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The activation of chick liver tyrosine transaminase *in vitro*

The several-fold activation of tyrosine transaminase (L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5) obtained by homogenizing chick livers in α-ketoglutarate was reported in a previous paper[1]. The purpose of the present study was to determine the requirements and the time course of this activation in order to assay the total enzyme. Some of these results were briefly reported[2].

Newly hatched White Rock chicks from a commercial supplier were fed a regular chick diet and used systematically at ages from 1 to 38 days. Since no age differences in the enzyme levels or the activations were observed, the different age groups are not separately reported. In half of the chicks the tyrosine transaminase activity was elevated by injecting hydrocortisone (2.4 mg per 100 g body wt.) intraperitoneally 4 h before killing. The livers were chilled and promptly homogenized in a Potter–Elvehjem homogenizer with 3 ml of cold 0.14 M KCl per g of liver. The homogenates were centrifuged at 100 000 $\times$ g for 1 h and the clear supernatant fractions withdrawn carefully with a pipette to avoid contamination with the particulate fraction. The conditions of the assay and the activation are described in Table I.

If pyridoxal phosphate and α-ketoglutarate were added to fresh homogenate of livers from either treated or untreated chicks, there was more than a threefold increase in tyrosine transaminase activity, to a maximum after a 1–2-h period, as previously reported[1]. A similar increase occurred if the additions were made to a liver supernatant

TABLE I

ACTIVATION OF TYROSINE TRANSAMINASE IN HOMOGENATE AND IN SOLUBLE FRACTION OF CHICK LIVERS

Tyrosine transaminase activity was determined in the supernatant fraction by the enol–borate method[3]. The final assay mixture was a total volume of 1 ml at pH 8, containing 3.4 μmoles of tyrosine, 22.8 μmoles of α-ketoglutarate, 0.03 μmoles of pyridoxal phosphate, 0.57 mM borate, and 0.15 k units of purified[4] arylpyruvate enol–keto tautomerase (EC 5.3.2.1). The enzymic reaction was initiated by the addition of 0.1 ml of the supernatant fraction equivalent to 25% liver extract. The reaction at 25° was followed by the increase in absorbance at 310 mμ due to the formation of the enol–borate complex of the *p*-hydroxyphenylpyruvate, using a Gilford Multiple Sample Recorder. Activities were measured before *(Initial Activity)* and after *(Final Activity)* incubations at 0° with 0.3 mM pyridoxal phosphate and 0.02 M α-ketoglutarate. The *Homogenate System"* was the original homogenate incubated with the additions for 1 h and centrifuged for 1 h before assay. The *"Soluble System"* was the original supernatant fraction incubated with the additions for 5 h before assay. Results are given for preparations from untreated and hydrocortisone-treated chicks, as the mean activity $\pm \sigma$, with the number of chicks used in parentheses.

Treatment of chicks	*Initial Activity (μmoles/h per g liver, 25°)*	*Final Activity (μmoles/h per g liver, 25°)*	
		Homogenate System (2 h)	*Soluble System (5 h)*
Untreated	4.7 ± 3.6 (32)	19.7 ± 9.6 (28)	14.8 ± 8.6 (32)
Hydrocortisone	19.9 ± 11.5 (33)	62.4 ± 25.2 (30)	51.3 ± 16.6 (33)

fraction instead of an homogenate (Table I). Activation in the supernatant fraction was slower than in the homogenate, reaching maximum activity after about 5 h (*cf.* Fig. 1). Supernatant fractions kept at 0° without additions were not activated (Fig. 1).

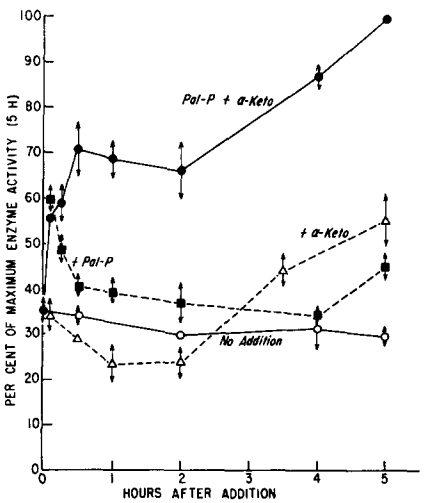

Fig. 1. Time curves of tyrosine transaminase activation in chick liver supernatant fractions at 0°, with no additions (○—○), with 0.3 mM pyridoxal phosphate (*Pal-P*) (■ — —■), with 0.02 M α-ketoglutarate (*α-Keto*) (△— —△), and with pyridoxal phosphate *plus* α-ketoglutarate (●—●). The average activity of six or more preparations at each point (± S.E. indicated by arrows) is expressed as the per cent of the maximum activity in the same preparations after treatment for 5 h with α-ketoglutarate *plus* pyridoxal phosphate. The maximum absolute activities of the preparations used for the different curves from untreated and hydrocortisone-treated chicks, respectively, averaged 11.7 and 40.0 μmoles per h per g liver.

The requirements and time course for activation of tyrosine transaminase in liver supernatant at 0° are shown in Fig. 1. Since preparations from normal and hydrocortisone-treated chicks increased tyrosine transaminase activity to the same degree (Table I), the results from these two kinds of chicks were pooled and expressed relative to the maximum activity. Although the assays were continued for 24 h, all the major changes occurred within the 5-h period. If only pyridoxal phosphate was added to the concentrated fraction, a very transient doubling of activity occurred within a few minutes, after which the activity fell. This occurred despite the presence of excess pyridoxal phosphate in the dilute assay system. When both pyridoxal phosphate and α-ketoglutarate were added there was a prompt increase, as with pyridoxal phosphate alone, but the activity remained high and later rose again, as with α-ketoglutarate alone.

If the enzyme was incubated with only α-ketoglutarate for 5 h and pyridoxal phosphate then added, within 5 min nearly full activation had occurred. If the sequence of addition was reversed, there was also prompt activation 5 h later, 5 min after α-ketoglutarate addition, but only to 60% of maximum.

Treatment of the supernatant fraction with α-ketoglutarate alone increased the apparent degree of conjugation of the tyrosine transaminase with its coenzyme, pyridoxal phosphate. This was measured under the standard conditions as the ratio of activities found without and with pyridoxal phosphate added to the assays. About a third of the total activity (37%) present in fresh or 5-h-old preparations was assayed in the absence of added pyridoxal phosphate. Treatment with α-ketoglutarate for 5 h doubled the fraction of activity (70%) assayable without added pyridoxal phosphate. Since this treatment with α-ketoglutarate also approximately doubled the absolute activity (Fig. 1), there was a fourfold increase in the apparent amount of enzyme conjugated with the coenzyme.

Concentrations of pyridoxal phosphate and of α-ketoglutarate one-tenth those routinely used caused less but still significant activations. Glutamate, tyrosine and tryptophan did not replace α-ketoglutarate in the activation, but they did not prevent the usual transient activation by pyridoxal phosphate alone. Addition of glutamate (0.02 M) with pyridoxal phosphate and α-ketoglutarate (0.002 M, one-tenth the usual concentration) reduced the activation to that with pyridoxal phosphate alone. Glutamate might have prevented activation by amination of the enzyme or the coenzyme. This is unlikely, however, since pyridoxamine phosphate, as well as pyridoxal phosphate, activated the enzyme.

Only about one-fourth of the total enzyme of chick liver homogenates is active in an optimal assay system without preliminary treatment. Since at least 75 or 80% of the total activity demonstrable in an incubated homogenate (Table I) can be obtained by treatment of the particle-free supernatant fraction, it is apparent that most of the inactive form is soluble. Since no additional enzyme could be extracted by various treatments of the particulate fraction from the homogenate, all of the inactive form might indeed be soluble. It may be incompletely, as well as slowly, activated in the soluble fraction in the absence of the particulate fraction.

The activation occurred in the concentrated enzyme fraction (25% liver extract) with the necessary additions, but with diluted enzyme (2.5% liver extract) at 25°, it either did not occur or occurred at a much slower rate. There appeared to be two aspects to the activation process: a slow action by α-ketoglutarate that was associated

with increase in the apparent degree of conjugation of endogenous coenzyme with the enzyme, and a prompt action by added pyridoxal phosphate.

The participation of factors besides pyridoxal phosphate and α-ketoglutarate can be inferred from the faster and greater activation occurring in homogenates, and the occurrence of activation only in supernatant fractions that are concentrated. A similar activation of crude rat liver transaminase to the extent of 10 to 30% over the initial activity has been reported. This occurred during incubation of soluble *plus* particulate fractions with a complex mixture that included α-ketoglutarate[5].

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Purification of human erythrocyte pyruvate kinase

Hereditary non-spherocytic hemolytic anemias in man may be classified on the basis of several different enzyme defects. The red cells of one such group of patients has a low ATP content and a specific deficiency of pyruvate kinase (ATP:pyruvate phosphotransferase, EC 2.7.1.40) (refs. 1–6). This report is concerned with purification and properties of this enzyme which catalyzes interconversion of ATP and pyruvate to ADP and phosphoenolpyruvate (PEP) in the presence of Mg^{2+} and K^+.

Pyruvate kinase was assayed by coupling the reaction to the DPNH-dependent reduction of pyruvate to lactate by lactate dehydrogenase (free of pyruvate kinase). Decrease in absorbance at 340 mμ provided a measure of pyruvate kinase activity. The assay system (3 ml) contained, in addition to the solution being assayed, the following reagents at 31°, pH 7.25: 0.04 M Tris, 0.075 M KCl, 0.008 M $MgSO_4$, 0.001 M

Abbreviation: PEP, phosphoenolypyruvate.