Journal of Bioenergetics and Biomembranes, Vol. 19, No. 4, 1987

MINI-REVIEW

Regulation of the Mitochondrial Outer Membrane Channel, VDAC

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Received February 13, 1987

Abstract

The channel-forming protein, VDAC, located in the mitochondrial outer membrane, is probably responsible for the high permeability of the outer membrane to small molecules. The ability to regulate this channel *in vitro* raises the possibility that VDAC may perform a regulatory role *in vivo*. VDAC exists in multiple, quasi-degenerate conformations with different permeability properties. Therefore a modest input of energy can change VDAC's conformation. The ability to use a membrane potential to convert VDAC from a high (open) to a low (closed) conducting form indicates the presence of a sensor in the protein that allows it to respond to the electric field. Titration and modification experiments point to a polyvalent, positively charged sensor. Soluble, polyvalent anions such as dextran sulfate and Konig's polyanion seem to be able to interact with the sensor to induce channel closure. Thus there are multiple ways of applying a force on the sensor so as to induce a conformational change in VDAC. Perhaps cells use one or more of these methods.

Key Words: Mitochondrion; outer membrane; permeability; regulation; voltage dependence; membrane channel; VDAC; gating process; polyanion; aluminum.

Introduction

A great deal of molecular traffic must cross the outer membrane in order for mitochondrial energy transduction to proceed. Not only must carbon sources such as succinate and citrate travel from the cell's cytoplasm to the mitochondrial matrix space but ADP is also needed for phosphorylation to proceed. The evidence is quite strong that all this traffic must cross the outer

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0145-479X/87/0800-0309$05.00/0

membrane via the channel called VDAC. VDAC channels are the only known constituents of the outer membrane with the appropriate permeability properties (Werkheiser and Bartley, 1957; Pfaff *et al.*, 1968; Wojtczak and Zaluska, 1969; Colombini, 1979; Zalman *et al.*, 1980), and in some organisms these channels are the predominant protein in the outer membrane (Mannella and Bonner, 1975; Mannella, 1982; Mannella and Colombini, 1984).

Beyond its function as a permeability pathway, there are suspicions that VDAC might regulate the permeability of the outer membrane and therefore mitochondrial function. VDAC has been found to be voltage dependent (Schein *et al.*, 1976; Colombini, 1979; Freitag *et al.*, 1982). It is highly permeable to ions at small membrane voltages (> 20 mV) and becomes less permeable at higher voltages. It does so by changing its conformation. Thus a relatively small amount of energy (equivalent to less than one H bond) provided by the electric field causes the conformational change. This shows that the structure of this channel is such that the protein can exist in quasi-degenerate conformational states with different permeability properties. This is a necessary condition for regulation to take place, and the electric field may only be one way to convert the channel from one conformational state to another. These properties are highly conserved in all mitochondria tested to date from protozoa (Schein *et al.*, 1976), mammals (Colombini, 1979), fungi (Colombini, 1980), and higher plants (Smack and Colombini, 1985). The conservation of these structural properties underlines their importance.

Despite the lack of direct evidence that the outer membrane regulates mitochondrial function, it is instructive to determine how VDAC can be regulated. This investigation may provide clues as to the regulation that may exist *in vivo* and ideas on how to look for such regulation. This report will summarize what is known about the ways in which VDAC's permeability to ions can be regulated.

Experimental

All experiments summarized here were performed on VDAC channels inserted into planar phospholipid membranes. These membranes were made by the monolayer technique of Montal and Mueller as modified by Schein *et al.* (1976). The experiments were performed under voltage clamp conditions at room temperature.

Results

In exploring the regulation of VDAC channels it will be assumed that there exists an entity called the sensor through which the channel can be

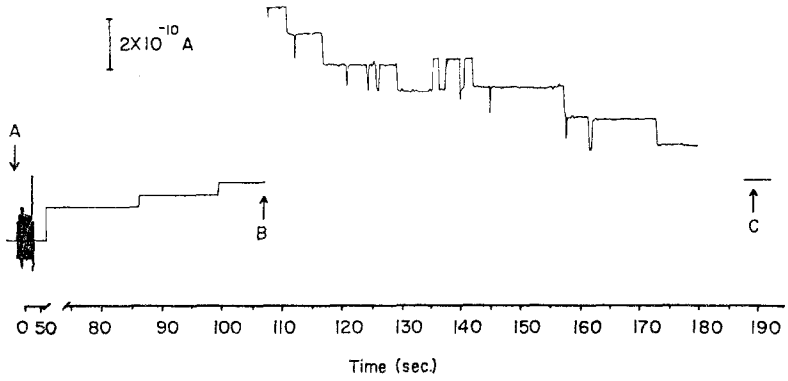

Fig. 1. The insertion of VDAC channels into a planar phospholipid membrane and their voltage-dependent closure (reprinted from Mannella *et al.*, 1983). VDAC channels were solubilized from *N. crassa* mitochondrial outer membranes and inserted into membranes made from soybean phospholipids. At point "A" the membrane extract was added to the aqueous phase bathing the membrane. At point "B" the membrane potential was raised from 10 to 40 mV, while at point "C" it was returned to 10 mV.

controlled. The sensor is part of the channel's structure and is located at a critical point. By applying a force on this sensor, a conformational change can be induced in the channel which results in a state of lower permeability. The evidence for the existence of such a sensor will be presented along with ways in which a force can be applied to this sensor.

Control via a Transmembrane Voltage

VDAC channels isolated from mitochondria can be solubilized in 1% (w/v) Triton X100 and inserted into planar phospholipid membranes. The insertions can be observed by monitoring increases in membrane permeability (as current increases). In the experiment (Mannella *et al.*, 1983) shown in Fig. 1, five channels spontaneously inserted. An aggregate of three channels caused the first increase in current and this was followed by two insertions of single channels. When the voltage was increased from 10 to 40 mV, instantaneously the current increased because of the 4-fold increase in driving force (no permeability increase occurred) but, with time, the current decreased as the voltage induced channel closure. Thus the applied voltage acted on the channels and induced them to undergo a conformational change to a less permeable conformation.

It is generally accepted that this type of voltage dependence indicates the presence of a charge or dipole in the structure of the channel that allows it to respond to the applied electric field. In the case of VDAC, several lines of evidence indicate that charges are involved.

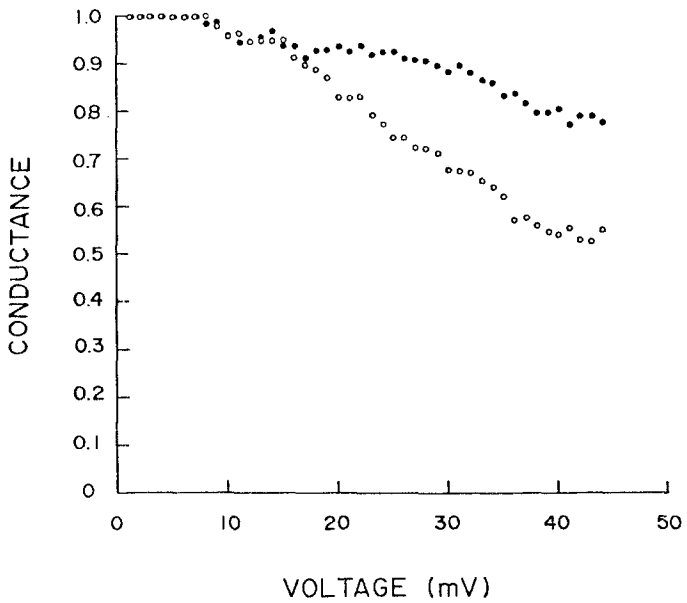

Fig. 2. Normalized conductance of a multichannel membrane as a function of membrane potential measured at pH 6.2 (open circles) and pH 10.7 (closed circles). (These results are reprinted with permission from Bowen *et al.*, 1985.) These results were obtained using VDAC-containing membranes (inserted as described in Fig. 1), but the channels were isolated from rat liver mitochondria.

Titration of the charged sensor

When the medium pH was raised from 6.2 to 10.7 (Bowen *et al.*, 1985), the voltage dependence of the channels was greatly reduced. Figure 2 shows how the membrane conductance or permeability (due to VDAC channels) decreases with applied voltage at the two pH levels. At the higher pH, higher voltages were required to reduce the membrane permeability by an equivalent amount. The reduction in voltage dependence can be quantitated in the usual way (Ehrenstein *et al.*, 1970; Schein *et al.*, 1976) by fitting to the Boltzmann distribution. This allows one to obtain two parameters: n, the steepness of the voltage dependence, and V_0, the voltage at which half the channels are open. The quantity n is also a measure of the number of charges that would have to traverse the entire membrane potential in order to account for the voltage dependence of the channels. If the charges on the sensor are titrated by raising the pH, the parameter n should decrease. This was indeed the case (Fig. 3). Moreover, if there is less charge on the sensor, more voltage should be needed to impart the same amount of energy to the channel and thus achieve the same degree of closure. Again, this was observed (Fig. 4). If the high pH were causing nonspecific conformational changes or

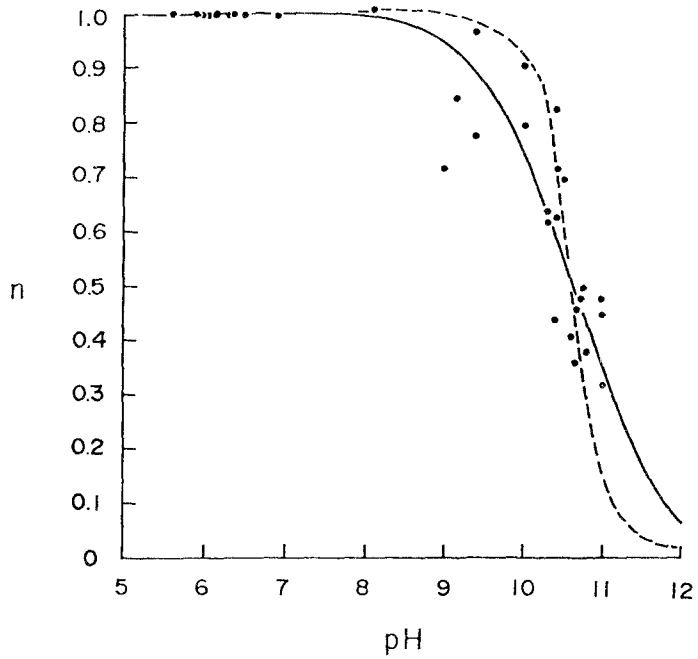

Fig. 3. The steepness of the voltage dependence, n, as a function of medium pH. (These results are reprinted as in Fig. 2.) The values were normalized to the n value measured at the starting pH (i.e., pH 6–7). The values of n at the starting pH averaged at 4.3. The dashed line represents a theoretical curve assuming a pK for the charges on the sensor of 10.6. The solid line assumes five dissociable groups whose pK ranges from 9.6 to 11.6.

partially denaturing the channels, the parallel changes in n and V_0 would probably not occur. Indeed, this parallel change indicates that the energy difference between the two conformations did not change.

Chemical Modification of the Sensor

Succinic anhydride reacts with amino groups and converts them to carboxyl groups. When VDAC was modified by adding succinic anhydride to VDAC-containing membranes, the voltage dependence was greatly reduced (Doring and Colombini, 1985). Figure 5 shows how the membrane conductance decreased with the applied voltage, before and after reaction with the anhydride. This looks very much like the results obtained after pH elevation and can be explained in the same way. The charged sensor is probably composed of amino groups and when these are neutralized by reaction with anhydride the voltage dependence is lost. Indeed, the parameter n decreased and V_0 increased in a similar manner to that described for the

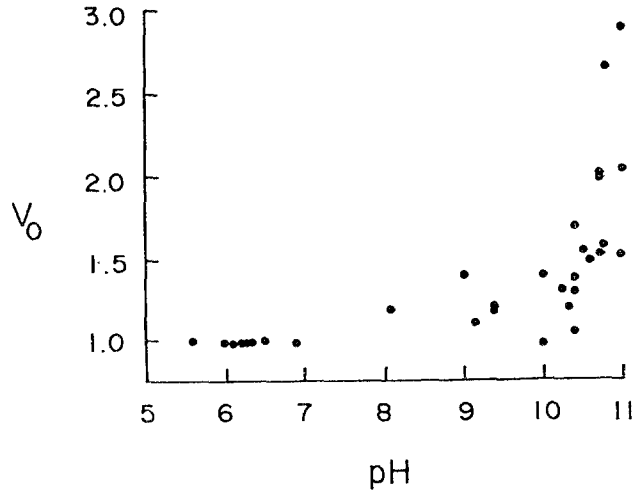

Fig. 4. The voltage at which half the channels are in the highly permeable, open, state as a function of medium pH. (These results are reprinted as in Fig. 2.) The V_0 values were normalized to the values measured at the starting pH (i.e., pH 6–7), which averaged 21 mV.

titration experiments (Doring and Colombini, 1985). However, since the anhydride converts amino groups to carboxyl groups, at high levels of reactivity the charge on the sensor could actually increase and the voltage dependence might be restored.

The restoration of voltage dependence by high degrees of modification with anhydride (Adelesberger-Mangan, 1986) is shown in Fig. 6. The voltage dependence of the permeability of a membrane containing many channels was quantitated by measuring the parameter n. Aliquots of succinic anhydride were added (the reaction is completed and excess anhydride is converted to succinate in about 10 min) and the voltage dependence was quantitated. Since succinic anhydride modification also results in a change in VDAC's ion selectivity (Doring and Colombini, 1985), this selectivity change was used as a measure of the degree of anhydride modification. The experiments were performed in the presence of a 10-fold gradient of KCl, and the voltage needed to bring the current to zero (i.e., the reversal potential) was used as a measure of the selectivity. Thus Fig. 6 shows how the voltage dependence varied with anhydride modification by plotting n as a function of the reversal potential. The voltage dependence shows a definite minimum and then increases with further modification. The restoration of voltage dependence with high levels of modification is consistent with the formation of a net negative charge on the sensor. It is worth noting that minimum voltage dependence does not coincide with zero reversal potential (i.e., zero selectivity).

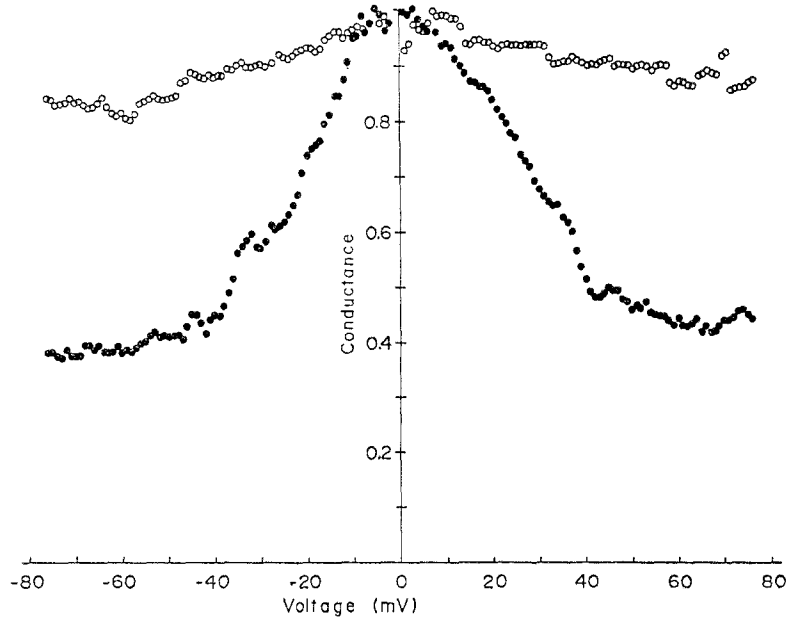

Fig. 5. Succinic anhydride modification reduces VDAC's voltage dependence. (These results are reprinted with permission from Doring and Colombini, 1985.) A multichannel membrane containing VDAC isolated from rat liver was used to obtain these results. The solid circles show the voltage dependence of the membrane conductance before modification, while the open circles show the voltage dependence after the addition of 8 μmol of succinic anhydride to each side of the membrane.

Although it is common to find uncharged channels that are highly selective, in view of VDAC's large pore size this finding might indicate a distinction between charges involved in the sensor and charges involved in imparting selectivity.

Application of Force on the Sensor by Means of Polyvalent Anions

The modification and titration experiments indicate that a positively charged sensor exists that is acted upon by the transmembrane potential resulting in a change in VDAC's conformation. If so, perhaps there are other ways of applying a force on the sensor. Polyvalent anions were used successfully to induce channel closure (Mangan and Colombini, 1987). Figure 7 shows how the voltage dependence of VDAC-containing membranes increased with the addition of dextran sulfate to the aqueous medium. Dextran sulfate increased the voltage dependence to unprecedented levels, 10 times greater than any previously reported. The dependence of n and V_o on dextran sulfate (Fig. 8) indicates that the effect of dextran sulfate depends on the applied

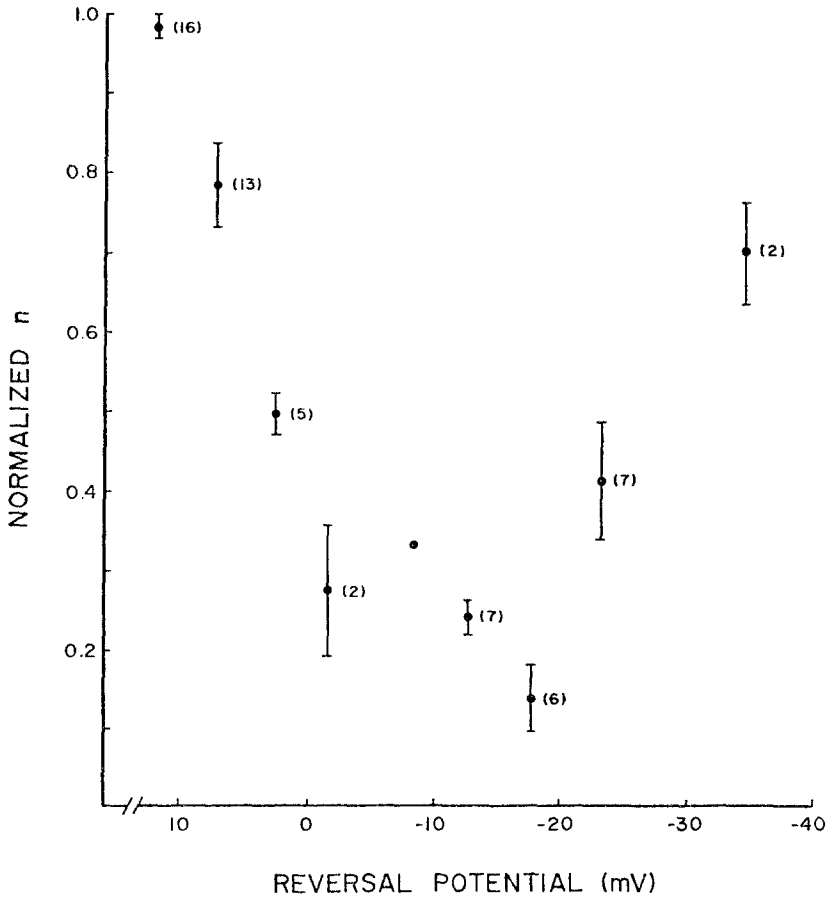

Fig. 6. The steepness of the voltage dependence, n, as a function of the open channel reversal potential. The changes resulted from the addition of succinic anhydride. The results were normalized to the value of n measured prior to anhydride modification. A total of 21 experiments are represented and the number of observations used for each point is indicated in parentheses. The points are means ± S.E. VDAC channels isolated from *N. crassa* were used to perform these experiments.

membrane potential. It is postulated that the membrane potential increases the probability of finding dextran sulfate adjacent to the channel. When it is next to the channel, dextran sulfate interacts with the sensor to induce channel closure. Figure 9 shows a schematic of what might be occurring. It is postulated that an access resistance exists at the mouth of the pore as was found for gramicidin (Lauger, 1976; Anderson, 1983). The field in this access region strongly attracts dextran sulfate (because of its polyvalent nature). Therefore, the ability of dextran sulfate to close the channel is postulated as

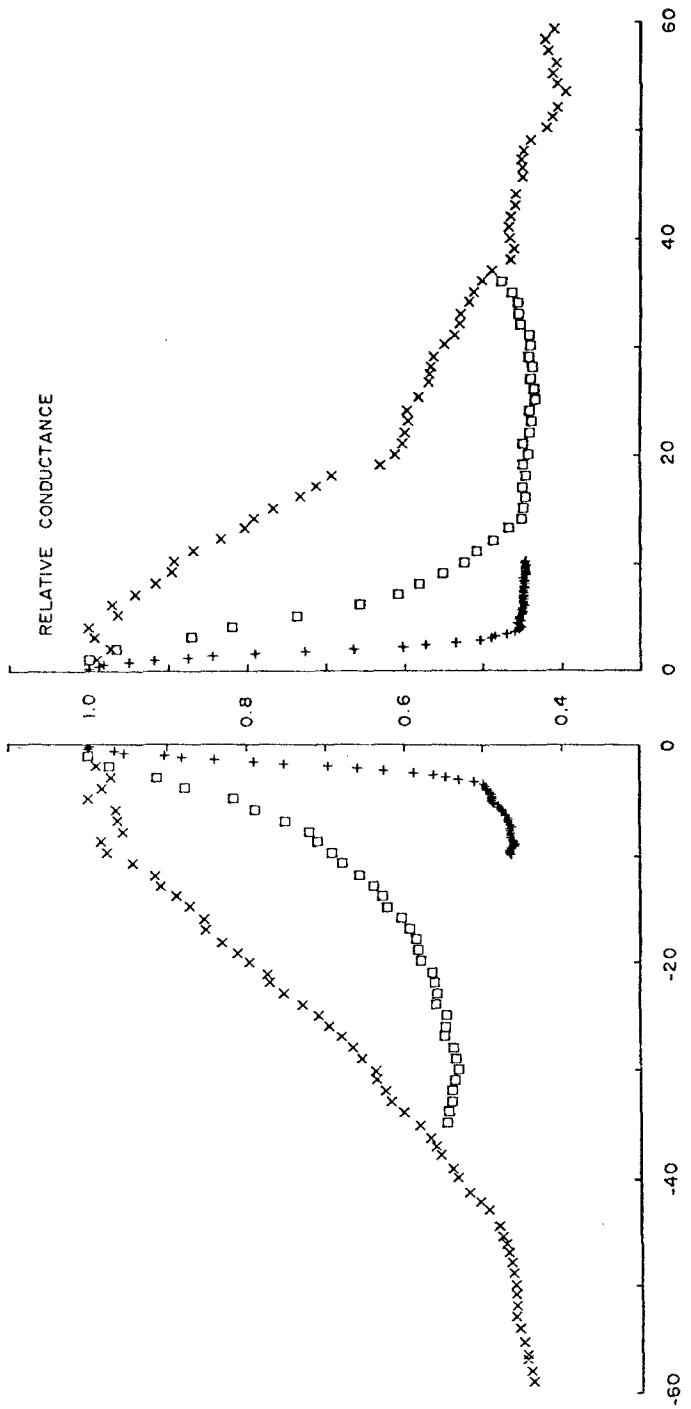

Fig. 7. Dextran sulfate 8000 increases VDAC's voltage dependence (from Mangan and Colombini, 1987). The reduction in the permeability of a VDAC-containing membrane (*N. crassa* was the source of the channels) in the absence (×) and in the presence of 6.2 μM (□) and 25 mM (+) dextran sulfate.

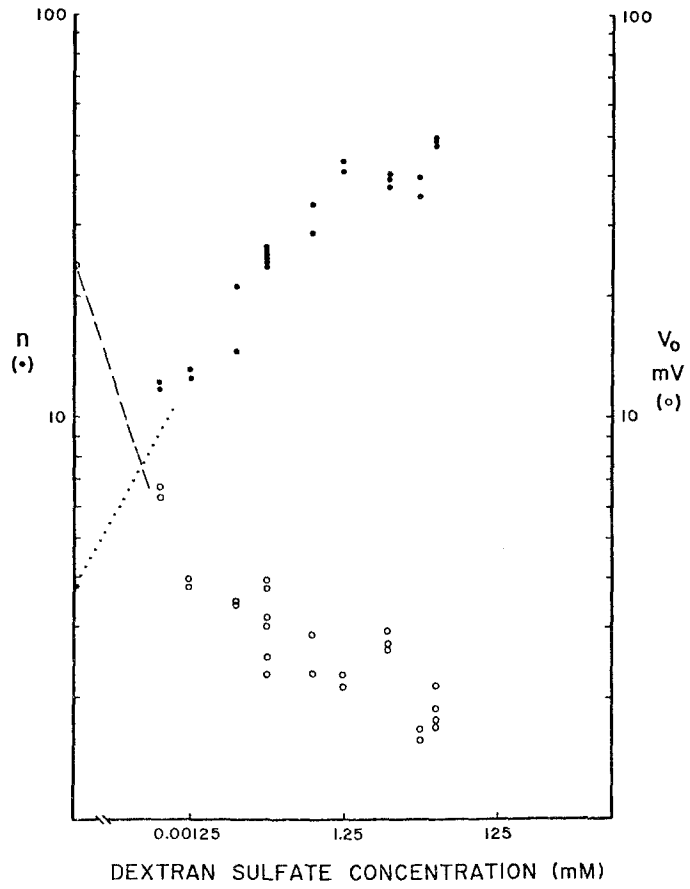

Fig. 8. The effect of dextran sulfate on the values of the parameters n and V_0 (from Mangan and Colombini, 1987). These were calculated from results such as those shown in Fig. 7.

being the result of an electrostatic interaction between this polyvalent anion and the sensor.

Konig and co-workers (Konig *et al.*, 1977, 1982) showed that a synthetic polyanion could interfere with the flux of molecules into mitochondria. This same polyanion acts similarly to dextran sulfate but does so at lower concentrations and is able to close VDAC channels in the absence of a membrane potential (Yeung *et al.*, 1986; Tung, 1986).

Discussion

The ability to control VDAC channels arises from the existence of quasi-degenerate conformational states with different permeabilities. The

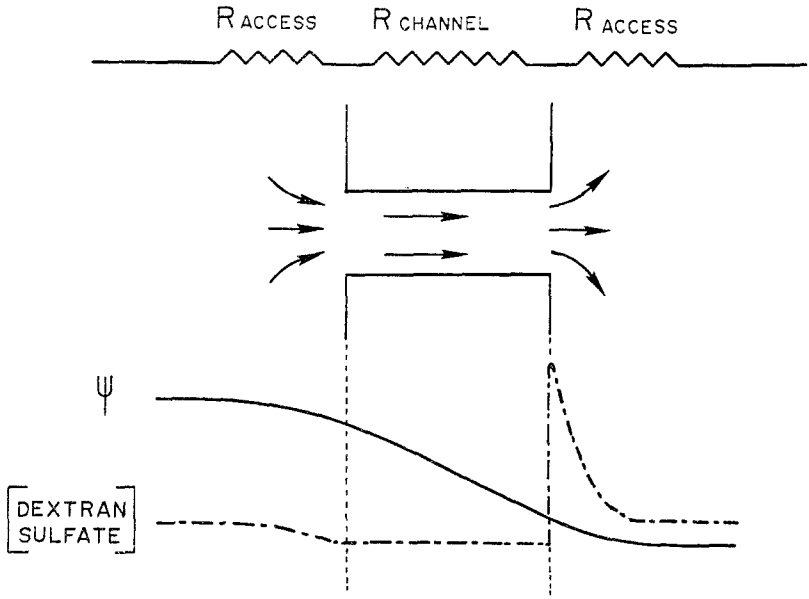

Fig. 9. Hypothesis for explaining the effect of dextran sulfate on VDAC channels (from Mangan and Colombini, 1987). The resistors on top represent the three resistances in series that an ion must traverse in order to cross the membrane through VDAC. The solid curve shows how some of the electric potential would change at the access resistance region. The dash-dot curve shows the dextran sulfate concentration and indicates how dextran sulfate should accumulate in the negative side of the membrane and be depleted on the positive side.

small energy differences between these can be altered by applying an external force because of the presence of a charged sensor. This sensor consists of a group of at least four amino groups (based on the steepness of the voltage dependence, n). A membrane potential or a polyvalent anion can exert a force on this sensor and induce the channels to close. The sensor might be viewed as an access point, a door knob, allowing external influences to open or close the channel.

Perhaps the cell uses a membrane potential to control VDAC's permeability. A Donnan potential resulting from impermeant proteins of opposite charge present on the two sides of the outer membrane could produce the small potential needed to close the channels without the problem of osmotic disequilibrium. A polyanion might serve equally well, and its degree of efficacy could be controlled by controlling its net charge (perhaps through protein phosphorylation). In principle, other agents could interact with the sensor and thus control the channel's permeability.

An example of a totally different substance that interacts with VDAC channels is the aluminate ion. Although probably not a physiological control

agent, it is found in the plasma of normal individuals. This ion seems to interact with the sensor on VDAC to reduce its net charge and thus reduce VDAC's voltage dependence (Dill *et al.*, 1986).

In summary, VDAC is a regulatable channel located in the outer mitochondrial membrane through which flows much of the traffic between the cytoplasm and the matrix space. Although no evidence presently exists which indicates that VDAC regulates mitochondrial function, such regulation may be present. Regulation by the outer membrane may be a way of controlling mitochondrial energy production, mitochondrial growth and division, and perhaps other mitochondrial functions.

Acknowledgments

This work was supported by NSF grant #DCB-85-10335 and ONR grant #N00014-85-K-0651.

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