INTERACTION OF γ-GLUTAMYL TRANSPEPTIDASE WITH *S*-ACYL DERIVATIVES OF GLUTATHIONE

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1. Introduction

Membrane-bound γ-glutamyl transpeptidase catalyzes transfer of the γ-glutamyl moiety of glutathione (and a variety of other γ-glutamyl compounds) to an amino acid or peptide acceptor. This enzyme is of considerable interest in relation to its proposed roles in mercapturic acid synthesis [1], in amino acid transport [2–4], and in renal ammoniagenesis [5,6]. Previous studies in this laboratory on purified rat kidney transpeptidase concerned its γ-glutamyl donor and amino acid and peptide acceptor specificity [7,8]. This communication deals with the interaction of the enzyme with *S*-acyl derivatives of glutathione. These compounds are excellent substrates; the *S*-acyl-cysteinyl-glycine products rearrange rapidly to the corresponding *N*-acyl derivatives. Determination of the exposed sulfhydryl groups provides a convenient assay procedure. The reaction also raises the possibility that metabolism of *S*-acyl-glutathione derivatives may involve transpeptidase-mediated breakdown.

2. Materials and methods

Glutathione, methyl-glyoxal, and glyoxalase 1 (yeast) were purchased from Sigma Chemical Co.; L-[methyl-^{14}C] methionine was obtained from New England Nuclear Corp. *S*-Acetophenone-glutathione was prepared as described [7]. Rat kidney γ-glutamyl transpeptidase (enzyme III) was purified as described [9].

S-Lactoyl-glutathione was prepared enzymically using glyoxalase 1 and methylglyoxal [10] essentially as described by Uotila [11]. *S*-Acetyl- and *S*-benzoyl-glutathione were made from glutathione and thiolacetic acid and thiobenzoic acid, respectively [11,12].

γ-Glutamyl transpeptidase assays using glutathione and *S*-acetophenone-glutathione were performed as described previously [7]. The activity with *S*-acyl-glutathiones was measured as follows: reaction mixture (1 ml) contained 0.1 M Tris (pH 7.5 with phosphoric acid), 1 mM *S*-acyl-glutathione, 20 mM glycyl-glycine (or other acceptor), 1 mM 5,5′-dithiobis (2-nitro benzoate) (DTNB; [13]). The increase in absorbance at 412 nm (due to thionitrobenzoate) was recorded at 37°C with a Cary Model 15 spectrophotometer. One unit of enzyme is defined as the amount which releases 1 μmol of product per min.

3. Results

The activity of transpeptidase towards glutathione and its *S*-derivatives and [^{14}C]-methionine is shown in table 1. The *S*-substrated glutathiones were more active than glutathione itself. Since thioesters of glutathione are unstable at pH values above 7 [14], the extent of hydrolysis of the *S*-acyl-glutathiones during transpeptidase assay was monitored by estimating the free sulfhydryl group so generated with DTNB. Results with *S*-acetyl-glutathione are shown in fig.1. Low rates of hydrolysis are observed in absence of enzyme (expt. 1) and with enzyme without added acceptor (expt. 2). In the presence of glycyl-glycine, an excellent acceptor, transpeptidase catalyzed a rapid release of free sulfhydryl groups (expt. 3); glycyl-glycine alone did not significantly alter the

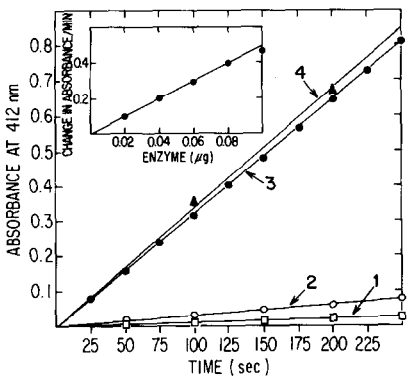

Fig.1. Release of free sulfhydryl groups from *S*-acetyl-glutathione. Expt. 1, *S*-acetyl-glutathione and DTNB; Expt. 2, as 1 plus enzyme (0.04 μg); Expt. 3, as 2 plus glycylglycine; Expt. 4 was similar to expt. 3 except that DTNB was omitted; the reactions were terminated with 0.1 ml of 2 N acetic acid. Then 0.4 ml of 0.5 M K-phosphate buffer (pH 6.8) was added, followed by 0.1 ml of 0.01 M DTNB and the absorbance at 412 nm determined. The inset shows the effect of enzyme concentration; assay solutions were similar to expt. 3.

non-enzymatic rate. The inset (fig.1) shows the dependence of the rate of release of sulfhydryls on enzyme concentration. Similar results were obtained with either *S*-lactoyl- or *S*-benzoyl-glutathione. DTNB itself does not influence the enzymatic release of free sulfhydryls as evidenced by results of expt. 4 (fig.1); also, preincubation of the enzyme with DTNB did not affect its activity.

The enzymatic rates of release of free sulfhydryls from *S*-acyl-glutathiones in the presence of glycyl-glycine and L-methionine are given in table 1. Studies with other amino acid and peptide acceptors showed that the relative rate of release of free sulfhydryls, in general, matched the specificity of transpeptidase towards these acceptors with either glutathione or *S*-acetophenone-glutathione [7]. Thus, the most active amino acids were L-glutamine and L-methionine; D-amino acids and L-proline were inactive. Glycyl-glycine was the most active dipeptide followed closely by L-alanyl-glycine, glycyl-L-alanine, L-α-aminobutyryl-glycine, and L-methionyl-glycine; glycyl-D-alanine and L-alanyl-L-alanine were poor, in agreement with earlier findings that these peptides were also poor acceptors of the γ-glutamyl moiety [7]. Examination of the data in table 1 indicate that the rate of γ-gluta-

Table 1
Activity of γ-glutamyl transpeptidase toward glutathione and its *S*-derivatives

γ-Glutamyl donor	Transpeptidase activity[a] (units/mg)			
	Acceptor			
	None	Glycylglycine	L-Methionine	
Glutathione	38	350	126	(115)[b]
S-Acetophenone-glutathione	5	547	193	(208)
S-Acetyl-glutathione	25	391	153	(168)
S-Lactoyl-glutathione	24	375	109	(120)
S-Benzoyl-glutathione	16	376	128	(142)

[a] The γ-glutamyl substrate was 1 mM and the acceptor, 20 mM (pH 7.5). The activities with *S*-acyl-derivatives are expressed in terms of μmoles of free sulfhydryl compounds produced (determined with DTNB).

[b] The values in parentheses are for experiments in which L-[^{14}C] methionine was used and formation of γ-glutamyl-methionine determined.

Table 2
Identification of the cysteinyl-glycine derivative produced by the action of γ-glutamyl transpeptidase on *S*-lactoyl-glutathione

Experiment[a]	Free sulfhydryl groups[b] (nmoles)		Amino acid composition of product eluted from Dowex 50 H^+ columns[c] (nmoles)		
	In reaction mixture	In eluates from Dowex 50 H^+ columns	Glutamate	Cysteic acid	Glycine
1. *S*-Lactoyl-glutathione	14	0	0	0	0
2. *S*-Lactoyl-glutathione + enzyme	40	30	0	25	28
3. *S*-Lactoyl-glutathione + glycyl-glycine + enzyme	350	342	0	307	321
4. Glutathione + glycyl-glycine + enzyme	980	0	0	0	0

[a] The reaction mixtures (1 ml) contained 1 mM *S*-lactoyl-glutathione (or glutathione) and, where indicated, 20 mM glycyl-glycine and 0.2 μg of enzyme. Following incubation at 37°C for 5 min, the reactions were terminated by the addition of 0.1 ml of 2 N acetic acid.

[b] Aliquots (0.1 ml) of the above solutions were taken for the determination of free sulfhydryl groups (with DTNB). Aliquots (0.8 ml) were also applied to Dowex 50 H^+ columns (0.5 × 2 cm) and the columns eluted with 2.2 ml of water. Portions were taken for sulfhydryl determinations and the remaining solutions lyophilized to dryness.

[c] The lyophilized residue was oxidized with performic acid (20), evaporated to dryness and hydrolyzed with 6 N HCl for 24 hr at 110°C. The hydrolyzates were analyzed for the constituent amino acids with a Durrum model D500 amino acid analyzer.

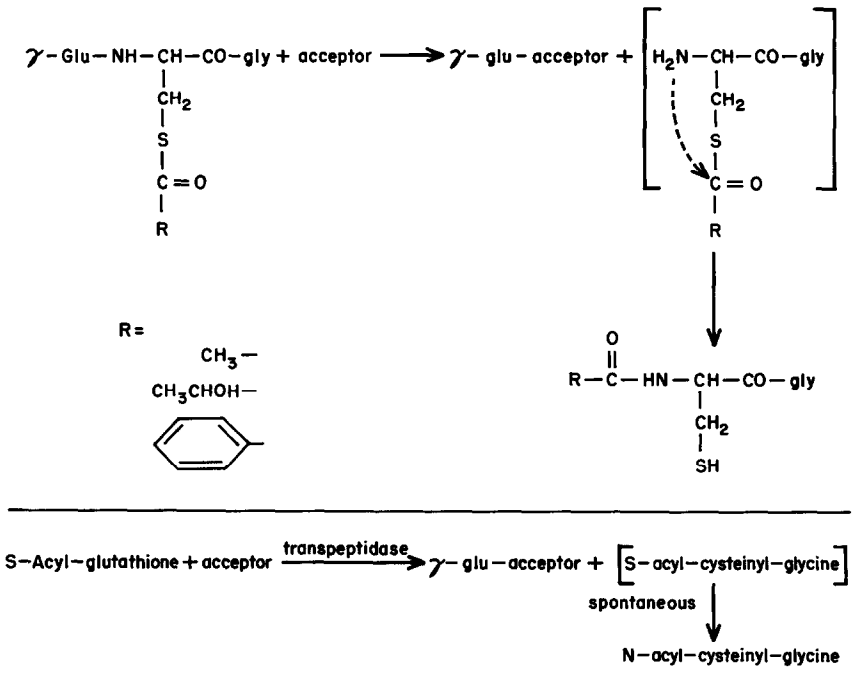

Fig.2. Action of γ-glutamyl transpeptidase on *S*-acyl-glutathione derivatives.

myl-[^{14}C] methionine formation in presence of [^{14}C] methionine and *S*-acyl-glutathiones, a reflection of the extent of transpeptidation, corresponds closely to the rate of release of free sulfhydryls. Since an equivalent amount of γ-glutamyl-methionine and thioesters of cysteinyl-glycine must be produced, it follows that the latter either hydrolyze rapidly to cysteinyl-glycine or spontaneously undergo transacylation to *N*-acyl-cysteinyl-glycine derivatives to account for the stoichiometry between transpeptidation products and free sulfhydryls produced. Rapid transacylation (S → N transfer) in *S*-acyl derivatives of 2-mercapto-ethylamine and cysteine have been observed by many workers [15,16; also see 17].

Products of hydrolysis (cysteinyl-glycine) and S → N transfer (*N*-acyl-peptide) are readily separated on a column of Dowex 50 H^+ since the former would adsorb to the cation-exchange resin whereas the latter would not. Results shown in table 2 demonstrate that the action of transpeptidase on *S*-lactoyl-glutathione leads to formation of a cysteinyl-glycine derivative with blocked α-amino group (presumably *N*-lactoyl-cysteinyl-glycine). Thus, the sulfhydryl compounds produced in experiments 2 and 3 did not bind to Dowex 50 H^+ and yielded equimolar amounts of cystedic acid and glycine upon oxidation followed by acid hydrolysis. In experiment 4 where glutathione was used, no sulfhydryl compounds emerged from Dowex 50 H^+ column. Results similar to experiment 3 were obtained when *S*-acetyl-glutathione was used.

4. Discussion

The results are consistent with reaction mechanism shown in fig.2 in which the *S*-acyl-cysteinyl-glycine products are rapidly and quantitatively converted to *N*-acyl-cysteinyl-glycine derivatives. It is possible that orientation of *S*-acyl-dipeptide in the enzyme's active site is such that the rapid S → N transfer is facilitated. The reaction provides a sensitive colorimetric technique for the assay of enzyme activity. Furthermore, transpeptidase may function in the metabolism of *S*-acyl-glutathiones; the latter are products of reactions catalyzed by glyoxalase 1 [10,18] and glutathione-dependent aldehyde dehydrogenases [19]. However, whether *S*-acyl-glutathiones are significant metabolites in vivo is uncertain. It is of interest also that glyoxalase II and other glutathione thiolesterases are widely distributed in animal tissues [see 18 and 11].

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References

[1] Boyland, E. and Chasseaud, L. F. (1969) Advan. Enzymol. 32, 173–219.
[2] Orlowski, M. and Meister, A. (1970) Proc. Natl. Acad. Sci. USA 67, 1248–1255.
[3] Meister, A. (1973) Science 180, 33–39.
[4] Meister, A. (1974) Life Sci. 15, 177–190.
[5] Tate, S. S. and Meister, A. (1974) Proc. Natl. Acad. Sci. USA 71, 3329–3333.
[6] Curthoys, N. P. and Kuhlenschmidt, T. (1975) J. Biol. Chem. 250, 2099–2105.
[7] Tate, S. S. and Meister, A. (1974) J. Biol. Chem. 249, 7593–7602.
[8] Tate, S. S. (1975) in: Isozymes (II): Physiology and Function (Markert, C. L., ed.), pp. 743–765, Academic Press, New York.
[9] Tate, S. S. and Meister, A. (1975) J. Biol. Chem. 250, in press.
[10] Racker, E. (1951) J. Biol. Chem. 190, 685–696.
[11] Uotila, L. (1973) Biochemistry 12, 3938–3943.
[12] Kielley, W. W. and Bradley, L. B. (1954) J. Biol. Chem. 206, 327–333.
[13] Ellman, G. L. (1959) Arch. Biochem. Biophys. 82, 70–77.
[14] Vignais, P. V. and Zabin, I. (1958) Biochim. Biophys. Acta 29, 263–269.
[15] Wieland, T. and Homig, H. (1956) Ann. Chem. 600, 12.
[16] Barnett, R. and Jencks, W. P. (1963) J. Am. Chem. Soc. 90, 4199–4200.
[17] Friedman, M. (1973) The Chemistry and Biochemistry of Sulfhydryl Group in Amino Acids, Peptides and Proteins, pp. 183–191, Pergamon Press, New York.
[18] Jocelyn, P. C. (1972) Biochemistry of the SH Group, pp. 207–211, Academic Press, New York.
[19] Uotila, L. and Koivusalo, M. (1974) J. Biol. Chem. 249, 7653–7663.
[20] Moore, S. (1973) J. Biol. Chem. 238, 235–337.