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Artificial transmembrane channels for sodium and potassium

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Transport of alkali metals, particularly sodium and potassium, across cell membranes is an essential function performed by special proteins that enable cells to regulate inter- and extracellular ion concentrations with exceptional selectivity. The importance of these channel-forming proteins has led to researchers emulating of their structural features: an ion-specific filter and conduction at rates up to 10^8 ions per second. Synthetic helical and cyclic polypeptides form channels, however, the specificity of ion transport is often low. Ion-specific macrocycles have been used as filters from which membrane-spanning derivatives have been prepared. Success has been limited as many compounds act as ion carriers rather than forming transmembrane channels. Surfactant compounds also allow ions to cross membranes but any specificity is serendipitous. Overall it seems possible to mimic either ion specificity or efficient transmembrane ion transport. The goal for the future will be to combine both characteristics in one artificial system.

Introduction

Several families of ion channel forming proteins allow transport of sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), protons (H^+) and chloride (Cl^-) across cell membranes. Similar structures almost certainly exist to selectively transport other ions such as the transition metals present in enzymes and metalloproteins.

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Although these ion channels are formed by immensely complex polypeptides some simple compounds, for example the bee sting protein melittin, also induce transmembrane ion transport. These observations indicate that small molecules can mimic natural systems and inform artificial models of ion channel formation.

All cells contain cations and anions at specific concentrations that may vary greatly from the extracellular concentrations of those same chemical species thus the management of transmembrane ion flux is a crucial biological process. For example, the extracellular concentration of Na^+ is typically 15 times greater than inside the cell. The reverse is true for K^+, which is 30 times more concentrated within cells than in the extracellular fluid. The difference between intra- and extracellular concentrations allows dynamic equilibria to power chemical reactions within cells. These simple ions are not the only species engaged in dynamic equilibria as water and small molecules also traverse cell membranes. This review will focus on examples of Na^+ and K^+ transport and discuss attempts to mimic Nature in the laboratory. Synthetic analogues of other transmembrane channels, particularly for Cl^-, have been made but most are not as amenable to study from a chemical perspective through their sheer complexity.

As small variations in the intracellular concentrations can mean the difference between the cell living or dying much work has been done to understand the mechanisms underlying transmembrane ion transport and, in particular, the activation and inactivation of transmembrane ion channels. The efficient function of ion channels is necessary if electrolytic homeostasis is to be maintained: when it is compromised through chemical attack or genetic misreading the consequences for the health of the organism can be profound[1]. Inactivation can be brought about by a wide range of toxins particularly those which block ion channels with positively charged groups. The effective operation of these channels is also compromised when compounds keep them open or induce repetitive firing. Na^+ channels are blocked by guanidinium-derived toxins such as saxitoxin from shellfish and kept open by aconitine (from wolfsbane) and the 'red tide' toxin, *Ptychodiscus brevis*. K^+ channels are subject to a wider variety of inactivating compounds including tolbutamide and glibenclamide, drugs used in the management of diabetes.

Conditions resulting from defective Na^+ channels range from muscle related conditions, including temporary paralysis and cardiac arrhythmia, to neuronal diseases, some of which may lead to epilepsy. Mutations in human skeletal muscle are associated with several forms of temporary, periodic and K^+-induced paralysis. Long QT syndrome, an arrhythmic cardiac disorder associated with fainting

and sudden death, is associated with mutations in both Na^+ and K^+ channels. Compromised K^+ channel function has also been linked to many conditions including certain forms of ataxia, diabetes mellitus, epilepsy and muscular dystrophy. A particular theme running through many of these conditions is the effect of channel inactivation on neuronal function. As neurones react to electrochemical stimuli induced by channel activity any loss of that activity reduces their ability to function effectively. Neurodegeneration often becomes progressive as response to neurotransmitters decreases and the conditions worsen. The development of artificial models to understand the mechanisms of ion channel function therefore has profound and wide reaching implications for health and a clear relevance to the design of therapeutics and strategies for management of disease.

Mechanism of ion transport across cell membranes

Ionophores and channels

Cell membranes consist of two layers of phospholipid molecules meeting 'tail-to-tail' to form a structure that has polar internal and external surfaces and a non-polar region in between. The bilayer has a thickness of approximately 40 Å giving a minimum distance to be traversed by an ion moving between aqueous regions (Figure 1). According to the fluid mosaic model[2], the membrane is host to a variety of macromolecules having specific functions that can penetrate, or bind to, its surfaces. The bilayer may move to incorporate these macromolecules but remains unbroken unless seriously compromised. Changes in polarity through the lipid bilayer ensure that it forms an effective barrier between the extra- and intracellular environments. How then do chemical species cross the cell membrane?

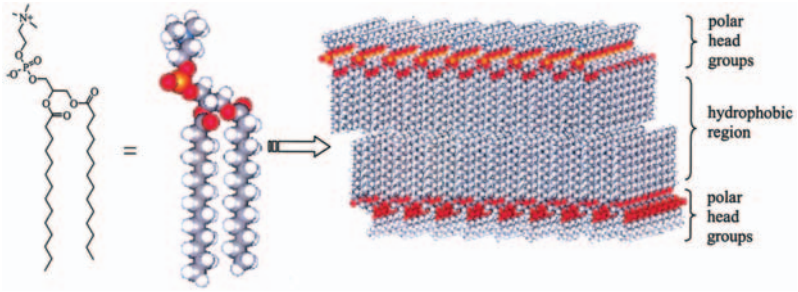


Fig. 1. *Phospholipid bilayer formation (chemical structure and space filling representation shown on left where oxygen atoms are depicted in red, phosphorus in orange, nitrogen in blue, carbons in grey and hydrogens in white).*

Two broad mechanisms for transmembrane transport exist: ionophore mediated migration and channel formation. In the former a carrier molecule, or ionophore, binds to an alkali metal cation and the complex passes through the bilayer into (or out of) the cell. For this to occur effectively the ionophore must bind specifically to only one type of ion. It must be soluble in the aqueous solution containing the ion yet be able, as the charged ion-ionophore complex, to traverse the non-polar region of the phospholipid bilayer. This is achieved by the ionophore having polarizable regions, to bind an ion or co-ordinate to water, and the ability to change conformation inverting these regions to present a hydrophobic face when passing through the local lipidic environment. Many naturally occurring ionophores are known and transport ions with high selectively at rates of 10^4 ions per second[3]. The K^+-valinomycin complex is a good example of this phenomenon, the ion-ionophore complex having a tubular shape, 9.5 Å high with an internal diameter of 6.3 Å and an outer diameter of 14.5 Å. This type of ion transport has been the mechanism by which many artificial ionophores are proposed to work, in particular, the crown ethers[4]. This family of small cyclic organic compounds, first prepared in the 1960s, has a high affinity with alkali metals and the ability to transport cations from aqueous solution to non-polar solvents. As each member of the crown family has a preference for a particular alkali metal, the principle has found widespread use in phase transfer catalysis and forms the basis of some artificial transmembrane channels. As will be seen, many channel models incorporate [18]crown-6 derivatives which bind K^+ (1.33 Å ionic radius) preferentially over the smaller Na^+ (0.97 Å). The complexes are usually smaller in size to those of naturally occurring analogues, for example, the K^+-[18]crown-6 complex has a disc-like shape, being a mere 2.8 Å high with an internal diameter of 5.1 Å and an outer diameter of 8.5 Å.

Channel formation occurs when one or more macromolecules insert into the cell membrane thereby opening a pore through the lipid bilayer. The size of the pore dictates the maximum size of species that may traverse its length. Transport is much faster than that observed for ionophores, with rates of 10^8 ions per second possible through some channels. To control the flow of ions the channel is often gated, opening only upon a certain electrical, physical or chemical signal, and may contain a constricted region to bar all but one ionic species. Simple channels such as gramicidin A are based on polypeptides that form membrane-spanning helices when two molecules meet across the membrane (Figure 2). More complex channels are formed when several molecules come together to form

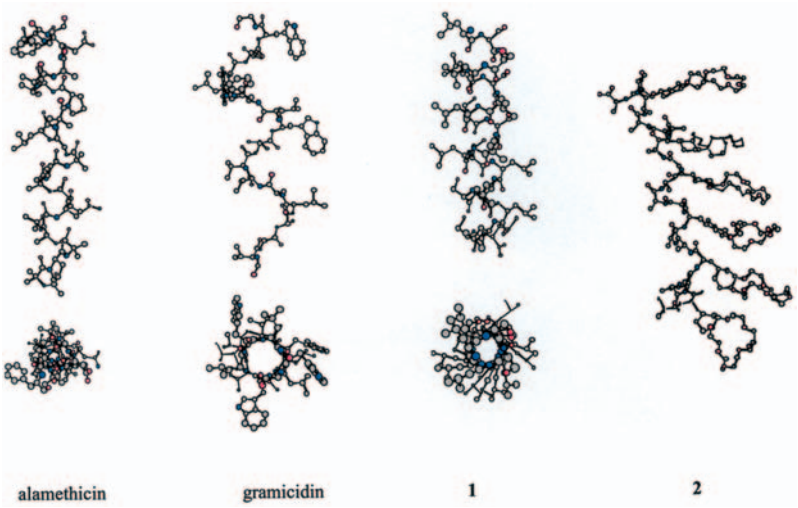


Fig. 2. *Natural membrane-spanning polypeptides alamethicin and gramicidin compared to the synthetic, pore-forming, helical peptide* ***1*** *and polypeptide* ***2,*** *which supports a channel made from aligned crown ether moieties. Oxygen atoms are depicted in red, nitrogens in blue and carbons in grey: hydrogen atoms have been removed for clarity.*

a 'barrel' with each molecule forming a 'stave' as in alamethicin, monensin or amphotericin B. These types of channels are often permeable to many species that diffuse through the central cavity, attracted to the polarized hydrophilic regions within. The channels of greatest relevance to this review are those which comprise several intertwined complex polypeptides incorporating finely tuned binding regions ('filters') for ion recognition and a gating mechanism.

Selectivity

Ionophores and transmembrane ion channels must both discriminate in favour of one ion to the exclusion of all others if they are to control intracellular ionic concentrations. Cyclic ionophores, like valinomycin (Figure 3), achieve selectivity through a match between cavity size and the target ion's preferred coordination geometry. The binding process must be reversible if the ion is ever to be released once across the lipid bilayer, however, the complex must remain intact during its passage through the hydrophobic centre. Two processes may be envisaged. Firstly, the ionophore binds to the ion with concomitant removal of key coordinated water molecules from the ion at the aqueous phase/bilayer interface. This is followed by a conformational change in the ionophore allowing it to 'smuggle' the ion

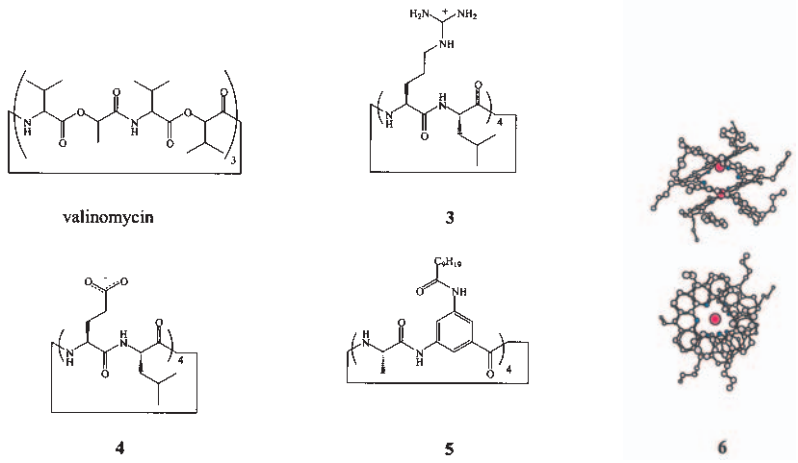


Fig. 3. *Valinomycin, a natural ionophore, compared to synthetic cyclopeptides* ***3****,* ***4****,* ***5*** *and synthetic helix* ***6****. Compounds* ***3*** *and* ***4*** *form membrane-spanning nanotubes,* ***5*** *imparts voltage-sensitive* K^+ *transport and* ***6*** *(shown in side and top views; sodium atoms in red, nitrogens in blue and carbons in grey) forms a double helix around* Na^+.

through the hydrophobic region of the bilayer. Once through this region the process is reversible provided the ion is not too tightly bound. Thus highly selective ionophores with low binding constants are the most effective.

Ion channels appear to achieve selectivity by incorporating a constricted region in the pore through which a particular ion may pass. If the recent crystal structure of a K^+ channel can be considered to be a general model for cation channels, then the amino acids in this region present incoming ions with a sequence of electron rich surfaces to which they are drawn[5,6]. In doing so they lose all but one of their coordinated water molecules. The influx of ions to the filter forces those in front to move to further regions of electron density until they complete their passage through the channel and emerge to regenerate their primary solvation shell.

Transmembrane ion channel architecture

It is now apparent that there is no common structure for all ion channels: voltage gated K^+, Na^+ and Ca^{2+} channels are composed of four identical subunits forming a central pore, Ca^{2+} release channels are similar but have fivefold symmetry and Cl^- channels are composed of two interlocking subunits each with a central pore[1]. The channels

of relevance to this review are those which transport Na^+ or K^+ and comprise several types.

Voltage-gated Na^+ channels, of which there are many kinds, are extremely sensitive to small changes in voltage and are seldom open. Since the advent of channel cloning and site-directed mutagenesis several mutant channels have had their Na^+ permeability probed through the actions of local anaesthetic molecules and toxin-binding studies. The use of tetrodotoxin, isolated from the puffer fish *Fugu*, has identified certain sequences that define the outer mouth of Na^+ channels to which the toxin binds causing channel failure. The activation of voltage-gated channels is believed to result from movements of charge within a membrane, as detected by charged amino acids, which is in turn transferred to residues in the channel protein that undergo conformational changes. The overall effect is that the mouth of the channel opens or becomes more accessible to incoming ions. Once ions enter the channel protein they then have to get past a second obstacle, the selectivity filter. The overall shape of the voltage-gated Na^+ channel isolated from the electric eel, *Electrophorus electricus*, was recently determined by electron microscopy to reveal a square-based bell shaped molecule with a large central cavity 40 Å high and 35 Å wide, four peripheral cavities (roughly 15 Å high and wide) and four small orifices in the hemispherical extracellular region of the channel protein connected to the central cavity[7]. The cavities connect to four helical regions containing constrictions, related to each other by fourfold symmetry. Presumably these features contain selectivity filters, however, at present the structure of the filter is unknown for Na^+ channels.

Transmembrane K^+ channels fall into two main categories. One group, the inwardly rectifying-K^+ (K_{ir}) channels, consists of two transmembrane domains; the other of six domains. The latter group includes voltage-gated-K^+ (K_v) channels and several Ca^{2+} activated-K^+ (K_{Ca}) channels. Electron microscopy and mass determinations indicate that K_v channels are hetero- or homotetramers. The outer mouth of K_v-channels have been shown to be inactivated by scorpion venom which binds to regions analogous to those in voltage-gated Na^+ channels targeted by tetrodotoxin. K^+ enters through a selectivity filter located about 5 Å from the extracellular face of the protein, however, mutagenesis experiments have shown that the absence of a tyrosine-glycine dipeptide sequence removes K^+ selectivity and allows other alkali metals to pass at rates similar to K^+ in the natural channel[8]. K_{Ca}-channels are categorised essentially on the magnitude of the calcium concentration necessary to obtain K^+ conductance. Little is known about their structures though some are

thought to contain separate sensor and conductance subunits while others resemble K_v channels. Fortunately for those involved in the design and synthesis of artificial ion channels, much more is known about the structure of K_{ir} channels following the remarkable work of MacKinnon to determine the crystallographic structure of KcsA[5] and by Roux to model K^+ movement through the selectivity filter[6]. KcsA is an inward rectifying K^+ channel but also has much in common with K_v and K_{Ca} channels and it appears that features of the selectivity determined for KcsA may well occur in many ion channels. The transmembrane protein is composed of four subunits that have extracelluar 'turrets'. Each subunit has an inner and outer α-helix, which interlock to narrow the channel as it enters the intercellular region. One K^+-binding site is found in the internal cavity of the channel protein which feeds four sites within the selectivity filter. Two final binding sites are present on the extracellular side of the protein. A combination of computational simulations and variable concentration crystal structure determinations has shown that not all sites are occupied simultaneously but that K^+ ions are linked by water molecules in an alternating pattern through the selectivity filter. As a cation approaches the internal opening to the filter it is bound to four water molecules from the hydrated internal cavity and to four carbonyl groups at the mouth of the filter thus conserving the preferred octahedral geometry of the K^+ primary coordination sphere. The resulting low energy pathway for transmembrane transport explains the high efficiency of the channel.

The importance of models

One of the most fundamental reasons for constructing a model is to simplify the processes involved and gain a greater understanding of the factors affecting the more complex system. This is particularly true for the chemist's mimicry of biological structures. Simplification of ion channels allows specific aspects of the model to be studied in a systematic manner and to examine the effects of structural modifications. Similar information may be obtained through site directed mutagenesis in ion channel proteins, however, the use of this approach to completely understand the mechanisms behind ion selection and transport requires immense effort and resources. By comparison, 'chemical' models can be generated to probe different aspects of ion channels. For example, a self-assembling artificial channel may be used to assess the importance of hydrogen-bonding in ion channel formation, or an ion specific binding agent could aid in determining the factors, such as cation-π interactions, which induce ions into the selectivity filter.

Information from models together with results from biological experiments can explain why particular compounds inactivate some ion channels while other channels allow toxins to pass through. This information can be used in the design of therapeutics to treat inactive channels or to deliver cytotoxic agents. An understanding of the fundamental processes in ion transport is therefore of interest beyond the chemist's laboratory.

Methods of investigation

How can the structures and dynamics of ion channels and their models be measured? Structures of some natural channels have been determined by protein crystallography at varying resolutions. Once high resolution (below 2 Å) has been achieved the relative positions of individual atoms becomes unambiguous, however, most structures have not been resolved to this level and some uncertainty will always exist regarding the exact orientations of peptides and levels of solvation. Computer simulations can add to this 'fuzzy' picture, taking X-ray data as a starting point to generate optimised geometries for protein sequences. This is even possible without X-ray data if the primary sequence is known to have similarities to regions of previously determined structures through homology mapping. There are drawbacks with both crystallographic and computational models. In the former a snapshot of a protein structure is obtained giving no information about structural dynamics over time. In addition, as a necessary part of the crystallisation process, solvent, usually water, may be lost. This has the potential to generate artefacts such as hydrogen bonds or unnaturally strong ion binding in the resultant structure leading to misleading interpretations. One method to improve resolution is to incorporate 'heavy' atoms like mercury to identify particular residue positions but this too may lead to structural artefacts.

Computational models suffer because of the sheer size of the proteins. Prediction of polypeptide geometries is accurate for small sequences and may be carried out at a high level of theory, however, large numbers of residues can only be modelled at present using simple molecular mechanics methods to give structural information. If good X-ray data are available then initial positions for atoms can be determined with some accuracy and the simulation conducted at semiempirical levels of theory thus enabling energetics to be investigated. Even this is computationally expensive: Roux's simulation of the KcsA channel contained over 40,000 atoms and required supercomputers to carry out the calculations[6]. Artificial systems are more

amenable to study by computational or single crystal X-ray diffraction experiments as the molecules are by and large smaller than their natural analogues.

The dynamics of ion transport can be studied either through a lipid bilayer or a cell membrane. In the former technique, useful for artificial channel mimics, a purified lipid is 'painted' across a small hole in a dividing wall between two compartments. The compounds to be studied are incorporated into the bilayer and the ion of interest introduced into one compartment. Transmembrane currents are measured across the bilayer which may itself be 'voltage clamped' where the transmembrane potential held at a constant value. The clamp may be applied at different potentials to determine the point at which ions permeate the lipid and will record the total current. Ion transport through a living cell, or through part of a cell membrane, is often measured as a function of single channel activity due either to opening of endogenous channels or to the effects of ion channel mimics. The technique of patch clamping, when used on whole cells, requires that a micropipette containing an electrode make a good seal with the cell membrane to generate a 10 GΩ resistance between the cell and the pipette wall. The resulting transmembrane currents, measured by microelectrodes inside and outside the cell, generate extremely low noise to allow single channel events to be monitored. This method allows for variation in the holding potential to investigate voltage-gated effects. As well as 'cell attached' experiments, whole cell currents can be measured by bursting the membrane with the pipette or the ion flux can be measured through an isolated 'patch' of cell membrane. There is always the danger that the events recorded are due to endogenous channel activity rather than the model compound, fortunately this is less of a problem with Na^+ channel models as there are far fewer Na^+ than K^+ channels present in cells.

The above methods are appropriate to study either natural or unnatural channels in a cellular or bilayer environment. Indeed, many channel-forming compounds have been tested using the planar lipid bilayer method as it is simple to interpret and gives unambiguous evidence of transmembrane ion currents. One other method available to model Na^+-channel mimics is the use of ^{23}Na NMR. Transport studies using Na^+ in solution have been reported for many years but advances in solid state NMR have implications for Na^+ binding within ion channels. Recently, magic angle spinning ^{23}Na NMR spectroscopy was used to determine the presence of Na^+ in three different environments within a G-quadruplex structure composed of guanosine derivatives. This technique relies on spinning a crystalline sample at 54.7° (the 'magic angle') to the applied magnetic

field thereby simplifying the analysis of the resulting spectrum. The interpretation was confirmed by X-ray crystallography[9].

Thus two facets of an artificial ion channel's operation must be evaluated to determine how closely it mimics natural channels: structure, particularly of the selectivity filter, and activity. The examples that follow have been chosen because they model one, and in some cases, both facets. None are as efficient as natural systems but all give a degree of chemical insight into the workings of Na^+ and K^+ channels.

Design of artificial transmembrane ion channels

The features which any synthetic analogue must have if it is to be a successful model for a natural transmembrane channel are threefold. Firstly, it must span the cell membrane, implying a concerted structure over 40 Å in length. Secondly, it must be able to discriminate in favour of one ion. Finally, it should achieve a transport rate in the region of 10^4 to 10^8 ions per second.

The following examples serve to illustrate the different approaches taken to synthesise artificial ion channels with an emphasis on Na^+- and K^+-selective systems. It will become clear that two synthetic philosophies exist. In one, linked helical molecules are used in an attempt to reproduce the channels either within the helix or between interlinked helices. In the other, compounds known to be selective for particular alkali metals are employed as selectivity filters and membrane-spanning molecules are attached to guide the ions to and from the filter. In general the first group is based on artificial sequences of peptides and the second on crown ethers or similar synthetic ionophores. In addition some systems have been designed primarily to generate membrane-spanning molecules, with the hope of ion specificity, and others are focused on the selectivity filter with the potential for transmembrane aggregation.

Classes of artificial systems

Peptide-based and helical channels

In 1988 DeGrado reported a group of helical compounds prepared from combinations of serine and leucine[10]. Heptapeptides were synthesised using varying sequences and linked to form dimers and trimers. The reasoning was simple: a 21-residue sequence should span a membrane and the combination of serine and leucine would imbue the polypeptides with a polar yet hydrophobic surface and α-helicity. Dimers failed to form stable channels, however, a

21-residue sequence, H_2N–(leu–ser–ser–leu–leu–ser–leu)$_3$-$CONH_2$ (**1**, Figure 2), had properties similar to the acetylcholine receptor. An analogue, in which the second serine is replaced with leucine, generated a proton selective channel. Computer simulations indicated that the first sequence formed interlocking hexamers with an internal channel diameter of approximately 8 Å, large enough to accommodate water molecules coordinating to the higher concentration of serine residues. The second sequence formed trimeric or tetrameric aggregates, in which the central cavity could transport protons but little else as the internal diameter was in the region of 1 Å. The large internal cavity formed by the first sequence was shown in lipid bilayer experiments to be permeable to a range of alkali metals but without any significant selectivity.

A variation on DeGrado's approach was introduced by Voyer in 1997[11]. A sequence of 21 amino acids was prepared from alanine and a synthetic ionophore [21]crown-7 inserted as a phenylalanine derivative positioned every third or fourth residue (**2**, Figure 2). The resulting compound resembled a peptide α-helix with a series of stacked crown ethers emanating from the external surface. Planar lipid bilayer experiments indicated Na^+ conductance, which could be reduced by guanidinium or Cs^+.

The use of an α-helix to form a channel or aggregate to leave a conducting core has obvious similarities with natural channels, however, an alternative peptide-based strategy has shown remarkable results. Ghadiri[12,13], and more recently Inoue[14], have explored the potential for cyclic peptides to self-assemble into hydrogen bonded tubes. Once the macrocycles insert into a membrane they continue to stack until they form a channel through the bilayer. This approach removes the necessity for long sequences of peptides with carefully tailored helical properties. Indeed, compounds as simple as cyclic tripeptides with an internal diameter of about 4 Å have been shown to be permeable to K^+.

Ghadiri has used alternating sequences of D- and L-amino acids to form structures such as cyclo[(L-arg-D-leu)$_4$-] (**3**) and cyclo[(L-glu-D-leu)$_4$-] (**4**) (Figure 3) with inner diameters of 7.5 Å. Initial experiments using the transmembrane porin α-hemolysin inserted into a planar bilayer showed that a cyclic peptide could lodge within a constriction inside the porin reducing the innate Cl^- flux and allowing K^+ to pass through[12]. The structures of these cyclic peptides have since been varied to give differential effects in cell membranes. Specifically, they have antibacterial activity against methicillin-resistant *Staphylococcus aureus* and *Escherichia coli*. It seems that they insert, stack and tilt at about 70° to the bacterial membrane. The

insertion compromises the membrane structure and leads to species dependent cell destruction[13].

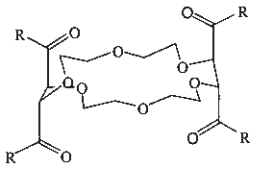
In a recent paper by Inoue[14], a series of cyclic compounds comprised of three to five dipeptide units alternating between natural and rigid, unnatural amino acids were prepared. Compounds, such as **5** in Figure 3, showed greater permeation to K^+ than Na^+ and were blocked by Ca^{2+} in a voltage-dependent fashion.

A further candidate for Na^+-selective ion channel mimicry from this class is a molecule designed by Bell[15] (**6**, Figure 3) that forms an intertwined double helix with Na^+. The dimer slowly unwinds around two Na^+ ions and when completely unwound would have a depth of 18 to 20 Å. Although this compound has yet to be tested for transmembrane ion transport it has features in common with helical peptides and may be a design worth pursuing.

Ionophore based channels

One of the first indications that synthetic ionophores could form the basis of artificial ion channels was the crystal structure of Lehn's tetracarboamido[18]crown-6 (**7**, Figure 4) as the potassium hydrate[16]. The solid state structure showed stacking of the ionophores with hydrated K^+ in channels formed by the crowns. As K^+ has a greater affinity for [18]crown-6 than for any other crown, the structure appeared to lend itself to ion channel formation.

To function as a true transmembrane channel the selectivity of [18]crown-6 had to be incorporated within a much larger structure. This challenge was taken up simultaneously by Lehn and Fyles. Lehn extended his crown ether derivative with polyamide groups terminating in polyethers (**8**, Figure 4), however, no transport data were determined[17]. Fyles used a modular approach to building up a channel mimic[18]. At the core was an [18]crown-6 di-, tetra- or hexaacid derivative to which polar wall units based on cyclic tetraesters were appended. At the termini were acid, alcohol or glucose groups all of which have the necessary hydrophilic character to intercalate into the polar surfaces of lipid bilayers. The natural torsion angles within the crown ether ensured that the arms alternated to span a membrane in a symmetrical and amphiphilic manner. The use of a glucose head group was particularly inspirational as it mimics the glycolipid structure found on the external surface of red blood cells. Some compounds showed ionophore-type behaviour, however, several, including **9** in Figure 4, exhibited transmembrane channel properties. It is assumed that the structures insert through the membrane with the crown ether moiety lying in the hydrophobic central region. In this region the ether oxygens are oriented inwards to form



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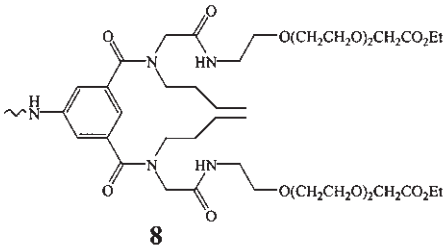

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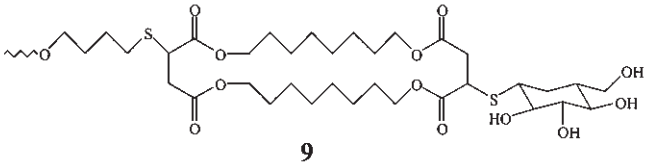

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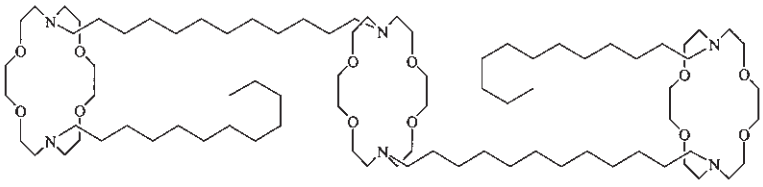

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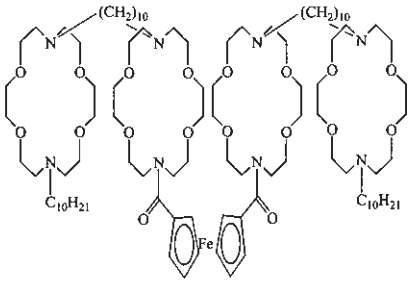

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Fig. 4. *Membrane-spanning compounds based on crown ethers. Compounds incorporating simple groups on the crown, such as dimethylamide* ***7****, are likely to act as ionophores whereas extensive sidechains, employed in* ***8*** *or* ***9****, give transmembrane activity. Compounds* ***10*** *and* ***11*** *use crown ethers as relays to facilitate cation transport.*

an ideal binding pocket for alkali metals. The large hydrophobic arms should collapse into impermeable cables when *in situ* yet aquated cations, initially attracted to the hydrophilic termini, pass through to the central crown ether filter and exit through the opposite end of the tubular molecule. The overall effect was memorably likened to "a snake swallowing an egg" by Professor Fyles when discussing these compounds in 1988. Of those compounds that acted as transmembrane channels, all had low transport rates for Li^+ (which appears to be a common theme in artificial transmembrane ion transport) and generally favoured either Na^+ or K^+.

Both Lehn's and Fyles' model compounds contain central selectivity filters which also function as staging posts where (aquated) cations are held in an energetically favourable environment prior to expulsion through a hydrophobic region and into aqueous solution. This principle has been used to great effect by Gokel whose 'hydraphiles' use one or more crown ethers as relays within membrane spanning structures[19]. The design is deceptively simple given its success. Rather than have several arms emanating from a crown ether, only two alkyl chains are employed. To generate a molecule long enough to span a bilayer, further sidearm-appended crowns are attached until an approximate length of 28 Å (as seen for **10** in Figure 4) is achieved.

Where transmembrane ion transport was observed, using either ^{23}Na NMR or planar lipid bilayer methods, Na^+ specificity was obtained. Initially this appeared to be at odds with the nature of the relay employed, diaza[18]crown-6, until the kinetics of the system were considered. Derivatives of [18]crown-6 are usually associated with K^+ selectivity yet Na^+ fluxes were being observed. Both can be bound by [18]crown-6 in aqueous solution with the selectivity (based on relative binding constants) for K^+ eighteen times that for Na^+. The selectivity is derived from two components, the rate of binding and the rate of release. What is important in this case is the rate of release, which is ten times greater for Na^+ than K^+. Thus these compounds transport Na^+ preferentially because K^+ is released more slowly.

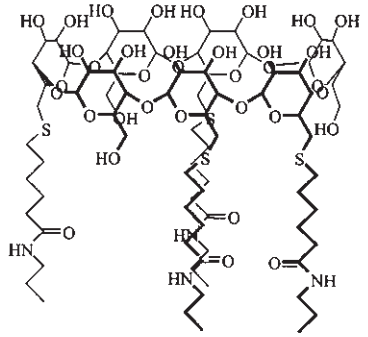
Using a similar principle, Hall has demonstrated voltage-dependent channel opening using a hydraphile[20]. In this model, a ferrocene group linked two crown ether relays thereby allowing the charge of the compound to be affected by the electrochemically active redox centre. Studies using patch clamp techniques on hamster neuronal cells indicated channel opening at -60 mV in the presence of hydraphile **11** (Figure 4), no activity at 0 mV and further activity at +60 mV. The implication was that channel formation and ion conductance varied in response to applied potentials.

Crown ethers make attractive platforms from which membrane-spanning channel mimics may be based but other cyclic compounds with well-defined central cavities have also been used to great effect. Two groups, the cyclodextrins and calixarenes, have been the subjects of several research groups. These compounds are characterised as having different functional groups at their upper and lower rims and are therefore often encountered as 'half channel' models. In most cases the macrocycle acts as a portal at the bilayer/water interface and hydrophobic groups intercalate with the lipophilic phospholipid 'tails'.

One of the earliest examples of this design strategy is Tabushi's β-cyclodextrin derivative[21]. The internal cavity size of a cyclodextrin is determined by the number of D-glucopyranoside units in its structure thus the three main homologues, α-, β-, and γ-cyclodextrin, comprising six, seven or eight units, have internal diameters of 5.7, 7.8 and 9.5 Å, respectively. In Tabushi's compound, **12** in Figure 5, one rim of the cyclodextrin bristled with 14 hydroxyl groups that allowed the molecule to sit in the polar region of the bilayer. Four alcohol groups on the other rim were modified to thiols to which amide containing alkyl chains were appended. Transition metal, although unfortunately no alkali metal, transport studies were undertaken.

Calixarenes, in particular calix[4]arene, have been seen as potential cation filters as they are readily functionalized on both upper and lower rims[22]. Calix[4]arenes have a second property which can be exploited to make ion channels: they exist in different conformers which can, in some instances, be interconverted. If an asymmetric filter is required it is possible to start from a cone conformer, however, a symmetric compound can be prepared from the 1,4-alternate conformer. The implication is that if chains which span a half channel are attached to the lower rim of a cone conformer, aggregation is necessary to span the entire membrane, however, if the same procedure is applied to the 1,4-alternate conformer the membrane can be spanned by a single molecule. This has been elegantly demonstrated by Gokel who appended four dodecyl ethers, terminating in *N*-benzyldiaza[18]crown-6, from calix[4]arene in both cone and alternate conformations[23]. The alternate derivative (**13**, Figure 5) allowed Na^+ to 'burst' through a planar lipid bilayer, however, the cone derivative exhibited no cation conduction as it could not span a bilayer fully.

Kobuke employed a calix[4]resorcarene extended with heptadecyl substituents (**14**, Figure 5) to span a lipid monolayer, demonstrating both Na^+ and K^+ conductance with a selectivity for K^+ over Na^+ by a factor of three[24]. Conduction of the latter could be blocked by addition



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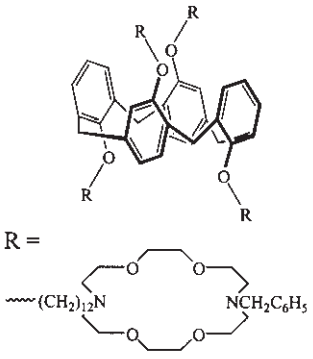

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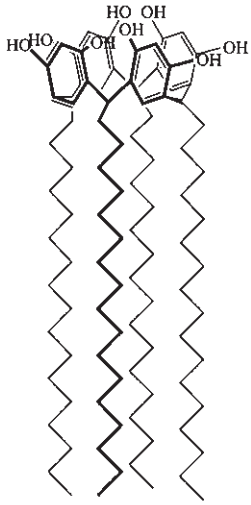

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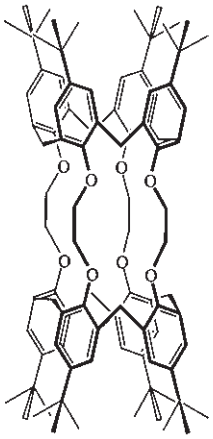

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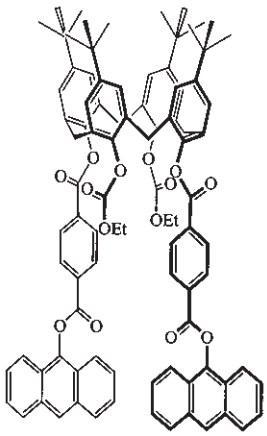

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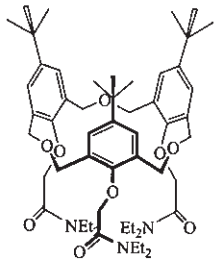

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Fig. 5. *Compounds based on rigid filters may form artificial ion channels (β-cyclodextrin derivative* ***12****, calix[4]arene* ***13*** *and calix[4]resorcarene* ***14****) or act as ionophores (calix[4]arenes* ***15*** *and* ***16****, and oxacalix[3]arene* ***17****).*

of Rb^+. Conductance was not observed by analogous compounds prepared with shorter undecyl substituents leading the authors to speculate that the compounds inserted in inner and outer phospholipid layers with cation transport occurring when two molecules met 'tail-to-tail'.

Three other calixarene-based systems deserve mention, even though they are not transmembrane channel forming compounds, as they highlight the use of calixarenes as ion filters. In 1997 Beer published a remarkable bis(calixarene) (**15**), shown in Figure 5, in which two calix[4]arenes in the cone conformer were linked by four ethyl bridges[25]. Two structures were determined by crystallography, in one the intercalixarene cavity was constricted and free from cations, in the other K^+ had been captured within the macrocyclic cavity which in turn had been forced in to a more expanded geometry. In a recent paper computational modelling indicated that the lowest energy pathway for the cation to enter the cavity was through the calixarene annulus rather than between the ethyl bridges[26].

In 2000 Jin published cation transport data for a bisanthroylcalix[4]arene (**16**, Figure 5) which selectively transported Na^+ over K^+ across a lipid bilayer[27]. One interesting aspect of the calixarene was that the anthracene groups were close enough to be photodimerized. The effect of this was to close the selectivity filter and greatly reduce Na^+ currents during and after irradiation.

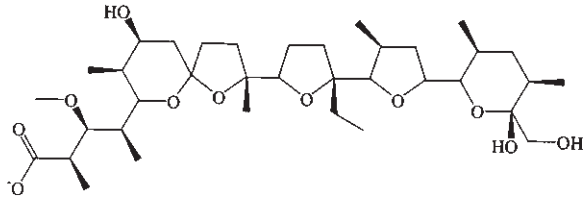
The other calixarene-based system of note is the oxacalix[3]arene derivative (**17**, Figure 5) of Cragg[28]. Oxacalix[3]arenes have similarities with both calixarenes and [18]crown-6, however, initial data indicated no particular preference for cation selectivity. Several attempts to crystallize **17** as a K^+ salt failed, even though the cavity size indicated a preference for this ion, and only when attention was switched to Na^+ was a crystal structure obtained. One water molecule was bound to both cations, supporting the current opinion of a cation-water-cation-water alternating chain through the filtering region of both Na^+- and K^+-selective channels. A patch clamp study of **17** introduced to mouse-rat hybrid glioma cells indicated ion transport, possibly due to membrane disruption, at potentials consistent with Na^+.

Amphiphiles and molecular harpoons

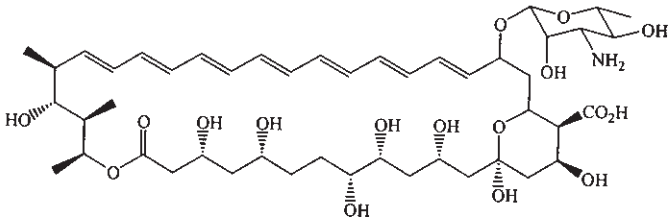
The final class of compounds promoting transmembrane ion transport contains no selectivity filter yet exhibits impressive transmembrane ion transport. The simplest compounds are the alkyl esters of polyethylene glycols containing benzyl head groups (*e.g.* **18** in Figure 6) reported by Menger in 1990[29]. These flexible compounds

were observed to have greater transporting abilities than gramicidin but were non-selective. Another group of compounds, consisting of bis(polyethyleneglycol)esters of alkanes (**19**, Figure 6) or alkenes, developed by Regan as 'molecular harpoons', selectively disrupts phospholipid bilayers and cell membranes in a similar fashion to the Triton class of surfactants[30]. Again there was no evidence of ion selectivity; membrane disruption alone was enough to compromise the intercellular ionic concentrations.

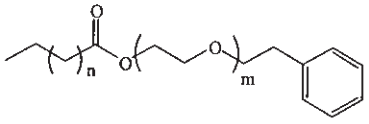
In an attempt to insert short amphiphiles into a phospholipid membrane, Kobuke linked alkylpolyether carboxylates to alkyl-



monensin A



amphotericin



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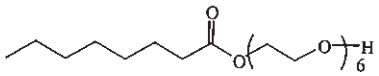

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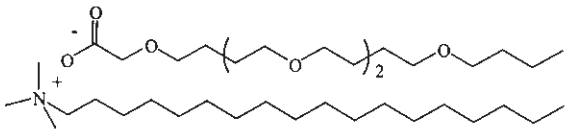

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Fig. 6. *Natural 'barrel stave' molecules monensin A and amphotericin that form transmembrane channels by aggregation, artificial molecular harpoons* ***18****,* ***19*** *that disrupt cell membranes, and channel forming* ***20****.*

ammonium cations (**20**, Figure 6) and demonstrated single channel opening, in the presence of both Na^+ and K^+, but without discrimination[31]. In later work the same group employed membrane spanning cholic acid derivatives that, despite having no clear filtering mechanism, were selective for Na^+ over K^+ and Li^+. The authors ascribed this to K^+ being held strongly by the channel forming compounds and allowing Na^+ through by reverse stability[32, 33]. It is interesting to compare this charge-based effect of reverse stability with the size-related effects in Gokel's diaza[18]crown-6 incorporating hydraphiles.

Conclusions

Consideration of the different classes of Na^+ and K^+ transmembrane transport systems described above leads to a realisation that certain features are required in the design of a successful ion selective artificial transmembrane channel. There are two basic requirements: a membrane spanning tube or ion guide and an effective mechanism for ion filtering. Based on the examples given and evidence from natural channel proteins an artificial channel should have a minimum span of 40 Å with a lipophilic central region and amphiphilic termini. The inner surface should be lined with weak regions of electron density, such as oxygen containing ethers and esters or aromatic groups, to facilitate the movement of ions under electrical or osmotic influences. The electron density should not be so great as to bind the ions irreversibly or they will block the channel. Selectivity can be achieved by matching the coordination environment of the selectivity filter to that of the solvated ion thus providing a low energy desolvation pathway for ions.

Future developments

Without the initial research on the structure and dynamics of natural alkali metal ion channels it is unlikely that synthetic chemists would have pursued the design and synthesis of mimetic systems. Improvements in techniques used to study cellular or *in vivo* ion channel activity will be vital if more realistic models of transmembrane ion channels are to be developed. Likewise, the incorporation of chemical functionality to reflect the natural architecture of ion channels relies on accurate, high resolution three dimensional structures. Advances in protein crystallography and structural elucidation by NMR will aid in this endeavour. What about the hardest task, the chemical synthesis of these complex systems? The field of supramolecular chemistry has, in recent years, led the way in the design

and synthesis of multicomponent structures of nanometre scale[34]. These 'supramolecules' sometimes rival Nature in their complexity, deriving tertiary structure from carefully designed hydrogen-bonding arrays and other non-covalent interactions. Perhaps the future of ion channel mimicry lies in the self-assembly of complementary small molecules, rather than one large moiety, to form channels terminating in ion specific filters.

The benefits of more accurate ion channel models will be twofold. Firstly, simple analogues of natural systems are relatively easy to study so more accurate models will give greater insight into the function of complex transmembrane proteins. Secondly, synthetic analogues may some day be used to augment naturally occurring ion transport activity which has been impaired by disease, genetic mutation or the effects of certain toxins. To date no channel model has been prepared which combines the rate of transmembrane ion transport with the ion selectivity seen in natural channels, however, it is clear that these two phenomena have been replicated individually. It remains to be seen if they can be combined in the laboratory to generate models that, through structural and dynamic analysis, help us to further understand the mechanisms by which ion channels function.

Acknowledgements

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