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MINI-REVIEW

Purification and Properties of the Voltage-Dependent Anion Channel of the Outer Mitochondrial Membrane

F. Palmieri[1] and V. De Pinto[1]

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Abstract

The methods for the purification of functionally active mitochondrial porin or voltage-dependent anion channel of the outer mitochondrial membrane are critically evaluated. Two rapid and efficient methods are now available. Both make use of a hydroxyapatite/celite column as a single chromatographic step. However, in one method with long polar head-group detergents, porin passes through the column, whereas in the other method, with shorter polar head-group detergents, porin is first bound to the column and then eluted by the addition of salts. On the basis of these results, a model for the arrangement of porin in the detergent–protein micelles is proposed.

Key Words: Porin; VDAC; purification; LDAO; cholesterol; reconstitution.

Introduction

The outer mitochondrial membrane contains pores which allow the free diffusion of low-molecular-weight solutes but not of larger molecules (Pfaff and Klingenberg, 1968). The presence of a protein, called mitochondrial porin or voltage-dependent anion channel (VDAC), is responsible for these permeability properties (Colombini, 1979; Zalman *et al.*, 1980; Benz, 1985).

Some functional properties and the biosynthesis of the mitochondrial porin from *Neurospora crassa* and yeast have been studied in detail. The gene

[1] Department of Pharmaco-Biology, Laboratory of Biochemistry, University of Bari and CNR Unit for the Study of Mitochondria and Bioenergetics, Traversa 200 Re David 4, 70125 Bari, Italy.

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of this protein is located in the nucleus of the cell, and the protein is synthesized on cytoplasmic ribosomes without a leader sequence (Freitag *et al.*, 1982b; Mihara *et al.*, 1982; Gasser and Schatz, 1983). The primary sequence of the mitochondrial porin from yeast and *Neurospora crassa* has been derived from the nucleotide sequence of the gene (Mihara and Sato, 1985; Kleene *et al.*, 1987). In contrast, little is known about the structure and the possible function of the pore-forming protein of the mitochondrial outer membrane of other organisms.

In this short review the methods for the purification of the mitochondrial porin or VDAC are critically evaluated.

Purification of Porin in the Presence of Triton X-100

A large variety of mitochondrial porins has been purified since the first report on the existence of a voltage-dependent channel in mitochondria appeared (Schein *et al.*, 1976). First, the most obvious approach based on a preliminary purification of the outer mitochondrial membrane was used (Freitag *et al.*, 1982a; Lindén *et al.*, 1982). The outer membrane was solubilized with nonionic detergents (Triton X-100 or Genapol), and the solution was subjected to at least two steps of ion-exchange chromatography. The main disadvantage of this method is its low-yield purification, caused by the preliminary isolation of outer membranes and the various chromatographic steps employed. A substantial improvement in yield was realized when porin was purified directly from whole mitochondria and hydroxyapatite and celite were introduced as chromatographic materials (De Pinto *et al.*, 1985). Based on this approach, a very simple and rapid procedure for the purification of the mitochondrial porin has recently been developed (De Pinto *et al.*, 1987b). This method, which allows the preparation of high amounts of pure porin in a short time, requires only a single chromatographic step on hydroxyapatite/celite after solubilization of the mitochondria in Triton X-100 (see Fig. 1, left panel). When Triton X-100 solubilized mitochondria are applied to hydroxyapatite/celite, only few very hydrophobic proteins, namely porin and some anion transport systems (see Kramer and Palmieri, 1989 for a review), are eluted from the column. Under appropriate conditions, however, protein elution may be limited to porin (Fig. 2, Triton X-100, lane A). For optimal purification of this protein, several factors are critical, such as the absence of salts, a low protein/detergent ratio, and an exact hydroxyapatite/celite ratio of 2 : 1. This procedure has been successfully applied by us to all the mitochondria tested derived from both primitive and advanced eukaryotic cells.

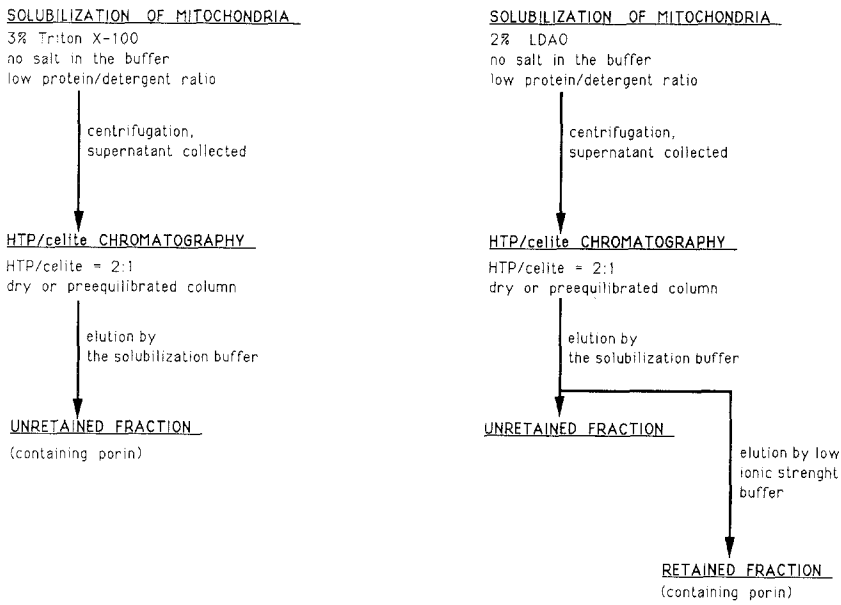


Fig. 1. Flow chart of porin purification in the presence of Triton X-100 (left panel) and in the presence of LDAO (right panel).

Purification of Porin in the Presence of LDAO[2] or Octylglucoside

In all the studies so far reported, the isolation of the mitochondrial porin was performed in the presence of Triton X-100 or Genapol. We have now investigated the effect of other detergents on the solubilization, purification, and functional properties of mitochondrial porin from bovine heart (De Pinto *et al.*, 1989). Table I reports the formulas and some characteristics of typical detergents. It should be considered that the length of the hydrophilic moiety of the detergent molecules has a great influence on the properties of the detergents. The most widely used nonionic detergents (i.e., the Tritons) have long hydrophilic portions. Relatively new nonionic detergents, which may be of great importance for the crystallization of membrane proteins (Michel, 1983), are small molecules with short hydrophilic portions.

When used at a concentration of 2%, all the detergents reported in Table I exhibit more or less the same solubilization power toward mitochondrial porin. In contrast, their effect on the purification process across the hydroxyapatite/celite column is strikingly different. In the presence of

[2]Abbreviations: Octyl-POE, octyl polydisperse oligooxyethylene; LDAO, lauryl-(dimethyl)-amine oxide.

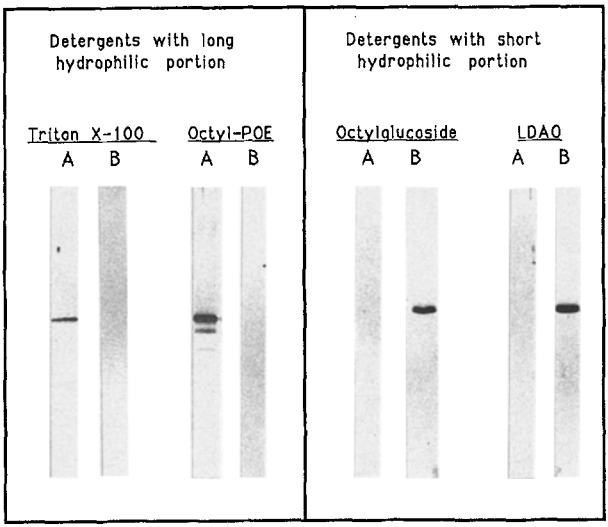
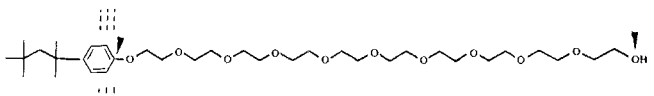
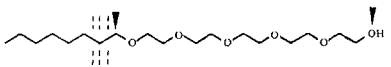
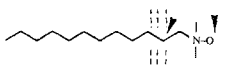
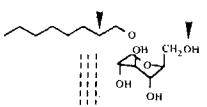


Fig. 2. Purification of the mitochondrial porin by chromatography on hydroxyapatite/celite in the presence of different detergents. SDS gel electrophoresis of unretained fraction (A) and retained fraction (B) eluted by low-ionic-strength buffer. Bovine heart mitochondria were solubilized (5 mg protein/ml) for 30 min at 0°C with 2% of the indicated detergents, 10 mM Tris/HCl, pH 7.0, and 1 mM EDTA. The solubilized fractions were loaded onto hydroxyapatite/celite columns (2 : 1, w/w, dry material). Elution was performed with the solubilization buffer (A), then with the same buffer supplemented with 5 mM KP_i and 50 mM KCl (B).

detergents which have long polar head groups, porin does not interact with hydroxyapatite/celite and is eluted in the pass-through. In the presence of the second type of detergents which have small polar head groups, on the other hand, porin is absorbed onto hydroxyapatite/celite. Figure 2 shows the contrasting effects of the two types of detergents (Triton X-100 and octyl-POE, on the one hand, and LDAO and octylglucoside, on the other hand) on the elution of porin from hydroxyapatite/celite. With Triton X-100 and octyl-POE, porin is present in the pass-through of the hydroxyapatite/celite column, whereas with LDAO and octylglucoside it is eluted only after the addition of salts. The difference between octyl-POE and octylglucoside, which have the same hydrophobic portion, is remarkable.

As a practical consequence of our studies on the interaction of different detergents with the mitochondrial porin, we have proposed a new purification procedure using the detergent LDAO (De Pinto *et al.*, 1989). The experimental conditions of this procedure are summarized in Fig. 1, right panel. The essential feature, and the advantage, of the new method is that, for the first time, porin is bound to a chromatographic material and then can be specifically eluted by the addition of a moderate-ionic-strength buffer. The

Table I. Formulas and Characteristics of Some Typical Detergents[a]

	Name	M.W.	CMC (mM)	Length of the hydrophilic part[c] (Å)
Detergents with long hydrophilic portion				
	Triton X-100	647	0.24–0.3	36.9
	Octyl-POE	350.5[b]	6.6	19.0
Detergents with short hydrophilic portion				
	LDAO	229.4	2.4	3.7
	Octylglucoside	292.4	23.4	5.5

[a] The dashed lines in the formulas represent an ideal boundary between the hydrophobic and the hydrophilic part of the detergent molecules.
[b] Molecular weight for an average polyoxyethylene unit number of 5.
[c] The distances between the atoms indicated by arrows have been calculated by the SYBYL 5.1 Molecular Graphic Software (TRIPOS Associates, St. Louis, Missouri) using standard geometries and assuming completely extended or 1,3 diaxyl conformation.

binding of porin to the hydroxyapatite/celite column allows the possibility of exchanging the detergent, which may represent an important step toward achieving the crystallization of this interesting mitochondrial protein. Furthermore, the use of LDAO instead of Triton X-100 has allowed investigation of the lipid bound to the porin. In the presence of Triton X-100, porin is eluted in the hydroxyapatite/celite pass-through together with all the lipid components of the mitochondrial membranes. In the presence of LDAO, on the other hand, all the lipids not tightly bound to the protein absorbed onto the hydroxyapatite/celite column can be removed during the extensive washing of the column in the presence of the detergent. In this way we have been able to show that the LDAO-purified porin contains no phospholipids nor other tightly bound lipids except 5 mol of cholesterol per mol 35-kDa porin polypeptide (De Pinto *et al.*, 1989).

Functional Properties

The mitochondrial porin from bovine heart, purified in the presence of detergents with long or short hydrophilic portions reported in Table I, was functionally characterized after reconstruction in planar lipid bilayers (in collaboration with R. Benz) (De Pinto *et al.*, 1989). Table II shows a comparison of the main structural and functional features of the bovine heart mitochondrial porin prepared in the presence of Triton X-100 or LDAO. Independent of the detergent used during the solubilization and purification of the protein, the functional properties of bovine heart porin can be summarized as follows: (a) incorporation of the protein into black lipid

Table II. Properties of the Mitochondrial Porin Purified in the Presence of Triton X-100 or LDAO

	Porin purified in Triton X-100	Porin purified in LDAO
Mr of monomer	35 kDa	35 kDa
Tightly bound lipids	not detectable	cholesterol (5 mol/mol 35 kDa)
Immunological reaction with:		
antiserum against rat-liver porin	positive	positive
antiserum against bovine-heart porin	positive	positive
antiserum against yeast porin	negative	not tested
Specific activity	$8 \cdot 10^{-7}$ S/mm^2	$1 \cdot 10^{-4}$ S/mm^2
Single-channel conductance	4 S	4 S
Calculated pore diameter	1.7 nm	1.7 nm
Voltage dependence	Yes	Yes
Gating charges	1.6	1.5
Ionic selectivity (P_c/P_a)	0.73	0.68

membranes results in a stepwise increase of the membrane conductance, due to the opening of pores; (b) the single-channel conductance is about 4 nS in 1 M KCl, corresponding to a calculated average pore diameter of 1.7 nm; (c) the size of the pores is voltage dependent since the single-channel conductance switches to substates on increasing the voltage; (d) the number of gating charges moving through the entire electrical field for channel gating is approximately 1.6; and (e) the pores in the open state are slightly anion selective. Similar results have been found for several other porins isolated from eukaryotic cells in the presence of Triton X-100 (De Pinto *et al.*, 1987). Surprisingly, the specific pore-forming activity of the bovine heart LDAO-purified porin was much higher than that shown by the protein purified in the presence of Triton X-100 (Table II). This result could indicate either a partial damage of the pore-forming unit in the presence of Triton X-100 or that the detergent–protein–lipid micelles did not completely dissociate under the experimental conditions, thus reducing the effective protein concentration in the aqueous phase in the presence of Triton X-100 as compared to that in LDAO. Also the histogram of the conductance fluctuations observed with the LDAO–porin was more homogeneous than that of the Triton–porin (De Pinto *et al.*, 1989), which is in favor of the idea that the action of LDAO is milder.

Conclusions

Two rapid and efficient methods are now available for the complete purification of the mitochondrial porin or the voltage-dependent anion channel of the outer mitochondrial membrane (Fig. 2). Both make use of a mixed hydroxyapatite/celite column as a single chromatographic step. However, in one method porin passes through the column, whereas in the other it is first bound to the column and then eluted by the addition of salts (Fig. 1). As documented above, the opposite chromatographic behavior of porin on hydroxyapatite/celite can be ascribed to the different length of the hydrophilic portion of the detergents used in the two methods: Triton X-100 and LDAO, respectively. Our interpretation of these findings is that, with long polar head-group detergents, the surface-exposed hydrophilic domains of the protein are shielded from the column material and porin is not absorbed. When, on the other hand, the polar head groups of the detergents are short, as in the case of LDAO, the water-exposed hydrophilic domains of the protein can interact with the chromatographic material and the protein is bound. On this basis we assume that the mitochondrial porin is arranged in the protein–detergent micelles as schematically shown in Fig. 3. The model considers that porin is deeply embedded in the micelles and that there are

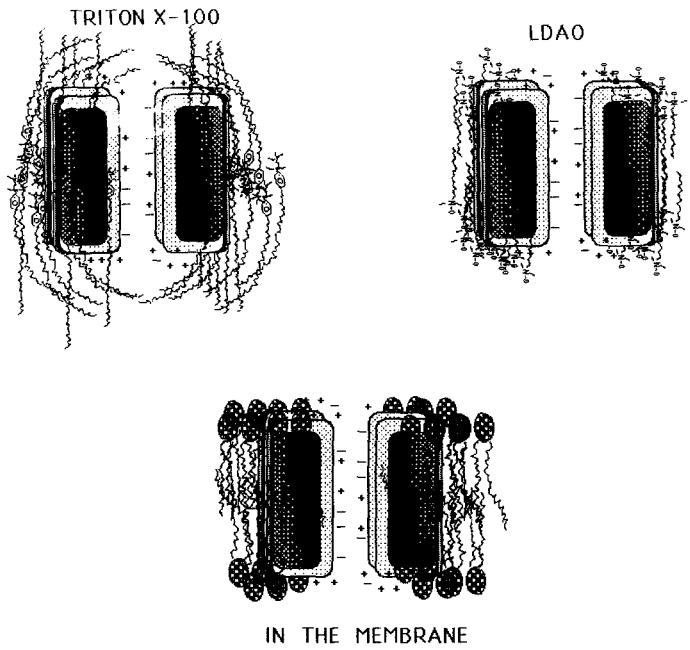


Fig. 3. A model describing the interactions of different detergents with the mitochondrial porin. Detergents with long hydrophilic portions (like Triton X-100) can shield the surface-exposed charges located at the mouth of the pore and hence prevent the binding of the protein to ion-exchange chromatographic material. In contrast, detergents with short hydrophilic portions (like LDAO) cannot cover the water-exposed hydrophilic domains of the protein. Presumably the arrangement of the protein in the LDAO–porin micelles resembles the physiological topography of porin in the outer mitochondrial membrane.

only few charges at the mouth of the pore which are available for the interaction with the chromatographic material unless they are not shielded by the hydrophilic portion of the detergent. This idea is supported by the observation that porin, bound to hydroxyapatite/celite in the presence of small polar head-group detergents, is easily eluted by the addition of a small amount of salts. Actually, porin is the first protein to be eluted when the ionic strength is increased, and this result also indicates that porin is one of the most hydrophobic mitochondrial proteins or, at least, one of the proteins with the smallest number of charged residues. Our model of porin arrangement in the micelles is also in agreement with the hypothesis that porin is largely or completely buried in the outer mitochondrial membrane too, since it is the only protein of this membrane which is not susceptible to the action of added proteases (Mihara *et al.*, 1982). As regards the nature of the charges responsible for the binding of porin to the hydroxyapatite/celite material,

there is no doubt that they are positive or prevailingly positive at neutral pH, since the LDAO-purified protein binds to cation but not to anion exchangers. It is interesting that several lysines are preserved in the primary sequence of porin from yeast and *Neurospora crassa* (Mihara and Sato, 1985; Kleene *et al.*, 1987) and that positively charged groups, presumably located on the mouth of the porin pore, are considered to be responsible for the voltage dependence of the channel (Bowen *et al.*, 1985). Experiments are now in progress in our laboratory to locate within the amino acid sequence of the protein the charged residues responsible for the binding of porin to cation exchangers.

References

Benz, R. (1985) *CRC Crit. Rev. Biochem.* **19**, 145–190.
Bowen, K. A., Tam, K., and Colombini, M. (1985) *J. Membr. Biol.* **86**, 51–59.
Colombini, M. (1979) *Nature (London)* **279**, 643–645.
De Pinto, V., Tommasino, M., Benz, R., and Palmieri, F. (1985) *Biochim. Biophys. Acta* **813**, 230–242.
De Pinto, V., Ludwig, O., Krause, J., Benz, R., and Palmieri, F. (1987) *Biochim. Biophys. Acta* **894**, 109–119.
De Pinto, V., Benz, R., and Palmieri, F. (1989) *Eur. J. Biochem.*, in press.
Freitag, H., Neupert, W., and Benz, R. (1982a) *Eur. J. Biochem.* **123**, 629–636.
Freitag, H., Jones, M., and Neupert, W. (1982b) *Eur. J. Biochem.* **126**, 197–202.
Gasser, M., and Schatz, G. (1983) *J. Biol. Chem.* **258**, 3427–3430.
Kleene, R., Pfanner, N., Pfaller, R., Link, T. A., Sebald, W., Neupert, W., and Tropschung, M. (1987) *EMBO J.* **6**, 2627–2633.
Lindén, M., Gellerfors, P., and Nelson, B. D. (1982) *Biochem. J.* **208**, 77–82.
Michel, H. (1983) *Trends Biochem. Sci.* **8**, 56–59.
Mihara, K., and Sato, R. (1985) *EMBO J.* **4**, 769–774.
Mihara, K., Blobel, G., and Sato, R. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7102–7106.
Pfaff, E., Klingenberg, M., Ritt, E., and Vogell, W. (1968) *Eur. J. Biochem.* **5**, 222–232.
Schein, S. J., Colombini, M., and Finkelstein, A. (1976) *J. Membr. Biol.* **30**, 99–105.
Zalman, L. S., Nakaido, H., and Kagawa, Y. (1980) *J. Biol. Chem.* **255**, 1771–1774.