THE INTERCONVERSION OF MULTIPLE FORMS OF TYROSINE AMINOTRANSFERASE

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Received April 30,1975

SUMMARY

Previous publications from this laboratory demonstrated that tyrosine aminotransferase from rat liver could be separated into three or four molecular forms by hydroxyapatite chromatography. The storage of soluble tyrosine aminotransferase for 60 hours at 4° caused a marked decrease in form II and consequent increase in form III and/or form IV while no change in total enzyme activity was seen. This effect was more marked when the liver homogenate was prepared in phosphate buffer, pH 6.9, than at pH 7.6. The pattern of multiple forms was altered by varying or changing the pH of the liver homogenate. The incubation of soluble tyrosine aminotransferase with components from the particulate fraction of a liver homogenate results in a pH dependent conversion of the multiple forms similar to that seen on storage. In addition, incubation of soluble tyrosine aminotransferase with a microsomal fraction of liver shows a similar, but less marked, effect. It is suggested, therefore, that a microsomal component whose catalytic action is dependent on pH is responsible for the interconversion of the multiple forms of tyrosine aminotransferase.

INTRODUCTION

Reports of several laboratories have shown the presence of multiple forms of tyrosine aminotransferase (L-tyrosine: 2-oxoglutarate aminotransferase, EC 2.6.1.5) (1-8). Earlier studies from this laboratory (1), and of Holt and Oliver (2) indicated that multiple forms of this enzyme were under the regulation of different hormones. In a subsequent report, Iwasaki et al. (9) suggested that the different forms of this enzyme may be due to post-translational modifications. It has been recently reported (10) that the proportion of different forms of the enzyme could be changed by varying the pH of the medium in which the liver is homogenized. The present study describes experiments

in vitro which suggest that the different forms of tyrosine aminotransferase from adult rat liver can be interconverted by a heat labile particulate fraction, a component of the microsomes, and that this interconversion is dependent on pH. While this work was being prepared for publication, Smith *et al.* (11) using livers of 10-12 day old rats, reported a heat labile hepatic factor that interconverts the multiple forms of tyrosine aminotransferase.

MATERIALS AND METHODS

Animals. Albino male rats, weighing about 180-200 g, were obtained from the Holtzman Rat Company, Madison, Wisconsin. They were housed in a light-dark regulated room, lighted from 20.00 to 8.00 hours, and allowed access to semi-purified diet containing 60% protein from 8.00 to 16.45 hours, for 10 days (12).

Preparation of liver homogenate and soluble tyrosine aminotransferase. Rats were sacrificed between 12.00 and 13.00 hours, by decapitation. The liver was homogenized, unless otherwise indicated, in 3 volumes of 0.1 M potassium phosphate buffer, pH 7.6, containing 0.4 mM pyridoxal-5'-phosphate (Sigma) and 1 mM dithiothreitol (Calbiochem), using 10 strokes of a Potter-Elvehjem homogenizer. All subsequent operations were at 4°. The homogenate was centrifuged at 105,000 x g for 90 min at 4° and the supernatant used directly.

Preparation of particulate fractions of liver homogenate. One rat cycled as above was sacrificed at 11.00 hours. The liver was homogenized in 3 volumes of 0.1 M KH_2PO_4 buffer, pH 6.9, containing 0.4 mM pyridoxal-5-phosphate and 1 mM dithiothreitol as above. The homogenate was centrifuged at 600 x g for 15 minutes. The supernatant was collected and centrifuged at 105,000 x g for 20 minutes. The pellet was washed, homogenized in the same buffer, and centrifuged again under the same conditions. The pellet was then resuspended in 7 ml of homogenizing buffer with a hand homogenizer. For some experiments this suspension was sonicated at 1.1 amps. for 60 sec. using an MSE sonicator followed by centrifugation at 105,000 x g for 90 minutes. The supernatant was used directly for incubation with soluble tyrosine aminotransferase.

The microsomal fraction was prepared by homogenizing the liver in 4 volumes of 0.44 M sucrose followed by a 10 min centrifugation at 12,500 x g. The supernatant was centrifuged 1 hour at 105,000 x g. The pellet was washed in the homogenizing medium and centrifuged at 105,000 x g for 90 minutes. The pellet was resuspended in 4 ml of 0.1 M KH_2PO_4 buffer, pH 6.5, containing 0.4 mM pyridoxal-5-phosphate and 1 mM dithiothreitol.

Hydroxyapatite chromatography. This chromatography was carried out at 4°. Hydroxyapatite (BioRad) columns (4.0 cm x 7.5 cm) were equilibrated with 3 volumes of 0.1 M potassium phosphate buffer, pH 6.9, containing 1 mM dithiothreitol. After applying the samples the columns were each washed with 64 ml of the initial buffer. This was followed by a linear gradient from 0.1 M to 0.5 M potassium phosphate concentration, pH 6.9, containing 1 mM dithiothreitol. The gradient was applied for 48 hours using an LKB Gradient Former with a flow rate of 26 ml/h. Fractions of 4 ml (280-300) were collected and assayed for tyrosine aminotransferase activity. Absorbance was determined at 280 nm. The phosphate gradient was measured by conductance. The relative amount

of the different forms of the enzyme was determined by measuring the area under the curves (Figures 1-3) using a planimeter.

Enzyme assay. Tyrosine aminotransferase activity was assayed by the modification described by Iwasaki and Pitot (1) of Diamondstone's method (13) using 0.2 ml of a 105,000 x g supernatant diluted 1:100 or 0.2 ml of the fractions from the hydroxyapatite columns. One unit of enzyme was taken to catalyze the formation of one µmole of p-hydroxyphenylpyruvate per minute at 37°. A value of 19,900 M^{-1} cm^{-1} was used for the molar absorption coefficient of p-hydroxyphenylpyruvate (13). Protein concentration was measured by the method of Lowry et al. (14).

RESULTS

Effect of the pH of the homogenizing buffer on the pattern of multiple forms of tyrosine aminotransferase. The supernatant of rat liver homogenates prepared at pH 7.6 and chromatographed on hydroxyapatite were shown to contain three forms of tyrosine aminotransferase (1). These forms were designated as II, III, and IV as in (1). This pattern was quite reproducible. Liver homogenates prepared at pH 6.9 were shown to contain the same three forms of the enzyme in a distribution which was not consistent; in some experiments the pattern was similar to that of preparations at pH 7.6 where forms II, III, and IV represented 57%, 35%, and 8% respectively, but more commonly at pH 6.9 the distribution was 14%, 27%, and 59%. When soluble tyrosine aminotransferase preparations in phosphate buffer, pH 6.9 and pH 7.6, were stored for 60 hours at 4°, a shift from enzyme forms II and III to form IV was noted for preparations in buffer at pH 6.9 (Table I). Dialysis at pH 6.9 did not prevent this alteration (Table I). At pH 7.6 the change was from form II to form III and in less proportion to form IV (Table I).

When samples from the same liver were homogenized at various pH's and chromatographed on hydroxyapatite columns, different multiple form patterns of the enzyme were obtained. Fig. 1 shows that homogenates prepared in buffers at pH 8.1 and pH 7.6 (A and B) contain predominantly form II of the enzyme (68%), while in homogenates prepared at pH 6.9 and pH 6.5, form IV predominated (C and D).

Effect of changes in the pH of the liver homogenate. The data in A and B of Fig. 2 demonstrate a significant modification in the pattern

Table I -- Effect of Storage for 60 Hours at 4° at pH 6.9 and 7.6 on the Multiple Forms of Tyrosine Aminotransferase.

Expt. No.	pH at which liver homogenate was prepared	Treatment of 105,000 x g supernatant	Tyrosine Aminotransferase Activity: Supernatant* (μmole/min)	% of chromatographic profile on hydroxyapatite column, Enzyme form II	III	IV
1	7.6	None	14	60	29	11
	7.6	Storage at 4° for 60 hours	14	28	45	27
2	6.9	None	24	59	33	8
	6.9	Storage at 4° for 60 hours	24	5	25	70
	6.9	Dialysis against homogenizing buffer for 60 hours at 4°	22	7	30	63
3	6.9	None	27	12	24	64
	6.9	Storage at 4° for 60 hours	25	0	10	90

*Tyrosine aminotransferase was prepared as described in Materials and Methods. Total enzyme activity was usually very stable during the storage. Occasionally losses of up to 8% of the total activity occurred, however.

of the multiple forms when the homogenate, prepared initially at pH 7.6, was brought to pH 6.5, without a change in the phosphate concentration, rehomogenized and then centrifuged. A decrease in form II of the enzyme from 73% to 54%, and an increase of form IV from 3% to 18% were noted. This change did not occur when the 105,000 x g supernatant was brought from pH 7.6 to pH 6.5.

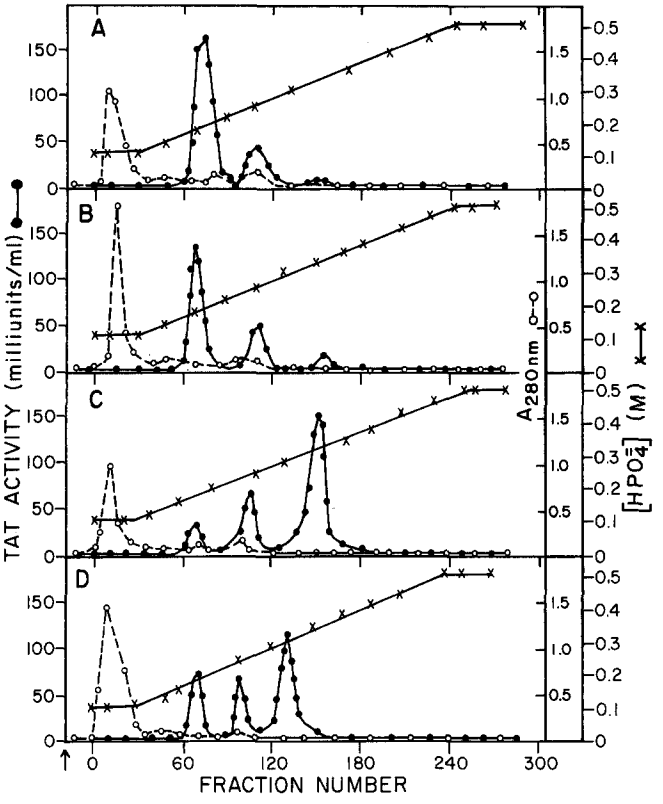

Fig. 1. The effects of homogenizing liver samples in phosphate buffer at various pH's on the distribution of multiple forms of tyrosine aminotransferase (TAT). Four 2 g samples of liver from a rat cycled on a 60% protein diet for 10 days were homogenized in 0.1 M potassium phosphate buffer, containing 0.4 mM pyridoxal-5-phosphate and 1 mM dithiothreitol at the following pH's: panel A, pH 8.1; panel B, pH 7.6; panel C, pH 6.9; panel D, pH 6.5. Each homogenate was centrifuged at 105,000 x g for 90 minutes at 4°. 2.5 ml aliquots of each supernatant were chromatographed on hydroxyapatite columns. Between the arrow and the point marked 0, the columns were washed with 64 ml (16 fractions) of the initial buffer. Total enzyme activity charged (μmoles/min at 37°): A, 11.6; B, 11.5; C, 12.7; D, 10.1.

Effect of particulate structures from rat liver on the pattern of multiple forms of tyrosine aminotransferase. In Table II is shown the effect of incubating soluble tyrosine aminotransferase prepared at pH 7.6 with the sonicate of a 105,000 x g pellet (See Methods). A marked shift from forms II and III to form IV occurred only on incubation at pH 6.9 or pH 6.5.

When a 105,000 x g supernatant was homogenized with the hepatic microsomal

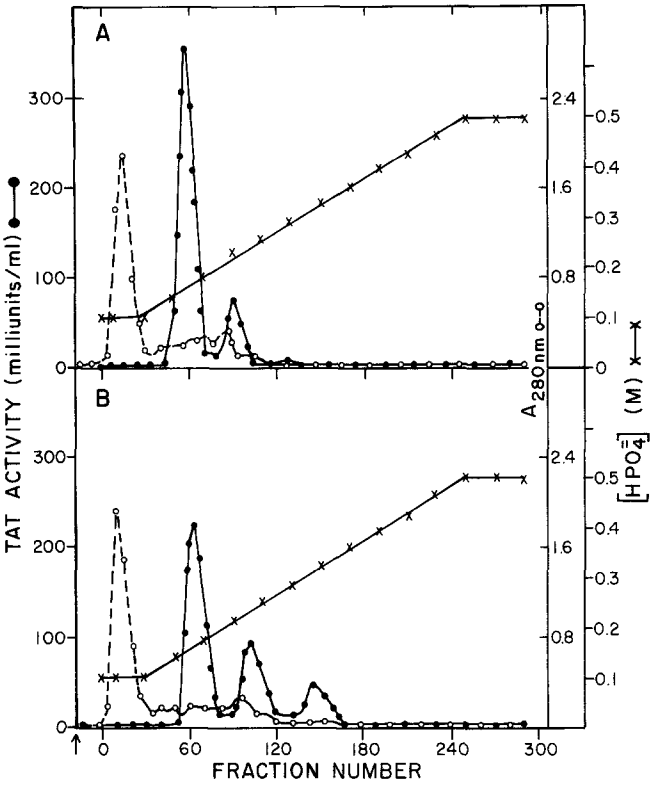

Fig. 2 Effect of changing the pH of the liver homogenate on the distribution of multiple forms of tyrosine aminotransferase (TAT). Liver from a rat cycled on a 60% protein diet for 10 days was homogenized in 0.1 M potassium phosphate buffer, pH 7.6, containing 0.4 mM pyridoxal-5-phosphate and 1 mM dithiothreitol. Half of the homogenate was brought to pH 6.5 without changing the concentration of potassium phosphate (B). Each half was homogenized again and centrifuged at 105,000 x g for 90 minutes at 4°. 3.5 ml aliquots of each supernatant were chromatographed on hydroxyapatite columns. From the arrow to the point marked 0, the columns were washed with 64 ml (16 fractions) of the initial buffer. Total enzyme activity charged (μmoles/min at 37°): A, 26.3 (pH 7.6); B, 26.5 (pH 6.5).

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fraction, incubated at pH 6.5 for 30 minutes at 25°, centrifuged at 105,000 x g and chromatographed on hydroxyapatite columns, an alteration in the chromatographic profile occurred. The data seen in Fig. 3 indicates that approximately 30% of enzyme form II was converted to forms III (increased from 20% to 31%), and IV (increased from 0% to 19%) on incubation with native microsomes. This interconversion did not occur when the enzyme was incubated with a heat denatured microsomal fraction (C, Fig. 3).

Table II -- Effect of Incubation at 0-4° for 60 Minutes of Soluble Tyrosine Aminotransferase with a Sonicate of the 105,000 x g Pellet.

Supernatant of 105,000 x g pellet after sonication added to assay	pH of incubation	Tyrosine Aminotransferase Activity*			
		Cytosol (μmole/min)	% of chromatographic profile on hydroxyapatite column		
			Enzyme form		
			II	III	IV
0.0 ml	--	7.3	27	42	31
1.8 ml (10.8 mg prt)	7.6	5.7	26	37	37
1.8 ml (10.8 mg prt)	6.9	8.2	13	26	61
1.8 ml (10.8 mg prt)	6.5	9.8	22	26	52

*The preparation of both supernatants and the details of the experiment are described in the Materials and Methods.

DISCUSSION

In this laboratory, Iwasaki et al. (1,9) separated four forms of soluble tyrosine aminotransferase from rat liver under various environmental conditions, in both crude extracts and purified preparations. Johnson et al. (6) described three forms of soluble tyrosine aminotransferase separable by CM-Sephadex chromatography, and suggested that they may be products of degradation of the enzyme. In contrast, recently Johnson and Grossman (10) have presented evidence that the distribution of multiple forms of tyrosine aminotransferase can be changed by varying the composition and pH of the medium in which the liver is homogenized. As the data of Fig. 1 indicate, we have also found similar alterations by using four different pH's in the homogenizing buffer, at a constant phosphate concentration. When the pH of the homogenate was brought from pH 7.6 to pH 6.5, a shift from form II to form IV occurred

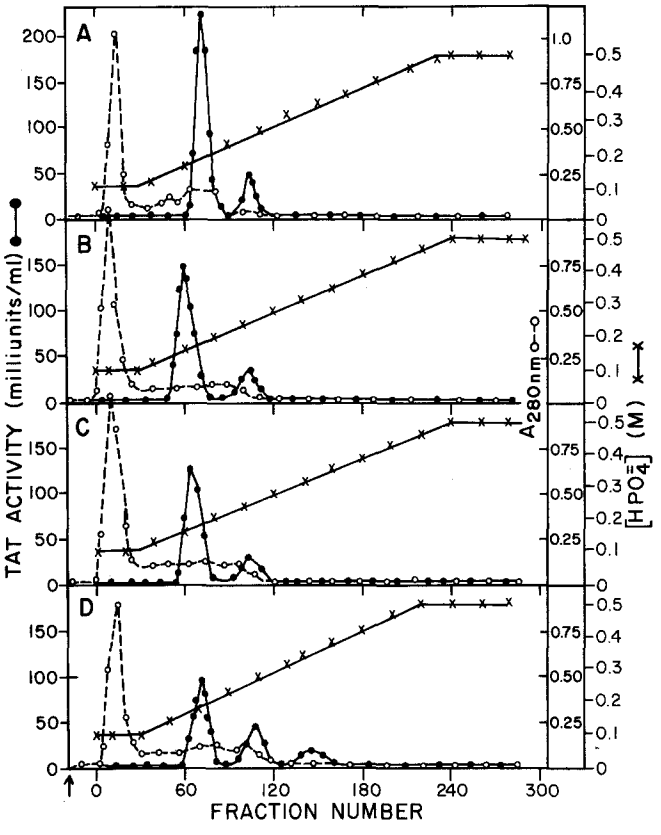

Fig. 3 The effect on the distribution of the multiple forms of tyrosine aminotransferase (TAT) of incubation with the microsomal fraction of a liver homogenate at 25° for 30 minutes. Liver from a rat cycled on a 60% protein diet for 10 days was homogenized as described in Fig. 2. The homogenate was centrifuged at 105,000 x g for 90 minutes at 4°. 5 ml aliquots of supernatant were mixed with: panel A, 2 ml of 0.1 M potassium phosphate buffer, pH 7.6; panel B, 2 ml of 0.1 M potassium phosphate buffer, pH 6.5; panel C, 2 ml of the microsome fraction in 0.1 M potassium phosphate buffer, pH 6.5, prepared as described in Materials and Methods, which had been heated at 100° for 5 minutes; panel D, 2 ml of the unheated microsome fraction (28.6 mg total protein). Each mixture (with exception of the sample corresponding to panel A) was brought to pH 6.5, rehomogenized, incubated at 25° for 30 minutes and centrifuged at 105,000 x g for 90 minutes at 4°. 3.5 ml aliquots of each supernatant were chromatographed on hydroxyapatite columns. Initially (arrow to point marked 0) the columns were washed with 64 ml (16 fractions) of the initial buffer. Total enzyme activity charged (μmoles/min at 37°): A, 10.8; B, 10.8; C, 10.7; D, 11.2.

(Fig. 2). This did not occur when a 105,000 x g supernatant was brought from pH 7.6 to 6.5, but incubation with a sonicate of cellular particulates or whole microsomes caused a shift from form II to form IV at pH 6.9 or pH 6.5; indicating

that the changes in the multiple form pattern of the enzyme described herein and reported by Johnson and Grossman (10) may be due at a factor present in these structures. The data of Fig. 3 demonstrate the factor's heat lability and that it is probably a component of microsomes or some other particulate structure such as lysosomes.

In light of these results the effect of storing the enzyme at 4° on the interconversion of multiple forms (Table I) may be due to the presence of the conversion factor in suboptimal amounts or to be a spontaneous interconversion.

ACKNOWLEDGEMENTS

The studies reported herein were supported by grants from the National Cancer Institute (CA-07175) and the American Cancer Society (E-588). Dr. J. M. Rodriguez is a Fogarty International Fellow of the N.I.H. (F05-TW-2012).

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