J. Membrane Biol. 157, 271–279 (1997)

The Journal of
Membrane
Biology

Regulation of Metabolite Flux through Voltage-Gating of VDAC Channels

T. Hodge, M. Colombini
Laboratories of Cell Biology, Department of Zoology, University of Maryland, College Park, MD 20742, USA

Received: 4 November 1996/Revised: 8 January 1997

Abstract. The mitochondrial outer membrane channel, VDAC, is thought to serve as the major permeability pathway for metabolite flux between the cytoplasm and mitochondria. The permeability of VDAC to citrate, succinate, and phosphate was studied in channels reconstituted into planar phospholipid membranes. All ions showed large changes in permeability depending on whether the channel was in the open or in the low conductance, ''closed'' state, with the closed state always more cation selective. This was especially true for the divalent and trivalent anions. Additionally, the anion flux when the voltage was zero was shown to decrease to 5–11% of the open state flux depending on the anion studied. These results give the first rigorous examination of the ability of metabolites to permeate through VDAC channels and indicate that these channels can control the flux of these ions through the outer membrane. This lends more evidence to the growing body of experiments that suggest that the outer mitochondrial membrane has a much more important role in controlling mitochondrial activity than has been thought historically.

Key words: Mitochondria — Outer membrane — Ion flux — VDAC — Channel — Gating

Introduction

For metabolites to gain access to the mitochondrial matrix, they must pass through both the inner and outer membrane. While transport through the inner mitochondrial membrane is mediated by specific carriers, translocation through the outer membrane is thought to be due to the presence of VDAC channels.

VDAC is a highly conserved, highly regulated channel (Colombini, 1979; Colombini, Blachly-Dyson & Forte, 1996). In addition to voltage, the channel has also been found to be regulated by various polyanions (Mangan & Colombini, 1987; Colombini et al., 1987; Colombini et al., 1989), a modulator protein (Holden & Colombini, 1988; Liu & Colombini, 1991, 1992*a*), and NADH (Zizi et al., 1994). By increasing the voltage dependence of the channel, these agents lower the change in membrane potential necessary to open/close the channel.

Channel closure results in both a selectivity change (more cation selective in the closed state) and a reduction in pore size. Thus, if anionic metabolites are more permeable in the open state and largely impermeable in the closed state, the activities of mitochondria could be affected by the conformational change or gating of VDAC channels.

Experiments with isolated mitochondria (Gellerich et al., 1987) and skinned muscle fibers (Saks et al., 1993) have provided evidence that the outer mitochondrial membrane limits the flow of adenine nucleotides. When tested, treatments that close channels in reconstituted conditions have also inhibited mitochondrial function, including the flux of nucleotides into the mitochondria (Colombini et al., 1987; Benz et al., 1988; Liu & Colombini, 1992*b*).

These experiments lend evidence to support the idea that VDAC can control the flux of metabolites through the outer mitochondrial membrane, thereby influencing the overall state of the mitochondrion itself. However, it is difficult to determine if the observed effects are due to effects of the channel alone, the channel interacting with other proteins, or some other effect. The actual situation in vivo may be very complex, as VDAC is known to interact with a variety of other proteins, including hexokinase (Linden, Gellerfors & Nelson, 1982; Fieck et al., 1982, Nakashima et al., 1986), creatine kinase (Brdiczka, Kaldis, & Wallimann, 1994), glycerol kinase (Fiek et al., 1982), the benzodiazepine receptor (McEnery, 1992),

Correspondence to: M. Colombini

and the VDAC modulator (Holden & Colombini, 1988; Liu & Colombini, 1991). To obtain more information on the permeability of metabolites through VDAC itself, we looked at the permeability of planar phospholipid membranes containing VDAC to citrate, succinate, and phosphate. We report that channel closure drastically lowers the permeability of VDAC to these metabolites.

Materials and Methods

All experiments were conducted using planar phospholipid membranes made by the method of Montal and Mueller (1972) as revised by Schein, Colombini and Finkelstein (1976) and Colombini (1987). The voltage was clamped and the current recorded. Calomel electrodes with saturated KCl bridges were used to interface with the aqueous solutions on either side of the membrane. Unless otherwise indicated, 1% asolectin, 0.2% cholesterol in hexane was used to form the monolayers and subsequently the membranes. The solutions contained only the salt of interest, although in some cases, a small amount of the Ca^{2+} or Mg^{2+} salt was added to get more stable membranes. Single-channel experiments were generated by reducing the amount of protein added to the chamber. The reversal potentials were determined by running a 5 mHz triangular wave from approximately +60 to −60 mV, and the point at which the current trace crossed zero was taken as the reversal potential for the open state. The closed state reversal potential was taken by extrapolating the current trace for the channel in the closed state to the zero current line. All chemicals were purchased from Sigma unless indicated otherwise. Results are reported as means ± SE with the number of observations in parentheses.

VDAC from *N. crassa* was used in these experiments. The mitochondria were isolated as previously described (Mannella, 1982).

Experimental Conditions

A twofold concentration gradient of 200 to 100 mM was used for all experiments. Solutions were made with either disodium succinate, trisodium citrate, sodium phosphate, or potassium chloride. The succinate and citrate experiments were brought to pH 7.2 with succinic acid or citric acid. In the case of citrate and succinate, 1 mM calcium succinate or 1 mM magnesium citrate were added to the solutions to help generate stable membranes.

The citrate experiments were performed using ultra-pure trisodium citrate from Amresco. For these experiments, the asolectin was replaced by diphytanoyl phosphatidylcholine (DPyPC) to generate more stable membranes. In addition, a separate set of experiments were performed using 150 mM KCl and 1 mM citrate. In the latter experiments, channels were inserted into the planar membrane in the presence of 150 mM KCl and then either trisodium citrate or magnesium citrate was added to the chamber in order to bring the final concentration of citrate up to 1 mM.

Two sets of experiments were conducted using sodium phosphate. In both experiments a twofold concentration gradient was used, with 200 mM salt solution on the cis side and 100 mM salt solution on the trans side. In the first set of experiments the pH was kept at 5.5, thereby generating primarily phosphate with a single negative charge. In the second set of experiments the pH was adjusted to 8.5 in order to generate primarily phosphate with two negative charges.

The reversal potential of an ideally cation-selective membrane was found by using an artificial cation-selective membrane (Ionac MC 3470). Alternatively, the reversal potential of gramicidin-doped membranes was measured. The cation-selective membrane separated the solutions of interest and the potential was measured with a voltmeter. For the gramicidin experiments, 10 μg/ml gramicidin in DMSO was added to a bilayer separating the solutions of interest. The reversal potential was monitored by determining the voltage that brought the current to zero.

Since the same electrodes and the same solutions were used to record reversal potentials for the ideally cation-selective membranes and the VDAC-doped membranes, electrode asymmetries and liquid junction potentials influence both sets of measurements in the same way. However, reversal potentials after the membrane broke were recorded to correct the measured potential values.

Results

The permeabilities of VDAC channels to citrate, succinate, phosphate, and chloride were examined in channels reconstituted into planar phospholipid membranes. Since current was the measured parameter it was crucial to distinguish between currents carried by the cation and that carried by the anion as both can permeate through VDAC. The zero-current or reversal potential was used to estimate the relative flux of anions and cations. A twofold gradient was used because the reversal potential is known to vary, in a highly nonlinear way with the size of the concentration gradient, at more elevated salt gradients (Zambrowicz & Colombini, 1993).

To convert reversal potential values into permeability ratios, activity ratios are needed. Since the counterions of the metabolic ions of interest were alkali metal ions, the activity ratio of these ions was determined experimentally and used as an estimate of the needed salt activity ratio.

Estimation of the Activity Ratio

The activity ratio of the cation was determined by measuring the equilibrium potential and using the Nernst equation to calculate the activity ratio:

$$\Delta V = \frac{RT}{zF} \ln \frac{a_2}{a_1} \tag{1}$$

Where R is the gas constant, T is the absolute temperature, z is the valency, F is Faraday's constant, a_2/a_1 is the activity ratio of the cation and ΔV is the equilibrium potential for the cation. The equilibrium potential for the cation was estimated by measuring the potential across an artificial cation-selective membrane separating solutions of the two different concentrations. Alternatively, it was measured by applying the same salt gradient to a phospholipid membrane doped with gramicidin, a cation-selective, pore-forming protein (Myers & Hayden, 1972). Since the results of these two different methods were very close, the artificial membrane experiment was taken as a good estimate of the equilibrium potential and the cation activity ratio was calculated using this number.

Table 1. Comparison of activity ratios

Compound	Literature salt activity ratio[1]	Measured cation activity ratios[2]	Debye-Huckel activity ratios[3]
Na_3citrate		1.68	1.65
Na_2succinate		1.71	1.75
Na_2HPO_4	1.69	1.69	1.72
NaH_2HPO_4	1.81	1.78	1.87
KCl	1.86	1.86	1.84

[1] from Stokes & Robinson
[2] calculated using the cation reversal potential determined from artificial membrane experiment
[3] calculated using Debye-Huckel equation and parameters from Lange's Handbook

The cation activity ratio was used to estimate the salt activity ratio. The relationship between the salt activity ratio and that of the anion and the cation for the salt $C_{v+}A_{v-}$ is:

$$\left[\frac{a_{1s}}{a_{2s}}\right]^{v_\pm} = \left[\frac{a_{1+}}{a_{2+}}\right]^{v_+}\left[\frac{a_{1-}}{a_{2-}}\right]^{v_-} \qquad (2)$$

where $v_\pm = v_+ + v_-$ and v_+ is the number of moles of cations and v_- the number of moles of anions produced by the dissociation of the salt. a_s, a_+, and a_- are the activities of the salt, the cation, and the anion, respectively, with 1 and 2 referring to the two different salt concentrations.

The relationships between activity coefficients are best seen for phosphate and KCl, where experimentally measured salt activity coefficients are available. The activity ratios for the individual ions and the salt were determined and compared in Table 1. Given the closeness of the numbers, it is reasonable to use the cation activity ratio as a good estimate of the salt activity ratio where this number is unavailable in the literature.

For comparison we include theoretical values calculated from the Debye-Huckel theory.

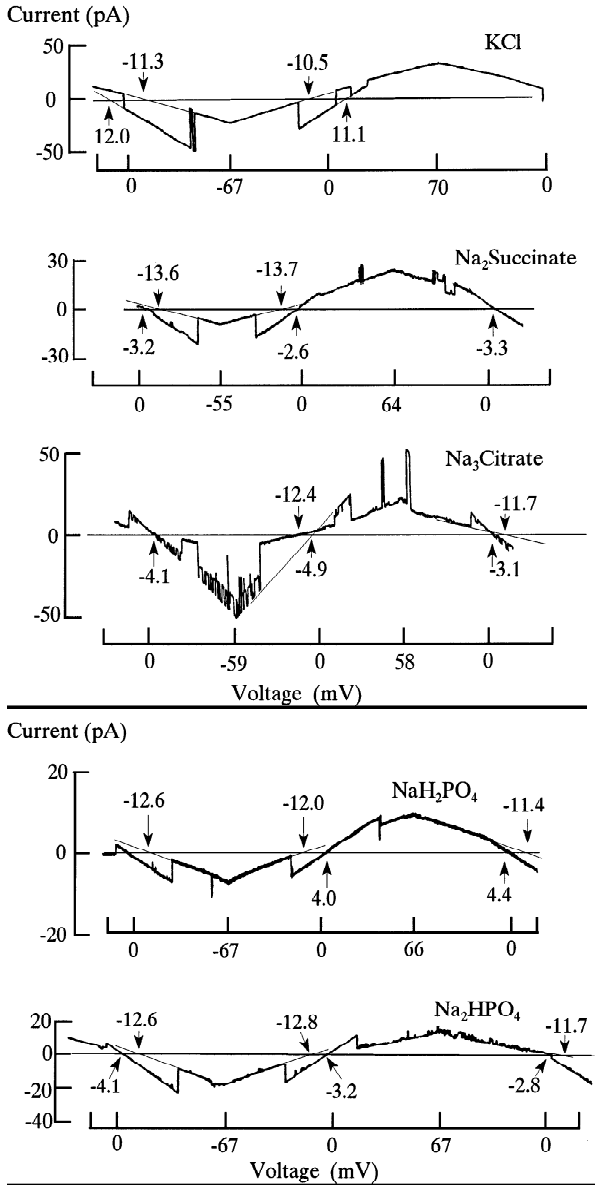

Fig. 1. Single-channel current records in response to applied triangular voltage waves. Twofold gradients of the indicated salt were present (*see* Materials and Methods). On the abscissa are indicated the peak voltages of the triangular voltage waves and the zero-voltage level. The arrows and associated numbers indicate the measured reversal potentials for the open and closed states of the channels. Note that the thin lines are extrapolations used to estimate the reversal potential.

Measurement of the Reversal Potentials for the Open and Closed States

These measurements were made on current records taken from membranes containing 1 VDAC channel. In a few instances, 2-channel membranes were used. Twofold gradients of the anion of interest were present. No significant levels of other anions were present in solution (only OH^- and trace contaminants in the salts). Triangular voltage waves were applied and the currents recorded (e.g., Fig. 1). The linear change of voltage with time produced linear segments in the current record whose slope is the conductance and zero-current intercept is the reversal potential. As the voltage changed, the channel underwent transitions from the high-conducting ''open'' state at low potentials to the low-conducting, ''closed'' state at high potentials. By extrapolation, reversal potentials were determined from the open and closed states. Although different closed states

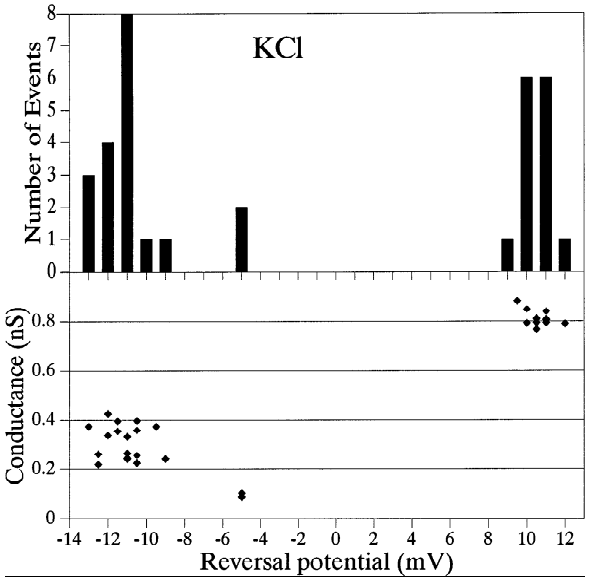

Fig. 2. Single-channel conductance and reversal potential for channels in KCl medium. In the lower panel, individual measurements of conductance of a particular channel are plotted *vs.* the corresponding reversal potential (some points are overlapped). No distinction was made between the open or closed state. However, events in the upper right correspond to the open state while those in the lower left correspond to the closed state. In the upper panel, these same observations (with reversal potentials rounded to the nearest whole number) are plotted in the form of a bar graph. The results plotted in each of Figs. 2–6 were collected on different membranes but multiple measurements were also made on the same channel in one membrane.

are known to exist, the reversal potential of the most frequently occupied closed states were recorded.

Figure 1 shows an example of a typical experiment conducted for each anion. For most of the experiments two relatively easy to identify states are evident: a lower conductive, closed state and a higher conductive, open state. Channel closure is indicated by an abrupt transition to a trace with a shallower slope. However, in some instances there were some states that did not seem to belong in either category and had different characteristics. In general, these were too infrequent to adequately classify.

The recorded traces from the citrate experiments were unusual in that oscillations were seen in all experiments, with only occasional closures of the channel to a much lower conducting state. These oscillations were not observed at physiological levels of citrate. Experiments were conducted in which 1 mM of either sodium citrate of magnesium citrate was added to the aqueous medium consisting of 150 mM KCl after VDAC had already inserted into the membrane. No oscillations were induced in the channel (*data not shown*). Thus the oscillation arises from the high citrate concentration.

Conductances and reversal potentials for the open and closed states of VDAC were measured for solutions containing succinate, citrate, chloride, and the two species of phosphate. The slopes of the linear segments of the single-channel records yielded the conductances of each state occupied by the channel (short-lived states were not measured). The corresponding zero-current potentials of each segment yielded the reversal potential of that state. Extrapolations were often needed to measure this reversal potential (thin lines on figures). The distributions of the reversal potentials of the states plotted with their corresponding single-channel conductance are illustrated in Figs. 2–6. One can clearly identify two populations corresponding to a higher conductive, open state and a lower conductive, closed state. It can also be seen that the lower conductive closed state has reversal potentials that are more negative than the open state, therefore more cation selective.

The distributions of the properties of the open and closed state are rather narrow in the case of chloride (Fig. 2) and broader for the other anions. For citrate, the distribution is the broadest (Fig. 4). Most of the variation is not due to error in the measurement but due to both intra- and interchannel variability. The oscillations evident in the citrate experiment are responsible for the wide variability.

Table 2 shows the average values. Note that all the closed state reversal potentials are negative, indicating a cation selective channel. In looking at these results it can be seen that the closed state reversal potentials are very close to the Nernst potential for the cation and that this represents a large change in selectivity from the open state. This also indicates that the closed state is indeed ''closed'' to the anionic metabolites.

Estimation of the Permeability Ratios

The permeability ratios were determined for each ion, using the Nernst-Planck flux equations. This theory assumes electrical neutrality in the permeability pathway. While this is not quite correct, for large channels it may be a better approximation than constant field. Starting with the standard general flux equations:

$$\phi_- = -p_- a_- \left(RT \frac{1}{a_-} \frac{da}{dx} + z_- F \frac{dV}{dx} \right) \tag{3}$$

$$\phi_+ = -p_+ a_+ \left(RT \frac{1}{a_+} \frac{da}{dx} + z_+ F \frac{dV}{dx} \right) \tag{4}$$

where ϕ is the flux, p is the mobility/permeability.

Assuming electroneutrality, Eqs. 3 and 4 can be combined to yield equations applicable to the situation when the membrane potential yields zero current through the channel. For the case of a monovalent cation and anion, the flux of the anion is equal to the flux of the

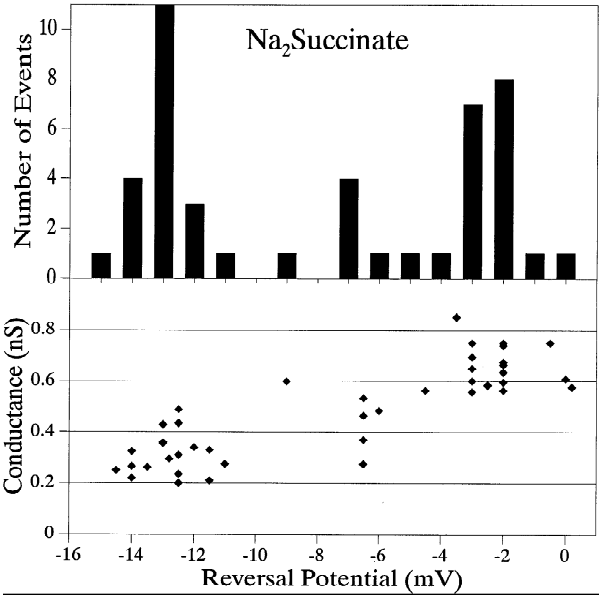

Fig. 3. Conductance and reversal potential for channels in sodium succinate medium. Details are as in Fig. 2.

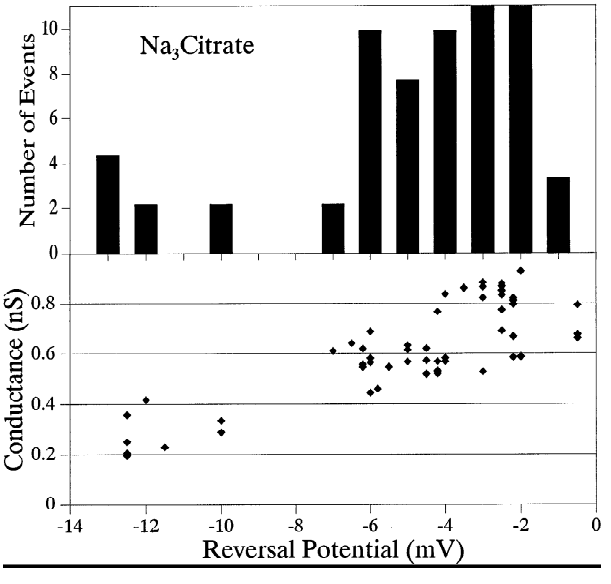

Fig. 4. Conductance and reversal potential for channels in sodium citrate medium. Details are as in Fig. 2.

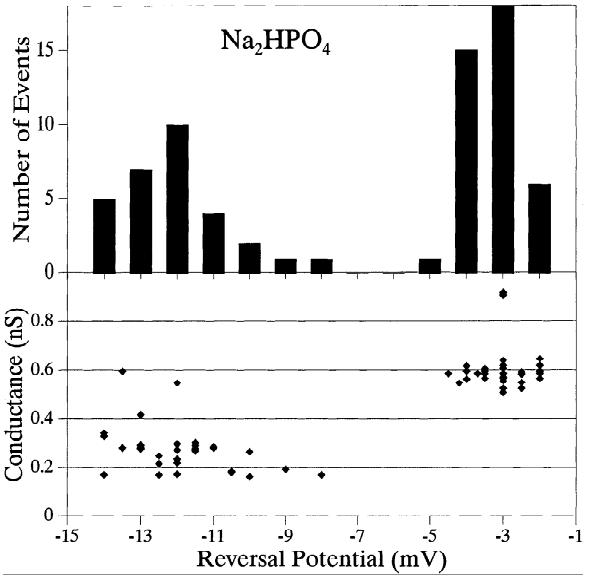

Fig. 5. Conductance and reversal potential for channels in Na_2HPO_4 medium. Details are as in Fig. 2.

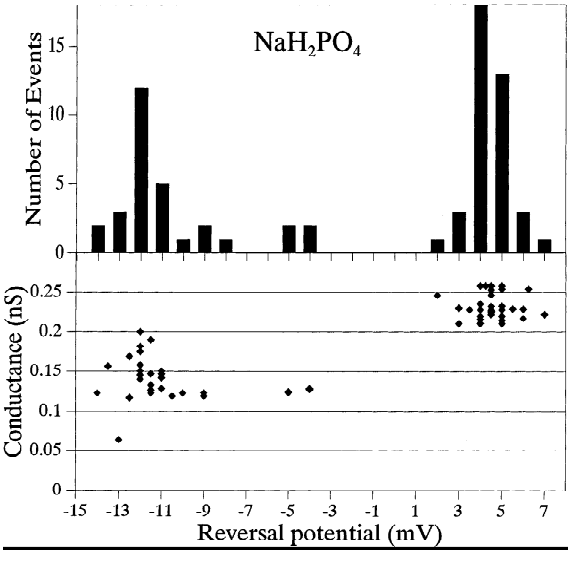

Fig. 6. Conductance and reversal potential for channels in NaH_2PO_4 medium. Details are as in Fig. 2.

cation ($\phi_- = \phi_+$), and the activities of ions are assumed to be equal ($a_+ = a_-$). The result is:

$$\Delta V = \frac{RT}{F}\frac{(p_- - p_+)}{(p_+ + p_-)} ln \frac{a_2}{a_1} \tag{5}$$

where V is the zero current potential. Knowing the activity ratio and the zero current potential, the permeability ratio (p_+/p_-) can be determined.

Likewise, the permeability ratios for the case of divalent and trivalent anions can be found. In general for a monovalent cation and an anion of valency z, $\phi_+ = |z|\,\phi_-$ and $a_+ = |z|a_-$. Thus the equation becomes:

$$\Delta V = \frac{RT}{F}\left(\frac{p_- - p_+}{p_+ + |z|p_-}\right) ln \frac{a_2}{a_1} \tag{6}$$

The permeability ratios shown in Table 3 confirm the high cation selectivity of the closed state for the divalent

Table 2. Reversal potentials of the open (E_o) and closed (E_c) channel, the conductances of the open (G_o) and closed (G_c) channel and the membrane potential of a cation selective membrane and the reversal potentials of gramicidin in the given solution.

Species	E_o (mV)	G_o (nS)	E_c (mV)	G_c (nS)	E_{cation} (mV)	$E_{Gramicidin}$ (mV)
Trisodium citrate	−3.7 ± 0.2 (25)	0.67 ± 0.02 (25)	−11.7 ± 0.4 (8)	0.28 ± 0.02 (8)	−13.1 ± 0.1 (4)	−13.8 ± 0.03 (3)
Disodium succinate	−3.3 ± 0.4 (20)	0.62 ± 0.02 (20)	−12.8 ± 0.2 (19)	0.30 ± 0.02 (19)	−14.0 ± 0.1 (3)	−14.5 ± 0.1 (5)
Na_2HPO_4	−3.1 ± 0.1 (40)	0.58 ± 0.004 (40)	−12.3 ± 0.2 (27)	0.29 ± 0.03 (27)	−12.9 ± 0.3 (5)	−13.4 ± 0.1 (4)
NaH_2PO_4	4.6 ± 0.1 (43)	0.23 ± 0.002 (43)	−11.6 ± 0.3 (29)	0.14 ± 0.01 (29)	−14.8 ± 0.03 (4)	−14.6 ± 0.1 (4)
KCl	10.7 ± 0.2 (15)	0.81 ± 0.01 (15)	−11.1 ± 0.2 (21)	0.31 ± 0.02 (21)	−15.9 ± 0.1 (3)	

Table 3. Permeability ratios for each compound calculated using the salt activity ratio (a_{2s}/a_{1s}) and/or the cation activity ratio (a_{2^+}/a_{1^+})

Compound	Calculated using the salt activity ratio		Calculated using the cation activity ratio	
	Open u_+/u_-	Closed u_+/u_-	Open u_+/u_-	Closed u_+/u_-
Na_3citrate			2.7 ± 0.2	46 ± 8
Na_2succinate			2.2 ± 0.2	93 ± 20
Na_2HPO_4	1.9 ± 0.04	91 ± 18	1.9 ± 0.04	91 ± 18
NaH_2PO_4	0.5 ± 0.01	8.8 ± 1.0	0.5 ± 0.02	10.5 ± 1.8
KCl	0.2 ± 0.01	6.1 ± 0.4	0.2 ± 0.01	6.1 ± 0.4

and trivalent anions. It is somewhat surprising that citrate, given its size and three negative charges is not the least permeable but this may be due to the oscillating behavior. All of the ratios, even that of the monovalent anions show a large change in permeability from the open to the closed states (Table 3).

Estimation of the Ion Fluxes

Estimates of individual ion fluxes were obtained using the current at zero voltage ($V = 0$) and the permeability ratios (p_+/p_-). The total current, I, is given by:

$$I = z_+F\phi_+ + z_-F\phi_- \tag{7}$$

By substituting in the flux equations (Eq. 3 and 4), and letting $V = 0$ for the case where both the anion and cation are monovalent:

$$I_{V=0)} = F\left[p_+RT\frac{da}{dx} - p_-RT\frac{da}{dx}\right] \tag{8}$$

defining $Y = p_+/p_-$, we obtain the following equation:

$$I_{V=0} = FRTp_+\left[\frac{da}{dx}Y(Y-1)\right] \tag{9}$$

Rearranging then gives:

$$\frac{da}{dx} = \frac{I_{V=0}}{FRTp_+Y(Y-1)} \tag{10}$$

The individual ion fluxes are then obtained by substituting Eq. 10 into the individual flux equations (Eq. 3 and 4), giving in the case of a monovalent cation and anion the following equations:

$$\phi_+ = \frac{I_{(V=0)}}{FY(Y-1)} \tag{11}$$

$$\phi_- = \frac{I_{V=0}}{F(Y-1)} \tag{12}$$

The solution for anions of valency, z, is:

$$\phi_z = \frac{I_{(V=0)}}{|z|F(Y-1)} \tag{13}$$

The individual ion fluxes calculated in this way are listed in Table 4. The large change in the calculated anion flux between the open and closed states is evident in the table and in Figure 7. In most cases the closed state only allows about 5 percent of the anion flux as compared to the open state. In contrast, the cation flux is similar in both the open and closed state. Even though the closed state has been shown to have a much smaller aqueous pore (Zimmerberg & Parsegian, 1986; Colombini, 1989), the selectivity change favors cation entry into the channel.

Discussion

The permeability of the mitochondrial outer membrane to nonelectrolytes has long been known to match that of the VDAC channel. However, the ability of VDAC to act as a conduit for the passage of metabolites, most of which are negatively charged was not carefully examined before this work. More importantly, the ability of VDAC to regulate the flow of metabolites had only been examined indirectly by looking at the effects of agents, known to close VDAC channels, on the permeability of the outer membrane. The present work shows that

Table 4. Ion fluxes ($\times 10^6$) in ions/sec

	Anion			Cation	
Species	Open	Closed	% of Open	Open	Closed
Na_3citrate	3.4 ± 0.2	0.24 ± 0.06	7.0	25 ± 0.7	21 ± 2
Na_2succinate	6.8 ± 0.4	0.31 ± 0.05	4.5	26 ± 1	25 ± 1
Na_2HPO_4	6.3 ± 0.1	0.29 ± 0.05	4.6	24 ± 0.3	23 ± 2
NaH_2PO_4	14 ± 0.2	1.6 ± 0.1	11.4	7.6 ± 0.1	12 ± 0.6
KCl	67 ± 0.5	4.5 ± 0.3	6.7	13 ± 0.5	26 ± 1

VDAC is a good conduit for the flow of succinate, citrate, and phosphate. It also shows that the transition of VDAC from the high-conducting, open state to the most easily accessible, lower conducting, ''closed'' state, results in a reduction in the flux of these metabolites that is out of proportion to the reduction in pore radius (from 1.4 to 0.9 nm; Colombini et al., 1996). Despite this ''closed'' state still being permeable to a tetrose (stachyose, Colombini et al., 1987), and even weakly to an 8-glucose cyclodextrin (gamma-cyclodextrin), it shows a remarkable reduced permeability to the metabolic anions. The channels become essentially ''closed'' to these anions. Clearly, electrostatic effects must be involved. This view is supported by the fact that the flow of cations is not reduced much at all by the closing event. The combination of the reduction in pore size and the permeability change resulting from channel closing accounts very well for these observations.

These findings correlate well with the current model of the channel. In the open state, the pore-forming region of VDAC is thought to consist of 12 beta strands and one alpha helix, with primarily nonpolar amino acid residues facing the lipid bilayer, while the polar and charged residues are in contact with water in the pore (Blachly-Dyson et al., 1989; Blachly-Dyson et al., 1990; Peng et al., 1992). In the open state, the net charge of the residues lining the inner wall of the pore, is positive thereby making the channel anion selective. Upon closure however, a subset of transmembrane strands are thought to be removed from the pore causing both the pore size to decrease and, by changing the net charge of the pore, the selectivity to be inverted (Peng et al., 1992). The large change in anion flux in these experiments may be explained in that both effects of channel closure (the change in size and selectivity) work together to limit anion flux. However, in the case of cation flux, these changes work in opposite directions and therefore the flux does not change much.

The decrease in anion flux would limit the availability of metabolites necessary for respiration. Even though this is just a sampling of metabolites that would need to gain access to the mitochondria, the evidence is compelling that other metabolites would have similar changes in permeability through VDAC, and therefore the outer

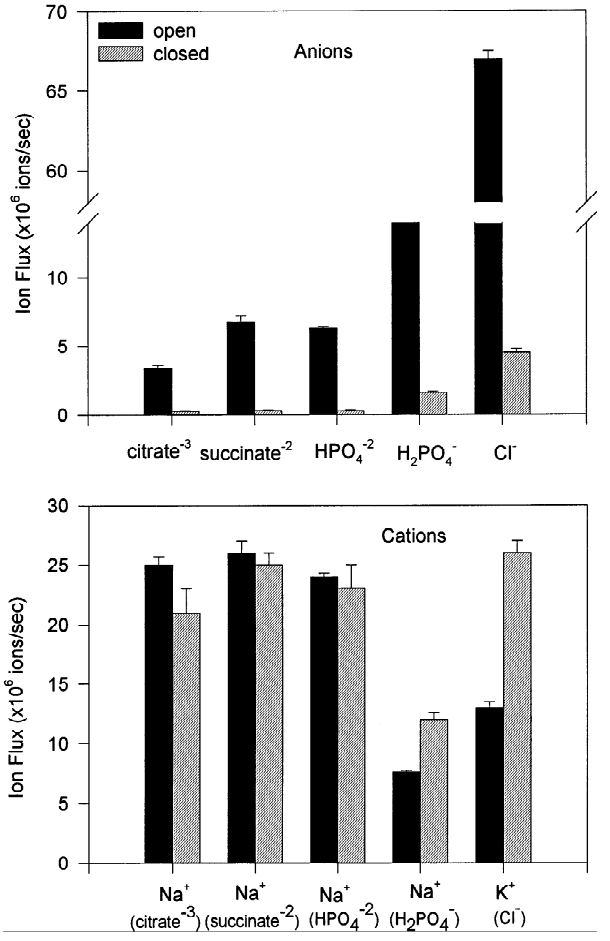

Fig. 7. Fluxes of cations (lower panel) and anions (upper panel) through VDAC channels in the open (solid bar) and closed (hatched bar) state. The error bars are standard errors.

membrane. It is important to note that the divalent ions succinate and HPO_4^{2-}, showed large changes in permeability in these experiments; clearly an additional negative charge drastically affects the permeability in the closed state. It is likely that similar divalent ions would show similar permeability characteristics. These results also correlate well with previous experiments on intact mitochondria in which agents that induce closure of reconstituted channels have been shown to reduce the respiration of mitochondria (Benz et al., 1988; Benz, Kottke & Brdiczka, 1990; Liu & Colombini, 1991; Lee, Zizi & Colombini, 1994). These experiments provide an important link between these two types of experiments in that the permeability was measured directly for the channel alone.

The permeability in vivo may be even more precisely regulated than these experiments indicate. In reality, depending on the actual voltage difference across the mitochondrial outer membrane, this reduction may even be greater. In the presence of the VDAC modula-

tor, VDAC is known to enter a closed state of lower conductance than that studied here. This may make the channel even less permeable to these anions. It is unclear how all the different proteins that bind to VDAC in vivo actually affect the function of the channel. Evidence has been presented that the binding of kinases to VDAC would allow the kinases to have direct access to metabolite flux through VDAC (Adams et al., 1989, Arora & Pederson, 1988; Rasschaert & Malaisse, 1990). However, how the system functions as a whole is unknown. It is possible, that given the local conditions, VDAC might control very precisely any traffic through the outer membrane, perhaps letting some substances in while stopping others. VDAC also shows some heterogeneity of closed states, (Zhang & Colombini, 1990), it is possible that in vivo these are very precisely regulated, which in turn would regulate metabolite flux into and out of mitochondria. VDAC could serve different roles depending on either the cell type or changing conditions in the cell. It has been speculated that VDAC present in the contact sites could be in a different state than VDAC located elsewhere on the outer membrane (Benz et al., 1990; Adams et al., 1989). The in vivo properties of VDAC probably depend on the integration of a number of factors: the voltage present across the outer membrane, the presence of modulator proteins, the binding of different kinases, and which VDAC gene is expressed (Blachly-Dyson et al., 1993).

While the entire system may be complex, the permeability characteristics of VDAC give the mitochondria the ability to control the flux of metabolites through the mitochondrial outer membrane, thereby giving it the ability to regulate the level of mitochondrial activity.

This work was supported by grants from the Office of Naval Research (N00014-90-J-1024) and the National Institutes of Health (GM 35759).

References

Adams, V., Bosch, W., Schlegel, J., Walliman, T., Brdiczka, D. 1989. Further characterization of contact sites from mitochondria of different tissues: topology of peripheral kinases. *Biochim. Biophys. Acta* **981:**213–215

Arora, K.K., Pedersen, P.L. Functional significance of mitochondrial-bound hexokinase in tumor cell metabolism. 1988. *J. Biol. Chem.* **263:**17422–17428

Benz, R., Kottke, M., Brdiczka, D. 1990. The cationically selective state of the mitochondrial outer membrane pore: a study with intact mitochondria and reconstituted mitochondrial porin. *Biochim. Biophys. Acta* **1022:**311–318

Benz, R., Wojtczak, L., Bosch, W., Brdiczka, D. 1988. Inhibition of adenine nucleotide transport through the mitochondrial porin by a synthetic polyanion. *FEBS Lett.* **231:**75–80

Blachly-Dyson, E., Peng, S.Z., Colombini, M., Forte, M. 1989. Probing the structure of the mitochondrial channel, VDAC, by site-directed mutagenesis: a progress report. *J. Bioenerg. Biomembr.* **21:**471–483

Blachly-Dyson, E., Peng, S.Z., Colombini, M., Forte, M. 1990. Selectivity changes in site-directed mutants of the VDAC ion channel: structural implications. *Science* **247:**1233–1236

Blachly-Dyson, E., Zambrowicz, E.B., Yu, W.H., Adams, V., McCabe, E.R.B., Adelman, J., Colombini, M., Forte, M. 1993. Cloning and functional expression in yeast of two human isoforms of the outer mitochondrial membrane channel, the voltage-dependent anion channel. *J. Biol. Chem.* **268:**1835–1841

Brdiczka, D., Kaldis, P., Wallimann, T. 1994. In vitro complex formation between the octamer of mitochondrial creatine kinase and porin. *J. Biol. Chem.* **269:**27640–27644

Colombini, M. 1979. A candidate for the permeability pathway of the outer mitochondrial membrane. *Nature* **279:**643–645

Colombini, M. 1987. Characterization of channels isolated from plant mitochondria. *Methods Enzymol.* **148:**465–475

Colombini, M. 1989. Voltage gating in the mitochondrial channel, VDAC. *J. Membrane Biol.* **111:**103–111

Colombini, M., Blachly-Dyson, E., Forte, M. 1996. VDAC, a channel in the outer mitochondrial membrane. *In:* Ion Channels Vol. 4, T. Narahashi, editor. pp. 169–202, Plenum Press, New York, NY

Colombini, M., Holden, M.J., Mangan, P.S. 1989. Modulation of the mitochondrial channel VDAC by a variety of agents. *In:* Anion Carriers of Mitochondrial Membranes. A. Azzi, K.A. Nalęcz, M.J. Nalęcz, and L. Wojtczak, editors. pp. 215–224. Springer-Verlag, New York

Colombini, M., Yeung, C.L., Tung, J., König, T. 1987. The mitochondrial outer membrane channel, VDAC, is regulated by a synthetic polyanion. *Biochem. Biophys. Acta* **905:**279–286

Fiek, C., Benz, R., Roos, N., Brdiczka, D. 1982. Evidence for identity between the hexokinase-binding protein and the mitochondrial porin in the outer membrane of rat liver mitochondrial. *Biochim. Biophys. Acta* **688:**429–440

Gellerich, F.N., Schlame, M., Bohnensack, R., Kunz, W. 1987. Dynamic compartmentation of adenine nucleotides in the mitochondrial intermembrane space of rat heart mitochondria. *Biochim. Biophys. Acta* **722:**381–391

Holden, M.J., Colombini, M. 1988. The mitochondrial outer membrane channel, VDAC, is modulated by a soluble protein. *FEBS Lett.* **241:**105–109

Lee, A., Zizi, M., Colombini, M. 1994. β-NADH decreases the permeability of the mitochondrial outer membrane to ADP by a factor of 6. *J. Biol. Chem.* **269:**30974–30980

Linden, M., Gellerfors, P., Nelson, B.D. 1982. Pore protein and the hexokinase-binding protein from the outer membrane of rat liver mitochondria are identical. *FEBS Lett.* **141:**189–192

Liu, M., Colombini, M. 1991. Voltage gating of the mitochondrial outer membrane channel VDAC is regulated by a very conserved protein. *Am. J. Physiol.* **260:**C371–C374

Liu, M., Colombini, M. 1992*a*. A soluble protein increases the voltage dependence of the mitochondrial channel, VDAC. *J. Bioenerg. Biomembr.* **24:**41–45

Liu, M., Colombini, M. 1992*b*. Regulation of mitochondrial respiration by controlling the permeability of the outer membrane through the mitochondrial channel, VDAC. *Biochim. Biophys. Acta* **1098:**255–260

Mangan, P.S., Colombini, M. Ultrasteep voltage dependence in a membrane channel. 1987 *Proc. Natl. Acad. Sci. USA* **84:**4896–4900

Mannella, C.A. 1982. Structure of the outer mitochondrial membrane: ordered arrays of porelike subunits in outer membrane fractions from *Neurospora crassa* mitochondria. *J. Cell. Biol.* **94:**680–687

McEnery, M.W. 1992. The mitochondrial benzodiazepine receptor: evidence for association with the voltage-dependent anion channel (VDAC). *J. Bioenerg. Biomembr.* **24:**63–69

Montal, M., Mueller, P. 1972. Formation of bimolecular membranes

from lipid monolayers and a study of their electrical properties. *Proc. Natl. Acad. Sci. USA* **69:**3561–3566

Myers, V.B., Hayden, D.A. 1972. Ion transfer across lipid membranes in the presence of gramicidin A. II. The ion selectivity. *Biochem. Biophys. Acta* **274:**313–322

Nakashima, R.A., Mangan, P.S., Colombini, M., Pederson, P.L. 1986. Hexokinase receptor complex in hepatoma mitochondria: evidence from N,N′-dicyclohexylcarbodiimide-labeling studies for the involvement of the pore-forming protein VDAC. *Biochemistry* **25:**1015–1021

Peng, S.Z., Blachly-Dyson, E., Forte, M., Colombini, M. 1992. Large scale rearrangement of protein domains is associated with voltage gating of the VDAC channel. *Biophys. J.* **62:**123–125

Rasschaert, J., Malaisse, W.J. 1990. Hexose metabolism in pancreatic islets: preferential utilization of mitochondrial ATP for glucose phosphorylation. *Biochim. Biophys. Acta* **1015:**353–360

Saks, V.A., Vasil'eva, E., Belikova, Yu, O., Kuznetsov, A.V., Lyapina, S., Petrova, L., Perov, N.A. 1993. Retarded diffusion of ADP in cardiomyocytes: possible role of mitochondrial outer membrane and creatine kinase in cellular regulation of oxidative phosphorylation. *Biochim. Biophys. Acta* **1144:**134–148

Schein, A.J., Colombini, M., Finkelstein, A. 1976. Reconstitution in planar lipid bilayers of a voltage-dependent anion-selective channel obtained from Paramecium mitochondria. *J. Membrane. Biol.* **30:**99–120

Zambrowicz, E.B., Colombini, M. 1993. Zero-current potentials in a large membrane channel: a simple theory accounts for complex behavior. *Biophys. J.* **65:**1093–1100

Zhang, D.-W., Colombini, M. 1990. Group IIIA-metal hydroxides indirectly neutralize the voltage sensor of the voltage-dependent mitochondrial channel, VDAC, by interacting with a dynamic binding site. *Biochem. Biophys. Acta* **1025:**127–134

Zimmerberg, J., Parsegian, V.A. 1986. Polymer inaccessible volume changes during opening and closing of a voltage-dependent ionic channel. *Nature* **323:**36–39

Zizi, M., Forte, M., Blachly-Dyson, E., Colombini, M. 1994. NADH regulates the gating of VDAC, the mitochondrial outer membrane channel. *J. Biol. Chem.* **269:**1614–1616