Human Ornithine Transcarbamylase

PURIFICATION AND CHARACTERIZATION OF THE ENZYME FROM NORMAL LIVER AND THE LIVER OF A REYE'S SYNDROME PATIENT

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Ornithine transcarbamylase was purified and characterized from normal human liver. The properties of this enzyme were compared to those of ornithine transcarbamylase purified from the liver of a patient with Reye's syndrome. The enzyme isolated from both sources appeared virtually identical for a variety of biochemical characteristics. The native molecular weight of ornithine transcarbamylase is 110,000 as determined by gel filtration. Electrophoresis of the enzyme, dissociated by sodium dodecyl sulfate, indicated that the enzyme exists as a trimer of identical or similar subunits of 38,500 daltons. Ornithine transcarbamylase from normal liver has an isoelectric point of 7.95, and the value for the enzyme from the Reye's syndrome liver was 8.05. No evidence of multiple species was found during the purification or subsequent characterization of the enzyme. The enzyme exhibited normal Michaelis-Menten kinetics, and the apparent Michaelis constants for L-ornithine and carbamyl phosphate are 0.20 mM and 0.09 mM, respectively. Inhibitor studies established the structural requirements for L-ornithine antagonists. L-Norvaline is the best competitive inhibitor of the enzyme with respect to L-ornithine. This study indicated that the reduced level of ornithine transcarbamylase activity commonly observed in Reye's syndrome is not necessarily due to structural or functional alterations of the enzyme.

The urea cycle consists of five enzymes, three of which are located in the cell's cytoplasm and two in the matrix of the mitochondrion (1–5). The main function of these enzymes is to eliminate ammonia through its conversion to urea. The two mitochondrial enzymes, carbamyl phosphate synthetase (EC 2.7.2.5) and ornithine transcarbamylase (EC 2.1.3.3), have been purified and characterized from both rat and bovine liver (6–14). However, studies on these two enzymes have been few and have relied primarily upon data obtained from crude enzyme preparations (15–17).

Both of these enzymes have been reported to be associated with metabolic disorders in man. Reye's syndrome, characterized by encephalopathy and fatty infiltration of the liver, is one such disease (18, 19). Numerous biochemical abnormalities, including hyperammonemia, are evident during the course of the disease. Although some of the biochemical imbalances are undoubtedly related and may have a common origin, determination of an initial causative metabolic lesion is difficult. However, Thaler (20) has described a sequence of clinical and pathological occurrences stemming from the hyperammonemic condition and encompassing a majority of the clinical and biochemical manifestations. Hyperammonemia could result from a dysfunction of one or more of the urea cycle enzymes. Since mitochondrial damage has been a consistent finding in the livers of Reye's syndrome patients (19), it seems plausible that one or both of the mitochondrial urea cycle enzymes could be structurally or functionally altered. Both of these enzymes have been reported to have reduced specific activities in crude liver homogenates prepared from patients with Reye's syndrome (21–28). The molecular basis for the reduction in the activities is unclear; less enzyme could be synthesized, increased proteolytic degradation may occur, or the enzymes may be altered in such a way as to decrease the turnover number. Previous studies to detect and define alterations in carbamyl phosphate synthetase and ornithine transcarbamylase from patients with Reye's syndrome have been complicated by the fact that neither enzyme has been purified. The need for purified enzyme for comparison of biochemical properties prompted the present investigation, which involves the purification and characterization of ornithine transcarbamylase, the second of the two enzymes.

EXPERIMENTAL PROCEDURES

Materials

Liver tissue was obtained 4 h after death from individuals with no history of liver disease and from a 5-month-old female with Reye's syndrome. The diagnosis was based on the following criteria: a typical prodromal illness, elevated blood ammonia and serum transaminases, increased prothrombin time, and a histological examination of liver tissue. The liver samples were stored at −70°C until homogenized.

Dilithium carbamyl phosphate, L-ornithine, Triton X-100, triethanolamine-HCl, amino acids, molecular weight standards, and Sephadex G-200 were purchased from Sigma Chemical Co. Hydroxylapatite (Bio-Gel HTP) and all chemicals for polyacrylamide gel electrophoresis were obtained from Bio-Rad Laboratories. Whatman was...
the source of the DEAE-cellulose (DE52). Amphotile carrier buffers were purchased from LKB (pH 5 to 8) and Brinkman (pH 7 to 9). All other chemicals were of the highest purity commercially available.

Measurement of Ornithine Transcarbamylase

Ornithine transcarbamylase activity was measured by a modification of the method of Ceriotti (29). Unless specified otherwise, reaction mixtures contained 0.5 μmol of carbamyl phosphate; 0.5 μmol of L-ornithine; 10 μmol of triethanolamine, pH 8.0, and enzyme in a final volume of 0.2 ml. Triethanolamine buffer was used in the assay mixture because consistently higher specific activities were obtained with this buffer compared to Tris or glycylglycine buffers. Following incubation for 10 min at 37°, the reaction was stopped by the addition of 3.0 ml of the chromogenic reagent described by Ceriotti (29). The composition of the chromogenic reagent is as follows: Solution A consists of 4 g of antipyrine, 50 mg of Pb(SO₄)₂, 9H₂O, and 100 ml of H₂SO₄ diluted to 1 liter; Solution B consists of 5 g of diacetyl monoxime, 3 g of Brij 35, and 50 ml of acetic acid diluted to 1 liter. Equal volumes of Solutions A and B are mixed before use. The reaction tubes were covered, heated at 90° for 15 min, and cooled at room temperature before absorption at 460 nm was determined with a Gilford 240 spectrophotometer. All assays were conducted under conditions such that citrulline production was directly proportional to the enzyme concentration for the time interval of the assay. Specific activity is expressed as micromoles of citrulline produced per min per mg of protein. Protein concentration was estimated as described by Lowry et al. (30) using bovine serum albumin (Fraction V) as the standard.

Isolation of Ornithine Transcarbamylase

**Step 1: Homogenate**—Five grams of liver were minced and homogenized (Ten-Broeck tissue grinder) in 15 ml of 10 mM triethanolamine buffer, pH 8.0, containing 0.3% Triton X-100. This and all subsequent procedures were carried out at 4°.

**Step 2: Extract**—Particulate debris was removed from the homogenate by centrifugation at 45,000 × g for 15 min. The pellet was washed with 5 ml of the homogenization buffer and centrifuged as before. The two supernatant fractions were combined.

**Step 3: DEAE-chromatography**—The extract was applied to a column of DEAE (18 by 25 cm) equilibrated in 10 mM triethanolamine buffer, pH 8.0, as described in Ref. 31. The ornithine transcarbamylase activity did not bind to the DEAE, and was completely eluted by washing the column with 30 ml of the equilibration buffer.

**Step 4: Hydroxylapatite Chromatography**—Fractions containing the ornithine transcarbamylase activity from the DEAE-column (35 ml) were combined and applied to a column containing Bio-Gel HTP (1.5 × 15 cm) equilibrated in 10 mM potassium phosphate buffer, pH 7.0. The column was washed with 50 ml of 0.2 M potassium phosphate buffer, pH 7.0. Ornithine transcarbamylase activity was eluted with a 300-ml linear gradient of potassium phosphate, pH 7.0 (0.2 to 0.5 M), and 2.0-ml fractions were collected. The fractions containing ornithine transcarbamylase activity were pooled, concentrated, and dialyzed against 10 mM potassium phosphate buffer, pH 7.0, in an Amicon Diaflo cell (PM-10 membrane). The final volume was 4 ml.

**Step 5: Gel Filtration**—The dialyzed preparation from hydroxylapatite was applied to a Sephadex G-200 column (2.5 × 90 cm) equilibrated in 10 mM potassium phosphate buffer, pH 6.0. The column was eluted (ascending) with the equilibration buffer, and 2.0-ml fractions were collected. Fractions containing ornithine transcarbamylase activity were pooled and concentrated in an Amicon Diaflo cell (PM-10 membrane).

Molecular Weight Determination

Initially the molecular weights were determined by calibration of the Sephadex G-200 column used for the final step in the purification scheme (Step 5). However, limiting amounts of available liver from the Reye's syndrome patient made multiple determinations impractical with enzyme of this purity. Thus, extracts were prepared from 200 mg of liver in 10 mM triethanolamine buffer, pH 8.0, containing 0.3% Triton X-100. The extracts were applied to a Sephadex G-200 column (1.5 × 55 cm) equilibrated in 10 mM triethanolamine buffer, pH 8.0. The column was eluted (ascending) with the equilibration buffer, and 2.0-ml fractions were collected. The Sephadex G-200 column was calibrated according to the method of Andrews (32) using the following molecular weight standards: γ-globulin (Mᵣ = 160,000), lipoxidase (Mᵣ = 102,000), bovine serum albumin (Mᵣ = 68,000), and chymotrypsinogen (Mᵣ = 25,000).

**Electrofocusing**

A sucrose-stabilized linear pH gradient (pH 5 to 8 or pH 7 to 9) of 2% ampholytes was prepared in an LKB (model 8101) electrofocusing column. Ornithine transcarbamylase (purified through the hydroxylapatite step) was added to the dense solution and distributed throughout the gradient. The electrofocusing was carried out at 4° at a final voltage of 700 V for 72 h. After completion of the run, 1-ml fractions were collected, and the pH was determined at 4°. The fractions containing ornithine transcarbamylase activity were concentrated and dialyzed against 10 mM triethanolamine buffer, pH 8.0, in an Amicon Diaflo cell (PM-10 membrane).

**Electrophoresis**

Electrophoresis was performed under dissociating conditions using 7.5% acrylamide precast gels and Tris/acetate/sodium dodecyl sulfate buffer, pH 6.6, as described in Ref. 33. Purified ornithine transcarbamylase (from electrofocusing, pH 7 to 9) was precipitated with 5% trichloroacetic acid, held at 4° for 20 min, and centrifuged at 800 × g for 10 min. The precipitate was washed once with acetone, and dissolved in Bio-Phore sodium dodecyl sulfate buffer diluted 1:5. The solubilized protein was then heated at 95° for 5 min. The dissociated protein (25 μg) was layered on the top of the gel and electrophoresed at 6 mA/gel at 15° for 5 h. After electrophoresis the gels were fixed in an isopropl alcohol:acetic acid:H₂O solution (40:10:50) overnight. The gels were stained in 0.05% Coomassie brilliant blue in 7% acetic acid for 3 h and destained in 7% acetic acid. The absorbance at 550 nm was determined by a Quick Scan gel scanner (Helena Laboratories). The molecular weight of the ornithine transcarbamylase polypeptide was determined by the method of Weber and Osborn (34). The molecular weight standards were bovine serum albumin (Mᵣ = 68,000), ovalbumin (Mᵣ = 45,000), and chymotrypsinogen (Mᵣ = 25,000).

**RESULTS**

Purification of Ornithine Transcarbamylase—Ornithine transcarbamylase was isolated and purified from both normal human liver and a liver from a Reye's syndrome patient. The purification procedure is described under "Experimental Procedures" and summaries of the results are shown in Tables I and II. The data in Table I are the averages of three experiments; those in Table II are from a single purification. Although ornithine transcarbamylase did not bind to DEAE, a 5- to 8-fold purification was achieved by passage of the extract through a column of DEAE. No additional enzyme activity

| Fraction | Total protein | Total units | Specific activity | Yield | Purification |
|----------|---------------|-------------|------------------|-------|--------------|
| Homogenate | 860 | 449 | 0.58 | 0.08 |
| Extract | 517 | 403 | 0.78 | 90 | 1.3 |
| DEAE | 103 | 332 | 3.2 | 73 | 5.6 |
| Hydroyxylapatite | 3 | 163 | 54.4 | 36 | 93.8 |
| Sephadex G-200 | 0.8 | 71 | 86.5 | 16 | 149.1 |

**RESULTS**

| Fraction | Total protein | Total units | Specific activity | Yield | Purification |
|----------|---------------|-------------|------------------|-------|--------------|
| Homogenate | 594 | 214 | 0.42 | 97 | 1.2 |
| Extract | 495 | 208 | 0.42 | 97 | 1.2 |
| DEAE | 51 | 142 | 2.8 | 66 | 7.8 |
| Hydroyxylapatite | 0.8 | 74 | 87.0 | 35 | 241.7 |
| Sephadex G-200 | 0.5 | 43 | 83.3 | 20 | 231.4 |
could be demonstrated when the column was eluted with increasing concentrations of KCl (0 to 1.0 M).

Ornithine transcarbamylase did bind to hydroxylapatite and was eluted with a potassium phosphate gradient (pH 7.0). The enzyme eluted from the hydroxylapatite column at a potassium phosphate concentration of 0.3 M. The fractions were assayed as previously described, but the carbamyl phosphate concentration was increased to 2 μmol/0.2-ml reaction volume in order to compensate for the competitively inhibitory effect of inorganic phosphate. Marshall and Cohen (11) found inorganic phosphate to be inhibitory by competing with carbamyl phosphate for a binding site on bovine ornithine transcarbamylase. The present investigators found the same to be true of the human enzyme from both normal and Reye’s syndrome livers. A 20- to 30-fold purification of the enzyme (with 50% recovery) was obtained by the hydroxylapatite procedure.

The final step of the purification procedure involved gel filtration of the enzyme through a Sephadex G-200 column. Following this procedure, the specific activities of the enzymes from both sources were virtually the same (Tables I and II) while the electrophoretic purity of both enzymes was improved over the previous step. The purification procedure described under "Experimental Procedures" represents the most rapid method for obtaining ornithine transcarbamylase of maximal specific activity in our laboratory.

**Kinetic Studies**—Ornithine transcarbamylase, purified from both normal and Reye’s syndrome livers, was utilized for kinetic studies. The Michaelis constants (K,') were determined for both substrates, L-ornithine and carbamyl phosphate, by Lineweaver-Burk plots (35). Snodgrass (15) and Cathelineau et al. (36) have demonstrated a dependence of the Michaelis constant for L-ornithine upon pH. Thus, in these studies, the pH was carefully maintained by 50 mM triethanolamine buffer, pH 8.0. The graphical determinations of the apparent K, values for L-ornithine and carbamyl phosphate of the normal enzyme are shown in Fig. 1. The double reciprocal plot of velocity versus substrate concentration yields a straight line for each substrate, indicating adherence to simple Michaelis-Menten kinetics. Upon extrapolation, the apparent K, for L-ornithine was determined to be 0.20 mM, and the apparent K, for carbamyl phosphate was 0.09 mM. Michaelis constants for ornithine transcarbamylase isolated from Reye’s syndrome liver are the same as those of the enzyme from normal liver (Fig. 2). In this study, the lower specific activity in the Reye’s syndrome liver homogenate and extract was not the result of an altered Michaelis constant for either substrate (Tables I and II). In addition the kinetic parameters of ornithine transcarbamylase in crude extracts were found to be the same as for the purified enzyme.

**Inhibitor Studies**—A variety of compounds structurally similar to L-ornithine was tested for the ability to inhibit ornithine transcarbamylase activity. The pattern of inhibition for the Reye’s syndrome enzyme is virtually identical with that of the normal enzyme (Table III). L-Norvaline is the most potent inhibitor listed. Kinetic analysis indicated that L-norvaline was a competitive inhibitor of human ornithine transcarbamylase activity (data not shown). Similar inhibition of the bovine enzyme by L-norvaline has been demonstrated (11). Inhibition of ornithine transcarbamylase activity by 4-pentenoic acid in crude homogenates of rat liver has been reported (25). No inhibition of purified human enzyme was observed. In addition, the possible presence of an inhibitor in the Reye’s syndrome liver was suggested from the data and a series of mixing experiments were performed in an effort to detect such an effect. No evidence of any endogenous inhibitor of ornithine transcarbamylase could be found.

**Electrofocusing**—Partially purified ornithine transcarbamylase from both normal and Reye’s syndrome livers was electrofocused as described under "Experimental Procedures." The enzyme was initially focused in a pH gradient of 5 to 8; the isoelectric point (pI) was determined to be approximately 8.0. Subsequently, a pH gradient of 7 to 9 was utilized to define

![Lineweaver-Burk plots of velocity versus substrate concentration for ornithine transcarbamylase from normal liver.](http://www.jbc.org/)

![Lineweaver-Burk plots of velocity versus substrate concentration for ornithine transcarbamylase from Reye's syndrome liver.](http://www.jbc.org/)

| Inhibitor          | Normal | Reye’s syndrome |
|--------------------|--------|-----------------|
| L-Norvaline        | 0.24   | 0.24            |
| L-2-Amino-4-pentenoic acid | 0.47   | 0.40            |
| L-2-Aminobutyrate  | 1.4    | 1.5             |
| L-2,4-Diaminobutyrate | 3.8    | 4.6             |
| L-Leucine          | 3.9    | 4.0             |
| L-Isoleucine       | NI     | NI              |
| L-Alanine          | NI     | NI              |
| L-Norleucine       | NI     | NI              |
| L-Lysine           | NI     | NI              |
| 4-Pentenoic acid   | NI     | NI              |
| 2,4-Diaminobutane  | NI     | NI              |

* Values listed are the concentrations required for 50% inhibition of activity.
* Noninhibitory (less than 50% inhibition at 20 mM).
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The lower gel and scan were obtained with ornithine transcarbamylase from normal and Reye’s syndrome livers were 7.95 and 8.05, respectively. Similar results were found when crude extracts of human liver were subjected to electrofocusing.

Molecular Weight and Subunit Composition—The molecular weight of human ornithine transcarbamylase from both normal and Reye’s syndrome livers was determined by gel filtration. No evidence was found in the present study for multiple species of the enzyme. The molecular weight of ornithine transcarbamylase from both sources was 110,000. The subunit composition was determined by polyacrylamide gel electrophoresis under dissociating conditions. Purified enzyme (from DEAE, hydroxylapatite, and electrofocusing) was dissociated as described under “Experimental Procedures” and subsequently electrophoresed. The subunit molecular weight of ornithine transcarbamylase isolated from normal liver is shown in the upper gel and Reye’s syndrome liver was found to be 36,500 (Fig. 4). The results of gel filtration and polyacrylamide gel electrophoresis indicate that the native enzyme is comprised of three similar or identical subunits of 36,500 daltons.

Discussion

The present study is concerned with the purification and characterization of ornithine transcarbamylase from human liver, and a comparison of the properties of the enzyme from normal human liver and from a liver of a patient with Reye’s syndrome. The purification of ornithine transcarbamylase from both normal and Reye’s syndrome livers was achieved by column chromatography. While previous investigators have relied mainly on the solubility and heat stability properties of the enzyme (10, 13, 14), the present procedure utilized DEAE-cellulose, hydroxylapatite, and Sephadex G-200 chromatography to achieve a simple and rapid purification. The enzyme was purified approximately 150-fold from intact liver within 2 days.

Abnormal kinetic properties of ornithine transcarbamylase have been reported for two diseases characterized by hyperammonemia, Reye’s syndrome and ornithine transcarbamylase deficiency disease. Investigators have speculated that in the latter disease a decreased affinity of the enzyme for L-ornithine or carbamyl phosphate results in the accumulation of toxic levels of ammonia (36, 37). Similar abnormal kinetic data have been reported for ornithine transcarbamylase from one Reye’s syndrome patient with an 18-fold increase in the $K_m$ for L-ornithine (21). No kinetic abnormalities of purified ornithine transcarbamylase from Reye’s syndrome liver were discerned in the present study (Figs. 1 and 2). The apparent $K_m$ values for L-ornithine and carbamyl phosphate were 0.20 and 0.09 mm, respectively. These values are similar to previously reported values (26, 27, 36, 38). There was no indication of any inhibition by either substrate as had been reported (15, 27).

The present study does not support the hypothesis of kinetic alterations in ornithine transcarbamylase which could result in the hyperammonemia characteristic of Reye’s syndrome; however, since it is based on the kinetic evaluation of the enzyme from only one patient, the data presented do not categorically eliminate the possibility that kinetic variations could play a role in some cases of the disease.

Various compounds were tested for possible modulating effects on ornithine transcarbamylase activity. L-Norvaline (2-amino-pentanoic acid) is structurally similar to L-ornithine (2,5-diaminopentanoic acid) and serves as a competitive inhibitor (data not shown) of the human enzyme (Table III). Similar inhibition of the bovine enzyme has been reported (11). Interestingly, 2,4-diaminobutyrate is a relatively poor inhibitor, while L-lysine (2,6-diaminohexanoic acid) was noninhibitory at 20 mM. These results, coupled with the potent inhibition afforded by L-norvaline, strongly suggest that the length of the side chain is more important than the presence of the amino group on the side chain. This is further illustrated by the lack of inhibition by L-norleucine (2-amino-hexanoic acid). The side chain of this aliphatic amino acid is only one methylene group longer than L-norvaline. The necessity for the $\alpha$-carboxyl and $\alpha$-amino groups is demonstrated by the noninhibitory nature of 2,4-diaminobutyrate and 4-pentenoic acid, respectively.

2-Amino-4-pentenoic acid is an analog of hypoglycin (39, 40). The symptoms of Jamaican vomiting disease are quite similar to those of Reye’s syndrome. Administration of 2-amino-4-pentenoic acid to rats induces hyperammonemia and clinical symptoms which parallel those of the two diseases (41).
Amino-4-pentenoic acid is structurally similar to l-norvaline, and is likewise a very potent inhibitor of human ornithine transcarbamylase activity (Table III). This compound also inhibits the enzyme in intact mitochondria.  

Methylsarcosylepropylacetic acid is a metabolite of hypoglycin and has been reported to be responsible for at least some of the symptoms of Jamaican vomiting disease (39). 4-Pentenoic acid is an analog of this compound, and when administered to rats, will produce most of the symptoms exhibited in Jamaican vomiting disease (42). 4-Pentenoic acid did not directly inhibit ornithine transcarbamylase from rat liver (25). Similarly, in the present study, no inhibitory effect was found with the purified human enzyme at concentrations up to 50 mM (data not shown).

Previous reports on the biophysical characteristics of ornithine transcarbamylase have demonstrated the presence of more than one species of the enzyme in the human liver. However, in the present study only one peak of enzyme activity was found by electrofocusing in either the normal or the diseased liver. The isoelectric points of ornithine transcarbamylase from normal and Reye's syndrome liver were 7.95 and 8.05, respectively. These values for purified human enzyme are in agreement with that reported by Reichard, who found a pI of 8.0 for the enzyme isolated from rat liver (14). Arashima et al. (17) have demonstrated two major species of ornithine transcarbamylase in a crude human liver homogenate by electrophoresis. The isoelectric points of the two species were 3.2 and 4.4. The reason for the discrepancies in isoelectric points is unclear.

The native molecular weight of human ornithine transcarbamylase from both normal and Reye's syndrome livers was determined by gel filtration to be 110,000. This is in good agreement with that reported by Reichard, who found a native molecular weight of 110,000 for the enzyme isolated from rat liver (14). Arashima et al. (17) have demonstrated two major species of ornithine transcarbamylase in a crude human liver homogenate by electrophoresis. The isoelectric points of the two species were 3.2 and 4.4. The reason for the discrepancies in isoelectric points is unclear.

Amino-4-pentenoic acid is structurally similar to L-norvaline, and is likewise a very potent inhibitor of human ornithine transcarbamylase activity (Table III). This compound also inhibits the enzyme in intact mitochondria.  

Preparations from both normal and Reye's syndrome livers were found to be identical for a variety of properties. Moreover, human ornithine transcarbamylase isolated from two other mammalian liver sources, rat and bovine, the rat and bovine ornithine transcarbamylase (10, 13).

The purification and characterization of the human enzyme revealed many similarities to ornithine transcarbamylase isolated from other mammalian liver sources, rat and bovine. The most striking of these properties are the native molecular weight, the apparent subunit structure, and the pattern of inhibition by L-ornithine-related compounds. In addition, ornithine transcarbamylase isolated from normal and Reye's syndrome livers was found to be identical for a variety of properties. Table IV summarizes the findings and demonstrates that no significant differences exist between the normal enzyme and that isolated from this particular case of Reye's syndrome.

The similarities in enzyme activity and biophysical properties studied here suggest that the biochemical lesion associated with Reye's syndrome does not involve a functional alteration of ornithine transcarbamylase. There are two possible alternatives: (a) an alteration in the first enzyme of the urea cycle, carbamyl phosphate synthetase, which is also located in the mitochondrion, and (b) a structural alteration of the mitochondrion which might affect enzyme activity or transport of the urea cycle intermediates. The regulation of carbamyl phosphate synthetase activity may be the most important step in the regulation of the urea cycle. McGivan et al. (43) have suggested that carbamyl phosphate synthetase may regulate urea synthesis in rats by virtue of its dependence upon N-acetyl glutamate for activity. On the other hand, since both carbamyl phosphate synthetase and ornithine transcarbamylase are situated in the intramitochondrial matrix in vivo, it may be more important to examine the overall reaction from ammonia to citrulline in intact mitochondria. It is possible that an organizational defect may exist in the mitochondria which may interfere with the carbamyl phosphate synthetase-ornithine transcarbamylase interaction, but may not be manifested in the purified enzymes. Mitochondrial swelling and intramitochondrial disorganization are commonly seen in cases of Reye's syndrome. These possible modes of regulation of the mitochondrial urea cycle enzymes are currently under examination.

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