Enforced Ion Release in Proton-driven Membrane Transport

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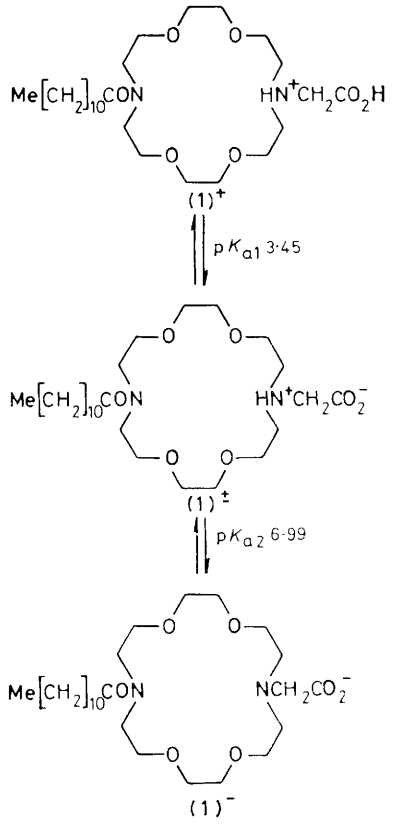
An aza-crown ether with a carboxylate cap acts as a new class of ion carrier for membrane transport, exhibiting rate acceleration due to enforced ion release in the neutral pH region and ion transport against its concentration gradient.

Cations are known to be transported through membranes with the aid of both synthetic macrocyclic polyethers and antibiotics. In particular, polyether antibiotics such as nigericin and monensin feature coupled counter-transport of cations and protons.[1-3] This phenomenon stems from *enforced* ion release which is mediated by interconversion of the cyclic and acyclic forms.[2-7] Although coupled counter-transport can be mimicked in an artificial system using crown ethers with an anionic cap,[6,7] the crown ether carriers do not permit as ready an enforced ion release as do the polyether antibiotics. In order to mimic this *enforced* ion release as well as the coupled counter-transport of cations and protons, we synthesised the ionophore (**1**) for which the following novel behaviour was expected. (i) Ion release from the membrane phase to the second (OUT) aqueous phase should be enforced by protonation of the ring nitrogen. (ii) The main species in the neutral pH region, $(\mathbf{1})^{\pm}$, should be highly lipophilic owing to its zwitterionic structure. (iii) The counter-current of protons should occur from the neutral OUT aqueous phase.

The carrier (**1**) was prepared by mono-*N*-alkylation of 1,10-diaza-4,7,13,16-tetraoxa-18-crown-6 with monochloroacetic acid, followed by acylation of the remaining amine with



dodecanoyl chloride. The product (**1**) (oil) was isolated by t.l.c. and was identified by n.m.r. spectroscopy and elemental analysis.

Potentiometric titration (30 °C) gave two pK_a values: pK_{a1} = 3.45 and pK_{a2} = 6.99. The fraction of zwitterionic $(\mathbf{1})^{\pm}$ thus becomes a maximum at pH 5.22 [= (pK_{a1} + pK_{a2})/2]. We estimated the partition of (**1**) between chloroform and aqueous solution by its absorbance at 300 nm (O.D.$_{300}$) which is on the lower slope of the absorption band of (**1**). The O.D.$_{300}$ values of the chloroform layer measured after agitation with equal volumes of following aqueous solutions were: 89.7% with 0.01 N HCl, 95.5% with pH 5.23 (0.05 M Tris–AcOH buffer), 72.7% with 0.01 M Et_4NOH, and 98.5% with 0.01 M KOH. The result indicates that the solubility of (**1**) in chloroform is in the order, $(\mathbf{1})^-$–K^+ complex > $(\mathbf{1})^{\pm}$ > $(\mathbf{1})^+$ > $(\mathbf{1})^-$.

The transport of alkali and alkaline earth metal cations across a chloroform membrane was studied using a U-tube apparatus immersed in a thermostatted water bath (30 °C). The result of the ion transport is summarized in Table 1. A number of points are worthy of mention. First, the rate of K^+ transport in the passive transport (entries 1—5) becomes a maximum when the OUT aqueous phase is maintained in the neutral pH region. When the OUT aqueous phase is strongly acidic or basic, the rates are significantly suppressed. These results indicate that the best K^+ transport system consists of the coupled counter-transport of K^+ and protons mediated by interconversion between the $(\mathbf{1})^-$–K^+ complex (IN → OUT) and $(\mathbf{1})^{\pm}$ (OUT → IN). One may envisage that the ion release is a slow, rate-limiting step when the OUT aqueous phase is basic, whereas the ion release is facilitated by enforced ion release due to protonation of the ring nitrogen when the OUT aqueous phase is neutral. A similar enforced ion release may be expected for $(\mathbf{1})^+$, the main species at acidic pH region. However, this species is less soluble in the membrane phase, and the overall transport efficiency is inferior to that of $(\mathbf{1})^{\pm}$.

Table 1. Initial concentrations and maximum rate of ion transport by carrier (**1**).[a]

Entry	IN aqueous phase (conc./mM)	[Carrier (**1**)] mM	OUT aqueous phase (conc./mM)	Relative rate of ion transport
1	KOH (10)	1.0	HCl (100)	1.9
2	KOH (10)	1.0	HCl (10.0)	2.2
3	KOH (10)	1.0	pH 5.24 with tartarate (50)–Tris	5.8
4	KOH (10)	1.0	pH 6.99 with Tris (50)–HCl	5.5
5	KOH (10)	1.0	Et_4NOH (10)	1.0[b]
6	NaOH (10)	1.0	pH 6.99 with Tris (50)–HCl	1.5
7	KOH (10)	0.50	KCl (10) in HCl (50.0)	0
8	KOH (10)	0.50	KCl (10) in pH 5.24 with AcOH (50)–Tris	0.57
9	$CaCl_2$ (8.9) $Ca(OH)_2$ (2.0) Picric acid (3.8)	1.0	HCl (10.0)	1.4
10	$CaCl_2$ (8.9) $Ca(OH)_2$ (2.0) Picric acid (3.8)	1.0	pH 5.25 with AcOH (50)–Tris	0.56
11	$CaCl_2$ (8.9) $Ca(OH)_2$ (2.0) Picric acid (3.8)	1.0	Et_4NOH (10)	0
12	$Ba(OH)_2$ (12.5) Picric acid (4.3)	1.0	pH 5.25 with AcOH (50)–Tris	13.3

[a] IN and OUT aqueous phase, 25 ml each; membrane phase, 50 ml of chloroform. [b] 1.7 μmol h^{-1} (absolute rate of ion transport).

The high efficiency of $(\mathbf{1})^{\pm}$ is more clearly demonstrated by the proton-driven active transport of K^+ (entry 7, 8). The net active transport to the neutral OUT aqueous phase took place at 0.97 μmol h^{-1}, whereas that to the acidic (0.05 M HCl) OUT aqueous phase was not detected at all. This result provides evidence that only $(\mathbf{1})^{\pm}$ is able to act as a recycle ion carrier and is able to carry K^+ against its concentration gradient.

In passive transport, Na^+ was carried less effectively than K^+, and Ca^{2+} less effectively than Ba^{2+} (entries 6, 9, 12).

Finally, entries 9—12 in Table 1 reveal that Ca^{2+} is transported to the acidic OUT aqueous phase more efficiently than to the neutral OUT aqueous phase. This implies that the enforced Ca^{2+} release occurs only from $(\mathbf{1})^+$ and the protonation of the ring nitrogen is not sufficient to eject the bound Ca^{2+} ion. The trend is quite different from that of K^+ transport and probably stems from the high affinity of Ca^{2+} with the carboxylate group.[2,6]

In conclusion, the present system provides a new class of ion transport involving rate acceleration due to the enforced ion release and ion transport against its concentration gradient due to the coupled counter-transport of protons. The results may provide important insight into natural ion-transport systems.

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