γ-GLUTAMYL TRANSPEPTIDASE IN Hydra littoralis

Suresh S. Tate and Alton Meister

Department of Biochemistry, Cornell University Medical College

New York, New York 10021

Received March 22,1976

SUMMARY

Hydra littoralis exhibits high γ-glutamyl transpeptidase activity, i.e., about 12% of the activity (determined with glutathione) of rat kidney. Histochemical studies show that the enzyme is located mainly in the gastric and sub-hypostome regions; the enzyme is also present in the tentacles and basal disc. These results and the presence of other enzymes of the γ-glutamyl cycle suggest that the cycle plays a role in the metabolism of glutathione in hydras and that γ-glutamyl transpeptidase may function in their digestive and absorptive processes and possibly also in the behavioral response to glutathione.

The functions ascribed to the widely distributed tripeptide glutathione appear to depend on its sulfhydryl group or its γ-glutamyl moiety (1). The γ-glutamyl group of glutathione can be transferred to a number of amino acids (or peptides) in a reaction catalyzed by γ-glutamyl transpeptidase; this is the initial step in what appears to be the major quantitative pathway of glutathione metabolism. Further reactions involving the activities of γ-glutamyl cyclotransferase, 5-oxoprolinase, and dipeptidase result in the formation of glutamate, cysteine, and glycine, which can be reutilized for the biosynthesis of glutathione catalyzed by the actions of γ-glutamylcysteine and glutathione synthetases. Thus, the glutathione-degrading and glutathione-synthesizing enzymes catalyze a cyclical process which has been called the γ-glutamyl cycle (1-4). It has been proposed that γ-glutamyl transpeptidase, which is membrane-bound in all mammalian tissues studied, functions in transepithelial transport of amino acids and that the other enzymes of the γ-glutamyl cycle function to release the amino acid from the γ-glutamyl carrier and to regenerate glutathione. The histochemical finding of γ-glutamyl transpeptidase activity in certain mammalian central nervous system neurons (e.g.,

Purkinje cells, anterior horn cells) has led to the suggestion that the enzyme may play a role in intracellular amino acid or amine transport in neurons (5,6).

Glutathione plays an interesting role in the coelenterates calledhydras; it activates the feeding response and either affects or elicits a variety of other behaviorial responses. Loomis (7) obtained evidence that glutathione is the specific chemical stimulator that evokes the feeding response; glutathione disulfide was found to be inactive. Subsequently, Cliffe and Waley (8) found that the activity of glutathione does not require the sulfhydryl group of this molecule; thus, norophthalmic acid (in which the sulfhydryl group is replaced by a hydrogen atom), and ophthalmic acid (in which the sulfhydryl group is replaced by a methyl group), are also very efficient activators of the feeding response. S-Methylglutathione also activates the feeding response (9); these and other studies have been reviewed by Lenhoff (10). Glutathione also affects hydras in other ways; it increases the rate of tentacle waving concerts and inhibits column and body contractions induced by light and mechanical stimulation (10,11).

The remarkable effect of glutathione (and of glutathione analogs that do not have a sulfhydryl group) on the behavior of hydras raises questions about the possible role of the γ-glutamyl cycle enzymes in these responses and also about the metabolic fate of glutathione in this organism. This report is concerned with γ-glutamyl transpeptidase which is active toward glutathione, ophthalmic acid, norophthalmic acid, and other γ-glutamyl compounds, but which is only slightly active toward glutathione disulfide (12, 13).

MATERIALS AND METHODS

Hydra littoralis (obtained from Carolina Biological Supply Co., Burlington, N.C.), were transferred to a medium containing 1 mM imidazole-HCl (pH 6.2), 1 mM $CaCl_2$, and 1 mM NaCl and starved for 24 hrs. These hydras showed the typical feeding reflex upon addition of glutathione to the medium. About 200 hydras were suspended in 1 ml of 0.05 M Tris-HCl buffer (pH 8) and homogenized in a Potter-Elvehjem homogenizer equipped with a Teflon pestle. The homogenates were assayed for enzyme activity as follows. γ-Glutamyl transpeptidase activity was determined with glycylglycine (50 mM) and four γ-glutamyl donors (glutathione (10 mM), S-acetophenone-glutathione

Table I

Activities of γ-Glutamyl Transpeptidase and Other γ-Glutamyl Cycle Enzymes in Hydra littoralis

Enzyme		Activity (nmoles/hr/mg of protein)	
γ-Glutamyl transpeptidase:			
Donor:	Glutathione	7,700	(66,000)
	S-Acetyl-glutathione	6,300	(73,800)
	S-Acetophenone-glutathione	6,050	(93,500)
	L-γ-Glutamyl-p-nitroanilide	3,050	(150,000)
γ-Glutamyl cyclotransferase		142	
Dipeptidase		9,100	
γ-Glutamylcysteine synthetase		25	

γ-Glutamyl transpeptidase activity was determined in the presence of the donors shown and glycylglycine. The values in parentheses are the corresponding activities found with rat kidney homogenate.

(5 mM), L-γ-glutamyl-p-nitroanilide (2.5 mM), and S-acetyl-glutathione (5 mM) as described (12,13). γ-Glutamyl cyclotransferase was assayed using L-γ-[U-^{14}C]-glutamyl-L-α-aminobutyrate (10 mM) as substrate (14), and γ-glutamylcysteine synthetase activity was determined as described (15). Dipeptidase was assayed using L-alanylglycine as substrate (5).

For histochemical localization of γ-glutamyl transpeptidase, the hydras were immobilized on a moist Millipore membrane filter as described by Lenhoff (16). Small pieces of the filter containing the immobilized hydras were placed on a glass slide and treated with acetone (at 0°) until the filter dissolved leaving the hydras affixed to the slide, which was then immersed in freshly prepared substrate solution (5) containing 1 mM L-γ-glutamyl-α-naphthylamide, 20 mM glycylglycine, and 0.05% o-aminoazotoluene diazonium salt (fast garnet GBC salt). After incubation for 10 min. at 37°, the slide was immersed in 0.1 M $CuSO_4$ for 2 min., rinsed with water, and dried in air.

RESULTS

Homogenates of hydras exhibited high levels of γ-glutamyl transpeptidase activity (Table I). The values for glutathione and γ-glutamyl p-nitroanilide are, respect-

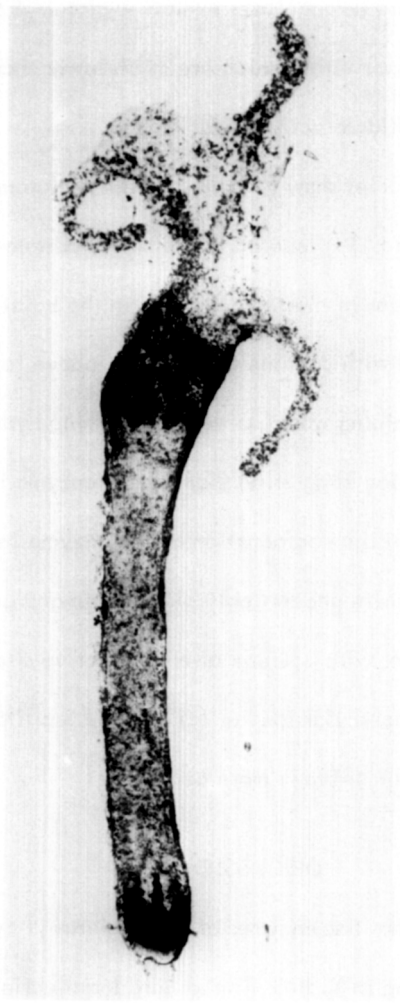

Figure 1. Histochemical localization of γ-glutamyl transpeptidase in Hydra littoralis. The dark areas represent enzyme activity.

ively, about 12% and 2% of the corresponding activities found in kidney homogenates (12). It may be significant that γ-glutamyl-p-nitroanilide is about 40% as active as glutathione with the hydra enzyme, while the rat kidney enzyme is about twice as active with γ-glutamyl p-nitroanilide as with glutathione; the rat kidney enzyme is also more active with S-acetophenone-glutathione than with glutathione. These findings suggest that the active site of hydra γ-glutamyl transpeptidase is more specific for glutathione than those of kidney and the other mammalian γ-glutamyl transpeptidases that

have been studied. The hydra homogenates exhibit γ-glutamyl cysteine synthetase and γ-glutamyl cyclotransferase activities which are much lower than those of transpeptidase. Relatively high dipeptidase activity was found.

The histochemical studies show that the highest γ-glutamyl transpeptidase activity (the dark areas in Figure 1) is located in the sub-hypostome and the upper gastric regions; activity is also present in the tentacles and in the basal disc. We found that the hydras had to be treated with acetone as described above to obtain a histochemical reaction. No significant staining was observed when living hydras were suspended in the histochemical substrate solution suggesting that (a) the enzyme is not located on the outer surface of the cells, (b) the conformation of the enzyme in the intact cell membrane is such that the active site accepts only glutathione and certain closely related derivatives and that treatment with acetone alters the active site so as to facilitate interaction with γ-glutamyl-α-naphthylamide, or (c) acetone facilitates penetration of the substrates by making the cell membrane more permeable.

DISCUSSION

The results suggest that the enzymes of the γ-glutamyl cycle may play a role in the metabolism of glutathione in hydra. The intense localization of the enzyme in the gastric region suggests a role in absorption of amino acids and peptides (1,2,4). It would be of interest to determine whether the enzyme is localized in the cells lining the gastric cavity. Such a localization would be analogous to that found in the housefly where the major site of enzyme localization is in the striated border of the epithelial cells of the mid gut (17). Similarly, in mammals high activity is located in the brush border of the jejunal epithelium (5,18,19). The finding of transpeptidase activity in the tentacles of hydra is consistent with a function in the behavioral response to glutathione. Lenhoff (20) found that the dissected head area responds to glutathione suggesting that this region has glutathione receptors involved in mediation of the feeding re-

sponse. Interaction of external glutathione with membrane-bound γ-glutamyl transpeptidase in this region might trigger the feeding response, possibly by leading to the formation of a γ-glutamyl derivative which functions as a specific neurotransmitter. The enzyme might also funation to inactivate glutathione, which could exert its neurohormonal effect in another way. Detailed studies on the cellular localization of the transpeptidase and on the metabolic fate of glutathione should provide further insight into the mechanism by which this tripeptide functions in hydra.

Supported in part by the National Science Foundation.

REFERENCES

1. For a recent review see Meister, A., in Metabolism of Sulfur Compounds, (Greenberg, D.M., ed) (Academic Press, New York, 1975), pp. 101-188.
2. Orlowski, M., and Meister, A., Proc. Natl. Acad. Sci. U. S., 67, 1248-1255 (1970).
3. Van Der Werf, P., Orlowski, M., and Meister, A., Proc. Natl. Acad. Sci. U.S., 68, 2982-2985 (1971).
4. Meister, A., Science 180, 33-39 (1973).
5. Ross, L.L., Barber, L., Tate, S.S., and Meister, A., Proc. Natl. Acad. Sci. U.S., 70, 2211-2214 (1973).
6. Meister, A., Research Publications of the Association for Research in Nervous and Mental Disease, 53, 273-291 (1974); Raven Press, New York, New York.
7. Loomis, W.F., Ann. N.Y. Acad. Sci., 62, 211-227 (1955).
8. Cliffe, E.E., and Waley, S.G., Nature, 182, 804-805 (1958).
9. Lenhoff, H.M., and Bovaird, J., Nature, 189, 486-487 (1961).
10. Lenhoff, H.M., Science, 161, 434-442 (1968).
11. Rushforth, N.B., in Biology of Hydra, (Burnett, A.L., ed.), pp. 3-41 (1973); Academic Press, New York, New York.
12. Tate, S.S., and Meister, A., J. Biol. Chem., 249, 7593-7602 (1974).
13. Tate, S.S., FEBS Lett., 54, 319-322 (1975).
14. Orlowski, M., Richman, P.G., and Meister, A., Biochemistry, 8, 1048-1055 (1969).
15. Orlowski, M., and Meister, A., Biochemistry, 10, 372-380 (1971).
16. Lenhoff, H.M., Exp. Cell Res., 17, 570-573 (1959).
17. Bodnaryk, R.P., Bronskill, J.F., and Fetterly, Jr. J. Insect. Physiol., 20, 167-181 (1974).
18. Greenberg, E., Wollaeger, E.E., Fleisher, G.A., and Engstrom, G.W., Clin. Chim. Acta, 16, 79-89 (1967).
19. Cohen, M.I., Gartner, L.M., Blumenfeld, O.O., and Arias, I.M., Pediat. Res., 3, 5-10 (1969).
20. Lenhoff, H.M., J. Gen. Physiol., 45, 331-344 (1961).