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DEGRADATION OF PHENYLALANINE:PYRUVATE TRANSAMINASE AFTER GLUCAGON TREATMENT

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Summary

Using a single-isotope and immune precipitation technique the half-life ($t_{1/2}$) of hepatic phenylalanine:pyruvate transaminase (aminotransferase, EC 2.6.1.—, number not yet assigned) from glucagon-treated rats was determined to be 2.8 days, similar to that of the control rats ($t_{1/2}$ = 3.3 days). The half-life of rat liver total soluble proteins also remained unchanged after glucagon treatment ($t_{1/2}$ = 2.7 days in glucagon-treated rats; $t_{1/2}$ = 2.8 days in normal). Thus, glucagon has no effect on the degradation of phenylalanine:pyruvate transaminase. Furthermore, the degradation rates are similar for both the holoenzyme and the apoenzyme of phenylalanine:pyruvate transaminase.

Introduction

Hepatic phenyalalanine:pyruvate transaminase (aminotransferase, EC 2.6.1.—, the exact EC number has not been assigned) catalyzes the transamination between phenylalanine and pyruvate [1] and is inducible by glucagon [2—5]. The enzymatic activity remains above control level for more than a week following a single subcutaneous injection of glucagon. The long duration of glucagon effect could be therapeutically useful in providing an alternative pathway for disposing of the excess phenylalanine in the human disease phenylketonuria. However, neither the functional significance of glucagon-induced phenylalanine:pyruvate transaminase nor its mechanism of glucagon induction are well understood at present. Our previous work showed that this enzyme was specific for pyruvate as an amino group acceptor and could be separated from phenylalanine:α-ketoglutarate transaminase by a Sephadex electrophoresis column [6]. Heat stability and glucagon inducibility experiments showed phenylalanine and histidine to be the primary amino acid sub-

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strates for this enzyme [4]. By pulse-labeling the enzyme with [^{3}H]leucine and using immunochemical precipitation, we demonstrated that the glucagon induction of this transaminase is via de novo synthesis (unpublished data).

It is generally accepted that degradation, as well as synthesis, is important in the regulation of enzyme level in animal tissues [7—9]. We therefore determined the effect of glucagon on the degradation rate of phenylalanine:pyruvate transaminase and rat liver total soluble proteins. Our results with single-isotope administration and immune precipitation techniques, suggest that normal and glucagon-induced transaminase is degraded at the same rate. Moreover, evidence is provided suggesting that this transaminase exists predominantly as the holoenzyme in the normal animals, but mostly as apoenzyme in the glucagon-treated rats. This finding implies that the apoenzyme and the holoenzyme are degraded at the same rate, and the enzyme-coenzyme interaction does not change the degradation rate of this enzyme.

Methods

Purification of phenylalanine:pyruvate transaminase and preparation of antiserum. Male Sprague-Dawley rats each weighing about 200 g were used in all experiments. The rats were maintained on regular laboratory chow and water ad libitum. The methods for purification and antibody preparation were reported in detail earlier [10]. Briefly, glucagon-induced hepatic phenylalanine:pyruvate transaminase was purified by DEAE-Sephadex A-50, hydroxyapatite and Sephadex G-100 chromatography and preparative gel electrophoresis. The purified enzyme was injected into rabbits for antibody production.

Isotopic and immunochemical procedure. The single-isotope administration method was used to determine the half-life and the degradation rate constant of phenylalanine:pyruvate transaminase. The same determination was performed for rat liver total soluble proteins. 1 mg/kg glucagon was injected subcutaneously and was repeated once more 8 h later in order to obtain maximum induction. 500 μCi uniformly labeled L-[4,5-^{3}H(N)]leucine in 1 ml was injected intraperitoneally at 24 h after the first glucagon injection. Rats were killed at 1, 2, 3, or 4 days after [^{3}H]leucine injection. Livers were removed, homogenized in 10 vols. (w/v) 0.05 M Tris · HCl buffer (pH 7.6) and centrifuged at 30000 × *g*. The supernatant fraction was partially purified by heating at 60°C for 10 min in the presence of 0.2 mM pyridoxal phosphate and then centrifuged. Sufficient antiserum to phenylalanine:pyruvate transaminase was added to the clear supernatant to precipitate 100% of the enzyme activity. 1 ml of antiserum was sufficient to precipitate 2368 units of transaminase activity. One unit of transaminase activity is defined as 1 nmol product formed/min per mg protein. The antibody/antigen mixture was incubated at 37°C for 30 min, then overnight in the cold. The immunoprecipitates were collected by centrifugation and washed three times with cold 0.9% NaCl in 0.05 M Tris · HCl buffer (pH 7.6). The washed immunoprecipitates were dissolved in a mixture of 1% sodium dodecyl sulfate/1% 2-mercaptoethanol/1% dithiothreitol, then subjected to gel electrophoresis [11]. After electrophoresis, the gel was sliced and digested for 12 h at 45°C in a toluene-based scintillation counting fluid containing 10% Nuclear

Chicago Scintillator and 1% H_2O. The radioactivity incorporated into the transaminase was determined by a Beckman LS-335 liquid scintillation counter. The same procedure was employed for the normal rat liver.

Radioactivity incorporated into the total soluble proteins was determined by precipitating an aliquot of the unheated liver supernatant with 5% trichloroacetic acid (final concentration) [12]. The protein precipitates were then spun down and resuspended in 5% trichloroacetic acid. After heating at 90°C for 15 min, the sample was cooled and filtered directly onto Millipore filters (type HA). The filter paper and precipitates were washed well with 5% cold trichloroacetic acid and counted in Aquasol 2 scintillation fluid. The degradation rate of the transaminase and rat liver total soluble proteins were determined assuming that the degradation follows first-order kinetics [13].

Enzyme assays. Phenylalanine:pyruvate transaminase was assayed by the spectrophotometric method described by Lin et al. [1]. The reaction mixture (1 ml) contained 90 mM L-phenylalanine/46 mM sodium pyruvate/0.12 mM pyridoxal phosphate/24 mM borate buffer (pH 8.1)/appropriate amount of enzyme. The keto-acid formed from L-phenylalanine was measured as the yellow enol · borate complex with an absorption maxima at 315 nm. The 30000 × *g* supernatant fraction was assayed in the absence and the presence of 0.12 mM pyridoxal phosphate in order to determine the percent holoenzyme of the transaminase. Protein concentrations were determined by the method of Lowry et al. [14]. Bovine serum albumin was used as the protein standard.

Results and Discussion

The specificity of immune precipitation. The specificity of our immune precipitation method with this enzyme has been documented before [6]. Fig. 1 shows the separation profiles of the radioactive immunoprecipitates after SDS-

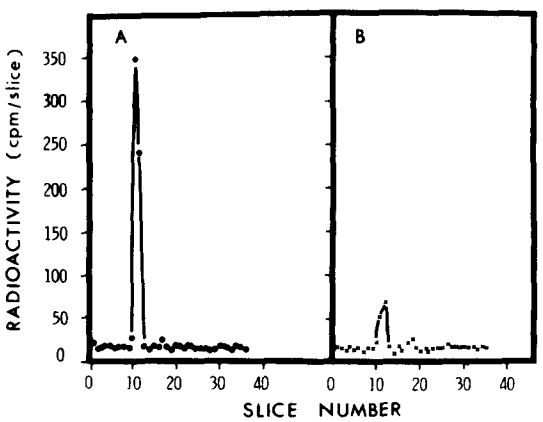

Fig. 1. Radioactive immunoprecipitates separated on polyacrylamide-sodium dodecyl sulfate gel electrophoresis. Rats weighing 180—200 g each were injected intraperitoneally with 500 μCi of L-[4,5-^{3}H(N)]-leucine 24 h after two glucagon injections. The rats were killed 24 h after the [^{3}H]leucine injection and the livers were homogenized. A 30000 × *g* supernatant fraction was heated and reacted with a phenylalanine:pyruvate transaminase-specific antiserum. See text for details. (A), 266 μg of precipitates from a glucagon-treated rat; (B), 266 μg of precipitates from a normal rat.

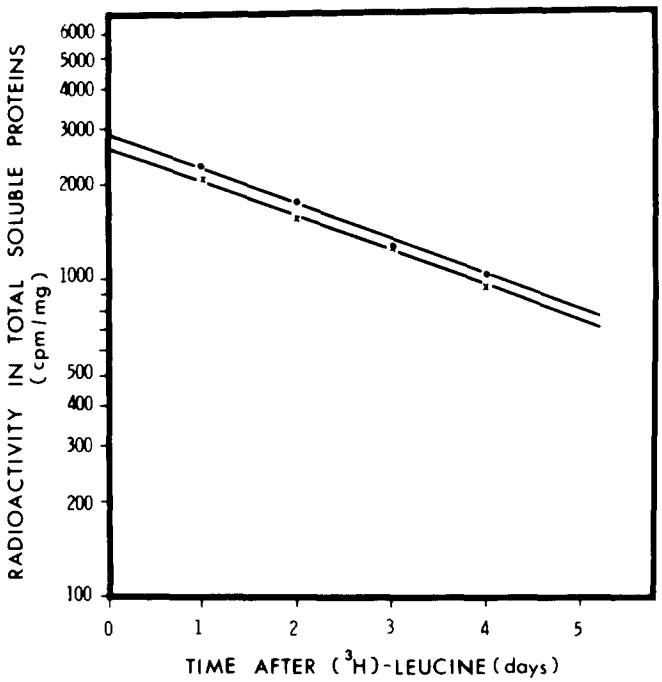

Fig. 2. The loss of radioactivity in rat liver total soluble proteins after glucagon treatment. Rats weighing 180—200 g each were injected with glucagon (1 mg/kg) subcutaneously at 0 and 8 h. L-[4,5-^{3}H(N)]-Leucine (500 μCi) was given to each rat at the 24th hour. Animals were killed at 1, 2, 3, or 4 days thereafter. Radioactivity incorporated into total soluble proteins was determined in 5% trichloroacetic acid precipitates. Each time point represents an average from two rats. Details are described in Methods. X———X, normal rats; ●———●, glucagon-treated rats.

polyacrylamide gel electrophoresis. It indicated that when the supernatant fraction from a 30000 $\times g$ centrifugation was heated at 60°C for 10 min, then reacted with the antiserum, only one radioactive peak was observed on the polyacrylamide gel for both glucagon-treated (Fig. 1A) and normal rats (Fig. 1B). Judging by the mobility on the gel, its subunit molecular weight was 42000, the same as that of the purified phenylalanine transaminase [6]. Under this condition, 90% of the radioactivity was recovered from the gel. As shown in Fig. 1, the amount of [^{3}H]leucine incorporated into the transaminase was much higher in glucagon-treated animals than in the normal rats. This result confirmed our previous report that the glucagon induction of phenylalanine transaminase is via de novo synthesis of the enzyme [6].

Degradation of total soluble proteins. Glucagon shows no effect on the degradation of total soluble proteins during the 4-day period. The half-life of liver total soluble proteins in normal rats is 2.8 days; in glucagon-treated rat liver is 2.7 days. Their apparent rate constants of degradation (K_d) remained unchanged (normal, $K_d = 0.25$ day^{-1}; glucagon treated, $K_d = 0.26$ day^{-1}, Fig. 2). However, the radioactivity incorporated into the total soluble proteins of glucagon-treated rats was 11% higher than that of the normal. This increase may be contributed by the inductive effect of glucagon on other hepatic enzymes [15], such as alanine aminotransferase, arginase and acetyl-CoA carboxylase [16].

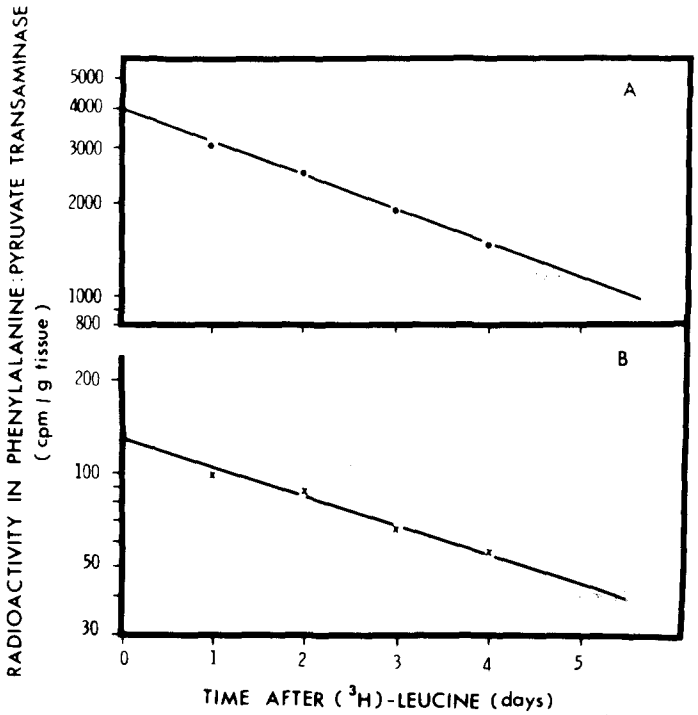

Fig. 3. The loss of radioactivity in hepatic phenylalanine:pyruvate transaminase from (A) glucagon-treated rats, (B) normal rats. The dosage, injection time of glucagon, and the amount of [H^3]leucine injected were the same as that described in Fig. 2. Radioactive phenylalanine:pyruvate transaminase was selectively precipitated with the specific antiserum after heating the 30000 × *g* supernatant fraction at 60°C for 10 min. The immunoprecipitates were separated on polyacrylamide-SDS gel. The radioactivity incorporated into the enzyme was determined by counting the corresponding gel slices. Each point represents an average of two rats, the error was less than 10%. See text for details.

The half-life of the soluble proteins in rat liver as determined in our experiment is comparable to that determined by Kim and Kim [17]. Using the poorly reutilized amino acid L-[guanidino-^{14}C]arginine, they reported that the half-life of normal rat liver total soluble proteins was 3.2 days. Thus, the reutilization of [^{3}H]leucine appears to be not markedly affecting our experimental measurements.

Degradation of phenyalalnine:pyruvate transaminase. The apparent half-life of phenylalanine:pyruvate transaminase from glucagon-treated rats is 2.8 days (K_d = 0.25 day^{-1}, Fig. 3A), similar to that of normal rats ($t_{1/2}$ = 3.3 days, K_d = 0.21 day^{-1}, Fig. 3B). The difference of 0.5 day is within experimental error. This finding suggests that glucagon has no effect on the degradation rate of the transaminase. Therefore, its inductive effect is entirely contributed by de novo biosynthesis of the enzyme as we have reported earlier [6]. The observed slow degradation rate of this transaminase may explain the previous finding [5,18, 19] that the transaminase is a slow inducing enzyme. The long lasting glucagon effect on this enzyme is clearly attributed to its slow degradation rate. A similar effect of glucocorticoid on glutamate:alanine transaminase [20] and tryptophan oxygenase [21] was reported previously. They have demonstrated that

TABLE I

GLUCAGON-INDUCED PHENYLALANINE : PYRUVATE TRANSAMINASE EXISTED MOSTLY AS THE APOENZYME FORM

The phenylalanine : pyruvate transaminase activity was determined by a spectrophometric assay [1]. The 30 000 × *g* supernatant fraction was assayed in the absence (holoenzyme activity) and presence (total enzyme activity) of 0.12 mM pyridoxal phosphate.

	Phenylalanine : pyruvate transaminase activity (nmol product formed/min per mg protein)		% Holoenzyme
	Holoenzyme	Total enzyme	
Normal	7.76	12.1	64
Glucagon treated	9.70	107.8	9

glucocorticoid did not change the degradation rate of the enzyme, thus only effect of glucocorticoid appears to be on the rate of enzyme synthesis.

It is interesting to note that after glucagon induction, the phenylalanine: pyruvate transaminase molecules are not all in the active holoenzyme form. The percent of holoenzyme was determined by measuring the catalytic activity in the absence and the presence of 0.12 mM pyridoxal phosphate, the saturation concentration under our experimental conditions. As indicated in Table I, 64% of the transaminase activity existed as holoenzyme in the normal rats, but only 9% as holoenzyme in the glucagon-treated rats. This phenomenon may be attributed to the inefficient association of the newly synthesized transaminase with pyridoxal phosphate after glucagon induction. Despite the fact that glucagon-induced transaminase is predominantly in its apoenzyme form, it is apparent from Fig. 3 that the incorporation of radioactivity into the glucagon-induced transaminase is much higher than that in the normal. A possible explanation is that the transaminase antibody does not distinguish between the holoenzyme and the apoenzyme of the transaminase. This conclusion was arrived at by using the immune precipitation technique described above to measure the [^{3}H]leucine incorporated into the apoenzyme of phenylalanine: pyruvate transaminase. The immune precipitation was done in the 30000 × *g* supernatant fraction from glucagon-treated rats with no pyridoxal phosphate added. Under these circumstances, similar separation profiles of radioactive immunoprecipitates (Fig. 1A) were obtained after electrophoresis. The amount of [^{3}H]leucine incorporated into the transaminase was also comparable. This finding indicates that the holoenzyme and the apoenzyme are equally well precipitated by the transaminase antibody. Nevertheless, we have found no significant difference in the degradation rate of glucagon-induced or normal phenylalanine:pyruvate transaminase, which would imply that the holoenzyme and apoenzyme are degraded at the same rate. This finding suggests that the interaction of the transaminase with its cofactor pyridoxal phosphate does not alter the degradation rate of the enzyme. On the contrary, Kominami and Katanuma [22] have discovered proteases which specifically cleave the apoenzyme forms of pyridoxal enzymes [22,23]; also Litwack and Rosenfield [24] have found the correlation between rates of in vivo degradation of several pyridoxal enzymes and their rates of coenzymes dissociation in vitro. Based on

these observations they suggested that dissociation of coenzymes to yield unstable apoenzymes may be rate limiting for degradation of pyridoxal enzymes. However, our finding is consistent with the report by Lee et al. [25], in which they demonstrated that the apoenzyme and active holoenzyme of tyrosine aminotransferase and alanine aminotransferase are degraded at the same rate.

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