Plant Physiol. (1993) 102: 615–621

Purification and Characterization of Porin from Corn (*Zea mays* L.) Mitochondria[1]

Jalal Ahmad Aljamal, Giuseppe Genchi, Vito De Pinto, Luisa Stefanizzi, Aurelio De Santis, Roland Benz, and Ferdinando Palmieri*

Department of Pharmaco-Biology, Laboratory of Biochemistry and Molecular Biology, University of Bari and Consiglio Nazionale delle Ricerche Unit for the Study of Mitochondria, Bari, Italy (J.A.A., G.G., V.D.P., L.S., F.P.); Department of Biology, University of Bologna, Italy (A.D.S.); and Lehrstühl fur Biotechnologie, Universität Würzburg, Würzburg, Germany (R.B.)

Mitochondrial porin from corn (*Zea mays* L. B 73) shoots was solubilized with lauryl(dimethyl)-amine oxide and purified by chromatography on a hydroxyapatite:celite column. On sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the purified protein had an apparent molecular mass of 35 kD. When reconstituted in planar lipid bilayer membranes the porin formed ion-permeable channels with single-channel conductance of 2.0 and 4.0 nanosiemens in 1 M KCl. At low transmembrane voltages corn porin had the properties of a general diffusion pore with an estimated effective diameter of 1.6 nm and a small selectivity for anions over cations. The primary structure of corn porin seems to be quite different from that of other mitochondrial porins, because it did not cross-react with monoclonal antibodies against human porin and with polyclonal antibodies against yeast porin. Furthermore, the peptide maps of corn and bovine heart porins were very different. A sequence of 21 amino acids obtained by Edman degradation of peptides generated by porin proteolysis with *Staphylococcus aureus* V8 protease did not show any significant homology with known sequences of mitochondrial porins. Results of our investigation suggest that corn porin possesses functional properties similar to those of other mitochondrial porins, despite major structural differences.

The mitochondrial outer membrane is freely permeable to hydrophilic solutes up to a well-defined molecular size because of the presence of a general diffusion pore (Pfaff et al., 1968). The protein responsible for the permeability properties of the mitochondrial outer membrane has been called porin (Benz, 1985) or VDAC, for voltage-dependent anion-selective channel (Colombini, 1979). The channel-forming protein has been isolated from mitochondrial outer membrane and whole mitochondria from a variety of eukaryotic cells (De Pinto et al., 1987a). When reconstituted in artificial lipid membranes, it forms large, slightly anion-selective channels in the open state. The channels are voltage dependent and switch to closed states of completely different permeability and selectivity at voltages larger than 20 to 30 mV (De Pinto et al., 1989b).

Biosynthesis, primary structure, and channel properties of the mitochondrial porins from *Neurospora crassa* and from *Saccaromyces cerevisiae* are known in detail. The primary structure of both porins is not particularly hydrophobic, and, according to predictions derived from the hydrophobic profiles, the secondary structure is composed mainly of membrane-spanning sided β-sheets (Mihara and Sato, 1985; Kleene et al., 1987). This finding has recently been confirmed for human porin and porin from *Dictyostelium discoideum* (Kayser et al., 1989; Troll et al., 1992). We have recently characterized and compared several porin channels from high- and low-eukaryotic cells (De Pinto et al., 1987a; Ludwig et al., 1988; Towbin et al., 1989; De Pinto et al., 1989a, 1989b, 1991b). Based on immunological and peptide-mapping experiments, the mammalian porins show strong structural similarities. The pore-forming proteins isolated from low-eukaryotic organisms, on the other hand, show different peptide maps and no immunological cross-reactivity with the mammalian porins (De Pinto et al., 1987a, 1989a).

Little is known about the properties of porin from plants. Only the presence of voltage-dependent channels in crude fractions of corn mitochondria has suggested the presence of porin (Smack and Colombini, 1985). The outer membrane of the chloroplast envelope also probably contains a porin channel (Flügge and Benz, 1984), but this channel has properties different from those exhibited by mitochondrial porins (Flügge and Benz, 1984).

In this study, we adapted the purification procedures developed for mammalian porins (De Pinto et al., 1989b) to mitochondria obtained from corn (*Zea mays* L. B 73) shoots. This procedure resulted in the preparation of an electrophoretically pure protein that was identified as *Z. mays* porin based on its ability to form ion-permeable channels when reconstituted in planar lipid membranes. In this paper we

[1] This work was supported by the National Research Council of Italy, Special Project Advanced Research for Innovations in the Agricultural System (RAISA), Subproject No. 2, Paper No. 810, by grants of the Deutsche Forschungsgemeinschaft (project B9 of the Sonderforschungsbereich 176), and by Fonds der Chemischen Industrie.

* Corresponding author; fax 39–80–242770.

Abbreviations: G, conductance; G_o, initial conductance; LDAO, lauryl (dimethyl)-amine oxide; N_o:N_c, ratio of the number of open channels to the number of closed channels; nS, nanosiemens; V_m, transmembrane potential.

describe the first isolation and purification of a porin from plants.

MATERIALS AND METHODS

Plant Material and Chemicals

Corn (*Zea mays* L. B 73) seeds were surface sterilized for 2 min in 1% (w/v) NaOCl and rinsed in distilled H_2O. Seedlings were grown in the dark at 30°C in moist vermiculite for 5 to 6 d. Hydroxyapatite (Bio-gel HTP) was obtained from Bio-Rad, and Triton X-100, acrylamide, and *N,N'*-methylenebisacrylamide were from Serva. Celite 535 was purchased from Roth and *Staphylococcus aureus* V8 protease was from Boehringer. Diphytanoyl phosphatidylcholine was purchased from Avanti Biochemicals (Birmingham, AL). All other reagents were of analytical grade.

Isolation and Purification of Corn Mitochondria

Mitochondria were isolated from etiolated corn shoots and purified by rapid centrifugation through a 0.6 M mannitol "cushion" as described by Day and Hanson (1977), with little modification. The grinding medium was 0.4 M mannitol, 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 0.05% Cys, and 0.1% BSA. In addition, a Braun mixer was used to disrupt the tissue rather than a mortar and pestle. The crude mitochondrial pellet was resuspended in a washing medium containing 0.3 M mannitol and 10 mM Tris-HCl (pH 7.2). The supernatant, after centrifugation at 1000*g* for 10 min, was gently underlaid with a cushion of 0.6 M mannitol and 10 mM Tris-HCl (pH 7.2) and centrifuged at 10,000*g* for 20 min.

Purification of Porin

Porin was purified from fresh or frozen corn mitochondria according to a method that has been described previously for the purification of porin from bovine heart mitochondria (De Pinto et al., 1989a). Corn mitochondria (80 mg) were lysed by osmotic shock in 10 mM Hepes and 1 mM EDTA (pH 7.2) for 10 min at 0°C. After centrifugation the pellet, composed mainly of mitochondrial membranes, was solubilized with 8 mL of a buffer containing 2% LDAO, 10 mM Hepes (pH 7.2), and 1 mM EDTA at a final concentration of 10 mg of protein mL^{-1}. After incubation for 30 min at 0°C, the solubilization mixture was centrifuged at 27,000*g* for 15 min, and the supernatant (approximately 8 mL) was loaded onto a dry hydroxyapatite:celite column (6 g, ratio 2:1). The elution was performed with the following buffers: (a) 50 mL of solubilization buffer, which gave the first collected fraction of 50 mL; (b) 200 mL of solubilization buffer supplemented with 1 mM KH_2PO_4 and 8 mM KCl, which gave two additional fractions of 100 mL each; (c) 500 mL of solubilization buffer supplemented with 2 mM KH_2PO_4 and 16 mM KCl, which gave another five fractions of 100 mL each. The flow rate of the chromatography was 50 mL h^{-1}. Pure porin was collected in the last four fractions.

Peptide-Mapping Experiments and Peptide Purification

Peptide-mapping experiments were performed on acetone-precipitated proteins dissolved in 4% SDS, 100 mM Tris-HCl (pH 7.0), 12% glycerol (w/v), and 20 mM DTE. Proteins were cleaved for 2 h at 4°C by *S. aureus* V8 protease at a protease:protein ratio of 1:20, and then the reaction was stopped by boiling for 10 min. The peptides obtained by proteolysis with *S. aureus* V8 protease were separated by the discontinuous Tricine system of Schägger and von Jagow (1987). For protein sequencing the peptides separated with the same method (Schägger and von Jagow, 1987) were transferred to polyvinylidene difluoride membranes (Immobilon from Millipore), detected by staining with Coomassie blue dye, excised, and subjected to Edman degradation in an Applied Biosystems 477A pulse liquid protein sequencer.

SDS Gel Electrophoresis

SDS-PAGE of acetone-precipitated samples was performed in the presence of 0.1% SDS according to the method of Laemmli (1970). The separation gel contained 17.5% acrylamide with an acrylamide:bisacrylamide ratio of 30:0.2. For peptide analysis, the discontinuous Tricine system of Schägger and von Jagow (1987) was used. We used 16.5% acrylamide with an acrylamide:bisacrylamide ratio of 15.5:1, with 13% glycerol included in the separation gel. The molecular mass markers used were the Bio-Rad low molecular mass markers (92.5, 66.2, 45, 31, 21.5, and 14.4 kD) and the molecular mass SDS-17 kit from Sigma, containing cyanogen bromide fragments of myoglobin (17, 14.4, 10.7, 8.2, 6.2, and 2.5 kD).

Immunoblotting Experiments

The antiserum against bovine heart porin was obtained from rabbit as described previously (De Pinto et al., 1989a). The antisera against the acetylated 19 N-terminal amino acids of human porin and against yeast porin were kind gifts of Dr. F. Thinnes (Göttingen, Germany) and Dr. M. Dihanich (Basel, Switzerland), respectively. The proteins separated on a 14% SDS-PAGE were transferred to nitrocellulose and incubated with the antisera and then with an anti-rabbit immunoglobulin horseradish peroxidase-linked antibody (purchased from Amersham). The peroxidase reaction was performed using 20 mL of a mixture of 0.05% 4-chloro-1-naphthol, 16% methanol, and 0.5% BSA in 0.14 M NaCl and 0.01 M phosphate (pH 7.0) with the final addition of 12 μL of 30% H_2O_2.

Reconstitution of Porin in Planar Lipid Bilayer Membranes

Reconstitution of porin into artificial lipid bilayer membranes has been described (Benz et al., 1978). Membranes were formed from a 1% (w/v) solution of diphytanoyl phosphatidylcholine in *n*-decane in a Teflon cell consisting of two aqueous compartments connected by a circular hole. The area of the hole was 0.2 mm^2 for single-channel experiments and 1 mm^2 for macroscopic conductance measurements. Membranes were formed across the hole and 10 to 100 ng of porin were added to 5 mL of the aqueous phase at one side of the membrane. The aqueous salt solutions were used unbuffered and had a pH of approximately 6.0. The temperature was kept at 20°C throughout. The membrane current was meas-

ured with a pair of calomel electrodes switched in series with a voltage source and an electrometer (Keithley 602). For single-channel recordings the electrometer was replaced by a current amplifier (Keithley 427). Zero-current membrane potential measurements were performed by establishing a salt gradient across membranes containing 100 to 1000 porin channels, as was described earlier (Benz et al., 1979).

Other Methods

Mitochondrial protein was measured by the biuret method, using potassium cyanogen to account for turbidity due to phospholipids (Kroger and Klingenberg, 1966). Purified protein was measured by the Lowry method modified for the presence of detergent (Kusov and Kalinchuk, 1978).

RESULTS

Purification of the Mitochondrial Porin

Two rapid methods have been established for the purification of functional mitochondrial porins to homogeneity (Palmieri and De Pinto, 1989). The detergent Triton X-100 is used with one method (De Pinto et al., 1987a), and the detergent LDAO is used with the other (De Pinto et al., 1989b). LDAO has a much shorter head group than Triton X-100. As a consequence, some of the hydrophilic domains of the LDAO-solubilized porin are exposed to the aqueous phase, and porin binds to cation-exchangers (De Pinto et al., 1990). Furthermore, mitochondrial porin purified in the presence of LDAO has a higher channel-forming activity and shows a more homogeneous channel distribution (De Pinto et al., 1989b).

We applied both purification procedures to corn mitochondria. When these mitochondria were solubilized in Triton X-100 and chromatographed on a dry hydroxyapatite:celite column as described previously (De Pinto et al., 1987b), five proteins with molecular masses between 20 and 35 kD were eluted just after the void volume of the column. These proteins also included porin, as could be shown by measurements of the pore-forming activity.

To avoid further chromatographic steps, which tend to decrease the pore-forming activity of the pure protein, we tried to apply the LDAO procedure to the purification of porin from corn mitochondria. In these experiments the LDAO solubilizate of corn mitochondria was applied to the dry hydroxyapatite:celite columns, and the eluates were analyzed by SDS-PAGE. In the case of bovine heart mitochondria, porin elutes as the first protein after the addition of 5 mM KH_2PO_4 and 50 mM KCl to the low ionic strength solubilization buffer. The application of the same procedure to corn mitochondria resulted in the elution of several different proteins from the dry hydroxyapatite:celite column. The most abundant of these proteins had an apparent molecular mass of 27 kD. We tried to optimize the purification of this protein by increasing the ion strength of the solubilization buffer in several steps. As shown in Figure 1, after the addition of solubilization buffer alone, the first fraction of 50 mL, collected from the dry hydroxyapatite:celite column (lane 1), contained the pure 27-kD protein. The addition of 1 mM KH_2PO_4 and 8 mM KCl to the solubilization buffer resulted

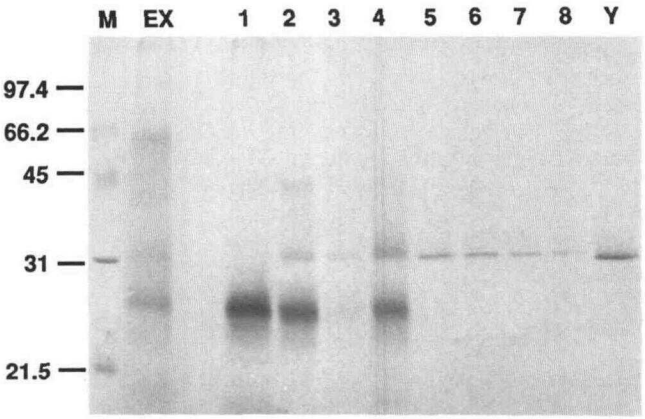

Figure 1. Purification of porin from corn mitochondria. SDS gel electrophoresis of fractions obtained by hydroxyapatite:celite chromatography of corn mitochondria solubilized with LDAO. M, Protein markers (from the top to the bottom: phosphorylase B, BSA, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor); EX, LDAO mitochondrial extract (5 μL; 1.5 μg of protein); 1 to 8, hydroxyapatite:celite fractions (400 μL); Y, purified yeast mitochondrial porin.

in the elution of two other proteins with molecular masses of 31.5 and 40 kD together with the 27-kD protein (lanes 2 and 3 in Fig. 1). The next fractions (lanes 4–8) were collected after the addition of 2 mM KH_2PO_4 and 16 mM KCl. Only fraction 4 contained two proteins; fractions 5 to 8 showed a single protein band with an apparent molecular mass of 31.5 kD. It has to be noted that the elution pattern of Figure 1 was highly reproducible for different samples of corn mitochondria. The total amount of the 31.5-kD protein purified with this procedure (lanes 5–8) was about 0.12% of the total mitochondrial protein. Lane Y of Figure 1 shows the electrophoretic mobility of purified yeast porin (apparent molecular mass 30 kD [Ludwig et al., 1988]) for comparison.

Lipid Bilayer Experiments

Lipid bilayer experiments were performed to study whether the 27- and/or the 31.5-kD proteins isolated from corn mitochondria showed any pore-forming activity. In a first set of experimental conditions we discovered that only the 31.5-kD protein, at concentrations of 10 to 100 ng mL^{-1}, was able to increase the specific conductance of lipid bilayer membranes by many orders of magnitude. The time course of the increase was similar to that described previously for different mitochondrial and bacterial porins (Benz, 1985). The addition of much smaller concentrations of the 31.5-kD protein to membranes with a surface area of about 0.2 mm^2 allowed the resolution of step increases in membrane conductance (Fig. 2). Again, these steps were specific for the addition of the 31.5-kD protein and were not observed when only the detergent LDAO or the 27-kD protein was present in the aqueous phase. These step increases in conductance define the 31.5-kD protein as the pore-forming component of the outer membrane of corn mitochondria, i.e. it is the corn porin.

A large number of channels had a single-channel conductance of 4 to 4.5 nS, in agreement with those of most mito-

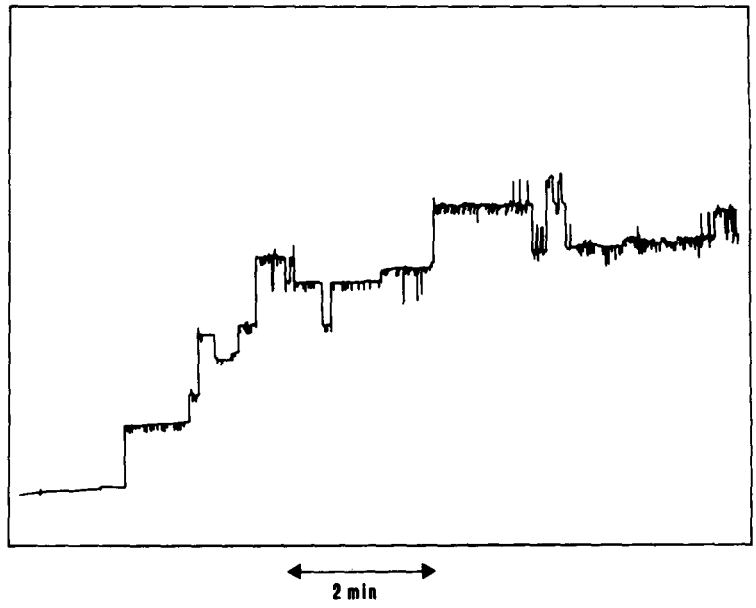

Figure 2. Stepwise increase of the membrane current after the addition of corn porin to a black lipid bilayer membrane. The aqueous phase contained 10 ng mL^{-1} of protein and 1 M KCl. The membrane was formed from diphytanoyl phosphatidylcholine/*n*-decane. The applied voltage was 10 mV; temperature was 25°C.

chondrial porins (Benz, 1985; De Pinto et al., 1987a). However, the single-channel conductance of a certain fraction of pores was smaller (2.0 nS in 1 M KCl) than that exhibited by the 4-nS channel (Fig. 3). We do not think that the small conductance steps are simply half of the large channel, although this possibility cannot be excluded. It seems that the smaller channel could represent a different configuration of the same protein. It is interesting to note that the smaller conductance steps have also been observed in all reconstitution experiments performed in our laboratory with eukaryotic porins (De Pinto et al., 1987a).

The single-channel conductance of the corn porin was studied as a function of the KCl concentration in the aqueous phase. The results, summarized in Table I, showed a linear relationship between conductance and salt concentration. Single-channel experiments were also performed with LiCl and KCH_3COO instead of KCl to get some insight in the selectivity of the corn porin channel. The conductance of the channel was smaller in 1 M KCH_3COO than in 1 M KCl or in 1 M LiCl. This result indicated some preferential movement of anions over cations, because acetate has a smaller aqueous mobility than chloride. The single-channel data suggest that the corn porin forms a wide, water-filled channel because of its large single-channel conductance and a linear conductance-concentration curve. Its diameter is probably very similar to that of other mitochondrial porins, which have, according to single-channel conductance data (De Pinto et al., 1987a), electron microscopic analysis (Mannella et al., 1989), and solute permeability data (Benz, 1985), a diameter between 1.7 and 3.0 nm.

Voltage Dependence

The common characteristic of all mitochondrial porins studied to date is their voltage dependence. To study whether corn porin was also voltage dependent in the reconstituted system we performed appropriate experiments under multi-channel conditions. The voltage across the membrane was set to different potentials ranging from 10 to 100 mV, and membrane currents were monitored on an oscilloscope screen. Immediately after application of the voltage, the current was a linear function of the applied membrane potential. For voltages larger than 30 mV, the membrane current decayed exponentially thereafter to smaller values. The maximal decrease of conductance was observed at a voltage of about 80 to 100 mV. At this potential, G was about 40 to 50% of G_o. Higher potentials did not cause a further conductance decrease.

Figure 4 shows the ratio $G:G_o$ as a function of V_m. The

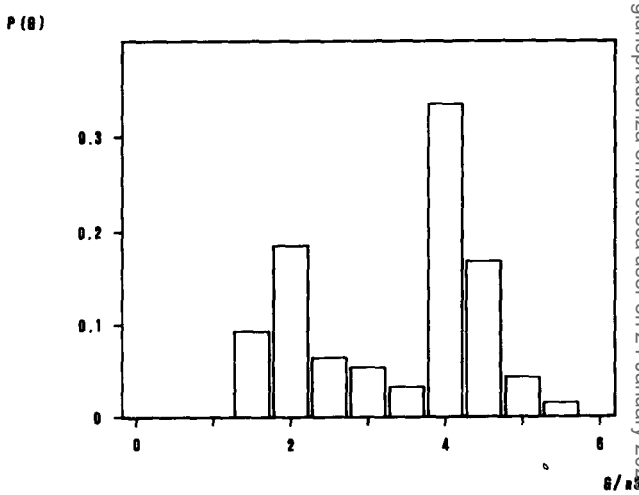

Figure 3. Histogram of fluctuations of G observed with membranes of diphytanoyl phosphatidylcholine/*n*-decane in the presence of corn porin. The aqueous phase contained 1 M KCl. The applied voltage was 10 mV. The mean value of all upward directed steps was 3.6 nS for 186 single events; temperature, 25°C.

Table I. *Average single-channel conductance, Λ, of porin from corn mitochondria in different salt solutions*

The aqueous solutions contained 5 to 10 ng mL^{-1} of porin and less than 0.1 μg mL^{-1} of LDAO; the pH was between 6.0 and 7.0. The membranes were made of diphytanoyl phosphatidylcholine/*n*-decane; temperature was 25°C; V_m = 10 mV. Λ was determined by recording at least 70 conductance steps and averaging over all values. c is the concentration of the salt solution, and Λ is its specific conductance.

Salt	c	Λ
	M	*nS*
KCl	0.03	0.13
	0.1	0.45
	0.3	1.2
	1	3.6
	3	10.0
LiCl	1	2.8
KCH_3COO	1	1.8

curve of Figure 4 could be fitted to a previously proposed formalism (Schein et al., 1976). A semilogarithmic plot of the ratio N_o:N_c as a function of V_m could be fitted to a straight line with a slope of 13 mV for an *e*-fold change of N_o:N_c (data not shown). This means that about two gating charges could be involved in the gating of corn porin, whereas the midpoint potential of the channel distribution, i.e. the potential at which 50% of the channels are in the closed configuration, is 42 mV. A similar number of gating charges have been found for other mitochondrial porins under similar conditions (De Pinto et al., 1987a).

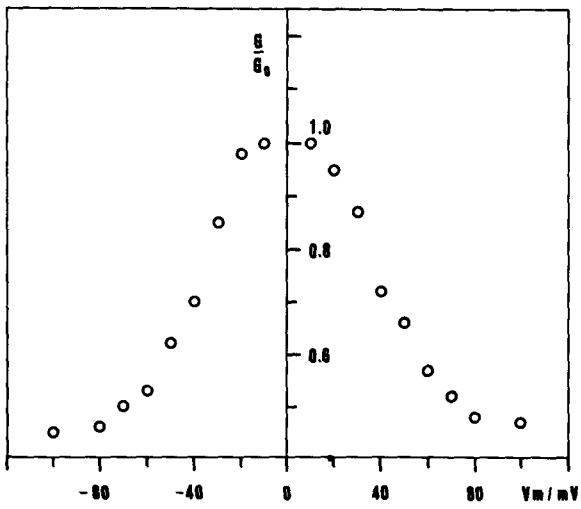

Figure 4. Bell-shaped curve for $C:C_o$ measured as a function of V_m. The aqueous phase contained 100 mM KCl and 50 ng mL^{-1} of mitochondrial porin from corn. Analysis of the data with a previously proposed formalism (Schein et al., 1976) suggested that the number, *n*, of gating charges moving through the entire membrane potential was about 2 and that the potential at which half of the channels are in the closed configuration was 42 mV. Means of experiments from three different membranes are shown.

Selectivity of Corn Porin

The single-channel data suggested that porin of *Z. mays* had a certain preference for anions over cations. To verify this, we investigated the ion selectivity of porin by measuring the membrane potential under zero-current conditions. After the incorporation of about 100 to 1000 porin channels into the membranes, the salt concentration on one side of the membrane was increased 10-fold to 500 mM, and zero-current potential was measured 5 to 10 min after the gradient was established. The results are summarized in Table II. For 10-fold gradients of KCl or LiCl the more dilute side (50 mM) was always negative, which suggested preferential movement of the anions through the porin channel in the open state, i.e. the channel is anionically selective for these two salts. However, the channel selectivity was not independent of the salt, and the more dilute side became positive in the case of potassium acetate. This result indicated preferential movement of the cation over the anion for the combination of the mobile potassium cation and the less mobile acetate anion. Analysis of the data of Table II using the Goldman-Hodgkin-Katz equation (Benz, 1985) gave some information about the ratios of the permeabilities for anions and for cations. They ranged between 0.63 for KCH_3COO and 1.8 for LiCl. Thus, the aqueous mobility of the ions plays a certain role in channels selectivity, which is not the case for channels exclusively selective for anions or cations.

Immunoblotting Experiments

Immunological analysis of corn porin was performed by western blotting using different antisera. These were raised against the N terminus (19 amino acids) of human porin, against *S. cerevisiae* porin, and against bovine heart porin. None of the three antisera cross-reacted with porin isolated from corn mitochondria even at an antiserum dilution of 1:400 (data not shown). These results indicated considerable structural differences between porin from plant and porins from other sources, such as mammals and yeast. It should be noted that fish and insect porins have been detected by the antiserum against the N-terminal end of human porin (De Pinto et al., 1989a, 1989b, 1991b), and fish porin has been

Table II. *Zero-current V_m observed in the presence of mitochondrial porin from corn*

The data were obtained in the presence of a 10-fold gradient of the indicated salts across membranes from diphytanoyl phosphatidylcholine/*n*-decane. V_m is defined as the potential of the dilute side (50 mM) relative to that of the concentrated side (500 mM). The temperature was 25°C. The ratio of the permeabilities for anions and cations (P_a:P_c) was calculated from the Goldman-Hodgkin-Katz equation (Benz, 1985) from at least four individual experiments.

Salt	V_m	P_a:P_c
	mV	
KCl (pH 6)	−4.2	1.2
LiCl (pH 6)	−12	1.8
KCH_3COO (pH 7)	+9.5	0.63

detected by both the antibodies against human porin and those against bovine heart porin (De Pinto et al., 1991b).

Peptide Maps

The peptide map of porin was obtained by fragmenting the purified protein with *S. aureus* V8 protease. The proteolytic pattern of corn protein is shown in Figure 5 together with the pattern obtained from bovine heart porin with the same protease. A comparison between the patterns indicates important differences in the primary structure between the corn and the bovine heart porins.

Two abundant peptides of about 16 and 5 kD obtained by proteolysis of corn porin with *S. aureus* V8 protease were purified and sequenced by Edman degradation. They had the same N-terminal sequence: LQYL(H)NYAGVDA(S)-VGLDANPG. It is interesting that this sequence does not show any clear homology to the known primary sequences of mitochondrial porins (Mihara and Sato, 1985; Kleene et al., 1987; Kayser et al., 1989).

DISCUSSION

The experiments described in this study demonstrate that we were able to isolate and purify a 31.5-kD protein (Fig. 1) from corn mitochondria that forms large ion-permeable pores when reconstituted in lipid bilayer membranes. The purification was achieved by using a well-established procedure (De Pinto et al., 1989b) with some modifications, i.e. the basic step was the chromatography across a dry hydroxyapatite:celite column. To obtain the 31.5-kD protein in a pure form, the original protocol had to be changed slightly; in particular the ionic strength of the elution buffer had to be decreased somewhat.

The most frequently observed single-channel conductance in 1 M KCl was 4.0 to 4.5 nS. A second peak in the histogram comprised values of approximately 2 nS (Fig. 3). The single-channel characteristics together with the weak anion selectivity of the channel are typical for all mitochondrial porins investigated, with the only exception being *Paramecium* porin, which forms exclusively a channel with the smaller conductance (Ludwig et al., 1989). Furthermore, the channels formed by the 31.5-kD protein of corn mitochondria are voltage dependent with a bell-shaped $G:G_o$ versus voltage curve, which represents another typical characteristic of mitochondrial porins and which has also been found for the voltage-dependent channel of crude extracts of corn root mitochondria (Smack and Colombini, 1985). These results strongly indicate that the 31.5-kD protein from corn mitochondria is the corn porin, although it does not cross-react with antibodies raised against porin from human B-lymphocytes or with antibodies against yeast porin.

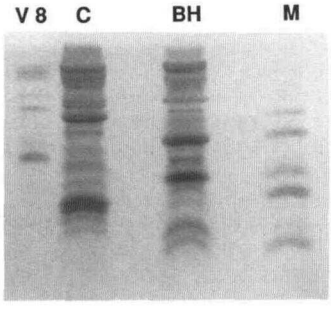

Figure 5. Peptide map of purified corn and bovine heart porins. Conditions were as described in "Materials and Methods." C, Corn porin; BH, bovine heart porin; V8, *S. aureus* V8 protease; M, the molecular mass SDS-17 kit from Sigma. Coomassie blue staining.

It has to be noted that the missing cross-reactivity of corn porin with mammalian and yeast porins and the completely different peptide maps of the bovine heart porin and of the 31.5-kD protein purified in this study do not mean that corn porin has a different structure and function compared to other mitochondrial porins. The β-barrel structure of membrane channels, which has been resolved for a bacterial porin by x-ray diffraction and which is very likely the main structural feature of all mitochondrial porins (De Pinto et al., 1991a; Weiss et al., 1991), tolerates extensive amino acid variations without substantial alterations in the secondary structure. The known sequences of mitochondrial porins (from *N. crassa* [Kleene et al., 1987], yeast [Mihara and Sato, 1985], human lymphocytes [Kayser et al., 1989], and *D. discoideum* [Troll et al., 1992]) have only 14 (of about 280) amino acids in common. Nevertheless, all of these channels exhibit a striking functional similarity.

Our data suggest that corn porin in its open state forms a wide, water-filled channel. This allows an approximate estimate of the effective diameter of the pore on the basis of a previously suggested formalism (Schein et al., 1976). Assuming that the pores are filled with a solution of the same specific conductivity as the bulk aqueous phase and have a cylindric shape with a length of 6 nm, the average pore diameter can be estimated to be about 1.7 nm ($G \approx 4.0$ nS). This value is also consistent with the results of EM studies (Mannella et al., 1989) and allows the permeation of solutes with molecular masses up to about 3 kD. On the other hand, it has to be noted that the diameter derived from single-channel experiments has to be taken as a lower limit of channel size, because the lipid bilayer technique tends to underestimate the channel size under certain conditions. In particular, this underestimation may be caused by the decrease of the maximal possible channel conductance (according to the dimensions of the channel) due to incomplete shielding of the channel interior against the low dielectric membrane interior.

Corn porin is responsible for the exchange of mitochondrial metabolites across the mitochondrial outer membrane. In its open state it is slightly anion selective, whereas preliminary data suggest that the closed state is cation selective (our unpublished data). This means that the closed state of corn porin may be part of the control of mitochondrial metabolism and restricts solute transport. In fact, it has recently been shown that an inhibitor of mitochondrial porin (a 10-kD copolymer from methacrylate, malate, and styrene in a 1:2:1 proportion) that shifts porin into its closed configuration blocks ATP and ADP exchange through rat liver porin in intact mitochondria (Benz et al., 1988, 1990). Similar investigations have not been performed with plant mitochondria, but there exists little doubt that the inhibitor has a similar

effect there. This may indicate that the function of the mitochondrial outer membrane could be an important one and deserves further investigation.

Received November 30, 1992; accepted March 1, 1993.

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