THE POLYPEPTIDES OF RAT LIVER MITOCHONDRIA: IDENTIFICATION OF A 36,000 DALTON POLYPEPTIDE AS THE SUBUNIT OF ORNITHINE TRANSCARBAMYLASE

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One of the major polypeptide bands seen after rat liver mitochondria are subjected to dodecyl sulfate gel electrophoresis is a component with a mass of 36,000 daltons that makes up 3 to 4% of the total mitochondrial protein. This band, designated Va (1), purifies with an enzyme of the urea cycle, ornithine transcarbamylase (E.C. 2.1.3.3). Evidence is presented that the band Va polypeptide is a single molecular species corresponding to the polypeptide chain of this enzyme.

When mitochondrial polypeptides from rat liver are separated by dodecyl sulfate tube gel electrophoresis, approximately 17 major components can be identified (1). Some of these bands are made up of polypeptides of similar molecular weight from different proteins, while other bands appear to contain only a single molecular species. One example of the latter class is the band I component, which has been identified with the polypeptide chain of carbamyl phosphate synthetase (2). While fractionating mitochondrial extracts, I observed that another polypeptide band behaved as a single species. This component (band Va), which is located in the matrix space or is loosely bound to the inner surface of the inner membrane (1), comigrated with another urea cycle enzyme activity, ornithine transcarbamylase. The purpose of this communication is to show that the material in band Va is the polypeptide chain of this enzyme.

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METHODS

Enzyme Assay: Ornithine transcarbamylase was assayed as described by Marshall and Cohen (3) except that glycylglycine was used to buffer the reaction mixture and trichloroacetic acid was used to terminate the reaction. Diammonium carbamyl phosphate was from Sigma. The concentration of the citrulline standard was established by amino acid analysis, using a color constant of $0.94 \times C_{avg}$ (Beckman Instruments).

Purification of Ornithine Transcarbamylase: Because previous purification schemes for the mammalian enzyme were designed for large amounts of tissue (3,4), a new purification was devised for obtaining mg amounts of the enzyme from one or two rat livers. Rat liver mitochondria were prepared as described (2). The enzyme was solubilized in 10 mM Tris·acetic acid pH 7.59 with 0.3 mg Lubrol WX 5070 (ICI America) per mg protein. The mitochondrial protein concentration was approximately 10 mg/ml. Membrane fragments were spun out, and the supernatant was applied to a 1.1 x 10 cm column of DEAE-Agarose (Biorad) equilibrated in the Tris buffer without detergent. The flow-through fractions were collected and adjusted to a final concentration of 5 mM L-ornithine, 100 mM sodium phos-

TABLE I
PURIFICATION OF ORNITHINE TRANSCARBAMYLASE FROM RAT LIVER

Fraction	Mg Protein	Specific Activity	Yield	Fold Purif.	% of Total Protein as Band Va
Mitochondria	640[a]	6.2[b]	100%[c]	1.0[d]	4.1
Lubrol Supernatant	422	8.7	95%	1.4	5.9
DEAE-Agarose	141	21.9	81%	3.5	12
Supernatant After Heating	57.4	44.8	69%	7.2	40
Salt-Elution Chromatography	5.7	197	31%	31.8	84
Glycerol Gradient	0.5	249	12%	40.1	96

[a] Determined from A_{280} in dodecyl sulfate (2) or by alkaline ninhydrin assay (6)

[b] μmol citrulline formed/min/mg protein at 37°, pH 8.5

[c] Corrected for amount not applied to the next step

[d] Determined by integration of scans of Coomassie-stained dodecyl sulfate gels

phate, 10 mM EDTA at pH 6.0. This solution was heated at 65° exactly as described by Marshall and Cohen (3). The supernatant after centrifugation was mixed with Celite 545 (Fisher) and precipitated with ammonium sulfate (Mann enzyme grade) at a final concentration of 3.5 M in 100 mM sodium phosphate, 10 mM EDTA, pH 6. This mixture was poured into a column and chromatographed as described by King (5) in a 300 ml gradient of 3.5 to 1.0 M ammonium sulfate in the same buffer. Enzyme activity eluted at approximately 2 M salt. This material was in some cases homogeneous by dodecyl sulfate gel electrophoresis; in other cases small amounts of 45,000 and 30,000 dalton polypeptides were also observed.

The salt-gradient enzyme could be further purified by glycerol gradient centrifugation after dialysis and concentration on Amicon XM-50 filters. Gradients (10 to 30%) were spun for 15 hr at 60,000 rpm in the SB 405 rotor of an IEC B60 ultracentrifuge.

All steps (except for the heat treatment) were performed at 0 to 4°. Table I summarizes a typical preparation.

Dodecyl Sulfate Gel Electrophoresis: Electrophoresis was performed in the borate buffer system as described (1). Tube gels contained 7.5% acrylamide, 0.2% methylenebisacrylamide, and 0.1% dodecyl sulfate. Slab gels (0.08 x 13.8 x 24 cm) contained a gradient (in the long dimension) of 7.5 to 15% acrylamide, 0.2 to 0.4% methylenebisacrylamide. The designation of polypeptide bands is as described (1).

RESULTS

As described in Methods, ornithine transcarbamylase has been purified from rat liver mitochondria to give a preparation with a specific activity of 250 μmol/min/mg protein. When subjected to dodecyl sulfate gel electrophoresis, a single polypeptide chain of 36,000 daltons is obtained (Fig. 1). This polypeptide chain comigrates with band Va, one of the major polypeptide components of rat liver mitochondria, on dodecyl sulfate disc gel electrophoresis and on slab gel electrophoresis (Fig. 2), where a better resolution of individual polypeptide bands is obtained.

Evidence presented in Fig. 3 suggests that no other mitochondrial proteins are components of band Va. All of the band Va material co-chromatographs with ornithine transcarbamylase activity on gel filtration and during glycerol gradient centrifugation. Analysis of these fractions, and of the various fractions obtained during the purification of the enzyme, indicates that the amount of material at the band Va position is proportional to the activity

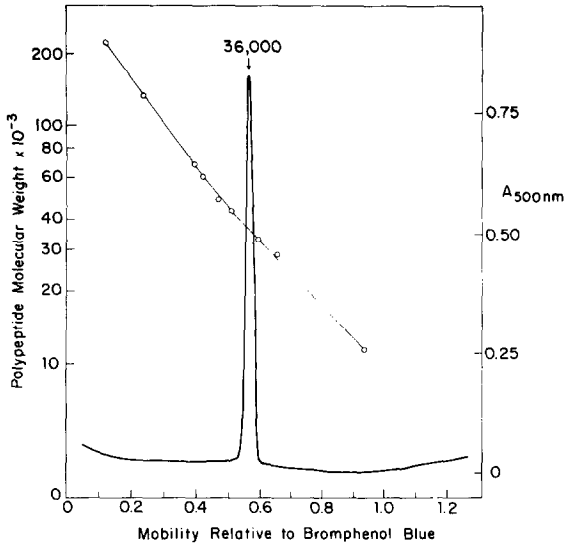

Fig. 1. Dodecyl sulfate disc gel electrophoresis of purified rat liver ornithine transcarbamylase. The gel was stained with Coomassie and scanned for optical density at 500 nm (scale at right). The polypeptide molecular weight was determined by comparison with the mobilities of marker proteins electrophoresed on separate gels. These standards include myosin (220,000), β-galactosidase (135,000), serum albumin (68,000), catalase (60,000), fumarase (48,500), ovalbumin (43,500), malate dehydrogenase (33,000), carbonic anhydrase (29,000), and cytochrome c (11,500).

of this enzyme. In contrast to this situation, in which a band on dodecyl sulfate gels appears to contain only a single molecular species, band IV polypeptides (51,000 daltons) are separated into several fractions by gel filtration and glycerol gradient centrifugation (Fig. 3).

The data obtained in Fig. 3 can also be used to determine the native molecular weight of the rat liver enzyme. From the interpolated sedimentation coefficient of 6.2 S and the diffusion coefficient of 5.4×10^{-7} cm^2/sec, a molecular weight of 107,000 is calculated ($\bar{v}$ assumed to be 0.74 cm^3/g). This value suggests that a trimeric structure is likely for this enzyme. The rarity of such quaternary structures (7) increases the significance of the coincident elution of the enzyme and the band Va polypeptide in systems that separate proteins by size.

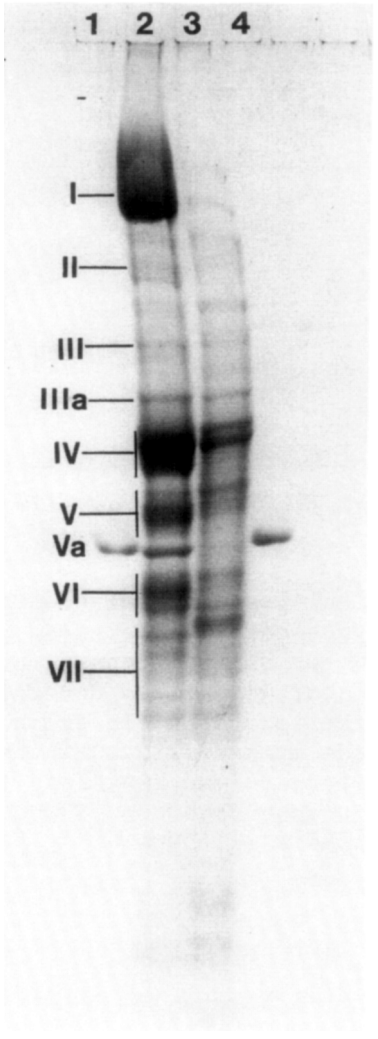

Fig. 2. Dodecyl sulfate slab gel electrophoresis of rat liver and kidney mitochondria which shows that purified ornithine transcarbamylase comigrates with band Va. 1,4, purified enzyme; 2, liver mitochondria; 3, kidney mitochondria. Labeling of bands is as described (1).

Fig. 2 shows that the band Va polypeptide is absent from rat kidney mitochondria. These mitochondria also lack ornithine transcarbamylase activity (8).

A single N-terminal amino acid residue (serine) has been detected in the purified enzyme preparation by the method of Weiner et al. (9).

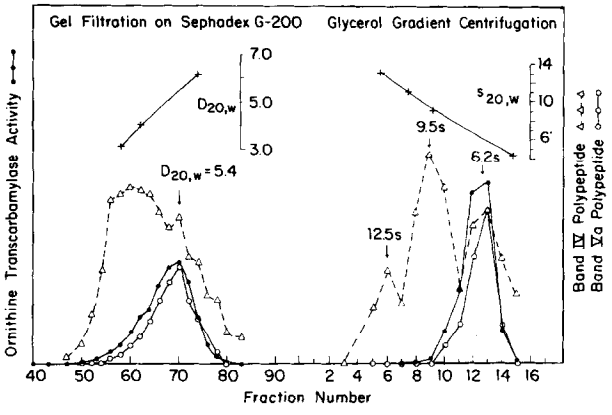

Fig. 3. Comigration of ornithine transcarbamylase and the band Va polypeptide on gel filtration and glycerol gradient centrifugation. Left, a freeze-thaw extract of rat liver mitochondria was chromatographed on a 2.5 x 86 cm column of Sephadex G-200 in 0.05 M Tris·H_2SO_4 pH 7.8. Fractions were assayed for enzyme activity and aliquots were electrophoresed on dodecyl sulfate gels. The amount of material in band IV and band Va polypeptides was quantitated by densitometry of Coomassie-stained gels. The apparent $D_{20,w}$ was determined from an interpolation of the peak positions of the activities of glutamate dehydrogenase (3.1×10^{-7} cm^2/sec), fumarase (4.0), and malate dehydrogenase (6.1) measured in the same fractions. Right, extract was centrifuged for 10 hr at 53,000 rpm on a glycerol gradient and the fractions were analyzed as above. Marker enzymes assayed in the same gradient to determine $s_{20,w}$ were glutamate dehydrogenase (13.2 S), catalase (11.1 S), fumarase (9.1 S), and malate dehydrogenase (4.3 S).

DISCUSSION

Although a homogeneous, well-characterized preparation of ornithine transcarbamylase has been obtained from bovine liver (3), the rat liver enzyme has not been so well described. The results presented here, however, indicate that the properties of the rat liver enzyme are very similar to those of the bovine enzyme. Both enzymes appear to be trimers of 36,000 dalton polypeptides. The bovine enzyme has aspartic acid as its N-terminal residue whereas the rat enzyme has serine. The specific activity measured here for the rat enzyme is approximately three-fold lower than that of the bovine enzyme.

Most importantly, however, the present results show that ornithine transcarbamylase is one of the major protein components of rat liver mitochondria, accounting for 3 to 4% of the total protein. Thus, like the other mitochondrial urea cycle enzyme carbamyl phosphate synthetase (2), this enzyme may be an ideal candidate for investigating the biosynthesis and transport of mitochondrial matrix enzymes. Furthermore, the unique migration position of this enzyme on dodecyl sulfate gels may facilitate the analysis of mutant forms of ornithine transcarbamylase described in mouse liver (10) and in human liver (11).

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