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Note

Occurrence of Kynurenine Aminotransferase in Extracts of Yeasts

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Kynurenine is a key intermediate in tryptophan metabolism and metabolized through two main alternative pathways.[1] One involves hydrolysis of kynurenine to alanine and anthranilic acid, which is converted to *cis,cis*-muconate and β-ketoadiptate (the aromatic pathway) and the other pathway involves degradation of kynurenine via kynurenic acid, which is dissimilated to 5-(β-carboxyethyl)-4,6-dihydroxypicolinate, (the quinaldine pathway). The first step of the aromatic pathway is catalyzed by kynureninase, which was purified to homogeneity and in a crystalline form from *Pseudomonas marginalis* to be characterized.[2,3] L-Kynurenine aminotransferase is the enzyme catalyzing the initial reaction in the quinaldine pathway, and has been demonstrated in mammalian liver, kidney, intestine, and brain, and *Neurospora crassa*.[4~7] Fujioka *et al.* reported that phenylalanine aminotransferase of *Achromobacter eurydice* catalyzes also transamination of kynurenine, though very slowly.[8] The experiments reported here are concerned with the first evidence for the occurrence of kynurenine aminotransferase in yeasts and some properties of the partially purified enzyme.

L-Kynurenine was synthesized by ozonolysis of L-tryptophan according to the method of Warnell and Berg.[9] Yeasts were cultivated in a medium containing 5% sucrose, 0.1% L-tryptophan, 0.2% KH_2PO_4, 0.2% K_2HPO_4, 0.1% $MgSO_4 \cdot 7H_2O$, 0.1% Na-L-glutamate, 0.1% urea, 0.1% $CaCO_3$, which was separately sterilized, and 0.01% yeast extract (pH 6.0–6.6) at 30° for 24 hr under aeration. The cells harvested by centrifugation were washed twice with 0.85% NaCl. The cell-free extracts were prepared by grinding the cells with levigated aluminium oxide in a chilled mortar followed by extraction with 0.02 M potassium phosphate buffer (pH 7.4) containing 0.1 mM pyridoxal 5′-phosphate (pyridoxal 5′-P) and 0.01% 2-mercaptoethanol. Kynurenine aminotransferase activity was determined as follows. The reaction mixture was composed of 200 μmoles of potassium phosphate buffer (pH 8.0), 1 μmole of L-kynurenine, 50 μmoles of pyridoxal 5′-P, 200 μmoles of sodium -α-ketoglutarate, and enzyme in a volume of 3.1 ml. After the mixture was incubated at 37° for 60 min, the reaction was terminated by addition of 0.2 ml of 25% trichloroacetic acid. The formed glutamate was separated by paper chromatography using Toyo filter paper No. 51 and a solvent system of methanol-butanol-benzene-water (2 : 1 : 1 : 1), and determined with ninhydrin.[10] One unit of enzyme is defined

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Table I. Distribution of L-Kynurenine Aminotransferase in Yeast.

Strain		Specific activity	Total activity
Saccharomyces cerevisiae (Awamori yeast)	AKU 4005	0.0040	0.020
Saccharomyces cerevisiae var. *sake* (Ozeki)	AKU 4014	0.0070	0.021
Saccharomyces fragilis	IFO 0288	0	0
Saccharomyces cerevisiae var. *sake* (Kikumasamune)	AKU 4027	0.0024	0.134
Saccharomyces cerevisiae	Y.U.W. -3	0.0040	0.109
Saccharomyces marxianus	IFO 0277	0.0083	0.139
Saccharomyces cerevisiae var. *sake*	AKU 4109	0.0021	0.063
Pichia polymorpha	IFO 0288	0.0014	0.014
Debariomyces hansenii	IFO 0039	0.0014	0.028
Debariomyces globosus	IFO 0016	0	0
Cryptococcus albidus	IFO 0378	0	0
Candida rugosa	IFO 0591	0	0
Tricosporon cutaneum	IFO 0116	0	0
Hansenula schneggii	IFO 0135	0.014	0.392
Schwanniomyces occidentalis	IFO 0371	0	0

as the amount of enzyme that catalyzes formation of 1 μmole of glutamate per min. The enzyme activity was assayed by measuring the rate of decrease in absorbance at 360 nm due to conversion of kynurenine throughout the purification. The reaction mixture was composed of 100 μmoles of Tris-HCl buffer (pH 8.0), 0.13 μmole of L-kynurenine, 0.1 μmole of pyridoxal 5'-P, 10 μmoles of sodium-α-ketoglutarate, and enzyme in a volume of 1.0 ml. L-Kynurenine was replaced by water in a blank. One unit of enzyme is defined as the amount of enzyme that is required to decrease 1.0 in absorbance at 360 nm per min at 25°. Specific activities are expressed as units per mg of protein.

The activity of kynurenine aminotransferase was investigated in various strains of yeasts. As shown in Table I the aminotransferase occurs in several strains of yeasts. Kynurenine aminotransferase was isolated and partially purified from *Hansenula schneggii* in which the enzyme was found most abundantly. All operations were performed at 5–10° unless otherwise specified. The washed cells were suspended in the same volume of 0.02 M potassium phosphate buffer (pH 7.4) containing 0.1 mM pyridoxal 5'-P and 0.01% 2-mercaptoethanol, and disrupted continuously by a DYNO-MILL aparatus (Willy A. Bachofen Mashinenfablik) with glass beads (0.25 mm in diameter). The supernatant obtained by centrifugation was used as the cell-free extract. To the cell-free extract 3 g of polyethyeneimine was added and the bulky precipitate formed was removed by centrifugation. The supernatant was fractionated with ammonium sulfate (0–70% saturation). The precipitate was dissolved in and dialyzed against 0.01 M potassium phosphate buffer (pH 7.4). The enzyme was applied to a DEAE-cellulose column (7×70 cm) buffered with the same buffer. After the column was washed thoroughly with the buffer containing 0.05 M NaCl, the enzyme was eluted with the buffer supplemented with 0.2 M NaCl and precipitated by addition of ammonium sulfate (0–70% saturation). The precipitate was dissolved in 0.01 M potassium phosphate buffer (pH 7.4) and then placed on a Sephadex G-150 column (5×100 cm) equilibrated with the same buffer. The column

Table II. Summary of Purification of L-Kynurenine Aminotransferase.

Step	Total protein	Specific activity	Total activity	Yield
	(mg)			(%)
Cell-free extract	35,040	0.07	2400	100
Polyethyleneimine treatment	22,800	0.08	1920	80
Ammonium sulfate fractionation	8,942	0.1	904	37
DEAE-cellulose chromatography	3,780	0.19	702	29
Sephadex G–150 chromatography	968	0.5	500	21

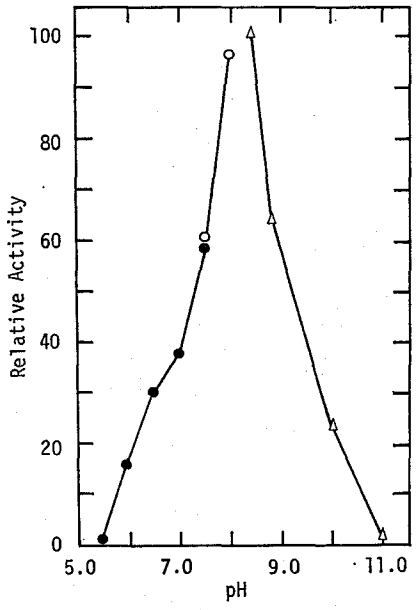

Fig. 1. Effect of pH on The Enzyme Activity.
The enzyme activity was determined by a paper chromatography-ninhydrin method as described in the text.
—●—: 0.2 M potassium phosphate buffer.
—○—: 0.2 M Tris-HCl buffer.
—△—: 0.2 M Glycine-KCl buffer.

was eluted with the buffer. The active effluents were combined and used as the partially purified enzyme, which was purified about 7-fold. The summary of purification is shown in Table II.

When the enzyme activity was examined in 0.2 M potassium phosphate, 0.2 M Tris–HCl, and 0.2 M glycine-KCl buffers, the enzyme had the maximum reactivity at about pH 8.2 (Fig. 1).

Michaelis constants for L-kynurenine and α-ketoglutarate were determined as 2.2 mM and 5 mM, respectively, according to a Lineweaver-Burk plot (Fig. 2).

Amino acceptor specificity was investigated with kynurenine and various keto acids. The following relative activities were obtained; 100, 90, 6, and 53, for α-ketoglutarate,

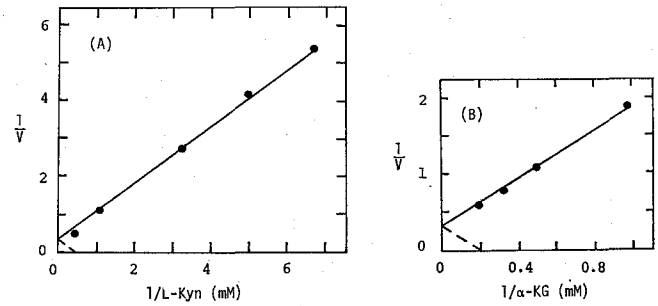

Fig. 2. Double-reciprocal Plots of The Velocity against L-Kynurenine (A) and α-Ketoglutarate Concentrations (B).
The reaction mixture was composed of 10 μmoles of α-ketoglutarate (or 5 μmoles of L-kynurenine), 0.1 μmole of pyridoxal 5′-P, 60 μmoles of Tris-HCl buffer (pH 8.2), the indicated amounts of L-kynurenine (or α-ketoglutarate), and enzyme in a volume of 1.0 ml. After the mixture was incubated at 37° for 10 min, 2.0 ml of 4 N HCl was added to stop the reaction. After addition of 1.0 ml of water, formation of kynurenic acid was followed by measuring absorbance at 340 nm. The velocity was expressed as increase in the absorbance at 340 nm per min.

pyruvate, oxalacetate, and glyoxylate, respectively.

The enzyme was inhibited 40% by D-cycloserine (1 mM), and 75% by hydroxylamine (1 mM), which are the typical inhibitors for pyridoxal 5′-P enzymes. Pyridoxal 5′-P partially reversed the inhibition by the reagents. This suggests that L-kynurenine aminotransferase of *H. schneggii* is a pyridoxal 5′-P enzyme. Mason reported that kynurenine aminotransferase of rat liver was inhibited with adipate competitively.[4] The aminotransferase of *H. schneggii* was also inhibited about 33% by adipate.

Further purification of the enzyme is now in progress to elucidate the properties of the enzyme.

REFERENCES

(1) R. Y. Stanier and O. Hayaishi, *Science,* **114,** 326 (1951).
(2) M. Moriguchi, T. Yamamoto, and K. Soda, *Biochemistry,* **12,** 2969 (1973).
(3) M. Moriguchi and K. Soda, *ibid.,* **12,** 2974 (1973).
(4) M. Mason, *J. Biol. Chem.,* **234,** 2770 (1959).
(5) H. Okamoto and O. Hayaishi, *ibid.,* **245,** 3603 (1970).
(6) W. B. Jakoby and D. M. Bonner, *ibid.,* **221,** 689 (1956).
(7) J. Nakamura, T. Noguchi, and R. Kido, *Biochem. J.,* **135,** 815 (1973).
(8) M. Fujioka, Y. Morino, and H. Wada, "Methods in Enzymology" Vol. 17A, Academic Press, New York, 1970, pp. 586–589.
(9) J. L. Warnell and C. P. Berg, *J. Amer. Chem. Soc.* **76,** 1708 (1954).
(10) K. Soda, T. Tochikura, and H. Katagiri, *Agr. Biol. Chem.,* **25,** 811 (1961).