L-Threonine Dehydrogenase of Chicken Liver

PURIFICATION, CHARACTERIZATION, AND PHYSIOLOGICAL SIGNIFICANCE*

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L-Threonine dehydrogenase catalyzes NAD⁺-dependent oxidation of L-threonine. The suspected product of the reaction is L-2-amino-3-oxobutyrate which spontaneously decomposes to yield aminoacetone and CO₂ (1). The dehydrogenase appears to function as the first step in a threonine degradation pathway in several microorganisms that use L-threonine as the main carbon and energy source (2-7). In microorganisms, 2-amino-3-oxobutyrate is further cleaved in a CoA-dependent reaction to produce glycine and acetyl-CoA (3).

L-Threonine dehydrogenase has been purified to homogeneity from chicken liver mitochondria. On the contrary, chicken liver mitochondria contain aminoacetone synthase (acetyl-CoA:glycine C-acetyltransferase (EC 2.3.1.29)). The enzyme has been partially purified. In the presence of L-threonine dehydrogenase and aminoacetone synthase, L-threonine is cleaved to glycine and acetyl-CoA via 2-amino-3-oxobutyrate. The result suggests that L-threonine dehydrogenase and aminoacetone synthase have a physiological role in L-threonine metabolism in vertebrates.

L-Threonine dehydrogenase (L-threonine:NAD⁺ oxidoreductase (EC 1.1.1.103)) has been purified to apparent homogeneity from chicken liver mitochondria. The presence of 2-mercaptoethanol and glycerol is necessary for stabilizing the enzyme during purification and storage. The enzyme is a monomer and has M₅₀ ≈ 88,000. The pH optimum is 8.6 to 8.7, and the isoelectric point of the enzyme is 5.9. The enzyme is specific for L-threonine and NAD⁺. The Kₘ values for L-threonine and NAD⁺ are 8.4 and 0.98 mM, respectively. Kinetic studies indicate that the reaction proceeds through an Ordered Bi Bi mechanism where NAD⁺ is added first, followed by L-threonine.

Chicken liver mitochondria contain aminoacetone synthase (acetyl-CoA:glycine C-acetyltransferase (EC 2.3.1.29)). The enzyme has been partially purified. In the presence of L-threonine dehydrogenase and aminoacetone synthase, L-threonine is cleaved to glycine and acetyl-CoA via 2-amino-3-oxobutyrate. The result suggests that L-threonine dehydrogenase and aminoacetone synthase have a physiological role in L-threonine metabolism in vertebrates.

RESULTS

Distribution of L-Threonine Dehydrogenase—When various chicken tissue homogenates were assayed for L-threonine dehydrogenase activity, liver proved to be the most active tissue by far. The tissue distribution is similar to that reported in rats (2).

The mitochondria were treated with 1.1 mg of digitonin/mg of mitochondrial protein and fractionated according to Schnaitman and Greenawalt (27). All the activity of L-threonine dehydrogenase was recovered in the inner membrane matrix fraction. In another experiment, the mitochondria were treated with varying amounts of digitonin and fractionated by centrifugation. Fig. 1 depicts the distribution of L-threonine dehydrogenase and other marker enzymes in the 12,000 × g pellet and in the supernatant fluid after centrifugation at 105,000 × g for 1 h.

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Purification of L-Threonine Dehydrogenase—The results of a typical purification are summarized in Table I. During the purification, the enzyme was stabilized by adding 14 mM 2-mercaptoethanol and 10% (v/v) glycerol to the buffers. Recovery of the enzyme was also improved in the earlier step of the purification by the addition of 1 mM L-threonine and 0.1 mM phenylmethylsulfonyl fluoride.

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The purified enzyme can be stored at −20°C with little loss of activity for at least a month in the presence of 14 mM 2-mercaptoethanol and 20% (v/v) glycerol. When 2-mercaptoethanol was removed from the enzyme solution by gel filtration with Sephadex G-25, the activity was completely lost and was not restored by the addition of the thiol.

Homogeneity—The purified enzyme was analyzed by polyacrylamide gel electrophoresis at pH 8.9. A single band was observed after staining with Coomassie blue and the activity was confined to the band (data not shown). The preparation on sodium dodecyl sulfate-polyacrylamide gel also showed a single protein band (Fig. 2).

Molecular Weight—Molecular weight was estimated in the presence of standards of known molecular weight by gel filtration (Fig. 3A), sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 3B), and glycerol density gradient centrifugation (Fig. 4). $M_r = 78,000$ was calculated by gel filtration, while a value of 88,000 was obtained from the gel electrophoresis. The density gradient ultracentrifugation gave a sedimentation coefficient of 5.8 S. The molecular weight was calculated as 88,000 according to Martin and Ames (30). These findings suggest that the L-threonine dehydrogenase of chicken liver consists of a single polypeptide. The molecular weight determined by gel filtration was smaller than those obtained by the other two methods. Presumably some interaction of the L-threonine dehydrogenase molecule with Sephadex may account for the difference.

Isoelectric Point—The isoelectric point of L-threonine dehydrogenase was determined by electrophoresis in 146 2,4-dimethyl-3-acetylpyrrole.

Effect of pH on the reaction was determined in 400 mM Tris-HCl buffer colorimetrically. The optimum pH for the enzyme is 8.6 to 8.7, but about 30 and 80% of the activity was found at pH 7.5 and 9.8, respectively.

Various metal chlorides were incubated for 5 min at 37°C with the enzyme that was passed through a column of Sephadex G-25 equilibrated with 50 mM Tris-HCl buffer, pH 8.3, 14 mM 2-mercaptoethanol, 30% glycerol, and activities were assayed colorimetrically. At concentrations of 0.05 and 1.0 mM Mg$^{2+}$, Ca$^{2+}$, Co$^{2+}$, Na$^+$, and K$^+$ ions showed essentially no effect on the activity, whereas Zn$^{2+}$, Cu$^{2+}$, and Cd$^{2+}$ inhibited the activity about 90%. Ni$^{2+}$ and Mn$^{2+}$ showed no effect at 0.05 mM, but caused 40 to 50% inhibition at 1.0 mM.

The stoichiometry of the L-threonine dehydrogenase reaction was determined in a single run. The reaction was conducted in a cuvette and changes in absorbance at 340 nm were recorded at 30°C for a period of time. At the end of the incubation, trichloroacetic acid was added and aminoacetone was estimated colorimetrically. As shown in Table II, aminoacetone and NADH production occurred in approximately equimolar amounts.

Reversibility of the Reaction—L-Threonine dehydrogenase was incubated with aminoacetone and NADH at 37°C. No change in absorbance at 340 nm was observed. When L-threonine dehydrogenase was incubated with aminoacetone synthase in the reaction mixture for the assay of aminoacetone synthase activity containing [2-14C]glycine plus NADH, considerable amounts of radioactivity were recovered in threonine after separation by paper chromatography (Table III). The reaction was dependent on NADH and L-threonine dehydrogenase. The results indicate that a product of the aminoacetone synthase reaction, presumably 2-amino-3-oxobutyrate which is a common suspected product of the L-threonine dehydrogenase reaction and the aminoacetone synthase reaction, serves as a substrate for L-threonine dehydrogenase and that the reaction catalyzed by L-threonine dehydrogenase is reversible.

Substrate and Coenzyme Specificity—L-Threonine appears to be the only substrate for L-threonine dehydrogenase of chicken liver. At a final concentration of 100 mM, d-threonine, L-allothreonine, DL-allothreonine, L-serine, L-homoserine, DL-2-amino-3-hydroxyvalerate, 2-aminobutyrate, and 3-hydroxybutyrate are all inactive for reducing NAD$^+$ in the spectrophotometric assay.

The enzyme requires NAD$^+$ as a coenzyme. NADP$^+$ and NADP$^+$ analogues such as 3-acetylpyridine adenine dinucleotide, 3-pyrindinealdehyde adenine dinucleotide, thionicotinamide adenine dinucleotide, and deaminoadenine dinucleotide (2 mM, final concentration) are inactive.

Kinetic Properties—The kinetic parameters for L-threonine dehydrogenase reaction were determined, at pH 8.0, colorimetrically according to Cleland (31). The double reciprocal plot was not a straight line, indicating the presence of nonlinearity in the reaction mixture. The reaction is reversible with respect to NAD$^+$ and aminoacetone.

TABLE I

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total protein</th>
<th>Total activity</th>
<th>Specific activity</th>
<th>Purification Yield</th>
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<td>Mitochondrial extract</td>
<td>23,900</td>
<td>218</td>
<td>0.009</td>
<td>1,100</td>
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<tr>
<td>DEAE-cellulose</td>
<td>5,400</td>
<td>155</td>
<td>0.029</td>
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<tr>
<td>Ammonium sulfate fraction</td>
<td>4,650</td>
<td>146</td>
<td>0.031</td>
<td>3.4</td>
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<tr>
<td>Second DEAE-cellulose</td>
<td>1,050</td>
<td>119</td>
<td>0.11</td>
<td>12</td>
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<tr>
<td>Blue dextran-Sepharose</td>
<td>36.0</td>
<td>84</td>
<td>2.3</td>
<td>256</td>
</tr>
<tr>
<td>Sephadex G-200</td>
<td>66</td>
<td>6.3</td>
<td>9.5</td>
<td>1,060</td>
</tr>
<tr>
<td>Amylamine-Sepharose</td>
<td>0.60</td>
<td>9.4</td>
<td>16</td>
<td>1,780</td>
</tr>
<tr>
<td>Second Sephadex G-200</td>
<td>0.49</td>
<td>9.1</td>
<td>19</td>
<td>2,100</td>
</tr>
</tbody>
</table>

The amounts of NADH and aminoacetone were determined simultaneously as described in the text.

Kinetic Properties—The kinetic parameters for L-threonine dehydrogenase reaction were determined, at pH 8.0, colorimetrically according to Cleland (31). The double reciprocal plot was not a straight line, indicating the presence of nonlinearity in the reaction mixture. The reaction is reversible with respect to NAD$^+$ and aminoacetone.

Expression of L-threonine by L-threonine dehydrogenase

<table>
<thead>
<tr>
<th>System</th>
<th>Threonine formed$^a$</th>
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</thead>
<tbody>
<tr>
<td>Complete</td>
<td>9.4</td>
</tr>
<tr>
<td>Minus L-threonine dehydrogenase</td>
<td>0</td>
</tr>
<tr>
<td>Minus aminoacetone synthase</td>
<td>0</td>
</tr>
<tr>
<td>Minus NADH</td>
<td>0</td>
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</tbody>
</table>

$^a$ The radioactivity recovered in threonine fraction at 0 time incubation was subtracted.


**Table IV**

| System                          | Glycine* | Acetyl-CoA (as citrate) | Aminoaconi-
tone |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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</tr>
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<tbody>
<tr>
<td>Complete</td>
<td>29.3</td>
<td>29.7</td>
<td>320</td>
</tr>
<tr>
<td>Minus threonine dehydrogenase</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Minus aminoacetone synthase</td>
<td>0</td>
<td>0</td>
<td>356</td>
</tr>
<tr>
<td>Minus CoA</td>
<td>0</td>
<td>0</td>
<td>351</td>
</tr>
</tbody>
</table>

*The radioactivity recovered in glycine or citrate fraction at 0 time incubation was subtracted.

plots of initial rates versus the concentration of the variable substrate at fixed level of the nonvaried substrate yielded a series of intersecting lines as shown in Figs. 5A and 6A. The Michaelis constants were determined with the replots of 1/v-axis to be 8.4 mM for L-threonine and 0.98 mM for NAD⁺ (Figs. 5B and 6B). When NAD⁺ was the variable substrate, NADH gave competitive inhibition, whereas noncompetitive inhibition was observed with L-threonine as the variable substrate (Fig. 7).

It is clear from Figs. 5A and 6A that the kinetic mechanism is sequential. The most direct kinetic approach to determine the substrate binding order in a sequential mechanism is the use of dead-end competitive inhibitors for each of the substrates (32). L-Allotriose was found to be a competitive inhibitor with respect to L-threonine (Fig. 8A). When concentration of NAD⁺ was varied, at several fixed concentrations of L-allotriose, the reciprocal plots of the initial velocity gave a series of parallel lines indicative of uncompetitive inhibition (Fig. 8B). Adenosine-5'-diphosphoribose, an inhibitor of a number of dehydrogenases, was selected as a competitive inhibitor with respect to NAD⁺. As shown in Fig. 9, it gave competitive inhibition with respect to NAD⁺ and noncompetitive inhibition with respect to L-threonine.

**Synthesis of Glycine and Acetyl-CoA from L-Threonine**

Recent reports indicated that in livers of guinea pig (10) and rat (11) L-threonine could be converted to glycine and acetyl-CoA with a series of reactions catalyzed by L-threonine dehydrogenase and aminoacetone synthase. We found that chicken liver mitochondria contain aminoacetone synthase, and the enzyme has been purified 270-fold over the mitochondrial extracts with a specific activity of 263 units/mg of protein. The possible participation of aminoacetone synthase in the metabolism of L-threonine was investigated incubating L-threonine, NAD⁺, and CoA with purified chicken liver L-threonine dehydrogenase and aminoacetone synthase. In these experiments, the acetyl-CoA formed was converted to citrate. As shown in Table IV, nearly equal amounts of glycine and acetyl-CoA (as citrate) were synthesized from L-threonine in addition to aminoacetone. The synthesis of glycine and acetyl-CoA was strictly dependent on CoA, L-threonine dehydrogenase, and aminoacetone synthase. The amounts of aminoacetone formed were decreased when glycine and acetyl-CoA were synthesized.

**Activities of Other L-Threonine metabolizing Enzymes in Chicken Liver**—Activities of L-threonine aldolase and L-threonine dehydratase were assayed using homogenate, disrupted mitochondria, and postmitochondrial supernatant of chicken liver. Even with high concentration of L-threonine (100 mM), we could not find any activity of these threonine-metabolizing enzymes in chicken liver.

**DISCUSSION**

We have purified L-threonine dehydrogenase from vertebrate liver for the first time to apparent homogeneity. Chicken liver L-threonine dehydrogenase is located in mitochondrial matrix and was readily solubilized. Like other dehydrogenases that utilize pyridine nucleotide coenzyme, chicken liver L-threonine dehydrogenase interacts with blue dextran-Seaphorose. It was essential to keep the concentration of glycerol at 30% during the elution of the enzyme from the affinity column. Lower glycerol concentration caused low recovery of the enzyme.

The purified L-threonine dehydrogenase is a monomer of $M_r \approx 88,000$. The enzyme requires the presence of an exogenous thiol and is irreversibly inactivated in the absence of the thiol. The chicken liver enzyme acts almost specifically on L-threonine and NAD⁺; other substrates and coenzymes tested showed no significant activity.

The dehydrogenase reaction is reversible. Although 2-amino-3-oxobutyrate was not tested for the substrate of L-threonine dehydrogenase, the evidence presented in Table III demonstrates that 2-amino-3-oxobutyrate serves as a substrate.

Recently, Boylan and Dekker obtained L-threonine dehydrogenase in homogeneous form from a mutant of *E. coli* K-12 (8). The properties of the enzyme from chicken liver reported in this communication are different in many respects from those reported for *E. coli* enzyme. *E. coli* enzyme has a native $M_r = 140,000$ and consists of four identical subunits. It utilized D-allothreonine, D-2-amino-3-hydroxyvalerate, and many NAD⁺-analogues which are all inactive for chicken liver enzyme. The optimum pH of *E. coli* enzyme (pH 10.3) is higher than that of chicken liver enzyme (pH 8.6 to 8.7). The activity of *E. coli* enzyme is stimulated by Mn⁺², whereas chicken liver enzyme exhibited no requirement for divalent cations.

Initial velocity indicate that the reaction is sequential, either ordered or random, involving the formation of a ternary complex. Since 2-amino-3-oxobutyrate is unstable (33), product inhibition studies were possible only with NADH. NADH was found to be a competitive inhibitor with respect to NAD⁺ and a noncompetitive inhibitor with respect to L-threonine, but the results did not distinguish the order of substrate binding. The problem of determining the order was approached by using L-allothreonine and adenosine-5'-diphosphoribose as substrate analogues. The inhibition patterns seen with these two compounds are consistent with ordered addition of NAD⁺ followed by L-threonine. Thus, the L-threonine dehydrogenase reaction proceeds via an Ordered BI Bi mechanism in which NAD⁺ binds to the enzyme prior to the binding of L-threonine, and 2-amino-3-oxobutyrate leaves from the enzyme before the release of NADH. $K_n$ value for L-threonine obtained from replots of 1/v-axis of Fig. 6A was 8.4 mM, the value similar to that reported for bullfrog enzyme (9). A little lower value has been obtained for *E. coli* enzyme (8). $K_n$ value for NAD⁺ was found to be 0.98 mM, the value 4 to 7 times higher than those reported previously (8, 9, 34).

**In vitro** synthesis of glycine and acetyl-CoA (as citrate) from L-threonine described in the present paper strongly supports the proposal of Dale (10) and Bird and Nunn (11) that L-threonine can be metabolized to glycine and acetyl-CoA via 2-amino-3-oxobutyrate in animal liver as has been found in a species of *Arthrobacter* (3). The obligatory requirement for L-threonine dehydrogenase and aminoacetone synthase in the conversion of L-threonine to glycine and acetyl-CoA (Table IV) indicates that the reaction is a sum of the following two reactions:

1. $L$-Threonine $+ NAD^+$ $→$ 2-amino-3-oxobutyrate $+ NADH + H^+$
2. $2$-Amino-3-oxobutyrate $+ CoA = glycine + acetyl-CoA
properties are presented in this communication. Reaction 2 is catalyzed by aminoacetone synthase which has long been known to catalyze the formation of aminoacetone from acetyl-CoA and glycine (17, 33). In animals, activity of aminoacetone synthase has been found in chicken reticulocytes (36), rat (11), and guinea pig liver (10, 17), and, in this communication, in chicken liver mitochondria. Aminoacetone synthase has been partially purified from the extract of chicken liver mitochondria. The enzyme preparation exhibited no threonine-metabolizing activities and did not utilize aminoacetone as substrate. As shown by Laver et al. (33), 2-amino-3-oxobutyrate is highly unstable and decomposes spontaneously to aminoacetone and CO₂. Since aminoacetone did not serve as a substrate for aminoacetone synthase, it is apparent that 2-amino-3-oxobutyrate formed from L-threonine is the immediate substrate for aminoacetone synthase to produce glycine and acetyl-CoA. Although the amounts of glycine and acetyl-CoA synthesized were far less than those of aminoacetone formed in the in vitro studies, it is plausible that L-threonine dehydrogenase and aminoacetone synthase are in a position where 2-amino-3-oxobutyrate is utilized effectively in mitochondria. In this connection, the two enzymes are reported to be located in the mitochondrial matrix (10, 22). The observation of Linstead et al. (6) also supports the above assumption. They reported that intact Trypanosoma brucei produced equimolar amounts of glycine and acetate and no aminoacetone when incubated in the presence of L-threonine. When extracts of T. brucei, prepared by either Triton X-100 lysis or sonication, were incubated with L-threonine, the formation of glycine and acetate was reduced and instead, aminoacetone was synthesized. Aminoacetone may be catabolized by the pathway proposed by Green and Elliott (2). In the same report they could not detect disappearance of aminoacetone in chicken liver homogenate.

In chicken liver, L-threonine dehydrogenase seems to be the only enzyme which degrades L-threonine because we could not find activities of L-threonine aldolase and L-threonine dehydratase in chicken liver. In a preliminary experiment, we found that the activity of L-threonine dehydrogenase is higher in livers obtained from chickens fed with 60% casein diet than fed with 20% casein diet.

In conclusion, L-threonine dehydrogenase has a physiologically significant role in the metabolism of L-threonine in chicken and presumably in vertebrate liver converting L-threonine to glycine and acetyl-CoA in collaboration with aminoacetone synthase.

Acknowledgments—We are indebted to Dr. Hitoshi Matsumoto of Tokushima University of Arts and Science and Dr. Tadashi Yoshida of Tohoku University for the synthesis of aminoacetone.

REFERENCES

L-Threonine Dehydrogenase of Chicken Liver

SUGGESTED MATERIAL

Supplemental Material

To L-Threonine Dehydrogenase of Chicken Liver: Purification, Characterization and Physiological Significance

By Taji Araya and Tatsuo Nakahara

Experimental Procedures

Materials—The following materials were purchased from commercial sources: L-threonine dehydrogenase from chicken liver (Sigma Chemical Co., St. Louis, Mo.); L-threonine dehydrogenase from chicken liver (Calbiochem-Behring Corp., Los Angeles, Calif.); L-threonine dehydrogenase from chicken liver (Biochemicals, Inc., New York, N.Y.); L-threonine dehydrogenase from chicken liver (Calbiochem-Behring Corp., Los Angeles, Calif.); L-threonine dehydrogenase from chicken liver (Calbiochem-Behring Corp., Los Angeles, Calif.).

Preparation of L-threonine dehydrogenase from chicken liver was carried out by the method of Nakahara et al. (19). L-threonine dehydrogenase was purified by ammonium sulfate precipitation and dialysis against 0.01 M sodium phosphate buffer, pH 7.0, containing 0.1 M NaCl. The L-threonine dehydrogenase was then concentrated by ultrafiltration, and the pure enzyme was dialyzed against 0.01 M sodium phosphate buffer, pH 7.0, containing 0.1 M NaCl. The enzyme was then freeze-dried and stored at 0°C.

The purified L-threonine dehydrogenase was dissolved in 0.01 M sodium phosphate buffer, pH 7.0, containing 0.1 M NaCl, and activity was determined according to the method of Nakahara et al. (19).

Results—The purified L-threonine dehydrogenase was found to be a single protein band on SDS-polyacrylamide gel electrophoresis. The molecular weight of the enzyme was determined to be approximately 50,000 by gel filtration on a Sephadex G-200 column.

Discussion—The L-threonine dehydrogenase from chicken liver was found to be a single protein band on SDS-polyacrylamide gel electrophoresis. The molecular weight of the enzyme was determined to be approximately 50,000 by gel filtration on a Sephadex G-200 column.
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L-Threonine Dehydrogenase of Chicken Liver

Fig. 1. Changes in the distribution pattern of L-threonine dehydrogenase, malate dehydrogenase, and cytochrome c oxidase caused by different concentrations of digitonin. Fractionation was carried out by the method of Scheraga and Greenway [2]. The percentage of the total recovered enzyme activity in 22,000 x g pellet (open symbols) or the 105,000 x g supernatant fraction (closed symbols) is plotted against the digitonin concentration. O, L-threonine dehydrogenase; O, malate dehydrogenase; and X, cytochrome c oxidase.

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of L-threonine dehydrogenase. The enzyme was subjected. The migration is from top to bottom.

Fig. 3. Molecular weight determination by gel filtration through Sephadex G-200 (Panel A) and by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Panel B). Standard molecular weights were: 1, myoglobin, 17,800; 2, ovalbumin, 45,000; 3, bovine serum albumin, 67,000; 4, phosphorylase a, 97,400; 5, lactate dehydrogenase, 142,000; 6, catalase, 240,000; and 7, apo-feritin, 480,000. The arrow indicates the fraction number and the relative mobility of L-threonine dehydrogenase.

Fig. 4. Sedimentation coefficient determination by glycerol density gradient centrifugation. Standard proteins used were: 1, bovine serum albumin; 2, lactate dehydrogenase; and 3, catalase. The arrow indicates the fraction number of L-threonine dehydrogenase.

Fig. 5. Double reciprocal plot of initial velocity study with L-threonine as the varied substrate at different fixed concentrations of NAD⁺ (Panel A), and a replot of the intercepts versus the reciprocal of the corresponding NAD⁺ concentrations (Panel B). Concentrations of NAD⁺ were: 0.1, 0.3, 1.0, and 2.0 mM.
Fig. 1. Double reciprocal plots of initial velocity in studies with 100 nM NADPH as the varied substrate at different fixed concentrations of L-threonine (Panel A) and 100 μM L-threonine as the varied substrate (Panel B). The concentrations of L-threonine were: ○, 7.0 mM; □, 12.0 mM; △, 27.0 mM; ▲, 0.02 mM; and ■, 0.05 mM.

Fig. 2. Double reciprocal plots of dead-end inhibition by L-threulose with L-threonine as the varied substrate (Panel A) and with NADPH as the varied substrate (Panel B). The concentration of L-threulose was held constant at 2.0 mM, and the concentration of L-threonine was held constant at 1.0 mM, and the concentrations of NADPH were: ○, none; □, 10 μM; and △, 20 μM.

Fig. 3. Double reciprocal plots of product inhibition by adenosine-5'-diphosphate with NADPH as the varied substrate (Panel A) and with L-threonine as the varied substrate (Panel B). The concentration of adenosine-5'-diphosphate was held constant at 1.0 mM (Panel A), while the concentration of NADPH was held constant at 1.0 mM (Panel B). The concentration of L-threonine was held constant at 1.0 mM, and the concentrations of NADPH were: ○, none; □, 0.1 mM; and △, 1.0 mM.

Fig. 4. Double reciprocal plots of product inhibition by adenosine-5'-diphosphate with NADH as the varied substrate (Panel A) and with L-threonine as the varied substrate (Panel B). The concentration of adenosine-5'-diphosphate was held constant at 1.0 mM (Panel A), while the concentration of NADH was held constant at 1.0 mM (Panel B). The concentration of L-threonine was held constant at 1.0 mM, and the concentrations of NADH were: ○, none; □, 0.1 mM; and △, 1.0 mM.