From Ion Channels Flow Fresh Discovery Approaches

Recent research on two channel types — potassium channels and water-selective aquaporins — revealed information about how channels work that could benefit the modeling of ligand–receptor interactions. The author discusses this research and its implications for drug design.

In this month's column, I will discuss recent breakthroughs in solving high-resolution structures of membrane proteins — in particular, channels that are selective for ions and water. These structural studies complement those of ligand–receptor interactions, and I believe that there are lessons here to be learned for rational drug design.

First, ion channels form water-filled pathways (pores) across cell membranes. High-resolution structural analyses of these pores provide information on multiple copies of the ligand bound to different binding sites on the same protein. While frozen in time, the multiple copies inside the pore present a time series of stable intermediates outlining the pathway of a molecule through the narrow pore. In other words, they provide snapshots on the movement of these solutes along a protein surface, giving a glimpse at the dynamics of surface binding.

Second, information on the location of binding sites of solutes and water molecules inside the pore allows accurate modeling of molecular interactions. Conformational restraints (i.e., a limited number of binding sites for a small number of solvent molecules) make modeling the solvent structure in these narrow pores possible. Because of their finite volume, these channels provide detailed information about local solvent structure, including details of solute–solvent, solvent–channel and solute–channel interactions. Knowledge gained from these interactions can be applied to pore-like binding pockets on enzymes and receptors.

Changing Our Thinking about Channels

Two channel types recently have gained prominence: potassium channels and water-selective aquaporins (1, 2). The importance of the structures of these cell membrane proteins lies in their ability to offer plausible mechanisms of channel selectivity, explaining puzzling observation from functional studies. Potassium-selective ion channels facilitate the diffusion of a large cation (potassium) at the expense of a smaller one (sodium), while aquaporins facilitate water transport at the expense of ions, particularly protons. Prior predictions about how these channel proteins accomplish their acts all have focused on plausible yet unsubstantiated models from independent studies (3). In recognition of the importance of these high resolution structures, Peter Agre and Roderick MacKinnon have been awarded the 2003 Nobel Prize in Chemistry for their work to elucidate the structure–function relationship of aquaporin and potassium channels, respectively, through X-ray crystallography.

The ability to study the high-resolution structures of channel types, as well as proton pumps and multi-drug resistance proteins, are changing the way we think about channels. For the most part, ion channel structure–function analyses have been based upon the study of model channels, such as toxins (e.g., alpha hemolysin, melittin), or channel-forming antimicrobial peptides, such as magainin, alamethicin or synthetic peptide nanotubes (4). These channels have been modeled as cylindrical, water-filled pores across a hydrophobic barrier. Polar and charged binding sites are placed along the permeation pathways to simulate energy barriers within the pore that could account for the observed ion selectivities and flux rates of cylindrical structures.

It now has become clear that this geometric criterion is not readily applicable to most membrane channels, which commonly exhibit asymmetrical features. These differences are unfortunate from a modeler's viewpoint, but might make sense; after all, toxin

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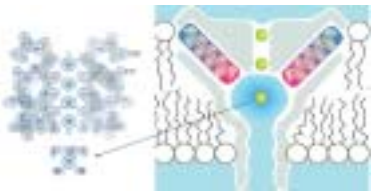
channels function by irreversibly perforating cell membranes, causing cells to die.

The emerging picture from aquaporin and potassium channels reinforces the importance of controlling the water structure surrounding a permeable ion. The ability to extract an ion from bulk water (partial dehydration) is a prerequisite for permeability, as most channels are too narrow for hydrated ions to pass through. Small differences in binding energies of water molecules bound to different ions (hydration shell) determine if ions can diffuse freely into the pore. Such an event is favored if the channel surface offers the ion a binding opportunity that matches the strength and geometry of bulk hydration.

The ability to control ion hydration is directly linked to the ability to control the structure of water inside channels. It has become evident that how the solvent behaves in the presence of an ion inside the pore is essential to permeability. In particular, the influence of hydrophobic amino acid residues on local water structure has been demonstrated at the atomic level of the bacterial K-channel KcsA at 2.0 Å resolution (5). The pore inside this channel is divided into three regions across the membrane (Figure 1); a narrow constriction called the selectivity filter faces the extracellular opening of the channel, followed by a wide central cavity that thins slightly toward the cytosolic opening. Eight water molecules inside the central cavity of the pore are grouped around a single potassium ion, forming a hydration shell with a dimension that perfectly mimics the selectivity filter structure — the narrowest region of the pore where ion selectivity is controlled by the channel. This allows a potassium ion to easily slip from its water shell into the selectivity filter, a narrow gauntlet of rings of backbone carbonyls (Figure 1).



The water–ion complex sits in a hydrophobic cavity made of isoleucines and phenylalanines. The use of hydrophobic residues — instead of polar or charged ones — eliminates hydrogen bonding between water and the protein surface. As a result, the water molecules are free to orient their dipoles toward the dominating, centrally-located ion. Thus, the mobile charge inside the channel determines the exact water structure, because water molecules

Figure 1. The ion pathway through a K-channel. The pore and ion binding sites (right) and high-resolution structure of the selectivity filter and central cavity (left) are shown. The central pore cavity contains eight water molecules surrounding the potassium ion (arrow). In the selectivity filter part, four ion binding sites (left) have been identified. However, no more than two ions can bind simultaneously, due to electrostatic repulsion between neighboring charges (right; upper narrow pore structure). (Adapted from references 2 and 5; © *Science AAAA*; Nature Publishing Group).

Fueled by cross-fertilization of ideas, the distinction made among channel, enzyme and receptor is starting to break down.

prefer electrostatic interactions over hydrophobic ones. Only if there is a perfect match between water structure and selectivity filter geometry does the ion encounter a minimal energy barrier, allowing it to jump into the filter. This will only be the case if a potassium ion sits in the cavity and the selectivity filter is in its open-gate conformation. Overall, the central cavity promotes rapid hydration/dehydration of potassium but not sodium ions, moving in and out of the selectivity filter of the channel.

A Better Understanding of Water Movement

Before the high-resolution structure described above became available, a widely accepted model of selectivity in potassium channels focused on ion binding to tyrosine residues, rather than hydration shell stability. The importance of hydration shell stability has been recognized for quite some time (6), and is the main model explaining cation selectivity of channels formed by the antimicrobial peptide, gramicidin. This peptide has been used as a model pore since the 1960s, for reasons that include its formation of the perfect cylindrical channel. Yet, its biochemical durability is due to a structural design and amino acid composition unlike any other naturally occurring protein or peptide channel, limiting its use as a model pore beyond the selectivity mechanism.

Referring back to K-channels, based on mutagenesis experiments, tyrosine residues were found to be essential for ion permeability. At the same time, organic chemists could show that potassium (but not sodium) ions form a preferred interaction to the aromatic ring of tyrosine, giving rise to a perfectly good model of ion selectivity based on tyrosine-ion interaction. Today we know that the role of these tyrosines in the selectivity filter is to stabilize subunit contact sites rather than to bind to ions.

While ion flux through channels is coupled to water movement, aquaporins require a mechanism that separates water from ion flux. A puzzling question in water selectivity is the prevention of proton flow. Unlike larger monovalent ions, proton flow is facilitated along hydrogen bond networks forming proton wires. The aquaporin structure shows that water molecules are reoriented by positively-charged residue at the center of the pore. It has been suggested that by reorienting the water dipole, the proton wire is broken inside the channel, interrupting proton conductance but not water flow (1). This model might not account for the whole story because all cations face electrostatic repulsion from the positively charged residues located at the pore constriction that measures a tight 3 Å.

There are no immediate medical breakthroughs expected from these structural studies, although both aquaporins and potassium channels are — like all membrane transport systems — involved in diseases (7). However, there are two explicit reasons why these channel structures are relevant for rational drug design. First, the structures reveal the importance of protein features that have been neglected when modeling ligand–receptor interactions. The structures help put modeling of solvent and ion interaction with proteins on a solid experimental foundation, based on the finding that backbone structures and hydrophobic amino acids — rather than polar and charged residues alone — play an important role in aqueous pathways. Second, the "snapshot" structures of multiple permeants (ions, sugars, glycerol and water) within pores allow molecular dynamics simulations of solute flux and calculations of energy landscapes that are of immediate relevance for computer-aided drug design.

Application to Enzymatic Systems

Lessons learned from water and proton flux at the microscopic level already are being applied to many enzyme systems (8), and the distinction made among channel, enzyme and receptor is starting to break down. Channels really are cavities with two entrances mimicking binding pockets on enzymes and receptors for substrate and ligands. Some examples will explain what I mean. Carbonic anhydrase is one of the most efficient enzymes, with a catalytic site (a zinc ion) buried several Ang-

stroms inside the protein. The substrates and products — water, carbon dioxide, protons and bicarbonate — need to rapidly diffuse in and out of the binding pocket. Water molecules are integral to the reaction mechanism, and proton flux is modeled along the line of a proton wire mechanism. An uninterrupted hydrogen bond network among water molecules in the enzymatic active site allows rapid equilibration of positive charges associated with free protons ("proton wire") between the bulk-phase and the catalytic site. In other enzyme systems, where metabolic pathways can be catalyzed in multi-enzyme complexes, substrate transfer reminiscent of membrane channel function is being studied. One example is the glutamine amidotransferase that carries out the fifth step of the histidine biosynthetic pathway. The enzyme complex promotes ammonia conductance between two catalytic subunits, bypassing cytosolic diffusion in a process known as substrate channeling. The channel inside the enzyme complex between adjacent catalytic sites shows properties similar to those found in membrane transporters, including permeability, selectivity and gating (9).

What makes the study of these ion and water channel structures intriguing is the availability of functional data. The electrophysiology and pharmacology literature is loaded with information about conductances, permeability ratios and selectivity for almost every available electrolyte combination. Without rich functional data, the best atomic details could not reveal such mechanisms (10). Thus, having a structure immediately conveys a mechanism for a functionally well-characterized protein system.

References

1. Murata et al., *Nature* **407(6804 SU-),** 599–605 (2000).
2. D.A. Doyle et al., *Science* **280(5360),** 69–77 (1998).
3. S.K. Silverman, H.A. Lester and D.A. Dougherty, *Biophys. J.* **75(3),** 1330–1339 (1998).
4. M.R. Ghadiri, J.R. Granja and L.K. Buehler, *Nature* **369(6478),** 301–304 (1994).
5. Y. Zhou et al., *Nature* **414(6859),** 43–48 (2001).
6. S.S. Sung and P.C. Jordan, *Biophysical Journal* **51(4),** 661–672 (1987).
7. National Center for Biotechnology, Online Mendelian Inheritance in Man (OMIM). Accessed at http://www.ncbi.nlm.nih.gov).
8. D.N. Silverman, *Biochem. Biophys. Acta.* **1458(1),** 88–103 (2000).
9. R. Amaro, E. Tajkhorshid and Z. Luthey-Schulten, *PNAS* **100(13),** 7599–7604 (2003).
10. L.K. Buehler, *PharmaGenomics* **3(5),** 20–21 (2003).**PG**