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Signal-Triggered Transmembrane Ion Transport through Synthetic Channels

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This review discusses recent advances in mimicry of transmembrane ion channel proteins using artificial systems. While modified protein channels and surface-functionalized nanoporous membranes are covered briefly, the primary focus is on synthetic ion channels that have been modified to introduce signal-responsive activity. Examples are presented wherein transmembrane ion transport occurs in response to irradiation, changes in transmembrane voltage, or the presence of additives that either promote assembly or block the pore.

Keywords: Synthetic ion channels; Gated ion channels; Ion channel sensors

INTRODUCTION

Ion channel proteins are pervasive in living systems, and are critical elements of cellular regulation and signal transduction, including nerve signaling [1]. Natural ion channels are classified according to the signals that activate them: ligand-gated channels are activated by small-molecule second messengers; voltage-gated channels are attenuated by changes in the transmembrane potential; and mechano-sensory channels open in response to mechanical deformation of the membrane. Following opening of the channel, either cations or anions flow through the channel down their concentration gradients. The resultant changes in ion concentration or transmembrane potential subsequently activate or deactivate other cellular processes.

Given the utility of this mode of signal transduction, wherein an applied signal is translated into an electrical signal, there has been significant interest in developing synthetic analogs for sensor and device applications [2,3]. These efforts have involved a number of different approaches, ranging from modification of natural ion channel proteins [4] to the preparation of artificial nanoporous membranes with signal-responsive permeabilities [5]. Intermediate between these two approaches is the use of synthetic ion channels. These are typically organic compounds that are able to insert into a phospholipid membrane and form a pore through which ions can pass. In order to prepare synthetic channels that are gated, several researchers have introduced clever design modifications that lead to changes in transmembrane current in response to an applied signal. This review will touch briefly on the use of modified proteins and nanoporous membranes for signal-responsive transmembrane ion transport, and will cover in more detail recent advances in the use of synthetic ion channels.

MODIFIED PROTEIN CHANNELS

α-Hemolysin is a heptameric protein with a 15 Å pore diameter that has been used in several sensor applications [6]. This protein can be inserted across a phospholipid bilayer separating two aqueous compartments such that current recordings represent ion flow through a single channel. Bayley and coworkers have developed several α-hemolysin mutants that allow the introduction of non-natural components such as metal-binding sidechains, signal-responsive polymers, or constricting macrocyclic gaskets [7]. In these systems, the flow of ions through the pore differs in the presence and absence of applied signals

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such as metal ions or small molecule pore binders, leading to a diagnostic conductivity pattern. Deamer has also used α-hemolysin to characterize DNA [8]. As each different nucleobase of the oligonucleotide moves through the pore, a different current is measured, leading to sequence analysis of a single DNA molecule.

Gramicidin is a bacterial toxin that forms pores upon tail-to-tail dimerization of two β-helical peptides within a membrane [1]. The smaller size of gramicidin (15 amino acid residues) renders it more readily modified in different ways. Some of these modifications include appending a light-sensitive diazobenzene to one terminus to afford photomodulated transmembrane current [9], or the addition of acidic or basic groups to confer pH sensitivity [10]. A further development toward functional sensors was first demonstrated by Cornell in which one leaflet of the phospholipid bilayer and the embedded gramicidin peptides were tethered to a gold surface [11]. The gramicidin in the top leaflet was modified to interact with different analytes. These interactions influenced the mobility of the gramicidin in the top leaflet, thus affecting the probability of dimerization to form active channels [12].

NANOPOROUS MEMBRANES

An attractive feature of the modified protein systems is the ability to analyze ion flux through a single pore; however, they suffer from the inherent instability of both proteins and phospholipid bilayers [3]. Much more robust sensors have been investigated wherein a nanoporous membrane of materials such as polycarbonate, glass, or polyethylene are coated with gold and thiols, or polymers that swell and contract in response to environmental changes [5]. This results in pores that are open or closed to transmembrane transport in response to the applied signals (changes in ion concentrations, pH, light, etc.) [13–17]. While these systems are more robust than the ion channel protein systems, they lack the ability to sense single molecules via formation of single pores. Furthermore, the pore dimensions and pore density vary considerably, resulting in channels that are less well-defined than protein pores.

SYNTHETIC GATED CHANNELS

In order to access signal-sensitive channels that are both robust and amenable to single channel recordings, a number of researchers are pursuing wholly synthetic compounds that can function as gated ion channels, but with properties that are designed de novo [18]. Most of the channel-forming compounds in this class are amphiphilic, which promotes favorable interactions with both the lipid bilayer and an aqueous pore. As shown in Figure 1, once the hydrophobic portion of the amphiphile drives insertion into the lipid bilayer, the hydrophilic regions tend to associate to minimize interactions with the hydrophobic lipid tails. The resulting aggregates form aqueous pores lined by hydrophilic surfaces. Signal sensitivity is introduced by modifying the amphiphile with functional groups that will respond to stimuli to modulate either formation of the active channels, or the flow of ions through the channel pore. Among the existing examples of fully synthetic signal-triggered ion channels, three modes of activation have been explored: switching by light, by change in applied voltage, and by the recognition of a ligand.

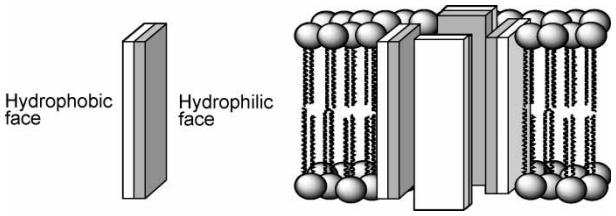

FIGURE 1 Amphiphilic molecules aggregate in hydrophobic membranes to form hydrophilic pores.

Light-gated Channels

Light-triggered gating has only been briefly explored with fully synthetic channels, although peptides have been modified to produce light-responsive channels. Dimers of gramicidin [19] and of a glutamic acid based peptide [20] have been linked with azobenzene to produce systems that show photomodulated ion transport. Woolley modified gramicidin with azobenzene endgroups to block the pore after isomerization to the *cis*- form [9]. The first non-peptidic channel with photomodulated activity was reported by Kobuke and coworkers, who utilized the photoisomerization of an azobenzene to switch synthetic aggregates between conducting and non-conducting states [21]. The researchers synthesized a *trans*-azobenzene-containing hydrophobic ammonium that was paired with a polar oligoether carboxylate to form a stable ion pair (**1**) capable of forming ion transporting aggregates in planar bilayer membranes (Figure 2). When the *trans*-azo ion pair **1** was irradiated at 367 nm and subsequently added to planar bilayers, only flickering currents were observed, presumably as a consequence of steric disruption of the conducting aggregate upon isomerization to *cis*-azo **1**.

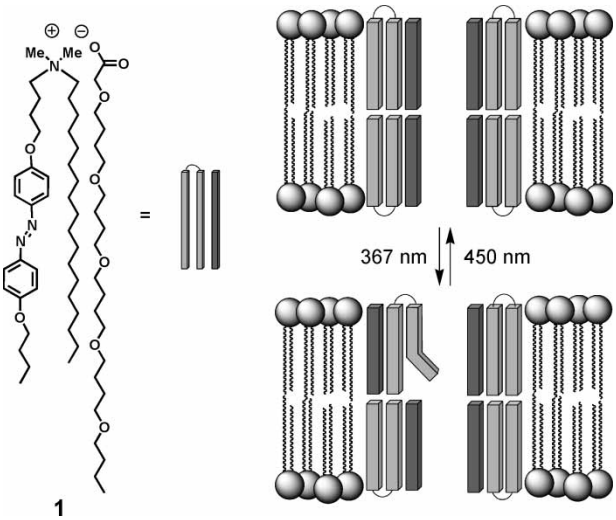

FIGURE 2 Azobenzene-containing ammonium carboxylate ion pair and proposed mechanism of gating.

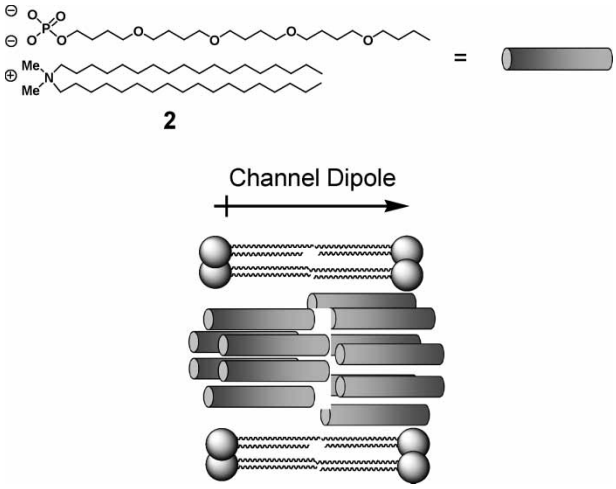

FIGURE 3 Ammonium phosphate amphiphilic ion pairs assemble to form asymmetric channels with a net dipole.

Voltage-Gated Channels

Voltage changes across the membrane have been explored more extensively as a trigger for channel formation. Most voltage-gating is accomplished by ion transporters having inherent molecular dipoles which react in predictable ways to applied voltages. The first application of this principle to synthetic channels appeared in 1995 with Kobuke's report on voltage-responsive ammonium phosphate ion pair **2** (Figure 3) [22]. Aggregation of multiple monomers of **2** forms half-channels in each leaflet of a lipid membrane; half-channels then come together in opposing leaflets to provide a structure capable of ion transport. Since aggregation is expected to occur independently in either membrane leaflet, the result is often uneven numbers of **2** in the two half-channels. When the amount of negative charge at opposite membrane surfaces differs, a net dipole is present in the channel aggregate. Applied voltages which act to stabilize the charged aggregate in the membrane will then produce a more stable transmembrane current.

Kobuke and coworkers elaborated on the concept of asymmetric charge distribution across a membrane with the synthesis of asymmetrically substituted bis-cholic acid derivatives (**3**) displaying a molecular dipole (Figure 4) [23]. These amphiphiles have the advantage of forming more stable aggregates than the ammonium phosphate pairs **2** described above, leading to more stable and longer-lasting open states. The asymmetric orientation of the monomers in the bilayer was ensured by applying a voltage during the membrane insertion process. The result is that more current is observed at positive applied voltage than at negative applied voltage, presumably due to interactions of the molecular dipole and the applied field.

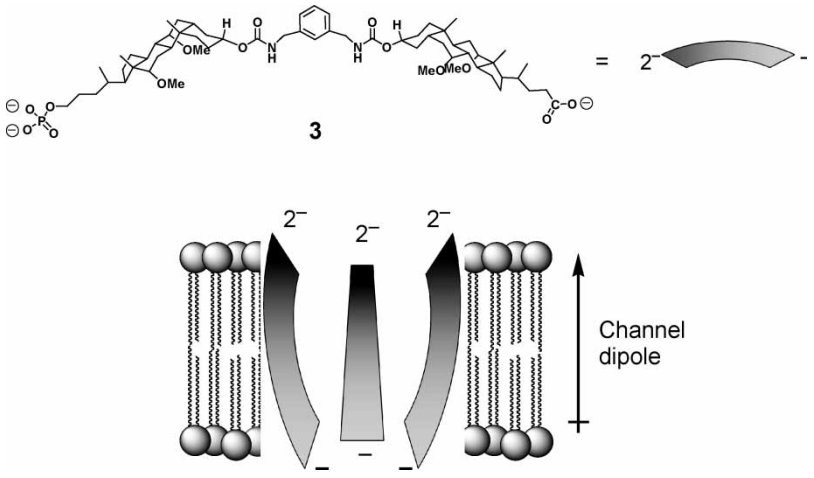

FIGURE 4 A bis-cholic acid amphiphile bearing a molecular dipole is inserted asymmetrically into a membrane to give voltage-responsive aggregates.

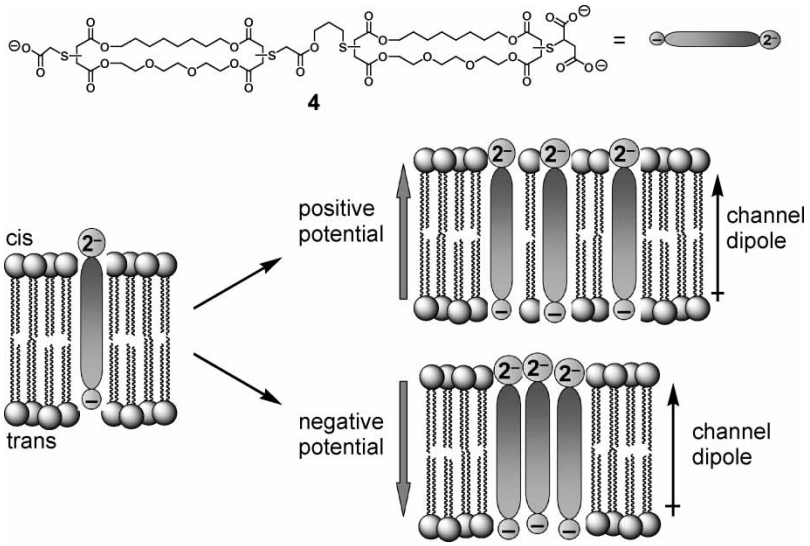

FIGURE 5 A bis-macrocyclic bolaamphiphile with a molecular dipole interacts preferentially with negative membrane potentials.

Fyles introduced another system of aggregating monomers with net molecular dipoles using a bis-macrocyclic bolaamphiphile (**4**) having a carboxylate on one end of the molecule and a succinate on the opposite end (Figure 5) [24]. Addition of a solution of **4** to the cis side of a membrane results predominantly in insertion so that the more highly charged endgroup remains on the cis side. Application of a negative membrane potential allows the formation of an aggregate due to the balancing of charge repulsion by the applied field. In contrast, application of positive potential does not ameliorate charge repulsion of the endgroups and the formation of a conducting aggregate is disfavored.

Systems in which uncharged monomers show voltage dependent behavior have also been reported. Recently, Gokel and coworkers detailed the synthesis and characterization of a voltage-gated, chloride-selective channel former, peptide conjugate **5** (Figure 6) [25]. The authors propose that the bent peptide portions of two monomer units **5** can form a half channel ~7 Å wide with the ability to conduct ions through the membrane. The channel currents show mild voltage dependence and a ten to one selectivity for chloride over potassium ions. Further studies on the mechanism of ion transport and the origin of chloride selectivity are underway [26].

Another approach to voltage gating using uncharged monomers is shown in Matile's development of voltage dependent β barrels [27,28]. The barrels comprise *p*-octiphenyl "staves" (**6**) which are functionalized by amino acid sequences engineered to form interdigitated β-sheet-like structures with adjacent stave monomers (Figure 7). The torsional

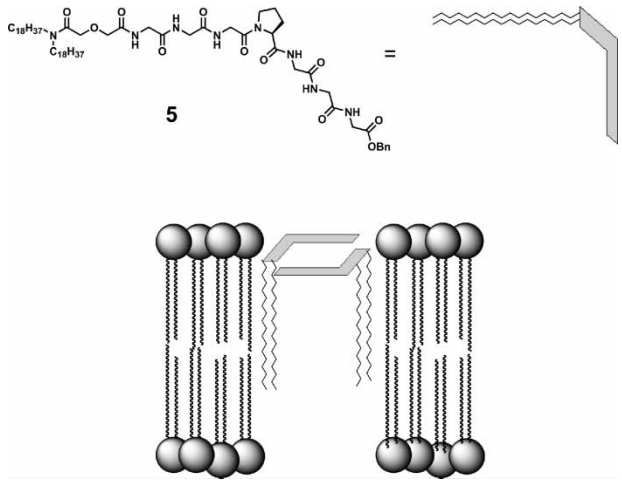

FIGURE 6 Proline containing peptide-lipid conjugates form a voltage-responsive pore in a lipid bilayer.

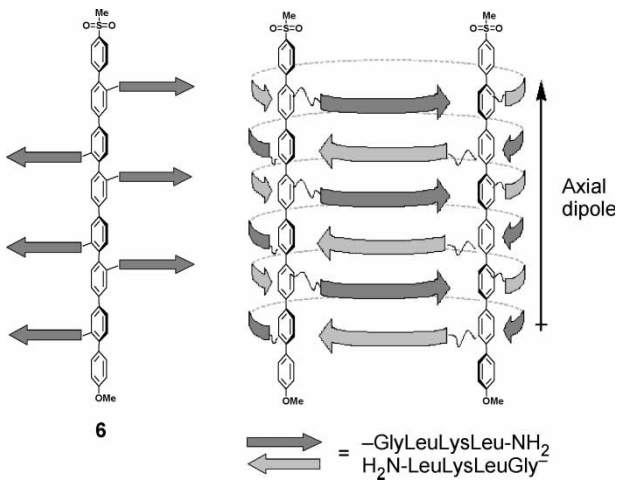

FIGURE 7 *p*-Octiphenyl staves with axial dipoles insert into polarized membranes to form β barrel pores.

requirements of octiphenyl stave **6** are such that consecutive peptide sequences are forced to radiate from the central rod in opposite directions so that the β-sheets form curved structures, completing a supramolecular barrel. *p*-Octiphenyl **6** is methyl sulfone-substituted at one end and methoxy-substituted at the other, providing an axial dipole. When stave molecule **6** is added to spherical bilayers polarized to be more negative in the interior, current increases exponentially with increased voltage. In this case, the applied voltage facilitates insertion of the more positive end of the staves to form open channels. Once in place, the interdigitated side chains provide stabilization of the channels in the membrane. By contrast, octiphenyl rods with symmetric axial substitution exhibit no such voltage dependent insertion and transport.

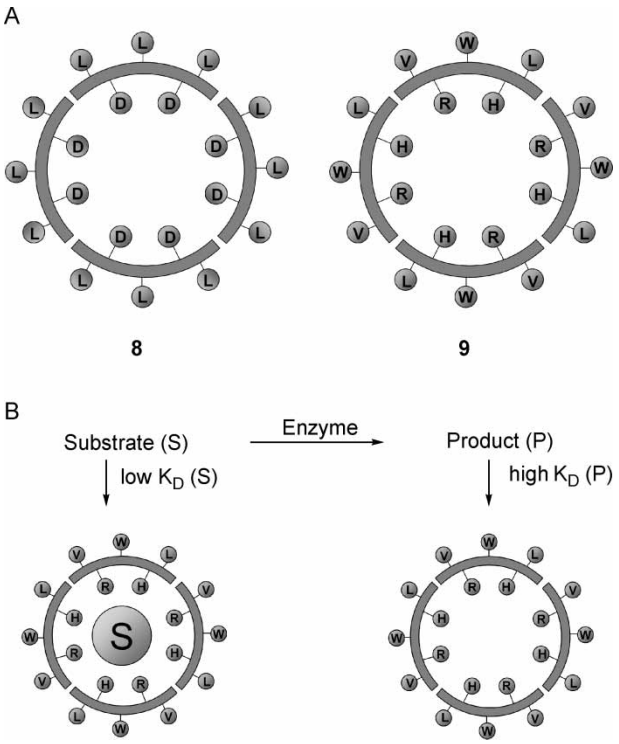

FIGURE 9 (A) Representative cross-sections of two pore-forming β barrels. (B) Schematic depiction of two possible modes of enzyme dependent gating with β barrel pores.

Ligand-gated Channels

Although ligand gating is a very common mechanism for natural ion channels, few researchers have reported synthetic ligand-gated channels. Matile reported the first example of a ligand-activated system, in which addition of a ligand triggers aggregation of monomers [29]. A *p*-septiphenyl chain substituted with an iminodiacetate-terminated diethylene glycol unit (**7**) inserts into bilayer membranes and remains an inactive monomer without external stimulus (Figure 8). The iminodiacetate moiety was chosen for its ability to form a stable complex with one copper (II) ion and imidazole. Thus the ligand used to activate the system is a polymer containing multiple histidine sidechains. Addition of $CuCl_2$ followed by the polyhistidine polymer to the exterior of a bilayer containing monomer **7** results in the formation of aggregates which facilitate transport of cations across the membrane.

Matile and coworkers have also expanded the scope of their β barrels by modifying the amino acid content of the peptide sidechains to allow binding of small molecules in the channel pore. Consequently, two β barrels with interiors functionalized with

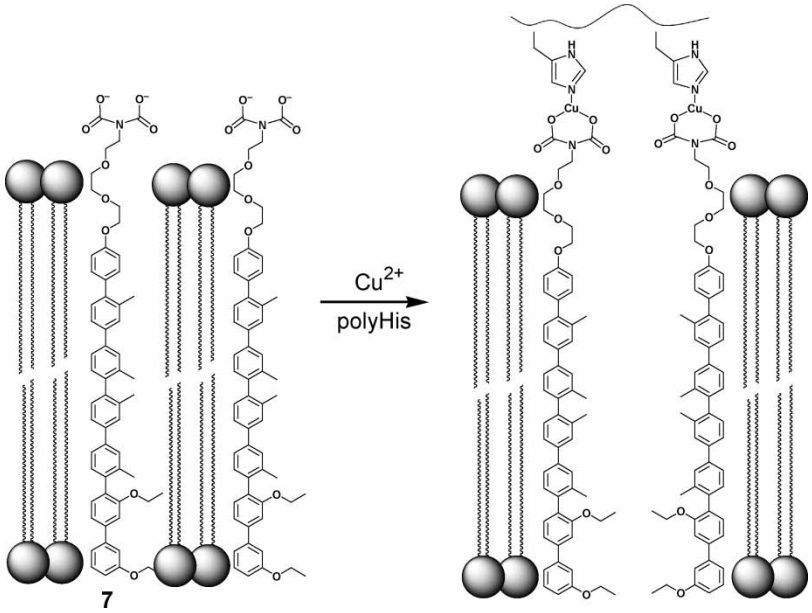

FIGURE 8 Iminodiacetate receptors bind copper and a histidine polymer to cause aggregation of oligophenyl channel-formers.

aspartates (**8**) or a combination of histidine and arginine (**9**) were synthesized and their binding with a number of substrates was evaluated (Figure 9) [30]. The pores were designed to differentiate between the substrates and products of enzymatic reactions by binding larger molecules better than smaller molecules with very similar structures; consequently, the larger molecule of a substrate-product pair will block the pore while the smaller does not. The pores can then be gated by removal of a good blocker by its enzymatic transformation into a poor one, or vice versa. For example, pore **9** binds ATP with a K_D of 2 μM while the K_D of ADP is 66 μM (25 °C). Hence, pore **9** blocked with ATP in lipid bilayers showed an appreciable increase in ion transport in the presence of apyrase, an enzyme that converts the strong pore blocker ATP into weak pore blocker ADP. The researchers have demonstrated the ability of the synthetic pores to bind numerous substrates, including nucleosides, carbohydrates, DNA, RNA, polysaccharides, and proteins and have detected the activity of a myriad of enzymes including phosphatases, glycosyltransferases, and protein kinases [30,31].

CONCLUSIONS AND OUTLOOK

Many exciting advances have been made toward the application of ion channels to sensing technologies. While much work has focused on either modification of ion channel proteins or on signal-responsive nanoporous membranes, an emerging area involves the use of robust but well-defined synthetic channels that have been designed to have signal-sensitive ion transport activity. The majority of these operate via signal-induced aggregation such that channels form in response to light, applied voltage, or addition of ligands. Matile's β barrel channels are the exception to this motif of signal-triggered assembly/disassembly. These comprise stable tetrameric aggregates that closely resemble natural ion channel proteins in that the channel, once assembled, is always present within the membrane. Whether it is opened or closed to transmembrane ion transport depends on the applied signal. Because of the robust nature of the β barrel channel, the reversibility of the gating modes, and the ease with which the pore can be modified, this approach to synthetic gated ion channels is likely to be the most successful for device applications.

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