Amino Acid Activation in Mammalian Brain

PURIFICATION AND CHARACTERIZATION OF TRYPTOPHAN-ACTIVATING ENZYME FROM BUFFALO BRAIN

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L-Tryptophan-activating enzyme [L-tryptophan-tRNA ligase (AMP), EC 6.1.1.2] of water-buffalo brain was purified to near homogeneity by heat and pH treatments, ammonium sulphate fractionation, column chromatography on DEAE-cellulose, hydroxyapatite and Amberlite CG-50, and gel filtration on Sephadex G-200. The purified enzyme catalyses tryptophanyl-tRNA formation with yeast tRNA, but not with *Escherichia coli* tRNA. The enzyme exhibits multiple peaks of activity in Sephadex gel filtration with molecular weights corresponding to 155000, 105000 and 50000. However, only one peak of activity with molecular weight of 155000 can be detected when the enzyme is subjected to gel filtration at high concentration. Disc gel electrophoresis in the presence of sodium dodecyl sulphate reveals a single band with molecular weight of 55000. The activity of the enzyme is concentration dependent. Different K_m and V_{max} values are obtained at different enzyme concentrations. These data suggest that this enzyme may exist in different quaternary structures, each with its own kinetic constants. The enzyme activity is inhibited by *p*-chloromercuribenzoate, and is not protected by the presence of the substrates, L-tryptophan, Mg^{2+} , ATP, in any combination.

In our preliminary experiments, we have determined the relative activities of amino acid-activating enzymes, or aminoacyl-tRNA ligases (EC 6.1.1.-), from water-buffalo brain, and have characterized some of their properties (C.-H. Chung, C.-C. Liu & M.-L. Lee, unpublished work). Tryptophan-activating enzyme interested us in that (1) it is by far the most active enzyme, (2) it requires an unusually low concentration of L-tryptophan in the catalysis of tryptophan-dependent ATP- $[^{32}P]PP_i$ exchange reaction, and (3) it is relatively stable at room temperature.

An important feature of tryptophan-activating enzyme in brain is that it shares a common substrate, L-tryptophan, with tryptophan hydroxylase, the first enzyme in the pathway of 5-hydroxytryptamine synthesis. The concentration of tryptophan normally present in the mammalian brain is below the K_m of tryptophan hydroxylase (Jequier *et al.*, 1967; McGeer *et al.*, 1968), and it has been demonstrated that the concentration of tryptophan in brain determines the rate of 5-hydroxytryptamine synthesis (Tagliamonte *et al.*, 1971; Knot & Curzon, 1972). Therefore, it is interesting to examine the kinetic properties of brain tryptophan-activating enzyme and especially the effect of L-tryptophan concentration on the activity of the enzyme.

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In this report we describe the purification and some properties of buffalo brain tryptophan-activating enzyme.

Experimental Procedure

Materials

Water-buffalo (*Bubalus buffelus* L.) brains were obtained from a slaughterhouse and stored at -22°C for as long as 5 months before use. Bovine pancreatic RNAase† (3000 units/mg) was purchased from Worthington Biochemical Corp., Freehold, N.J., U.S.A. Ox heart cytochrome *c*, bovine serum albumin, sodium dodecyl sulphate and dithiothreitol were from Sigma Chemical Co., St. Louis, Mo., U.S.A. Polyethylene glycol (molecular weight 4000) was purchased from Wako Chemical Co., Tokyo, Japan. *p*-Chloromercuribenzoate was obtained from Calbiochem, Los Angeles, Calif., U.S.A. Unfractionated *Escherichia coli* and brewers' yeast tRNA were from Schwarz/Mann, Orangeburg, N.Y., U.S.A. Materials for disc-gel electrophoresis were purchased from Canalco, Rockville, Md., U.S.A. Hydroxyapatite was prepared by the method of Main *et al.* (1959). Amberlite CG-50 was a product of Rohm and Hass, Philadelphia, Pa., U.S.A., and was prepared as described by Hirs *et al.* (1953).

† Abbreviation: RNAase, ribonuclease.

Methods

Assay of tryptophan-activating enzyme. Enzyme activity was measured by the rate of L-tryptophan-dependent ATP- ^{32}P PP_i exchange reaction. One unit of enzyme incorporates 1 nmol of ^{32}P PP_i into ATP in 15 min at 37°C. The reaction mixture (1 ml) contains: 100 mM-Tris-HCl, pH 8.0, 5 mM-MgCl₂, 6 mM-ATP, 50 μM -L-tryptophan and 2 mM- ^{32}P PP_i (10^4 – 10^5 c.p.m./ μmol). The reaction was initiated by the addition of 30–400 units of enzyme. Portions of 0.2 ml were withdrawn at 2.5, 5, 10 and 15 min, or shorter intervals when necessary, and added to 0.1 ml of stopping solution [7% (w/v) perchloric acid in 0.4 M-PP_i]. ^{32}P ATP was collected and counted for radioactivity as described by Muench (1971). Under these conditions the reaction is proportional to the time and amount of enzyme added. On some occasions the enzyme was assayed by aminoacylation of tRNA with L- ^{14}C tryptophan as described by Joseph & Muench (1971a).

Polyacrylamide-gel disc electrophoresis. Polyacrylamide-gel disc electrophoresis at pH 8.5 was performed as described by Davis (1964). The electrophoresis was done with 7.5% (w/v) gel with 25 mM-Tris-glycine buffer, pH 8.5. Proteins were stained with 1.0% Amido Black (Schwartz) in 7.5% (v/v) acetic acid for 2 h and destained with 7% (v/v) acetic acid. A separate column electrophoresed at 4°C was sliced into 2 mm slices and the enzyme was eluted with 50 mM-potassium phosphate buffer, pH 6.8, containing 10 mM-2-mercaptoethanol and 10% (v/v) glycerol and was assayed as described in 'Methods'.

Polyacrylamide-gel electrophoresis in sodium dodecyl sulphate. This was performed by the method of Weber & Osborn (1969). A sample (10–20 μg) of the Sephadex G-200 fraction of tryptophan-activating enzyme was used for each column. The standard curve was obtained by plotting the mobility of protein markers against the logarithm of their molecular weights. Markers used were bovine serum albumin, subunit molecular weight 68 000 (Tanford *et al.*, 1967),

E. coli K244 tryptophan-activating enzyme, subunit molecular weight 37 000 (Joseph & Muench, 1971b), RNAase, molecular weight 13 700 (Weber & Osborn, 1969) and cytochrome *c*, molecular weight 11 700 (Weber & Osborn, 1969).

Results and Discussion

Purification of tryptophan-activating enzyme

The purification is summarized in Table 1. All steps were at 0–4°C except the DE-52 DEAE-cellulose, hydroxyapatite and Amberlite chromatography steps, which were performed at room temperature (12–15°C).

Purification procedures

Three frozen brains (1.7 kg) were thawed and homogenized with 1700 ml of 50 mM-potassium phosphate buffer, pH 6.8, containing 10% (v/v) glycerol, 10 mM-MgCl₂ and 10 mM-2-mercaptoethanol. The homogenate was centrifuged at 17 000 *g* for 1 h and the clear supernatant fraction was collected (1600 ml). The extracts were placed in two 1-litre flasks and were incubated at 50°C for 1 h with constant stirring. The turbid heat-treated extract was clarified by centrifugation at 34 000 *g* for 30 min to yield 1500 ml (heat fraction). To the heat fraction, solid (NH₄)₂SO₄ (425 g, 47% satn.) was added with constant stirring. The precipitate was removed by centrifuging at 34 000 *g* for 30 min, then 140 g of solid (NH₄)₂SO₄ (62% satn.) was added. The precipitate was collected by centrifugation as described above and dissolved in 140 ml of solution A [10 mM-2-mercaptoethanol, 10% (v/v) glycerol] containing 50 mM-potassium phosphate buffer, pH 6.8, and dialysed against 20 volumes of the same buffer.

The (NH₄)₂SO₄ fraction (150 ml) was adjusted to pH 4.7 with 1 M-acetic acid with constant stirring. The suspension was further stirred for 15 min and the precipitate was removed by centrifugation as des-

Table 1. Purification of tryptophan-activating enzyme

The details are described in the text.

Fraction	Volume (ml)	$10^{-3} \times$ Total units	Recovery (%)	Total protein (mg)	$10^{-3} \times$ Specific activity (units/mg)	Purification
Extract	1600	5900	100	26000	0.22	1.0
Heat	1500	5000	85	14000	0.28	1.3
Ammonium sulphate pH 4.7	150	4500	76	2800	1.6	7.3
	162	3000	51	1800	1.7	7.7
DE-52 DEAE-cellulose	40	650	11	110	5.9	27
Hydroxyapatite	6	620	10.5	21	26	120
Amberlite CG-50	4	600	8.5	12	50	220
Sephadex G-200	3.1	430	7.3	4	110	500

cribed above. The clear supernatant was adjusted to pH 6.8 with 1M-K₂HPO₄ and dialysed against 200 volumes of solution A containing 50mM-potassium phosphate, buffer pH 6.8.

The pH 4.7 fraction (162 ml) was then placed over a 2.4cm × 16cm DE-52 DEAE-cellulose column previously equilibrated with 75mM-potassium phosphate buffer, pH 6.8, in solution A. The column was washed with 100ml of the same buffer, then developed with a linear gradient from 100 to 200mM-potassium phosphate buffer, pH 6.8, in a total of 800ml of solution A and at a flow rate of 150ml/h. The enzyme emerged as a symmetrical peak centered at a buffer concentration of about 150mM. Fractions comprising the activity peak were pooled and concentrated by dialysis against 20 volumes of solution A containing 100mM-phosphate buffer, pH 6.8, and 15% polyethylene glycol. The final solution was dialysed again against 50 volumes of solution A with 50mM-potassium phosphate buffer, pH 6.8.

The DE-52 pool (40ml) was then applied to a 1cm × 14cm hydroxyapatite column equilibrated with 50mM-potassium phosphate buffer, pH 6.8, in solution A. After a wash with 25ml of the same buffer, the column was developed at a flow rate of 50ml/h by a linear gradient from 50 to 200mM-potassium phosphate buffer, pH 6.8, in a total of 100ml of solution A. The enzyme was eluted as a single peak at a buffer concentration of about 130mM, and was pooled, and concentrated as described above. The hydroxyapatite pool was dialysed against 500 volumes of 10mM-potassium phosphate buffer, pH 6.0, in solution A and then adsorbed on a 1.0cm × 11cm Amberlite CG-50 column equilibrated with the same buffer. After a wash of 20ml of the same buffer, the enzyme was eluted by a linear gradient from 10 to 500mM-potassium phosphate buffer, pH 6.0, in a total of 100ml of solution A. The flow rate was 50ml/h. The enzyme, which appeared as a single, symmetrical peak at a phosphate concentration of about 250mM, was pooled, and concentrated to 3mg/ml or greater as described above.

Sephadex G-200 gel filtration was performed at 0–4°C. After dialysis against 500 volumes of solution A in 100mM-potassium phosphate buffer, pH 6.8, the enzyme (4ml) was applied to a 1.8cm × 110cm Sephadex column equilibrated with the above buffer. The column was developed at a flow rate of 4ml/h. The fractions containing enzyme activity were pooled and concentrated as described above. The enzyme exhibited multiple peaks of activity if input enzyme concentration was low (see below).

Comments on purification

1. Sephadex G-200 fraction (100–120μg) exhibits a single band on 7.5% (w/v) polyacrylamide-gel disc electrophoresis at pH 8.5 (Davis, 1964), with relative mobility of 0.35 to the dye front.

2. Activities of the other amino acid-activating enzymes in the Sephadex G-200 fraction were measured by ATP-[³²P]PP_i exchange reaction (C.-H. Chung, C.-C. Liu & M.-L. Lee, unpublished work). Histidine- and isoleucine-activating enzyme activities were 2% relative to tryptophan-activating enzyme. No other activities were measurable.

3. The low recovery (20–40%) from the DE-52 chromatography step was not due to the effect of temperature, as it was unchanged when the chromatography was done at 0–4°C. The presence of 0.5mM-L-tryptophan in the buffer during chromatography improved the recovery slightly (40–50%). The high recovery of enzyme after hydroxyapatite and Amberlite column chromatographies at room temperature indicated that this enzyme is rather stable under the conditions applied (neutral pH, in the presence of glycerol and 2-mercaptoethanol at room temperature).

Aminoacylation of tRNA

Aminoacylation of tRNA was performed by the method of Joseph & Muench (1971a); 90 exchange-units of purified enzyme were used per assay. The

Fig. 1. Effect of pH on the rate of L-tryptophan-dependent ATP-[³²P]PP_i exchange reaction

Each reaction mixture contained 250 units of enzyme from the Amberlite fraction in 1ml of 100mM-potassium acetate (●), potassium phosphate (○) or Tris-HCl (□) buffer, and other components of the reaction mixture as described in 'Methods'. Each point represents an initial rate (2.5, 5.0 and 10.0 min) at 37°C.

reaction was done at 37°C for 30 min. Under these conditions the enzyme charged 10–12 pmol of L-tryptophan on to one E_{260} unit of brewer's yeast tRNA but less than 1 pmol on to *E. coli* B tRNA. (One E_{260} unit of tRNA has an absorbance of 1.0 at 260 nm in a 1.0 cm optical path in a neutral buffer.) With the homologous synthetases the acceptances of L-tryptophan of brewer's yeast and *E. coli* B tRNA were 49 and 16 pmol/ E_{260} unit respectively. We were unable to isolate tRNA from buffalo brain by the method of von Ehrenstein (1968) and of Gonano *et al.* (1971).

Optimal assay conditions

The rate of L-tryptophan-dependent ATP-[32 P]PP_i exchange reaction as a function of pH was determined in the presence of 100 mM-potassium acetate buffer, potassium phosphate buffer, or Tris-HCl buffer (Fig. 1). The exchange reaction has two pH optima, 6.5 and 8.0. Similar results have been observed for the

tryptophan- and proline-activating enzymes of *E. coli*, whether the enzymes were assayed by the exchange reaction or by aminoacylation of tRNA (Joseph & Muench, 1971a; M.-L. Lee & K. H. Muench, unpublished work). Tryptophan-activating enzyme at pH 6.0 has a much lower activity when assayed in potassium phosphate buffer than in potassium acetate buffer, and at pH 7.5 the enzyme has a lower activity in potassium phosphate buffer than in Tris-HCl buffer (Fig. 1). Such an inhibition by potassium phosphate buffer was also reported for the same enzyme from *E. coli* (Joseph & Muench, 1971a).

Molecular weight and quaternary structure

The subunit molecular weight of tryptophan-activating enzyme, as determined by gel electrophoresis in the presence of sodium dodecyl sulphate (Weber & Osborn, 1969), is 55 000. This value is close to that reported for the same enzyme from bovine pancreas (108 000) (Gros *et al.*, 1972), and human placenta (116 000) (N. S. Penneys & K. H. Muench, personal communication).

The molecular weight for native tryptophan-activating enzyme was estimated by gel filtration on Sephadex G-200 (Fig. 2). When a high concentration

Fig. 2. Apparent molecular weight of tryptophan-activating enzyme

A Sephadex G-200 column, 1.8 cm $\times$ 110 cm, was prepared and equilibrated with 100 mM-potassium phosphate buffer, pH 6.8, with 10 mM-2-mercaptoethanol, and 10% (v/v) glycerol at 0–4°C. The column was calibrated as described by Whitaker (1963). In a typical experiment, enzyme at concentration of 4 mg/ml (●) or 1 mg/ml (○) was applied to the column. The column was developed with downward flow at 4 ml/h, 2.5 ml/fraction. The enzymes were then assayed as described in 'Methods'. The molecular weights of marker proteins and enzymes were determined as above (Whitaker 1963). The three components and their molecular weights are indicated.

Fig. 3. Effect of enzyme concentrations on the rate of the L-tryptophan-dependent ATP-[32 P]PP_i exchange reaction

Enzymes were diluted with 50 mM-potassium phosphate buffer, pH 6.8, with 10 mM-2-mercaptoethanol and 10% (v/v) glycerol so that the enzyme concentrations in the reaction mixture were as indicated. Enzyme activities were assayed as described in 'Methods'. Each point represents an initial rate (samples assayed at 5, 10, 15 and 20 min for the concentration range A and 1, 2, 3, 5 and 10 min for the concentration ranges B and C) at 37°C.

of enzyme (3mg/ml) or higher was present in the column input, the enzyme exhibited a single symmetrical peak with an apparent molecular weight of 155000. However, when a more diluted enzyme (1.0mg/ml or less) was present in the column input multiple peaks of activity were observed. The molecular weights were estimated to be 155000, 105000 and 50000 for the enzyme in peaks I, II and III respectively. At present, we do not know whether the enzyme in peaks I, II and III represents the trimer, dimer and monomer. The fact that multiple peaks of activity can be detected in gel filtration does not prove that the enzyme in all of its quaternary structures is active, since conversion from one state into the other may take place during the assay. Such a conversion has been documented for the *E. coli* proline-activating enzyme (Lee & Muench, 1969). Different kinetic parameters can be obtained with different enzyme concentrations (see below), giving further support to the idea that the tryptophan-activating enzyme can exist in more than one kind of quaternary structure.

Kinetic studies

When the initial rates of exchange reaction were determined at various enzyme concentrations, a non-linear plot was obtained (Fig. 3). The curve was arbitrarily divided into three segments. In segment A the enzyme has a very low activity. In segment B the enzyme has a much higher initial rate. Further increase in the enzyme concentration (segment C) decreases the initial rate. The decrease was not due to the limitation of substrates in the reaction mixture, for addition of substrates did not improve the activity. There are at least two explanations for changes in enzyme activity: (1) enzyme in a dilute state undergoes an irreversible denaturation more rapidly than does enzyme in a concentrated state, and the rate of denaturation is fast. (2) The enzymes in segments A, B and C represent different quaternary structures, namely, the enzymes undergo a concentration-dependent dissociation-association between monomer and oligomer(s). At different states of association

the enzyme has different kinetic constants, resulting in the differences in catalytic activity. Possibility (1) is unlikely, since the enzyme showed no detectable loss of activity when incubated at 37°C for 15 min over a 40-fold difference in enzyme concentration (1.6–0.04mg/ml). To see if possibility (2) is the case, we determined the apparent kinetic constants in the L-tryptophan-dependent ATP-[³²P]PP_i exchange reaction, with enzyme concentrations in the range of B or C of Fig. 3. (The *K_m* and *V_{max}* for the enzyme at concentration range A were not determined, because the activities at low substrate concentrations were too low to be accurately determined.) The *K_m* and *V_{max}* values for the enzyme at 7 and 20 μg/ml, at pH 8.0, are shown in Fig. 4 and Table 2. Although the difference for the constants obtained at 7 and 20 μg/ml is not great, it is reproducible (Table 2).

Fig. 4. Effect of (a) tryptophan and (b) ATP substrates on the L-tryptophan-dependent ATP-[³²P]PP_i exchange reaction

(a) All the components, except L-tryptophan, in the reaction mixture were as described in 'Methods'. The enzyme concentrations in the reaction mixture were 7.0 μg/ml for curve (B) and 20 μg/ml for curve (C). The initial rates were obtained by incubation at 37°C for 2.5, 5.0 and 10.0 min for (B) and 1, 2 and 5 min for (C). The initial rates (*v*) were then plotted against *v*/[S] (substrate) as described by Dowd & Riggs (1965). (b) Components other than ATP were as described in 'Methods'.

Table 2. *K_m* and *V_{max}* of tryptophan-activating enzyme for tryptophan and ATP

The *K_m* and *V_{max}* values were calculated from Fig. 4. *V_{max}* was obtained from the intercept of the line with ordinate and *K_m* from the negative of the slope. B and C represent the concentrations used in the Fig. 4.

Substrate	Enzyme concentration (μg/ml)	<i>K_m</i> (μM)			<i>V_{max}</i> (nmol/min per μg)
		Expt. I	Expt. II	Expt. III	
L-Tryptophan	7.0 (B)	2.1	2.5	2.7	9.2
	20 (C)	1.3	1.3	1.0	8.0
ATP	7.0 (B)	2200	2600	—	10.2
	20 (C)	1300	1000	—	8.0

The K_m for tryptophan is about 10^{-6} M, but most of the K_m values reported for amino acids, as determined by the ATP- $[^{32}\text{P}]\text{PP}_i$ exchange reaction, are about 10^{-4} – 10^{-5} M (Peterson, 1967). Under the same

Fig. 5. Inhibition of L-tryptophan on the L-tryptophan-dependent ATP- $[^{32}\text{P}]\text{PP}_i$ exchange reaction

All the components, except L-tryptophan, were as described in 'Methods'. The enzyme concentration in the reaction mixture in the experiment was $7.0\text{ }\mu\text{g/ml}$ (B) and $20\text{ }\mu\text{g/ml}$ (C). Each point represents an initial rate at different L-tryptophan concentrations (0.05–2.5 mM).

Fig. 6. Effect of *p*-chloromercuribenzoate

The enzyme was passed through a Sephadex G-25 column, $0.9\text{ cm} \times 10\text{ cm}$, equilibrated with 50 mM -potassium phosphate buffer, pH 6.8, with 10% (v/v) glycerol. The breakthrough fractions which contained enzyme activities were pooled. *p*-Chloromercuribenzoate was added to the final concentrations of $0\text{ }\mu\text{M}$ (●), $1\text{ }\mu\text{M}$ (○), $3.3\text{ }\mu\text{M}$ (■) and $10\text{ }\mu\text{M}$ (△) and the enzymes were incubated at 0°C for the time indicated. Samples were withdrawn and assayed for enzyme activity as described in 'Methods'. The enzyme activity before the addition of inhibitor was normalized to 100%.

experimental conditions used for buffalo brain tryptophan-activating enzyme the K_m for tryptophan for the same enzyme from *E. coli* is 1.4×10^{-5} M (M.-L. Lee, unpublished work). It is therefore reasonable to assume that tryptophan-activating enzyme from buffalo brain can operate at a lower tryptophan concentration than can the *E. coli* enzyme. The normal concentration of tryptophan in mammalian brain is about 10^{-5} M (Williams *et al.*, 1950; Eccleston *et al.*, 1965; Perry *et al.*, 1971). Although this is lower than the K_m of tryptophan for the tryptophan hydroxylase of brain (Jequier *et al.*, 1967; McGeer *et al.*, 1968), it is above the K_m of tryptophan for the brain tryptophan-activating enzyme. A similar low K_m of tryptophan has been observed with the same enzyme from other mammalian tissues. For example, the K_m of tryptophan for the enzyme from bovine pancreas is 2.5×10^{-6} M (Dorizzi, 1972), from human skin is 2.7×10^{-6} M (Penneys & Muench, 1971) and from a human chronic lymphocytic leukemia lymphocyte is 2.9×10^{-6} M (Tchou *et al.*, 1971).

Concentrations of tryptophan greater than 0.2 mM inhibit enzyme activity (Fig. 5). Again the enzyme behaves differently at 7 and $20\text{ }\mu\text{g/ml}$.

Our kinetic data, obtained with the pure enzyme, confirms our initial observation that tryptophan-activating enzyme can be detected at unusually low tryptophan concentrations (C.-H. Chung, C.-C. Liu & M.-L. Lee, unpublished work).

Table 3. Effect of *p*-chloromercuribenzoate on tryptophan-activating enzyme in the presence or absence of substrate(s)

Enzyme of Sephadex G-200 fraction was treated as described in Fig. 6, and was then incubated with substrate or substrates, as indicated below, at 0°C for 30 min. The concentrations in the incubation mixture were $50\text{ }\mu\text{M}$ -L-tryptophan, 5 mM - Mg^{2+} and 6 mM -ATP. *p*-Chloromercuribenzoate was then added to the final concentration of $5\text{ }\mu\text{M}$ (see Fig. 6) and incubation was continued for 10 min at 0°C , before the enzyme was assayed as described in 'Methods'. The activity with enzyme incubated without inhibitor (control) was taken as 100%.

Substrates added before <i>p</i> -chloromercuribenzoate	Activity (% of control)
—	1.7
Tryptophan	2.5
Mg^{2+}	0
ATP	0
Tryptophan + Mg^{2+}	6.0
Tryptophan + ATP	2.9
Mg^{2+} + ATP	3.2
Tryptophan + Mg^{2+} + ATP	4.8

Effect of p-chloromercuribenzoate

Most of the amino acid-activating enzymes thus far studied require free thiol(s) for their activities, with a few exceptions such as lysine-activating enzyme from *E. coli* (Stern *et al.*, 1966), methionine-activating enzyme of *Sarcina lutea* (Hahn & Brown, 1967) and alanine-activating enzyme from pig liver (Webster, 1961). Tryptophan-activating enzyme from buffalo brain is strongly inhibited by *p*-chloromercuribenzoate as shown in Fig. 6. The inhibition was not prevented by the presence of substrate or substrates (Table 3). On the contrary, the same enzyme from *E. coli* or bovine pancreas is protected from *p*-chloromercuribenzoate inhibition by the presence of tryptophan and ATP (Joseph & Muench, 1971b; Lemaire *et al.*, 1967).

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