Acta Chemica Scandinavica B 34 (1980) 607–621

Short Communications

Identification of a New Glutathione *S*-Transferase in Human Liver *

MARGARETA WARHOLM,[a] CLAES GUTHENBERG,[a] BENGT MANNERVIK,[a] CHRISTER von BAHR [b] and HANS GLAUMANN [c]

[a] Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden and [b] Departments of Clinical Pharmacology and [c] Pathology, Huddinge University Hospital, Karolinska Institute, S-141 86 Huddinge, Sweden

The group of glutathione *S*-transferases (EC 2.5.1.18) has in recent years attracted great interest owing to its possible physiological role in detoxication of electrophilic compounds in the cell.[1] The enzymes have been identified in various organisms including plants, invertebrates and vertebrates. From the medical point of view it is desirable to learn more about these enzymes in human tissues. Glutathione *S*-transferases have been isolated and characterized from human liver,[2] human erythrocytes[3] and human placenta.[4,5] In liver five basic enzyme forms were identified, which appeared identical in all respects investigated except for the isoelectric points.[2] In erythrocytes an acidic transferase is present in small amounts,[3] whereas in placenta a similar acidic transferase, which appears to be identical with the former enzyme is present in larger quantities.[4] The availability of human liver from kidney donors[6] made possible an investigation of glutathione *S*-transferases in several human subjects, and the present communication shows the presence in some individuals of a new transferase with a substrate specificity which differs from the liver enzymes previously described.

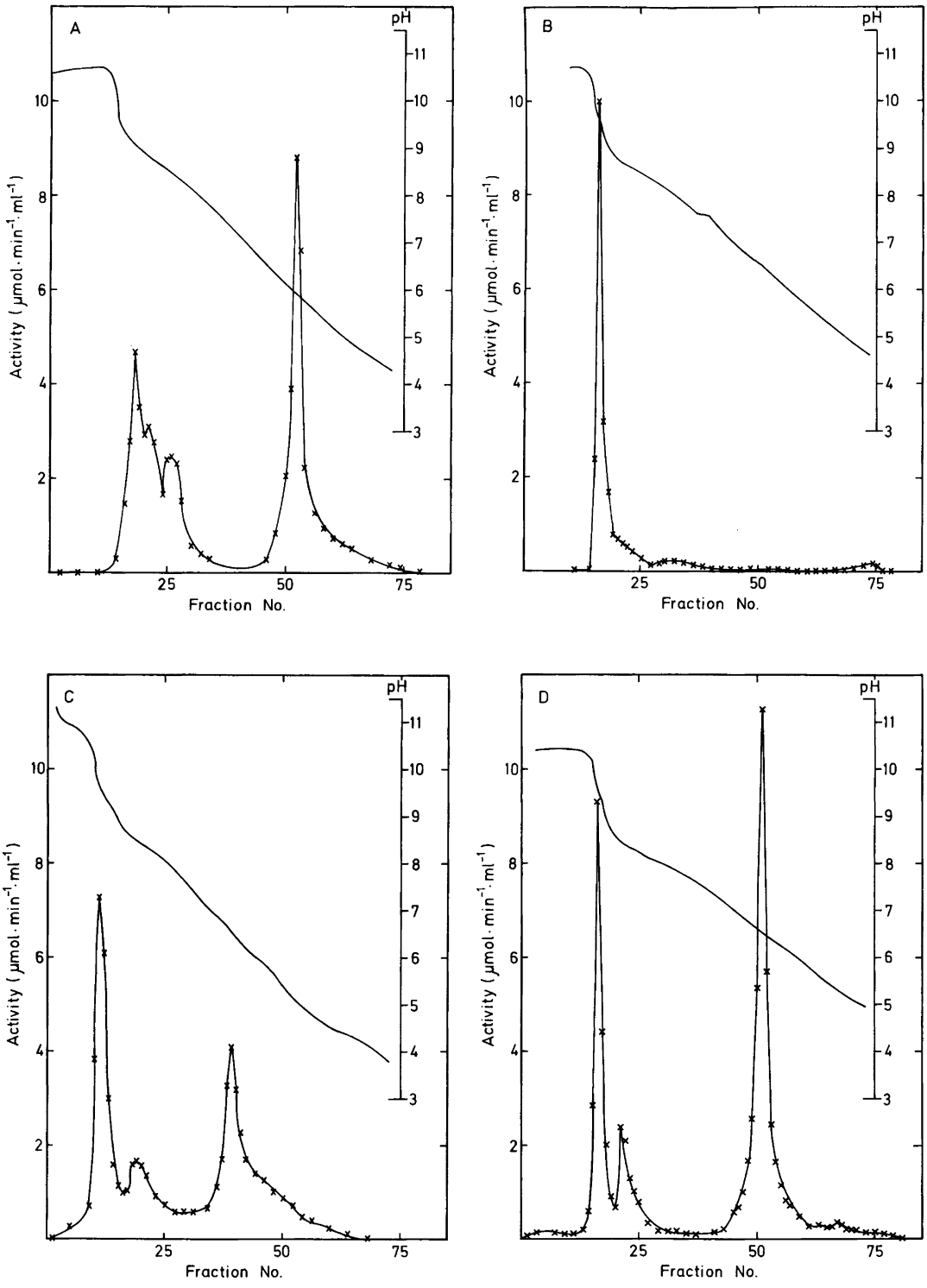
Experimental. Human liver tissue was obtained from kidney donors with normal morphological appearance of their livers. The handling and storage of the samples have previously been described (Ref. 6). The tissue was homogenized in 0.3 M sucrose and 100 000 *g* (post-microsomal) supernatants were obtained by centrifugation as described.[6] Before use in the present investigation the supernatant fractions were stored at −80 °C. Glutathione *S*-transferase activities with various electrophilic substrates were measured essentially as described.[7] Notable differences were in the concentration of 1,2-epoxy-3-(*p*-nitrophenoxy)-propane, which was 0.5 mM, and in the pH value used (pH 8.0) for the assays involving 1,2-dichloro-4-nitrobenzene. Protein concentrations were determined by a microbiuret method.[8] Isoelectric focusing was performed according to instructions of the manufacturer (LKB Produkter, Stockholm) using Ampholine pH 3.5–10 as the ampholyte. The samples were dialyzed against 10 mM sodium phosphate (pH 7) overnight before isoelectric focusing.

Results and discussion. Post-microsomal supernatant fractions from human livers were analyzed with respect to the glutathione *S*-transferase activity obtained with various electrophilic substrates. Table 1 shows the data obtained using supernatants from 5 different livers. 1-Chloro-2,4-dinitrobenzene was found to give the highest acticity of the substrates used; specific activities ranging between 600 and 1630 nmol/min per mg of protein. Other halogen-containing aromatic substrates were 1,2-dichloro-4-nitrobenzene and sulfobromophthalein which gave activities in the order of 1 nmol/min per mg. The latter compound has been used in liver function tests. The aliphatic epoxide 1,2-epoxy-3-(*p*-nitrophenoxy)propane gave activities in the range of 3.2–10.6 nmol/min per mg, and the alkene substrate ethacrynic acid ([2,3-dichloro-4-(2-methylenebutyryl)phenoxy]acetic acid) gave values of similar magnitude. The only substrate tested which displayed large differences between the glutathione *S*-transferase activities of the different livers was *trans*-4-phenyl-3-buten-2-one. Three preparations showing high activity with this substrate had about 10-fold higher values than the two preparations showing low activity.

The different supernatant fractions were also subjected to isoelectric focusing in order to

* Communication at the Meeting of the Swedish Biochemical Society in Lund, 5–6th June, 1980.

0302-4369/80/080607-04$02.50


A
B
C
D
pH
Activity (μmol · min⁻¹ · ml⁻¹)
Fraction No.
25
50
75
2
4
6
8
10
3
4
5
6
7
8
9
10
11

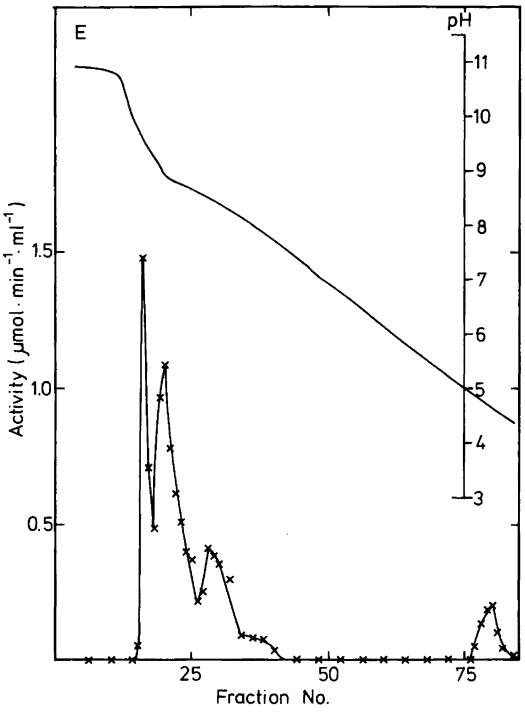


Fig. 1. Glutathione *S*-transferase activity profiles obtained after isoelectric focusing of post-microsomal supernatant fractions from human livers. Enzymatic activities (×) were determined by use of 1-chloro-2,4-dinitrobenzene as electrophilic substrate. Panels A – E illustrate experiments with supernatant fractions from livers Nos. 3, 4, 5, 13 and 15,[6] respectively (*cf.* Table 1).

evaluate possible differences in terms of number and properties of multiple forms of glutathione *S*-transferase. The activity profiles obtained by use of 1-chloro-2,4-dinitrobenzene as electrophilic substrate after isoelectric focusing are shown in Fig. 1. All preparations had at least one transferase with an isoelectric point at pH > 8. In Fig. 1E three such basic forms can clearly be identified. These forms probably correspond to the basic transferases earlier characterized.[2] In some preparations a minor acidic form was found (see especially Fig. 1E). Its isoelectric point was close to pH 4.7, which is the value reported for glutathione *S*-transferase from erythrocytes.[3] Even though the livers were perfused to remove remaining blood from the tissue[6] it is uncertain whether this acidic form is endogenous to the hepatic tissue or originates from contaminating blood.

The activity profiles of the three liver preparations displaying the highest activities with *trans*-4-phenyl-3-buten-2-one (Figs. 1A, C, D) showed a peak at about pH 6 – 6.5 that was not found in the other preparations. The activity profiles were obtained by use of 1-chloro-2,4-dinitrobenzene as electrophilic substrate, but the transferase activity was also tested with *trans*-4-phenyl-3-buten-2-one in the different peaks obtained after isoelectric focusing. Only the peak at pH 6 – 6.5 was found to have any detectable activity with the latter substrate. This glutathione *S*-transferase, which has an isoelectric point at pH 6 – 6.5 and a relatively high activity with *trans*-4-phenyl-3-buten-2-one, has not previously been identified and characterized. The fact that this enzyme is present only in some individuals may explain why it has previously escaped notice. However, examination of a published activity

Table 1. Glutathione *S*-transferase activities of post-microsomal supernatant fractions of five human livers. Activities are reported in units of nmol/min per mg protein.

Supernatant fractions from liver no.[a] (sex)	Electrophilic substrate					
	1-Chloro-2,4-di-nitro-benzene	1,2-Dichloro-4-nitro-benzene	Sulfo-bromo-phthalein	1,2-Epoxy-3-(*p*-nitro-phenoxy) propane	Ethacrynic acid	*trans*-4-Phenyl-3-buten-2-one
3 (♀)	1330	2.7	0.72	9.2	3.7	1.5
4 (♂)	600	2.0	0.31	3.2	4.4	0.16
5 (♀)	990	2.2	0.34	5.0	4.6	1.0
13 (♀)	1630	3.7	0.83	10.6	4.7	3.1
15 (♀)	610	1.8	0.30	5.7	7.1	0.07

[a] The numbers refer to the individuals listed in Ref. 6.

profile after isoelectric focusing (Fig. 2 in Ref. 9) indicates that an individual having this transferase in the liver cytosol has previously been encountered. All preparations analyzed in the present investigation were obtained from livers judged to be normal by morphological and biochemical criteria. Analyses of various microsomal enzymes indicated that no major *post mortem* inactivation or degradation had occurred,[6] and it therefore seems unlikely that the multiple forms of glutathione *S*-transferase have arisen by *post mortem* alterations. No dependence of the appearance of the activity on the time of storage of the liver tissue could be noted. It remains to clarify if the variation in activity profiles from individual to individual originates from genetic differences, induction of enzyme or other causes. Each of the glutathione S-transferases A, B and C in rat liver are inducible.[10]

Acknowledgements. This work was supported by the Swedish Cancer Society and the Swedish Medical Research Council.

1. Chasseaud, L. F. *Adv. Cancer Res. 29* (1979) 175.
2. Kamisaka, K., Habig, W. H., Ketley, J. N., Arias, I. M. and Jakoby, W. B. *Eur. J. Biochem. 60* (1975) 153.
3. Marcus, C. J., Habig, W. H. and Jakoby, W. B. *Arch. Biochem. Biophys. 188* (1978) 287.
4. Guthenberg, C., Åkerfeldt, K. and Mannervik, B. *Acta Chem. Scand. B 33* (1979) 595.
5. Polidoro, G., Zulli, P., DiIlio, C., Del Boccio, G., DiCola, D. and Federici, G. *Boll. Soc. Ital. Biol. Sper. 55* (1979) 840.
6. von Bahr, C., Groth, C.-G., Jansson, H., Lundgren, G., Lind, M. and Glaumann, H. *Clin. Pharmacol. Ther. 27* (1980) 711.
7. Habig, W. H., Pabst, M. J. and Jakoby, W. B. *J. Biol. Chem. 249* (1974) 7130.
8. Goa, J. *Scand. J. Clin. Lab. Invest. 5* (1953) 218.
9. Benson, A. M., Talalay, P., Keen, J. H. and Jakoby, W. B. *Proc. Natl. Acad. Sci. U.S.A. 74* (1977) 158.
10. Guthenberg, C., Morgenstern, R., DePierre, J. W. and Mannervik, B. *Biochim. Biophys. Acta 631* (1980) 1.

Received June 24, 1980.