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A SET OF INHIBITORS FOR DISCRIMINATION BETWEEN THE BASIC ISOZYMES OF GLUTATHIONE TRANSFERASE IN RAT LIVER

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SUMMARY: A set of inhibitors including hematin, bromosulfophthalein, and triethyltin bromide was used for discrimination and identification of the major basic isozymes of glutathione transferase in rat liver cytosol. Six enzymes are formed as binary combinations of 4 protein subunits: A, B, C, and L. Discrimination between the transferases can be based on the differences of the subunits in susceptibility to the inhibitors. The identification of transferase subunits is further supported by the combined use of specific substrates and inhibitors.

Various isozymes of glutathione transferase (EC.2.5.1.18) have been demonstrated in rat liver cytosol (1,2) The quantitatively dominating isozymes are six dimeric proteins with high isoelectric points (3). In addition, at least three glutathione transferases with lower isoelectric points exist in lower concentrations. It was recently found that two subunits, B and L, in binary combinations form the three most basic isozymes: glutathione transferases B_2, BL, and L_2 (4). Likewise, subunits named A and C account for glutathione transferase A_2, AC, and C_2 with lower isoelectric points. The four subunits are distinguished by their substrate specificities in addition to their physical properties (4). For example, subunit A has high relative activity with 1,2-dichloro-4-nitrobenzene (DCNB)[1]; subunit B with ethacrynic acid; subunit C with trans-4-phenyl-3-buten-2-one (tPBO); and subunit L with p-nitrophenyl acetate. However, in

[1] Abbreviations: BSP, bromosulfophthalein; CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene; tPBO, trans-4-phenyl-3-buten-2-one

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view of partially overlapping substrate specificities and the averaging of the activities in the heterodimers, the activity patterns appear insufficient for identification and discrimination of isozymes in different samples from liver and other tissues. The present communication shows how the use of a set of inhibitors is a powerful tool for such purposes.

MATERIAL AND METHODS

The six basic isozymes of glutathione transferase in rat liver cytosol were purified to homogeneity as described previously (4). Glutathione transferase activities were determined at 30°C by published procedures (5) by measuring the reaction of glutathione and the following substrates: 1-chloro-2,4-dinitrobenzene, 1,2-dichloro-4-nitrobenzene, ethacrynic acid, trans-4-phenyl-3-buten-2-one, p-nitrophenyl acetate. For example, the assay system with glutathione and 1-chloro-2,4-dinitrobenzene as substrates contained: 500 µl of 0.2 M sodium phosphate, pH 6.5, containing 2 mM EDTA, 50 µl of 20 mM GSH in deionized water, 50 µl of 20 mM CDNB in 95% ethanol, a suitable amount of enzyme, and deionized water to final volume of 1 ml. The reaction was always started by addition of enzyme. The progress was monitored spectrophotometrically as the increase in absorbance at 340 nm (ε_{340} = 10 $mM^{-1}cm^{-1}$). A correction for the spontaneous reaction was made by measuring and subtracting the rate in the absence of enzyme.

The effect of an inhibitor on the catalytic activity was determined by measuring the reaction rate in the presence and in the absence of inhibitor. The concentration of the inhibitor producing 50% inhibition of enzymatic activity (I_{50} value) was determined by using a series of dilutions from a stock solution. The stock solutions used were: BSP, 50 mM in deionized water; triethyltin bromide, 100 mM in 95% ethanol; hematin, 1 mM in dilute NH_4OH, and tributyltin acetate, 1 mM in 95% ethanol. The volume of the additions of inhibitor solutions was 10 µl.

RESULTS

Several compounds were screened for strong inhibition of the basic glutathione transferases of rat liver cytosol. A condition for selecting an inhibitor was that the effect should be differential for the four types of subunits: A, B, C, and L (for nomenclature - see Ref. 4). Three compounds previously recognized as inhibitors of glutathione transferases were finally chosen: bromosulfophthalein (6), hematin (7), and triethyltin bromide (8,9). Table 1 shows the concentration of inhibitor effecting 50 per cent inhibition of enzymatic activity (I_{50}) tested in 0.1 M

Table 1. Differential inhibition of hepatic glutathione transferases measured with 1-chloro-2,4-dinitrobenzene as electrophilic substrate

Glutathione transferase isozyme	I_{50} (μM)		
	BSP	triethyltin bromide	hematin
L_2	2	500	0.1
BL	10	100	10
B_2	200	3	>10
A_2	10	1	2
AC	6	1.5	2
C_2	0.5	100	1

sodium phosphate (pH 6.5) containing 1 mM EDTA using 1 mM CDNB and 1 mM GSH as substrates. It should be added that the I_{50} values for tributyltin acetate are in the range of 0.5 to 2 μM for the six isozymes, but the values are not separate enough to discriminate between the various enzyme forms.

The I_{50} values were obtained by interpolation in graphs of remaining enzyme activity at different concentrations of inhibitor. Fig. 1 exemplifies such titration curves.

The data should be considered primarily for the differential effects on the different subunits, which are expressed by the homodimeric glutathione transferases. Glutathione transferase A_2 is most specifically inhibited by triethyltin bromide, transferase C_2 by BSP, and transferase L_2 by hematin (Table 1). Transferase B_2, like transferase A_2, is also strongly inhibited by triethyltin bromide, but can be distinguished from the latter by being much less sensitive to BSP. Actually tributyltin acetate was the best inhibitor for transferase B_2 (I_{50} = 0.5 μM), but the inhibitory effect was not considered discriminatory enough with respect to the other isozymes to be practically useful.

Table 1 shows that the differential inhibitory effects allow the discrimination between the homodimeric basic glutathione transferases A_2, B_2, C_2, and L_2, but the heterodimeric transferases

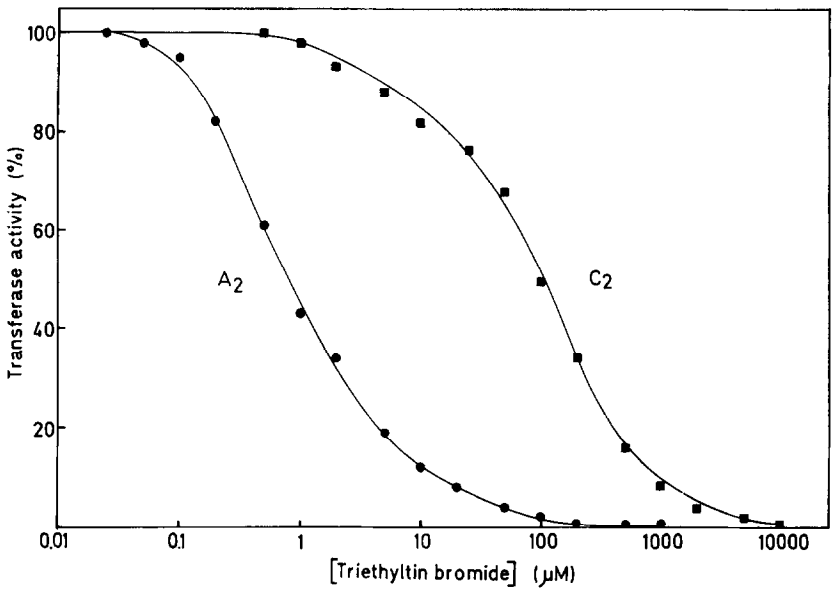

Fig. 1. Inhibition of glutathione transferases A_2 (●) and C_2 (■) as a function of triethyltin bromide concentration. Remaining transferase activity was measured at pH 6.5 with 1-chloro-2,4-dinitrobenzene as the electrophilic substrate.

AC and BL are somewhat less facile to identify. In particular, it would appear difficult to distinguish between transferases A_2 and AC or between transferases BL and L_2 (cf. Table 1). However, increased accuracy in discrimination between these pairs of isozymes is obtainable by use of alternative electrophilic substrates that display the differences in specificity between subunits A and C or subunits B and L, respectively. DCNB is highly specific as substrate for subunit A, whereas tPBO is highly specific for subunit C. Accordingly, transferase AC assayed with DCNB displays the catalytic activity of subunit A; the same isozyme assayed with tPBO displays the activity of subunit C. Table 2 shows the results of inhibition experiments using these substrates for the isozymes containing subunits A or C. Similar assays were carried out with the isozymes containing subunits B or L. In this case p-nitrophenyl acetate and ethacrynic acid were used as electrophilic substrates. They give high relative activity with

Table 2. Inhibition of glutathione transferase isozymes using substrates specific for different transferase subunits

Inhibitor	Substrate	I_{50} of Glutathione transferase isozyme		
		A_2	AC	C_2
Triethyltin bromide	DCNB	0.2	0.2	
Triethyltin bromide	tPBO		100	100
BSP	DCNB	8	10	
BSP	tPBO		1	0.8

subunits L and B, respectively, but are less specific for one kind of subunit than are DCNB and tPBO (4).

DISCUSSION

The results of the present study show that the discrimination between the six basic isozymes of glutathione transferase in rat liver cytosol (4) can be based on the inhibition characteristics of the individual subunits when subjected to a set of three inhibitors, viz. BSP, hematin, and triethyltin bromide. The inhibition should preferably be assayed not only with the universal substrate, CDNB, but also with substrates characterizing the different subunits.

It is evident that the inhibitors could serve as useful tools in studies of the distribution of the various isozymes of glutathione transferase in different tissues of the rat. Such an application has already been made in the identification of enzyme forms in rat testis (10). Another application is in the study of the biological regulation of the transferase activities, e.g., as expressed by the induction of enzyme activity in the liver (cf. Ref. 11). The set of inhibitors may also help clarify the relationship between the isozymes in rat liver with the various enzyme forms in other organisms. Thus, the inhibition experiments will be a valuable complement to a battery of tests required to identify individual isozymes of glutathione transferase.

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