New and Notable

Why Can't Protons Move through Water Channels?

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Aquaporins are proteins with a hole down their middle that allow the flow of water (Agre and Kozono, 2003) through otherwise impermeable membranes and thus make the life of (animal) cells possible, as we know it (Agre et al., 1998). The membranes of animal cells are weak, not able to maintain significant pressures, and so water must flow easily out of cells if they are to survive with membranes intact. Torn membranes are often the immediate cause of death from disease or dysfunction because torn membranes cannot maintain the gradients of concentration and electrical potential necessary for cell function. As in so many other cases, evolution controls a vital cellular function by a single class of proteins, and so it is fitting that the discoverer of these proteins—Peter Agre—was one of the recipients of this year's Nobel Prize in chemistry.

When a positive charge is added to a water molecule, the resulting water ion becomes the fundamental aqueous cation, called a ''proton'' here to beg the question of its precise chemical identity. The flow of these protons is as fundamental to life as the flow of water (DeCoursey, 2003), because the flow of protons is coupled to the energetics that fuel metabolism. It seems advantageous for the cell to have separate transport mechanisms for water and protons so it can control cell volume and metabolism independently. From this biological point of view, it is not surprising that protons are unable to flow through aquaporins. The chemical point of view is different, however. Protons hardly move through protein channels filled with water, but they move very easily through water, and ice, by some variation of the so-called Grotthuss mechanism involving proton/charge exchange, rather than electrodiffusion of a cationic water moiety. It is necessary then to explain why protons cannot move easily through a water channel as they do through an aqueous solution or ice. The explanation should reside, one imagines, in the structure of the channel protein or some special physical property of the protein and lipid surrounding it.

The structure of several important channels is now known, thanks to Roderick MacKinnon. His pioneering work in crystallizing channel proteins and determining their structure was recognized with the award of a Nobel Prize this year, shared with Peter Agre. Following these studies, Fu et al. (2000) and Sui et al. (2001) determined the structures of some aquaporins. It is natural to look at these structures seeking an answer to the question: ''Why can't protons move through a water channel?'' But the answer is not clear. The structure tells much but it does not immediately predict permeation and selectivity. The structure only hints at the special physical properties of the protein and surrounding lipid.

Theoretical attempts to address the water/proton selectivity in aquaporins (e.g., de Groot and Grubmüller, 2001; Tajkhorshid et al., 2002) have actually studied only water transport. Water transport is much simpler to simulate than proton transport because water has no net charge. Many effects of the electric field seem safe to ignore when studying water transport. Most theoretical studies—building on earlier conceptual models of proton transport (e.g., Nagle and Morowitz, 1978)—have more or less assumed that proton flow in channels is controlled by a one-dimensional version of the Grotthuss mechanism, with a column of waters forming a proton wire threading through the channel protein (e.g., Fu et al., 2000; de Groot and Grubmüller, 2001; Kong and Ma, 2001; Law and Sansom, 2002; Tajkhorshid et al., 2002; DeCoursey, 2003). Protons are then thought not to flow through aquaporin because the protein disrupts the specific arrangement of water molecules necessary for proton exchange.

A recent paper of Burykin and Warshel (2003) challenges this long-held belief by examining the actual energetics of *proton* transport in aquaporin, seeking to evaluate the electrostatic energy needed to transfer a proton through the protein. Warshel and co-workers have studied the role of the electric field in determining many properties of proteins, including proton transport, for many years (Warshel, 1979; Warshel and Russell, 1984; Warshel, 1986; Sham et al., 1999), and recently they have been joined by many others who seek to explain important functions of proteins and channels starting with their electrostatics (see the classical papers of Davis and McCammon (1990), Honig and Nichols (1995), and Levitt (1991); and see the early papers of Eisenberg (1990, 1996)).

Burykin and Warshel (2003) calculate the energetics of a proton wire in the electrostatic environment of a channel. They use a mesoscopic model of the electric field together with a simplified empirical valence bond type effective potential to describe proton exchange in a proton wire and calculate stable estimates of the free energies of the different steps in proton transport. Burykin and Warshel (2003) found (see their Fig. 4) that the barrier for proton transport is enormous (~15 kcal/mol), whereas the barrier for water transport is small (<2 kcal/mol). The main source of the barrier was the (mostly electrostatic) desolvation penalty of moving the proton charge from bulk solution to water molecules in the channel interior. The dielectric properties of the protein dominate this electro-

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static barrier, and the protein permanent dipoles and ionized groups contribute to its shape. The effects of perfect water orientation are *relatively small in membrane proteins* embedded in lipid bilayers because the electrostatic barriers are much larger in such systems. The same effects of water orientation are *relatively large in bulk water and ice*, which do not have these electrostatic barriers because the water and ice are not part of a membrane system.

The importance of electrostatic effects in proton transport is increasingly recognized. de Groot et al. (2003) present qualitative free energy profiles that led to a significant barrier at the center of the channel, which they attribute to the effect of helix macrodipoles. This finding is in some conflict with the finding of Burykin and Warshel who show minimal contribution from the helix macrodipoles. Jensen et al. (2003) suggest that that lack of proton transport depends on the dipolar water arrangement, but argue that electrostatic interactions between the proton and the channel play a major role.

The finding of Burykin and Warshel (2003) seems to be of general relevance to channels and transporters, where it is likely that electrostatic effects are one of the main factors (Eisenberg, 1996; Cardenas et al, 2000; Corry et al, 2000; Eisenberg, 2000; Im and Roux, 2002) that control transport, along with finite volume effects of crowded charge (Nonner et al, 2000; Eisenberg, 2003) so important in determining selectivity.

It seems clear that understanding the biological role of aquaporin requires reliable and calibrated calculations of the energetics of proton movement in aquaporin. Burykin and Warshel (2003) show that electrostatic energies dominate proton movement. If so, the task of understanding biological function is much easier: the chemical processes involved in proton exchange need be studied with only enough resolution to verify their relative unimportance. Understanding proteins and channels would be much easier if all their energetics were dominated by mesoscale electrostatics and physics that can be calculated without keeping track of the trajectories of myriads of atoms on a femtosecond timescale.

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