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TYROSINE AMINOTRANSFERASE FROM CHICK LIVER

HEAT ACTIVATION AND COLD INACTIVATION OF THE ENZYME *

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Summary

The reversible heat activation and cold inactivation of tyrosine aminotransferase (L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5) of chick liver were investigated. When the enzyme obtained by gel filtration was preincubated at 37°C for 10 min with 50 μM pyridoxal 5'-phosphate (pyridoxal-5'-*P*), a 7-fold increase in enzyme activity was detected. When the preincubated enzyme was cooled to 0°C, it lost its activity. Furthermore, the dramatic cyclical changes in enzyme activity occurred by sequential heating at 37°C and cooling to 0°C of the enzyme, in the presence of pyridoxal-5'-*P*, over shorter periods of time without loss of enzyme activity. However, when α-ketoglutarate was added to the enzyme during cold exposure, no further decrease in activity was observed. This protective effect was seen at a concentration of 5 μM.

Introduction

With respect to its regulatory properties, tyrosine aminotransferase (L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5) is probably the most intensively investigated enzyme in current biochemical literature [1]. Accordingly, its induction by the administration of a large number of substances has been investigated. Furthermore, the purification and properties of the enzyme from rat liver have been well established [2—4]. On the avian enzyme, however, very few reports have been published. Knox and Eppenberger found that "activa-

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Abbreviation: pyridoxal-5'-*P*, pyridoxal-5'-phosphate.

tion" of the enzyme occurred if a 100 000 × *g* supernatant of the chick embryo livers homogenized in KCl alone was allowed to stand for 2 h at 0° C after the addition of α-ketoglutarate [5]. Recently DeVivo and Peterkofsky observed that the tyrosine aminotransferase activity of chick embryo liver increased 1.5—2.0-fold by heating in the presence of α-ketoglutarate [6]. These reported data suggest that the properties of the chick liver tyrosine aminotransferase seem to differ from those of the rat liver enzyme.

While investigating on the regulation of tyrosine aminotransferase activity, we observed that the chick liver enzyme could be rapidly activated by preincubation at 37° C with pyridoxal-5′-*P*, but not α-ketoglutarate. Furthermore, enzyme activated by preincubation could be easily inactivated by exposure to cold unless protected by α-ketoglutarate.

The present report treats the effects of pyridoxal-5′-*P* and α-ketoglutarate on the reversible heat activation and cold inactivation of chick liver tyrosine aminotransferase.

Materials and Methods

All the chemicals used were commercial preparations. One-day-old male chicks (White Leghorn) were obtained from Sato Farms, Kyoto. The livers were removed from intact chick and either homogenized immediately or stored at −20° C for periods up to ten days and thawed just before homogenizing.

Assay of tyrosine aminotransferase

Tyrosine aminotransferase was measured by a modification of Diamondstone's method [7]. The reaction mixture containing 80 nmol of pyridoxal-5′-*P*, 10 μmol of α-ketoglutarate, 30 nmol of diethyldithiocarbamate, 100 μmol of Tris/Cl^- buffer, pH 7.5, 6 μmol of tyrosine and appropriate amounts of enzyme preparation in a total volume of 1.0 ml. The reaction was started by heating at 25° C. After incubation for 10 min, the reaction was stopped by the addition and rapid mixing of 4 ml of 1 M KOH. The KOH-treated samples were incubated for 30 min at 37° C. The absorbance of the samples was read at 331 nm in a Hitachi spectrophotometer (Type 124). To calculate the amount of *p*-hydroxyphenylpyruvate formed, an effective extinction coefficient of 19 900 M^{-1} was used [2]. One unit of enzyme was defined as the amount of enzyme producing 1 nmol of *p*-hydroxyphenylpyruvate in 10 min. Protein was determined by the method of Lowry et al. [8].

Results

Effect of preincubation on the enzyme activity

In the experiments on the stability of the tyrosine aminotransferase of chick embryo liver, DeVivo et al. [6] observed that α-ketoglutarate not only completely protected the enzyme activity but in addition activated the enzyme, when the enzyme was preincubated at 45° C. Furthermore, they suggested that the effect of pyridoxal-5′-*P* was variable and appeared to be independent of the action of α-ketoglutarate. We reinvestigated this point with a 105 000 × *g* supernatant as enzyme and confirmed that the enzyme activity represented a 4-

TABLE I

EFFECT OF PREINCUBATION WITH PYRIDOXAL-5′-*P*, α-KETOGLUTARATE OR BOTH ON THE ENZYME ACTIVITY

The liver was homogenzied with 4 vols. chilled 0.08 M Tris/Cl^- buffer containing 0.14 M KCl and 1 mM dithiothreitol (pH 7.5). The 105 000 × *g* supernatant was diluted 8-fold with same buffer. 0.4 ml of the diluted samples were preincubated at 45°C for 10 min with pyridoxal-5′-*P*, α-ketoglutarate or both. The mixture was chilled for 10 min. The assay was carried out at 37°C for 10 min in the standard reaction mixture as described in text. The data are the mean ± S.E. for 6 observations.

Additions in preincubation mixture	Activity (units/g tissue)
Non-preincubated	168 ± 19
Preincubated	
No addition	88 ± 26
$5 \cdot 10^{-4}$ M pyridoxal-5′-*P*	339 ± 19
$5 \cdot 10^{-4}$ M α-ketoglutarate	220 ± 25
Both	611 ± 59

fold increase over the activity recovered when the enzyme was preincubated with both α-ketoglutarate and pyridoxal-5′-*P* (Table I). Pyridoxal-5′-*P* alone showed a 2-fold increase in enzyme activity. However, as shown in Table I, it appears likely that α-ketoglutarate by itself may not significantly activate the enzyme, but it does protect the loss of the enzyme activity by heat treatment.

To clarify these phenomena further, the following experiments were carried out. 5 ml of the 105 000 × *g* supernatant used in the above experiment were applied to a Sephadex G-100 column, 2.5 × 40 cm, equilibrated with 0.08 M Tris/Cl^- buffer containing 1 mM dithiothreitol, pH 7.5. The elution was carried out with the same buffer. Fractions of 5 ml were collected. Under these conditions the enzyme activity was recovered in one single peak (tubes 12—20). The enzymes were pooled and referred to as the native enzyme.

When the native enzyme was preincubated at 37°C for 10 min in the presence of 50 μM pyridoxal-5′-*P* plus 50 μM α-ketoglutarate, and 50 μM pyridoxal-5′-*P* alone, respectively, 8-fold and 7-fold enzyme activities could be observed, compared to the value obtained without preparation. Again, significant increase in enzyme activity was not detected by preincubation in the presence of α-ketoglutarate alone (data not shown). Therefore, our data disagree with those of DeVivo et al. [6], at least on the effect of α-ketoglutarate.

Kinetic studies

The time course of *p*-hydroxyphenylpyruvate formation was examined at different temperature. Using the enzyme preincubated with both pyridoxal-5′-*P* and α-ketoglutarate, the rate of *p*-hydroxyphenylpyruvate formation became linear with increasing assay time at all temperature tested (Fig. 1A). Similar results were also obtained with the enzyme preincubated in the presence of pyridoxal-5′-*P* alone, although the enzyme activity was about 80% of that seen in Fig. 1A. Using the native enzyme, however, the reaction rate was clearly nonlinear at 37 or 30°C, showing an upward curvature (Fig. 1B). Therefore, we presumed that increase in enzyme activity by preincubation in the presence of pyridoxal-5′-*P* plus α-ketoglutarate or pyridoxal-5′-*P* alone is due to

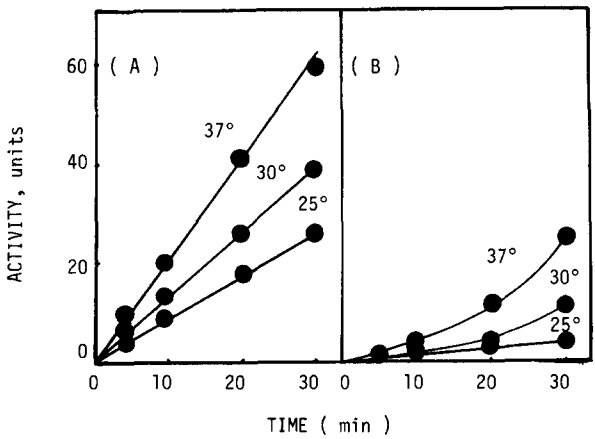


Fig. 1. Time course for *p*-hydroxyphenylpyruvate formation at 25, 30, and 37°C with native and preincubated tyrosine aminotransferase. Enzyme preincubation was carried out in the presence of both 50 μM pyridoxal-5′-*P* and same concentrations of α-ketoglutarate at 37°C for 10 min with 0.14 mg native enzyme. The enzyme activity was measured by the method described in text except temperature used.

enzyme activation occurred during preincubation. At 25°C, the reaction was linear with both enzymes (Fig. 1A and B). Therefore, the following experiments were carried out at 25°C:

To see the optimal temperature for the reaction: inactive → active enzyme, the native enzyme was preincubated at different temperatures for 10 min. It was found that the maximal activation would occur at 37°C when the enzyme was preincubated with 50 μM pyridoxal-5′-*P* plus the same concentrations of α-ketoglutarate or pyridoxal-5′-*P* alone. The time required for enzyme activation was investigated. It was found that maximum activation was reached within 10 min at 37°C in the presence of 50 μM pyridoxal-5′-*P* plus the same concentrations of α-ketoglutarate (data not shown).

Activation as a function of pyridoxal-5′-*P* concentration in the preincubation system was investigated. When the native enzyme was preincubated with increasing concentrations (0—50 μM) of pyridoxal-5′-*P* and 50 μM α-ketoglutarate at 37°C for 10 min, the concentration required for maximum activation was found to be 5 μM. Furthermore, the K_a for pyridoxal-5′-*P* was calculated to be 0.1 μM.

Stability of activated enzyme

The stability of activated enzyme at 0°C was investigated. The activity promoted by preincubation at 37°C in the presence of both pyridoxal-5′-*P* and α-ketoglutarate remained constant for at least 6 h (Fig. 2), but the enzyme activated in the presence of pyridoxal-5′-*P* alone lost about 60% of its activity during the first 1 h. If, however, 50 μM α-ketoglutarate was added to the activated enzyme during the cold exposure, no further decrease in enzyme activity was observed. The effect of increasing concentration of α-ketoglutarate on the stabilization of the activated enzyme at 0°C for 40 min was investigated. It was found that the most complete protection against cold inactivation was observed in the presence of α-ketoglutarate at a concentration of 50 μM, and the half

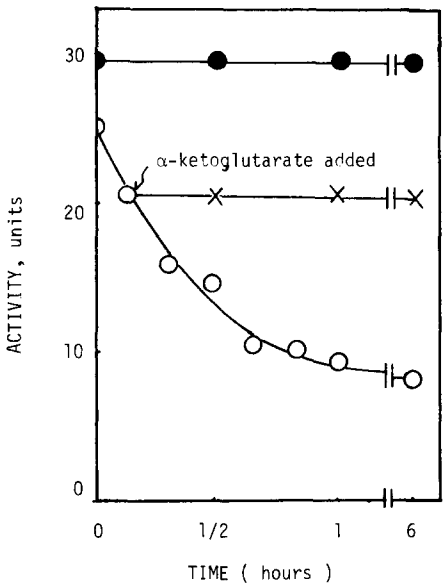


Fig. 2. The protective effect of α-ketoglutarate on tyrosine aminotransferase against cold inactivation. The native enzyme was preincubated with either pyridoxal-5′-*P* and α-ketoglutarate or pyridoxal-5′-*P* alone. Aliquots of the preincubated enzyme were chilled at 0°C for the times indicated, and then their activities were assayed at 25°C as described in text. •———•, preincubated with 50 μM pyridoxal-5′-*P* plus 50 μM α-ketoglutarate; ○———○, preincubated with 50 μM pyridoxal-5′-*P* alone; X———X, α-ketoglutarate added at a concentration of 50 μM.

maximum protection by α-ketoglutarate was calculated to be 10 μM. The protective values of other keto acids under the same conditions were also tested. As seen in Table II, the oxaloacetate, α-ketovalerate, and α-ketobutyrate were found effective at a concentration of 10 mM. This concentration is more than 100 times that of α-ketoglutarate. Such α-keto acids as pyruvate and α-ketoadi-

TABLE II

RELATIVE PROTECTION OF TYROSINE AMINOTRANSFERASE ACTIVITY BY VARIOUS α-KETO ACIDS DURING COOLING AT 0°C

The enzyme, obtained by gel filtration and preincubated with 50 μM pyridoxal-5′-*P*, was immediately chilled at 0°C in the presence of various α-keto acids. The pH of the keto acids was adjusted to pH 7.5 just before use. After standing for 40 min at 0°C, the enzyme was assayed at 25% as described in text. The enzyme activity protected by 0.1 mM α-ketoglutarate during cold exposure was defined as 100%. The activity of the enzyme exposed to 0°C for 40 min in the absence of α-keto acid was 40% compared to that in the presence of 0.1 mM α-ketoglutarate. Each reaction mixture contains 0.12 mg enzyme protein.

Keto acids	Activity (%)		
	0.1 mM	1 mM	10 mM
Oxaloacetate	48.0	60.0	80.0
α-Ketovalerate	39.0	49.4	90.3
α-Ketobutyrate	48.9	76.2	98.4
Pyruvate	44.4	44.4	66.0
α-Ketoadipate	50.6	74.7	72.3

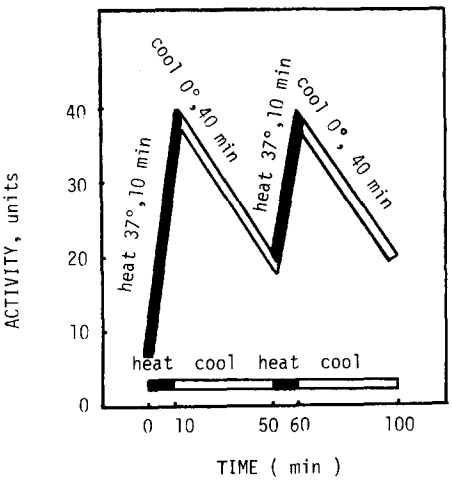


Fig. 3. Reversibility of heat activation and cold inactivation of tyrosine aminotransferase. The native enzyme was assayed without preincubation. The remaining enzyme was preincubated with 50 μM pyridoxal-5′-*P* at 37°C for 10 min. The rest of the preincubated enzyme solution was chilled at 0°C for 40 min and immediately assayed. The heating and cooling steps were repeated on the remaining samples. All the assays were carried out at 25°C with 0.12 mg protein of the enzyme.

pate offered only a slight protective effect at high concentrations, and β- and γ-keto acids such as β-ketoglutarate and levulinate were inactive (data not shown).

Reversibility of activated and inactivated enzyme

It was interest to see the reversibility of heat activation and cold inactivation of tyrosine aminotransferase. As depicted in Fig. 3, dramatic cyclical changes in enzyme activity were observed by sequential heating and cooling of the enzyme over shorter periods of time. Heating resulted in a 7-fold increase; cooling to 0°C resulted in significantly decreased activity, which could again be reversed to its original high level without any irreversible loss.

Discussion

We demonstrated the reversible heat activation and cold inactivation of chick liver tyrosine aminotransferase. The following observations led us to re-investigate the effect of pyridoxal-5′-*P* and α-ketoglutarate on the enzyme stability: (1) using native enzyme the rate of *p*-hydroxyphenylpyruvate formation at the temperature above 30°C is not linear; and (2) a rapid loss in enzyme activity due to cold exposure occurs in the enzyme activated in the presence of pyridoxal-5′-*P* alone.

DeVivo et al. [6] reported evidence of the activation of chick liver tyrosine aminotransferase by preincubation with α-ketoglutarate. Since their experiments were carried out using crude extract (probably contains sufficients of pyridoxal-5′-*P* for heat activation) or using gel filtrate obtained in the presence of pyridoxal-5′-*P*, α-ketoglutarate could quite probably have appeared to be the enzyme activator. Since the heat activation of the chick liver enzyme can occur only in the presence of pyridoxal-5′-*P* concentrations as low as 0.1 μM, the

trace amounts of cofactor could be effective in manifesting the α-ketoglutarate effect on enzyme activity.

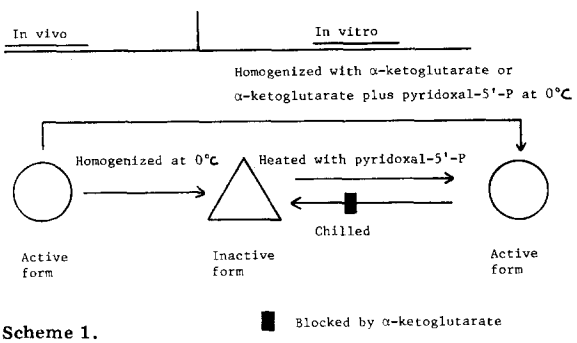
Apart from the results of in vitro experiment, tyrosine aminotransferase would present in an active form in vivo, because the concentration of pyridoxal-5′-*P* in the chick liver is sufficient for heat activation [8]. If this is the case, it is possible that the active form in vivo would be inactivated during enzyme preparation at low temperatures, in the absence of α-ketoglutarate. In fact, DeVivo et al. [6] have demonstrated that high enzyme activity can recover when the chick liver is homogenized with chilled homogenizing medium containing α-ketoglutarate. We also confirmed this point (data not shown).

Furthermore, DeVivo et al. [6] observed that other α-keto acids such as α-ketoadipate can also affect enzyme activity. We also confirmed this point with several α-keto acids. The results show that not only α-ketoglutarate but also other α-keto acids offer protection against cold inactivation, although α-ketoglutarate proved the most active among the several α-keto acids tested here (Table II).

Many investigators have described the details of the properties of rat liver tyrosine aminotransferase [2—4]. They demonstrated the protective nature of α-ketoglutarate against the heat inactivation of the enzyme. Hayashi et al. found that α-ketoglutarate affords protection to the holoenzyme by preventing the tightly bound cofactor pyridoxal-5′-*P* being converted to the more loosely bound form, pyridoxamine phosphate [2]. Whether the stabilizing effect on this enzyme in chick liver differs from that in mammals is as yet unknown. However, it is true that no such cold inactivation and heat activation occur in rat liver (data not shown).

Reversible cold lability has been documented for several enzymes [10—17]. These enzymes inactivation involves a concomitant molecular dissociation into subunits [10,12—16]. Furthermore, Toyama et al. [18] observed the activation of the crystalline taurine-α-ketoglutarate transaminase from *Achromobacter superficialis* by heating at 45—60°C in the presence of pyridoxal-5′-*P*. The detailed mechanism of this activation is unknown. Therefore, it is necessary to investigate the state of coenzyme, its extent of binding, the state of association of the enzyme, and the nature of the products of the activation process using the purified chick liver tyrosine aminotransferase.

The present data together with those reported by DeVivo et al. [6] would read the following scheme on the possible change of active and inactive tyrosine aminotransferase from chick liver in vivo and in vitro (Scheme 1).



Scheme 1.

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