THE INACTIVATION OF CHICK TYROSINE AMINOTRANSFERASE BY ACID AND ALKALINE PHOSPHATASES

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Received April 22, 1971

SUMMARY

Chick tyrosine aminotransferase is inactivated by incubation in the presence of acid or alkaline phosphatases from various sources. The inactivating activity as well as the phosphatase activity of these preparations was inhibited by NaF in the case of acid phosphatase and stimulated by $MgCl_2$ in the case of intestinal alkaline phosphatase. The mechanism of inactivation appears to be initial dephosphorylation of loosely bound pyridoxal phosphate followed by denaturation of the labile apoenzyme.

The enzyme tyrosine aminotransferase (EC 2.6.1.5) turns over very rapidly in rat liver (1) and hepatoma tissue culture cells (2,3) but specific enzymes which catalyze its degradation or inactivation have not been found. It was therefore of interest when we observed that tyrosine aminotransferase activity in crude extracts of chick embryo liver was rapidly lost while the enzyme activity in heated extracts or DEAE cellulose fractionated extracts was quite stable when stored at 4°. A similar observation had been made for the enzyme alanine-phenylglyoxal aminotransferase of Rhodopseudomonas spheroides and in that instance the inactivation could be duplicated by treating the purified enzyme with an alkaline phosphatase preparation (4). This report describes the inactivation of tyrosine aminotransferase by acid and alkaline phosphatases from various sources.

MATERIALS AND METHODS

Livers from 19-day-old embryos, 10-day-old chicks or 80 g rats were homogenized in a buffered medium containing 10 mM α-ketoglutarate and 0.08 mM pyridoxal-5'-phosphate as described previously (5). The supernatant solution obtained after centrifugation at 105,000 x g for 1 hour was fractionated batchwise with DEAE cellulose by a method which will be described elsewhere (6). Three fractions were obtained: (I) unadsorbed protein, (II) protein eluted with 0.1M KCl and

(III) protein eluted with 0.3 M KCl. Fractions I and II contained acid phosphatase activity and tyrosine aminotransferase inactivating activity measured at pH 4.1, while fraction III contained tyrosine aminotransferase purified approximately thirtyfold.

Partially purified wheat germ acid phosphatase, chromatographically purified E. coli alkaline phosphatase and pig heart aspartic aminotransferase were purchased from Worthington Biochemical Corp. Chicken intestine alkaline phosphatase was purchased from Sigma Chemical Corporation.

Tyrosine aminotransferase was assayed by measuring the formation of p-hydroxyphenyl pyruvate as described previously (5). Phosphatase activities against p-nitrophenyl phosphate were measured essentially as described by Lowry (7) except that a total volume of 0.30 ml was used and time points were taken at 0 and 20-60 min. Acid phosphatase was measured at pH 4.1 using 0.1N acetate buffer and alkaline phosphatase at pH 8.9 using 0.1N 2-amino-2-methyl-propanediol buffer in the presence of 0.01 M $MgCl_2$. Aspartate aminotransferase was measured as described by Jenkins and Sizer (8).

Inactivation of aminotransferases was measured by carrying out a preliminary incubation at pH 4.1 or 8.9 at 37°. Reaction mixtures for the preincubation contained 0.1N buffer, 200-300 milliunits per ml of chick or rat tyrosine aminotransferase (Fraction III) or aspartic aminotransferase, 0.01M $MgCl_2$ (if chicken intestine alkaline phosphatase were used) and an enzyme fraction containing phosphatase activity or appropriate buffer in no-enzyme controls. Results presented have all been corrected for losses observed in control tubes. The aminotransferase solutions were dialyzed before use against 0.01M triethanolamine buffer, pH 8, containing 2mM α-ketoglutarate. The components of the reaction were mixed at 0°, a sample removed before initiating the reaction at 37° and at 45 and 90 min afterwards. Samples to be assayed for tyrosine aminotransferase activity were placed directly into reaction mixtures equilibrated at 37°. The conditions of the assay (pH, pyridoxal phosphate concentration) were sufficient to stop the inactivation reaction. Samples to be assayed for aspartic aminotransferase activity were

added to reaction mixtures containing all the components except NADH and aspartate. NADH was added initially to detect any NADH oxidase activity and then aspartate was added to initiate the aminotransferase reaction.

RESULTS

Initial observations which suggested that phosphatase activity might be responsible for the inactivation of tyrosine aminotransferase by embryo liver fraction I were that inactivation was optimal at pH 4.1 and that pyridoxal-5'-phosphate inhibited the inactivation, but with time the inhibition was reversed. In Table I it may be seen that at a low concentration of pyridoxal phosphate, 0.04 mM, and a near saturating level of tyrosine aminotransferase, there was only partial inhibition of the inactivation after 90 min. At a higher concentration of the cofactor and a lower concentration of aminotransferase, however, inhibition was complete even after 90 min. In addition, inactivation by wheat germ acid phosphatase was observed at pH 4.1 and this activity was also inhibited by pyridoxal phosphate.

Table I. Effect of pyridoxal-5'-phosphate on tyrosine aminotransferase (TAT) inactivation by acid phosphatase

Expt.	Enzyme Source	pH	PLP mM	TAT munits/ml	TAT inactivated in 90 min. munits/ml	Percent Inhibition
1	liver, fraction I	4.1	0	249	96.0	-
			0.04		29.8	69.0
2	liver, fraction I	4.1	0	140	116.8	-
			1.0		0	100
3	wheat germ	4.1	0	220	138.5	-
			0.04		78.3	43.5

Preincubations to measure inactivation were carried out in a total volume of 0.30 ml as described under Methods and the protein concentration of the phosphatase-containing fractions used were: expt. 1, 6.7; expt. 2, 6.7 and expt. 3, 3.3 mg/ml.

The dephosphorylation of pyridoxal phosphate by phosphatases from various sources has been reported (9,10,11). Chick embryo and wheat germ acid phosphatases and chicken intestine and E. coli alkaline phosphatases were checked for such activity indirectly by testing the inhibitory effect of pyridoxal phosphate on the dephosphorylation of p-nitrophenylphosphate. The dephosphorylation of the substrate by all of the phosphatases was inhibited to some extent but embryo liver acid phosphatase and chicken intestine alkaline phosphatase could be substantially inhibited at relatively low concentrations of pyridoxal phosphate (0.5 mM) and saturating levels of substrate (6.7 mM). The inhibition appeared to be of the competitive type.

Inactivation of tyrosine aminotransferase was catalyzed by the four phosphatase preparations as shown in Table II. Sodium fluoride, a known inhibitor of acid phosphatases (12) inhibited both acid phosphatase activity against p-nitrophenyl phosphate and tyrosine aminotransferase inactivating activity of liver

Table II. Relationship between phosphatase activity and tyrosine aminotransferase inactivating activity

Enzyme Source	pH	Additions	Phosphatase Activity units/ml	TAT inactivated in 90 min munits/ml	TAT inactivated $\times 10^{-1}$ / phosphatase
liver, fraction I	4.1	-	0.45	25.3	5.6
	4.1	NaF	0.26	7.2	-
wheat germ	4.1	-	2.85	160.4	5.6
	4.1	NaF	0.18	59.2	-
chicken intestine	8.9	-	0.02	2.0	-
	8.9	$MgCl_2$	0.18	50.2	27.8
E. coli	8.9	-	0.69	88.6	12.8

The volume of reaction mixtures in inactivation assays was 0.30 ml and the following concentrations of enzymes were used: embryo liver, 3.92 mg/ml; wheat germ acid phosphatase, 3.33 mg/ml, chicken intestine alkaline phosphatase, 6.67 μg/ml; E. coli alkaline phosphatase, 6.67 μg/ml. Chick TAT (fraction III) was used at a concentration of 180 munits/ml. Phosphatase assays were carried out for 20 min. as described in the Methods section.

fraction I and wheat germ acid phosphatase. The activity of intestinal alkaline phosphatase towards both substrates was greatly stimulated by $MgCl_2$. When activity was compared on the basis of milliunits of aminotransferase inactivated per unit of phosphatase activity, it may be seen that intestinal alkaline phosphatase was most active. This enzyme was also most sensitive to pyridoxal phosphate inhibition.

Further evidence that the inactivation is a specific effect of phosphatase was shown by testing several other proteins under the same conditions. Lysozyme, crude histone, crystalline serum albumin and purified ribonuclease (Sigma, protease free) were tested at 0.1 mg/ml and compared to 3.33 mg/ml of wheat germ acid phosphatase at pH 4.1 and 6.7 μg/ml of intestinal alkaline phosphatase at pH 8.9. No significant effect on tyrosine aminotransferase was seen except with ribonuclease which had approximately 20% of the activity of acid phosphatase at pH 4.1. It showed no inactivating activity at pH 8.9. No acid phosphatase activity against p-nitrophenyl phosphate was detected in the ribonuclease solution and the reason for its ability to inactivate is at present unexplained.

No inactivation of rat liver tyrosine aminotransferase by acid phosphatase could be detected when the usual assay system containing pyridoxal phosphate was

Table III. Effect of acid and alkaline phosphatases on rat tyrosine aminotransferase

Enzyme Source	pH	PLP in TAT assay	TAT inactivated in 90 min. munits/ml
Wheat germ	4.1	-	155.1
Wheat germ	4.1	+	2.7
Chicken intestine	8.9	-	113.9
Chicken intestine	8.9	+	93.0

The initial concentration of rat tyrosine aminotransferase was 240 munits/ml; otherwise conditions were exactly the same as described in Table II. TAT was measured in the presence or absence of pyridoxal phosphate (PLP) as indicated.

employed (Table III). If the cofactor was omitted from the assay system, however, an apparent loss of activity was observed, indicating that acid phosphatase had dephosphorylated the cofactor and that the remaining apoenzyme was stable at pH 4.1. Treatment with alkaline phosphatase resulted in inactivation.

Purified aspartic aminotransferase was neither inactivated nor converted into apoenzyme after treatment of the pyridoxal phosphate form of the enzyme with either of the alkaline phosphatases. Interpretation of data obtained with wheat germ acid phosphatase was complicated by the extreme instability of the aspartic aminotransferase holoenzyme at pH 4.1 and the presence of endogenous aspartic aminotransferase activity in the acid phosphatase preparation.

DISCUSSION

Previous brief reports have suggested that E. coli alkaline phosphatase could inactivate alanine-phenylglyoxal aminotransferase (4) and resolve E. coli tryptophanase (13). This report has shown that inactivation of tyrosine aminotransferase initially observed in crude extracts of chick embryo liver may be catalyzed by an acid phosphatase present in these extracts and that, in addition, acid and alkaline phosphatases from various sources can carry out such inactivation to varying degrees.

The results suggest that inactivation of chick tyrosine aminotransferase occurs by a mechanism which may be tentatively outlined as follows:

The conclusion that pyridoxal phosphate is the site of phosphatase attack is supported by the observation that rat tyrosine aminotransferase, was converted into the apoenzyme form, which is apparently stable at pH 4.1, by treatment with acid phosphatase. The observation that aspartic aminotransferase, which contains a tightly bound cofactor (14), is not affected by either of the alkaline phosphatases tested suggests that free rather than bound cofactor is attacked. Thus chick tyrosine aminotransferase, which has a very labile apoenzyme form (5), would first

be resolved and its apoenzyme rapidly inactivated by incubation at 37°.

These observations suggest the possibility that phosphatases may be involved in the intracellular regulation of pyridoxal phosphate dependent enzymes. One of the current theories regarding protein degradation is that lysosomes play a role in this process (15). Both alanine aminotransferase (16) and tyrosine aminotransferase (17) of rat liver have been reported to be inactivated by crude lysosomal preparations at pH 5. A possible function of acid phosphatase, a lysosomal enzyme, might be to form apoenzymes which are more susceptible to lysosomal proteolysis. This would be analogous to the susceptibility of tryptophan oxygenase to degradation in rat liver in the absence of tryptophan (18).

Recently (3) it has been observed that fluoride prevents the decrease of induced levels of tyrosine aminotransferase which normally occurs when steroid is removed from rat hepatoma tissue culture cells. Although it was shown that there was a concomitant decrease in ATP levels and this decrease was suggested as the factor affecting the turnover of the enzyme, the possibility exists that fluoride may also be affecting turnover through its inhibitory action on acid phosphatase.

Acknowledgements. I wish to thank Drs. T. R. Breitman and A. Peterkofsky for valuable discussions and Mrs. H. Bergman for excellent technical assistance.

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