STUDIES ON KYNURENINE TRANSAMINASE

I. PURIFICATION AND SOME PROPERTIES

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Purified preparations of glutamic-aspartic, glutamic-alanine and tyrosine- α -ketoglutarate transaminases have been made available and a number of studies have been carried out using these enzymes. Kynurenine transaminase is the enzyme studied extensively by Mason *et al.* (1—6), but it was not obtained in a state of high purity. In the course of the investigations on the relation between vitamin B₆ and the increase of xanthurenic acid, the author has developed a procedure for preparing the highly pure kynurenine transaminase of rat kidney. In this paper the purification and some properties of the enzyme will be described.

EXPERIMENTAL

L-Kynurenine sulfate monohydrate and pyridoxal phosphate were purchased from California Corporation for Biochemical Research. α -Ketoglutaric acid was obtained from Wako Pure Chemical Industries, Ltd. All other reagents were of reagent grade. Hydroxyl apatite was prepared by treating brushite with alkali (7).

Kynurenine transaminase was assayed in a reaction mixture containing the following components in 0.5 ml: 1.0 μ mole of L-kynurenine, 3.0 μ moles of α -ketoglutaric acid, 50 μ g of pyridoxal phosphate, and 100 μ moles of potassium phosphate buffer, pH 6.8. After 10 minutes at 37°, the reaction was stopped by adding 10 volumes of 1 per cent boric acid in ethanol. Transamination was expressed as the change of the optical density at 333 m μ (maximum of kynurenic acid), A_{333} , after correction for the absorption due to kynurenine as described by Knox (8). Specific activity was given as A_{333} per mg of protein.

Protein concentrations were determined by the biuret method and ultraviolet absorption.

RESULTS AND DISCUSSION

1. Purification of Rat-Kidney Kynurenine Transaminase

Step 1—Rat kidneys, removed immediately after stunning and decapitation, were chilled in ice and homogenized in 10 volumes of ice-cold distilled water in a Waring Blendor. The homogenate was centrifuged for 20 minutes at 20,000 $\times$ g and the sediments were discarded.

Step 2—The pH of the supernatant fluid was adjusted to 5.0 by dropwise addition of 1 *M* acetic acid. The resulting inactive precipitate was removed by centrifugation at $20,000 \times g$ and the liquid phase was adjusted to pH 7.0 by dropwise addition of 1 *N* KOH.

Step 3—Solid ammonium sulfate was added to 0.35 saturation, and the suspension was stirred for 1 hour before centrifugation at $20,000 \times g$. The precipitate was discarded and the ammonium sulfate concentration was then increased to 0.50 saturation by adding the solid salt. The precipitate was collected by centrifugation at $20,000 \times g$ for 15 minutes and dissolved in 0.01 *M* potassium phosphate buffer, pH 7.0. The solution was dialysed for 3 to 4 hours against the same buffer.

Step 4— α -Ketoglutaric acid and pyridoxal phosphate were found to afford considerable protection of kynurenine transaminase against thermal denaturation, but it was not so marked as found by Jenkins *et al.* (9) for glutamate-aspartate transaminase. After addition of α -ketoglutaric acid and pyridoxal phosphate up to 1.0×10^{-2} and 2.0×10^{-4} *M* respectively, the temperature of preparation was brought to 60° for 5 minutes and then cooled rapidly in an ice bath. The denatured protein was removed by centrifugation and the sediment was washed with one-fifth the original volume of the buffer. The enzyme was precipitated by addition of solid ammonium sulfate to 0.60 saturation. The precipitate was collected by centrifugation and dialysed against 0.01 *M* potassium phosphate buffer, pH 6.8.

Step 5—The dialysed enzyme was subjected to hydroxyl apatite chromatography. The adsorbent was packed into a column and equilibrated with 0.01 *M* potassium phosphate buffer, pH 6.8. The enzyme was placed on the column and eluted with increasing concentrations of phosphate buffer, pH 6.8. The elution pattern of the enzyme is shown in Fig. 1. The fractions having the transaminase activity were pooled and concentrated by addition of solid ammonium sulfate to 0.60 saturation. The results of the purification procedure are summarized in Table I.

FIG. 1 *Hydroxyl Apatite Column Chromatography of Kynurenine Transaminase* Potassium phosphate buffer of various concentrations, pH 6.8, was used.

TABLE I
Purification of Kynurenine Transaminase

Step of purification	Total activity	Total protein	Specific activity
1. 20,000×g supernatant	1430	6810	0.21
2. pH 5.0 treatment	1520	1788	0.85
3. Ammonium sulfate	1160	555	2.09
4. Heating	926	212	4.37
5. Hydroxyl apatite I	312	9	34.6
6. " " II	262	3.6	38.4

FIG. 2 *Stability of the Enzyme to Five-Minute Heating*

0.05 *M* potassium phosphate buffer, pH 6.8, was used; ×, enzyme only; o, 0.0002 *M* pyridoxal phosphate; ●, 0.0002 *M* pyridoxal phosphate + 0.01 *M* α -ketoglutarate; Δ, 0.0002 *M* pyridoxal phosphate + 0.01 *M* L-kynurenine.

the assumption that the prosthetic group has not been dissociated completely in the purification procedure described in this paper, and in fact, the present purified enzyme preparation shows a little activity without pyridoxal phosphate or pyridoxamine phosphate.

4. Optimal pH

The pH dependence of the enzyme activity was measured at a constant concentration of potassium phosphate buffer, and the result was found not to be identical with the value reported by Mason (3). As shown in Fig. 4, pH optimum of the partially purified enzyme was approximately 6.8.

2. Stability to Heat

For this investigation, the enzyme in Step 3 was used. Pyridoxal phosphate can afford a considerable protection against the thermal denaturation, for the prosthetic group is dissociated in this step according to Mason (4). As shown in Fig. 2, the enzyme is more stable to heat in the presence of α -ketoglutarate but less stable to heat in the presence of L-kynurenine. These results are in good accord with the case of glutamate-aspartate transaminase (9).

3. Absorption Spectrum

Fig. 3 shows the absorption spectra of kynurenine transaminase from rat kidney. The enzyme exhibited maxima at 280 and 410 $m\mu$. A shoulder at 330 $m\mu$ was also noted in the spectrum. Mason (4) has shown that the kynurenine transaminase is readily inactivated, and pyridoxal phosphate and pyridoxamine phosphate reactivate this enzyme.

The spectra have been interpreted on

FIG. 3 Absorption Spectra of Kynurenine Transaminase in 0.01 M Potassium Phosphate Buffer, pH 7.0

FIG. 4 Effect of pH on Kynurenine Transaminase Activity

5. Effect of Adding Various Reagents on Kynurenine Transaminase

The effect of adding various ions was investigated. In this case, the enzyme solution was dialysed against 0.01 M Tris-HCl buffer, pH 7.0, and activity was assayed in a reaction mixture containing 100 μ moles of Tris-HCl buffer instead of potassium phosphate buffer. Stimulation was not observed by the addition of any ions. Na^+ , K^+ , NH_4^+ , Mg^{2+} , Ca^{2+} and Mn^{2+} at $1.0 \times 10^{-2} M$ were found to have no effect on the rate of enzyme activity. Hg^{2+} , Sn^{2+} , Co^{2+} , and Ni^{2+} ions inhibited kynurenine transaminase completely.

TABLE II
Effect of Some Cations on Kynurenine Transaminase

Cation ($10^{-2} M$)	Relative activity	Cation ($10^{-2} M$)	Relative activity
None	100	Mn^{2+}	102
Na^+	103	Hg^{2+}	4
K^+	102	Sn^{2+}	1
NH_4^+	98	Zn^{2+}	1
Mg^{2+}	101	Co^{2+}	3
Ca^{2+}	105	Ni^{2+}	0

Inhibition of 95 and 6 % of control activity was produced by 10^{-2} and $10^{-3} M$ potassium cyanide respectively. Hydroxylamine at $10^{-3} M$ inhibited the reaction completely.

Ethylene-diamine tetraacetate ($10^{-2} M$) and thyroxine ($10^{-5} M$) were without effect. The effect of various concentrations of α -ketoglutarate on kynurenine transaminase was also investigated. In Fig. 5 Lineweaver-Burk plots of the reaction velocity as a function of α -ketoglutarate concentration are shown. The Michaelis constant derived from these plots was found to be $1.2 \times 10^{-3} M$.

FIG. 5 Effect of α -Ketoglutarate Concentration on Kynurenine Transaminase (Lineweaver-Burk plot)

SUMMARY

A purification method of kynurenine transaminase is described. The steps consist of pH 5 treatment, ammonium sulfate fractionation, heating at 60° for 5 minutes, and hydroxyl apatite column chromatography. About 200-fold purification has been achieved starting from the supernatant of rat kidney homogenate.

α -Ketoglutarate and pyridoxal phosphate have been found to afford considerable protection of kynurenine transaminase against thermal denaturation.

Spectrophotometric measurements on the purified enzyme revealed the absorption bands at 280 and 410 m μ . This enzyme has an optimum at approximately pH 6.8.

The effect of adding various reagents on the activity of the purified enzyme has been determined.

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