This paper presents the first purification of the branched chain aminotransferase (EC 2.6.1.42) from rat heart mitochondria. The enzyme has been purified from the 100,000 x g supernatant obtained after sonication and ultracentrifugation of rat heart mitochondria. A combination of open column chromatography, high pressure liquid chromatography (HPLC), and discontinuous polyacrylamide disc gel electrophoresis was used. The key step in the procedure was hydrophobic interaction chromatography on HPLC. The final purification step was polyacrylamide disc gel electrophoresis where the enzyme appeared as a doublet. When electroeluted from the gel, each of these bands had the same specific activity demonstrating that there are two forms of the purified enzyme which differ slightly in electrical charge. On sodium dodecyl sulfate-polyacrylamide gel electrophoresis, these two enzyme forms appeared as a single band with a molecular mass of 43 kDa. Size exclusion chromatography on Sephacryl S-100 identified the enzyme as a 50-kDa protein. These experiments argue against the existence of a dimeric form of this enzyme. The ratio of enzyme activity with leucine (0.84), valine (0.88), or glutamate (0.66) as amino acid substrate versus isoleucine remained constant throughout the purification procedure. Specific activity of the final preparation was 66 units/mg of enzyme protein.

Polyclonal antibodies against the purified enzyme were raised in rabbits. On an immunoblot the antisera recognized a 43-kDa protein in the 100,000 x g supernatant from a rat heart mitochondrial sonicate but did not recognize any proteins in rat brain cytosol. Quantitative immunodot assay resulted in an estimated enzyme content of about 100 µg of branched chain aminotransferase protein/g of heart, wet weight. Finally, 97% of the heart branched chain aminotransferase activity could be neutralized by the antisemum, but the antisemum would not neutralize aminotransferase activity in brain cytosol. These data suggest that close sequence homology does not exist between the two proteins.

Purification of Branched Chain Aminotransferase from Rat Heart Mitochondria*

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\[ \text{EC 2.6.1.42} \]

**Experimental Procedures**

Preparation of Mitochondria and Brain Cytosol

Male Sprague-Dawley rats were used. Heart mitochondria were prepared as described by LaNoue et al. (12). The isolation medium contained 0.225 M mannitol, 0.075 M sucrose, 0.1 mM EDTA, 5 mM Mops, pH 7.0. For purification of the branched chain aminotransferase, 1 mM DIFP was added to the 600 x g supernatant before

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1. The abbreviations used are: Mops, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Heps, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DIFP, diisopropyl fluorophosphate; DT, dl-dithiothreitol; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; CHAPS, 3-[[(3-cholamidopropyl)dimethylammonio]1-propanesulfonic acid; KIV, a-ketoisovaleric acid.

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isolation of the mitochondria at 8,000 x g. DIFP was also included in the medium during the two wash steps. The final mitochondrial pellet was resuspended in a minimal volume of isolation medium (60–80 mg of mitochondrial protein/ml) containing DIFP. Mitochondrial protein was measured by the method in the presence of 0.95% deoxycholate using bovine serum albumin as the standard. The mitochondria were diluted with deionized water (1.3, v/v) in the presence of 1 mM DIFP and allowed to sit at 4 °C. The hypotonic mitochondrial suspension was then sonicated for 5 min at 70% duty cycle using a Branson model 250 sonifier. Subsequently, the mitochondrial sonicate was centrifuged at 100,000 x g for 1 h. DTT (1 mM) was added to the 100,000 x g supernatant before it was frozen at −70 °C. For samples prepared from tissue, the brains were removed rapidly and placed in the standard isolation medium containing 1 mM DIFP. The brains were blotted, weighed, diluted (1 g of tissue/2 ml of medium), and homogenized, and differential centrifugation was performed as described by Booth and Clark (13) except the 600 x g supernatant step was omitted for preparation of the 100,000 x g supernatant (cytosol).

**Determination of Branched Chain Aminotransferase Activity**

Amino transferase activity was measured at 37 °C in 50 mM Hepes buffer, pH 7.8 (KOH), which contained 50 μM pyridoxal phosphate, 0.5 mM DTT, and 0.5 mg/ml bovine serum albumin as described by Hutson et al. (10). The standard assay contained 1 mM L-[1-14C]KIV and 12 μM isoleucine. When amino transferase activity was determined in isolated mitochondria, 0.4% CHAPS was included in the assay to disrupt the mitochondrial membranes. A unit of enzyme activity was defined as 1 μmol of valine formed per min at 37 °C.

**Purification of Branched Chain Aminotransferase**

Steps 1–3 were carried out at 4 °C. Steps 4–7 were carried out at room temperature (22 °C). All buffers and solutions used for the purification procedure contained 1 mM DIFP and 1 mM DTT.

**Step 1. DEAE-cellulose Ion-exchange Chromatography**—The 100,000 x g supernatant of the mitochondrial sonicate was thawed, and fresh DTT (1 mM) and DIFP (1 mM) were added. The 100,000 x g supernatant was centrifuged at 10,000 x g in a Sorvall J5 centrifuge (Du Pont-Sorvall, Wilmington, DE) to remove any precipitated proteins before the supernatant was loaded onto a column of DEAE-cellulose (Whatman DE52) equilibrated with 10 mM potassium phosphate buffer, pH 7.5 (Buffer A). The column was washed extensively with Buffer A before the aminotransferase was stepwise released from the column in Buffer A containing 0.3 M NaCl.

**Step 2. Hydroxylapatite Chromatography**—The branched chain aminotransferase containing fraction from the ion exchanger was desalted on Sephadex G-25 in Buffer A. The desalted enzyme fraction in Buffer A was applied to a column of hydroxylapatite (Bio-Gel HT, Bio-Rad) equilibrated in the same buffer. The hydroxylapatite column was washed with 4-bed volumes of Buffer A before retained proteins were eluted with a phosphate gradient ranging from 10 mM (Buffer A) to 0.3 M potassium phosphate, pH 7.5. The branched chain aminotransferase was eluted at 0.1 M phosphate (see "Results").

**Step 3. Ammonium Sulfate Precipitation**—The branched chain aminotransferase-containing fraction from the hydroxylapatite column was mixed with an equal volume of ammonium sulfate which had been saturated at 4 °C and adjusted to pH 7.4 with NaOH. The mixture was left on ice for 15 min before precipitated proteins were removed by centrifugation at 10,000 x g. The supernatant which contained the branched chain aminotransferase (see "Results") was allowed to warm up to room temperature before the enzyme was subjected to HPLC chromatography.

**Step 4. Hydrophobic Interaction Chromatography Using HPLC**—The branched chain aminotransferase in 50% ammonium sulfate was applied to a Hydropre column supplied by Rainin Instrument Co. (Woburn, MA). The column was attached to a Rainin gradient HPLC system equipped with a Gilson 704 HPLC system manager and a variable wavelength detector. The column was equilibrated in 0.1 M potassium phosphate buffer, pH 7.0, containing 2 mM ammonium sulfate. Proteins retained by the column were eluted with a descending gradient of ammonium sulfate in the phosphate buffer. The gradient profile used was as follows: *Results.* The branched chain aminotransferase activity was determined on the initial sample and the void volume from each column. The hydrolyzed column was gel-filtrated on Sephadex S-100 packed in a fast protein liquid chromatography column supplied by Pharmacia (Uppsala, Sweden). The fast protein liquid chromatography column was attached to a Rainin gradient HPLC system for delivery of buffer. The Sephadex S-100 was equilibrated and eluted with 0.01 M potassium phosphate buffer, pH 7.5.

**Step 5. Discontinuous Electrophoresis at pH 9.1 and Electrodetection**—The gel electrophoresis system described by Davis (14) was used. Tube gels were made with a separating and concentrating gel. The branched chain aminotransferase-containing fraction from the Sephadex S-100 column was made 10% in sucrose and loaded in 100-μl aliquots onto premade gels. Electrophoresis was carried out at a constant current of 1.25 mA/gel. One gel was stained briefly with Coomassie Brilliant Blue to locate the marker for the position of proteins that had migrated into the gel. Gel pieces containing such proteins were sliced from the tube gel and placed in an isoceo model 1750 sample concentrator (Lscso, Inc., Lincoln, NE) for electrodetection in 0.01 M Tris acetate buffer, pH 8.6. The procedure provided by the manufacturer was followed in detail. Electrophoresed branched chain aminotransferase was used immediately for activity measurements. In steps 5 and 6 protein was measured with the micromethod described by Schaffner and Weissmann (15).

**SDS-PAGE**

SDS-PAGE was carried out according to Laemml (16) in 10% gels. Prior to electrophoresis, all samples were boiled for 2 min in the presence of 1% SDS, with or without 5% mercaptoethanol. Standard proteins used for molecular mass determinations were obtained from Bio-Rad. Gels were stained with Coomassie Brilliant Blue as described by Fairbanks et al. (17).

**Preparation of Antiserum**

Fifty μg of electrophoretically branched chain aminotransferase was mixed 1:1 (v/v) with Freund’s complete adjuvant and given intradermally at multiple sites on the back of a 5-week-old female New Zealand White rabbit (Franklin, Wake Forest, NC). After 4 weeks, 25 μg of enzyme emulsified in Freund’s incomplete adjuvant was given as a booster dose. One week after the last injection, 30 ml of blood was drawn from the rabbit by ear vein puncture, and serum was prepared by standard methods. Serum was stored at −20 °C.

**Immunoblotting**

Proteins in SDS-PAGE gels were transferred to Immobilon P membrane (Millipore Corp., Bedford, MA) for immunoblotting. The transfer was carried out in a Bio-Rad Trans Blott cell as described recently (18). The Immobilon P membrane was treated with 5% milk fat and reacted with antibodies as described by Reiderer et al. (18). Immunoreactive protein bands were visualized after horseradish peroxidase reduction of 4-chloro-1-naphthol (19).

**Immunofluorescence Chromatography of Branched Chain Aminotransferase**

An immunofluorescence resin was prepared by coupling the purified IgG fraction from antiserum against branched chain aminotransferase to CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden). IgG was purified on Protein G-Sepharose according to this manufacturer’s suggestion (Pharmacia, Uppsala, Sweden). IgG-Sepharose was prepared by coupling purified IgG (Sigma) to the CNBr-activated Sepharose. Sodium chloride (0.3 M final concentration) was added to fresh heart mitochondrial 100,000 x g supernatant and brain cytosol before loading each sample on the immunofluorescence assay. The membrane was treated with 5% nonfat dried milk as a blocking agent. Nonspecific binding was determined by using preimmune serum. These values were subtracted from values with specific antibodies to obtain the specific binding.

**Quantitative Immunodot Assay**

Defined amounts of purified branched chain aminotransferase (in the nanogram range) and 0.5, 1, and 2 μl of the mitochondrial matrix 100,000 x g supernatant were spotted in duplicate onto nitrocellulose membranes and incubated with antibodies and 125I-Protein A as described by Reiderer et al. (18). The membranes were treated with 5% nonfat dried milk as a blocking agent. Nonspecific binding was determined by using preimmune serum. These values were subtracted from values with specific antibodies to obtain the specific binding.
Mitochondrial Branched Chain Aminotransferase

Chemicals and Reagents

L-[1-14C]Valine was obtained from Amersham Corp. The radioactive [1-14C]KIV was synthesized from [1-14C]valine as described (20), and purity was checked routinely. Naseg was from Enzyme Research Corp. (New York). Pansorbin was obtained from Calbiochem. Bovine serum albumin (essentially fatty-acid- and globulin-free) was obtained from Sigma. All other reagents were reagent grade or better.

RESULTS

Branched Chain Aminotransferase Purification—The data presented in Table I describe purification of branched chain aminotransferase isolated from rat heart mitochondria. From 0.5 to 1 g of rat heart mitochondria was used routinely as starting material. The purification protocol included six steps and provided an electrophoretically homogenous enzyme preparation.

When sonicated in hypotonic media at 4 °C, the branched chain aminotransferase was extracted easily from mitochondria in high yield (see Table I). A significant purification of the enzyme was also obtained at this point. There was almost a 10-fold increase in specific enzyme activity in the supernatant recovered after centrifugation of the mitochondrial sonicate at 100,000 × g when the supernatant was assayed after freezing at −70 °C (see Table I). When stored at −70 °C in the presence of DTT, aminotransferase activity in the 100,000 × g supernatant was stable for at least 4 weeks. Aminotransferase activity was not stable to freezing without addition of DTT.

All enzyme activity was retained by the ion exchanger when the 100,000 × g supernatant from the mitochondrial sonicate was loaded onto a DEAE-cellulose DE52 column which had been equilibrated in 10 mM phosphate buffer, pH 7.5, at 4 °C (Buffer A) (Fig. 1). When a gradient of NaCl in the phosphate buffer was used for elution of the enzyme from the ion exchanger, only trace amounts of enzyme activity could be measured in column fractions. On the other hand, when the enzyme was stepwise-eluted from the column with 0.3 M NaCl dissolved in Buffer A, recovery of activity was >80% (Table I). DEAE-cellulose ion exchange chromatography yielded about a 3-fold purification of the enzyme (see Table I).

The next step in the procedure was hydroxylapatite chromatography carried out at 4 °C. The fractions shown in Fig. 1 which contained active enzyme were pooled and desalted on Sephadex G-25 in Buffer A. This pooled fraction was loaded onto a column of hydroxylapatite equilibrated in Buffer A. The hydroxylapatite column retained all branched chain aminotransferase activity. The enzyme eluted from the column at 0.1 M phosphate, when a 200-ml gradient ranging from 10 mM to 0.3 M phosphate was applied to the column. As shown in Fig. 2, hydroxylapatite chromatography separated the enzyme from the main protein peak eluting from the column.

This step resulted in about a 3-fold purification of the enzyme.

After hydrophobic interaction chromatography on HPLC, which was the next step in the procedure, a significant amount of enzyme activity was lost (see Table I). This step was, nevertheless, the key step in the procedure because it separated the branched chain aminotransferase enzyme from most of the contaminating proteins (Fig. 3). A precipitate formed when the enzyme-containing fractions from the hydroxylapatite column (Fig. 2) were mixed (1:1, v/v) with saturated ammonium sulfate on ice. However, no enzyme activity was measured in the precipitate, and all enzyme activity could be accounted for in the supernatant that was recovered after centrifugation. Therefore, the low recovery obtained at this step did not appear to result from enzyme loss by precipitation or inactivation by the ammonium sulfate. The ammonium sulfate gradient shown in Fig. 3 was designed to achieve the best separation of the enzyme from contaminating proteins. This chromatography step usually resulted in a 4–6-fold purification of the enzyme.

Two-ml aliquots of the pooled enzyme containing fractions from the Hydropore column (Fig. 3) were chromatographed on a Sephacryl S-100 column which was attached to the HPLC system. The column, which was equilibrated in phos-
was removed by centrifugation. The supernatant was allowed to warm up to room temperature before it was loaded in 2-ml aliquots onto a Hydropore HPLC column which had been equilibrated at 4 °C with 0.1 M potassium phosphate buffer, pH 7.0, containing 2 M ammonium sulfate. The column which was attached to a Rainin gradient HPLC system was eluted with a descending gradient of ammonium sulfate. The flow rate was 1 ml/min. —, optical density at 280 nm; W, branched chain aminotransferase activity.

The experimental Procedures), was eluted with 0.1 M phosphate buffer at a constant flow rate of 2 ml/min. V, indicates the elution volume versus log molecular mass. The following proteins were used as molecular mass standards: bovine fibrinogen, 340 kDa (V0); bovine albumin, 66 kDa; egg albumin, 45 kDa; carbonic anhydrase, 29 kDa; trypsinogen, 24 kDa. —, optical density at 280 nm; W, branched chain aminotransferase activity.

SDS-PAGE of proteins isolated at the various steps in the purification procedure and nondenaturing electrophoresis as the final step in the procedure resulted in an 800-fold purification of the enzyme (average specific activity of 66) with a final recovery of 4%.

After the Sephacryl S-100 column, DTT was not effective in stabilizing enzyme activity. Neither addition of substrate nor pyridoxal phosphate prevented the slow loss of activity. Thus, the specific activity of the final preparation after electrophoresis and electroelution was often lower than at the Sephacryl S-100 step. Instability of the purifed enzyme to freezing in the presence of β-mercaptoethanol has been reported (1, 21).

SDS-PAGE of the purified enzyme when carried out both under reducing (5% mercaptoethanol) and nonreducing conditions yielded a protein band of 43 kDa (Fig. 5, lane E). These two proteins had the same specific enzyme activity and thus appeared to be enzyme isoforms which differed in electrical charge. In support of this conclusion was the finding that both proteins, when subjected to sequence analysis, had the same N-terminal sequence.2 Discontinuous electrophoresis as the final step in the procedure resulted in an 800-fold purification of the enzyme (average specific activity of 66) with a final recovery of 4%.

After the Sephacryl S-100 column, DTT was not effective in stabilizing enzyme activity. Neither addition of substrate nor pyridoxal phosphate prevented the slow loss of activity. Thus, the specific activity of the final preparation after electrophoresis and electroelution was often lower than at the Sephacryl S-100 step. Instability of the purified enzyme to freezing in the presence of β-mercaptoethanol has been reported (1, 21).

Effects of Polyclonul Antibodies—Immunization of rabbits with 50 μg of purified enzyme protein resulted in an antiserum which neutralized enzyme activity and reacted with the enzyme protein after immunoblotting (see Table III and Fig. 6).

* T. R. Hall and S. M. Hutson, unpublished observations.
TABLE II
Relative activities for branched chain amino acids and glutamic acid during purification of branched chain aminotransferase

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Activity relative to isoleucine*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart mitochondria</td>
<td>Valine 0.92</td>
</tr>
<tr>
<td>100,000 × g supernatant</td>
<td>Leucine 0.90</td>
</tr>
<tr>
<td>DEAE-cellulose column</td>
<td>Glutamic acid 0.56</td>
</tr>
<tr>
<td>Hydroxylapatite column</td>
<td></td>
</tr>
<tr>
<td>Hydropropore column</td>
<td></td>
</tr>
<tr>
<td>Sephacryl S-100 column</td>
<td></td>
</tr>
<tr>
<td>Electrophoresis</td>
<td></td>
</tr>
<tr>
<td>Band I</td>
<td></td>
</tr>
<tr>
<td>Band 2</td>
<td></td>
</tr>
</tbody>
</table>

* Branched chain aminotransferase activity was determined using the standard assay with 1 mM [14C]KIV and 12 mM amino acid.

TABLE III
The effect of immunoaffinity chromatography on branched chain aminotransferase activity in heart mitochondria and brain cytosol

Antibodies against the purified rat heart mitochondrial enzyme were coupled to CNBr-activated Sepharose 4B to prepare an immunoaffinity column. The 100,000 × g supernatants from the heart mitochondrial sonicate and brain cytosol were applied to the immunoaffinity column or an IgG-Sepharose column which was used as a control. The heart mitochondrial sonicate and brain cytosol were prepared as described under "Experimental Procedures," and the 100,000 × g supernatants from the heart mitochondria were used immediately (see "Experimental Procedures"). Branched chain aminotransferase activity was measured before and after chromatography (see "Experimental Procedures").

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Heart mitochondrial supernatant</th>
<th>Brain cytosol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting material</td>
<td>0.294</td>
<td>0.136</td>
</tr>
<tr>
<td>Void from</td>
<td>0.010</td>
<td>0.134</td>
</tr>
<tr>
<td>A. Immunoaffinity column</td>
<td>0.275</td>
<td>0.141</td>
</tr>
<tr>
<td>B. IgG-Sepharose column</td>
<td>0.275</td>
<td>0.141</td>
</tr>
</tbody>
</table>

As shown in Table III, when the mitochondrial 100,000 × g supernatant was passed through an anti-branched chain aminotransferase affinity column or an IgG-Sepharose column prepared identically to the affinity column, the affinity column, but not the IgG-Sepharose column, retained the enzyme that was present in the heart mitochondrial supernatant. The antibodies recognized a 43-kDa protein when it was used to identify protein in the mitochondrial supernatant after immunoblotting (Fig. 6, lane B). As also shown in Table III, the enzyme in brain cytosol was not retained by the affinity column nor was it recognized by the antibodies upon immunoblotting (Fig. 6, lane A). In addition, partially purified brain enzyme did not react with these antibodies after immunoblotting (data not shown). These experiments raise questions about a close structural relationship between these enzymes since common epitopes did not appear to be present.

Quantitative immunodot assays were performed in order to estimate the amount of enzyme protein in isolated heart mitochondria. A standard curve obtained with purified branched chain aminotransferase was shown in Fig. 7. Enzyme concentrations as low as 2.5–3 μg/ml could be measured reliably using this assay. An average value of 1.98 μg of branched chain aminotransferase/mg of mitochondrial protein was determined using two different mitochondrial preparations which represents about 0.2% of total mitochondrial protein. Based on published values for the mitochondrial protein content in heart muscle of 53–60.9 mg of mitochondrial protein/g of heart, wet weight (10, 11, 22), this would amount to approximately 105–120 μg of branched chain aminotransferase enzyme protein/g of heart muscle, wet weight.

DISCUSSION

This paper presents the first protocol for purification of the branched chain aminotransferase from rat heart mitochondria. The enzyme migrates in SDS-PAGE as a single protein band of 43 kDa, both in the presence and absence of the thiol reductant mercaptoethanol, which suggests that the enzyme exists as a single polypeptide in the mitochondrial matrix. In earlier work on the hog heart and brain enzymes, the existence of dimeric forms of branched chain aminotransferase was proposed (3). Experiments with branched chain aminotransferase from rat heart do not support this hypothesis, although there is evidence that pancreatic branched chain aminotrans-
ferase(s) may be a dimer(s) (5). That dimeric forms of branched chain aminotransferase do not exist in rat heart mitochondria is supported by our finding that the enzyme eluted from a Sephacryl S-100 size exclusion column with an apparent molecular mass of 50 kDa. In addition, antibodies raised against the purified enzyme did not recognize any proteins in the mitochondrial 100,000 × g supernatant other than a 43-kDa protein. Brain was selected for comparison with heart because in this tissue the aminotransferase is predominantly cytosolic (7, 8, 11) and thought to consist primarily of the Enzyme III form (3) (for review, see Ref. 6). Rat heart has been reported to contain only the Enzyme I form of branched chain aminotransferase (7, 8). The antibodies did not recognize any proteins present in cytosol from rat brain. In addition, the brain enzyme activity in the cytosol and a partially purified enzyme preparation eluted from the same Sephacryl S-100 column with an apparent molecular mass of 66 kDa. Therefore, the antibodies appear to be specific for the mitochondrial form of branched chain aminotransferase.

Our data confirm earlier reports (9) which suggested the existence of different enzyme forms of branched chain aminotransferase. Indeed, the rat heart mitochondrial enzyme appears to be a polypeptide which is approximately 20 kDa smaller than the native molecular mass of the brain enzyme. Since aminotransferases, specifically aspartate aminotransferase, are known to be significantly glycosylated (23), a difference in the size of the polypeptide gene product is likely to be responsible for the molecular mass difference. Furthermore, two independent genes for branched chain aminotransferase have been identified in humans (24). More work is required, however, to verify whether or not the branched chain aminotransferases present in various tissues constitute a family of proteins that possibly originated from a common gene.

The protocol for branched chain aminotransferase purification from rat heart mitochondria presented in this paper results in a 3–5% yield of active enzyme. The major loss of activity occurred after hydrophobic interaction chromatography in ammonium sulfate. This HPLC step was conducted at room temperature. We showed that chromatography at room temperature conducted within the time limits needed for this step did not result in any significant loss of activity, which argues against a temperature triggered loss of activity. Loss of active enzyme may have resulted from removal of the pyridoxal phosphate cofactor during chromatography in high concentrations of ammonium sulfate. We were, however, never successful in reconstituting lost activity by dialyzing column fractions against buffers containing pyridoxal phosphate and/or α-ketoisocaproate. Purification of the enzyme was tried on several different chromatographic supports as alternatives to the Hydroapore column that was used for hydrophobic interaction chromatography. However, we found that separation of the enzyme from contaminating proteins was unique on this column, so it became the key step in the successful purification of the rat heart enzyme.

Classic discontinuous electrophoresis according to Davis (14) separated the purified 43-kDa branched chain aminotransferase into two bands that appeared somewhat diffuse after staining. Taylor and Jenkins (1) have reported the appearance of a diffuse protein band when branched chain aminotransferase from hog heart was subjected to starch electrophoresis in the absence of substrate (α-ketoglutarate). A more focused protein band was obtained when α-ketoglutarate was included in their electrophoresis buffer (1). Incubation of our purified branched chain aminotransferase preparation from rat heart mitochondria with substrate prior to discontinuous electrophoresis did not change the electrophoretic pattern (data not shown). Although the presence of carbohydrate cannot be excluded at this time, it is also possible that the more negatively charged form of the branched chain aminotransferase which appears in this electrophoresis system may have resulted from deamidation of glutamine residues during the purification procedure. Deamidation could create a more acid enzyme form without causing any change in the catalytic capacity of the enzyme.

In conclusion, purification of branched chain aminotransferase from rat heart mitochondria has provided the necessary tools for a more detailed investigation of the branched chain aminotransferase enzyme family.

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R Wallin, T R Hall and S M Hutson


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