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Characterization of the mitochondrial porin from *Drosophila melanogaster*

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Mitochondrial porin was isolated from the fruit fly *Drosophila melanogaster* at different developmental stages, starting from whole mitochondria. The porin from adults' mitochondria was fully characterized. The protein had a molecular mass of 31 kDa as judged from sodium dodecylsulfate electrophoretograms. It was very resistive against digestion with V8 proteinase of *Staphylococcus aureus* and a larger number of fragments were only obtained after digestion with papain. *Drosophila* porin showed little interaction with antibodies raised against mitochondrial porins from mammalia and *Neurospora crassa*, but a strong reactivity with antibodies raised against yeast porin. Reconstitution experiments with planar lipid bilayer membranes showed that the protein was able to form ion-permeable pores with a single-channel conductance of 0.41 nS in 0.1 M KCl. At low transmembrane voltages *Drosophila* porin had the properties of a general diffusion pore with an estimated effective diameter of about 1.7 nm and a small selectivity for anions over cations. Voltages larger than 20 to 30 mV resulted in a closure of the pore. The closed states of the pore were found to be cation-selective. The addition of a synthetic polyanion to the aqueous phase on one side of the membrane resulted in an asymmetric shift of the voltage dependence and the pore became already closed at very small voltages negative at the cis-side (the side of the addition of the polyanion).

Introduction

The outer membranes of mitochondria and Gram-negative bacteria contain general diffusion pores (porins or VDAC) which are responsible for the free permeation of small hydrophilic solutes through these membranes [1,2]. The pores are impermeable for large molecular weight solutes with an exclusion limit of about 600–800 dalton in enteric Gram-negative bacteria [3] and of about 2500 dalton in mitochondria [4]. The general diffusion pores of the mitochondrial outer membrane are slightly anion selective in the open state [5]. Transmembrane voltages larger than 20 to 30 mV shift the pores in a closed state, which has completely different properties than the open state [2].

We have recently characterized several porins from mammalian tissues [5–7]. All mammalian porins exhibit strong similarities as judged from immunological cross-reaction, peptide-mapping and pore-forming characteristics. On the other hand, mammalian porins on one side and porins from yeast or *Paramecium* on the other side show no immunological cross-reaction and have completely different peptide maps [5]. In this investigation we describe the characterization of the porin of *Drosophila melanogaster* and its comparison to other mitochondrial porins. Reconstitution experiments with *Drosophila* porin showed that it formed a general diffusion pore in the open state. The closed state of the pore is cation selective as judged from experiments performed at voltages larger than 30 mV and from the interaction with a synthetic polyanion [8]. We describe here the first characterization of a porin from an insect cell.

Materials and Methods

Materials. Hydroxyapatite (Bio-Gel HTP) was obtained from Bio-Rad, Triton X-100, acrylamide and

Abbreviations: Mes, 4-morpholineethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane; SDS, sodium dodecylsulfate; HTP, hydroxyapatite; VDAC, voltage-dependent anion channel.

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N,N'-methylenebisacrylamide from Serva. Celite 535 was usually purchased from Roth, *Staphylococcus aureus* V8 proteinase from Miles laboratories and papain from Sigma. Diphytanoylphosphatidylcholine was purchased from Avanti Biochemicals (Birmingham, AL) and all salts used in lipid bilayer experiments were obtained from Merck, Darmstadt, F.R.G. and were analytical grade.

Isolation of Drosophila mitochondria and purification of porin. The mitochondria of *Drosophila* Oregon R wild type at different developmental stages were isolated as in Ref. 9, with the only exception that the first centrifugation at $300 \times g$ was repeated twice. The isolated mitochondria were frozen or immediately used for the protein purification.

The purification of the *Drosophila* porin was performed essentially as in Ref. 10. The mitochondria were lysed by osmotic shock; after centrifugation the pellet, mainly composed of mitochondrial membranes, was solubilized by 3% Triton X-100, 10 mM Tris-HCl (pH 7.0) and 1 mM EDTA at a final concentration of 10 mg protein/ml. After 30 min at 0°C, the solubilization mixture was centrifuged at $40000 \times g$ for 15 min and the supernatant (0.6 ml) loaded onto a dry hydroxyapatite/celite column (0.6 g, ratio 2:1). The elution was performed with the solubilization buffer. The first 0.6 ml eluted were collected.

SDS gel electrophoresis. Polyacrylamide slab gel electrophoresis of acetone-precipitated samples was performed in the presence of 0.1% SDS. The separation gel contained 17.5% acrylamide with a ratio acrylamide/bisacrylamide of 30:0.2.

Peptide mapping experiments. Peptide mapping experiments were performed on slices of SDS gel stained with Coomassie blue as in Ref. 11. The protein bands corresponding to porins were cut out and equilibrated with 0.125 M Tris-HCl (pH 6.8), 10% glycerol, 0.3% 2-mercaptoethanol, 1 mM EDTA and 0.1% SDS. They were then pushed to the bottom of the wells of a 17% acrylamide (acrylamide/bisacrylamide ratio 30:0.8) gel and covered with the same buffer containing 20% glycerol, on top of which the solution of proteinase was overlayered. To allow porin digestion by proteinase, the electrophoretic run was very slow in the stacking gel. Furthermore, when the samples had stacked, the power was turned off for 30 min. Finally the rate of migration was increased in the separating gel.

Immunoblotting experiments. Antiserum against bovine heart porin was obtained from rabbit. After a first injection of 200 μg of purified porin mixed with Freund's complete adjuvant, two more portions of 200 μg of porin were injected after three and then two weeks. Blood was taken from rabbit ear. The proteins separated on a 12% SDS-PAGE were transferred to nitrocellulose, incubated with the antiserum and then incubated with an anti-rabbit Ig horseradish peroxidase linked antibody (purchased from Amersham). The peroxidase reaction was performed by 20 ml of a mixture of 0.05% 4-chloro-1-naphthol, 16% methanol, 0.5% BSA in 0.14 M NaCl, 0.01 M phosphate (pH 7.0) with the final addition of 12 μl of 30% H_2O_2.

Lipid bilayer experiments. The methods used for the black lipid bilayer experiments were described previously [12]. The membranes were formed across circular holes (surface area about 0.1 mm^2 in the case of the single-channel experiments or 1 mm^2 for the macroscopic conductance and the selectivity measurements) in the thin wall of a Teflon cell separating two aqueous compartments. The membranes were formed by painting onto the holes a 1% (w/v) solution of diphytanoylphosphatidylcholine dissolved in *n*-decane. Bilayer formation was indicated when the membrane turned optically black in reflected light. Polyanion (a copolymer of methacrylate, maleate and styrene in 1:2:3 proportion [8]) was a kind gift from Dr. L. Wojtczak. Small amounts of a concentrated solution were added to the aqueous salt solutions to get final concentrations between 0.3 and 10 μg/ml. The aqueous solutions were used unbuffered and had either a pH around 6 or as indicated.

The current through the membranes was measured with two calomel electrodes connected in series with a voltage source and a current amplifier. The amplified signal was monitored with a storage oscilloscope and recorded on a strip chart or a tape recorder. For the macroscopic conductance measurements the current amplifier was replaced by a Keithley electrometer (model 610 C). The zero-current membrane potentials were measured using a Keithley 610 C electrometer as the result of a 5-fold salt gradient (the more dilute side was 10 mM) across a membrane in which between 10^2 and 10^4 pores were reconstituted [13]. The membrane potential reached a steady-state within 5 to 10 min after the addition of the concentrated salt solution to one side of the membrane.

Other methods. Mitochondrial protein was measured by the biuret method, using KCN to account for turbidity due to phospholipids [14]. Purified protein was measured by the Lowry method modified for the presence of detergent [15].

Results

Purification and biochemical characterization of Drosophila melanogaster porin

We have recently published a rapid method for the purification of mammalian porins [10]. A single chromatographic step on hydroxyapatite/celite is required, after mitochondrial solubilization by the non-ionic detergent Triton X-100. The application of the same conditions to mitochondrial membranes obtained from the insect *Drosophila melanogaster* at different developmen-

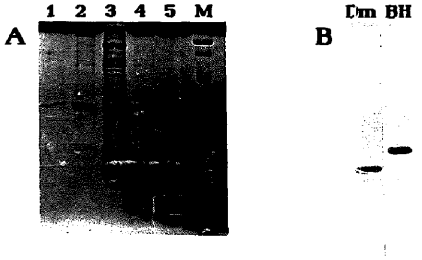

Fig. 1. Purification of *Drosophila melanogaster* porin. (A) 17.5% SDS gel electrophoresis of *D. melanogaster* mitochondria solubilized with Triton X-100 and chromatographed on HTP/celite columns at different developmental stages. 1, embryos; 2, I and II instar larvae; 3, III instar larvae; 4, early pupae; 5, adults. M, Bio-Rad molecular mass markers (from the top to the bottom: 92.5 kDa, 66.2 kDa, 45 kDa, 30 kDa, 21.5 kDa, 14.4 kDa). (B) 12% SDS gel electrophoresis of Dm, *D. melanogaster* adults mitochondrial porin, used in the characterization experiments, and of BH, bovine heart mitochondrial porin. Coomassie blue staining.

tal stages gave the results shown in Fig. 1A. With all the mitochondria tested a single protein band, or a major one contaminated with few other protein was obtained. The apparent molecular mass was determined to be 31 000 daltons on standard SDS gel electrophoresis by molecular weight markers (Fig. 1). The pure or the major band had the same electrophoretic mobility for all the developmental stages used. Pure protein preparations were tested for pore-forming activity (see below). Therefore the 31 kDa protein has been identified as the *Drosophila melanogaster* mitochondrial porin. Embryos, early pupae and adults mitochondria resulted in the most pure porin preparations. The yield of their purification was between 0.5 and 1% of total mitochondrial protein. Since it is easier to collect adults than insects at other developmental stages, all the characterization experiments described in this paper were performed with porin purified from adults' mitochondria (Fig. 1B).

The peptide maps of the *D. melanogaster* 31 kDa polypeptide and, for comparison, of bovine heart porin, were obtained by fragmenting the protein contained in gel slices by the proteinases V8 from *S. aureus* and papain. Fig. 2A shows the peptide maps obtained by incubating in parallel bovine heart (BH) and *D. melanogaster* (Dm) porins with proteinase V8. The proteolytic patterns are completely different, indicating important differences in the structure of the two porins. The porin from *D. melanogaster* was mostly uncleaved by the proteinase. In contrast the peptide map of bovine heart porin was similar to that described elsewhere (see Ref. 5). Fig. 2B shows a similar experiment performed in the presence of papain. Also in this case the differences in the proteolytic patterns are very clear: the cleavage of bovine heart porin gives a cloudy pattern in the low molecular weight region, while the *D. melanogaster* porin is again much less sensitive to the action of the proteinase. The peptide maps of bovine heart and *D. melanogaster* porin do not show any meaningful similarity.

Immunological experiments

We have performed immunological analysis of *Drosophila melanogaster* porin by means of several antisera raised against porin purified from different organisms. Fig. 3A shows the result of an immunoblot with an antiserum against yeast porin (a kind gift from M. Dihanic). The same amount of *D. melanogaster* porin was blotted in several nitrocellulose lanes and the antiserum at different dilution used. *D. melanogaster* porin strongly reacts with the anti-yeast porin antiserum. The reaction is still clearly visible at an antiserum dilution of 1 : 800. A similar experiment was performed with an antiserum raised against the bovine heart porin (Fig. 3B). The cross-reactivity is very slight: only at an antiserum dilution of 1 : 100 a faint staining reaction can be observed. Surprisingly there is no cross-reactivity of the 31 kDa *D. melanogaster* protein with an antiserum raised against *Neurospora crassa* porin (a kind gift from Dr. Ralf Kleene).

Incorporation of the Drosophila porin into lipid bilayer membranes

When the porin was added in small quantities (10 to 100 ng/ml) to one or both aqueous solutions bathing a

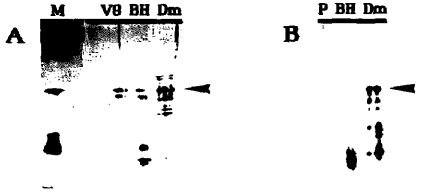

Fig. 2. Peptide maps of *Drosophila melanogaster* and mammalian porin obtained by incubating gel slices corresponding to purified porins with different proteinases. (Panel A) Gel slices containing 2–3 μg of porin purified from bovine heart or *D. melanogaster* were re-run in the presence of 1 μg *Staphylococcus aureus* proteinase V8 as described in Methods. M: molecular weight markers (from the top to the bottom: bovine serum albumin, 68000; carbonic anhydrase, 30000; cytochrome c, 12500); BH: peptide map obtained by digestion of purified bovine heart porin; Dm: peptide map obtained by digestion of purified *D. melanogaster* porin; V8: 1 μg of *S. aureus* proteinase V8. Silver staining. (Panel B) The same experiment described above was performed, with the exception that 0.2 μg of papain instead of proteinase V8 were present. BH and Dm: as above; P: 0.2 μg papain. Silver staining. The arrows show the migration of the uncleaved *D. melanogaster* porin.

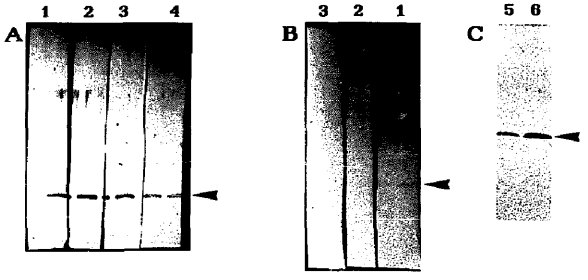

Fig. 3. Immunological analysis of *Drosophila melanogaster* adults' mitochondrial porin. (Panel A) *Drosophila melanogaster* adults porin was transferred from a standard SDS gel to nitrocellulose and the paper immuno-stained as described in Methods by an antiserum raised against yeast porin. 1 μg of *Drosophila* porin was present in each lane of the gel. The dilutions of the antiserum were: 1, 1:100; 2, 1:200; 3, 1:400; 4, 1:800. (Panel B). The same procedure described above was repeated with a second gel. Now the immunological reaction was performed by an antiserum raised against bovine heart porin. 1 μg of *Drosophila* porin was present in each lane of the gel. The dilutions of the antiserum were: 1, 1:100; 2, 1:200; 3, 1:400. (Panel C) Control of the anti-bovine heart porin antiserum activity. 0.5 μg (5) and 1 μg (6) of bovine heart porin were run on a SDS gel and transferred to nitrocellulose. The immunological reactivity was shown by the antiserum raised against bovine heart porin at a dilution of 1:1000. The arrows show the bands corresponding to porin.

lipid bilayer membrane, the specific conductance of the membrane increased by several orders of magnitude. The time course of the conductance increase was similar to that described previously for other mitochondrial porins [16]. The addition of the detergent Triton X-100 alone in a similar concentration to that used in the presence of the protein did not lead to any appreciable increase in the membrane conductance above the specific conductance in the absence of the protein (10^{-7} to 10^{-8} S/cm^2).

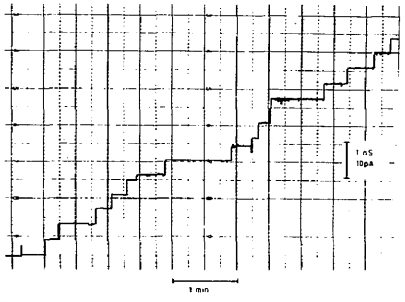

Fig. 4. Stepwise increase of the membrane current after the addition of 2 ng/ml *Drosophila* porin to the aqueous phase bathing a black lipid bilayer membrane. The aqueous phase contained 0.1 M KCl, pH 6. The membrane was formed from 1% diphytanoylphosphatidylcholine/*n*-decane. The applied voltage was 10 mV; $T = 25\,^{\circ}C$.

Single-channel analysis

The addition of smaller amounts of the *Drosophila* porin (1 to 10 ng/ml) to lipid bilayer membranes having small surface areas (0.1 mm^2) allowed the resolution of step increases in conductance (see Fig. 4 for an experiment of this type). Most of the conductance steps were directed upwards and closing steps were only rarely observed at transmembrane potentials of 5 or 10 mV. The most frequent value for the single-channel conductance of the *Drosophila* porin was 0.41 nS in 0.1

TABLE I

Average single-channel conductance of Drosophila porin in different salt solutions of concentration c

The aqueous solutions contained 5 ng/ml *Drosophila* porin and less than 0.1 μg/ml Triton X-100; the pH was between 6.0 and 7.0. The membranes were made from a 1% (w/v) solution of diphytanoylphosphatidylcholine in *n*-decane; $T = 25\,^{\circ}C$; $V_m = 10$ mV. Λ was determined by recording at least 70 conductance steps and averaging over the distribution of the values, σ is the specific conductance of the aqueous salt solutions.

Salt	C (M)	Λ (nS)	Λ/σ (10^{-8} cm)
KCl	0.01	0.05	3.6
	0.03	0.15	3.6
	0.1	0.41	3.7
	0.3	1.24	3.6
	1	4.5	4.1
	3	9.9	3.9
LiCl	1	3.0	4.8
KCH_3COO	1	2.4	3.8
$MgCl_2$	0.5	1.0	4.5

M KCl. At 10 mV transmembrane potential, the closing events represented only a minor fraction of the total number of conductance fluctuations. However, at higher potentials the closing events became more and more frequent. Furthermore, the closing events had in almost all cases a smaller amplitude than the initial on-steps. This result indicated that the *Drosophila* porin pore switched to substates at high voltages. The pore may have different conductance states depending on the magnitude of the applied membrane potential. A similar voltage dependence has been observed with a variety of other mitochondrial porins [5,16].

Estimation of the pore diameter

The channel in the mitochondrial outer membrane of *Drosophila* was permeable for a variety of different ions. Table I shows the single channel conductances in the presence of different salt solutions. Although there existed a considerable influence of the salt on the pore conductance, the ratio Λ/σ varied less than a factor of two, i.e. the ions seemed to move inside the pore in a manner similar to the way they move in an aqueous environment. This finding together with the results obtained below from the selectivity measurements allowed a rough estimate of the effective diameter of the pores. Assuming that the porin pores are filled with a solution of the same specific conductivity σ as the external solution and assuming a cylindric pore with a length l of 6 nm (which is very likely according to electron microscopic studies of the porin pores of *Neurospora crassa* [17,18]), the average pore diameter $d\ (=2r)$ can be obtained according to the equation:

$$\Lambda = \sigma\pi r^2/l \qquad (1)$$

The effective diameter of *Drosophila* pores was estimated from the average single channel-conductance of the pores in 0.1 M KCl ($\Lambda = 0.41$ nS; $\sigma = 13$ mS/cm) to be about 1.7 nm. This was very similar to the diameter of most mitochondrial pores (except for *Paramecium* porin [19]). Such a diameter would allow the permeation of hydrophilic solutes up to molecular weights of about 2500–3000 through the pores.

Properties of the closed state

The single-channel conductance of the closed state of the *Drosophila* porin was investigated in detail to get some insight in its ion selectivity. The experiments were performed in the following way. Membranes were formed in 0.5 M salt solutions composed of cations and anions of different aqueous mobility, and the pH adjusted to 7.2. 30 mV transmembrane potential was applied to the membrane and porin was added in a small concentration (5 ng/ml). The open state had under these conditions only a limited lifetime which means that it was possible to measure the single channel conductance of the closed state by subtracting the conductance of the closing events from those of the open states. It was found that the single-channel conductance in the presence of 0.5 M KCl, the reference solution, was 2.1 nS in the open state and 1.2 nS in the closed state (mean of at least 50 single events). The single-channel conductance was 0.85 nS for K-Mes and 1.4 nS for Tris-HCl in the open state, while the closed state of the pore was for Tris-HCl considerably smaller (0.25 nS) than for K-Mes (0.70 nS), despite a similar aqueous mobility for K^+ and Cl^-. This result suggested that the closed state of the *Drosophila* porin could be cation-selective. Polyanion added to one side of the membrane led to a smaller single-channel conductance (very similar to those described above for the closed state) but only when the sign of the voltage was negative at the cis-side, the side of the addition of the protein. For opposite polarity (positive at the cis-side) the single-channel conductances obtained under these conditions were identical to those of the open state.

Voltage-dependence

The voltage dependence of the *Drosophila* porin was studied as follows. The voltage across a membrane bathed in 1 M KCl and containing a large number of pores was switched from zero to a given voltage, V_m. The initial current, i.e., the current immediately after application of the voltage, was a linear function of the membrane potential. Subsequently, the membrane current showed an exponential decay with a defined time constant. This effect was first noticeable at about 20 mV and the maximum conductance decrease reached was about 50% of the initial conductance, G_0, at a transmembrane potential of about 90 mV. Fig. 5 shows the mean of the ratio G/G_0 (G is the membrane conductance at a given voltage, V_m after the decay was complete) as a function of the applied voltage. The data of Fig. 5 could be explained by the same formalism used earlier for the fit of the voltage-dependence of other mitochondrial porins [5,20]. The ratio of the open to the closed pores, N_o/N_c, showed an exponential dependence on the applied membrane potential. The number of the gating charges involved in the gating process of *Drosophila* porin was approximately 2. Half of the channels were in the closed state at a potential difference of about 45 mV. Similar values have also been found for other mitochondrial porins [5].

A polyanion of M_r 10000 has been shown to act as a potent inhibitor of the mitochondrial pore [21,22]. To study if this polyanion has a similar influence on the *Drosophila* porin we added in similar experiments as described above polyanion in a concentration of 1 μg/ml to one side (the cis-side) of the membrane. The results are also shown in Fig. 5. Voltage negative at the cis-side resulted in a strong decay of the membrane conductance, whereas voltages with opposite polarity did no

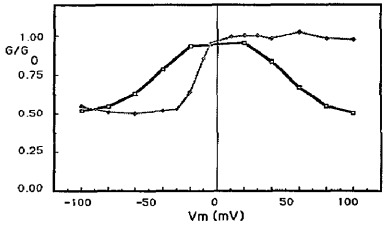

Fig. 5. Ratio of the conductance G at a given membrane potential divided by the conductance G_0 at zero potential as a function of the applied membrane potential V_m with respect to the cis-side. The membranes were formed of diphytanoylphosphatidylcholine/*n*-decane. The aqueous phase contained 1 M KCl. The cis-side contained 20 ng/ml porin (plain line) and 20 ng/ml porin and 1 μg/ml polyanion (dashed line). Note that the conductance decreased in the presence of the polyanion only when the cis-side was negative. Means of at least three individual experiments are shown; $T = 25\,^{\circ}C$.

longer influence the pore conductance. Even voltages up to +100 mV (with respect of the cis-side) were not able to close the pores.

Zero-current membrane potentials

Further information about the structure of the pore formed by *Drosophila* porin may be obtained from zero-current membrane potential measurements in presence of salt gradients. A 5-fold KCl gradient, across a lipid bilayer membrane in which porin was incorporated, resulted in an asymmetry potential of about -8 mV (negative at the more diluted side). This result indicated some preferential movement of anions over cations through the pore at neutral pH. The zero-current membrane potentials were analysed using the Goldman-Hodgkin-Katz equation [13]. The ratio of the anion permeability P_a divided by the cation permeability P_c was about 1.6, suggesting a small anion selectivity of the mitochondrial porin from *Drosophila* in the case of the equally mobile potassium and chloride ions. The use of LiCl in similar experiments resulted in an even higher anion selectivity and P_a/P_c was calculated to be 2.6 from an asymmetry potential of −16 mV at the diluted side of a 5-fold LiCl gradient. To test whether the anion had a similar influence on the zero-current potential we performed also measurements in the presence of potassium acetate. In these experiments the asymmetry potential became positive at the more diluted side. We calculated a permeability ratio $P_a/P_c = 0.6$ from an asymmetry potential of 9 mV observed for a 5-fold potassium acetate gradient. This result represented another support for the function of the *Drosophila* porin as general diffusion pore in the open state.

Discussion

We have purified the 31 kDa major protein component of the mitochondrial outer membrane of *Drosophila*. This protein was isolated using a previously proposed simple isolation procedure [10]. This method is also applicable to *Drosophila* and the porin had an apparent molecular mass of 31 kDa on SDS electrophoretograms in all the developmental stages tested (Fig. 1), indicating that porin is not subjected to major modification in the development. The molecular mass of 31 kDa is very close to those of mitochondrial porins from other eukaryotic cells which range from 30 to 35.5 kDa [5,19]. The peptide mapping of the *Drosophila* porin showed marked differences in the structure with respect to mammalian porins. In fact, *Drosophila* porin had only little immunological cross-reactivity with antibodies raised against bovine heart porin. There was also no cross-reaction with antibodies raised against porin from *Neurospora crassa*. In contrast, the porin from *Drosophila* showed a strong cross-reactivity with an antiserum against yeast porin (Fig. 3). The structural similarity between the *Drosophila* and yeast porin is confirmed by the similar proteolytic pattern produced by the V8 proteinase digestion (cf. Fig. 2A with Fig. 4A of Ref. 5).

The pore-forming properties were, on the other hand, very similar to those of other eukaryotic porins. Reconstitution at very low protein concentrations in the aqueous phase resulted in an increase of the membrane conductance in distinct steps. The single-channel conductance is 0.41 nS in 0.1 M KCl. With the usual assumptions, the pore diameter has been calculated to be 1.7 nm according to Eqn. 1. Other mitochondrial porins have a similar effective diameter as judged from their single channel conductance in 0.1 M KCl [2,5]. Only porin of *Paramecium* mitochondria seems to have a smaller diameter [19].

The mitochondrial porin from *Drosophila* forms voltage-dependent pores in lipid bilayer membranes. This result is in agreement with what was observed earlier for mitochondrial porins from rat liver [16] and *Neurospora crassa* [23,24]. The pore conductance is reduced at higher voltages. This also results in the restriction of the permeability of the pore for larger solutes. In fact, single-channel experiments with *Drosophila* porin at 30 mV showed that the closed state of the pores had a largely reduced single-channel conductance for salts composed of a mobile anion and a less mobile cation (for example Tris-HCl). This means that the pore appears to be cation-selective in the closed state while it is slightly anion-selective in the open state. The permeability of the porin pore from *Drosophila* is also restricted by a polyanion of M_r 10000, copolymer of methacrylate, maleate and styrene, which was shown to inhibit oxidative phosphorylation [8] and mitochondrial car-

riers [25]. This result is consistent with the possibility that the polyanion may act by closing the mitochondrial porin pore for the penetration of ADP and ATP [22].

The overall picture coming from our experiments indicates that the function of the porin from *Drosophila* is preserved though its primary sequence may largely be different from those of other mitochondrial porins.

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