Identification of the Isozymes of Glutathione Transferase Induced by *trans*-Stilbene Oxide *

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It is well established that glutathione transferase (EC 2.5.1.18) activity in rat liver can be induced by various chemical agents (see Refs. 1 and 2). One of the most powerful inducers discovered is *trans*-stilbene oxide, which is capable of increasing the total glutathione transferase activity 3–4-fold.[2] Studies involving the use of antibodies for quantitation of enzyme protein demonstrated that the increased specific activities of transferases "A", "B" and "C" were in all cases due to increased concentrations in the cytosol.[2] However, more recent studies have demonstrated that the above transferases are but 3 of the group of 6 isozymes with basic isoelectric points and that binary combinations of 4 different subunits account for the 6 isozymes.[3] The subunits belong to two groups within which they may be combined: One contains subunits B and L, and the other contains subunits A and C. The isozymes are now named according to their subunit composition as glutathione transferase B_2, BL ("B" above), L_2, A_2 ("A" above), AC ("C" above) and C_2. Thus, it has become important to investigate whether the previously observed induction by *trans*-stilbene oxide involves all 4 subunits or only some. The results demonstrate that 2 subunits are selectively induced and that the 4 isozymes containing these subunits increase in amount.

Results and discussion. The technique that made possible the resolution of the basic glutathione transferases in rat liver cytosol was chromatofocusing.[4] By use of this technique, the isozymes in the hepatic cytosol fraction of a rat treated with *trans*-stilbene oxide (400 mg per kg

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Table 1. Activity ratios ($\times 10^3$) of substrates for identification of isozymes of glutathione transferases in rat liver cytosol of control and induced animals.

Animal	Glutathione transferase isozyme (peak No. in Fig. 1)					
	L_2(I)	BL(II)	B_2(III)	A_2(IV)	AC(V)	C_2(VI)
Ethacrynic acid/1-chloro-2,4-dinitrobenzene						
Control	5	23	70			
Induced	5	26	66			
trans-4-Phenyl-3-buten-2-one/1-chloro-2,4-dinitrobenzene						
Control				1	18	80
Induced				2	18	n.d.[a]

[a] n.d., not determined; the amount of glutatione transferase C_2 was too small to give accurate data.

Table 2. Fraction of total glutathione transferase activity of the isozymes separated by chromatofocusing (Fig. 1).

Animal	Glutathione transferase isozyme (peak No. in Fig. 1)					
	L_2(I)	BL(II)	B_2(III)	A_2(IV)	AC(V)	C_2(VI)
Control	0.15	0.29	0.08	0.14	0.30	0.04
Induced	0.32	0.19	0.04	0.27	0.17	0.01

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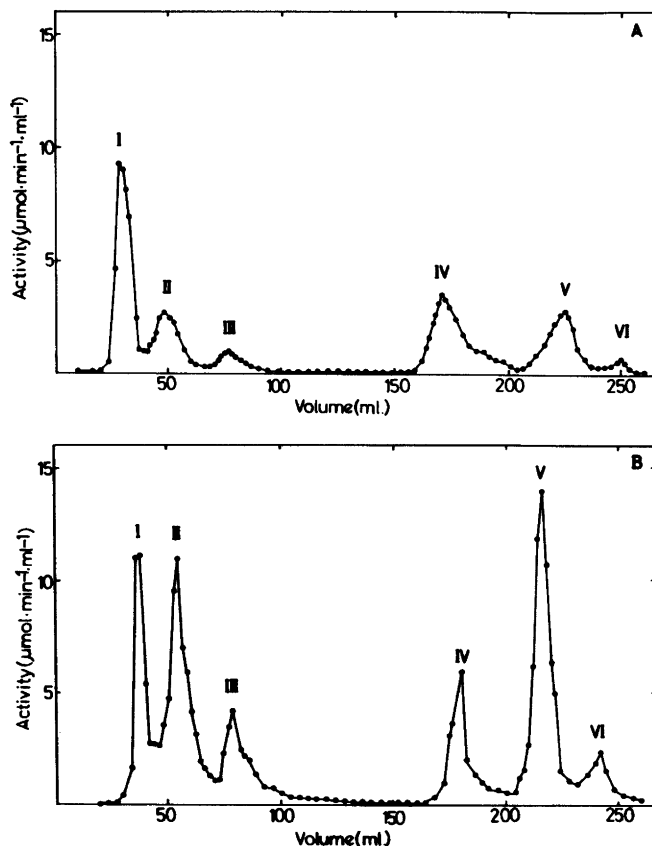

Fig. 1. Separation of basic glutathione transferases from rat liver cytosol of (A) induced and (B) control rats by chromatofocusing. Transferase activity was measured with 1-chloro-2,4-dinitrobenzene as electrophilic substrate. Approximately equal amounts of total transferase activity were loaded onto the chromatofocusing columns, even though the induced rat had a 3-fold higher cytosolic specific activity.

body weight for 5 days) could be separated and compared with those of a control animal. Fig. 1 shows that in the chromatogram of a treated rat peaks I and IV increase whereas the other peaks decrease in relative activity in comparison with the corresponding peaks of the control. Peaks I–VI correspond to transferases L_2, BL, B_2, A_2, AC and C_2 in this sequence. The transferases of the induced rat were identified by, in addition to their elution position, their subunit molecular weights and substrate specificities (*cf.* Ref. 3). Some particularly discriminating ratios of activities with different substrates are listed in Table 1.

Quantitation of the relative amounts of activity in the different peaks obtained by chromatofocusing was made by summing the activities of the individual fractions. Table 2 shows that the relative amounts of transferases L_2 and A_2 increase, whereas those of the other transferases decrease, in the sample from the induced rat, as compared with the control. It may be added that more acidic glutathione transferases,[3,4] which are eluted from the chromatofocusing column by 1 M NaCl, are not induced by *trans*-stilbene oxide.

The simplest, and most likely, explanation of the above findings is that essentially only subunits A and L rise in concentration upon treatment of the rat with *trans*-stilbene oxide. As a result, not only the homodimers, transferases A_2 and L_2, but also the heterodimers, transferases AC and BL, increase in absolute amounts. The formation of the heterodimers takes place at the expense of the homodimers, transferases B_2 and C_2, thus providing an explanation for the decrease of their

relative amounts.

Thus, the induction of glutathione transferase activity in rat liver by *trans*-stilbene oxide [2] can be explained by increases in the levels of transferase subunits A and L resulting in increased amounts of the four transferases L_2, BL, A_2 and AC of the several isozymes identified in the cytosol.

Experimental. Male Sprague Dawley rats (weighing 180–200 g) of the same litter, reared to be free of specific pathogens, were used in the investigation. The cytosol fraction of the liver of control and induced rats was prepared as earlier described; [3,4] one animal was used for each separation. After affinity chromatography on *S*-hexylglutathione Sepharose, a sample was analyzed by chromatofocusing on gel PBE 118 (Pharmacia Fine Chemicals) as described.[3,4] Glutathione transferase activity was measured at 30 °C in 0.1 M sodium phosphate (pH 6.5) with 1 mM glutathione and 1 mM 1-chloro-2,4-dinitrobenzene as substrates. Assays with ethacrynic acid or *trans*-4-phenyl-3-buten-2-one as electrophilic substrate were carried out by published procedures.[5]

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