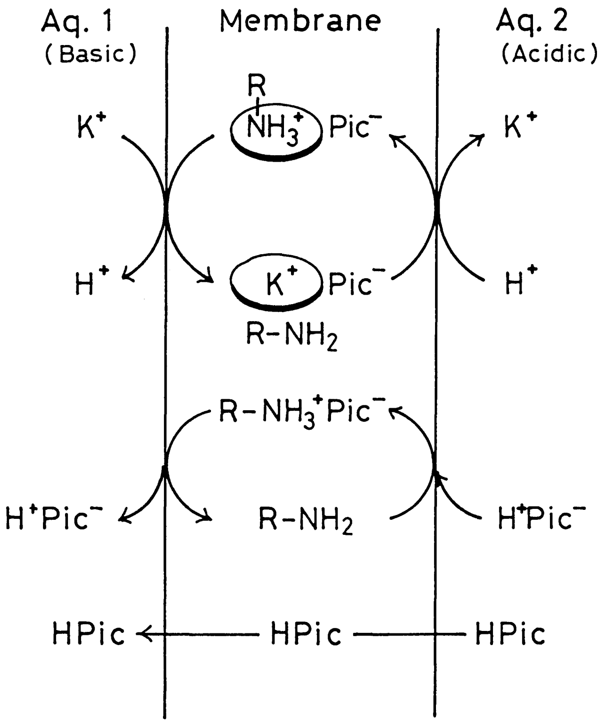

Fig. 10. Three processes occurring simultaneously in the active transport of K^+ and Pic^- through a chloroform membrane containing a DC18C6-$C_{18}NH_2$ mixture.

rationalized on the basis of these three steps. At pH's below 3, three processes occur simultaneously, so that a large amount of Pic^- is counter-transported. In a pH range 3—9, two processes other than the HPic diffusion are taking place simultaneously. The lowering Pic^- transport with increasing pH may be due to a reduction in the fraction of the protonated amine, $C_{18}NH_3^+$, at the acidic interface. This is compatible with the diminishing %E of Pic^- with increasing pH for the HPic/$C_{18}NH_2$ extraction system (Fig. 6). At pH's above 9, only the top process in Fig. 10 is possible, namely the K^+ transport should be coupled to the H^+ transport because no Pic^- transport occurs. The K^+ transport tends to diminish above pH 9, because of the lowering H^+ flux due to a decrease in the fraction of the $C_{18}NH_3^+$-DC18C6 complex at the acidic interface, as seen in Fig. 6. When the 1 : 1 K^+/H^+ stoichiometry is no longer maintained, part of the $[C_{18}NH_3\text{-DC18C6}]^+Pic^-$ complex carries Pic^- with proton to the source phase and takes up no K^+ at the basic interface. If the $[C_{18}NH_3\text{-DC18C6}]^+Pic^-$ complex carries Pic^- to Aq. 1, the lowered Pic^- transport results in a decrease in the H^+ transport which is the driving force for the uphill transport of K^+. This is in conflict with the results shown in Fig. 5. However, the presence of $C_{18}NH_3^+Pic^-$ at the acidic interface could not be confirmed from the optical spectra of picrate salts given in Fig. 3 and Table 1.

In the present system, H^+ may be transported from Aq. 2 to Aq. 1 in the forms of $(C_{18}NH_3^+\text{-DC18C6})Pic^-$, $C_{18}NH_3^+Pic^-$, and HPic. The transport of an ion-pair with Cl^-, PO_4^{3-}, or MES^- across the low polarity chloroform membrane is negligible, since these anions are more hydrophilic than Pic^-. The amines employed as buffer, TMAOH, TEA, Tris, and EA, are capable of H^+-carrying based on their protonation, but these amines are again very hydrophilic. Consequently, the amount of H^+ transported should be equal to the sum of those of K^+ and Pic^- transported, as seen in Fig. 10. However, the transport of H^+ or OH^- was not detectable due to the high buffer capacity of the aqueous phases. Although the Pic^- concentration gradient probably acts as part of the driving force for the K^+ uphill transport in a pH range where Pic^- is transported, the main process should be the proton-coupled transport of K^+. In the present transport system, the pumping up of Pic^- is not a dominant factor for the K^+ uphill transport, since the efficiency of the latter remains constant in a wide pH range where the Pic^- transport diminishes with increasing pH. The Pic^- transport unfortunately consumes the H^+ concentration gradient. In a "double carrier" membrane system[13] where the metal ion transport and the Pic^- pumping up using the H^+ gradient proceed in parallel, the pumping up of Pic^- plays an essential role in transporting the metal ion against its concentration gradient. Hence the transportability of Pic^- would directly control the uphill transportability of the metal ion in that case.

Conclusions

A selective uphill transport of a metal ion can be achieved in a wide range of the receiving phase pH by use of a lipophilic amine-neutral crown ether mixture as a cooperative carrier in the liquid membrane system. The complexation of crown ether with amine enhances the release of K^+ at the acidic interface, resulting in an effective transport of K^+. The main process in the present system is a K^+/H^+ coupled counter-transport, though three processes are occurring simultaneously. In contrast, the uphill transport of a metal ion by neutral crown ether alone is driven by the Pic^- concentration gradient which is built up by the diffusion of picric acid molecule when the receiving phase is strongly acidic. This is however an unwanted phenomenon in quantitating the proton-coupled uphill transport by a carrier bearing a proton-ionizable moiety. The cooperative carrier system may find applications in designing a synthetic ionophore capable of active-transporting a specific ion, or in examining fundamental transport behaviors of various amine-ionophore combinations.

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