PARTICIPATION OF THE TRYPTOPHAN SYNTHASE INACTIVATING SYSTEM FROM YEAST IN THE ACTIVATION OF CHITIN SYNTHASE

Andrej Hasilik and Helmut Holzer

Institut für Biochemie der Gesellschaft für Strahlen- und Umweltforschung mbH and Biochemisches Institut der Universität Freiburg im Breisgau (Germany)

Received May 31,1973

SUMMARY

The previously described tryptophan synthase "inactivase II", a proteolytic enzyme from yeast, exhibits high activity in the activation of chitin synthase. Tryptophan synthase inactivase I shows essentially no activity.

The purified, heat-stable inhibitor of the tryptophan synthase inactivating enzymes also inhibits the activation of chitin synthase. We take these results to mean that the proteolytic inactivation of tryptophan synthase and the proteolytic activation of chitin synthase are catalyzed and regulated by the same protease/inhibitor system

INTRODUCTION

Cabib et al. (1,2,3) have shown that chitin synthase from yeast, which participates in the formation of the septum between the mother cell and the daughter cell during budding, is converted from an inactive preenzyme to the active enzyme by an "activating factor", which proves to be a proteinase. The activating factor is found predominantly at late growth stages, and more activity is present in cells grown in minimal than in rich media. The proteolytic activation of chitin synthase is controlled by a heat-stable protein, which acts as an inhibitor of the activating proteinase. In all these respects the chitin synthase activating proteinase behaves analogously to the tryptophan synthase inactivating enzyme found by Manney (4) and purified (5) and characterized as two different proteinases (5,6) in our laboratory. The aim of the present paper was to show whether any of the tryp-

tophan synthase inactivases prepared in our laboratory can also activate chitin synthase, and if so, whether this activation is also sensitive to the purified inhibitor of the inactivases (7).

MATERIALS AND METHODS

Baker's yeast (Pleser, Darmstadt) was used in the purification of tryptophan synthase inactivases (5). Saccharomyces cerevisiae S288C grown in YEPD medium (4) to an optical density of about 6 (Zeiss PM 4, 650 nm, 1 cm, diluted ca. 20 x) was used for the preparation of chitin synthase. The protoplasts were obtained by the method of Schatz and Kováč (8) with 0.5 ml of Juice d'Helix pomatia (Ind.Biol.Franç., Genevilliers) per 2 g wet yeast, EDTA was omitted in the last washing. Pre-chitin synthase was subsequently prepared as in the ref. 1.

The "AF assay" of Cabib and Farkas (1) was employed to measure chitin synthase activation: total volume in the activating step was 42 µl inclusive up to 12 µl of activating enzymes and/or inhibitor. Incubations for 15 min and for 30 min (both at 30° C) were used routinely in the activation and incorporation steps, respectively. Controls were run with 12 µl water, trypsin (1) or inhibitor solution, 125 µg after ethanol fractionation (7). The activation reaction was stopped by addition of 125 µg of this inhibitor preparation when not initially present, or by 15 µg of soya bean trypsin inhibitor (Serva, Heidelberg), both in 5 µl. The incorporation reaction was started by addition of 5 µl of a mixture of 0.32 M N-acetyl glucosamine and 12.5 nCi of uridine diphospho-N-acetyl [glucosamine-^{14}C (U)] , 257 Ci/mol, purchased as ammonium salt from Amersham Buchler, Braunschweig. The incorporation was linear with time and amount of enzyme up to 6000 cpm. The test was stopped and the incorporated label

recovered as in the ref. 9 except that the final sediment was dissolved in 0.2 ml NC solvent (Nuclear Chicago, Heusenstamm) and counted with 92 % efficiency in 10 ml of scintillation solution, 4 g DPO and 0.1 g POPOP per 1 l toluene.

The tryptophan synthase inactivase activity was determined with holo-tryptophan synthase by the method of Katsunuma et al. (5) unless otherwise stated.

The esterolytic activity was determined by the method of Hata et al. (10) with acetyl tyrosine ethyl ester (ATEE) or benzoyl arginine ethyl ester (BAEE) at 10 and 20 mM final concentrations, respectively, at pH 8.0 and 25° C, using the pH-stat assembly ABU 12/ TTT 11/ PHM 26/ SBR 2/ TT31 (Radiometer, Copenhagen).

Acid proteinase activity was determined at pH 3.0 and 25° C by the method of Hata et al. (10) as modified by Saheki & Holzer (11), with HCl-denatured hemoglobin.

RESULTS AND DISCUSSION

Fig. 1 shows the elution pattern of a partially purified preparation of the tryptophan synthase inactivating enzymes from a DEAE-sephadex column. The chitin synthase activating activity appears in two peaks at fractions 10 and 54. Both activity peaks correspond to those of tryptophan synthase inactivase and of an esterase, which has a low activity quotient for ATEE and BAEE recalling that of proteinase B described by Hata et al. (12). These peaks also resemble protinase B in their heat lability: about 90 % of the inactivase activity in fractions 9 through 54 was lost upon incubation for 45 min at 37° C. The ratio of chitin synthase activating to ATEE esterolyzing activity of the two peaks is 330-350 (see Fig. 1). Another peak of ATEE esterolyzing

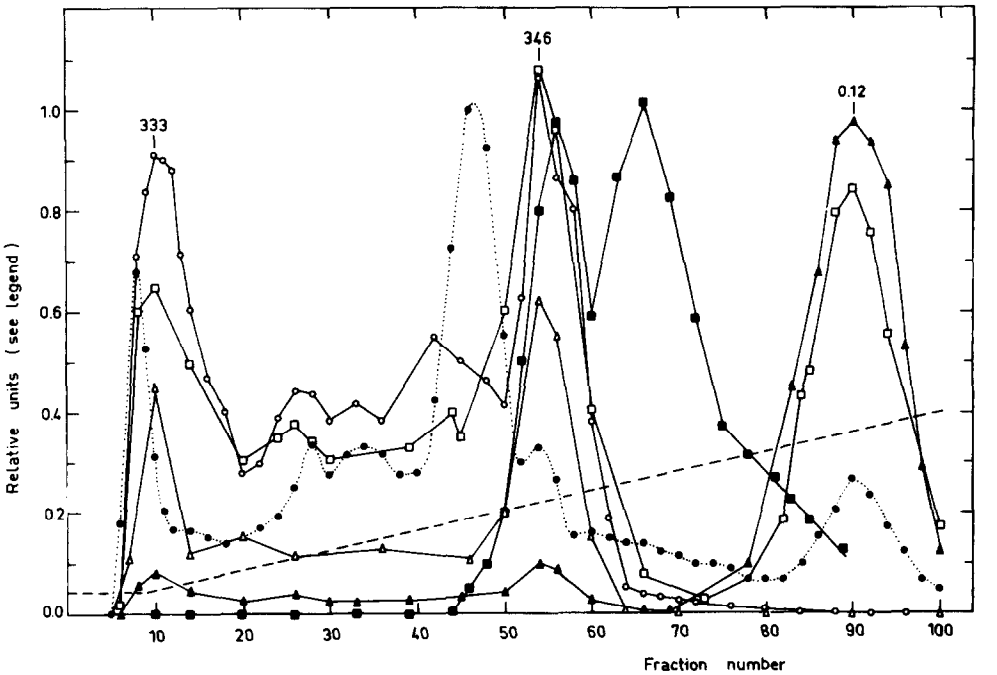

Fig. 1 Elution pattern of tryptophan synthase inactivase and of the chitin synthase activating factor from DEAE Sephadex A 50. The column, 1x14 cm was equilibrated with 10 mM potassium phosphate, pH 6.0, containing 1 mM mercaptoethanol and 40 mM KCl, and eluted by a linear gradient of KCl up to 0.4 M. Tryptophan synthase inactivase was prepared according to Katsunuma et al. (5), with modifications, through chromatography on hydroxyl apatite. The activity which appeared in two close peaks was pooled, concentrated and dialyzed against equilibrium buffers. 110 mg from this preparation was applied on the column. An ordinate unit represents molarity of KCl (---), an absorbance at 280 nm of 2 (.●.) and the following enzyme activities expressed per ml: 250 units of tryptophan synthase inactivase (-o-), one unit represents first order inactivation rate constant as determined with 15 µg of 25-fold purified tryptophan synthase in apo form; the activation of 1 mU chitin synthase per minute (-Δ-); 20 U ATEE esterase (-▲-); 1 U BAEE esterase (-□-); 90 µg of tyrosine equivalents appearing per minute in the 4.5 % TCA supernate of the Hb assay (-■-).

activity at fraction 90 shows a ratio less than 1/1000th of these: 0.12.

This peak with essentially no tryptophan synthase inactivating activity but a high ATEE esterolytic activity (80 I.U./mg), probably corresponds to proteinase C described by Hata (12). Proteinase C is characterized by a high ratio of ATEE to BAEE esterolysis (13) and no tryptophan synthase inactivating activity (11).

TABLE 1

Tryptophan synthase inactivation and chitin synthase activation by tryptophan synthase inactivases I and II

	Tryptophan synthase inactivation (units/mg)	Chitin synthase activation (Δ mU ChS/min•mg)
Inactivase I	3.7	$\leq$ 0.5
Inactivase II	5.2	3240

Tryptophan synthase inactivase was determined with holo-tryptophan synthase as substrate (5), and the chitin synthase activating factor as given under methods.

The coincidence of inactivation of tryptophan synthase and activation of chitin synthase prompted an experiment with the two tryptophan synthase inactivating enzymes described earlier (5). As shown in Table I, the inactivases I and II, prepared according to Katsunuma et al. (5) are similarly effective in the inactivation of tryptophan synthase, however, only inactivase II can activate chitin synthase. Further support for the idea that inactivase II is identical with the "chitin synthase activating factor" of Cabib et al. (1,3) is offered by an experiment with the purified inhibitor of the tryptophan synthase inactivating enzyme (cf. Table II). Small amounts of the electrophoretically homogeneous inhibitor, prepared by the method of Ferguson et al. (7) as modified by Betz (unpublished), inhibit completely inactivation of tryptophan synthase and activation of chitin synthase by inactivase II. In a separate determination the esterolytic activity of the same inactivase II preparation with either BAEE or ATEE was also found to be fully supressible by the partially purified inhibitor (through ethanol fractionation, ref.

TABLE 2

Effect of the inhibitor of the tryptophan synthase inactivating enzymes (7) on inactivation of tryptophan synthase and activation of chitin synthase by tryptophan synthase inactivase II

	Tryptophan synthase inactivase (units/mg)	Chitin synthase AF[+] (Δ mU ChS/min·mg)
Inactivase II	4.9	990
Inactivase II + inhibitor	≤ 0.01	- 80

The reaction mixture was that for the assay of chitin synthase with addition of 1.5 mM pyridoxal phosphate, 50 mM L-serine, 0.33 mU chitin synthase as preenzyme, 3.3 mU of 25-fold purified tryptophan synthase, and 6.4 µg inactivase II plus 1.6 µg inhibitor, in 84 µl. Aliquots were tested for tryptophan synthase and chitin synthase activities after 5 and 15 minutes at 30°, respectively.

[+]The true activity is underestimated, because of inhibition by the added tryptophan synthase, a competing substrate for inactivase II (cf. Table 1). A negative value arises by suppression of a basal activation attributed to small amounts of activating factor in chitin synthase preparation.

(7). According to Saheki and Holzer (11) inactivase II is identical or closely related to proteinase B described by Hata et al. (12) as judged from a comparison of the respective preparations. On the other hand the esterolytic activity with BAEE and proteolytic activity with Azocoll of chitin synthase activating factor prompted Cabib and Ulane (3) to point out its similarity to proteinase B.

The evidence for a biological significance of the chitin synthase activating protease and its inhibitor in the septum formation in budding yeast ((14) is convincing. In the case of the tryptophan synthase inactivating system further evidence for its involvement in the regulation of cellular tryptophan syn-

thase activity needs to be furnished. Nevertheless it seems to us an interesting idea, that one and the same proteolytic enzyme may function in the regulation of cell metabolism as an activating principle by proteolyzing a preenzyme to an active enzyme, and as an inactivating principle by proteolyzing an active enzyme to inactive fragments. One and the same substance might then inhibit enzyme activation as well as inactivation.

Specific changes in the proteolytic activity in yeast batch culture and during the cell cycle have been described (15,16). Until recently, however, little attention was paid to interactions of yeast proteinases with substrates present in yeast cells. The physiologically conditioned appearance of the tryptophan synthase inactivating activity (5) and of the chitin synthase activating factor (1) should lend impetus to studies of factors controlling the synthesis, activation, and degradation of proteinases and their inhibitors, substrate specificity of the proteinases, influences on proteinase-inhibitor interactions. These examples of possible regulatory phenomena are to be considered in the light of compartmentation, found recently for the chitin synthase activating factor (14) as well as for the tryptophan synthase inactivating activity (17).

ACKNOWLEDGEMENTS

The technical assistance of Miss Hannelore Müller is appreciated. Preparations of tryptophan synthase inactivases I and II, and of the inhibitor were kindly donated by Dr.E.Schött and Dr.H.Betz, respectively. Dr.R.Wohlhueter assisted with preparation of the manuscript.

REFERENCES

1. Cabib, E., and Farkas, V., Proc.Nat.Acad.Sci.U.S.A. 68, 2052 (1971).
2. Cabib, E., and Keller, F.A., J.Biol.Chem. 246, 167 (1971).
3. Cabib, E., and Ulane, R., Biochem.Biophys.Res.Commun. 50, 186 (1973).

4. Manney, T.R., J.Bacteriol. 96, 403 (1968).
5. Katsunuma, T., Schött, E., Elsässer, S., and Holzer, H., Eur.J.Biochem. 27, 520 (1972).
6. Holzer, H., Katsunuma, T., Schött, E., Ferguson, A.R., Hasilik, A., and Betz, H., in: Weber, G. (ed.) Advances in Enzyme Regulation, Vol.11, in press, Pergamon Press, Oxford (1973).
7. Ferguson, A.R., Katsunuma, T., Betz, H., and Holzer, H., Eur.J.Biochem. 32, 444 (1973).
8. Schatz, G., and Kováč, L., in: Fleischer, S., Packer, L., and Estabrook, R.W. (eds.) Methods in Enzymology, in press, Acad.Press, New York (1973).
9. Cabib, E., and Keller, F.A., J.Biol.Chem. 246, 160 (1971).
10. Hata, T., Hayashi, R., and Doi, E., Agr.Biol.Chem. 31, 357 (1967).
11. Saheki, T., and Holzer, H., submitted to Eur.J.Biochem.
12. Hata, T., Hayashi, R., and Doi, E., Agr.Biol.Chem. 31, 150 (1967).
13. Doi, E., Hayashi, R., and Hata, T., Agr.Biol.Chem. 31, 160 (1967).
14. Cabib, E., and Ulane, R., J.Biol.Chem. 248, 1451 (1973).
15. Matile, Ph., Wiemken, A., and Guyer, W., Planta (Berlin) 96, 43 (1971).
16. Sylvén, B., Tobias, C.A., Malmgren, H., Ottoson, R., and Thorell, B., Exper.Cell.Res. 16, 75 (1959).
17. Hasilik, A., in preparation.