Vol. 87, No. 3, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 13, 1979 Pages 657-663

ACTIVATION OF MICROSOMAL GLUTATHIONE S-TRANSFERASE ACTIVITY BY SULFHYDRYL REAGENTS

Ralf Morgenstern, Joseph W. DePierre, and Lars Ernster

Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

Received February 7, 1979

SUMMARY

Rat liver microsomes exhibit glutathione S-transferase activity with 1-chloro-2,4-dinitrobenzene as the second substrate. This activity can be stimulated 8-fold by treatment of the microsomes with N-ethylmaleimide and 4-fold with iodoacetamide. The corresponding glutathione S-transferase activity of the supernatant fraction is not affected by such treatment. These findings suggest that rat liver microsomes contain glutathione S-transferase distinct from those found in the cytoplasm; and that the microsomal transferase can be activated by modification of microsomal sulfhydryl group(s).

INTRODUCTION

Glutathione S-transferases (E.C.2.5.1.18) play an important role in the biotransformation of many xenobiotics, including pharmacologically active compounds and, especially, potential alkylating agents (1). These enzymes have broad substrate specificities; but most substrates resemble one another in being both electrophilic and hydrophobic. Even though the occurrence of microsomal glutathione S-transferase activity has been reported (2,3), most studies to date have concentrated on the cytoplasmic enzymes (for a recent review see 4). In our efforts to demonstrate that the microsomal glutathione S-transferase activity is not due to cytoplasmic contamination, we discovered that the microsomal activity towards 1-chloro-2,4-dinitrobenzene (CDNB) can be stimulated greatly by treatment of the microsomes with sulfhydryl reagents; whereas the corresponding activity of the supernatant fraction is not affected by such treatment.

Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; NEM, N-ethylmaleimide; IAA, iodoacetamide; DTNB, 5,5'-dithiobis-(2-nitrobenzoic acid).

0006-291X/79/070657-07$01.00/0

MATERIALS AND METHODS

Glutathione, N-ethylmaleimide (NEM), and iodoacetamide (IAA) were obtained from the Sigma Chemical Co. All other chemicals were of reagent grade and purchased from common commercial sources.

Liver microsomes and the supernatant fraction from 180-200 g male Sprague-Dawley rats that had been starved overnight were prepared as described previously (5), with the minor modification that the microsomes were washed twice with 0.15 M Tris-Cl, pH 8, in order to remove contaminating cytoplasm (additional washes had no effect on the microsomal glutathione S-transferase activity). All experiments were performed on material prepared the same day.

Treatment with NEM and IAA was carried out as follows: 1.5-2.2 mg protein of the microsomal or supernatant fractions was incubated at room temperature in 90 (or 80) mM sodium phosphate, pH 7.5-25 (or 12.5) mM sucrose in a total volume of 1 ml and with IAA (or NEM) at the concentrations indicated in the tables. Before treatment with NEM the supernatant fraction was passed through a Sephadex G-25 M column equilibrated with 0.25 M sucrose in order to remove low molecular weight thiols (chiefly glutathione). Aliquots were withdrawn at the times indicated and assayed for glutathione S-transferase activity with CDNB as described by Habig et al. (6), except that 3 mM glutathione was used in the assay in order to saturate the microsomal enzyme. The aliquots taken were small enough so that the concentration of NEM (or IAA) in the assay medium was never more than 1/60 (or 1/6) of the glutathione concentration.

Determination of fast-reacting sulfhydryl groups was carried out with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) (7).

Protein was determined by the method of Lowry et al. (8) with bovine serum albumin as the standard.

RESULTS

Treatment of fresh rat liver microsomes with 1 mM NEM at pH 7.5 increases the glutathione S-transferase activity assayed with CDNB about 8-fold in 1 min. (Table I). When lower concentrations of NEM were used, maximal activation was assured by incubation for 10 min. With concentrations as low as 50 µM, the activation by NEM remains about 6-7-fold; and 10 µM NEM still gives nearly half-maximal activation. The microsomal content of fast-reacting sulfhydryl groups, as measured with DTNB, was found to be about 40 nmoles/mg protein. These observations suggest that the sulfhydryl group(s) involved in the activation of microsomal glutathione S-transferase activity is more reactive towards NEM than microsomal sulfhydryl groups in general. No change in the microsomal glutathione S-transferase activity was found in

Table I

Effect of NEM treatment on Glutathione-S-transferase activity in rat liver microsomes[a]

Conditions:		Activity	
NEM, mM	Incubation time min.	nmol/mg min.[b]	treated/control
0	1	94.3 ± 0.8	
0	10	96.3 ± 1.1	
0.001	10	112 ± 0.6	1.16
0.005	10	187 ± 8	1.94
0.01	10	321 ± 4	3.33
0.05	10	591 ± 16	6.14
0.1	10	646 ± 26	6.71
1	1	733 ± 44	7.77
1	10	664 ± 16	7.04

[a] 1.5 mg of microsomal protein/ml

[b] Quadruplicate determinations were performed on each of three rats and averages of these quadruplicates were calculated. The values shown are the means of these averages ± the standard error of the mean.

controls incubated in the absence of NEM or when a 10-fold excess of glutathione was added before the addition of NEM (not shown).

Originally, these experiments were performed in attempt to distinguish between the microsomal and soluble proteins catalyzing glutathione S-transferase activity. NEM is known to be an inhibitor of the cytoplasmic glutathione S-transferases of rat liver (9). However, treatment of the supernatant fraction with NEM for the short time periods used here did not affect its glutathione S-transferase activity assayed with CDNB (Table II).

Treatment of microsomes with 10 mM IAA (another sulfhydryl reagent) for 20 min. enhances the microsomal glutathione S-transferase activity about

Table II

Effect of NEM and IAA treatment on glutathione S-transferase activity of the supernatant fraction[a] of rat liver

Conditions:		Activity
NEM, mM	Incubation time min.	nmol/mg min.[b]
0	1	1017 ± 1
0	10	1006 ± 28
1	1	1044 ± 33
1	10	993 ± 13
0.1	10	1077 ± 32
0.01	10	966 ± 18
IAA, mM		
0	1	1333 ± 44
0	60	1297 ± 10
10	60	1410 ± 17
1	60	1383 ± 49

[a] IAA treatment was performed with 1.5 mg of soluble protein/ml. Thiol concentration (not precipitable by 5 % TCA (Trichloroacetic acid)) as measured with DTNB was 60-80 µM.
NEM treatment was performed with 1.6-1.7 mg of desalted soluble protein/ml. Thiol concentration (not precipitable by 5 % TCA) was not more than 4 µM. Liver soluble protein -SH is around 100 nmol/mg protein (11).

[b] Quadruplicate determinations were performed on each of two rats and averages of these quadruplicates were calculated. The values shown are the means of these averages ± the standard error of the mean.

4-fold (Fig. 1). The use of lower concentrations of IAA both prolongs the time required to reach maximal activation and decreases the extent of this activation (see also Table III). When the supernatant fraction is treated with 10 mM IAA in a corresponding manner, very little change in glutathione S-transferase activity is seen (Table II).

DISCUSSION

Microsomal glutathione S-transferase activity with CDNB as the second substrate is enhanced many-fold by treatment with both NEM and IAA. The ac-

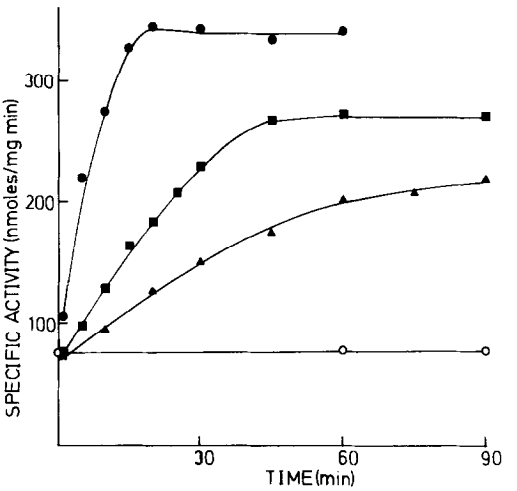

Fig. 1. Effect of IAA on glutathione S-transferase activity in rat liver microsomes (2.2 mg protein/ml). Measurements were done in quadruplicate on material from 1 rat and performed as described in the methods section. ● = 10 mM, ■ = 1 mM, ▲= 0.5 mM, o = 0 mM IAA.

Table III

Effect of IAA treatment on Glutathione-S-transferase activity in rat liver microsomes[a]

Conditions:		Activity	
IAA, mM	Incubation time min.	nmol/mg min[b]	treated/control
0	1	64.8 ± 2.6	
0	90	71.3 ± 3.5	
0.5	90	207 ± 6.5	2.90
1	90	246 ± 7.0	3.45
10	60	274 ± 12	3.96

[a] 2.2 mg of microsomal protein/ml

[b] see footnote b to table I.

tivation thus apparently results from attack on microsomal sulfhydryl group(s); but whether this sulfhydryl group(s) is actually localized on the protein which catalyzes the glutathione S-transferase activity or on some other protein, e.g. an inhibitor, remains to be established. In the case of fructose

1,6 diphosphatase [E.C.3.1.3.11] activation has been shown to occur by modification of -SH groups on the protein itself (10).

In either case the possibility arises that microsomal glutathione S-transferase activity is being regulated in vivo by a mechanism involving sulfhydryl group modification. It can be calculated that in the non-activated form, the microsomal fraction accounts for only 5-10 % of the total liver glutathione S-transferase activity with CDNB; whereas after maximal activation this figure increases to about 30 %. Since most substrates for the glutathione S-transferases are hydrophobic and would be expected to dissolve extensively in membranes; and since many hydrophobic xenobiotics become substrates for the glutathione S-transferases only after conversion to electrophilic intermediates by the cytochrome P-450 system of the endoplasmic reticulum, the microsomal activity may have an even greater role to play than is indicated by these figures.

It may be that activation of the microsomal glutathione S-transferase in vivo occurs by addition of glutathione to the sulfhydryl group involved. Indeed, it has been demonstrated that disulfide formation between the sulfhydryl groups of proteins and glutathione occurs, varies diurnally, and is affected by cyclic AMP levels (11,12). Another possibility is that the microsomal enzyme is activated by its own substrates, which being electrophilic, can react with sulfhydryl groups both enzymically and, to a certain extent, non-enzymically.

The greatly different responses of soluble and microsomal glutathione S-transferase activities to sulfhydryl reagents probably reflect the presence of a different enzyme(s) in the microsomal fraction. Of course, this question can only be answered by isolation of the microsomal enzyme(s).

ACKNOWLEDGEMENTS

We would like to thank Drs. T. Bartfai and B. Mannervik for their helpful suggestions and advice.

This study was supported by National Cancer Institute Contract No. NO1 33363, "Polycyclic Hydrocarbon Metabolism in the Respiratory Tract".

REFERENCES

1. Grover, P.L. (1977) in Drug Metabolism - Microbe Man, Symp. (Parke, D.V., Smith, R.L., eds.) pp. 105-122. Taylor & Francis, London.
2. Booth, I., Boyland, E. and Sims, P. (1961) Biochem. J. 79, 516-524.
3. Kraus, P. (1978) in Conjugation Reactions in Drug Biotransformation. (A. Aitio, Ed.) p. 503, Elsevier/North Holland Biomedical Press, Amsterdam.
4. Jakoby, W.B. (1978) Advances in Enzymology (Meister, A., ed.) Vol. 46, pp. 383-414.
5. Ernster, L. Siekevitz, P. and Palade, G.E. (1962) J. Cell Biol. 15, 541-562.
6. Habig, W.H., Pabst, M.I. and Jakoby, W.B. (1974) J. Biol. Chem. 249, 7130-7139.
7. Ellman, G.L. (1959) Archiv. Biochem. Biophys. 82, 70-77.
8. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
9. Askelöf, P., Guthenberg, C., Jakobson, I. and Mannervik, B. (1975) Biochem. J. 147, 513-522.
10. Pontremoli, S. and Horecker, B.L. (1970) in Current Topics in Cellular Regulation (Horecker, B.L., Stadtman, E.R., eds.) Vol. 2, pp. 173-199. Academic Press, New York and London.
11. Isaacs, I. and Binkley, F. (1977) Biochim. Biophys. Acta 497, 192-204.
12. Isaacs, J.T. and Binkley, F. (1977) Biochim. Biophys. Acta 498, 29-38.