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MINI-REVIEW

Water Movement During Channel Opening and Closing

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Abstract

By applying the osmotic stress of a nonpenetrating polymer, we have measured the change Δv in polymer-inaccessible internal water volume of a voltage-gated ionic channel. The voltage-dependent anion channel (VDAC) from mitochondrial outer membranes shows a Δv comparable in magnitude to the full channel volume estimated from solute penetrability, single-channel conductance, or image reconstruction. It thus appears that channel "gating" involves significant structure reorganization and water movement rather than the minimal changes caused by a local constriction or blockade. Hydration of the inner channel surface may be an important factor in channel gating as is the hydration of molecular surfaces in controlling macromolecular interaction in solution.

Key Words: Osmotic stress; hydration; voltage-gating; VDAC; mitochondrial outer membrane; aqueous volume changes; ionic channels; anion channel; hexokinase binding protein.

Although there is still no detailed understanding of how channels open and close, the term "gating" is commonly heard. That may be prejudicial. The idea of a swinging gate is given as a pictorial representation of an unknown structure (Harris, 1984; Spray *et al.*, 1984). Other representations (Fig. 1) include some local blockage; a slotted structure that rotates within the channel space; plugs, balls, or corks on the ends of chains; a guillotine that closes down; and the rotation of an amino acid side group to prevent ion flux (Kosower, 1984). Considered together, these form a class of models that

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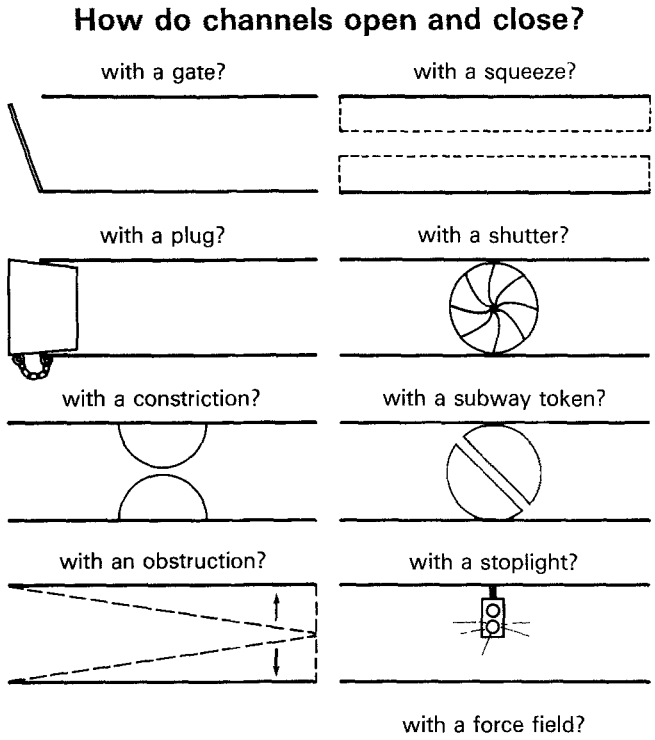

Fig. 1. Cartoon of possible models for channel opening and closing.

assumes a local constriction, i.e., a change in the accessibility of this channel to ion flow, arising from a very small change in the internal aqueous volume of that channel. One problem we encounter in imagining channel crossings produced by local constrictions is that the entire transmembrane voltage will drop across that constriction, resulting in a very large field ($\sim 10^6$ V/cm), the kind of field strength that can cause dielectric breakdown if not somehow delocalized (e.g., Auckland *et al.*, 1968).

Another class of models is characterized by a significant change in the internal aqueous volume of the channel as a result of macromolecular rearrangement. We have been able to distinguish between these two general classes of channel opening and closing, those with small volume changes and those with large volume changes, by stressing the channel with osmotic pressure (Zimmerberg and Parsegian, 1986). If we think of the transition of a channel (at certain voltages) between open and closed states as being an equilibrium change between two conformational states of a protein, applying osmotic tension (trying to pull water out of this space) would bias the

equilibrium toward the state where more water was available to the excluded osmotic agent. For the channel to open in the presence of solutes inaccessible to that pore, the channel would have to do work to extract water from the solution.

The osmotic pressure difference Π_{osm} between the outside solution and the inside of the channel is set by the polymer osmotic pressure of the bulk solution. When there is a change in volume Δv of the pore between the open and closed states, the work being done to change the volume in the presence of the pressure Π_{osm} is $\Pi_{osm}\Delta v$. This will be seen as an extra work of opening a channel due to osmotic stress. We feel comfortable with the notion of osmotic tension (negative pressure as effected by osmotic stress) inside of channels because of experiments done with porous membranes under different osmotic conditions (Mauro, 1957; Mauro, 1965; Mauro, 1981). As to the physical basis of the phenomenon of osmotic stress, no further work has explored Onsager's intriguing explanation: a momentum deficiency at the mouth of the channel facing inaccessible species (Mauro, 1965).

We can introduce this work as energy into an earlier formalism for channel opening (Ehrenstein *et al.*, 1970) to compare it with the electrical work of channel regulation. If we look only at the equilibrium between the open and closed state as a function of transmembrane voltage Ψ,

$$\text{closed} \overset{K(\Psi)}{\rightleftharpoons} \text{open}$$

that equilibrium can be expressed as a Boltzmann relation between equilibrium constant and work energy ΔW

$$-kT \ln K = \Delta W$$

or

$$K(\Psi) = \text{open/closed} = e^{-\Delta W(\Psi)/kT}$$

where k is the Boltzmann constant and T is temperature. We usually consider the electrical work energy to be a function of the number of equivalent gating particles, n, times the unitary charge, q, times the voltage Ψ across the membrane:

$$\Delta W(\Psi) = nq(\Psi - \Psi_0)$$

where Ψ_0 is the voltage at which the channel is equally likely to be open or closed. The polymer adds a new work term to give the energy change

$$\Delta W(\Psi, \Pi_{osm}) = nq(\Psi - \Psi_0) + \Pi_{osm}\Delta v$$

The open-to-closed ratio is going to be a function not only of voltage but of osmotic pressure.

If the presence of polymer changes only the probability of the open and closed states but does not perturb the structure of the channel in those states, then we can extract the volume change from the change in those probabilities. We first measure (in terms of Ψ and n) the electrical energy needed to open and close the channel; we then add polymer and measure again. The shift in open/closed vs. voltage gives the change in volume.

It is important that one add polymer to both sides of the membrane. Otherwise one has no idea what osmotic pressure to use to calculate Δv.

Also one must worry whether osmotic stress is affecting some part of the membrane away from the channel, for example creating a more rigid membrane by dehydrating membrane lipids. To exclude this kind of indirect effect, we stress the system with a small osmotic agent capable of exerting some strong pressure but small enough to penetrate the channel and therefore unable to act osmotically on the channel cavity itself. Using glucose, sucrose, or stachyose and going to pressures 50 times that needed for effects by polymer, we saw no action whatsoever of these small peptides.

Our polymer experiments were designed to obtain a thermodynamic measurement of channel statistics: to determine the equilibrium constant between open and closed states as a function of voltage. The limitation of the experiment has to do with getting good statistics. If one is looking at an opening and closing process of a single channel, one needs more than 50 fluctuations per voltage setting in order to get a good probability estimate. VDAC is very slow, too slow to allow one to collect data with the required wait at each voltage and then to repeat with added polymer. It takes several hours to collect the necessary data, by which time a membrane will usually break and ruin the whole procedure.

The best experiments came from membranes that had five to eight channels, where we could see discrete jumps, directly determine the open/closed statistics, and also measure single-channel conductances. Membranes containing enough channels to measure macroscopic conductances were also used. The ambiguity arising from macroscopic experiments stems from the observation that VDAC can be in many different substates, although the single largest open state and the largest closed state predominate. In macroscopic measurements we cannot tell exactly which proportion of the channels is in any substate.

While single-channel measurements are not practical because there are not enough transitions during the time of a reasonable experiment, they are critically useful in showing that the characteristic conductance of the various states is not noticeably affected by the applied osmotic stress. The single-channel size does not change, but the amount of time the channel is in a given state does.

One worry we had is that we were dealing with a contaminant in our solution that was binding and chemically altering VDAC. Since an alteration

of VDAC might change n (Colombini, 1987), we looked at the change in the slope of this curve as a function of osmotic pressure because this would indicate that somehow we were altering the VDAC itself. We found no consistent change in n in three different polymer solutions: polyethelene glycol, polyvinyl-pyrolidine, and dextran. We think it unlikely that the three different polymer solutions, prepared in three different ways, would contain the same contaminants in the solution. In addition, we dialyzed the polymers for about a week before using them to get rid of low molecular-weight contaminants.

We measure a large volume change. From the change in Ψ_0 in a number of experiments we calculated Δv to be between 2 and 4 $\times$ 10^4 Å^3.

This volume change is not consistent with a gate, local constriction, guillotine, or a demon of the first, second, or any kind. Rather, it is consistent with a large molecular rearrangement substantially altering the polymer-inaccessible aqueous volume of VDAC.

How does this relate to other estimates of the molecular dimensions of VDAC? Using image reconstruction, Mannella and co-workers (Mannella *et al.*, 1986; Mannella and Tedeschi, 1987; Forte *et al.*, 1987) describes a channel 50–60 Å long and 10 Å in radius. Benz and co-workers have calculated the radius of an equivalent volume having the same bulk conductivity as the bathing solution and speaks of a radius of about 10 Å (Benz *et al.*, 1985). Colombini measured the permeability of VDAC to polyethylene glycol molecules (Colombini, 1980). The largest PEG which gets through, 3400 MW, has a diffusion constant which corresponds to a hydrodynamic sphere of radius 19 Å. Thus, the range of calculated total channel volumes is comparable to the volume change we measure.

It is possible that there are conformations accessible by polymer but inaccessible by voltage alone. If one thinks of opening and closing channels with applied voltage, and then thinks of independent stresses opening and closing channels (such as polymer osmotic pressure), then one might imagine different sets of transitions: some conformational changes which are coupled to charged movements, and other movements which do not cause gating charge movement. In other words, there may be degeneracies in channel conformations. By stressing channels with other parameters, we might start seeing some splitting in these degeneracies. One example of this is the emergence under stress of a second, smaller open state. Here osmotic stress biases the channel toward a substate, as if the open state itself has a little bit of elasticity. The change is insensitive to applied voltage because that small change in the molecule does not engender any motion of charge. The conductance of a closed substate that we saw under polymer-induced osmotic stress was only 10% of the open-channel conductance. We estimate the volume change between the closed state and this closed substate to be 7900 Å^3.

It may help, to remove some of the mystery about channels, to think of them as specialized proteins rather than peculiar organelles. To this end, one might think of a chemical potential for each of the conformational states $\{i\}$

$$\mu_i = \mu_i(\Psi, \{L\}, \Pi, P, \ldots)$$

as a function of applied voltage Ψ, activities $\{L\}$ of liganding species, osmotic stress $\{\Pi\}$ effected by various agents, and hydrostatic pressure P. Conjugate to each of these are, respectively, q, the charge displaced, n_L, the number of ligands bound, v_Π, the volume accessible to the particular osmotic agent, and V, the total volume of the system.

There are other pairs of variables one may use to probe channels. For example, hydrostatic pressure and the entire density of the system form another pair, which has been used to probe the sodium channel in the squid axon. The volume change in response to a hydrostatic pressure which acts to change density is a very different quantity from the exchange of water between membrane channel and bathing medium in response to osmotic pressure.

Is the polymer-inaccessible space strictly a measure of the channel volume? We are in fact measuring an aggregate polymer-inaccessible space which can include water far from the site of an ion's progress. Our operational definition of channel volume seems to work well with VDAC, but the distinction between this operational volume, sensitive to stress, and the actual channel path should be kept in mind.

Channels are probably not the cylindrical or rectangular solids that they are often imagined to be for schematic convenience. Probes of different molecular size will in practice stress different parts of oddly shaped channels and give different volume estimates that might teach us more about actual channel shape. An extreme example of this is shown in the above-mentioned nonresponse to sugars up to the size of stachyose; there are probably no aqueous pockets smaller than ~ 10 Å across that change size with the opening of VDAC (Scherrer and Gerhardt, 1971).

Seen from the perspective of molecular hydration, so important in macromolecular interaction, the lumen of VDAC should be thought of as bounded by a water-perturbing surface (LeNeveu *et al.*, 1976; Prouty *et al.*, 1985; Rau *et al.*, 1984). We know, from the direct measurement of forces between membranes or between macromolecules, that below 10–15 Å separation these forces are dominated by surface hydration. It is entirely likely that virtually all the water inside VDAC, whose diameter is of the order of 20 Å, is similarly perturbed and that one must begin to think of the work of dehyrating the protein lining the pore as an important, even paramount, factor in the structural changes associated with channel "gating." Of possible further relevance to channel gating is the fact that some divalent or

polyvalent cations condense DNA helices by entropic forces (Rau and Parsegian, 1986). Not only is there a repulsive hydration force between similar surface but possibly an equally strong attractive force between complementary surfaces (Parsegian *et al.*, 1987).

Perhaps a change in transmembrane voltage changes surface hydration within the pore, leading to a new molecular configuration of protein structure such that attractive and repulsive forces are balanced once again at a new separation of molecular surfaces. A small change in energy gives rise to a large movement of water out of that channel. This might be related to ion selectivity or to effects of specific ions on freezing in any particular conformational state (e.g., aluminum on VDAC; see Colombini, 1987).

There have been measurements reported recently of the work of dehydrating cylindrical cavities in the inverted hexagonal phase of phospholipids. The radii of these cylinders cover the range inferred for VDAC. The work of removal of water from hexagonal phases of dioleylphosphatidylethanolamine brought from 10 to 5 Å radius is an order of magnitude larger than the $7kT$ one estimates for VDAC from the work $nq\Psi_0$ that it would take to close it osmotically at zero applied voltage (theoretically to be effected by an osmotic stress of 7–14 $\times$ 10^6 dyn/cm^2). That VDAC can close down more easily than a spontaneously formed cavity in lipids might be evidence for attractive forces within the channel or for a structure engineered to enable closure of a relatively large space.

The role of VDAC in the outer mitochondrial membrane is unknown. Planar bilayers in salt solutions are not biological membranes bounding cells. Cytoplasmic polymer can exert oncotic stress within the cell similar to that applied artificially here. The perimitochondrial space is a protein-rich space and cytoplasm has a high protein concentration. The actual voltage curve for VDAC that may be relevant (in the absence of specific binding factors) is probably much closer to what we measure with PEG than in 1.0 M KCl. In fact, one might speculate that things are just balanced so that VDAC is sensing the aggregate protein concentration of the cell and controlling protein synthesis by controlling mitochondrial activity.

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