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Purification and Characterization of the Voltage-Dependent Anion Channel from the Outer Mitochondrial Membrane of Yeast

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Summary. The outer mitochondrial membranes of all organisms so far examined contain a protein which forms voltage-dependent anion selective channels (VDAC) when incorporated into planar phospholipid membranes. Previous reports have suggested that the yeast (*Saccharomyces cerevisiae*) outer mitochondrial membrane component responsible for channel formation is a protein of 29,000 daltons which is also the major component of this membrane. In this report, we describe the purification of this 29,000-dalton protein to virtual homogeneity from yeast outer mitochondrial membranes. The purified protein readily incorporates into planar phospholipid membranes to produce ionic channels. Electrophysiological characterization of these channels has demonstrated they have a size, selectivity and voltage dependence similar to VDAC from other organisms. Biochemically, the purified protein has been characterized by determining its amino acid composition and isoelectric point (*pI*). In addition, we have shown that the purified protein, when reconstituted into liposomes, can bind hexokinase in a glucose-6-phosphate dependent manner, as has been shown for VDAC purified from other sources. Since physiological characterization suggests that the functional parameters of this protein have been conserved, antibodies specific to yeast VDAC have been used to assess antigenic conservation among mitochondrial proteins from a wide number of species. These experiments have shown that yeast VDAC antibodies will recognize single mitochondrial proteins from *Drosophila*, *Dictyostelium* and *Neurospora* of the appropriate molecular weight to be VDAC from these organisms. No reaction was seen to any mitochondrial protein from rat liver, rainbow trout, *Paramecium*, or mung bean. In addition, yeast VDAC antibodies will recognize a 50-kDa mol wt protein present in tobacco chloroplasts. These results suggest that there is some antigenic as well as functional conservation among different VDACs.

Key Words ion channels · voltage sensitivity · outer mitochondrial membrane

Introduction

Voltage-dependent ion channels are integral membrane proteins responsible for the generation of membrane excitability and thus such phenomena as nerve excitation, nerve conduction and muscle excitation. Despite intense biochemical and physiological characterization over the last 30 years, our understanding of these channels remains very incomplete. In particular, a molecular description of the distinguishing property of this class of channels, voltage gating of ion flow, has remained particularly elusive. How exactly is the voltage stimulus sensed and how does this cause the channels to open or close? Increasingly elaborate kinetic descriptions of gating have been developed, yet *no* biochemical correlates exist. An understanding of gating in molecular terms can only come as molecular structures of ion channels are elucidated and tested.

A unique and attractive system in which to study the basic molecular characteristics of voltage gating is provided by the voltage-dependent anion channel (VDAC) present in the outer mitochondrial membrane of all organisms. VDAC was first identified in hexane extracts of *Paramecium* and *Paramecium* mitochondrial (Schein, Colombini & Finkelstein, 1976). Subsequently it has been found in the outer mitochondrial membranes from all sources examined to date (e.g., Colombini, 1979; Colombini, 1980; Zalman, Nikaido & Kagawa, 1980; Freitag, Neupert & Benz, 1982; Linden, Gellerfors & Nelson, 1982*b*; Roos, Benz & Brdiczka, 1982). The biophysical properties of VDAC have been studied after insertion into planar phospholipid bilayers. Its characteristic single-channel conductance, ion selectivity and the voltage dependence distinguish VDAC from other transport proteins and channels.

In this report, we describe the purification to virtual homogeneity of VDAC from the outer mitochondrial membrane of the yeast *Saccharomyces cerevisiae*. The protein has a molecular weight of 29,000 daltons and is a major component of the yeast outer mitochondrial membrane. The single-channel conductance, ion selectivity and voltage dependence of the purified protein have been analyzed following incorporation into planar lipid bilay-

ers. Biochemically, the amino acid composition and pI of the protein have been determined. In addition, we have determined that the purified protein can bind hexokinase in a glucose-6-phosphate-dependent manner when reconstituted into liposomes. We also show that antibodies specific for yeast VDAC will also cross-react with specific mitochondrial proteins from *Neurospora*, *Dictyostelium*, and *Drosophila* and a 50-kDa mol wt protein found in tobacco chloroplasts.

Materials and Methods

Isolation of Mitochondrial and Outer Mitochondrial Membranes

Mitochondria were routinely isolated from 4 lbs. of commercial yeast cakes (Red Star) essentially by the method of Trembath and Tzagoloff (1979). Briefly, yeast cells, frozen in liquid N_2, are disrupted on a large scale by homogenization in a Waring blender. The homogenate is then centrifuged at low speed (2000 $\times$ g, 30 min) to remove unbroken cells and large particles. The supernatant is centrifuged at 17,000 $\times$ g for 25 min to pellet the mitochondria. Mitochondria are then washed three times by resuspension in buffer and recentrifugation. Outer mitochondrial membranes are purified by osmotic shock of the mitochondria and sucrose density gradient centrifugation essentially as described by Mihara, Blobel and Sato (1982).

Isolation of VDAC from Yeast Outer Mitochondrial Membranes

The procedures used for the isolation of yeast VDAC were modifications of the protocols described by Freitag, Benz and Neuert (1983) for the isolation of VDAC from *Neurospora* mitochondrial membranes. Outer mitochondrial membranes prepared as described above were mixed with an equal volume of 20 mM Tris-HCl at pH 7.5, 60 mM octylglucopyranoside and shaken for 45 min at 4°C. This mixture was then centrifuged at 150,000 $\times$ g for 30 min in a Spinco SW50.1 rotor. The resulting pellet was resuspended in 20 mM Tris-HCl at pH 7.5, 2% Triton X-100, shaken for 45 min at 4°C and recentrifuged as described above. The supernatant was immediately applied to a 10-ml DEAE-Sephadex column equilibrated in 20 mM Tris-HCl at pH 7.5, 0.1% Triton X-100 at room temperature. Essentially pure VDAC eluted with the void volume.

Production of Antibodies to Yeast VDAC

Antibodies to yeast VDAC were produced by injecting 200 μg of purified yeast VDAC denatured with 6 M urea and 1% SDS into a New Zealand White rabbit. One month after the first injection, a second, similar, injection was administered followed by a third injection two weeks later. Seven days after the third injection, the rabbits were bled and decomplemented serum prepared. From this serum, a population of affinity-purified antibodies has been prepared. Approximately 5 mg of pure yeast VDAC was coupled to CNBr activated-Sepharose 4B in the presence of 1% SDS. Serum from immunized rabbits was then passed over the affinity column and washed extensively to remove nonspecific antibodies. Antibodies specific for VDAC were then eluted from the affinity column with 4 M Mg/Cl_2, followed by dialysis and concentration of the antibodies by vacuum filtration.

Biochemical Analysis

Isoelectric focusing gels were prepared and run essentially as described by O'Farrell (1975). Protein amino acid compositions were determined using a Waters Pico-Tag system as described by the manufacturer. The ability of purified VDAC to bind hexokinase was assayed essentially as described by Felgner, Messer and Wilson (1979).

Mitochondria for Western Blots were prepared from cell homogenates by differential centrifugation and banding to equilibrium on sucrose gradients. Chloroplasts were prepared from tobacco leaf homogenates by differential centrifugation and sucrose gradient sedimentation.

SDS polyacrylamide gel electrophoresis was performed by the method of Laemmli (1970). Proteins for Western blots were transferred to nitrocellulose electrophoretically and developed by the method of Glass, Briggs and Hnilica (1981). Protein concentrations were determined by the method of Lowry as modified by Markwell (1978) using bovine serum albumin as a standard.

Physiological Characterization of Yeast VDAC

Planar phospholipid bilayer membranes were generated as described previously (Schein et al., 1976) following the method of Montal and Mueller (1972). The aqueous phase of the *cis* side (i.e., the side to which the sample was to be added) was 1.0 M KCl + 5 mM $CaCl_2$ while that on the *trans* side was 0.1 M KCl + 5 mM $CaCl_2$. The transmembrane voltage was clamped in such a way that the voltage on the *cis* side was controlled with respect to the *trans* side (the *trans* side was virtual ground). The diameter of the membrane was approximately 0.15 mm.

The fraction to be tested was supplemented with Triton X 100 to a final concentration of 1% (vol/vol). Ten microliters of this solution were added to the *cis* side under constant stirring. The volume of the aqueous phase on the *cis* side was approximately 4 ml. Channel insertion was usually monitored in the absence of an external electric field. The number of channels inserting per unit time was measured by actually counting channels (for samples with low activity) or by dividing the observed current by the single-channel current for high activity samples.

Results

Purification of VDAC from the Outer Mitochondrial Membrane of *Saccharomyces cerevisiae*

VDAC from mung beans (Zalman et al., 1980), *Neurospora* (Freitag et al., 1982) and rat liver (Linden et al., 1982*a*; Roos et al., 1982; Colombini, 1983) has

been identified as a 31,000 mol wt protein of the outer mitochondrial membrane. In contrast, the major constituent of the outer mitochondrial membrane of yeast is a 29,000 mol wt (29K) protein (e.g., Fig. 1) which, on preliminary investigation, was tentatively designated as the yeast outer mitochondrial membrane porin (Mihara et al., 1982). Extending these observations, we have developed procedures for the large-scale purification of VDAC to homogeneity (Fig. 1) from purified yeast outer mitochondrial membranes. The procedures used are basically modifications of the method of Freitag, Benz and Neupert (1983). Briefly, commercial baker's yeast, *Saccharomyces cerevisiae*, frozen in liquid N_2, are disrupted on a large scale by homogenization in a Waring Blender® (Trembath & Tzagoloff, 1979). Mitochondria are then prepared by differential centrifugation, followed by purification of outer mitochondrial membranes by osmotic shock of the mitochondria and sucrose gradient centrifugation. As shown in Fig. 1, such outer membrane preparations are enriched for a 29K protein, which has tentatively been identified as yeast VDAC by Mihara et al. (1982). The 29K protein can be isolated in essentially pure form from these membranes by differential detergent extraction. The membranes are first resuspended in octylglucoside, which solubilizes most of the outer membrane proteins. The octylglucoside insoluble material, which contains the majority of the 29K protein (Fig. 1), is then resuspended in Triton X 100, releasing the 29K protein from the octylglucoside insoluble material. Minor contaminants are then removed by passing the Triton soluble material over a DEAE Sephadex column, the flow-through fraction consisting of essentially pure 29K protein (Fig. 1). By these procedures, we are able to isolate relatively large quantities of the 29K protein. Starting with 4 lbs. of commercial yeast we are able to isolate up to 2 mg of 29K protein representing about 10% of the total protein in the outer membrane. The degree of purification achieved by these procedures is difficult to access given the difficulty and variability of the bilayer assay. However, the quantities of protein recovered from yeast mitochondria in our hands are consistent with the observation that VDAC represents 0.2 to 0.4% of the total mitochondria protein, as is the case in both *Neurospora* (Freitag et al., 1982) and rat liver (Roos et al., 1982).

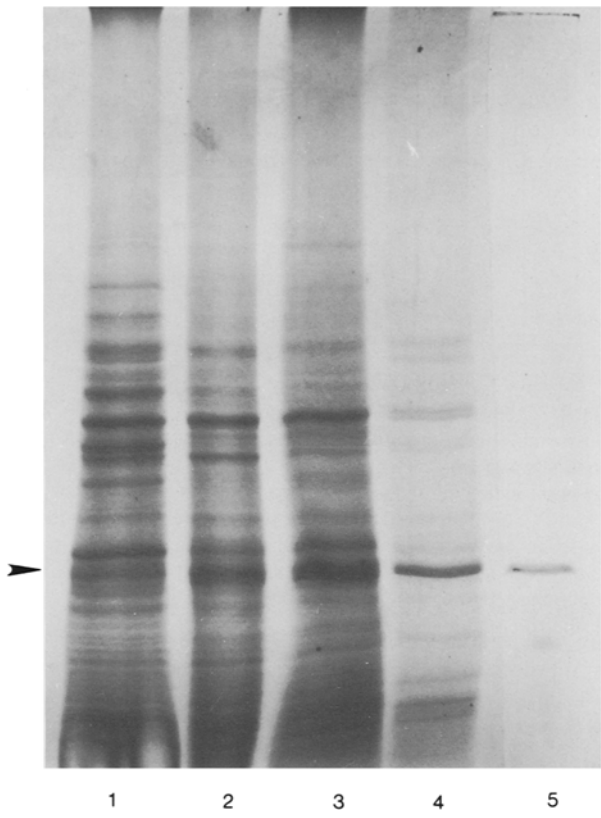

Fig. 1. SDS-polyacrylamide gel of the various fractions obtained during the purification of VDAC from yeast outer mitochondrial membranes. (*1*) Whole yeast mitochondria; (*2*) outer mitochondrial membranes; (*3*) octylglucoside pellet; (*4*) Triton X-100 supernatant; (*5*) DEAE flow-through fraction. Gel is 7.5–15% gradient of acrylamide. Lanes *1–4* were stained with Coomassie blue. Lane 5 was stained with silver. Arrow indicates the position of a 29,000 mol wt protein as determined from the migration in this gel of molecular weight markers

Electrophysiological Properties of the Pure 29-kDa Protein When Inserted into Phospholipid Bilayers

The identification of the 29K outer mitochondrial membrane protein as the functional equivalent of yeast VDAC was determined after the insertion of the purified protein into a planar phospholipid membrane. The monolayer method of Montal and Mueller (as described in Schein et al., 1976) was used to generate phospholipid membranes using soybean phospholipids. When purified 29K protein was added to one side of the membrane, channels spontaneously inserted into the membrane. The membrane's permeability to ions was monitored under voltage-clamp conditions as previously described (Schein et al., 1976). The ionic current flowing across the membrane, in response to an applied voltage, was used to measure the ability of the membrane to conduct ions. Prior to 29K addition, the membrane's permeability to ions was extremely low (a conductance of about 10 pS). The insertion of channels into the membrane by the addition of 29K protein was followed by observing discrete increases in current in the presence of a 9.6-mV trans-

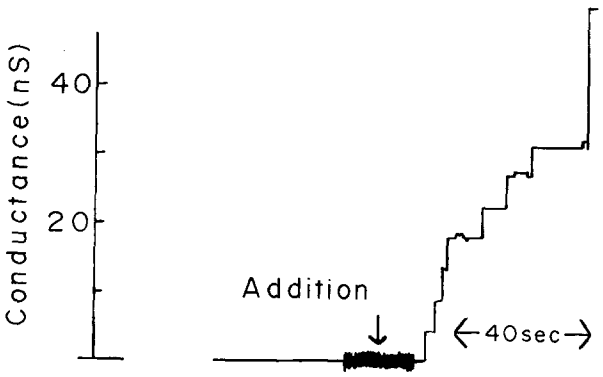

Fig. 2. The insertion of VDAC channels into a planar phospholipid membrane. The membrane was made using soybean phospholipids and 1.0 M KCl, 5 mM $CaCl_2$ for the aqueous phase. At the point indicated by the noisy record (stirring artifact), 10 μl of Triton X-100 solubilized yeast VDAC was added to the aqueous phase on one side of the membrane. The membrane was constantly voltage clamped at −9.6 mV (negative on the side to which the VDAC was added). The current increments were converted to conductance increments

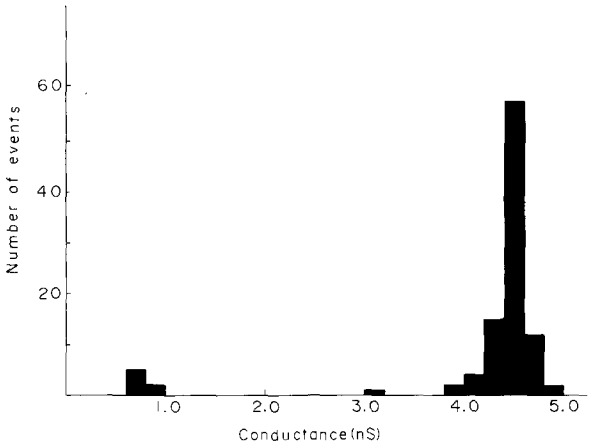

Fig. 3. A histogram of the conductance increments observed when yeast VDAC inserts into the phospholipid membranes. The data obtained from several experiments, such as that shown in Fig. 2, were used to generate this histogram. All clearly discernable conductance increments have been included in the histogram except those that were multiples of the single-channel conductance

membrane potential. The current increments were converted to conductance and a tracing is shown in Fig. 2. These increases in membrane conductance were rather uniform in magnitude (4.5 nS) as shown by their histogram (Fig. 3).

The ion selectivity of the channels was determined by examing the current flow through the channels in the presence of a salt gradient (1.0 M KCl, 5 mM $CaCl_2$ *vs.* 0.1 M KCl, 5 mM $CaCl_2$). From the potential needed to bring the current to zero and the Goldman-Hodgkin-Katz equation, the selectivity of the channels for Cl^- over K^+ was calculated to be 1.8 (Table 1).

Table 1. Ion selectivity of yeast VDAC

Medium[a]	Activity ratio	Reversal[b] potential	Cl^- Permeability/ K^+ Permeability (constant field approximation)
cis 1 M KCl *trans* 0.1 M KCl	6.7	11.0 ± 0.2(6)	1.8

[a] Both solutions also contained 5 mM $CaCl_2$.
[b] Mean ± SD ($\frac{1}{2}$ of experiments).

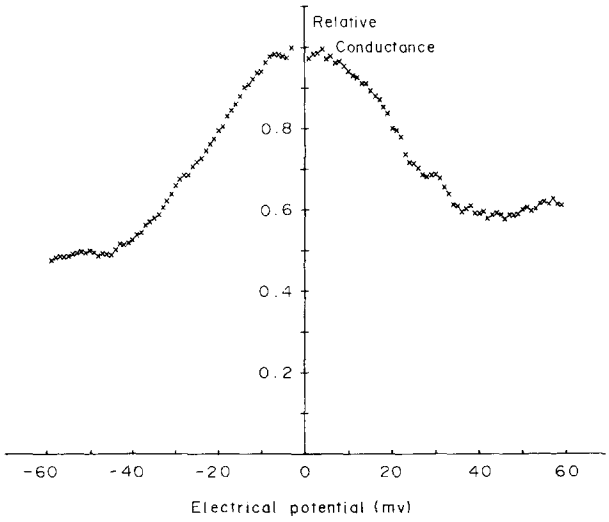

Fig. 4. The voltage dependence of yeast VDAC. The experimental conditions were similar to those described in Fig. 2 except that many channels had been allowed to insert into the membrane. The applied voltage was varied continuously (0.52 mV per sec) in the form of a triangular wave. The current flow across the membrane was recorded and converted into conductance. Only recordings in which the voltage was decreasing with time were used to generate this figure

The voltage dependence of yeast VDAC was investigated by varying the transmembrane voltage continuously with time and monitoring the ionic current. From these measurements, the conductance of the 29K protein-containing membrane was calculated as the applied voltage was changed (Fig. 4). The highest conductance was observed at zero potential, and the conductance decreased as the membrane potential was increased either in the positive or negative direction. The steepness of the voltage dependence is a measure of how sensitive

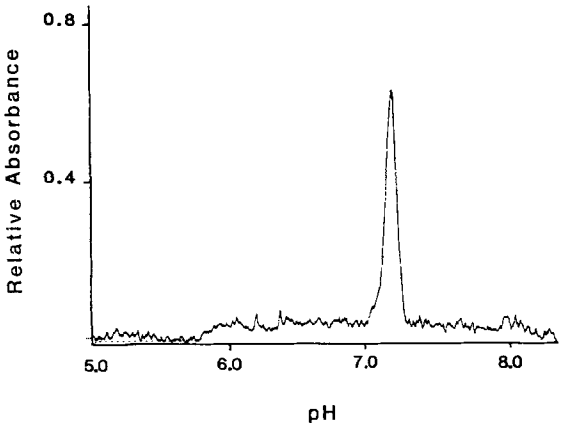

Fig. 5. Isoelectric focusing gel of purified yeast VDAC. Sample (1 μg) was prepared, run, stained with Coomassie Blue and scanned as described in Materials and Methods

the channel is to changes in the applied electrical potential. This steepness is expressed in terms of a parameter, n, which is derived based on a two-state model, each channel existing in either an open or a closed state (as in Schein et al., 1976). n is a measure of the number of gating charges needed on the protein to account for the voltage dependence if the charges were able to respond to the entire applied potential. Therefore, the larger the value of n, the steeper the voltage dependence. For yeast VDAC, an n of 4.4 was calculated for positive potentials and 3.9 for negative potentials. The location of the switching regions on the voltage axis was estimated by determining the voltages at which half the channels were open (V_o). These were found to be +21 and −24 mV.

Biochemical Characterization of Yeast VDAC

The outer mitochondrial membrane 29K protein isolated from yeast has a single-channel conductance, voltage sensitivity and ion selectivity similar to VDAC examined in other systems. Based on this functional characterization the 29K protein is assumed to be yeast VDAC. Three additional biochemical characteristics of purified yeast VDAC have been determined.

1. Protein pI

The pI of yeast was determined by standard isoelectric focusing gel electrophoresis in the presence of Triton X100. As shown in Fig. 5, yeast VDAC runs as a single band at pI = 7.35.

Table 2. Amino acid composition of purified yeast VDAC

Amino acid	Yeast	Neurospora	Rat liver	AS-30D
Polar				
LYS	7.3	10.5	9.4	7.7
HIS	1.7	3.9	1.4	1.1
ARG	3.0	3.5	2.3	4.8
ASX	8.6	12.4	11.6	6.4
THR	9.2	9.6	9.0	9.1
SER	7.7	6.4	5.8	6.9
GLX	8.0	3.7	8.3	8.6
Total polar	45.5	50.0	47.8	44.6
Nonpolar				
PRO	5.2	2.8	2.5	3.6
GLY	7.2	5.0	11.7	8.9
ALA	5.8	9.0	7.4	8.0
CYS	N.D.	*	*	N.D.
VAL	6.9	7.2	5.9	6.6
MET	1.3	2.5	0.6	N.D.
ILE	4.4	4.3	3.7	4.8
LEU	13.2	6.5	10.0	13.2
TYR	2.6	5.2	4.4	4.0
PHE	7.3	9.3	6.2	6.4
TRP	N.D.	*	*	N.D.
Total nonpolar	53.9	51.8	52.4	55.5

Composition in all cases is listed as mole %. Values for *Neurospora* (Freitag et al., 1982), rat liver (Linden et al., 1982*a*) and AS-30D cells (Nakashima et al., 1986) are shown for comparison. Calculation of the polarity index was according to Capaldi and Vanderkooi (1972). Gln and Asn are converted to Glu and Asp during processing of yeast protein samples for amino acid analysis. N.D., not detected; *, not determined.

2. Amino Acid Composition

The amino acid composition of yeast VDAC has been obtained following acid hydrolysis of the purified protein and analysis using a Waters Pico-Tag HPLC system. The results shown in Table 2 are expressed as mole %. The results indicate that yeast VDAC has a high concentration (45.5%) of polar amino acids. Also shown in Table 2 are the amino acid compositions of VDAC from *Neurospora*, rat liver and AS-30D cells for comparison. The amino acid composition of the purified protein is very close to the amino acid composition derived from the nucleotide sequence of the cloned yeast VDAC gene (Mihara & Sato, 1985; Forte, Guy Mannella, 1987).

3. Hexokinase Binding

Another property of VDAC, at least in rat liver mitochondria and AS-30D cells, is its ability to bind hexokinase in a glucose-6-phosphate dependent

Table 3. Binding of hexokinase to outer mitochondrial and to asolectin vesicles containing yeast VDAC

	Outer mitochondrial membrane	Asolectin vesicles	
		With VDAC	Without VDAC
10 mM $MgCl_2$	7.0	136	0.00
10 mM $MgCl_2$, 10 mM glucose-6-phosphate	1.3	1.2	0.00
Ratio −/+ glucose-6-phosphate	5.38	113	

Yeast hexokinase was purchased from Boehringer Mannheim. Values represent mU hexokinase bound/mg protein. Bound hexokinase was determined following centrifugation at 100,000 × *g* for 60 min. Glucose-6-phosphate sensitivity and hexokinase were determined exactly as described by Felgner et al. (1979).

manner (Fiek et al., 1982; Linden et al., 1982*b*; Nakashima et al., 1986). The ability of purified yeast VDAC to bind yeast hexokinase was assessed after the protein had been reincorporated into asolectin vesicles. Reincorporation and vesicle formation was achieved using Bio-Beads SM-2 to remove the Triton in the presence of excess asolectin. Under these conditions, at least 80% of the yeast VDAC becomes associated with the vesicles. The binding of hexokinase to these vesicles was then assayed essentially as described by Felgner et al. (1979). As shown in Table 3, when reincorporated into lipid vesicles, yeast VDAC is able to bind hexokinase in a glucose-6-phosphate sensitive manner.

Antibodies to Yeast VDAC

Antibodies to yeast VDAC have been elicited and affinity purified as described in Materials and Methods. As shown in Fig. 6*A*, such affinity-purified antibodies will specifically recognize VDAC as assayed by standard Western blots of whole yeast cell protein and purified yeast VDAC. Figure 6*A* also shows that control blots using pre-immune serum or antisera which had been absorbed with purified yeast VDAC showed no reaction with any proteins in similar samples (*data not shown*). These antibodies have been used to assess whether the functional conservation of the biophysical properties of VDAC from different species (Benz, 1985) is reflected in a conservation of VDAC epitopes. Affinity-purified yeast VDAC antibodies were tested for their ability to cross-react with mitochondrial proteins from a wide variety of species. As shown in Fig. 5*B*, the yeast VDAC antibodies show signifi-

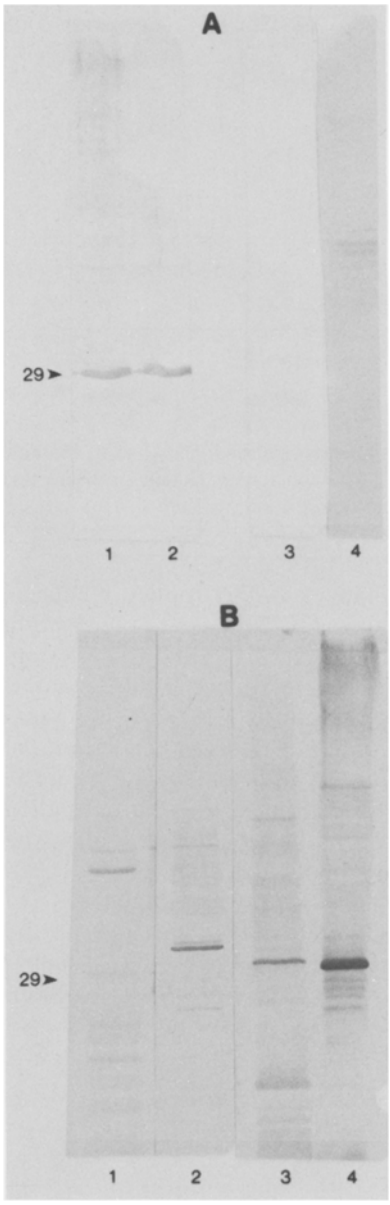

Fig. 6. (*A*) Western blots of total yeast cell protein (lane *1*) and purified yeast VDAC (lane *2*) probed with affinity-purified antibodies generated to yeast VDAC. Control incubations of total yeast cell protein with preimmune serum (lane *3*) and affinity purified antibodies to which excess purified yeast VDAC had been added (lane *4*) are also shown. (*B*) Western blots of whole tabacco chloroplast (lane *1*), *Drosophila* (lane *2*), *Dictyostelium* (lane *3*) and *Neurospora* (lane *4*), mitochondria probes with affinity-purified antibodies generated to yeast VDAC. Control blots probed with preimmune serum or antibodies to which excess purified yeast VDAC had been added back showed no staining. In *A* and *B*, proteins were separated on 7.5–15% SDS polyacrylamide gels and transferred electrophoretically to nitocellulose paper. Approximately 50 μg of whole organelle and 0.5 μg of purified yeast VDAC were run in each case. Arrow indicates the position of a 29,000 mol wt protein as determined by the migration of molecular weight standards

cant cross-reactivity on Western blots with a single mitochondrial protein from *Neurospora*, *Dictyostelium*, and *Drosophila* of a molecular weight expected for VDAC from these organisms. In addition, the antibodies recognize a protein (50K) present in tobacco chloroplasts. No significant cross-reactivity was observed with *Paramecium*, mung bean, rainbow trout, walleyed pike, or rat liver mitochondrial proteins (*data not shown*).

Discussion

In this report, we describe the purification of the anion VDAC present in the outer mitochondrial membrane of the yeast, *Saccaromyces cerevisae*. The protein was purified by assuming initially that, as is the case in another fungus, the major low molecular weight protein of the outer mitochondrial membrane is VDAC. In yeast the major outer mitochondrial membrane protein has a molecular weight of 29K. This protein, when purified by procedures similar to those used for VDAC from rat liver and *Neurospora*, will form channels on introduction into planar lipid bilayers. The single-channel conductance (4.5 nS), ion selectivity and voltage sensitivity are similar to those observed for both *Neurospora* and rat liver. Biochemically, the protein is smaller than that from *Neurospora* (30K) and rat liver (32K) but is composed of roughly the same percentage of polar amino acids as VDAC from these organisms. Such a relatively polar amino acid composition is surprising in light of the apparent hydrophobicity of VDAC as evidenced by the inaccessibility of the protein to proteases in situ and in detergent extracts (Freitag et al., 1982; Gasser & Schatz, 1983) and to antibodies (Mannella & Colombini, 1984) in situ. As has been shown for rat liver and AS-30D cells (Fiek et al., 1982; Linden et al., 1982*b*; Nakashima et al., 1986), VDAC from yeast can bind hexokinase in a glucose-6-phosphate dependent manner. On isoelectric focusing gels, however, a single form with a *pI* of 7.35 is observed in contrast to *Neurospora* and rat liver, where multiple isoelectric forms of the protein are found in the PI range of 7.2 to 7.9.

Since the biophysical properties of VDAC are essentially the same in all organisms examined to date (Benz, 1985), we have used a population of affinity-purified antibodies raised to yeast VDAC to investigate whether this functional conservation may also be reflected in a conservation of antigenic sites. When purified mitochondria from a wide variety of organisms are tested on Western blots, single proteins from the mitochondria of *Neurospora*, *Dictyostellium* and *Drosophila* are observed to cross-react. These proteins have molecular weights consistent with that expected for VDAC from these organisms. Notably, a higher molecular weight protein in chloroplasts also cross-reacts strongly with the yeast VDAC antibodies. This may represent cross-reaction with a pore-forming component recently found in the outer chloroplast membrane which forms the largest general diffusion pathway (7 nS) observed so far in the outer membrane of gram-negative bacteria and cell organelles (Flugge & Benz, 1984). No cross-reactivity at this level was observed with mitochondrial proteins from rat liver, rainbow trout, *Paramecium* or mung bean. The lack of cross-reactivity with *Paramecium* VDAC protein is surprising since we have recently been able to use the cloned yeast VDAC gene as a nucleic acid probe to isolate the *Paramecium* VDAC gene, suggesting significant homology at the nucleic acid and presumably the amino acid level (M. Forte, *unpublished observations*).

Recently, we have been able to use the high specificity antibodies described in this report to isolate both *c*DNA and genomic clones encoding the VDAC gene in yeast (Forte et al., 1987). Nucleic acid sequencing of one of these clones has established the complete amino acid sequence of the yeast VDAC protein. The analysis of VDAC function can now be extended on a molecular level using the modern molecular genetic tools offered uniquely by the yeast experimental system. Combined with the protein biochemistry and biophysical analysis described here, we hope to generate a powerful multidisciplinary approach to the study of the molecular characteristics of voltage gating of ion flow.

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