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REVERSIBLE INACTIVATION OF TYROSINE AMINOTRANSFERASE FROM GUINEA PIG LIVER BY THIOL AND DISULFIDE COMPOUNDS

G. Federici, D. Di Cola, P. Sacchetta, C. Di Ilio, G. Del Boccio and G. Polidoro.

Institutes of Biological Chemistry and Chemistry, Faculty of Medicine, University " G. D'Annunzio", Chieti, Italy.

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SUMMARY: Tyrosine aminotransferase from guinea pig liver is strongly inactivated by a variety of natural thiols and disulfides. L-cysteine was used as a model compound in the study of inactivation. Inactivation is due to the disulfide produced by spontaneous oxidation of thiol during incubation. Binding studies with [^{35}S]-cysteine revealed simultaneous incorporation of [^{35}S] into tyrosine aminotransferase and loss of enzyme activity. The reversibility demonstrates that the inactivation is the result of the formation of mixed disulfide between the disulfide and the sulfhydryl group of tyrosine aminotransferase. Some features of the enzyme active site are showed by the inactivation reaction.

Tyrosine aminotransferase (L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5) has several distinctive features which have made this enzyme a convenient model for studies of regulatory processes in enzyme induction and protein degradation in mammalian cells.

An extensive literature has appeared on the regulation of synthesis of tyrosine aminotransferase, while only few reports appeared concerning regulation of inactivation or degradation of this enzyme. Ten years ago Holten et al. (1) first reported that the enzyme lost activity in the presence of a mixture of L-cysteine, L-histidine and L-threonine. Since a partial protective effect was observed in the presence of pyridoxal-5'-phosphate (PLP) they concluded that the amino acids function by removing the PLP from the enzyme. Others have reported that incubation of this enzyme with L-cysteine, L-homocysteine and penicillamine brought about a loss of activity when the enzyme was assayed in the absence of PLP (2). Auricchio et al. (3) have described a system in rat liver homogenate which inactivate tyrosine aminotransferase in the presence of cysteine. They propose that cysteine acts by activation of lysosomal cathepsin B and B_1. Reynold and Thompson have per-

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formed a study on the inactivation of rat liver tyrosine aminotransferase by cysteine and some analogs. They observed an irreversible inactivation not attributable to a simple loss of PLP. A strict correlation between the structure of various cysteine analogs and the ability to inactivate the enzyme was also observed by these authors (4). More recently another investigation on the enzyme from the same tissue shows a reversible inactivation by cysteine (5).

Although various hypotheses have been proposed on the inactivation of tyrosine aminotransferase by cysteine and analogs, the exact mechanism has not yet been recognized. The present paper investigates the inactivation of tyrosine aminotransferase from guinea pig liver by some thiol and disulfide compounds and a new mechanism for their action is proposed.

MATERIALS AND METHODS : Male albino guinea pig (400-500 g) were obtained from Istituto Zooprofilattico of Teramo (Italy) and maintened on regular laboratory chow and water ad libitum. Tyrosine aminotransferase was purified from liver by a new convenient and rapid method (6). All the thiols and disulfides were obtained from Fluka (Buchs); Sephadex G-150 was purchased from Pharmacia (Uppsala) and [^{35}S]-L-cysteine hydrochloride (24.4 mCi/mmole) from Radiochemical Centre (Amersham). Other products were of the best available grade. Radioactivity was determined by a 3380 Packard Tricarb spectrometer using a conventional PPO-POPOP-toluene scintillation liquid. The incubations with the compound tested were carried out in a water bath, at 37°C, in 0.1 M phosphate buffer pH 7.4. The method of Diamondstone (7) was used for tyrosine aminotransferase activity. Proteins were determined by the method of lowry et al. (8) or by the biuret assay, using crystalline bovine serum albumin as a standard.

RESULTS : The tyrosine aminotransferase activity decreases after incubation of the enzyme in the presence of cysteine at concentration between 0.1-10 mM (fig. 1), while the incubation in the presence off all others amino acids, at mM final concentration, gives no loss of activity. The addition of 50 mM mercaptoethanol after two hours incubation with cysteine is able to restore the initial activity. PLP and α-ketoglutarate both at 1 and 10 mM concentration are unable to remove the loss of enzyme activity caused by cysteine. Only 1 mM EDTA exerts a protective effect in any condition of incubation.

In order to establish if the inactivation is due to the -SH moiety of cysteine various thiols and disulfides were assayed. The results are reported in table 1 . Cysteine ethylesther, cysteamine, homocysteine and reduced

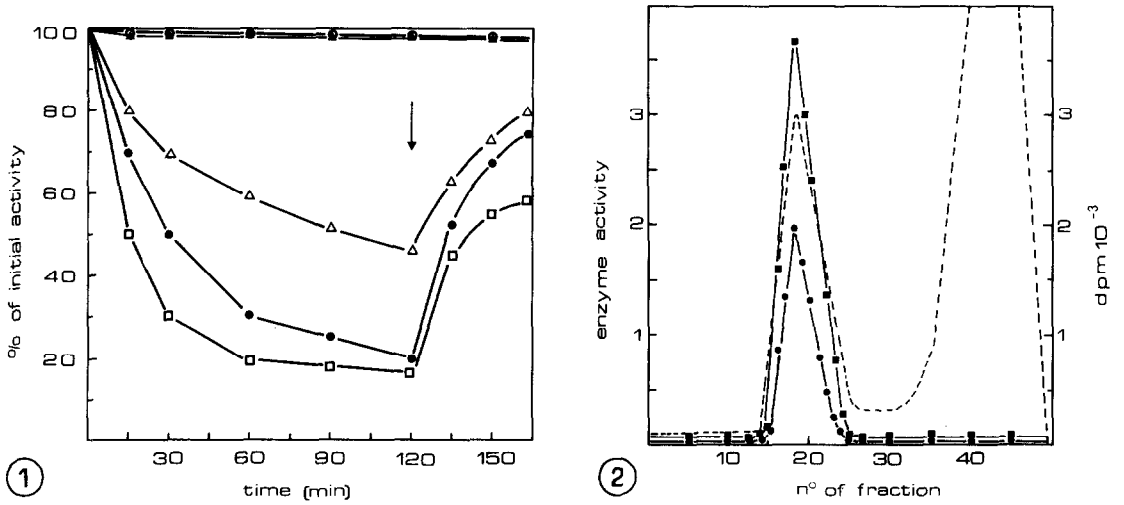

Figure 1

Time course of tyrosine aminotransferase inactivation.
(0.2 mg of enzyme were incubated as described in the text)

△—△ with 0.1 mM cysteine

□—□ with 10 mM cysteine

●—● with 1 mM cysteine

○—○ with 1 mM cysteine and 1 mM EDTA

▲—▲ with 1 mM solution off all amino acids, without cysteine

at the arrow 50 mM mercaptoethanol was added.

Figure 2

Gel filtration of tyrosine aminotransferase inactivated by $\left[{}^{35}S\right]$ -cysteine

●—● enzyme activity in arbitrary units

■—■ enzyme activity after addition of 50 mM mercaptoethanol

----- radioactivity

glutathione give different time courses of inactivation, while mercaptoethanol and mercaptopropionic acid are uneffective. Cystine ethylesther, cystamine, homocistine, oxidized glutathione and cystine are able to produce loss in enzyme activity at the same concentrations of their respective reduced analogs. EDTA is able to remove the inactivation of all thiols tested but cannot exert protective effect when the enzyme is incubated in the presence of disulfides.

In another experiment 0.5 mg of tyrosine aminotransferase were incu-

TABLE I

Inactivation of tyrosine aminotransferase by thiols and disulfides

The incubation were carried out as described under experimental. All the reactants were used at 1 mM concentration. The results are reported as percent of initial activity after two hours incubation, and are the mean of two determinations.

	-SH	-S-S-
Cyst(e)ine	24	22
Cyst(e)ine + 1 mM EDTA	100	24
Cyst(e)ine ethylesther	12	14
Cyst(e)ine ethylesther + 1 mM EDTA	100	14
Cyst(e)amine	16	17
Cyst(e)amine + 1 mM EDTA	100	16
Homocyst(e)ine	80	81
Homocyst(e)ine + 1 mM EDTA	100	76
Mercaptopropionic acid	100	100
Glutathione	92	90
Mercaptoethanol	100	--

bated with 0.5 mM cysteine containing 1 μCi of $[^{35}S]$-L-cysteine hydrochloride, in a total volume of 1 ml. Enzyme activity was assayed every ten minutes. When tyrosine aminotransferase had lost about 50% of the initial activity. the incubation mixture was subjected to gel chromatography on a Sephadex G-150 column, 1.5 x 60 cm. Both tyrosine aminotransferase activity and radioactivity were determined in the eluate. The results are reported in fig. 2. Two peaks of radioactivity were obtained; the first corresponding to the peak of enzyme activity, while the second major peak, eluted at the total volume of column, corresponds to unreacted cysteine, as determined by nitroprusside-cyanide test. All the fractions containing tyrosine aminotransferase activity were treated with 50 mM mercaptoethanol and, after 45 minutes at room temperature, the activity was again determined. The results are reported in the same fig. 2. The inhibition was removed in all the fractions and the effect of mercaptoethanol gives a restoration of about 90-95 % of initial activity. The active fractions were pooled and concentrated up to 1 ml and then dialyzed against the same buffer of gel chromatography. The tyrosine aminotransferase losses all the bound radioactivity that was quantita-

tively recovered in the ultrafiltrate and in the dialysate. If the treatment with mercaptoethanol was omitted and the active fractions were pooled, concentrated and dialyzed, tyrosine aminotransferase shows both bound radioactivity and inhibition.

DISCUSSION : The involvment of disulfide exchange as a possible regulatory system for sulfhydryl enzymes has been extensively studied in rabbit liver fructose diphosphatase by Pontremoli and Horecker (9) and in rat liver glycogen synthetase by Ernest and Kim (10).

All the results so far obtained in the study of interactions between cysteine and tyrosine aminotransferase did not clarify the mechanism of the enzyme inactivation (1-5). The data reported in fig. 1 and table I show that the enzyme is inhibited by the disulfide form of reactants. In fact if the reaction with thiols was carried out in the presence of 1 mM EDTA no loss in enzyme activity was observed after two hours incubation, while the disulfide reagents are able to inactivate the enzyme also in the presence of EDTA. The hypothesis that the inactivation proceeds by a sulfhydryl-disulfide exchange was confirmed by the experiment of gel filtration, after the reaction of the enzyme with $\left[^{35}S\right]$ -cysteine in the absence of EDTA. Under the experimental conditions used the oxidation of cysteine to cystine was rapid, as checked by the nitroprusside test in the presence or absence of cyanide, and was paralleled by enzyme inactivation. In fact after treatment of the active fractions with mercaptoethanol we have observed that all bound radioactivity was lost and the initial enzyme activity restored.

Some idea of the geometry of the site(s) of action of disulfides, possibly the active site, can be gained from the data reported. In fact no protective effect against inactivation was exerted by PLP or by substrates and the various thiols and disulfides assayed inhibit at various extent tyrosine aminotransferase activity. Cyst(e)ine was less effective than the corresponding ethylesther while cyst(e)amine was the most physiological effective reagent. These data implicate that charged carboxyl groups must partially block the access to the critical sulfhydryl group(s) of the enzyme. The greater stability of tyrosine aminotransferase towards homocyst(e)ine indicates the importance of the distance between the disulfide bond and the α-carbon of the molecule. Furthermore the inability of mercaptopropionic acid to inactivate

the enzyme demonstrates the importance of the α-amino group for the interaction of inhibitors with the reactive -SH group(s) of tyrosine aminotransferase. As expected, the least effective physiological compound tested was glutathione, where the α-amino and α-carboxyl groups of cysteine are involved in peptide linkage with glutamic acid and glycine, respectively.

The physiological significance of the in vitro inactivation system described herein requires further investigations, nevertheless it may play a significant role in the modulation or even removal of tyrosine aminotransferase from liver cells.

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