On the Mechanism of Inactivation and ATP-Dependent Reactivation of Rat Liver Tyrosine Aminotransferase

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The mechanism of *in vitro* inactivation and ATP-dependent rapid reactivation of rat liver tyrosine aminotransferase by a membrane-bound system from rat liver and kidney cortex and the nucleotide specificity of this process was investigated using partially purified tyrosine aminotransferase as a substrate. Adenosine 5′-triphosphate (ATP) could be replaced by guanosine 5′-triphosphate (GTP), whereas inosine 5′-triphosphate (ITP) was less effective. During reactivation [γ-^{32}P]ATP was incorporated into the enzyme and not excorporated by incubation of the labeled enzyme with excess non-radioative ATP. Inactivation of labeled tyrosine aminotransferase by a particulate fraction led to a decrease protein-bound radioactivity concomitant with an increase of [^{32}P]orthophosphate. This points to a phosphorylation and dephosphorylation mechanism in the regulation of tyrosine aminotransferase activity.

As previously reported [1], tyrosine aminotransferase from crude rat liver extracts is rapidly inactivated in the presence of rat liver particulate fractions. This inactivation is counteracted by ATP. Addition of ATP to inactivated tyrosine aminotransferase in the presence of particulate fractions led to a rapid reactivation of the enzyme. In order to gain further insight into the mechanism of the above system, rat liver tyrosine aminotransferase was partially purified; the dependence of this process on Mg^{2+} and ATP and the ^{32}P-incorporation from [γ-^{32}P]ATP weres tudied.

Materials and Methods

Male Sprague Dawley rats (250 – 300 g) were kept on a 50% protein diet (Altromin C 1001) in a room illuminated between 7:00 a.m. and 7:00 p.m. For tyrosine aminotransferase induction, 22 rats were starved for 10 h, injected intraperitoneally with 4 mg dexamethasone per rat and starved for an additional 12 h. The animals were sacrificed in light ether anaesthesia and the livers rapidly excised. Tyrosine aminotransferase was partially purified following the procedure of Valeriote *et al.* [2] up to the G 200 step. The final product had a specific activity of 86 U/mg protein.

Nuclear (N), heavy mitochondrial (M), light mitochondrial (L) and microsomal (P) fractions from rat liver and kidney cortex, respectively, were isolated according to De Duve's method [3].

Soluble liver extracts were prepared by homogenization of rat liver with 4 volumes of 0.1 M Tris/HCl buffer, pH 8.1, in a Potter Elvehjem glass homogenizer [4] with a loosely fitting teflon pestle and subsequent centrifugation at 100 000 × *g* for 30 min.

Incubations were performed in Warburg vessels (3 ml) at 37 °C with constant shaking. To avoid oxidation during the experiments 5 mM cysteine was added. At the times indicated 1.0 ml aliquots were removed, kept in an ice-bath and centrifuged for 30 min at 100 000 × *g*.

Tyrosine aminotransferase activity was assayed in the supernatants by a slight modification of the method of Diamondstone [5]. The reaction was started by addition of 1.25 mM or 4.38 mM tyrosine as indicated. Protein was measured according to Lowry *et al.* [6].

^{32}P-labeled tyrosine aminotransferase was prepared as follows: 18.1 units of partially purified tyrosine aminotransferase were inactivated by incubation (45 min at 37 °C) with 0.8 ml of a light mitochondrial (L) fraction (20.2 mg protein in 0.25 M sucrose) supplemented with 100 mM Tris/HCl, pH 8.1, and 5 mM cysteine in a total volume of 1.033 ml. To the inactivated dephosphorylated enzyme 8 μmoles of ATP together with 1 mCi [γ-^{32}P]ATP in a total volume of 0.1 ml were added. After 7 min at 37 °C, the sample was rapidly cooled in an ice-bath and centrifuged for 30 min at 100 000 × *g*. The supernatant was dialyzed 4 times against 10^3 volumes of buffer (0.05 M potassium

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phosphate, pH 6.5, 1 mM EDTA, 1 mM dithiothreitol, 2 mM 2-oxoglutarate and 0.2 mM pyridoxal phosphate) and chromatographed on Sephadex G 200 (Pharmacia).

Radioactivity was measured in a Packard Tricarb 3385. Because of the short half-life of ^{32}P, the cpm were corrected to the value at the time of preparation of ^{32}P-labeled tyrosine aminotransferase. ^{32}P-radioactivity in the eluates of chromatography on G 200 was measured in fractions of 0.7 ml after addition of 20 ml Aquasol (NEN) and thermal equilibration. For the measurement of protein-bound ^{32}P-radioactivity the specimens were diluted with an equal volume of ice-cold 10% trichloroacidic acid and filtered through filter discs (Membranfilter Göttingen, 0.1 μm mesh) and the residue was washed with additional trichloroacidic acid.

Results and Discussion

Rat liver tyrosine aminotransferase of crude liver homogenates can be rapidly inactivated by incubation at 37 °C [1]. Addition of kidney cortex homogenates enhances this inactivation, though the specific activity of tyrosine aminotransferase in this organ is approximately 160 times less than in liver [7]. Addition of 1 mM ATP together with an ATP-regenerating system abolishes the inactivation at pH 8.1 [1]. The inactivation of partially purified tyrosine aminotransferase by the nuclear fraction (N) from kidney cortex was dependent on the amount of added particulate protein (Fig. 1). To investigate the specificity of the protective effect of trinucleotides, soluble liver extracts containing tyrosine aminotransferase were incubated at 37 °C with the 33 000 × g sediment from kidney cortex and various concentrations of nucleotide triphosphates: GTP protected as well as ATP, but in the presence of 5 mM ITP, 20% of tyrosine aminotransferase activity in the supernatant was lost after 90 min.

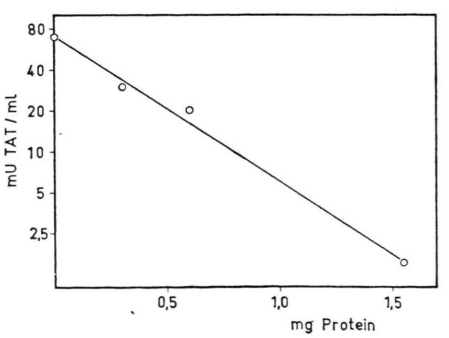

Fig. 1. Inactivation of partially purified tyrosine aminotransferase by a nuclear fraction (N) from rat kidney cortex. Enzyme activity was determined after 90 min incubation in the presence of 1.25 mM tyrosine.

As previously reported [1], protection against inactivation at suboptimal levels of ATP could be significantly improved by 10 μM cAMP or cGMP. The inactivation of tyrosine aminotransferase in soluble liver extracts by the light mitochondrial

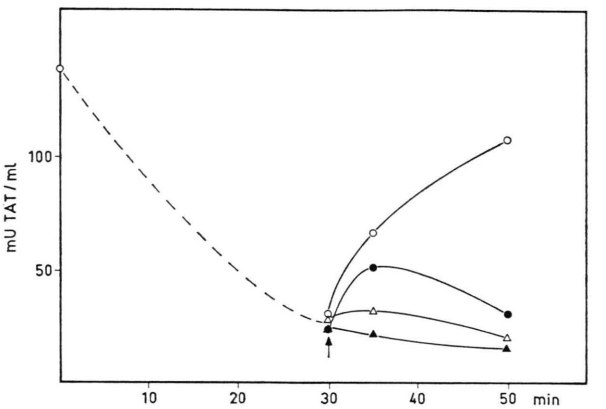

Fig. 2. Inactivation and ATP-dependent reactivation of partially purified tyrosine aminotransferase by a light mitochondrial fraction (L) from rat liver. 13.3 mg protein of the L-fraction, 0.4 U tyrosine aminotransferase, 1 mM Mg^{2+}, 0.3 mM EDTA and 1.6 mg bovine serum albumin per ml were incubated in a total volume of 3 ml. Enzyme activity was determined in the presence of 4.38 mM tyrosine. ▲—▲ 0.5 mM ATP, △—△ 1.0 mM ATP, ●—● 2 mM ATP, ○—○ 5 mM ATP added as indicated by arrow.

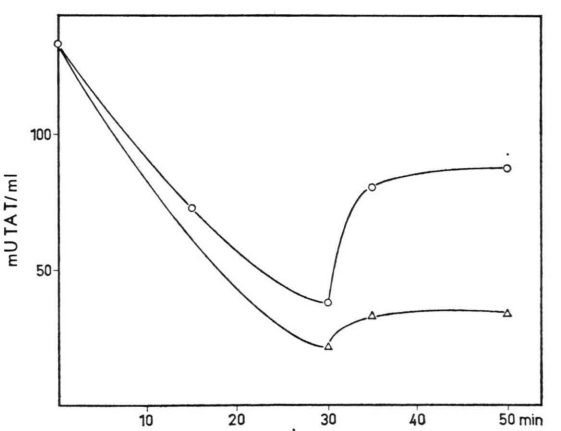

Fig. 3. Mg^{2+}-dependence of the ATP-dependent reactivation of partially purified tyrosine aminotransferase. 10.2 mg protein of the L-fraction and 0.4 U tyrosine aminotransferase were incubated at 37 °C. After 30 min 2.5 mM ATP, 30 mM acetylphosphate and 3 U acetokinase were added. Other experimental conditions as in Fig. 2. △—△ without Mg^{2+}, ○—○ with 1 mM Mg^{2+}.

fraction (L) could also be prevented by the addition of 20 mM fluoride.

Partially purified tyrosine aminotransferase inactivated by a light mitochondrial fraction (L) from rat liver required for complete reactivation at least 5 mM ATP (Fig. 2) or 1 mM ATP and an ATP-regenerating system (acetylphosphate and acetokinase).

Mg^{2+}-ions are necessary for optimal ATP-mediated reactivation of rat liver tyrosine aminotransferase. As shown in Fig. 3, addition of 1 mM Mg^{2+} stimulated the ATP-dependent reactivation of the enzyme by rat liver particles. This makes an effect of ATP as a complexing agent unlikely and points to a possible role of the nucleotide as a phosphate donor. It is also consistent with the fact that adenylyl-(β,γ-methylene)-diphosphonate (AMP-PCP), an ATP-analog which cannot provide a phosphate group for phosphorylation, failed to inhibit inactivation or to promote reactivation in the presence of rat liver or kidney particles.

To obtain more direct evidence for this mechanism, 18.1 units of tyrosine aminotransferase were first inactivated in the presence of a rat liver light mitochondrial fraction (L) and then reactivated in the presence of [γ-^{32}P]ATP and Mg^{2+}. After centrifugation (100 000 × g for 30 min) all soluble components of the sample were applied to Sephadex G 200. The fractions containing both activity and protein-bound ^{32}P-radioactivity were pooled and rechromatographed on Sephadex G 200 (Fig. 4).

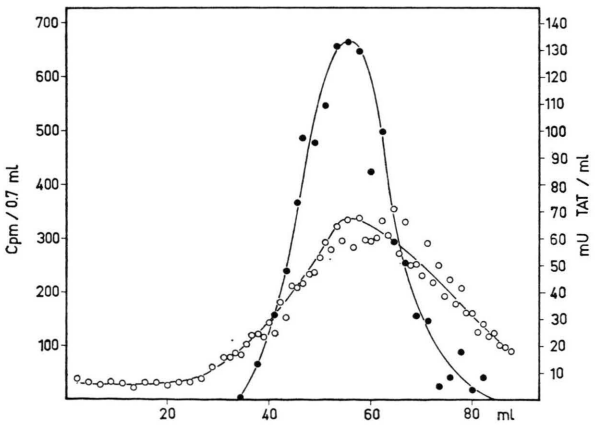

Fig. 4. Rechromatography of ^{32}P-labeled tyrosine aminotransferase on Sephadex G 200. Enzyme activity was determined in the presence of 4.38 mM tyrosine. Experimental conditions are indicated under "Materials and Methods". ○—○ radioactivity; ●—● tyrosine aminotransferase activity.

Table I. Characterization of ^{32}P-labeled tyrosine aminotransferase. Incubation with the light mitochondrial fraction (L) of rat liver (79.2 mg protein) was performed in the presence of 2 mM Mg^{2+} and 0.028 mM pyridoxal phosphate (PLP) in a total volume of 4.29 ml. Tyrosine aminotransferase activity was measured in the presence of 4.38 mM tyrosine.

Pretreatment	Time [min]	Activity [U/sample]	cpm/sample after acid precipitation	
			Supernatant	Sediment
I * Incubation	0	0.25	98	819
+4.8 mM ATP +0.095 mM PLP	70	0.28	103	735
II * Incubation	0	2.57	1068	2726
+L-Fraction	60	1.05	1646	1693

* Differently labeled tyrosine aminotransferase was used in experiments I and II.

As shown in Table I, most of the protein-associated radioactivity is trichloroacidic acid-insoluble, pointing to a covalent linkage to the enzyme.

A possible adsorption of [γ-^{32}P]ATP to tyrosine aminotransferase can be excluded, because even after 70 min of incubation in the presence of 5 mM unlabeled ATP the trichloroacidic acid-insoluble portion of radioactivity remained unchanged. Only during tyrosine aminotransferase inactivation by a rat liver light mitochondrial fraction did the trichloroacidic acid-insoluble radioactivity decrease concomitant with an increase of trichloroacidic acid-soluble radioactivity. The release of protein-bound ^{32}P together with a decrease in tyrosine aminotransferase activity points to a protein phosphatase activity in the light mitochondrial fraction.

It is concluded that hepatic tyrosine aminotransferase activity is subject to regulation by chemical modification. The fully active enzyme is obtained by ATP or GTP-dependent phosphorylation in the presence of a particle-bound kinase; inactivation occurs as a result of dephosphorylation catalyzed by an enzyme of the light mitochondrial fraction. The interconversion of tyrosine aminotransferase by a protein kinase/phosphatase system is possibly under hormonal control.

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