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Topical Review

Voltage Gating in the Mitochondrial Channel, VDAC

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Introduction

Of the many properties that are displayed by membranes, none is more unique and intimately related to this quasi-two-dimensional structure as the ability to respond to very small electrical potentials. The ultra-thin, high-resistance structure that is the biological membrane is ideal for separating aqueous compartments in such a way that small potential differences are translated into enormous electric fields (within the membrane). The 5 nm of insulation converts a 50-mV potential difference into an electric field of 100 kV per cm, sufficient to induce conformational changes in intrinsic membrane proteins. Like most other properties of proteins (such as binding of small molecules or catalysis) certain proteins are specially designed to respond to these electric fields, and their presence in membranes allows these membranes to respond to small changes in transmembrane potential.

It used to be thought that this electrical excitability/responsiveness was confined to nerve and muscle cells, or at least to the plasma membrane. Although the phenomenology associated with electrical excitability is most easily observed in these cell types, it is now clear that cells, in general, exploit this property for a variety of tasks. Subcellular organelles also contain the fundamental unit needed for electrical excitability, the voltage-gated channel (Schein, Colombini & Finkelstein, 1976).

As evidenced in the well-studied structures of enzymes and immunoglobulins, in order for a protein to be finely tuned to perform a specific function some portion of the protein must have special structural features that perform these functions. Natural selection results in the formation of structures that are optimized to perform the protein's function. In the case of voltage-gated channels these structures are the voltage sensor and the water-filled pore. While theoretical considerations suggest how proteins may form such structures (and indeed provide constraints on what these structures might be) it is only by examining the actual proteins that we will begin to understand how they are constructed and how they function.

This article will focus on the voltage-gated channel-forming protein, called VDAC, located in the outer membrane of mitochondria. This protein has been isolated from mitochondria of all eucaryotic kingdoms and its properties are highly conserved. The Table shows the remarkable conservation of VDAC's basic properties: single-channel conductance, selectivity, and voltage dependence (*see* below for meaning of the parameters "n" and "V_0"). Despite this conservation, some differences have been observed, and therefore observations on VDAC from one organism may not apply totally to VDAC from another source. The observations presented in this article will focus on the voltage-gating and ion-selecting properties of VDAC; little will be said about other aspects (such as: structure as determined by electron microscopy, hexokinase binding properties, biogenesis and insertion into membranes; e.g., *see* Mini-Review Series, 1987). Although some investigators refer to this protein as mitochondrial porin, it should not be confused with the unrelated group of proteins located in bacterial outer membranes and collectively referred to as porins. None of the properties presented here should be misconstrued as being applicable to the porins.

VDAC Channels Close when Electrical Potentials Are Applied

Figure 1 shows the insertion of single VDAC channels from rat liver mitochondria (monitored as dis-

Key Words channel · VDAC · voltage gating · mitochondria · outer membrane · protein structure

Table. Properties of VDAC from various sources

Organism	Single channel conductance (nS)		Selectivity (1 M *vs.* 0.1 M KCl) $P(Cl^-)/P(K^+)$	Voltage dependence		Reference
	1 M KCl	0.1 M KCl		n	V_0 (mV)	
Protist						
(*P. aurelia*)		0.55	1.9			(p1)
				4.5	20	(p2)
Fungus						
(*N. crassa*)	4.5					(f1) (f2) (f3)
			1.9			(f4)
				3.5	23	(f5)
(*S. cerevisiae*)	4.5		1.8	4	22	(f6)
		0.45		2		(f7)
Mammal						
(*R. norvegicus*)	4.5, 4.4	0.48				(m1) (m5)
	4.2		1.8	4.5	31	(m2)
			1.7	3.6	24	(m3)
				4.3	21	(m4)
		0.58				(m6)
Plant						
(*Z. mays*)	3.9		1.7	3.8	20	(z1)

The values listed in this table are meant to show the high degree of conservation of these basic properties of VDAC. The differences may or may not be significant because some values (especially the voltage-dependence parameters) are somewhat variable. Also the experimental conditions were not all the same.

In the selectivity experiments, in addition to KCl, 5 mM $CaCl_2$ was also present in most cases.

References: (p1) Colombini, M., Schein, S.J. (1975) *unpublished*; (p2) Schein et al., 1976; (f1, m1) Colombini, 1980; (f2) Freitag, Neupert & Benz, 1982; (f3) Pfaller et al., 1985; (f4) Adelsberger-Mangan & Colombini, 1987; (f5) Mangan & Colombini, 1987; (f6) Forte, Adelsberger-Mangan & Colombini, 1987; (f7) Ludwig et al., 1988; (m2) Colombini, 1983; (m3) Doring & Colombini, 1985; (m4) Bowen et al., 1985; (m5) Roos, Benz & Brdiczka, 1982; (m6) Colombini, M., *unpublished*; (z1) Smack & Colombini, 1985.

See De Pinto et al. (1987) for more variable results than those presented here. The reasons for the higher variability is unclear and may have to do with regulation of the channel.

continuous increases in the flow of ions due to the insertion of individual channels) while the inset shows a histogram of single channel conductances. The channels are typically very uniform in conductance with little variation from one source to another (corn VDAC has the lowest conductance, Table). The conductance is linear[1] with KCl activity up to 4 M KCl (Colombini, 1986), showing the remarkable ability of VDAC to conduct these ions with no sign of saturation (only true for KCl). Once inserted, the channel remains in a high conducting, "open" state at low potentials but enters one of a variety of lower-conducting "closed" states at high potentials (the "closed" states are still permeable to small ions but the permeability is reduced).

A typical recording for a single channel (Fig. 2) shows that voltage-dependent channel closure occurs at both positive and negative potentials. In the figure, the records of both the applied voltage and the resulting current flow are displayed. Channel closure results in an abrupt transition from a line with a steep slope (open channel conductance $\Delta I/\Delta V$) to one with a shallower slope (closed channel conductance). The presence of only one channel in the membrane results in stochastic variability in the voltage at which the transitions occur. However, with a multi-channel membrane a clear switching region is observed. The location of the switching region is somewhat variable, depending on, for example, the membrane lipid composition (Holden & Colombini, 1988), but in mixed phospholipids is around 20 mV. To a first approximation, each channel closes in a symmetrical manner, i.e., closure at positive potentials is the same as closure at negative potentials. Different closed states are reached with

[1] The less than 10-fold difference in the single-channel conductance in 1 M compared to 0.1 M KCl is due to the smaller activity coefficient for 1 M KCl.

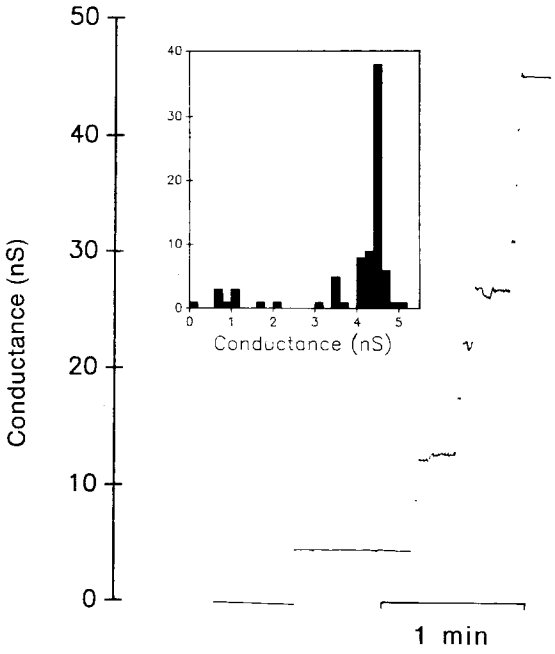

Fig. 1. The insertion of single rat liver VDAC channels into a planar phospholipid membrane. The channels were solubilized from mitochondrial membranes and purified. When an aliquot of the Triton X100 solubilized channels was added to the aqueous phase (1 M KCl, 5 mM $CaCl_2$) bathing a membrane made with soybean phospholipids, individual channels spontaneously inserted into the membrane, as indicated by the discontinuous increases in conductance (a 10-mV potential was applied). The inset shows a histogram of the frequency of observing insertion events of a particular conductance. *See* Colombini (1980) for more detail

the opposite potentials, probably resulting from two distinct voltage-gating processes (Colombini, 1986). However, often one observes that closure of a single or a population of channels occurs at lower potentials for one sign of the potential. The origin of this asymmetry is unclear and may result from the generation of asymmetric planar bilayers (asymmetry with respect to protein orientation, lipid composition, detergent concentration, etc.). The possibility exists that VDAC itself is asymmetric, but no convincing evidence is available.

VDAC channels show rapid rates of opening (μsec to msec time range depending on the source of VDAC (Colombini, 1979; Dill, 1987)), while the rates of closure are relatively slow (seconds) and voltage dependent (Schein et al., 1976; Dill, 1987). Moreover, the rates of opening slow down dramatically the longer the channels are held in a closed conformation by applying an elevated potential. Both the kinetics and this adaptation, this memory, are aspects of the gating mechanism that must, at some point, be accounted for.

VDAC's Sensor Responds to Electrical Potentials Whether Applied Locally or to the Bulk Phase

In order for a voltage-gated structure to function, the device, in this case the protein, must contain a structure that both detects and responds to the external field. This structure, the sensor, could be a highly charged protein domain or a set of strong dipoles that move in a concerted fashion. Both these processes would result in a voltage-dependent energy change that would result in a voltage-dependent change in the probability of finding the protein in a particular conformational state. The magnitudes of the voltage-dependent changes are proportional to the magnitude of the charge responding to the field, or the total change in dipole moment in the direction of the field. Thus, if charges are involved, changing the net charge will change the degree of voltage dependence.

In the case of VDAC, evidence that the sensor contains a group of positive charges comes from the successful efforts to neutralize these charges. Simple titration to high pH (Bowen, Tam & Colombini, 1985) reduces the voltage-dependence in a way that is consistent with sensor neutralization. Other possible interpretations can be largely eliminated by analyzing the energetics of the system.

The voltage dependence of the probability of finding a channel in the open state can be fit to a Boltzmann distribution as follows (Ehrenstein, Lecar & Nossal, 1970; Schein et al., 1976):

$$P_o = P_c \exp(-\Delta G/RT) \tag{1}$$

where P_o and P_c are the open and closed states probabilities, ΔG is the free energy difference between the states (open-closed), R is the gas constant and T is the absolute temperature. The free energy difference is divided into a voltage-independent and a voltage-dependent component

$$\Delta G = \Delta G_{\text{in}} + nFV \tag{2}$$

where n in the valency of the sensor, F is Faraday's constant, and V is the potential difference across the membrane. Let the voltage at which $P_o = P_c$ be V_0. Thus at $V = V_0$, $\Delta G = 0$ and $\Delta G_{\text{in}} = -nFV_0$. Therefore

$$P_o = P_c \exp(-nF(V - V_0)/RT). \tag{3}$$

Thus the larger the valency, n, the steeper the voltage dependence. Conversely, if n were 0 then the channel would be voltage independent. Most importantly, if a treatment were directed specifically at neutralizing the sensor, the voltage-independent en-

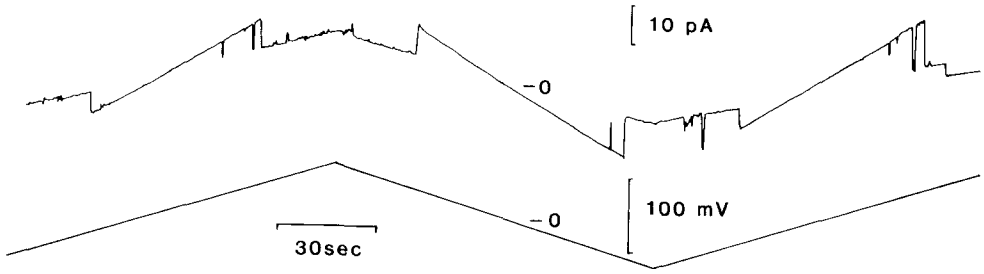

Fig. 2. The voltage dependence of VDAC as illustrated by the behavior of a single VDAC channel from *P. aurelia*. The channel was inserted into a planar phospholipid membrane by incorporating VDAC into the monolayers used to make the membrane (as described in Schein et al., 1976). The salt conc. was 0.1 M KCl, 5 mM $CaCl_2$. The recordings were made (Colombini, M., and Schein, S.J., 1975, *unpublished*) under voltage-clamp conditions with the voltage changing continuously as shown (a triangular wave which varied linearly from +54 to −64 mV; lower trace). The upper trace shows the recorded current. A steep slope in the current trace indicates a high conductance, and thus the channel is in the open state. Transitions to lines with shallower slopes are channel closures. The reverse is an opening. Time proceeded from left to right, and the zero current and voltage levels are indicated. The channel was open at low potentials (steep slope of current record) but closed (entered a lower-conductance state) at both high positive and negative potentials. This behavior is typical of VDAC channels from all organisms

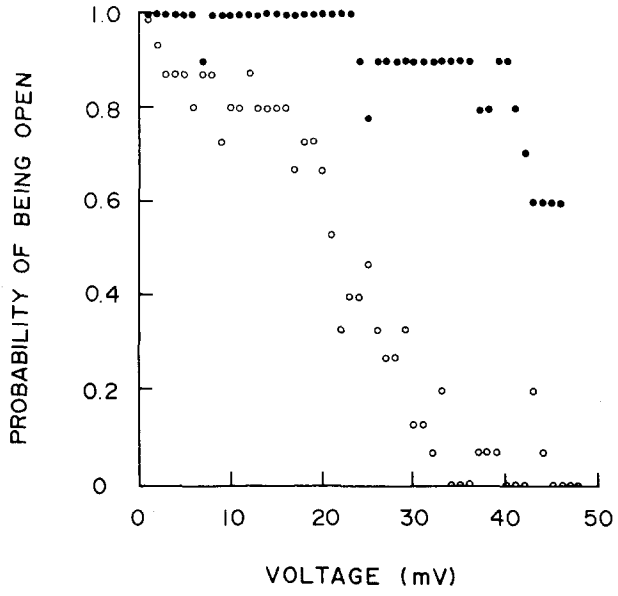

Fig. 3. At elevated pH the voltage dependence of the channels is reduced. The probability of finding the channel open as a function of voltage was determined from records such as those shown in Fig. 2 (except that rat liver VDAC was used). Many single channel records (15 at pH 6.1, open circles, and 10 at pH 10.7, filled circles), taken on single channels before and after pH elevation, were averaged in order to obtain fairly smooth curves showing the voltage dependence of the probability of being open. (Reproduced from Bowen et al., 1985)

ergy difference between the states, $\Delta G_{in} = nFV_0$, should not change. Thus if n were reduced by a treatment, V_0 should increase in a proportionate manner resulting in no change in nFV_0. This criterion allows one to distinguish between sensor neutralization and other effects such as preferential stabilization of a particular state (nFV_0 would change), or change in the height of the energy barrier (neither n nor V_0 should change).

From experiments such as the one shown in Fig. 2, one can estimate the probability of finding a channel open at a given voltage. By adding up the results from many observations at pH 6.1 (open circles) and 10.7 (filled circles), Bowen et al. (1985) generated the data in Fig. 3. At the elevated pH, channel closure still occurred but at higher voltages and with a shallower voltage dependence. By using multi-channel membranes and fitting to Eq. (3) they determined values for "n" and "V_0" as a function of pH (Fig. 4). Note that the decrease in n (filled circles) was paralleled by an increase in V_0 (open circles) so that the voltage-independent conformational energy difference (nFV_0, diamonds) changed very little. Thus the criterion described in the previous paragraph is satisfied, strongly supporting the conclusion that elevating the pH reduces the charge on the voltage sensor. Further, the voltage-dependence can be restored by returning the pH to neutrality. Thus, although titration probably changes the charge on many groups on VDAC, the changes that influence the voltage dependence seem to be limited to the charge on the sensor. The findings are consistent with an apparent mean pK of 10.6.

Similar results were obtained when the sensor was neutralized by chemical modification with succinic anhydride (Doring & Colombini, 1985). This agent reacts with amino groups, converting them to carboxyl groups. As the positive charge of the sensor is presumably neutralized, the voltage dependence is lost. As was the case with titration, the decrease in the value of n was matched by an increase in V_0, resulting in very little change in the

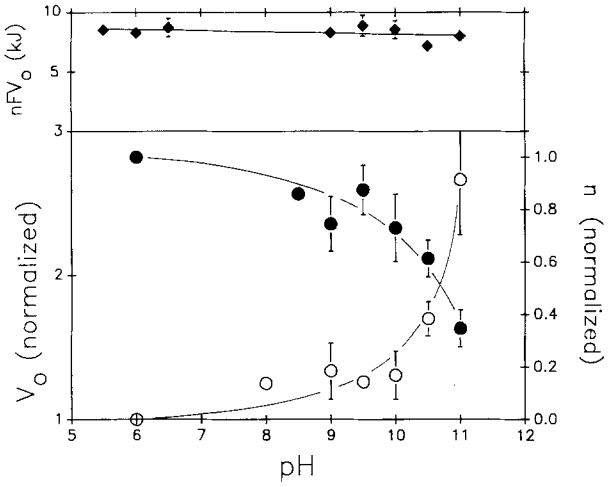

Fig. 4. The pH dependence of VDAC's voltage gating. By analyzing the recordings made from multi-channel (from rat liver) membranes, the voltage-dependence parameters, "n" (filled circles) and "V_0" (open circles), were determined (*see* text) at control pH (around 6) and then after the pH was elevated. To reduce membrane-to-membrane variability, the results at elevated pH were normalized for the values observed at control pH. The symbol for n and V_0 at pH 6 is exactly at 1 due to the normalization, and although located exactly at pH 6 it contains values from 5.5 to 7. All other points are pools of results at pH values ± 0.25 pH units from the indicated point. The values of nFV_0 are in kilojoules

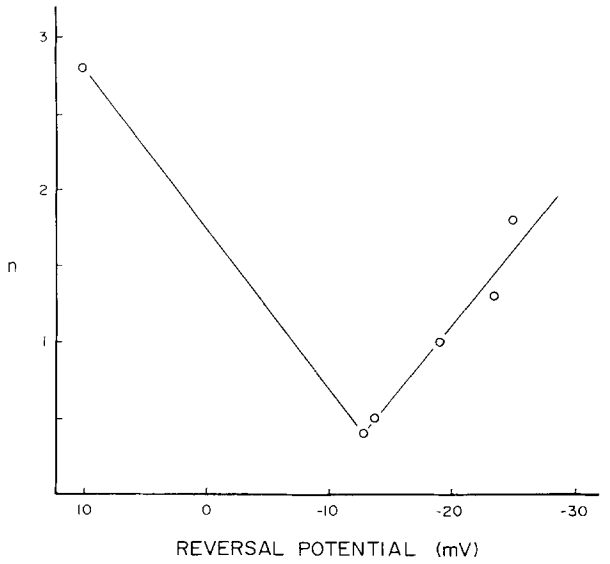

Fig. 5. Elimination and restoration of voltage dependence in VDAC with graded succinic anhydride treatment. The steepness of the voltage dependence of VDAC (from *N. crassa*) channels "n" was determined prior to and following five successive additions of succinic anhydride. The experiments were performed on channels inserted in membranes in the presence of a salt gradient (1 M KCl *vs.* 0.1 M KCl, plus 5 mM $CaCl_2$ and 5 mM HEPES (K^+ salt at pH 7.5 initially and >7 after anhydride addition) to each side) in order to simultaneously monitor changes in the selectivity of the channels. The reversal potential (potential needed to bring the current through the channels to zero) is a measure of the selectivity and of the degree of anhydride modification. Although this figure represents the results from just one experiment, many experiments were performed with similar results (from Adelsberger-Mangan & Colombini, 1987)

voltage-independent energy, nFV_0. Further succinylation would be expected to generate a net negative charge on the sensor. If the sign of the charge were not important, merely the existence of charge in the right location of the molecule, then charge restoration should restore the voltage dependence (Adelsberger-Mangan & Colombini, 1987). In Fig. 5, the steepness of voltage dependence, as indicated by the value of "n," was plotted against a measure of degree of succinylation, the reversal potential of the current flowing through the channels in the presence of a salt gradient (succinylation markedly changes the selectivity of VDAC, which is estimated by the reversal potential). Low levels of succinylation virtually eliminate the voltage dependence while higher levels result in partial restoration.

Remarkably, the voltage dependence can be dramatically increased by a variety of polyvalent anions such as dextran sulfate, 8 kDa (Mangan & Colombini, 1987; Colombini et al., 1987; Colombini, Mangan & Holden, 1988). Figure 6 shows how the voltage dependence of the conductance of a multichannel membrane changes when dextran sulfate is added to both aqueous phases. The control record (×) shows steep voltage dependence but 6.2 μM (squares) and 25 mM (+) dextran sulfate produce ultra-steep voltage dependence. The results are consistent with an action that is specifically targeted to VDAC's sensor. Following the criteria presented for the titration experiments, the value "n" increased 14-fold (from 3.5 to 50) (Fig. 7) while V_0 decreased by a comparable amount, resulting in no noticeable change in the value of nFV_0. At first glance it appears that these polyvalent anions somehow increase the charge on the sensor. How could the highly charged dextran sulfate anion increase the charge on a positively charged sensor? Alternatively, the polyvalent anion interacts with the sensor, in a voltage-dependent manner, inducing VDAC closure. This interaction is proposed (Mangan & Colombini, 1987) to be voltage dependent due to a voltage-dependent increase in the probability of finding the polyvalent anion next to the channel (an increase in the local effective concentration). As shown in the inset to Fig. 6, the highly conductive VDAC channel is proposed to be able to process ions at such a rate that diffusion to

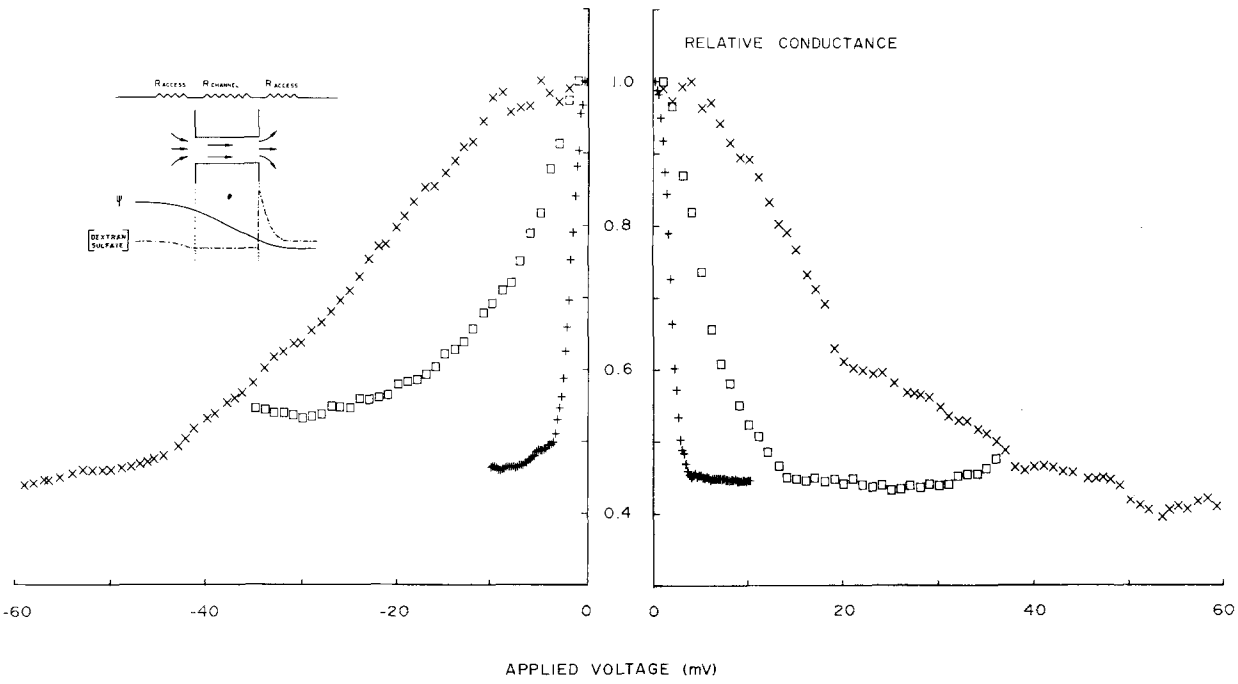

Fig. 6. Increase in the voltage dependence of VDAC by dextran sulfate (8 kDa). The voltage dependence of the conductance of a multichannel (from *N. crassa*) membrane prior to (×) and after dextran sulfate addition (to both sides to final concentrations of 6.25 μM (squares) and 25 mM (+)). The conductances were normalized to the value at 0 potential. The inset is a model to explain the ultra-steep voltage dependence (*see* text). The transmembrane potential (solid curve) and the dextran sulfate conc. (broken curve) are those proposed through the pore not through the lipid bilayer. The arrows indicate the flow of positive charge. (Adapated from Mangan & Colombini, 1987)

the mouth of the pore[2] is insufficient to maintain the ion concentration at the mouth equal to that in the bulk phase. This access resistance results in a potential drop between the bulk phase and the mouth of the pore. The impermeant polyvalent anion partitions into this phase according to the potential difference. Accumulation occurs on the side of the membrane that is made negative (the pore's mouth would be more positive). This hypothesis is supported by a variety of experimental observations including the fact that the polyanion acts only when the side of the membrane to which it is added is made negative (Mangan & Colombini, 1987).

Charges on the Wall of VDAC's Pore Seem to be the Major Ion Selectivity Filter

From VDAC's large pore size (3 nm) and its low selectivity for ions of simple salts (2 : 1 for Cl^- over K^+, ions of nearly equal mobility in aqueous solution), one might conclude that VDAC is a channel lacking a selectivity filter. However, considering the size and valency of the ions that should be using VDAC to cross the membrane in vivo (ATP^{4-}, $citrate^{3-}$ . . . or their Mg^{2+} complexes), it is clear that the nature of the selectivity filter in VDAC may be rather different from that expected for narrow pores. It is reasonable to expect, in the case of large pores, that charges on the walls of the pore may be the most important factor determining the selectivity of the channel. Succinylation results in a dramatic reversal in VDAC's selectivity (Fig. 5) from 2 : 1 Cl^- over K^+ to 8 : 1 K^+ over Cl^- (based on GHK analysis, Adelsberger-Mangan & Colombini, 1987). This is attributable to the generation of negative charges on the walls lining the pore[2] of the channel.

The importance of selectivity in this channel is indicated by the fact that upon voltage-dependent closure, the channel undergoes a large change in ion selectivity. In the closed state the channel almost always prefers cations. However, the variety of closed states precludes the assignment of a single selectivity value (Zhang & Colombini, Fig. 8).

[2] The terms "pore" and "channel" are usually used interchangeably. In this article I will use "pore" to refer to the aqueous pathway through the membrane formed by the protein. The term "channel" will refer to the protein that forms the pore.

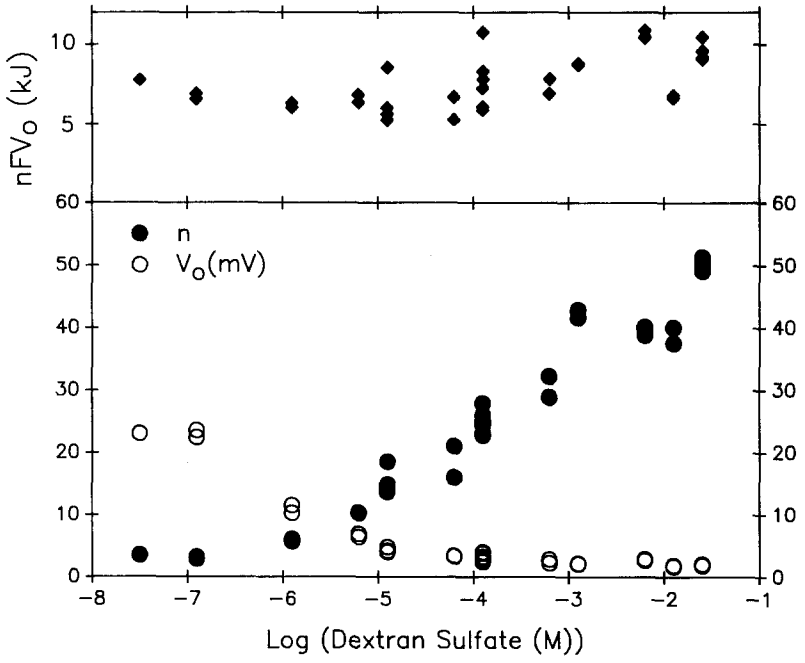

Fig. 7. Quantitation of the increase in voltage dependence by dextran sulfate. The voltage-dependent parameters, n and V_0, were obtained from results such as those shown in Fig. 6

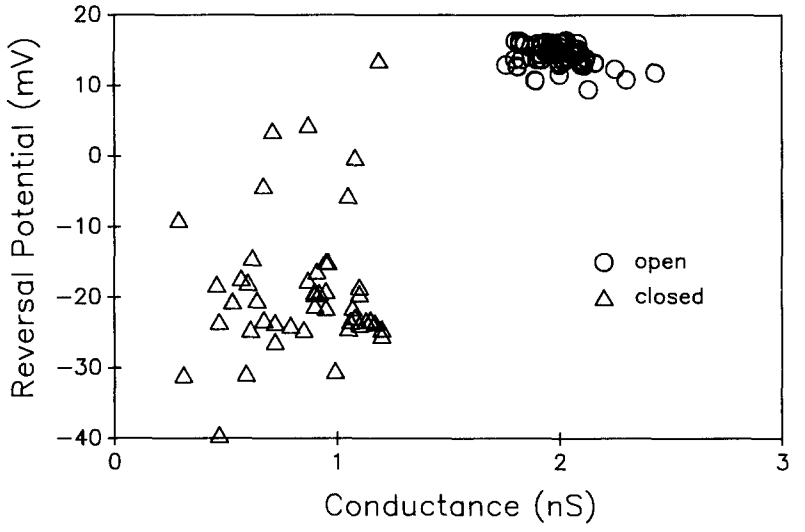

Fig. 8. The variability of the closed-state selectivity of VDAC channels. The data were collected from many membranes containing one or a few channels. The reversal potential (a measure of selectivity) was recorded for channels in their open or closed state (the salt gradient was 1 M KCl *vs.* 0.1 M KCl, 5 mM $CaCl_2$) as was the conductance of the same channel. (D.W. Zhang and M. Colombini, *unpublished*)

While the range of selectivities of individual VDAC channels in the open state (circles) is very narrow, that for the "closed" conformations (triangles) is extremely broad! This breath is comparable even when one channel is observed by repeated opening and closure. Indeed, by monitoring the channel's selectivity one can observe many more closed states than can be distinguished by conductance measurements alone. The heterogeneity is overwhelming. Surprisingly, there appears to be no correlation between selectivity and conductance.

A direct way of determining which groups contribute to VDAC's selectivity is to replace individual amino acid residues with others possessing different properties. The replacement of a positively charged amino acid side chain with a negative residue should alter the channel's selectivity if the group is in the appropriate location. If charge on the walls of the pore is the primary determinant of selectivity then any change in charge of side chains lining the pore should result in an appropriate change in selectivity. Ongoing experiments (*see* Blachly-Dyson et al., 1989) are yielding results consistent with this notion. Substituting a negative for a positive charge results in a 5 to 12 mV change in the reversal potential (10-fold KCl conc. gradient) when certain locations are altered. At other locations, no change was observed. This approach is proving to

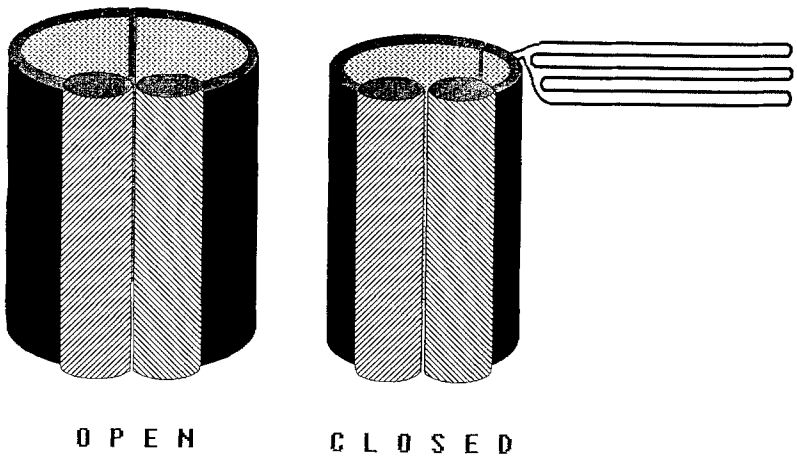

Fig. 9. Model of the structure of the open and one closed state of VDAC. The striped structures are α helices oriented in opposite directions while the cylindrical walls represent antiparallel β sheets. The open channel is a dimer with twofold rotational symmetry in the plane of the membrane (or an axis perpendicular to the paper and in the middle of the two α helices. In the closed conformation, one monomer has lost six transmembrane strands of the β sheet lining the wall of the pore. These strands now lie on the surface of the membrane. The exact opposite would result for closure with the opposite potential

be a powerful tool for determining the overall structure of the channel.

A Large Structural Change Between Quasi-Degenerate Conformations

In considering the nature of the voltage-dependent conformational change that results in channel opening and closure, one wonders whether the change is subtle or extensive. In order for these structural changes to occur at low potentials, the difference in the energy levels between the open and closed conformations must be very low. For VDAC it is about 8 kJ/mole (Schein et al., 1976; Bowen et al., 1985; Doring & Colombini, 1985), equivalent to the change in the structure of 1/3 of a hydrogen bond! This small energy change argues either for a subtle conformational change or finely-tuned structures (i.e., large structural differences but whose overall energies match almost perfectly).

A number of observations argue for extensive conformational changes. The effective diameter of the pore has been estimated to change from 3 to 1.8 nm (Colombini et al., 1987). More dramatically, the internal volume of the pore is reduced by about 30 nm^3 (Zimmerberg & Parsegian, 1986). In addition, the channel's selectivity can change from 2 : 1 Cl^- over K^+ to 8 : 1 K^+ over Cl^- (Fig. 8). Finally, in order to account for the steepness of the voltage dependence, at least four charges must be translocated all the way through the transmembrane potential. All these argue for an extensive change in conformation rather than a subtle change.

A working model for this conformational change (based on functional, structural, and sequence data) is shown in Fig. 9. The channel is proposed to be a dimer of two identical subunits (Colombini, 1986). The subunits are aligned in opposite directions in the membrane[3] forming a channel with twofold rotational symmetry about an axis in the plane of the membrane. This would account for closure at both positive and negative potentials if one subunit responded at positive while the other at negative potentials. The translocation of charge (required by the voltage dependence) would occur by the translocation of six transmembrane strands (three beta loops) through the membrane driven by the displacing charge. The removal of these transmembrane strands would reduce both the pore diameter and volume by amounts that fit the experimental values. The strands that move out of the pore remain on the surface of the membrane. Therefore, there is no movement of side chains into or out of the membrane environment. This minimizes the conformational energy differences between the states of the channel.

Conclusion

The mitochondrial channel, VDAC, is proving to be a good system in which to understand the molecular basis for voltage gating. Insights have been gained on the nature of the voltage sensor, the basis for selectivity, and the nature of the conformational change. A working model has been presented that is consistent with observations made thus far.

[3] In the proposed structure, the subunits are aligned in opposite directions. This poses a problem with regard to insertion of the subunits into the membrane. However, the proposed structure is the simplest way of accounting for the symmetrical behavior of VDAC, including two gating processes acting at opposite potentials.

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