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A Light-Gated Synthetic Ion Channel

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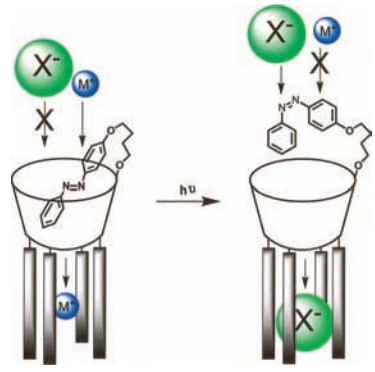
ABSTRACT



A gated synthetic ion channel with *β*-cyclodextrin as the pore and azobenzene as the gate is reported. Irradiation converts a tethered *trans*-azobenzene to *cis*-azobenzene which likely transforms the channel from a self-inclusion complex to a dissociated structure. This transformation results in an increase in anion transport and a decrease in cation transport across a phospholipid vesicle membrane.

There has been longstanding interest in the design of systems to achieve controlled ion transport across biological membranes.[1] Gated synthetic ion channels,[2] in which external stimuli such as temperature fluctuations, pH of the medium, ligand binding, or light trigger changes in ion transport rates, offer an attractive approach for the transduction of the applied signal into an electronic signal, with an eye toward the development of biosensing devices.[3] Among these, light-gated ion channels are of particular interest because of their short response times.

Photosensitive groups which have previously been used as photoswitches in other contexts include azobenzene, spiropyran/merocyanine, and a naturally occurring photoswitch, retinal.[4] Azobenzene is one of the most commonly used photoswitches due to a large change in azobenzene length and geometry upon *cis*−*trans* isomerization, in addition to its short response time.[5] Additionally, the ability of *trans*-azobenzene to bind into the hydrophobic cavity of *β*-cyclodextrin (*β*-CD) with greater affinity than *cis*-azobenzene[6] offers an attractive method for attenuating the accessibility of the cyclodextrin pore to ion diffusion.

We have previously reported the synthesis of a modified *β*-CD ion channel and its ability to mediate rapid trans-

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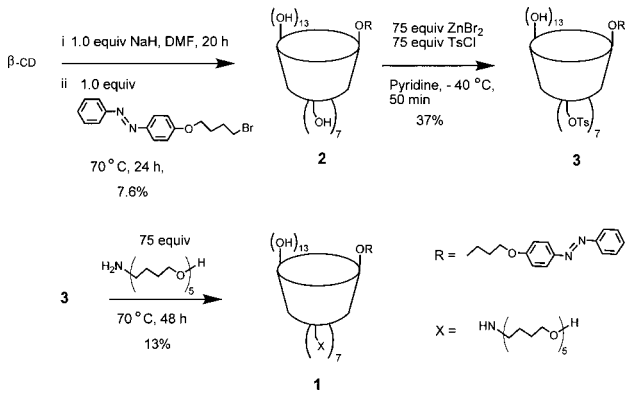
membrane ion transport.[9] We report here the attachment of azobenzene to the secondary face of the β-CD channel (**1**, Scheme 1), which acts as a light-sensitive gate. This gated

Scheme 1



synthetic ion channel shows a significant difference in the ion transport rates for both cations and anions depending on the *cis*−*trans* isomerization of the azobenzene. Remarkably, in the presence of *cis*-azobenzene, anions display a higher transmembrane transport rate while cations show a lower transport rate relative to the *trans*-azobenzene channel. This results in a distinctive change in transmembrane current in response to irradiation.

Incorporation of the gate onto the secondary face of β-CD was planned by attaching an azobenzene unit to a secondary hydroxyl of the β-CD by a tether of appropriate length. Molecular mechanics calculations[7] (using Molecular Operating Environment Software) indicated that a tether length of 4−5 carbon atoms would result in a reasonable low-energy structure of a planar azobenzene unit, which can move freely between a bound and a free state from the β-CD cavity. On the basis of this, 4-phenylazo-phenol and the four-carbon chain 1,4-dibromobutane were chosen as a gate−tether combination for the synthesis of the gated ion channel (Scheme 1).

Attachment of this gate−tether combination to the secondary face of β-CD was challenging due to the lower reactivity of the secondary hydroxyls versus the primary hydroxyls lining the lower rim. Nonetheless, the use of a modified literature procedure[8] to attach the bromobutyl azobenzene to the β-CD secondary face following deprotonation with sodium hydride afforded azobenzene cyclodextrin **2** in modest yield (Scheme 1). The attachment of the tether at the secondary face of the β-CD was confirmed[8] using ^{13}C NMR spectroscopy (see Supporting Information).

The previously reported nongated channel[9] was prepared by attaching the oligoether amine chains at the primary face of β-CD via 7-*O*-heptaiodo β-CD, which in turn was prepared from β-CD using iodine in the presence of triphenylphosphine. However, several attempts to synthesize the corresponding heptaiodo anologue of **2** using the same method were unsuccessful, perhaps due to occlusion of the cavity interior by the azobenzene. A change in leaving group to *p*-toluenesulfonate by sulfonation of the primary hydroxyls in **2**[10] in the presence of zinc bromide and pyridine at low temperature provided the intermediate product **3** (37%), which upon treatment with an excess of pentabutylene glycol amine with mild heating provided gated synthetic ion channel **1** in 13% yield. Purification of the heptatosylate **3** was achieved by flash column chromatography, whereas ion channel **1** was purified by dialysis to remove the excess pentabutylene glycol amine from the reaction mixture. The presence of **1** was confirmed based on the peak of the desired molecular weight in MALDI-MS and the lack of peaks corresponding to underreacted material.

To achieve the desired gated response of the synthetic ion channel **1** upon application of the external stimulus of light, three things were critical. First, binding of the *trans*-azobenzene unit to the hydrophobic cavity of β-CD was needed to "close" the channel to ion flow; second, photoisomerization of *trans*-azobenzene to *cis*-azobenzene with a concomitant decrease in the binding affinity of *cis*-azobenzene was needed to "open" the channel and allow the ions to pass through the pore; and third, reversibility of the above-mentioned processes, namely, isomerization of *cis*-azobenzene to *trans*-azobenzene and thus increased *trans*-azobenzene−β-CD binding, was also essential. The fact that *trans*-azobenzene was indeed isomerized to the *cis* isomer was confirmed by UV−visible spectroscopic studies of ion channel **1** (see Supporting Information). Changes in the binding interactions of *trans*-azobenzene versus *cis*-azobenzene with the β-CD were evidenced by downfield shifts of the *trans*-azobenzene aromatic signals and desymmetrization of the β-CD methine proton signals in the ^{1}H NMR spectra (10 mM in D_2O). Lastly, the reversibility of both the processes, azobenzene isomerization and azobenzene-β-CD binding, was also confirmed from UV and NMR studies, respectively.

To study the ion transport properties of channel **1**, a fluorescence-based assay was used (Figure 1).[9] In this assay, phospholipid vesicles which have pH-sensitive HPTS (8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt) dye entrapped were prepared. HPTS dye shows an increase in fluorescence intensity at 425 nm upon deprotonation. In the experiment, a Na^+ concentration gradient across the vesicle membrane was introduced by the addition of 2 N NaOH which was followed by the addition of a solution of **1** in methanol. If the channel mediates ion transport, sodium ions should flow inside the vesicles, along with OH^- (symport) or H^+ (antiport) to maintain charge balance. The resultant increase in pH would cause a deprotonation of the HPTS

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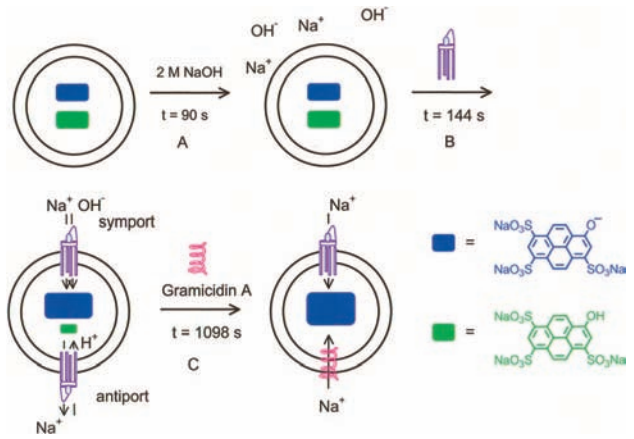

Figure 1. Schematic diagram of a typical ion transport assay.

dye. A change in fluorescence intensity of this HPTS dye at 425 nm was then observed over time. At the end of a typical experiment, complete equilibration of the protonated and nonprotonated HPTS dye was achieved by the addition of gramicidin (a natural ion channel). A schematic diagram of a typical assay is shown in Figure 1, and a plot of an observed change in fluorescence intensity of the HPTS dye with time upon addition of NaOH, suggesting the ion flow across the membrane, is shown in Figure 2. For each set of experiments,

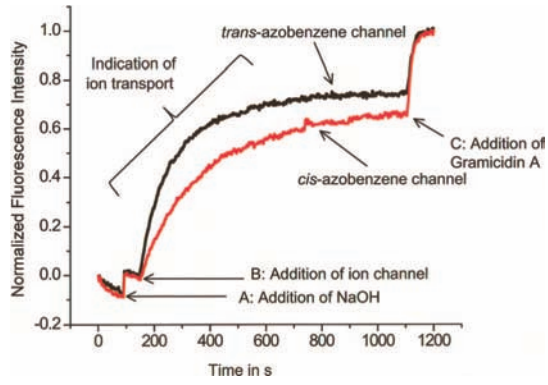

Figure 2. Observed change in fluorescence intensity of HPTS upon addition of NaOH and channel **1**. The lower rate of increase in fluorescence intensity for *cis*-**1** indicates a lower rate of cation transport.

although the vesicle concentration is not known precisely, it is constant throughout the experiment. For this reason, the amount of channel added (nanomoles) is reported rather than the concentration.

As noted in our earlier report,[9] a linear correlation between the rates of ion transport with the amount of channel added would indicate formation of a monomolecular ion channel, whereas deviation from linearity, especially at higher concentration, might suggest dimerization or aggregation of the ion channel during the assay. Initial fluorescence studies with channel **1** at low concentration and NaOH concentration gradients indicated rapid ion transport rates that increased linearly with the amount of channel added. However, an exponential increase in the rate of ion transport was observed in preliminary experiments when the amount of the channel added was greater than 0.7 nmol (see Supporting Information). This could be an indication of a dimerization or aggregation of the ion channel during the assay at higher concentrations, but further work is necessary to evaluate this behavior in detail. To ensure the presence of a monomolecular channel in this work, all the experiments were performed with amounts of channel added in the range of 0.15−0.62 nmol (<0.7 nmol).

For each sample, the ion transport rate[11] was obtained when the channel solution contained predominantly *trans*-**1** (gate closed state). To obtain the ion transport rate with channel solution containing *cis*-**1** (gate open state), the channel solution was irradiated with UV light (∼ 350 nm) for 1 min prior to addition to the vesicle suspension in the assay.

The efficiency and selectivity of ion transport through both *trans*-**1** and *cis*-**1** were studied using the fluorescence experiment described above (Figures 1 and 2). To test the cation selectivity, separate fluorescence experiments were performed with alkali hydroxides LiOH, NaOH, and KOH. There was not significant selectivity among the cations studied through either *trans*-**1** or *cis*-**1** (Table 1). Interestingly,

Table 1. Relative Rates of Ion Transport for Alkali Hydroxides

	k_{obs} (s^{-1})		
metal hydroxide	k_{trans} [a]	k_{cis} [b]	k_{cis}/k_{trans}
LiOH	0.0062	0.0037	0.59
NaOH	0.0063	0.0041	0.65
KOH	0.0066	0.0043	0.65

[a] *trans*-Azobenzene predominant (closed gate). [b] *cis*-Azobenzene predominant (open gate)

for each cation studied, the rate of ion transport through the closed channel (*trans*-**1**) was greater than through the open channel (*cis*-**1**), which is discussed later.

In our earlier study,[9] considerable anion selectivity was observed with a nongated version of the ion channel **1**; hence, further study was undertaken to study the effect of gating on anion transport using channel **1**. Halide salts of sodium (NaCl, NaBr, and NaI) were used in this study. The shape of the fluorescence curves observed for NaBr and NaI were identical to those reported previously[13] in which an initial rapid decrease in fluorescence was ascribed to either X^-/H^+ symport or X^-/OH^- antiport, which was followed by a steady increase due to a slower Na^+ influx. For NaCl, a gradual decrease in fluorescence intensity was observed indicating

(11) Cation transport rates were obtained by fitting the observed fluorescence curve to a linear equation in the Origin program, and anion ion transport rates were obtained by the same curve fitting procedure to exponential equations in the MS Excel spreadsheet program (see Supporting Information for details).

Table 2. Comparison of Ion Transport Rates of NaX Salts with Gate Open and Closed States

	rate of ion transport [k_{obs} in (s^{-1})]							
	trans-**1**[a]			*cis*-**1**[b]			cations (M^+)	anions (X^-)
halide salts	k_M	k_X	k_X/k_M	k_M	k_X	k_X/k_M	k_{cis}/k_{trans}	k_{cis}/k_{trans}
NaCl	0.0071	0.0073	1.0	0.0059	0.018	3.1	0.83	2.5
NaBr	0.0051	0.032	6.2	0.0034	0.051	15	0.66	1.6
NaI	0.0047	0.044	9.3	0.0041	0.055	13.4	0.9	1.2

[a] *trans*-Azobenzene predominant. [b] *cis*-Azobenzene predominant.

similar rates of ion transport for Na^+ and Cl^- (see Supporting Information). The rates of ion transport for all of the anion selectivity studies (NaCl, NaBr, and NaI) were obtained by fitting the curves to bi- or triexponential equations.[9,12] As expected, the differences in the ion transport rates among the anions (k_X) for both the closed (*trans*-**1**) and open (*cis*-**1**) states were substantial for all the anions studied and showed an increase in anion transport rate in going from Cl^- to I^- (Table 2). However, if a comparison is made for the rate of anion transport through *trans*-**1** vs *cis*-**1**, then the data indicate that the azobenzene gate works most efficiently for chloride ion transport (2.5 times greater ion transport rate with *cis*-**1** vs *trans*-**1**).

The ion transport rate difference (k_{trans} vs k_{cis}) supports the hypothesis that the increased binding of *trans*-azobenzene partially blocks the pore of the channel **1**. The selectivity observed with ion channel **1** (both for $X^- > M^+$ and $I^- > Br^- > Cl^-$) is likely due to the lower energetic cost for the transition from water to the membrane, as discussed in previous work.[9] In aqueous solution, the coordinating ability of water decreases with an increase in molecular radii, which results in a decrease in dehydration energy of the salt. For the salts studied, the dehydration energy decreases in the following order: NaCl > NaBr > NaI.[13] These observations indicate two things: one, Cl^- can have a larger hydration shell than that of the hydrated I^- ion, and second, the energy required to shed the water molecules and enter the membrane would increase in the order $I^- < Br^- < Cl^-$. While more studies are needed to ascertain the role of anion hydration on transport rates, the relative hydration energies are consistent with the observed behavior of ion transport rates for all of the halide salts studied. For example, both in the gate open and the gate closed states, if we assume that the size of the hydrated Cl^- ion is actually greater than the I^- ion and that the energy required to shed the water molecules and enter the membrane would be greater for the Cl^- ion than for I^-, then this would explain the observed decrease in the rate of ion transport for the Cl^- ion compared to both Br^- and I^- ions through channel **1**. Now, in the gate open state, the ion transport rate increase for Cl^- ions (2.5 times compared to gate closed state) is again consistent based on the bigger size of hydrated Cl^- ions, for which the absence of a gate would make the most remarkable difference compared to Br^- or I^- ions (1.6 and 1.2 times increase in rate, respectively, for gate open vs gate closed channel, Figure 3a). In the case of cation transport rates, additional studies are required to understand why cation transport is faster through *cis*-**1**. One possible explanation is that the bound azobenzene actually reduces the energetic cost of dehydration through cation$-\pi$ interactions with the much smaller cations (Figure 3b).

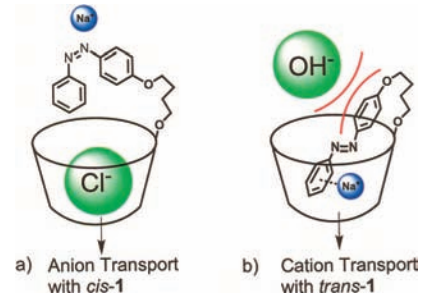

Figure 3. Schematic diagram of possible anion and cation transport through ion channel **1**.

In conclusion, a gated synthetic ion channel with β-CD as a pore and azobenzene as a gate has been synthesized. Photoisomerization of the azobenzene gate using UV light results in the attenuation of ion transport rates such that conversion of *trans*-**1** to *cis*-**1** leads to faster anion transport and slower cation transport. Further study and development of these robust synthetic gated ion channels should allow their use in more complex biomimetic photoactive materials in various nanotechnology and biomechanical applications.

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Supporting Information Available: Complete experimental details along with characterization of compounds, in addition to UV- visible spectroscopic studies and fluorescence assay results. This material is available free of charge via the Internet at http://pubs.acs.org.

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