STUDIES ON SOLUBLE AND MITOCHONDRIAL TYROSINE AMINOTRANSFERASE: EVIDENCE FOR A PHYSICAL CHANGE IN THE CYTOSOL ENZYME DURING INDUCTION

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SUMMARY: Tyrosine aminotransferase acts on tyrosine or monoiodotyrosine (MIT) as substrate. The ratio of the initial velocities for the reactions with tyrosine and monoiodotyrosine has been measured under various conditions *in vivo*. When the enzyme is induced by cortisol, this ratio increases due to the reduction of the relative rate of MIT transamination. In contrast, purification of the cytosol enzyme decreases the tyrosine:MIT ratio. The mitochondrial enzyme, by its utilization of oxaloacetate as amino acceptor and its selective inhibition by aspartate, can be distinguished from the cytosol enzyme. Since the mitochondrial enzyme is not responsible for the change in ratio of activities with the two substrates, induction *in vivo* may involve the formation of physical conformers of the cytosol tyrosine aminotransferase.

Earlier studies from this laboratory demonstrated that as tyrosine aminotransferase (EC 2.6.1.5) is purified from liver cytosol, the initial velocity with monoiodotyrosine relative to the initial velocity with tyrosine increases proportionately to the degree of purification (1). This indicated that monoiodotyrosine was becoming a better substrate as the purification progressed. Since it is possible to enrich rather specifically the quantity of this enzyme in liver by hormonal induction, this process can be viewed as a purification *in vivo*. Therefore, it was of interest to learn if the type of change connected with purification of the cytosol enzyme occurred in relation to enzyme induction.

Adrenalectomized, male Sprague-Dawley rats were injected with tyrosine, monoiodotyrosine, cortisol, or cortisol plus monoiodotyrosine and sacrificed 4 hours later. Rats were used for experiment between 7 and 12 days after adrenalectomy. Treated animals and controls were always decapitated at noon to minimize diurnal variations of activity (2,3). Livers were removed

quickly, rinsed, weighed and homogenized in ice cold 0.25 M sucrose. Nuclei and cellular debris were removed by centrifugation at 700 x g for 10 min. and washed once. Mitochondria were obtained by centrifugation at 14,500 x g for 10 min. and washed once. Liver cytosol was the supernatant obtained after centrifugation at 100,000 x g for 60 min.

Tyrosine aminotransferase activity, expressed as mμmoles p-hydroxyphenylpyruvate or 3-iodo-p-hydroxyphenylpyruvate formed per min. per mg protein, was determined by measuring p-hydroxyphenylpyruvate or 3-iodo-4-hydroxyphenylpyruvic acid formation by a modified Briggs reaction (1,4,5). The reaction mixture contained 10 mM α-ketoglutarate, 40 μM pyridoxal-P, 1.8 mM diethyldithiocarbamate, 170 mM potassium phosphate buffer, pH 7.6, and 4 mM tyrosine or monoiodotyrosine for cytosol and 9 mM tyrosine or monoiodotyrosine for mitochondria unless otherwise indicated. Protein was measured by the Biuret method (6).

The effects of the various treatments on the relative levels of enzymatic activity, and the activity ratios for the two substrates can be seen in Table I. As observed previously (7) injection of amino acids into adrenalectomized rats does not result in induction. Cortisol greatly stimulated enzymatic activity and the combination of hormone plus amino acid results in a still higher level of activity. The ratio of the velocity of tyrosine transamination to the velocity of monoiodotyrosine transamination after purification of the cytosol tyrosine aminotransferase is unity (1). In the cytosol of an untreated animal the ratio is 2.0. The ratio changes even more after induction, increasing to 2 to 3 times that of the basal state. Although the mitochondrial enzyme can be induced slightly with cortisol (8) or cortisol plus monoiodotyrosine (Table I), the stimulation is small and ratio changes were not observed. Hence the mitochondrial enzyme serves as a standard with which the changes in the cytosol enzyme can be compared.

The utilization of monoiodotyrosine by mitochondrial and cytosol systems becomes of interest because monoiodotyrosine inhibits tyrosine hydroxylase

Table I

Activity of Tyrosine Aminotransferase in Cell Compartments of Liver After Induction

Treatment* of Adrex Rats	Cytosol			Mitochondria		
	n	$\frac{v_{Tyrosine}}{v_{MIT}}$+	Fold Stimulation‡	n	$\frac{v_{Tyrosine}}{v_{MIT}}$	Fold Stimulation
Saline	19	2.0		3	0.8	
Tyrosine	16	2.7	1.5	4	0.9	1.0
MIT	13	2.2	1.5	2	0.7	1.2
Cortisol	14	4.4	5.1	3	0.9	1.2
Cortisol plus MIT	8	5.8	7.5	2	0.8	1.9

*Cortisol, 60 mg/kg body weight. Amino Acids, 600 mg/kg body weight. Injections are intraperitoneal.
+MIT = monoiodotyrosine; n = number of experiments; v = initial velocity.

‡Fold stimulation refers to initial velocity with tyrosine in the basal state compared to the activity with tyrosine as substrate after treatment with the indicated substances. In the basal state the the activity of the cytosol enzyme is 0.86 mμmoles/min/mg protein with tyrosine as substrate and 0.44 mμmoles/min/mg protein with MIT as substrate. The mitochondrial enzymatic activity in the basal state is 0.7 mμmoles/min/mg protein with tyrosine and 0.9 mμmoles/min/mg protein with MIT.

Table II

Keto Acid Specificity of Mitochondrial and Soluble

Tyrosine Aminotransferase From Liver

Keto Acid	Tyrosine		MIT	
	Mitochondria (4)	Cytosol (2)	Mitochondria (2)	Cytosol (2)
	mμmoles/min/mg protein (%)			
α-Ketoglutarate	0.38 (100)	1.0 (100)	0.25 (100)	0.49 (100)
Oxaloacetate	0.28 (75)	0.05 (5)	0.17 (68)	0.04 (8)
Pyruvate	0.12 (32)	0.05 (5)	0.03 (14)	0.02 (5)

Keto acid concentrations were 5 mM. Tyrosine (or MIT) was 4 mM in these studies. Similar data for mitochondrial tyrosine aminotransferase were observed for each of the treated conditions listed in Table I using 9 mM tyrosine (MIT). Data for oxaloacetate and pyruvate are compared to that for α-ketoglutarate by setting the activity with α-ketoglutarate equal to 100 per cent.

in vitro (9,10) and depletes tissue norepinephrine in vivo. Also, tyrosine is a precursor of norepinephrine and a substrate for tyrosine hydroxylase. Norepinephrine levels have been correlated with the diurnal cycle of liver cytosol tyrosine aminotransferase (2). Therefore, the utilization of monoiodotyrosine by tyrosine aminotransferase as a regulatory mechanism is being explored.

The possibility that more than one enzyme contributes to the observed responses in soluble tyrosine aminotransferase activity needs clarification. Small amounts of tyrosine aminotransferase have been observed to be associated with mitochondria (8,11,12) and other cell fractions (13) but the question of cross contamination clearly has not been resolved. The mitochondrial enzymes from liver, brain and heart are being purified in our laboratory, and these enzymes have keto acid specificities and kinetic properties different from the liver cytosol enzyme which persist after several hundred fold purification. The keto acid specificity was used to distinguish between tyrosine aminotransferase activity of mitochondria and cytosol from normal rat liver with tyrosine or monoiodotyrosine as substrate (Table II). The mitochondrial enzyme readily utilized oxaloacetate and pyruvate to a lesser extent while the cytosol enzyme showed negligible activity with either keto acid as expected (1,14,15). There appears to be no contamination of the cytosol enzyme by the mitochondrial form. In addition to the keto acid specificity which eliminates the possibility of cross contamination is a potent non-competitive inhibition of the mitochondrial enzyme by aspartate (50% inhibition at 0.8 mM aspartate) with respect to tyrosine. This mode of inhibition results when the data are plotted by the method of Hunter and Downs (16) (Figure 1) or of Lineweaver and Burk (17). Aspartate does not inhibit the cytosol enzyme in the concentration range where inhibition of the mitochondrial enzyme occurs. These two enzymatic activities represent different enzyme forms as demonstrated by pH isoelectric focusing of the cytosol enzyme (500 fold) and mitochondrial enzyme (35 to 700 fold). The

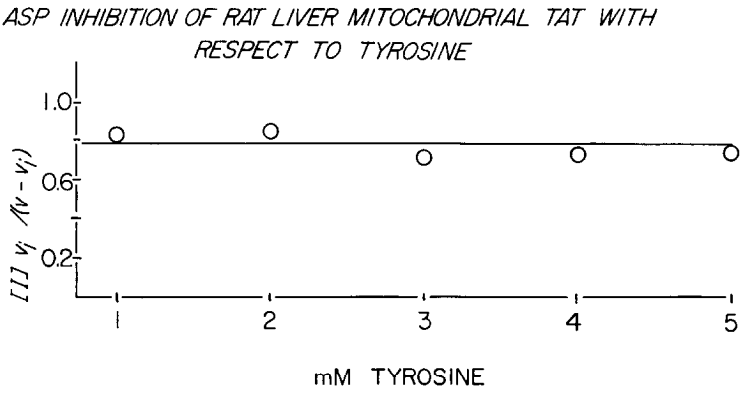

Fig. 1.

Non-competitive mode of inhibition of mitochondrial tyrosine aminotransferase by 0.8 L-aspartate. The plot is made according to the method of Hunter and Downs (16).

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cytosol enzyme was anionic (focused at pH 4.1) while the mitochondrial enzyme was cationic (focused at pH 9.4). Thus on the basis of keto acid specificity, aspartate inhibition, and charge the two enzymes are different. In a subsequent paper on the purification and properties of the mitochondrial enzyme more details will be given concerning these differences. Since the mitochondrial form does not appear to contribute to the activity observed in cytosol (Table II) due to loss of enzyme from mitochondria during homogenization and centrifugation, physical changes in the cytosol enzyme must be occurring. Studies by Litwack et al. (18) on pyridoxal-P protection against urea inhibition and a report by Holten et al. (19) on pyridoxal-P protection under various conditions suggest that the cytosol enzyme undergoes physical changes. The utilization in these studies of monoiodotyrosine, with its iodide atom acting as a bulky substituent on the substrate, should monitor the dimensions of the catalytic center with regard to substrate binding. We conclude, therefore, that the cytosol enzyme can exist in different conformations and that induction produces a form with a more constricted active site of the cytosol tyrosine aminotransferase.

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