J. Enzyme Inhibition, 1989, Vol. 3, pp. 77–80
Reprints available directly from the publisher
Photocopying permitted by license only

INACTIVATION OF BOVINE LIVER GLUTATHIONE *S*-TRANSFERASE BY SPECIFIC MODIFICATION OF ARGININE RESIDUES WITH PHENYLGLYOXAL

KAZUO ASAOKA

Department of Biochemistry, Primate Research Institute, Kyoto University, Inuyama, Aichi 484

and

KENJI TAKAHASHI*

Department of Biophysics and Biochemistry, Faculty of Science, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

(Received 8 November, 1988)

KEY WORDS: Glutathione *S*-transferase, modification, inactivation, arginine residues, phenylglyoxal.

INTRODUCTION

To date a number of glutathione *S*-transferases have been purified from various sources and their properties investigated[1]. However, little information is available in regard to the amino acid residues involved in catalysis or specific binding of substrates or reduced glutathione as a cosubstrate. Previously, we purified glutathione *S*-transferase from monkey liver[2] and investigated the effects of various chemical modification reagents on the activity[3]. In that study, we observed a rapid inactivation of the enzyme by treatment with phenylglyoxal, an arginine-specific reagent[4–6]. This led us to speculate that the inactivation might be due to modification of an arginine residue(s) at the glutathione-binding site, essential for specific binding of the carboxyl group(s) of reduced glutathione. The correlation of the extents of reaction of phenylglyoxal and enzyme inactivation, however, remained to be elucidated. Meanwhile, we also purified glutathione *S*-transferase from bovine liver[7]. The bovine liver enzyme was similar to the monkey enzyme in various properties, but was thought to be more suitable for study on the structure-function relationship of a glutathione *S*-transferase since the bovine enzyme can be prepared in much larger quantities. As a first step, we have examined the effect of phenylglyoxal on the activity of the bovine enzyme using the ^{14}C-labeled reagent. The results obtained suggest that the bovine enzyme has a specific arginine residue at the active site that presumably binds to one of the carboxyl groups of reduced glutathione.

* Correspondence.

MATERIALS AND METHODS

Bovine liver glutathione *S*-transferase was purified to homogeneity as described previously[2] and the isozyme II was used in the present study. Phenylglyoxal hydrate was obtained from K and K Laboratories, New York, and [^{14}C]phenylglyoxal (5900 cpm/mol) was prepared as described previously[4]. *o*-Dinitrobenzene, sulfanilamide, and *N*-(1-naphthyl)ethylenediamine dihydrochloride were purchased from Wako Pure Chem. Ind., Tokyo. Reduced glutathione was obtained from Boehringer, Mannheim, 1-chloro-2,4-dinitrobenzene from Tokyo Kasei Kogyo Co., Tokyo, and bromosulfophthalein from Daiichi Pure Chem. Co., Tokyo.

To react phenylglyoxal with the enzyme, a 3 μM enzyme solution was treated with 7.5 mM phenylglyoxal in 0.07 M N-ethylmorpholine acetate buffer, pH 8.0, at 37°C. Aliquots were removed at appropriate intervals and the reaction was stopped by the addition of arginine as an aqueous solution to a final concentration of 60 mM. Part of the reaction mixture was used for the assay of the remaining activity. The enzyme activity was determined at pH 7.0 and 37°C with *o*-dinitrobenzene as a substrate as described previously[8]. The reaction was also performed under the same conditions using [^{14}C]phenylglyoxal. The reaction mixture was passed through a column (1.5 × 70 cm) of Sephadex G-25 equilibrated and eluted with 0.01 M acetic acid and part of the pooled protein fraction was used for radioactivity counting and amino acid analysis. The molecular weight of the modified enzyme was estimated by passage through a column (1.6 × 100 cm) of Sephadex G-150 equilibrated and eluted with 0.01 M *N*-ethylmorpholine acetate buffer, pH 8.0, containing 0.1 M NaCl.

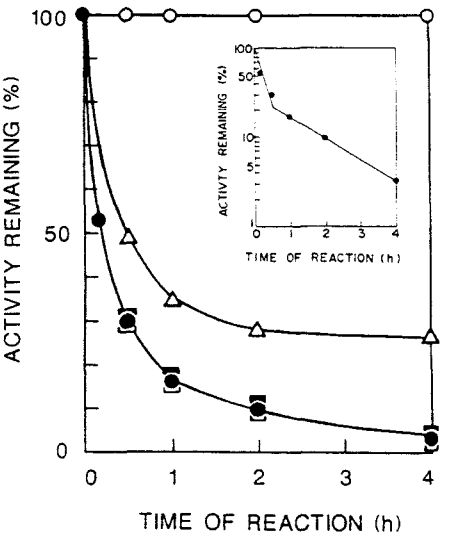

FIGURE 1 Rates of inactivation of glutathione *S*-transferase by reaction with phenylglyoxal in the presence and absence of substrates. The enzyme (3 μM) was treated with phenylglyoxal (7.5 mM) at pH 8.0 and 37°C. o, control (no phenylglyoxal);●, no substrate;△, reduced glutathione (1 mM),□, 1-chloro-2,4-dinitrobenzene (1 mM);■, bromosulfophthalein (0.1 mM). Activity was measured with *o*-dinitrobenzene as a substrate. Inset: a semilogarithmic plot of the rate of inactivation of the enzyme by reaction with phenylglyoxal.

RESULTS AND DISCUSSION

As can be seen from Figure 1, the enzyme was rapidly inactivated by reaction with phenylglyoxal. Under the conditions used, the half life of the activity was about 15 min and nearly 90% inactivation occurred in 2 h. This profile of inactivation is similar to that obtained with the monkey enzyme[3]. The inactivation appears to be biphasic (Figure 1, inset), and the first, rapid inactivation is thought to be due to specific modification of a critical residue at or near the active site of the enzyme. The rate of inactivation was markedly slowed down by the presence of 1 mM reduced glutathione, whereas no protective effect was observed in the presence of 1 mM 1-chloro-2,4-dinitrobenzene or 0.1 mM bromosulfophthalein (Figure 1). The latter two compounds are good substrates of the enzyme and are thought to bind to the substrate (*o*-dinitrobenzene) binding site as well. These results thus seem to indicate that the residue(s) modified is presumably located at or near the binding site for reduced glutathione rather than at the substrate binding site of the enzyme.

In order to obtain further information about the residue(s) modified by phenylglyoxal, the correlation between the extent of inactivation and the number of phenylglyoxal molecule introduced into the protein was investigated. As shown in Figure 2, the loss of activity occurred in parallel with the reaction of phenylglyoxal, and the extrapolation of the results in the first rapid phase to complete inactivation showed the incorporation of 4.1 mol of the reagent per mol of enzyme. Since it is known that one arginine residue reacts with two phenylglyoxal molecules[4], this incorporation corresponds to the modification of two arginine residues per molecule of protein. The bovine enzyme was shown to be composed of two identical or nearly identical subunits[7], so that the modification of one arginine residue per subunit had occurred. The results of amino acid analyses of the native and the modified enzymes indicated that no amino acid residue other than arginine had been significantly modified although the loss of arginine residue could not be determined correctly due to a rather

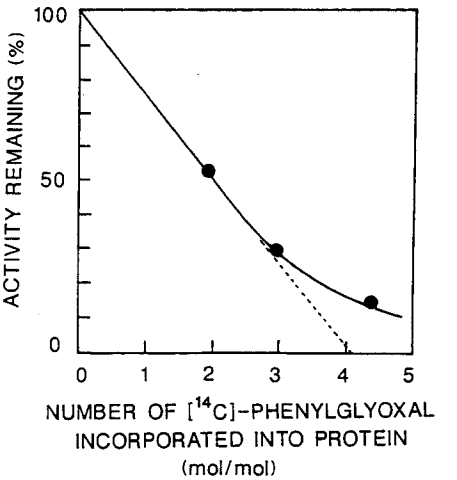

FIGURE 2 Correlation between the extents of [^{14}C]-phenylglyoxal incorporation and inactivation of glutathione *S*-transferase.

high content of this residue (25 residues per molecule of protein[7]) (data not shown). The thiol group which is potentially reactive with phenylglyoxal was not analyzed. The bovine enzyme contains two half-cystine residues per molecule of protein[7], possibly as two cysteine residues, as in the case of the monkey enzyme[3], and the thiol reagents were generally without much effect on the activity of the monkey enzyme[3]. Therefore, presumably there may be no critical thiol group in the bovine enzyme either. Further, the bovine enzyme has no α-NH_2 group which is potentially reactive with the reagent since its NH_2-terminal residue is proline[5,7]. The modified enzyme showed a molecular weight of about 49,000 as examined by gel filtration. This value is essentially the same as that of the native enzyme[7], indicating that the modification did not affect the dimeric structure of the original enzyme.

Taken together, these results seem to show that phenylglyoxal reacted specifically with a critical arginine residue, presumably at the glutathione binding site, of each subunit. It is tempting to assume that this arginine residue specifically binds one of the negatively charged carboxyl group(s) of reduced glutathione and so orients the glutathione molecule to participate in the catalytic reaction. A similar result was reported[9] for yeast glyoxalase I, suggesting that a specific arginine residue may be involved in the binding of reduced glutathione to the enzyme. Further studies are necessary, however, to get a definite conclusion on the role of arginine residue(s) in glutathione *S*-transferases.

Acknowledgements

This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

References

1. Jakoby, W.B. (1985) *Methods Enzymol.*, **113**, 495.
2. Asaoka, K. Ito, H. and Takahashi, K. (1977) *J. Biochem.*, **82**, 973.
3. Asaoka, K. and Takahashi, K. (1977) *J. Biochem.*, **82**, 1313.
4. Takahashi, K.(1968)*J. Biol.Chem.*, **243**, 6171.
5. Takahashi, K. (1977) *J. Biochem.* **81**, 395.
6. Takahashi, K. (1977) *J. Biochem.* **81**, 403.
7. Asaoka, K. (1984) *J. Biochem.* **95**, 685.
8. Asaoka K. and Takahashi, K. (1983) *J. Biochem.* **94**, 1685.
9. Schasteen C.S. and Reed, D.J. (1983) *Biochim. Biophys. Acta*, **742**, 419.