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# SUBCELLULAR DISTRIBUTION OF RAT LIVER PORIN

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**The subcellular distribution of rat liver porin was investigated using the immunoblotting technique and monospecific antisera against the protein isolated from the outer membrane of rat liver mitochondria. Subfractionation of mitochondria into inner membranes, outer membranes and matrix fractions revealed the presence of porin only in the outer membranes. Porin was also not detected in highly purified subcellular fractions, including plasma membranes, nuclear membranes, Golgi I and Golgi II, microsomes and lysosomes. Thus, liver porin is located exclusively in the outer mitochondrial membrane.**

## Introduction

The outer membrane of mitochondria contains voltage-dependent channels which are created by porin, a peptide with a molecular weight of approx. 30000 [1–6]. However, neither the structure of these channels nor their physiological functions are well characterized. Furthermore it is not known if porin is present in mammalian membranes other than the outer mitochondrial membranes. Particularly interesting in this regard are the gap junctions of plasma membranes, which contain pores (see Ref. 7 for review) composed of a major polypeptide with an apparent molecular weight of approx. 28000 [8,9], and which are in physical contact with the outer membrane of the mitochondria [10]. Pore structures are also found in nuclear membranes, but these seemingly have very different structural characteristics from those in the mitochondria [11,12]. In the present study we have used specific antibodies against rat liver porin to assess the subcellular distribution of this peptide. Analysis of a number of highly purified subcellular membrane fractions from rat liver as well as the different compartments of the mitochondria show that porin is located exclusively in the outer mitochondrial membrane.

## Methods

Porin was isolated from the outer membranes of rat liver mitochondria as described in Ref. 1. Antibodies were raised in rabbits by injecting 250 $\mu$g protein intramuscular, followed 6 weeks later by a booster of 160 $\mu$g protein given intramuscular. A second booster was given 2 weeks later, 14 days after which the animals were bled. The antigen was dissolved in 6 M urea and 500 $\mu$l Freunds adjuvant according to Wielburski and Nelson [13]. Immuno transfer onto nitrocellulose was done according to Ref. 14. The antigen-IgG complexes were visualized with the aid of $^{125}$I-protein A and autoradiography. SDS-polyacrylamide gel electrophoresis was run in the buffer system of Laemmli [15]. Rough and smooth microsomes [16], Golgi I and Golgi II [16], lysosomes [17], nuclei [18] and plasma membranes (bile canaliculi and contiguous face) [19] were isolated as described previously. Mitochondria and the various mitochondrial subfractions were prepared as in Ref. 20. Marker

enzymes for the different subcellular fractions were measured as in Ref. 10.

## Results

Antisera raised against rat liver porin is highly specific as shown by immunoblotting experiments (Fig. 1). The outer membrane exhibited a single reactive peptide with an apparent molecular weight of 30000 (Fig. 1, lane 2) which co-migrates with purified porin (Fig. 1, lane 3) on SDS-polyacrylamide gels. The only other antigen detected was a minor component which appeared occasionally in mitochondria (Fig. 1, lane 1). This had an apparent molecular weight of about 60000 and might represent a dimeric form of porin. As will be seen below, this peptide was not observed in the inner membrane, outer membrane or matrix fractions, or the other subcellular fractions studied.

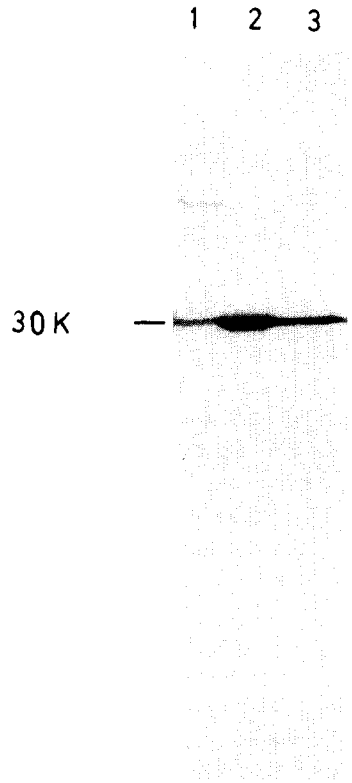


Fig. 1. Immunoblotting of rat liver porin. Mitochondria, 100 μg (lane 1), isolated outer mitochondrial membranes, 100 μg (lane 2) and isolated porin, 1 μg (lane 3) from rat liver were separated by SDS-polyacrylamide gel electrophoresis and immunoblotted as described in Methods.

TABLE I

DISTRIBUTION OF PORIN IN SUBFRACTIONS OF RAT LIVER MITOCHONDRIA

The various fractions were resolved on SDS-polyacrylamide gels and then transferred onto nitrocellulose filters. The filter was incubated with antisera against porin and then decorated with $^{125}$I-protein A. Radioactive bands were located by autoradiography, cut from the nitrocellulose, and counted.

| Fraction | μg protein blotted | Radioactivity | |
|---|---|---|---|
| | | Total cpm [a] | cpm/mg protein |
| Isolated porin | 1 | 4250 | 4250000 |
| Outer membrane | 5 | 715 | 143000 |
| Mitochondria | 300 | 3400 | 11400 |
| Matrix | 50 | 58 | 1000 |
| Inner membrane | 50 | 112 | 2200 |

[a] The values are corrected for background (154 cpm) obtained by counting pieces of the filter not containing porin.

Table I shows the semiquantitative analysis of the distribution of porin in the various compartments of rat liver mitochondria. In these experiments the antigens were transferred onto nitrocellulose filters by electrophoresis and then incubated with antisera followed by $^{125}$I-protein A, both of which were in excess of the antigen. Porin was detected autoradiographically and the bands were cut from the nitrocellulose filter and counted. Analysis of the outer membranes, inner membranes and matrix fractions show that porin is located predominantly, if not exclusively, in the outer membrane.

The subcellular distribution of porin was analysed in the same manner as described above for Table I. Porin was not detected in fractions highly enriched in plasma membranes, derived mainly from bile Canaliculi and the contiguous face containing junctional complexes (Table II). Neither was porin detected in nuclear membranes, Golgi, or rough and smooth microsomes. In several cases, as much as 300 μg of membrane protein was subjected to electrophoresis without detectable amounts of porin being observed (not shown). The small amount of porin detected in lysosomes (Table II, Expt. 2) is accounted for by mitochondrial contamination. Antibodies against porin re-

TABLE II

DISTRIBUTION OF PORIN IN DIFFERENT SUBCELLULAR MEMBRANE FRACTIONS FROM RAT LIVER TISSUE

| Expt. | Fraction | μg protein blotted | Radioactivity | |
|---|---|---|---|---|
| | | | Total cpm [a] | cpm/mg protein |
| I | Porin | 1 | 2615 | 2615000 |
| | Nuclear membrane | 200 | 0 | 0 |
| | Plasma membrane | 200 | 0 | 0 |
| | Rough microsomes | 200 | 0 | 0 |
| II | Outer mitochondrial membranes | 5 | 457 | 91400 |
| | Mitochondria | 300 | 2140 | 7140 |
| | Lysosomes | 100 | 0 | 0 |
| III | Golgi I | 100 | 0 | 0 |
| | Golgi II | 100 | 0 | 0 |
| | Lysosomes | 100 | 44 | 440 |
| | Rough microsomes | 100 | 0 | 0 |
| | Smooth microsomes | 100 | 0 | 0 |

[a] Corrected for background radioactivity (196 cpm).

cognized no additional peptides in the different membrane fractions tested. The distribution of marker enzymes in the membrane fractions used in immunoblotting experiments is summarized in Table III. The increase in the specific activity of marker enzymes of microsomes (2-fold), plasma membranes (16-fold), Golgi I (50-fold), Golgi II (55-fold), lysosomes (20-fold) agrees well with previously reported values for the purified membranes [16,17,19].

TABLE III

ENZYMATIC CHARACTERIZATION OF THE SUBCELLULAR FRACTIONS USED FOR IMMUNOBLOTTING

Values are the means ± S.D. of six determinations. n.d., not determined.

| | NADPH-cytochrome *c* reductase (μmol/min per mg protein) [a] | UDP-galactose: ovomucoid galactosyl-transferase (nmol/30 min per mg protein) [b] | Acid phosphatase (μmol $P_i$/10 min per mg protein) [c] | 5′-Nucleotidase (μmol $P_i$/20 min per mg protein) [d] |
|---|---|---|---|---|
| Total particulate fraction | 0.048 ± 0.005 | 7.1 ± 0.4 | 0.08 ± 0.005 | 0.30 ± 0.02 |
| Nuclei | 0.008 ± 0.001 | n.d. | n.d. | n.d. |
| Microsomes | 0.082 ± 0.006 | 16.6 ± 0.9 | 0.07 ± 0.003 | 0.18 ± 0.01 |
| Golgi I | 0.020 ± 0.002 | 398.2 ± 60 | 0.15 ± 0.011 | 1.05 ± 0.06 |
| Golgi II | 0.015 ± 0.002 | 367.2 ± 55 | 0.21 ± 0.014 | 2.85 ± 0.13 |
| Lysosomes | 0.004 ± 0.0005 | 63.0 ± 9 | 1.5 ± 0.14 | 1.47 ± 0.07 |
| Plasma membranes | n.d. | n.d. | n.d. | 5.05 ± 0.26 |

[a] μmol cytochrome *c* reduced/min per mg protein.
[b] nmol galactose transferred/30 min per mg protein.
[c] μmol $P_i$ released/10 min mg per protein.
[d] μmol $P_i$ released/20 min per mg protein.

## Discussion

The present study shows that porin is located exclusively in outer mitochondrial membranes in rat liver. It was not detected by immunoblotting methods in mitochondrial inner membranes or matrix fractions, or in fractions highly enriched in Golgi membranes, rough and smooth microsomes, lysosomes, nuclear membranes or plasma membranes. The absence of porin from the latter two membranes is of interest since both contain pore structures [7–10,12]. Nuclear pores differ from those in the outer mitochondrial membranes in several important respects, including size, composition and function. Pore structures in mitochondria and in the gap junctions of the plasma membrane are, however, similar in that both are voltage regulated, water-filled pores, and both are composed of a major peptide with an apparent molecular weight of between 28 000 and 30 000 on SDS-polyacrylamide gel electrophoresis electrophoresis [8,9]. In spite of these superficial similarities, the present results show that the pore structures of the two membranes are composed of different peptides.

The exclusive location of porin in the outer mitochondrial membrane raises questions regarding the ability of porin to recognize and insert itself into the target membrane. Porin is synthesized on free ribosomes [22] in the cytosol [21,23]. Since the initial translation product of porin apparently lacks a signal sequence [22,23], the exclusive recognition of the outer membrane by porin requires a mitochondrial-specific receptor protein or a unique mitochondrial structure. The presence of a specific porin receptor raises, in turn, the question of how such a receptor could become exclusively located in the mitochondrion. The possibility that a porin receptor might be synthesized within the mitochondria was rejected since no mitochondrial translation products could be found in the outer membrane in rat liver [21]. Thus, it is likely that the porin 'receptor' is a structural state associated with the functional proteins of the mitochondrial membranes.

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