A dual host approach to transmembrane transport of salts

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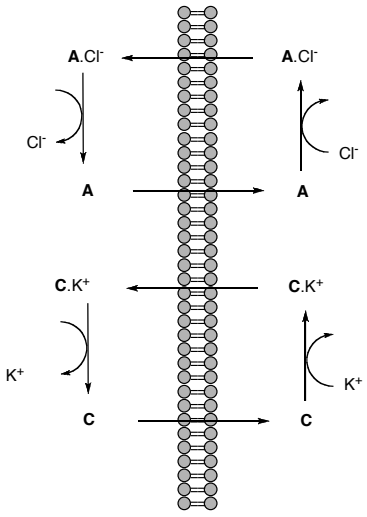
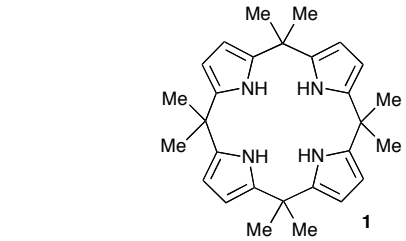
A dual host approach for M^+/Cl^- co-transport has been shown to be effective in lipid bilayers consisting of POPC using fluorescence-based transport assays.

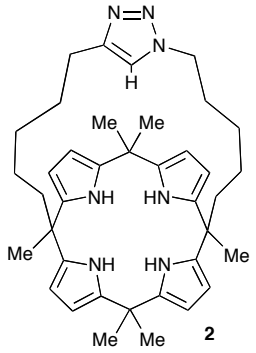
Ion pair complexation has attracted much attention recently both in the development of receptors that can complex both components of a salt and also compounds that can transport salts across lipid bilayer membranes.[1] This latter application is driven by the need to find future approaches to the treatment of diseases such as cystic fibrosis that are caused by the misregulation of chloride transport across epithelial cell membranes.[2] Cross talk occurs between different cation and anion transport processes in epithelial cell membranes[3] and there are a number of examples of coupled cation anion transport in biological membranes.[4] Gokel and co-workers has previously shown that valinomycin, a naturally occurring cyclic depsipeptide which has been shown to transport potassium cations across cell membranes, can be used with synthetic chloride channels to effect release of salts from liposomes[5] and similar effects have been observed with biological chloride channels.[6] The combination of separate anion and cation receptors in the extraction of metal salts from aqueous to organic solution as proven to be a successful strategy.[7] We wished to explore whether the same approach could be used to co-transport metal salts across lipid bilayers by carriers. This is shown schematically in Scheme 1 and is achieved *via* two synergistically coupled uniport processes in which a cation transporter **C** and an anion transporter **A** each transport an ion resulting in effective co-transport of a salt.

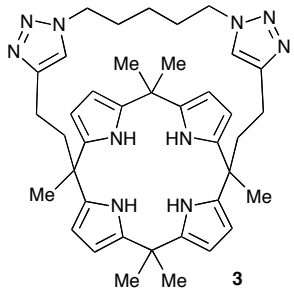


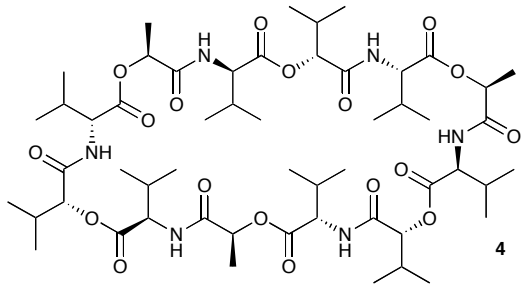
Scheme 1 A dual host approach to the co-transport of KCl by cation transporter **C** and anion transporter **A**.

To achieve this we examined the chloride transport properties of calix[4]pyrroles **1**-**3** in the absence and presence of valinomycin **4**.[8] K^+/H^+ exchange by valinomycin and protonophores such as chlorophenols have previously been shown to be coupled processes.[9]









meso-Octamethylcalix[4]pyrrole **1** has been shown to transport chloride across lipid bilayers only as part of a caesium chloride ion-pair[10] whilst triazole-strapped calix[4]pyrroles **2** and **3** additionally transport chloride *via* an antiport process with nitrate, in which cations are not involved.[11] So whilst compounds **2** and **3** and valinomycin could co-transport metal salts such as KCl *via* the mechanism shown in Scheme 1, this should not be possible for compound **1** and valinomycin.

POPC vesicles were prepared in 71mM sodium sulfate solution buffered to pH 7.2 with sodium phosphate and containing 1mM lucigenin (a chloride sensitive fluorescent dye). The vesicles were passed through a Sephadex G-50 column to remove unencapsulated dye and then diluted with a solution containing 5 mM sodium phosphate and 71 mM sodium sulfate solution to form a stock solution of vesicles containing encapsulated lucigenin.

A pulse of KCl (such that the concentration of added metal salt in the extravesicular solution was 100 mM) was added and the solution allowed to equilibrate for 30s. The carriers were then added in acetone to give 2 % molar carrier to lipid concentration. Chloride influx was monitored by following fluorescence changes of the encapsulated lucigenin (see ESI). The results for addition of potasium chloride are shown in Figures 1 – 3 for compounds **1** – **3** respectively. In each case the results show the effect of adding calixpyrrole and valinomycin alone on the internal chloride concentration of the vesicles and the effect of adding valinomycin and calixpyrrole together. Additionally the 'sum' of the change in concentration of chloride by adding valinomycin and calixpyrrole separately is shown. This illustrates in the case of compounds **2** and **3** the significant synergistic effect on chloride transport of adding the cationophore and anionophore together (there is a significant difference between the sum of the individual concentration changes for adding the valinomycin and calixpyrrole separately *vs.* the concentration change when calixpyrroles **2** or **3** are added together with valinomycin.

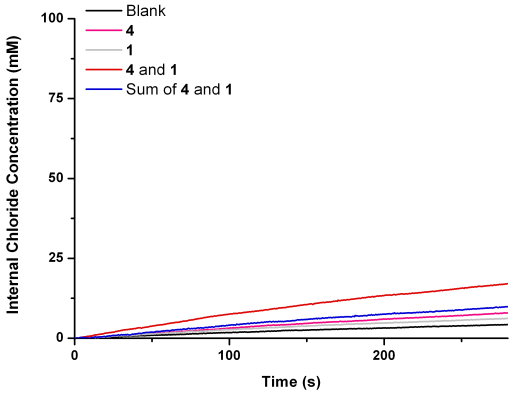


Figure 1 Change in internal chloride concentration of unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **4** (2mol%), **1** (2mol%) and both **4** and **1** (2mol% each) following a KCl pulse bringing the extravesicular KCl concentration to 100 mM.

As *meso*-octamethylcalixpyrrole predominantly transports chloride *via* an ion-pair transport process with CsCl then only a relatively small increase in the internal chloride concentration was observed when compound **1** and valinomycin were added together (Figure 1).

In the case of compounds **2** and **3**, the internal concentration of chloride increases significantly more quickly when the valinomycin and triazole strapped calixpyrroles are added together, evidence supporting a co-transport process occurring as shown in Scheme 1. Upon addition of compound **2** alone there is an increase in the internal chloride concentration. Chloride transport by this compound under similar conditions has been observed previously.[8] However the enhancement in the rate of chloride transport when valinomycin and calixpyrrole **2** are added together is still significant in this case (Figure 2). Addition of calixpyrrole **3** and valinomycin together gives a very clear enhancement in chloride transport rate over addition of the individual component (Figure 3). Experiments conducted with NaCl and RbCl show similar results (see ESI).

It should be noted that in previous work, Smith and co-workers had shown that salt transport could be achieved using an isophthalamide-based receptor containing a crown ether strapped across the anion binding site.[12] In that case when a simple isophthalamide and a benzyl-functionalised diazozcrown ether, which represented the individual binding sites in the ion pair receptor, were added together no transport occurred.

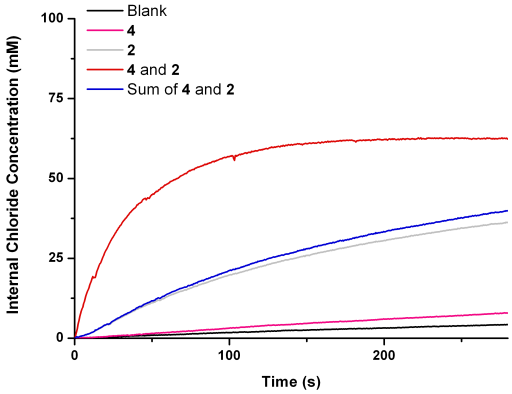


Figure 2 Change in internal chloride concentration of unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **4** (2mol%), **2** (2mol%) and both **4** and **2** (2mol% each) following a KCl pulse bringing the extravesicular KCl concentration to 100 mM.

Conclusions

We have shown that addition of both a cationophore and anionophore together can result in a signficnatly enhanced rate of anion transport through a lipid bilayer membrane. By separating the components of a co-tranport process (or in the future an antiport process) it may be possible to design receptors to optimise the transport of each ion transported and hence enhance transport rates and selectivity. These studies are currently underway in our laboratory.

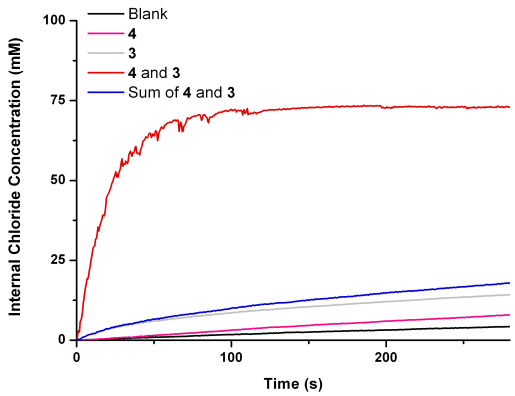


Figure 3 Change in internal chloride concentration of unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **4** (2mol%), **3** (2mol%) and both **4** and **3** (2mol% each) following a KCl pulse bringing the extravesicular KCl concentration to 100 mM.

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Notes and references

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† Electronic Supplementary Information (ESI) available: details of the membrane transport studies and graphs showing change in internal chloride concentrations for analogous experiments conducted with NaCl and RbCl. See DOI: 10.1039/b000000x/

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