INACTIVATION AND ATP-DEPENDENT REACTIVATION OF TYROSINE AMINOTRANSFERASE IN VITRO BY MEMBRANE BOUND ENZYMES FROM RAT LIVER AND KIDNEY CORTEX

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Summary:

In an attempt to learn more about the physiological significance of a membrane bound system responsible for the inactivation and ATP-depending stabilization of renal PEP-carboxykinase in vitro (5, 6), similar studies were extended to hepatic tyrosine aminotransferase (TAT). Like in the case of PEP-carboxykinase the subcellular fractions from liver and kidney cortex also inactivate TAT. ATP causes full protection in the presence of the membraneous fractions from liver with an optimum in the microsomal fraction. The inactivation is inhibited by fluoride. Reactivation of TAT by ATP after inactivation favors interconversion of TAT into different forms as the primary cause of the protective effect of ATP. Cyclic AMP and GMP potentiate the protective effect of ATP.

The physiological significance of the membrane bound system in control of gluconeogenesis is discussed.

Increasing experimental evidence from various laboratories points to a synergistic control of gluconeogenesis in liver and kidney by short term acting hormones via interaction with β- and α-receptors of the cell membrane, respectively (1-4). Both cyclic AMP and GMP have been discussed as possible second messengers of these hormones (4). Inactivation and nucleoside triphosphate dependent stabilization of renal phosphoenolpyruvate (PEP) carboxykinase in vitro by a membrane bound enzyme system from liver and kidney (5, 6) locate one of the possible regulatory attacks of the above hormones at the pyruvate/PEP level. In view of numerous reports on a cyclic AMP mediated control of tyrosine aminotransferase (TAT) and PEP-carboxykinase in liver, and of gluconeogenesis in both kidney and liver, the specifity studies with the membrane bound enzyme systems from both organs

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were extended to TAT from liver. The present study on TAT represents a first experimental support for a broad specifity of the membrane bound system for at least the glucogenic enzymes.

MATERIAL AND METHODS

Male Sprague Dawley rats (140 - 180 g), kept on a 50 % protein diet (Altromin C-1001), were used as kidney and liver donors.

Liver and kidney cortex homogenates were prepared by homogenization with 4 volumes of 0.1 M Tris-HCl buffer pH 8.1 supplemented by 5 mM cysteine. Soluble extracts were obtained by subsequent centrifugation for 30 min at 100 000 x g.

Fractionation of subcellular components from 10 g liver into nuclei, heavy and light mitochondria and microsomes was carried out according to De Duve (7). The nuclear fractions were resuspended in 20 ml 0.25 M sucrose, and the resulting fractions in each 10 ml of isotonic sucrose solution. All fractions were supplemented with 5 mM cysteine and 1 % Triton. The latter detergent causes a 2 to 3fold stimulation of the inactivation by the subcellular fractions.

When the effects of cyclic nucleotides were investigated, because of an apparent sensitivity of these effects to the mode of preparation, the addition of the detergent was omitted.

Tyrosine aminotransferase was assayed according to Diamondstone (8) at suboptimal concentrations of 1.23 mM tyrosine.

Incubations were performed either in Warburgs vessels (3 ml) or 80 ml vessels (6 ml) in 0.1 M Tris-buffer pH 8.1 at 37°C under shaking. Additional components are indicated in the figures and tables.

When indicated, ATP was kept at constant levels in a system containing acetyl-P, ATP, Mg^{++} and acetokinase.

Protein was determined according to Lowry et al. (10).

RESULTS

Fig. 1 illustrates the inactivation of hepatic TAT by the subcellular fraction from liver (curve II) and liver + kidney (curve IV). The necessity of the subcellular fraction is evident from unchanged activities of TAT when the soluble extract alone is incubat-

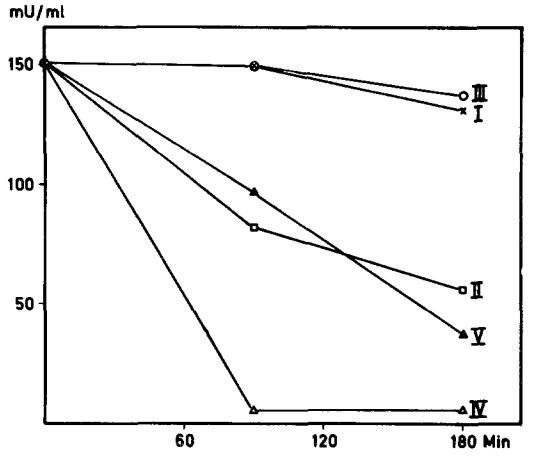

Fig. 1: Inactivation and ATP-dependent stabilization of tyrosine aminotransferase in the subcellular fraction from liver and kidney cortex. 4 ml liver homogenate or soluble extract (o.9 units TAT) were incubated in an end volume of 6 ml at 37°C under shaking (o.1 M Tris-buffer pH 8.1; 5 mM cysteine). Aliquots of each 1 ml were centrifuged at the intervals indicated, and TAT assayed (o.o3 - o.o6 ml) in the supernatant. Where indicated, ATP was regenerated with 2o mM acetyl-P, 7 mM Mg^{++}, 1 mM ATP, and 1.2 units acetokinase per ml.

Curve I: Liver extract, no addition. Curve II: Liver homogenate, no addition. Curve III: Liver homogenate + ATP. Curve IV: Liver homogenate + o.7 ml kidney cortex homogenate. Curve V: Liver homogenate + kidney cortex homogenate + ATP.

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ed under identical conditions (curve I). Optimal protection of TAT by ATP is only achieved in the liver homogenate (curve II vs. curve III). In the combined homogenates there is only a partial protection (curve IV vs. curve V).

This indicates high activities of the inactivating system in kidney cortex membranes as compared to the stabilizing system. This interpretation is supported by the failure to demonstrate a reactivation of PEP-carboxykinase in former studies with the kidney system (5). On the contrary, reactivation of TAT can be demonstrated with the liver system (Fig. 3).

In figure 2 the subcellular distribution of the membrane bound inactivation and ATP-dependent stabilization of TAT is illustrated. It is evident, that almost all subcellular fractions show activity with a preference for the microsomes (P). As shown in the study

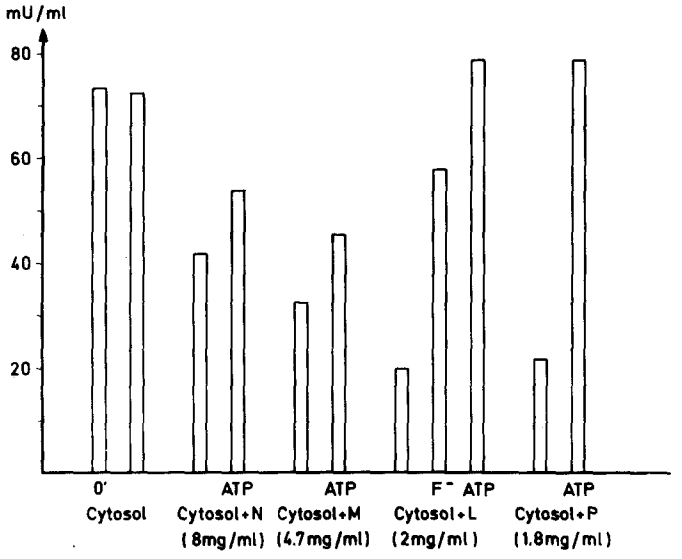

Fig. 2: Subcellular distribution of TAT inactivating and stabilizing system in rat liver.
1 ml liver extract was incubated with the various fractions and o.5 mM Mg^{++} in an end volume of 3 ml. All incubations - except where indicated - were incubated for 6o minutes. Where indicated, the reaction mixture was supplemented with 5 mM ATP (not regenerated) and 3.3 mM fluoride, respectively.
N = nuclei; M = heavy mitochondria; L = light mitochondria; P = microsomes.

with the L-fraction, fluoride inhibits the inactivation of TAT. The relative distribution of inactivation and stabilization as illustrated in figure 2 has been confirmed in at least 5 additional experiments.

In view of a control of glycogen synthetase by a membrane bound protein kinase from kidney (1o), contrary to our original assumption of a protection by ATP against proteolytic inactivation (5) also an interconversion of TAT into different forms had to be considered. This interpretation is further substantiated by the demonstration of a reactivation of TAT by ATP and Mg^{++} subsequent to its inactivation by the same subcellular fraction (Fig. 3).

In view of recent report by Kneer (4) on a regulatory role of cyclic AMP and cyclic GMP in gluconeogenesis, possible effects of these nucleotides on the inactivation and ATP-dependent stabilization of TAT by the subcellular fraction from liver were investi-

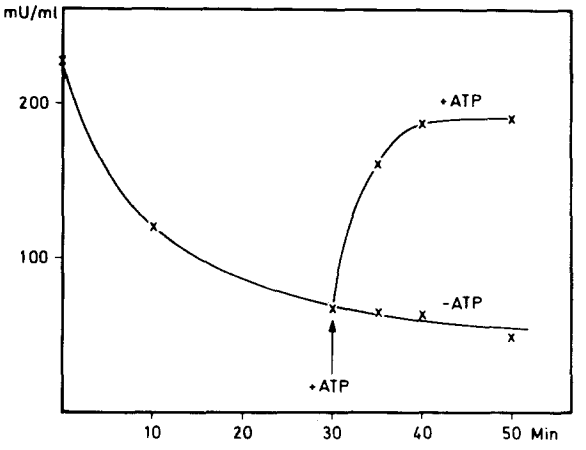

Fig. 3: Inactivation and ATP-dependent reactivation of TAT by the L-fraction.
2 ml liver extract were incubated with 20.4 mg of the L-fraction and 0.2 mM Mg^{++} in an end volume of 6 ml. Aliquots of each 1 ml were centrifuged and TAT assayed in the supernatant. Where indicated, 1 mM ATP was regenerated with 20 mM acetyl-P and 1.2 units acetokinase per ml.

gated. Reproducible effects as summarized in table 1 were only observed in the nuclear fraction at suboptimal concentrations of ATP and after ommission of the Triton treatment of particles. The inactivation rates were not altered by the cyclic nucleotides (not shown).

The present studies are consistent with an interconversion of TAT into an active and inactive form catalyzed by a membrane bound enzyme system from liver. Whether this interconversion involves dephosphorylation and an ATP-dependent phosphorylation or other mechanisms is subject of a current investigation with purified enzymes and γ-labelled ATP. The rather great unspecifity of this system is supported by the fact that the subcellular fractions from liver and kidney can substitute for each other in inactivation and ATP-dependent stabilization of both hepatic TAT (Fig. 1) and renal PEP-carboxykinase (not shown). These properties and especially the wide distribution among all subcellular fractions rise the question as to the physiological significance of the membrane bound system in control of gluconeogenesis. Only a study of the relative con-

Addition	Incubation period (min)	Tyrosine amino transferase in mU/ml ± S.E.M. (N)	P
None	0	560 ± 29 (3)	—
None	60	246 ± 8 (2)	—
ATP-regenerating system	60	442 ± 16 (3)+	<0.005
ATP-regen. system + 10^{-6} M, 3',5'-cAMP	60	540 ± 11 (3)++	<0.01
ATP-regen. system + 10^{-6} M, 3',5'-cGMP	60	544 ± 11 (3)++	<0.01

Table 1: Effects of cyclic nucleotides on ATP-dependent stabilization of TAT by the nuclear fraction from liver
1 ml liver extract was incubated with 24.5 mg of the N-fraction in an end volume of 3 ml. Where indicated, 0.5 mM ATP (suboptimal) was regenerated with 5 mM acetyl-P, 0.5 mM Mg^{++} and 0.7 units acetokinase/ml.
+ Significance vs. 60 min incubation without ATP.
++ Significance vs. 60 min incubation with 0.5 mM ATP.

tents of inactive and active forms of PEP-carboxykinase and TAT at various metabolic states can give an answer to this question. This study is currently in progress in our laboratory.

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