COMPARATIVE STUDIES ON THE INACTIVATING ENZYMES FOR PYRIDOXAL ENZYMES FROM YEAST AND RAT

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SUMMARY

With protein fractionating methods an apo-ornithine transaminase[1] inactivating activity from yeast has been partially purified. The kinetics of the inactivating reaction and the behaviour of the active principle during purification make it very probable that we are dealing with an "inactivating enzyme". Comparison of the OTA inactivating enzyme from yeast with the previously described OTA "splitting enzyme" from rat tissues was established by cross reacting the substrate OTA's and other substrate enzymes with the inactivating enzymes from yeast and rat intestine. It has been found that the group specificity of the inactivating enzyme towards the apo-forms of pyridoxal enzymes is not only observed when the inactivating enzyme and the substrate enzymes are prepared from the same species but also when the inactivating enzymes and the substrate enzymes from yeast and rat tissues are cross exchanged.

INTRODUCTION

Enzyme-inactivating proteolysis has been considered to participate in the control of enzyme levels (for reviews see (1,2,3)). Usually more or less unspecific proteases are assumed to participate in these regulatory mechanisms. In a recent publication N. Katunuma and coworkers, however, have

[1] Abbreviation: OTA = ornithine transaminase

been able to demonstrate in small intestine and some other rat tissues 3 types of "splitting enzymes" which inactivate specifically the apo-enzymes of pyridoxalphosphate-dependent enzymes (4), NAD-dependent enzymes (5), and FAD-dependent enzymes (6). The most carefully studied enzyme of these groups is the OTA inactivating enzyme from rat intestine. This "splitting enzyme" inactivates the apo-enzymes of OTA as well as tyrosine transaminase and serine dehydratase from rat liver. It is, however, inactive with four other pyridoxal phosphate-independent enzymes from rat tissues. To get more information on the specificity of this pyridoxal enzymes splitting enzyme comparative studies on the enzyme from rat intestine and from yeast have been undertaken.

MATERIALS AND METHODS

Purification of OTA from yeast. For extraction 1 kg of commercial baker's yeast (Sinner-Hefefabrik, Karlsruhe) was suspended in 300 ml 0.5 M phosphate buffer pH 7.0 containing 50 γ/ml PALP, 2.5 mM α-oxoglutarate, 2 mM MET, and 0.5 μM EDTA and passed six times through a Manton-Gaulin Laboratory homogenizer (Manton Gaulin Manufactoring Corp., Everett, Mass., USA) at maximum pressure. The homogenate was centrifuged at 27000 g for 30 min. A freshly prepared solution of protamine sulfate was added with stirring to the supernatant giving a final ratio of 1 mg protamine sulfate per 10 mg of protein. The precipitate was removed by centrifugation. The supernatant was adjusted to pH 7.0 and heated in four batches for 1 min at 60° C, rapidly cooled in an ice bath and centrifuged. The supernatant was treated by ammonium sulfate fractionation. The 30-50 % fraction was dissolved in the extraction buffer and used for all experiments. The enzyme exhibits a sharp maximum of activity at pH 7 (16). This is in contrast to

the rat enzyme, which shows maximal activity at pH 8. The biological significance of this difference is obvious: the overall pH of yeast cells is about 6.5 - 7.0, whereas rat tissues show more alkaline overall pH values.

Preparation of pyridoxal enzymes inactivating enzyme from yeast. For extraction 0.5 kg of baker's yeast were suspended in 50 mM phosphate buffer pH 7.5 and homogenized in a Manton-Gaulin Laboratory homogenizer. The homogenate was centrifuged and a 3 % solution of protamine sulfate was added to the supernatant (protein:protamine sulfate = 1:10). The precipitate was removed by centrifugation. The 50-70 % ammonium sulfate fraction of the supernatant was dissolved in 50 mM phosphate buffer pH 7.5 and dialyzed 4 hours against destilled water. The dialyzed protein was kept in the refrigerator over night, and precipitated protein was removed by centrifugation. The supernatant was again treated by ammonium sulfate and the 50-70 % fraction was dissolved in 50 mM phosphate buffer pH 7.5 and used for all experiments. In the test described below, samples containing between 5 and 25 mg protein gave a linear response in inactivation of yeast OTA.

Purification of other enzymes. OTA from rat liver and pig liver was purified and crystallized according to Sanada et al. (7). The "splitting enzyme" from rat intestine was purified until the acetone fractionation step according to Katunuma et al. (4). Serine dehydratase from rat liver was purified according to Nakagawa et al. (8). Aspartate aminotransferase from rat and pig liver was purified and crystallized as described by Morino et al. (9). Serine-(threonine)-dehydratase from yeast was purified according to Katsunuma et al. (10). Aspartate transaminase from yeast was purified as described by Schreiber and Holzer (11) with slight modification. Instead of the sucrose-density ultracentrifugation

a G-150 gelfiltration step was performed.

Preparation of apo-enzymes. Apo-OTA of yeast, rat liver and pig liver was prepared according to Matsuzawa et al. (12). Conversion to the apo-forms of serine dehydratase and aspartate transaminase was carried out following the technique of Nishii et al. (13). In the absence of PALP all apo-enzymes showed less than 1 % of the activity with PALP.

Assay of enzymes. OTA and "splitting enzyme" from rat were tested as described by Katunuma et al. (4). The yeast enzymes were tested in the same way, however, instead of buffer pH 8.0 a 0.2 M phosphate buffer pH 7.0 was used.

RESULTS AND DISCUSSION

As can be seen from Fig.1A, incubation at 37° C of the partially purified inactivating fraction from yeast with apo-OTA leads, in the absence of pyridoxalphosphate, to a nearly constant rate of inactivation for at least 15 min. This observation makes it appear probable that the active principle in the inactivating

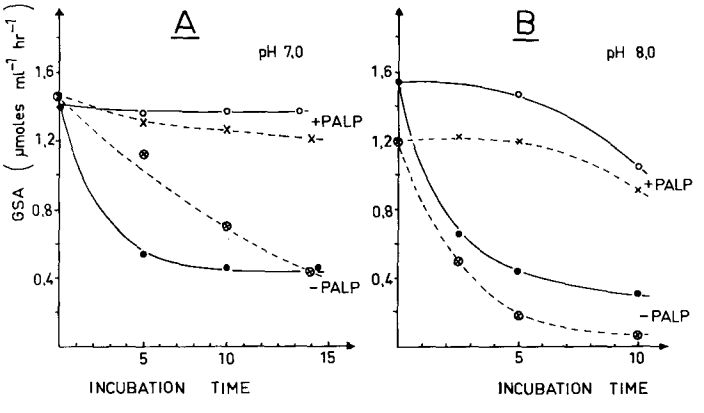

Fig. 1 Time course of inactivation of yeast and rat OTA by "inactivating" enzyme from yeast (A) and "splitting" enzyme from rat (B). T = 37° C, GSA = glutamic semialdehyde. ● = Apo-OTA from rat; o = Holo-OTA from rat; ⊗ = Apo-OTA from yeast; x = Holo-OTA from yeast.

fraction from yeast is not an inhibitor, but an enzyme. Further evidence for the enzyme nature of the active principle are the temperature dependence of the inactivation reaction and the fact that the active principle is heat labile and can be enriched with usual methods for purification of enzymes.

Addition of pyridoxal phosphate protects against inactivation of OTA (see Fig.1A). An analogous dependence on pyridoxal phosphate was observed in the earlier experiments of Katunuma et al. (4) on the OTA "splitting enzyme" from rat intestine. In this latter case Katunuma et al. (4,6) have demonstrated that the molecular mechanism of inactivation is a splitting off of a nonapeptide from rat OTA. Because of the analogies in the properties of the inactivating fractions from intestine and yeast it is very probable that the yeast enzyme described here is also a "splitting enzyme". In the present paper, the enzyme is, however, called "inactivating enzyme" because "splitting" has as yet not been demonstrated.

As shown in Fig.1A the yeast inactivating enzyme not only inactivates OTA from yeast but also OTA from rat liver. Analogous to the situation in yeast the inactivation is prevented by addition of pyridoxal phosphate. To look further for this type of specificity the experiments with "splitting enzyme" from rat intestine, depicted in Fig.1B, were made. As the figure shows, there is again inactivation of the substrate OTA from both sources and in both cases protection by the addition of pyridoxal phosphate. The results in Table I show that also OTA as well as aspartate aminotransferase from pig liver, and aspartate aminotransferase and serine dehydratase from rat liver are inactivated by purified "splitting enzyme" from rat intestine. In contrast aspartate aminotransferase and serine dehydratase from yeast are not at-

TABLE I

Activities of "Splitting Enzyme" from Rat Intestine for Several B_6-Enzymes from Different Sources

	Apo-Aspartate aminotransferase	Apo-Serine dehydratase	Apo-Ornithine aminotransferase
Rat liver	100% (23%)	100% (140%)	100% (100%)
Pig liver	34%	-	170%
Yeast	0%	0%	300%

(...%) indicates relative splitting coefficient apo-ornithine aminotransferase:apo-serine dehydratase:apo-aspartate aminotransferase from rat liver

tacked. From this observations it can be assumed that the inactivating enzymes from yeast and rat are not species-specific, but specific for the catalytic (or coenzyme-binding) site of the substrate enzyme independent of its origin. This assumption becomes plausible if one takes into consideration that the amino acid sequences of the active or coenzyme-binding sites of enzymes with the same function from different species are usually identical or very similar (for examples see (14,15)).

Evidence for the biological significance of the apo-OTA inactivating enzymes in yeast and in rat tissues is given by the dependence of the activities of these enzymes from pH (16). The yeast-inactivating enzyme exhibits maximal activity at pH values between 6 and 7 with substrate OTA from yeast as well as from rat liver. The rat "splitting enzyme", however, exhibits maximal activity at a pH higher than 8 with OTA from rat as well as from yeast. It is well known that the overall pH in yeast is between 6 and 7 (Conway et al. (17), Neal et al. (18)), whereas the overall pH values of mammalian tissues are in a more alkaline range.

At present two possible explanations for the metabolic function of "splitting enzymes" are available. 1) As proposed by

Katunuma et al. (6) "splitting enzymes" might have an emergency function in vitamin deficiency by making available the coenzyme (formed from the respective vitamin) by splitting and thereby introducing degradation of the respective apo-enzymes. Experimental evidence for this hypothesis is the appearance of coenzyme-specific "splitting enzmes" in vitamin deficiency in mammalian tissues (Katunuma et al. (4)), and the specificity of "splitting enzymes" for the coenzyme of the substrate enzyme which is split. 2) "Splitting enzymes" might be useful for making available amino acids from enzymes which are not longer necessary after the transition of well-fed (growing) cells to fasting (resting) cells. (In the case of mammals the transition might be from "well-fed" to "fasting", in the case of microorganisms from "growing" to "resting"). Experimental evidence for such a function has been demonstrated by Katsunuma et al. (19) with the finding that the tryptophan synthase inactivating enzyme in yeast is inactive in exponentially growing cells and becomes active in the stationary phase. Similar to the OTA "splitting (or inactivating) enzymes", described in the present paper, the 1500-fold purified tryptophan synthase inactivating enzyme from yeast, described by Katsunuma et al. (19), is only active with the apoform of tryptophan synthase as a substrate, and inactivation is completely inhibited by the addition of a mixture of pyridoxal phosphate and serine.

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