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HYDRODYNAMIC PROPERTIES OF PORIN ISOLATED FROM OUTER MEMBRANES OF RAT LIVER MITOCHONDRIA

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The hydrodynamic properties of purified porin (M_r = 30000), isolated from outer membranes of rat liver mitochondria has been studied. After gel filtration, active porin was eluted in a symmetrical peak with an estimated Stokes radius of 5.4 nm. The sedimentation coefficient (s) and partial specific volume ($\bar{v}$) were found to be 2.6 S and 0.908 cm^3/g, respectively, for the purified porin-Triton X-100 complex. Based on these determinations, a molecular weight of 170000 for the porin-Triton X-100 complex was calculated. Correcting for bound Triton X-100, 1.8 g/g of protein, a molecular weight of 60000 was estimated for the protein portion of the complex. Thus, isolated active porin appears to exist as a dimer.

Introduction

Mitochondrial porin is located in the outer mitochondrial membranes. This protein forms aqueous channels which allows the passage of polysaccharides up to 8 kDa [1,2]. Conductance measurements performed by Roos et al. [3] as well as by Colombini [4], showed that porin could also form voltage-dependent, anion-selective channels.

Porin has recently been isolated in an active form both from rat liver [2,3] and *Neurospora crassa* [5]. The purified porin from these sources displayed an apparent molecular weight of 30000 upon SDS-gel electrophoresis [2,5]. Rat liver porin could be further resolved into a major (pI 7.9) and two minor components by isoelectric focusing [2]. The amino acid composition of rat liver porin [2] (polarity 47.8%) is significantly different from that reported for *Neurospora crassa* porin [5] as well as the OmpF protein [6] of Gram-negative bacteria, suggesting structural dissimilarities between the various porins.

Very little is known about the quarternary structure of the active outer membrane pore complex. The well known resistance of porin to protease digestion [7], suggests that it is deeply imbedded in the membrane. Electron microscopic studies have revealed pore structures in the outer membrane with a diameter of 2 nm [7], in agreement with the calculated diameter based on conductance measurements [5].

In this paper, we describe some hydrodynamic properties of the porin-Triton complex purified from rat liver mitochondria [2]. Based on these measurements, a molecular weight of 171000 has been determined for the active porin-Triton complex. Correcting for bound Triton, we suggest that porin is a dimeric protein, with a molecular weight of 60000.

Materials and Methods

[6,6′(n)-^{3}H]Sucrose (1–5 mCi/mmol) was purchased from the Radiochemical Centre, Amersham. [*carboxyl*-^{14}C]Dextran (M_r 70000) (1.1 mCi/g) and [^{3}H]Triton X-100 (1.58 mCi/mg) were purchased from New England Nuclear. Sephacryl S-300 superfine and Blue dextran 2000 were

bought from Pharmacia, Sweden. Triton X-100, reference proteins and other biochemicals used in this study were all reagent grade, obtained from commercial sources.

Rat liver porin was isolated as described before [2]. Pore forming activity was determined according to Zalman et al. [1] with the modification of Lindén et al. [2].

Determination of the Stokes radius for the porin-Triton complex

50 μg porin was incubated together with marker enzymes, Blue dextran and [^{3}H]sucrose in 1 ml of 40 mM potassium phosphate buffer, pH 7.0, containing 0.05% (w/v) Triton X-100 at 4°C for 2 h. After incubation, the sample was applied on a Sephacryl S-300 superfine column (1.4 × 85 cm) equilibrated with 40 mM phosphate buffer, pH 7.0, 0.05% (w/v) Triton X-100 and 0.1 mM dithioerythritol. The column was eluted with the equilibration buffer at a rate of 4 ml/h.

Fractions were collected and analyzed for porin as well as for marker enzymes. Due to Triton X-100 absorbance at 280 nm, the elution position of porin and the marker enzymes were monitored by running an aliquot of each fraction on an SDS-polyacrylamide gel. After Coomassie blue staining or silver staining [8], the amount of each protein was quantitated by a Zeiss KM3 gel scanner at 400 nm. Marker enzymes used were thyroglobulin (a = 8.2 nm), bovine serum albumin (a = 3.5 nm), ovalbumin (a = 2.7 nm), catalase (a = 5.2 nm), yeast alcohol dehydrogenase (a = 4.6 nm), and hemoglobin (a = 2.5 nm). Stokes radii were taken from Ref. 9. Porin [2], catalase [10] and yeast alcohol dehydrogenase [11] were assayed as described. Hemoglobin was assayed spectrophotometrically at A_{415} nm. Blue Dextran and [^{3}H]sucrose were used to estimate the void volume and the total volume of the column, which were determined to be 52.5 ml and 129 ml, respectively. The Stokes radius was estimated as described by Porath [12].

Determination of partial specific volume of the porin-Triton complex

The partial specific volume of the porin-Triton complex was determined by equilibrium centrifugation. 15 μg of purified porin was introduced into a 4.4 ml 10–40% (w/w) sucrose gradient containing 40 mM potassium phosphate, pH 7.4, and 0.05% (w/v) Triton X-100. Centrifugation was performed at 230000 × g (average) in a Beckman SW56 rotor for 72 and 96 h to ensure that equilibrium had been reached. After centrifugation, 0.2 ml fractions were collected. The concentration of sucrose in the various fractions was determined refractometrically. Porin was located as described above.

Determination of sedimentation coefficient of the porin-Triton complex

10 μg of purified porin was dissolved together with the marker enzymes, bovine serum albumin, and cytochrome c, in 150 μl 40 mM potassium phosphate, pH 7.0, and 0.3% (w/v) Triton X-100. Centrifugation was performed at 230000 × g for 16 h in a Beckman SW56 rotor. 0.2 ml fractions were collected and subjected to SDS-gel electrophoresis. Densitometric analysis and location of porin was done as outlined above. Standard proteins with known sedimentation coefficients were: bovine serum albumin (4.6 S) and cytochrome c (1.7 S).

Detergent binding

100 μg of purified porin was layered on top of a 5–20% (w/v) sucrose gradient containing 0.05% (w/v) [^{3}H]Triton X-100 (0.2 mCi/μg) and 40 mM potassium phosphate, pH 7.0. Samples were centrifuged at 230000 × g for 24 h in a Beckman SW 56 rotor. After centrifugation, 100 μl fractions were collected from the bottom of the tube. The specific radioactivity and the amount of protein was determined in each fraction. The radioactivity over the base line of the plateau region for a given fraction was divided by the specific activity of the labeled detergent and the amount of protein in the same sample volume, according to Clarke [13].

Other procedures

SDS-gel electrophoresis was done according to Laemmli [14]. Protein was determined by the method of Petterson [15].

Results

Hydrodynamic properties of the porin-Triton complex

The rat liver porin preparation used in this study had a high pore-forming activity ($^{14}C/^{3}H$ of over 30) (for details see Ref. 2). The preparation revealed only one band (M_r 30000) on SDS-gel electrophoresis.

The purified porin-Triton complex, when subjected to sucrose gradient centrifugation, sedimented as a sharp band with a sedimentation coefficient of 2.6 S. The protein retained about 70–80% of its pore-forming activity after sucrose gradient centrifugation, indicating that it was not altered during centrifugation. Inactivation of Triton X-100 solubilized porin preparations during sucrose gradient centrifugation has been reported [1].

The Stokes radius of the porin-Triton complex was determined by gel filtration on a Sephacryl-300 column in the presence of 0.05% Triton X-100. Porin eluted in a symmetrical peak indicating a homogenous preparation. The position of the porin-Triton complex peak migrated just ahead of the Triton X-100 micelle peak, indicating a slight difference in size between the protein-detergent micelles and pure micelles. By comparing the K_d for the porin-Triton complex with the K_d for standard proteins with known Stokes radii, the Stokes radius for the porin-Triton complex was estimated to 5.4 nm. The same value was obtained for three experiments.

The partial specific volume ($\bar{v}$) for the porin-Triton complex was determined by equilibrium density centrifugation in a sucrose gradient containing 0.05% Triton X-100. The porin-Triton complex banded after both 72 h and 96 h at a sucrose concentration of 24% (refractive index 1.371) (Fig. 1). This corresponds to a density of 1.101 g/ml, giving a partial specific volume for the porin-Triton complex of 0.908 cm^3/g. Since the partial specific volume of porin determined from its amino acid composition [2] is only 0.733 cm^3/g, a substantial amount of Triton X-100, or possibly lipid, is probably bound to the protein giving rise to the observed high value. Lipid analysis of the porin-Triton complex revealed less than 5% phospholipid (w/w) hence the component responsible for the high partial specific values observed is most likely Triton X-100. To be able to estimate the molecular weight of the active porin, the amount of bound detergent was determined.

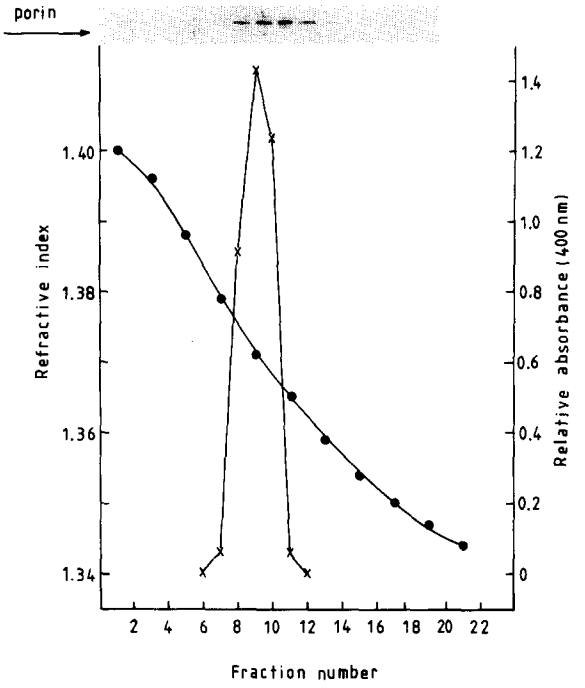

Fig. 1. Determination of the partial specific volume for the porin-Triton complex by sucrose gradient centrifugation. 15 μg of purified porin-Triton complex was centrifuged for 72 h in a 10–40% sucrose gradient containing 0.05% Triton X-100. Collected fractions (0.2 ml) were analyzed for refractive index and subsequently subjected to SDS-polyacrylamide gel electrophoresis (see top part of the figure). Polypeptide bands were quantitated by gel scanning at 400 nm (for details, see Materials and Methods).

Triton X-100 binding to porin

Fig. 2 shows an experiment in which bound Triton X-100 was determined. The porin-Triton complex was subjected to sucrose gradient centrifugation in the presence of [^{3}H]Triton X-100. The amount of radioactive Triton X-100 associated with the porin peak was subtracted from the background plateau level, a mean value of 1.78 ± 0.23 mg Triton X-100 bound per mg of protein (mean $\pm$ S.E., $n = 6$) was calculated. This value is in good agreement with the values determined for other purified membrane protein [16,17].

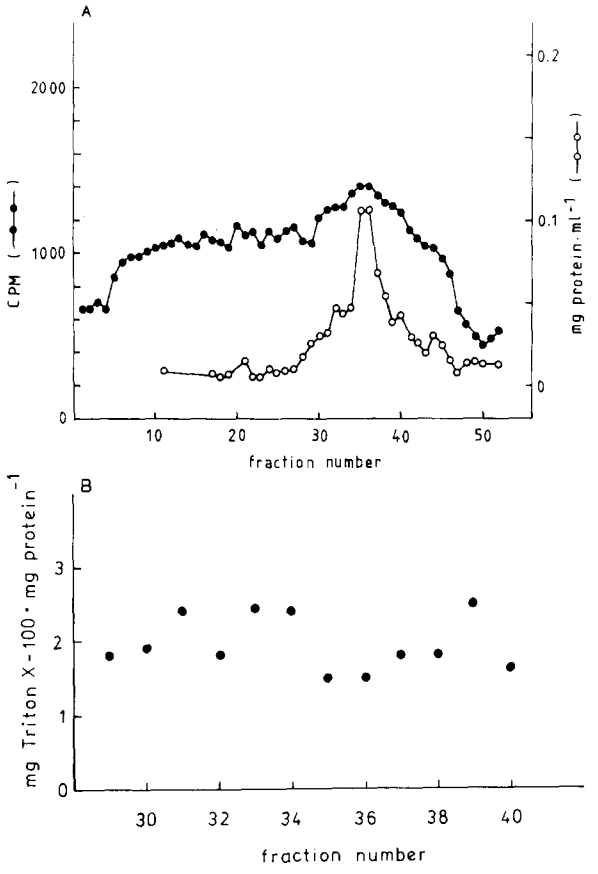

Fig. 2. Determination of Triton X-100 binding to porin. (A) Purified porin-Triton complex (100 μg) was centrifuged in a 5 to 20% sucrose gradient containing 0.05% [^{3}H]Triton X-100 (spec. act. 87 cpm/μg). 100-μl fractions were collected and Triton X-100 binding was determined by measuring the radioactivity above the base line (1100 cpm) and divided by the amount of protein in the same volume for each fraction [13]. (B) The amount of Triton X-100 (mg) bound per mg porin is illustrated in an expanded scale. The values are mean values from three different experiments.

Molecular weight and oligomeric structure of porin

Table I summarizes the hydrodynamic properties determined for the porin-Triton complex. The molecular weight of a protein or a protein-detergent complex may be calculated from the Svedberg equation.

$$M = \frac{6\pi \cdot \eta_{20,w} \cdot s_{20,w} \cdot a \cdot N}{(1 - \bar{v} \cdot \rho_{20,w})} \quad (1)$$

where M, $\eta_{20,w}$, $s_{20,w}$ a, N, $\bar{v}$, and $\rho_{20,w}$ are the molecular weight of the protein detergent complex, viscosity of water at 20°C, standard sedimentation coefficient, Stokes radius, Avogadros number, partial specific volume of the protein detergent complex and the density of water at 20°C, respectively.

TABLE I

PROPERTIES OF PURIFIED PORIN-TRITON X-100 COMPLEX

Parameter	
Stokes radius	5.4 nm
Sedimentation coefficient	2.6 S
Partial specific volume [a]	0.908 cm^3/g
Partial specific volume (protein part) [b]	0.733 cm^3/g
Calculated partial specific volume [c]	0.881 cm^3/g
Minimal molecular weight of porin (SDS-PAGE)	30000
Molecular weight porin-Triton X-100 complex	171000
Triton X-100 binding	1.8 g/g
Meric form of porin	dimer (α_2)

[a] Experimentally determined.
[b] Calculated from amino acid composition of porin [2] according to Ref. 20.
[c] Calculation based on a dimeric form of porin (M_r 60000) and a Triton X-100 binding of 1.8 g/g.

Introduction of the experimentally determined values for a, $s_{20,w}$, and $\bar{v}$, in Eqn. 1 yields an estimated molecular weight of 171000. This is a composite value of protein and bound detergent. After correcting for bound Triton X-100 (1.8 g/g protein), the molecular weight of the protein portion is calculated to approx. 60000. Thus, these results suggest that isolated porin (minimal molecular weight 30000) exists as a dimer.

Discussion

In this paper, we have determined the Stokes radius (5.4 nm), the sedimentation coefficient (2.6 S), and the partial specific volume (0.908 cm^3/g) for rat liver porin in the presence of Triton X-100. By insertion of these parameters into the Svedberg equation, a molecular weight of 171000 was calculated for the porin-detergent complex.

To be able to estimate the molecular weight of porin, we had to determine the amount of bound Triton X-100. A value of 1.8 g Triton/g protein was obtained. If porin (minimal molecular weight 30000) exists as a monomer, approx. 82% of the total mass of the protein detergent complex should be Triton X-100. This is equal to a Triton X-100

binding of 4.7 g/g. However, if porin is a dimer (M_r 60000), a calculated Triton X-100 binding of 1.8 g/g should theoretically be found. This value is in good agreement with that found experimentally. Porin in a trimeric form would bind only 0.9 g Triton X-100/g protein. Hence, by this criteria, porin is most likely a dimer, however, Triton X-100 has recently been found to cause modification of proteins into dimers [18].

The molecular weight of porin can also be estimated by comparing the experimentally determined value of the partial specific volume ($\bar{v}$) for the porin-Triton complex with the theoretical value, assuming different meric forms of porin. The $\bar{v}$ of a multicomponent complex can be calculated from the sum of its individual components' weight fraction and partial specific volume of the individual components [19]. To calculate $\bar{v}$ for the porin-Triton complex, assuming no other major component is present in the complex, the $\bar{v}$ for porin and the $\bar{v}$ for Triton X-100, as well as their relative contribution to the total mass of the complex, must be known. The $\bar{v}$ for Triton X-100 used in these experiments was determined to 0.960 cm^3/g by equilibrium density centrifugation. By using a value of 0.733 for porin (for details see Table I) and assuming either a monomeric, dimeric or a trimeric form or porin, $\bar{v}$ values of 0.920, 0.881 and 0.853, respectively, are obtained. Comparing these values with those obtained experimentally (0.908 cm^3/g), porin would appear to exist either as a monomer or a dimer. Since a monomeric form of porin is not compatible with its Triton X-100 binding properties (see above) and as an overestimation of $\bar{v}$ due to hydration of the protein detergent micelle has been observed [13], the theoretically value of 0.881 (dimeric form of porin) is probably the more correct.

The molecular architecture and regulation of the outer mitochondrial membrane pores are still not yet understood. Recent experiments showing that porin binds hexokinase [21] and also glycerol kinase [22] during certain metabolic states, e.g. high aerobic glycolysis, for example in tumor cells [23], suggests that the physiological pore complexes might be constructed of a membrane segment (dimeric porin) with additional molecules bound to it. The loosely bound, water soluble molecules might be involved in the regulation of the pore channel. Further structural and functional work is obviously needed to be able to understand this in more detail.

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References

1 Zalman, L.S., Nikaido, H. and Kagawa, Y. (1980) J. Biol. Chem. 255, 1771–1774
2 Lindén, M., Gellerfors, P. and Nelson, B.D. (1982) Biochem. J. 208, 77–82
3 Roos, N., Benz, R. and Brdiczka, D. (1982) Biochim. Biophys. Acta 686, 204–214
4 Colombini, M. (1980) Ann. N.Y. Acad. Sci. 341, 552–563
5 Freitag, H., Neupert, W. and Benz, R. (1982) Eur. J. Biochem. 123, 629–636
6 Chen, R., Krämer, C., Schmidmayr, W., Chen-Schmeisser, U. and Henning, U. (1982) Biochem. J. 203, 33–43
7 Mannella, C. and Bonner, W. Jr. (1975) Biochim. Biophys. Acta 413, 213–225
8 Merril, C.R., Goldmann, D., Sedmann, S.A. and Ebert, M.H. (1981) Science 211, 1437–1438
9 Siegel, L.M. and Monty, K.J. (1966) Biochim. Biophys. Acta 112, 346–362
10 Beers, R.F., Jr. and Sizer, I.W. (1952) J. Biol. Chem. 195, 133–140
11 Vallee, B.L. and Hoch, F.L. (1955) Proc. Natl. Acad. Sci. U.S.A. 41, 327–337
12 Porath, J. (1963) Pure Appl. Chem. 6, 233–244
13 Clarke, S. (1975) J. Biol. Chem. 250, 5459–5469
14 Laemmli, U.K. (1970) Nature (London) 227, 680–685
15 Petterson, G. (1977) Anal. Biochem. 83, 346–356
16 Lin, C.S., Hackenberg, H. and Klingenberg, M. (1980) FEBS Lett. 113, 304–306
17 Hackenberg, H. and Klingenberg, M. (1980) Biochemistry 19, 548–555
18 Pappert, G. and Schubert, D. (1983) Biochim. Biophys. Acta 730, 32–40
19 Tanford, C., Nozaki, Y., Reynolds, J.A. and Makino, S. (1974) Biochemistry 13, 2369–2376
20 Cohn, E.J. and Edsall, J.T. (1943) in Proteins, Amino Acids and Peptides as Ions and Dipolar Ions (Cohn, E.J. and Edsall, J.T., eds.), pp. 370–381, Reinhold Publishing Corporation, New York
21 Lindén, M., Gellerfors, P. and Nelson, B.D. (1982) FEBS Lett. 141, 189–192
22 Fiek, C., Benz, R., Roos, N. and Brdiczka, D. (1982) Biochim. Biophys. Acta 688, 429–440
23 Bustamente, E., Morris, H.P. and Pedersen, P.L. (1981) J. Biol. Chem. 256, 8699–8704