Vol. 98, No. 2, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 30, 1981 Pages 512-519

PURIFICATION OF A NEW GLUTATHIONE S-TRANSFERASE (TRANSFERASE μ) FROM HUMAN LIVER HAVING HIGH ACTIVITY WITH BENZO(α)PYRENE-4,5-OXIDE

Margareta Warholm*+, Claes Guthenberg*, Bengt Mannervik*, and Christer von Bahr‡

*Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, +Unit of Occupational Toxicology, National Board of Occupational Safety and Health, S-171 84 Solna, and ‡ Department of Clinical Pharmacology, Huddinge University Hospital, S-141 86 Huddinge, Sweden

Received December 5,1980

SUMMARY: A new glutathione S-transferase from human liver has been purified to homogeneity in good yield by use of ion-exchange chromatography on DEAE--cellulose, affinity chromatography on S-hexylglutathione coupled to epoxy--activated Sepharose 6B, and chromatography on hydroxyapatite. This new enzyme, transferase μ, is present in high concentration, but only in some individuals. It has an isoelectric point at about pH 6 to 6.5 and a different substrate specificity than the previously described alkaline transferases α-ε from human liver. Especially noteworthy is the finding of high activity against benzo(α)pyrene-4,5-oxide. Glutathione S-transferase μ has about 20--fold higher activity with this substrate than have the alkaline transferases. The most pronounced difference was found with trans-4-phenyl-3-buten-2-one which was > 100-fold better as substrate for transferase μ than for the previously described transferases.

The glutathione S-transferases, a group of cytosolic proteins, play an important role in the biotransformation and detoxication of a variety of hydrophobic electrophilic substances (1). They have been found in all mammals tested, including man (1). Human glutathione S-transferases (or ligandins) have been purified from liver (2,3), erythrocytes (4) and placenta (5) and have also been shown to be present in kidney, lung, adrenal cortex, testis, skin, ovary and the gastrointestinal tract (6). Five different glutathione S--transferases (named α, β, γ, δ, ε) have been purified from human liver (2). They are all alkaline proteins with very similar substrate specificities, amino acid compositions, and immunological properties. The presence in human liver of two acidic forms (pI 4.6 and 5.4, respectively) has also been reported (3,7). In contrast to the multiplicity of human liver glutathione S-trans-

0006-291X/81/020512-08$01.00/0

ferases, only one acidic form (pI 4.7) has been found in erythrocytes (4) and placenta (5,8). This paper describes the purification of a new form (μ) of glutathione S-transferase in human liver. It was discovered in the middle of the pH range covered by isoelectric focusing (isoelectric point at about pH 6 to 6.5) and is only present in some individuals (9). The high specific activity of transferase μ with benzo(α)pyrene-4,5-oxide suggests that the new enzyme may be of great importance in the detoxication of metabolites of carcinogenic polycyclic hydrocarbons.

MATERIALS AND METHODS

Human liver tissue was obtained from kidney donors, judged to have normal livers (by morphological criteria) (10). The supernatant fraction was prepared as described (10) and stored at -80°C prior to use. S-Hexylglutathione Sepharose 6B was prepared as described in (11). [^{3}H]-Benzo(α)pyrene-4,5-oxide (specific radioactivity 10 mCi/mmol) was a gift from Dr. J.R. Bend, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA, and Δ^5-androstene-3,17-dione was a gift from Dr. A.M. Benson, Johns Hopkins University, Baltimore, MD, USA. All other chemicals used were of the highest purity commercially available. The glutathione S-transferase activities were measured by published methods (12-16). Activity with [^{3}H]-benzo(α)pyrene-4,5--oxide was measured essentially as in (17). The reaction was started by addition of 15 nmol [^{3}H]-benzo(α)pyrene-4,5-oxide in 1 μl dimethylsulfoxide. The incubation was carried out at 30°C and the reaction was stopped after 1 or 2 min.

Purification of glutathione S-transferases

Liver tissue was homogenized in 0.3 M sucrose and the homogenate fractionated as described (10). The 100 000 g supernatant (∿ 80 ml) was passed through a Sephadex G-25 column (4 x 32 cm) packed in 10 mM Tris/HCl (pH 8.0) containing 1 mM EDTA and then passed through a DEAE-cellulose column (4 x 9 cm) equilibrated with the same buffer. The column was washed with the starting buffer until glutathione S-transferase activity ceased to appear in the effluent. The pooled effluent was applied on a column of S-hexylglutathione Sepharose 6B (2 x 10 cm) equilibrated with the same Tris-buffer. The column was then washed with the same buffer fortified with 0.2 M NaCl until no protein could be detected in the effluent. Glutathione S-transferases were eluted with 5 mM S--hexylglutathione dissolved in 10 mM Tris/HCl (pH 8.0) containing 0.2 M NaCl. The active fractions were pooled, desalted on a Sephadex G-25 column (4 x 32 cm), packed in 10 mM sodium phosphate (pH 6.7) containing 1 mM EDTA, and subsequently adsorbed on a column of hydroxyapatite (2 x 10 cm) equilibrated with the same buffer. After washing with about two volumes of starting buffer the glutathione S-transferases were eluted by a linear concentration gradient (10-350 mM, total volume 400 ml) of potassium phosphate (pH 6.7). During the purification glutathione S-transferase activity was measured at pH 6.5 with 1 mM 1-chloro-2,4-dinitrobenzene and 1 mM glutathione as substrates and protein content determined by the method of Kalckar (18). The protein concentration was otherwise measured as described by Lowry et al. (19). Disc gel electrophoresis was performed as previously described (12). Sodium dodecyl--sulfate-polyacrylamide slab gel electrophoresis was performed essentially as described by Maizel (20).

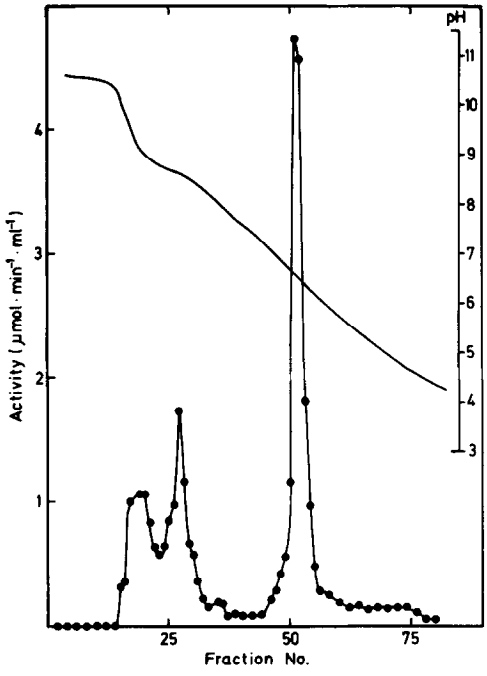

Fig. 1. Isoelectric focusing of a cytosolic fraction from human liver (no. 10 in Ref. 10). Enzymatic activity was measured at 30°C and pH 6.5 by use of 1 mM 1-chloro-2,4-dinitrobenzene and 1 mM glutathione as substrates.

RESULTS

Isoelectric focusing of liver supernatants from different individuals had revealed the existence of a new form of glutathione S-transferase present in only some individuals (9). Fig. 1 shows the activity profile obtained after isoelectric focusing of liver supernatant no. 10 (see Ref. 10). In addition to the peaks in the alkaline region, there is a peak at about pH 6.5 near the middle of the gradient. This new form (μ) has now been purified to homogeneity. The results of the purification of enzyme from liver no. 10 are summarized in Table 1. The glutathione S-transferase activity did not bind to DEAE-cellulose, but was slightly retarded. Affinity chromatography on S-hexyl-glutathione Sepharose 6B proved to be a very efficient step, as previously found in work on rat tissues (21). It resulted in a 10-fold purification with almost no loss of activity. The new form of glutathione S-transferase, transferase μ, was separated from the alkaline forms by chromatography on hydroxy-

Table 1. Purification of glutathione S-transferases from human liver.

Fraction	Volume (ml)	Total activity (μmol)min)	Specific activity[a] (μmol/min per mg protein)	Yield (%)
Liver supernatant	75	926	1.3	100
DEAE-cellulose	169	826	5.6	89
S-Hexylglutathione Sepharose 6B + Sephadex G-25	77	905	58	98
Hydroxyapatite				
Peak 1 (μ)	23.5	240	104 (180)[b]	26
Peak 2 (α-ε)	34	406	41	44

[a] Activity measured at 30°C and pH 6.5 using 1 mM 1-chloro-2,4-dinitrobenzene and 1 mM GSH as substrates. Protein calculated from the absorbance at 260 and 280 nm (18).

[b] Protein determined by the method of Lowry et al. (19).

apatite (Fig. 2). The first peak contained glutathione S-transferase μ and the second peak the alkaline forms. The purified glutathione S-transferase μ displayed only one protein band when analyzed by disc gel electrophoresis, whereas peak II from the hydroxyapatite chromatography contained two bands, both showing glutathione S-transferase activity. These results indicate that peak I consists of homogeneous transferase μ and peak II of a mixture of two alkaline forms of glutathione S-transferase. The latter have not been separated from each other and it is not known which of transferases α-ε they represent. However, the human liver used in this work seems to contain only two alkaline forms in appreciable amounts. Sodium-dodecyl-sulfate polyacrylamide slab gel electrophoresis confirmed the homogeneity of the new glutathione S--transferase (μ) and indicated that the enzyme was composed of two identical subunits.

The substrate specificity of homogeneous glutathione S-transferase μ was examined (Table 2), and the results were compared with those reported for the

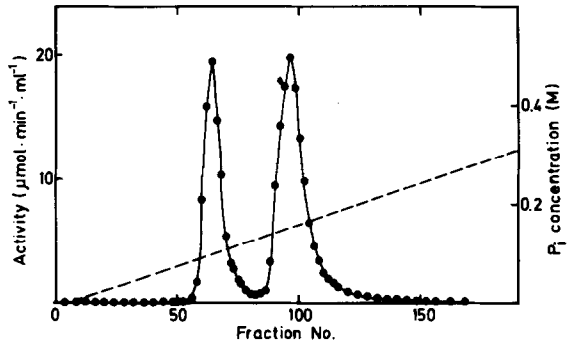

Fig. 2. Separation of glutathione S-transferases by chromatography on a hydroxyapatite column eluted with a linear concentration gradient of potassium phosphate (pH 6.7). Enzymatic activity was measured with 1-chloro-2,4-dinitrobenzene and glutathione. The first peak contains homogeneous transferase μ and the second peak two separable alkaline transferases.

five alkaline transferases α-ε (2). Transferase μ has considerably higher activity than transferases α-ε with 1-chloro-2,4-dinitrobenzene, the substrate most commonly used to measure glutathione S-transferase activity. Another difference is the comparatively high activity with trans-4-phenyl-3-buten-2-one. This activity originally distinguished transferase μ from the alkaline forms (9), because the latter transferases display negligible activity with this substrate. Benzo(α)pyrene-4,5-oxide proved to give about 20-fold higher activity with transferase μ than with the basic forms. This finding is of special significance in relation to detoxication of reactive metabolites of benzo(α)pyrene.

DISCUSSION

The purification procedure for glutathione S-transferases from human liver cytosol presented in this paper yields the new enzyme (9) in apparently homogeneous form in three days. The value of the affinity matrix used has previously been documented in preparations of transferases from rat tissues (21) and human placenta (5). The alkaline transferases (α-ε) were not resolved in the present procedure, but were distinctly separated from the new enzyme (Fig. 2).

Table 2. Specific activities of human liver glutathione S-transferases with various substrates

Substrate	Specific activity ($\mu mol \cdot min^{-1} \cdot mg^{-1}$) Transferases	
	μ [a]	α-ε [b]
1-Chloro-2,4-dinitrobenzene	187	16-37
1,2-Dichloro-4-nitrobenzene	0.032	0.035-0.065
Bromosulfophthalein	< 0.002	0.001-0.010
Ethacrynic acid	0.081	0.017-0.044
trans-4-Phenyl-3-buten-2-one	0.36	0.001-0.002
1,2-Epoxy-3-(p-nitrophenoxy)-propane	0.11	0
Benzo(α)pyrene-4,5-oxide	0.92	0.025-0.030
Cumene hydroperoxide	0.63	-
Δ^5-Androstene-3,17-dione	0.36	0.19-10.2
p-Nitrophenyl acetate	0.22	15.0 (δ)

[a] Present study.

[b] Values from Ref. 1 except for p-nitrophenyl acetate (14) and Δ^5-androstene-3,17-dione (22) which were taken from the original publications.

Several lines of evidence show that a new form (μ) of glutathione S--transferase has been isolated. The molecular properties of transferase μ differ significantly in important respects from those of transferases α-ε (2); the molecular weight of the enzyme and its subunits and the amino acid composition show clear differences (M. Warholm, C. Guthenberg, and B. Mannervik, unpublished data). The kinetic properties of each of transferases α-ε are very similar for most substrates (1,2), but differ significantly from those of transferase μ with several substrates. Transferase μ is >100-fold and about 20-fold as active as forms α-ε with trans-4-phenyl-3-buten-2-one and benzo(α)-pyrene-4,5-oxide, respectively (Table 2). Transferase μ also has significantly higher activity with 1-chloro-2,4-dinitrobenzene. On the contrary, the alkaline transferases have approximately 20-fold higher glutathione peroxidase

activity than transferase μ with cumene hydroperoxide (M. Warholm, C. Guthenberg and B. Mannervik, unpublished data). The existence of transferase μ only in some individuals, in contrast to the presence of at least one form of alkaline transferase in all subjects examined (9), also points to the uniqueness of the new enzyme.

The full significance of glutathione S-transferase μ in human liver remains to be clarified. It has been found in both males and females (9). Whether the presence of the new enzyme is related to induction or solely to genetic differences cannot yet be decided. It should be noted that, when present, transferase μ constitutes 15-20% of the protein having glutathione S--transferase activity (see Table 1). In terms of total activity in the human liver cytosol fraction, transferase μ represents 98% of the activity with trans-4-phenyl-3-buten-2-one and about 75% of the activity with benzo(α)pyrene-4,5-oxide. The latter activity of transferase μ may be discriminatory in defining the capacity of different individuals to metabolize and detoxify various carcinogenic polyaromatic hydrocarbons.

ACKNOWLEDGEMENTS

We thank Drs. J.R. Bend and A.M. Benson for gifts of substrate. Dr. H. Glaumann, Department of Pathology, Huddinge University Hospital, contributed by supplying liver supernatant fractions. This work was supported by grants from the Swedish Cancer Society (to B.M.) and the Swedish Medical Research Council (to C.v.B.).

REFERENCES

1. Jakoby, W.B. and Habig, W.H. (1980) In "Enzymatic Basis of Detoxication" (W.B. Jakoby, ed.), Vol. 2, pp 63-94, Academic Press, New York.
2. Kamisaka, K., Habig, W.H., Ketley, J.N., Arias, I.M. and Jakoby, W.B. (1975) Eur. J. Biochem. 60, 153-161.
3. Awasthi, Y.C., Dao, D.D. and Saneto, R.P. (1980) Biochem. J. 191, 1-10.
4. Marcus, C.J., Habig, W.H. and Jakoby, W.B. (1978) Arch. Biochem. Biophys. 188, 287-293.
5. Guthenberg, C., Åkerfeldt, K. and Mannervik, B. (1979) Acta Chem. Scand. B 33, 595-596.
6. Campbell, J.A.H., Bass, N.M. and Kirsch, R.E. (1980) Cancer 45, 503-510.
7. Koskelo, K. and Valmet, E. (1980) Scand. J. Clin. Lab. Invest. 40, 179-184.
8. Polidoro, G., Di Ilio, C., Del Boccio, G., Zulli, P. and Federici, G. (1980) Biochem. Pharmacol. 29, 1677-1680.
9. Warholm, M., Guthenberg, C., Mannervik, B., von Bahr, C. and Glaumann, H. (1980) Acta Chem. Scand. B 34, 607-610.
10. von Bahr, C., Groth, C.-G., Jansson, H., Lundgren, G., Lind, M. and Glaumann, H. (1980) Clin. Pharmacol. Ther. 27, 711-725.

11. Aronsson, A.-C., Marmstål, E. and Mannervik B. (1978) Biochem. Biophys. Res. Commun. 81, 1235-1240.
12. Askelöf, P., Guthenberg, C., Jakobson, I. and Mannervik, B. (1975) Biochem. J. 147, 513-522.
13. Habig, W.H., Pabst, M.J. and Jakoby, W.B. (1974) J. Biol. Chem. 249, 7130-7139.
14. Keen, J.H. and Jakoby, W.B. (1978) J. Biol. Chem. 253, 5654-5657.
15. Benson, A.M. and Talalay, P. (1976) Biochem. Biophys. Res. Commun. 69, 1073-1079.
16. Lawrence, R.A. and Burk, R.F. (1976) Biochem. Biophys. Res. Commun, 71, 952-958.
17. Mukhtar, H. and Bend, J.R. (1977) Life Sci. 21, 1277-1286.
18. Kalckar, H.M. (1947) J. Biol. Chem. 167, 461-475.
19. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
20. Maizel, J.V. Jr., Summers, D.F. and Scharff, M.D. (1970) J. Cell. Physiol. 76, 273-287.
21. Guthenberg, C. and Mannervik, B. (1979) Biochem. Biophys. Res. Commun. 86, 1304-1310.
22. Benson, A.M., Talalay, P., Keen, J.H. and Jakoby, W.B. (1977) Proc. Natl. Acad. Sci. USA 74, 158-162.