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Catechols as Membrane Anion Transporters

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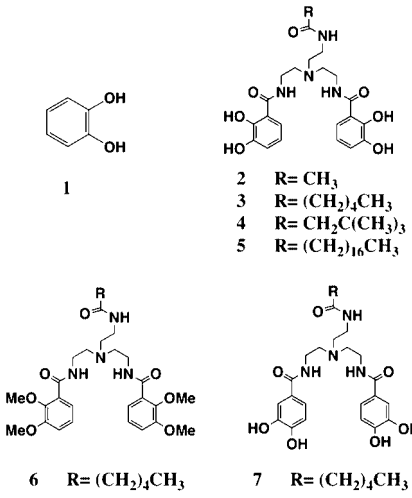
Compounds that transport ions across cell membranes may function as sensors, signal transducers, or antimicrobials. Synthetic transporters may also help us understand how natural systems move ions across hydrophobic barriers.[1] There is an interest in identifying compounds that transport anions, especially Cl^-, across lipid membranes.[2] Reports that catechol **1** binds Cl^- in organic solvents prompted us to use this group to form new anion transporters.[3,4] While bacterial siderophores and synthetic analogues use catecholates to move Fe^{3+} across membranes,[5] this paper is the first report, to our knowledge, of catechols facilitating transmembrane transport of anions.

We report that bis-catechol **3** is an anion transporter whose activity depends on the catechol's substitution and amphiphilicity. We also describe a liposomal assay that allows one to readily measure anion transport selectivity. This assay shows that transport facilitated by **3** follows the Hofmeister sequence,[6] wherein anions that are easier to dehydrate are made more permeable to the membrane by this bis-catechol.

Chart 1. Structures of Catechol **1** and Bis-catechols **2**−**7**



ESI-MS analysis showed that a dimer, $\mathbf{1}_2{\bullet}Cl^-$, was the major complex formed when TBA^+Cl^- was mixed with excess catechol (**1**) (Figure S2). Based on this finding we attached two catechols, as either 2,3-dihydroxy or 3,4-dihydroxy benzoates, to a TREN scaffold.[7] An alkyl amide group was linked to TREN's third position. Syntheses of analogues **2**−**7** in Chart 1 are described in the Supporting Information (SI). The membrane transport activity of these amphiphiles was first evaluated using an assay with the pH-sensitive HPTS dye.[8] EYPC liposomes filled with $NaNO_3$ and HPTS were suspended in a buffer containing Na_2SO_4 as an external electrolyte. Fluorescence measurements monitored changes in intravesicular pH after adding analogues **2**−**7**. Figure 1a indicates that intravesicular alkalinization occurred upon addition of **2**−**5**. Because sulfate is poorly membrane permeable, the increase in intravesicular pH is likely caused by ligand-facilitated NO_3^- efflux coupled with cotransport or diffusion of H^+ out of the liposome.

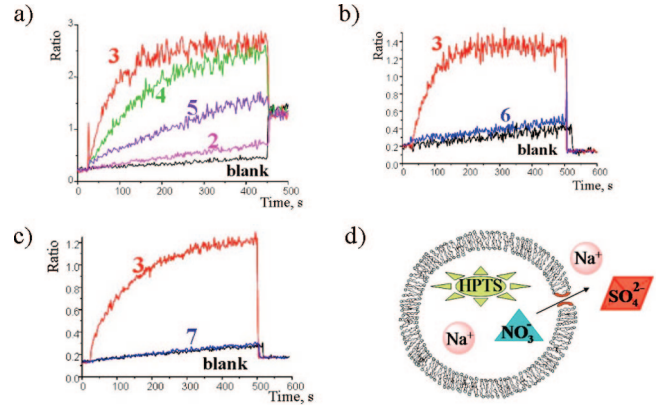


Figure 1. NO_3^- transport assays: *y*-axis "Ratio" in (a−c) refers to the ratio of HPTS fluorescence emission at 510 nm (I_0/I_1), where I_0 is excitation at 460 nm (basic form of HPTS) and I_1 is excitation at 403 nm (acidic form of HPTS).[8] Intravesicular 100 mM $NaNO_3$, 10 mM phosphate buffer (pH = 6.4), extravesicular 75 mM Na_2SO_4, 10 mM phosphate buffer (pH = 6.3), ratio of EYPC: **2**−**7** = 10:1, solutions of amphiphiles **2**−**7** in MeOH were injected at t = 25 s, and aqueous 10% Triton-X was injected at t = 500 s. Data for (a) 2,3-catechol analogues **2**−**5**, (b) **3** vs **6**, and (c) **3** vs **7**. (d) Schematic showing the experimental assay.

Analogues **2**−**5** showed major differences in their ion transport activity (Figure 1). Bis-catechol **3**, with a medium-length alkyl chain, was the most active of the 2,3-catechols, indicating that transport activity depends on the compound's ability to partition into the membrane. Compound **5** with the longer C17 chain likely aggregates in water, limiting its partitioning into the liposomes. Compound **2**, with the shortest chain, was the least active analogue. Presumably, the most active analogue **3** does not aggregate too much in water and is hydrophobic enough to partition into liposomes. The bis-catechol's alkyl chain length was not the only structural determinant for transport function. Figure 1b/c shows that the catechol's substitution pattern is also crucial for ion transport. Both 2,3-*O*-methyl analogue **6** and 3,4-substituted catechol **7** were inactive, indicating that 2,3-OH diols are essential for membrane transport activity. Simple changes in structure can clearly result in profound differences in function.[7]

In the course of determining whether intra- and extravesicular anions influence the membrane activity of **3**, we devised a method to measure the selectivity of ligand-mediated anion transport.[9] This assay relies on incorporating the salt of a weak acid, such as NaN_3 (pK_A of HN_3 = 4.7), into the liposomes.[10] In the presence of a transmembrane anion and/or pH differential, azide is protonated and the resulting neutral hydrazoic acid readily diffuses out of the vesicle. We then monitored intravesicular pH as a function of the extravesicular anion after addition of transporter **3**.

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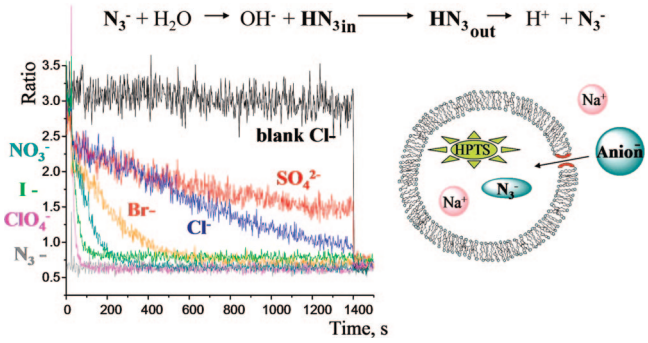

Figure 2. Anion transport assays. Solution of **3** in MeOH was injected at $t = 25$ s, ratio EYPC/**3** = 10:1, aqueous 10% Triton-X was injected at $t = 1400$ s. Extravesicular 100 mM Na^+ A^- ($A^- = Cl^-$, Br^-, NO_3^-, I^-, ClO_4^-, N_3^-) or 75 mM Na_2SO_4, 10 mM phosphate (pH = 7.15); intravesicular 100 mM NaN_3, 10 mM phosphate (pH = 5.5).

When strong electrolytes were encapsulated within liposomes no pH changes were observed in the absence of **3** because of the poor membrane permeability of those ions. In contrast, when liposomes loaded with NaN_3 were exposed to a transmembrane anion and pH gradient, the intravesicular pH immediately rose due to outward diffusion of the neutral acid HN_3 (the starting I_0/I_1 ratio of 3.2 indicates an intravesicular pH near 8.5).[8] Addition of amphiphile **3** then enabled a compensating influx of extravesicular anions back into the liposome. The consequent decrease in intravesicular pH shown in Figure 2 reflects the receptor-facilitated change in the chemical gradients of the N_3^- anion and the particular extravesicular anion. The time dependence of this change in intravesicular pH can be described by first-order kinetics, allowing one to determine the rate constant for ligand-mediated anion influx, k_{Anion}, and the turnover number, n_{Anion}, the number of anions transported per liposome per second (Table 1).

Table 1. Anion Transport Rates (k_{Anion}), Turnover Numbers (n), and Differences in Activation Energy for Transmembrane Transport by Bis-catechol **3** Relative to Cl^- ($\Delta\Delta G^{\ddagger}$)[a]

Anion	k_{Anion} $s^{-1} \times 10^3$	n, s^{-1}	$\Delta\Delta G^{\ddagger}$ kJ·mol^{-1}	$\Delta\Delta G^{\ddagger}_{CFTR}$ kJ·mol^{-1}	$\Delta\Delta G_{hydr}$ kJ·mol^{-1}
Cl^-	1.29 ± 0.01	44	0	0	0
Br^-	4.40 ± 0.07	150	2.9	0.49	26
NO_3^-	11.3 ± 0.10	384	5.3	0.89	41
I^-	36.2 ± 0.80	1230	8.1	1.72	64
ClO_4^-	69.5 ± 1.9	2400	9.7	N/A	133

[a] The last two columns in the table list differences in activation energy for anion permeation relative to Cl^- in the CFTR channel ($\Delta\Delta G^{\ddagger}_{CFTR}$ values from ref 11) and differences in anion hydration energy relative to Cl^- ($\Delta\Delta G_{hydr}$ values from ref 12). See SI for more detailed explanation of the $\Delta\Delta G$ values.

Table 1 shows that the selectivity of ionic permeability across the membrane in the presence of **3** follows a Hofmeister sequence with k_{Anion} decreasing in the order $ClO_4^- > I^- > NO_3^- > Br^- > Cl^-$. This weak dependence of transport rates on the anion's hydration energy indicates that the anions only need to be partly dehydrated to pass across the membrane in the presence of bis-catechol **3**. This selectivity pattern for the synthetic transporter **3**, where anion transport rates correlate with the anion's hydration energy, is also seen for some of Nature's anion transporters. Thus, anion permeation in the CFTR chloride channel shows a weak dependence on the anion's hydration energy (Table 1).[11,13] We observed a nonlinear dependence of the rate constant for anion transport on the concentration of transporter **3** (Figure S11). This result indicated that anion transport became more efficient when bis-catechol **3** self-associates in the membrane. We propose that self-association of **3** provides pathways that increase anion permeability across the bilayer without requiring complete dehydration of the transported anion.

In conclusion, we have shown that anion permeation across a phospholipid bilayer can be catalyzed by amphiphilic bis-catechols such as **3**. This anion transport process, which depends significantly on both the catechol's substitution pattern and its amphiphilicity, follows a Hofmeister sequence that shows that permeability depends on the ion's hydration energy. Future efforts will include incorporating selectivity filters into these catechol transporters to help overcome this Hofmeister bias in an effort to make Cl^- selective transporters.[14] We also believe that the liposomal assay described in Figure 2 will be useful for those interested in determining the selectivity and mechanism for other synthetic and natural transmembrane ion transporters.

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Supporting Information Available: Experimental details and spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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