Inhibitors for distinction of three types of human glutathione transferase

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A set of inhibitors that are useful for distinction of three types of human cytosolic glutathione transferase is presented. The near-neutral transferase is inhibited most effectively by Cibacron blue (I_{50} = 0.05 μM), the acidic transferase by Cibacron blue (I_{50} = 0.5 μM), and the basic transferase by tributyltin acetate (I_{50} = 0.1μM). The use of any of these two compounds makes possible differentiation between all three types of human transferase.

Human glutathione transferase *Discriminatory inhibitor* *Isoenzyme* *Cibacron blue* *Organotin compound*

1. INTRODUCTION

Glutathione transferase (EC 2.5.1.18) occurs in multiple forms in most biological sources investigated [1]. Like the family of cytochrome P-450 [2], the glutathione transferases are considered to be involved in detoxication of xenobiotics as well as of compounds endogenous to the organism. A possible explanation for the existence of isoenzymes of detoxication enzymes is that an ensemble of proteins with somewhat different substrate specificities can metabolize a broader spectrum of toxic compounds than could a single enzyme.

Several isoenzymes of glutathione transferase have been isolated from the cytosol fraction of human tissues, and three classes of the enzyme have been distinguished [1,3]. These classes have been referred to as basic (or α–ϵ), near-neutral (or μ), and acidic (or π/ϱ) [4]. The members of the three classes have clearly different substrate specificities, indicating distinct roles of the various enzymes in detoxication reactions. In view of the non-uniform tissue distribution of the isoenzymes, and their different catalytic capacities, it is important to be able to distinguish the types of transferase in previously uncharacterized samples. Investigations of the multiple forms of glutathione transferase in the rat have shown that a battery of tests are necessary for an accurate identification of the isoenzymes [4]. One such test involves the determination of the sensitivity to a set of inhibitors [5]. The present communication provides such data for the three types of human glutathione transferase.

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2. MATERIALS AND METHODS

2.1. *Chemicals*

S-Hexylglutathione and *S*-*p*-bromobenzylglutathione were synthesized by method A of Vince et al. [6] as detailed earlier [7]. All other chemicals were standard commercial products used without further purification.

Stock solutions of *S*-hexylglutathione, *S*-*p*-bromobenzylglutathione, and the dye Cibacron blue F3GA (Color Index 61211) were made by dissolving the inhibitor in the minimal amount of 0.1 M NaOH, followed by neutralization with 0.1 M HCl and dilution with deionized water to the desired concentration. Hematin was similarly dissolved in 0.1 M NH_3. Triphenyltin chloride was

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dissolved in acetone, but dilutions were made with ethanol to avoid the comparatively higher inhibitory action of acetone. Stock solutions as well as dilutions of all other inhibitors were made in 95% (v/v) ethanol.

2.2. *Enzymes*

The three types of human glutathione transferase were obtained by published procedures: acidic transferase (π) from placenta [7]; near-neutral (μ) and basic transferases (α–ϵ) from adult liver [8]. Each of the three enzyme preparations was homogeneous on dodecylsulfate–polyacrylamide gel electrophoresis, but it is known that the basic fraction may contain up to at least 5 components with similar enzymatic properties [9,10]. In view of the reported similarities, no attempt at separating basic components was made.

2.3. *Assay of enzyme activity*

Glutathione transferase activity was measured at 30°C using 1 mM GSH and 1 mM 1-chloro-2,4-dinitrobenzene as substrates. The latter substrate was added from a 20 mM stock solution in 95% ethanol to the reaction system (final volume 1 ml) containing 0.1 M sodium phosphate (pH 6.5). The reaction was started by the addition of the enzyme and was monitored spectrophotometrically at 340 nm [11]. The effect of inhibitors on the catalytic activity was studied by comparing the reaction rate in the presence and the absence of inhibitor. The concentration of inhibitor giving 50% inhibition, the I_{50} value, was determined by interpolation from plots of remaining activity *vs* inhibitor concentration. Inhibitor solutions were diluted appropriately before addition to the reaction system in order to maintain a constant concentration of organic solvent (if used) when the inhibitor concentration was varied.

3. RESULTS

Table 1 gives a set of I_{50} values for each of the three types of human glutathione transferase assayed with a variety of inhibitors. Most compounds listed inhibit at least one enzyme form significantly at 1 μM. The exceptions are triethyltin bromide and indomethacin, which were included because they are strong inhibitors of rat glutathione transferases 3–3 [5] and 4–4 (called VII by the authors, [12]), respectively. The data also show that virtually all inhibitors distinguish at least one isoenzyme from the other two. With

Table 1

Inhibition parameters, I_{50} values (μM)[a], for the three types of human GSH transferase

Inhibitor	Isoenzyme		
	Basic (α–ϵ)	Near-neutral (μ)	Acidic (π)
Cibacron blue	5	0.05	0.5
Gossypol acetic acid	50	2	>100
Tributyltin acetate	0.1	0.5	4
Triethyltin bromide	10	5	6
Triphenyltin chloride	0.25	0.5	>10
Bromosulfophthalein	75	2	100
Hematin	0.5	1	5
S-Hexylglutathione	3	10	20
S-(*p*-Bromobenzyl)glutathione	4	1	4
Indomethacin	100	40	100
Rose Bengal	1	1	15

[a] The I_{50} value is the concentration of inhibitor giving 50% inhibition of the enzyme activity assayed at pH 6.5, 30°C, with 1 mM 1-chloro-2,4-dinitrobenzene and 1 mM GSH as substrates

some inhibitors, all three enzyme forms have discrete I_{50} values. Fig.1 shows the differential effects of tributyltin acetate and Cibacron blue on the three types of transferase. Tributyltin acetate was the most potent inhibitor of the basic transferase and Cibacron blue the most effective with the near-neutral and the acidic types. However, in no case was a compound found to inhibit the acidic transferase more strongly than both of the other enzyme forms.

4. DISCUSSION

The primary object of this investigation was to find a suitable set of inhibitors to be used for identification of the three different types of cytosolic human glutathione transferase. For example, inhibitors have been used (unpublished) to verify the conclusion that the acidic transferase in human fetal liver [13], is of the same type as the placental enzyme rather than of the category including the

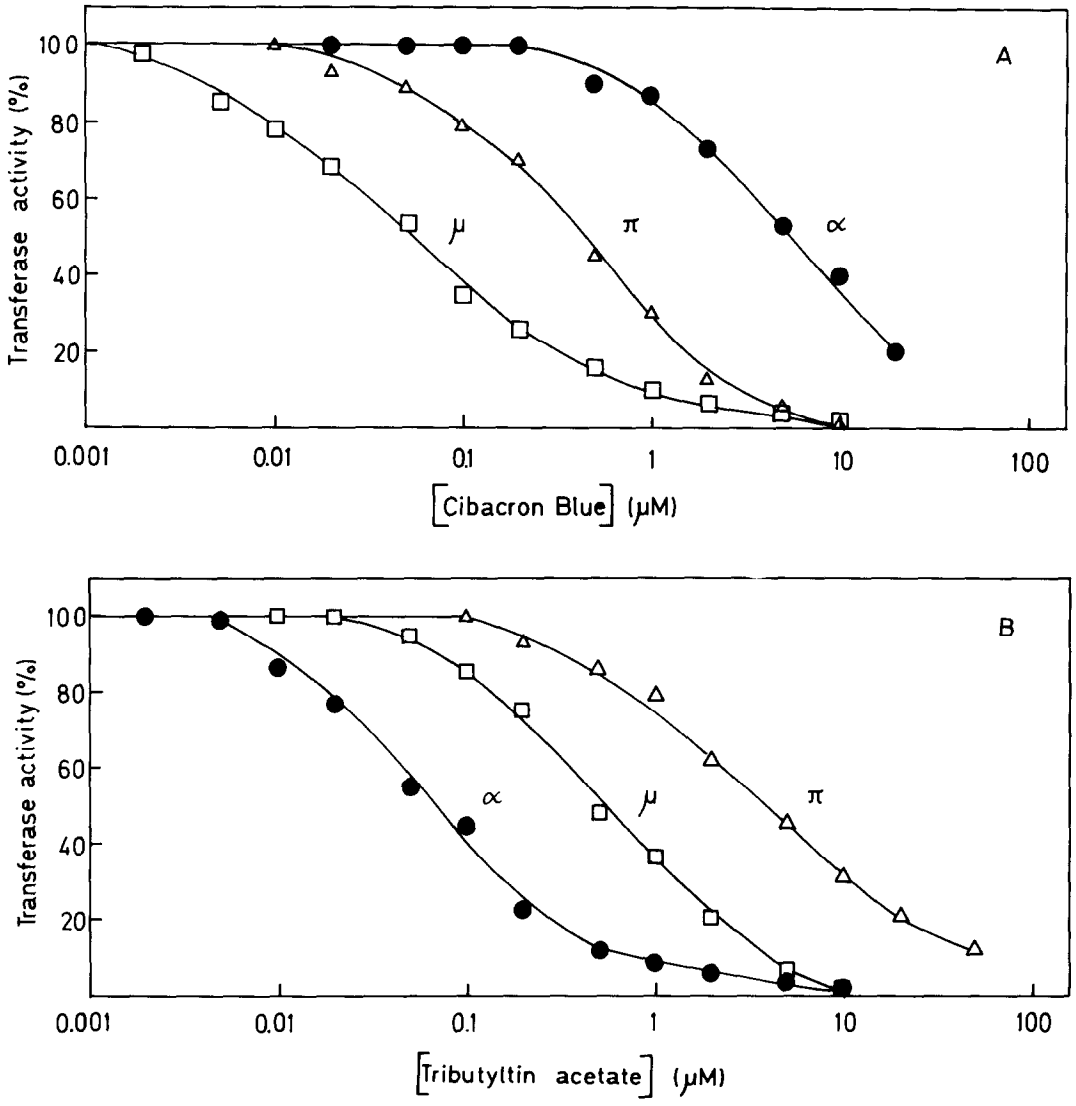

Fig.1. Inhibition of the three types of human glutathione transferase as a function of the concentration of Cibacron blue (A) and tributyltin acetate (B). Remaining transferase activity of the basic (α) (●), the near-neutral (μ) (□) and the acidic (π) (△) enzyme forms was measured at pH 6.5 with 1-chloro-2,4-dinitrobenzene as electrophilic substrate.

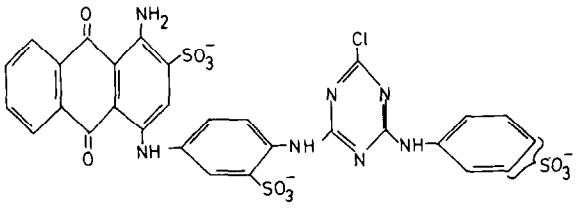

Fig.2. Structure of Cibacron blue F3GA.

acidic forms detected in adult human liver [14]. This example shows that the criterion of isoelectric point is not adequate for identification, since both the fetal and the adult enzyme forms considered are acidic proteins. Table 1 gives data that could be used for discrimination between the human isoenzymes so far characterized.

The information in table 1 could also be used in the design of metabolic studies with isolated cells. By judicious choice of inhibitors, one type of glutathione transferase may be selectively inhibited, allowing evaluation of its contribution to metabolism and to protection of the cell.

More detailed studies are required for characterizing the interaction of the inhibitors with the different enzyme forms. Nevertheless, the data in table 1 lead to the conclusion that both hydrophobic interactions and steric effects have decisive roles in determining the inhibitory strength of a given compound. For example, the related organotin compounds on the one hand, and the glutathione derivatives on the other hand, exhibit such differential inhibitory effects that support this conclusion. Cibacron blue (fig.2) is one of the most potent glutathione transferase inhibitors, but the possible interpretation that binding of this compound signifies the presence of a dinucleotide fold in the protein [15] is contradicted by the lack of inhibition of the transferases by NAD^+ or $NADP^+$. However, further investigations of inhibitor binding may give information about the molecular topography of the different isoenzymes of glutathione transferase.

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