γ-GLUTAMYL TRANSPEPTIDASE IN HYDRA

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SUMMARY

γ-Glutamyl transpeptidase (EC 2.3.2.2) activity is described in the coelenterate, Hydra attenuata, using the substrate γ-glutamyl-p-nitroanilide. The properties of the γ-glutamyl donor required for binding to the transpeptidase were investigated by measuring the ability of GSH analogs to inhibit the release of p-nitroaniline. Whereas no binding was observed when the γ-glutamyl moiety was altered, analogs with substitution in the Cys residue were capable of binding to the enzyme. A specificity for the Gly residue was indicated because analogs containing Leu or Tyr in place of Gly exhibited decreased binding capacities for the hydra transpeptidase. A comparison of these data with those obtained using the same analogs in the GSH induced feeding response bioassay shows that γ-glutamyl transpeptidase activity and the GSH receptor for the hydra feeding response have different specificities.

INTRODUCTION

γ-Glutamyl transpeptidase (EC 2.3.2.2), thought to act on GSH in vivo (1), is described in a number of animal tissues (2-7). Studies on the specificity of the enzyme show that the glutamyl donor requires the γ-peptide linkage (3) but not the thiol group (7). In this paper we describe the presence of γ-glutamyl transpeptidase activity in extracts of the fresh water coelenterate Hydra attenuata. In order to examine the specificity of the hydra enzyme, we used a number of GSH analogs, some of which have not been previously described. We found that analogs in which Gly was substituted by Leu or Tyr exhibited decreased affinities for the enzyme relative to GSH. We used these analogs to distinguish between two GSH requiring activities in hydra, each of which requires the γ-glutamyl moiety but not the thiol group, i.e., γ-glutamyl transpeptidase and that activity responsible for initiating

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the GSH-induced feeding response (8). [After this work had been completed, γ-glutamyl transpeptidase was described in H. littoralis by Tate and Meister (9).]

MATERIALS AND METHODS

Laboratory reared specimens (10) of Hydra attenuata were not fed for 1-2 days and then placed in rifampicin [50 μg/ml in "M" solution (10)] for another 1-2 days. Next, the animals were washed 4 times with 500 ml portions of "M" solution and homogenized in 0.05 M phosphate, pH 6.5, using a glass homogenizer and teflon pestle. The homogenate was centrifuged at 1000 x g for 3 min and the resulting supernatant was dialyzed against the buffer for 18 hours at 4°.

γ-Glutamyl transpeptidase activity was assayed by the procedure of Orlowski and Meister (11). The one ml reaction mixture contained 2.5×10^{-3} M γ-glutamyl-p-nitroanilide in 0.1 M tris at pH 9.0 + 0.01 M $MgCl_2$. The reaction was initiated with 0.05-0.2 mg of protein extract (12). After 20 min at 38°, 0.9 ml of 1 N acetic acid was added followed by 0.1 ml 2% SDS. Release of p-nitroaniline was determined by measuring absorbance at 410 nm.

The data described in Fig. 1 were obtained by carrying out this same assay for γ-glutamyl transpeptidase in the presence of 5×10^{-4} M to 4×10^{-3} M of the specified tripeptides or GSSG, and with 0.02 M Met added as an acceptor.

The GSH analogs used for inhibition studies were synthesized as described elsewhere (manuscript in preparation). GSH and GSSG were obtained from Sigma (St. Louis, Mo.). γ-Glu-Met was purchased from Bachem (Marina Del Rey, Calif.) and γ-Glu-Leu from Vega-Fox (Tucson, Ariz.).

The feeding response bioassay was carried out according to the method of Lenhoff (13), except that the temperature was set at 28-29°.

RESULTS

Presence of γ-glutamyl transpeptidase activity: The specific activity (nmoles p-nitroaniline formed • min^{-1} • mg $protein^{-1}$) of the dialyzed supernatant in the absence of acceptor was 25 (N=4). Met, Leu and Gly-Gly, all known to function as acceptors of Glu from either GSH or γ-glutamyl-p-nitroanilide (5-7), were tested with the hydra extract. Table I shows that Gly-Gly, Met and Leu enhanced enzyme activity as they do with transpeptidases from other sources (5-7).

Abbreviations: SDS, sodium dodecylsulfate; GSMe, S-methylglutathione; Abu, α-amino-n-butyrate.

TABLE I

Effect of Acceptors on γ-Glutamyl Transpeptidase Activity

0.02 M Acceptor	Enhancement of Activity
Leu	1.2
Met	1.6
Gly-Gly	2.2

Enzyme activity was assayed as described in the text. Enhancement of activity is defined as the ratio of enzyme activity in the presence of added amino acid or peptide to that in its absence.

Thin layer chromatography (n-butanol/pyridine/water=1/1/1) of the reaction products formed in the absence of added acceptor amino acids revealed a spot which was UV absorbing, ninhydrin positive and corresponded to none of the starting materials or to the hydrolysis products Glu or p-nitroaniline. The addition of Leu or Met to the reaction reduced or eliminated this spot with the concomitant appearance of a new one. The spot formed in the absence of added acceptor was probably due to the acceptor activity of γ-glutamyl-p-nitroanilide (6). A comparison of the relative mobilities of the additional spots with those of γ-Glu-Met and γ-Glu-Leu markers indicated that they were γ-glutamyl transpeptidation products of Met and of Leu.

Effect of GSH analogs on the release of p-nitroaniline: A number of glutamate derivatives, GSH and S-substituted GSH analogs bind to γ-glutamyl transpeptidase (7, 14, 15). In order to determine the degree of binding of several new GSH analogs, we measured their ability to inhibit the release of p-nitroaniline from γ-glutamyl-p-nitroanilide. Several peptides previously studied with other transpeptidases were also investigated in this manner (see Materials and Methods). As shown in Fig. 1, GSH, GSSG,

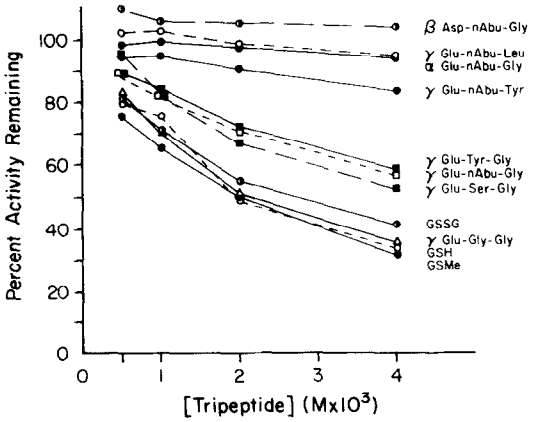

Fig. 1. Effect of GSH Tripeptide Analogs on the Release of p-Nitroaniline Catalyzed by γ-Glutamyl Transpeptidase Activity.

Reaction conditions are described under Materials and Methods. Percent activity remaining refers to the amount of p-nitroaniline released in the presence of a peptide compared to that amount released in its absence. With respect to GSSG, the concentration is expressed in terms of tripeptide units. (nAbu refers to α-amino-n-butyrate.)

γ-Glu-Gly-Gly, γ-Glu-Ser-Gly, γ-Glu-Abu-Gly, and γ-Glu-Tyr-Gly, peptides unaltered in the first and third positions, effectively inhibited (40% or more) the transpeptidase catalyzed release of p-nitroaniline; little or no inhibition (0-14%) of p-nitroaniline release was observed with peptides altered in the γ-glutamyl moiety (β-Asp-Abu-Gly and α-Glu-Abu-Gly), or in the glycyl residue (γ-Glu-Abu-Leu and γ-Glu-Abu-Tyr).

In order to eliminate the possibility that γ-Glu-Abu-Leu and γ-Glu-Abu-Tyr were also functioning as acceptors and therefore masking the inhibition of p=nitroaniline release, these peptides were tested in the presence of Gly-Gly, a better acceptor than Met. In the presence of 0.02 M Gly-Gly, 4×10^{-3} M γ-Glu-Abu-Leu inhibited p-nitroaniline release by 8%, whereas 4×10^{-3} M γ-Glu-Abu-Tyr inhibited 21% (compared to 14% inhibition with Met). Hence, these acceptor activities are not of sufficient magnitude to account for the inability of the analogs to inhibit the release of p-nitroaniline (see Fig. 1).

Effect of GSH analogs on the GSH-induced feeding response: There exists in hydra another GSH requiring system, the GSH-induced feeding response, known to have the unique specificity for GSH (8, 16, 17) later reported for the transpeptidase (7). In order to determine whether or not the specificity for activation of this behavioral response is the same as that shown in this paper for γ-glutamyl transpeptidase, the animals were exposed to four different tripeptides. GSH and γ-Glu-Abu-Gly, which activate feeding responses in H. littoralis (16, 17), were used as controls. In addition, γ-Glu-Abu-Gly served as a control for the other two tripeptides tested, γ-Glu-Abu-Leu and γ-Glu-Abu-Tyr, in which Abu was substituted for Cys, and Leu and Tyr substituted for Gly. We found that all four tripeptides initiated a feeding response at a minimum concentration of about 10^{-6} M. Furthermore, the response was maximal at about 10^{-5} M and did not increase with further increases in peptide concentration.

DISCUSSION

We describe the presence of γ-glutamyl transpeptidase activity in extracts of the coelenterate Hydra attenuata. Its specific activity was comparable to that found in homogenates of the housefly Musca domestica (5) and bovine choroid plexus (18), but was 3.3% that of the rat kidney homogenate (7) and 7.8% that of the rabbit choroid plexus extract (18). In addition, we now note the activity with γ-glutamyl-p-nitroanilide, when carried out in the presence of glycyl-glycine and when expressed in nmoles/hr/mg protein, is slightly higher than that recently reported in H. littoralis under similar conditions by Tate and Meister (9).

The characteristics of the γ-glutamyl tripeptide donor required for binding to the hydra transpeptidase can be deduced by an analysis of the data of Fig. 1. Analogs of GSH in which Cys was replaced by a number of amino acids [Cys(Me), Gly, Ser, Abu, Tyr] appeared to bind to the enzyme. Our results with GSMe and γ-Glu-Abu-Gly are consistent with those obtained

by Tate and Meister (7) who showed that these analogs are glutamyl donors with rat kidney transpeptidase. Whereas γ-Glu-Abu-Gly was able to bind to the hydra transpeptidase (Fig. 1), α-Glu-Abu-Gly (an analog of isoglutathione -- see also ref. 3) and β-Asp-Abu-Gly (an analog of asparthione) were not, indicating a requirement for the γ-glutamyl moiety in binding to the hydra γ-glutamyl transpeptidase. Our results with the tripeptide β-Asp-Abu-Gly are consistent with conclusions drawn from experiments on the enzyme from beef kidney (3) and from hog kidney (6) using β-Asp-Tyr and Asn, respectively.

The results of Fig. 1 also demonstrate that γ-glutamyl tripeptides with the Gly residue substituted by Leu or Tyr exhibited decreased binding capacities for the hydra enzyme. Such apparent specificity for the Gly residue of the γ-glutamyl tripeptide donor has hitherto not been reported for γ-glutamyl transpeptidases obtained from other sources.

The feeding response, on the other hand, we show is activated by the same tripeptides with Leu and Tyr substituted for the Gly residue. Thus, the use of these new analogs allows us to distinguish between these two activities in hydra, both requiring the intact γ-glutamyl moiety of GSH and not requiring the thiol group of GSH. Accordingly, it would appear that the γ-glutamyl transpeptidase is not involved in the initial activation of the GSH-induced feeding response in hydra.

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