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# LEUKOTRIENE $C_4$ FORMATION CATALYZED BY THREE DISTINCT FORMS OF HUMAN CYTOSOLIC GLUTATHIONE TRANSFERASE

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The ability of three distinct types of human cytosolic glutathione transferase to catalyze the formation of leukotriene $C_4$ from glutathione and leukotriene $A_4$ has been demonstrated. The near-neutral transferase (μ) was the most efficient enzyme with $V_{max}$ = 180 nmol x $min^{-1}$ x $mg^{-1}$ and $K_m$ = 160 μM. The $V_{max}$ and $K_m$ values for the basic (α-ε) and the acidic (π) transferases were 66 and 24 nmol x $min^{-1}$ x $mg^{-1}$ and 130 and 190 μM, respectively. The synthetic methyl ester derivative of leukotriene $A_4$ was somewhat more active as a substrate for all the three forms of the enzyme. 

Cytosolic glutathione transferase (EC 2.5.1.18) occurs in three distinct forms in human tissues (1). The three types of the human enzyme have been referred to as basic (α-ε), near-neutral (μ), and acidic (π) glutathione transferase and can be distinguished by physico-chemical and catalytic properties (1), sensitivities to inhibitors (2), and primary structure (3). The different isoenzymes catalyze the conjugation of a variety of electrophilic compounds with glutathione (4-6). Cysteine-containing leukotrienes, formed via reaction of glutathione with the epoxy group of leukotriene $A_4$, are biologically active molecules with smooth-muscle stimulating and edema inducing properties (7-10). These compounds are also presumed to mediate allergic and anaphylactic reactions. The enzyme(s) involved in the conversion of leukotriene $A_4$ to leukotriene $C_4$, have not been extensively studied. In contrast, the metabolic reactions giving rise to leukotrienes $D_4$ and $E_4$ are considered to be catalyzed by γ-glutamyltransferase (EC 2.3.2.2) and a dipeptidase, respectively (11-13). Reports from two laboratories show that leukotriene $C_4$ may be formed

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by a membrane-bound enzyme from rat basophilic leukemia cells (14,15). In view of the high intracellular concentration and the broad substrate specificities of the ubiquitous glutathione transferases (5), the possibility that also these enzymes may contribute to the biosynthesis has been investigated (16,17). Indeed, our recent work has demonstrated that a synthetic derivative, the methyl ester of leukotriene $A_4$, is a good substrate for some of the cytosolic isoenzymes of glutathione transferase in the rat (17). Preliminary experiments indicated that also leukotriene $A_4$ is a substrate for the same isoenzymes (17).

In view of the medical importance of the biological responses elicited by the cysteine-containing leukotrienes, the ability of human glutathione transferases to catalyze the formation of leukotriene $C_4$ from leukotriene $A_4$ has been investigated. Kinetic constants are reported that distinguish the three different types of human glutathione transferase in their capacity to catalyze the conjugation of leukotriene $A_4$ with glutathione.

## MATERIALS AND METHODS

Leukotriene $A_4$ methyl ester and leukotriene $C_4$ were generous gifts of Dr. J. Rokach, Merck-Frosst, Canada. Inc. [14,15-$^3H_2$]Leukotriene $A_4$ methyl ester was purchased from New England Nuclear. Glutathione transferases μ and α-ε were isolated from human liver cytosol (18), and transferase π was isolated from from human placenta as described earlier (19).

Leukotriene $A_4$ methyl ester was hydrolyzed with 0.05 ml of 0.1 M LiOH(aq) in 0.2 ml of tetrahydrofuran. The mixture was stirred at room temperature overnight. Before use, the hydrolysis mixture was brought to dryness under a stream of argon and dissolved in 0.05 ml of ethanol. The standard assay system contained glutathione transferase (0.2-14 μg), 25 mM potassium phosphate buffer, pH 7.0, 0.5 mM EDTA, 5 mM glutathione, and 35 μM leukotriene $A_4$ lithium salt or 12 μM leukotriene $A_4$ methyl ester. The mixtures were preincubated at 30°C for 2 min, 1 min before and 1 min after the addition of glutathione. Reactions were started by addition of leukotriene $A_4$ and terminated after 1 min by addition of 0.1 ml methanol. The total reaction mixtures were analyzed by reverse-phase HPLC on a $C_{18}$ Nucleosil column (250 x 4.6 mm) using, as mobile phase, methanol/water 6.5:3.5 (v/v) plus 0.07% acetic acid/0.03% phosphoric acid, adjusted to pH 5.4 with $NH_4OH$ (flow rate: 1 ml/min). The formation of leukotriene $C_4$ was determined from the radioactivity in the peak that emerged at the same retention time as synthetic leukotriene $C_4$. In the incubations with leukotriene $A_4$ methyl ester as substrate, the conversion into leukotriene $C_4$ monomethyl ester was determined after ethyl acetate extraction. The enzyme activity was calculated from the radioactivity remaining in the aqueous phase in relation to the total radioactivity added; the value was corrected for the

non-enzymatic reaction (17). Apparent $K_m$ values and $V_{max}$ values were determined by non-linear regression analysis (20).

## RESULTS AND DISCUSSION

The basic, near-neutral, and acidic forms of human cytosolic glutathione transferase were incubated with glutathione and leukotriene $A_4$. Formation of leukotriene $C_4$ was demonstrated in the reaction system with each of the isoenzymes. In the absence of enzyme no synthesis of leukotriene $C_4$ could be detected. Fig. 1 shows the elution profile of a reverse-phase HPLC analysis of the incubation mixture after reaction catalyzed by the acidic transferase. Peaks corresponding to leukotriene $C_4$ (38 min) as well as two major degradation products of leukotriene $A_4$, (46 and 52 min) can be identified. Similar chromatograms were obtained for the reaction mixtures containing the basic or the near-neutral transferases. Identification of leukotriene $C_4$ as a reaction product was based on co-chromatography with synthetic leukotriene $C_4$ (Fig. 1B). Further support for the identification was obtained by the coincidence of

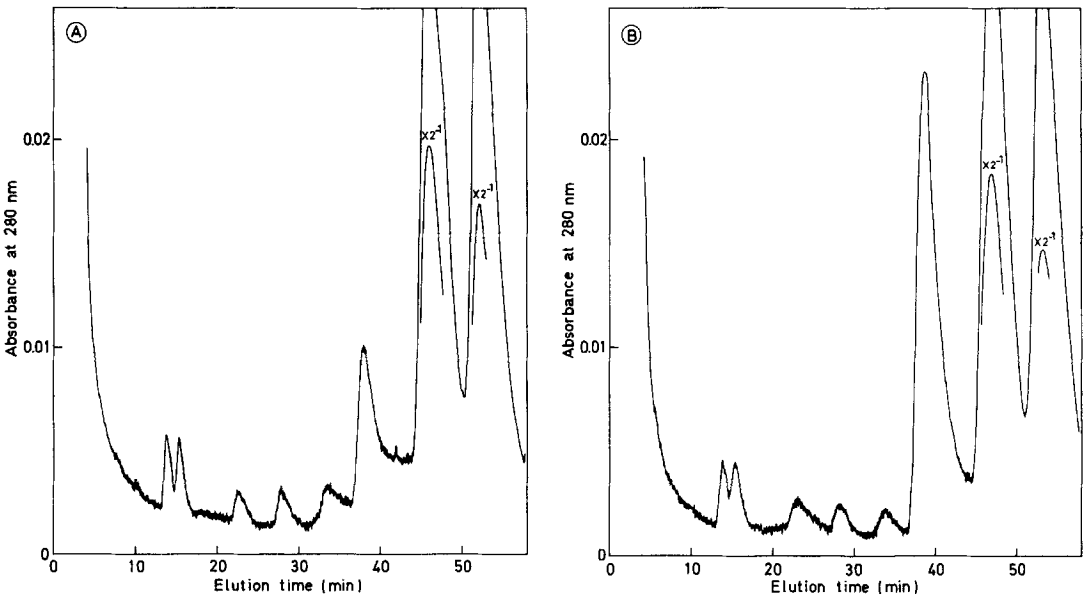


Fig. 1. Reverse-phase HPLC of the products formed from leukotriene $A_4$ in the presence of glutathione and glutathione transferase π from placenta. The separation was carried out on a 250 x 4.6 mm $C_{18}$ Nucleosil column. Experimental details are described in the Materials and Methods section. (A) Incubation mixture; (B) incubation mixture supplemented with synthetic leukotriene $C_4$ (0.2 nmol).

radioactivity from labeled leukotriene $A_4$ with light absorbance at 280 nm in the 38-min peak. The ratio between radioactivity and $A_{280}$ was that expected for leukotriene $C_4$. Finally, in the absence of glutathione, no peak corresponding to leukotriene $C_4$ appeared in the chromatogram.

The steady-state kinetics of the conjugation of glutathione with leukotriene $A_4$ were studied with the three forms of human glutathione transferase. The concentration of leukotriene $A_4$ was varied between 6 and 60 μM at a fixed glutathione concentration of 5 mM. The latter value is representative of intracellular glutathione concentrations (21). It was found that under these conditions the kinetics could be described by the Michaelis-Menten equation. Table 1 gives the $K_m$ and $V_{max}$ values determined by non-linear regression analysis as well as the specific activities determined in the standard assay system. In addition to the values obtained with the natural substrate, data for the methyl ester of leukotriene $A_4$ are included. The concentration of this substrate was varied between 2 and 24 μM in the kinetic studies.

Table 1 shows that the near-neutral transferase has the highest $V_{max}$ value of the human isoenzymes with leukotriene $A_4$ as well as with leukotriene $A_4$

Table 1

Kinetic constants for three different types of human glutathione transferase using leukotriene $A_4$ and leukotriene $A_4$methyl ester as electrophilic substrate[a]

| Glutathione transferase | Leukotriene $A_4$[b] | | | Leukotriene $A_4$ methyl ester[c] | | |
|---|---|---|---|---|---|---|
| | Specific activity (nmol/min per mg) | $K_m$ (μM) | $V_{max}$ (nmol/min per mg) | Specific activity (nmol/min per mg) | $K_m$ (μM) | $V_{max}$ (nmol/min per mg) |
| Basic (α-ε) | 9 | 130 | 66 | 12 | 19 | 75 |
| Near-neutral (μ) | 44 | 160 | 180 | 210 | 19 | 550 |
| Acidic (π) | 2 | 190 | 24 | 22 | 23 | 77 |

[a]The kinetic data were obtained at 30°C and pH 7.0 in a reaction system containing 5 mM glutathione. The standard deviations were estimated as 30% of the values cited.
[b]The concentration of leukotriene $A_4$ was 35 μM. [c]The concentration of leukotriene $A_4$ methyl ester was 12 μM.

methyl ester. The catalytic efficiency, expressed as $V_{max}/K_m$, is also highest for the near-neutral transferase. No significant differences in the $K_m$ values of the three human isoenzymes were obtained. All of the kinetic parameters in Table 1 are comparable in magnitude to parameters determined with other substrates of human glutathione transferase (1).

The data obtained with leukotriene $A_4$ methyl ester can be compared with corresponding values available for isoenzymes of rat glutathione transferase (17). The $V_{max}$ values determined for the reaction catalyzed by the rat transferases range from 17 to 615 nmol x $min^{-1}$ x $mg^{-1}$ and the $K_m$ values vary between 2.3 and 15 μM. The most efficient rat isoenzyme, transferase 4-4, gave a $V_{max}$ value of 615 nmol x $min^{-1}$ x $mg^{-1}$ and a $K_m$ value of 11 μM, similar to the corresponding values of 550 and 19, respectively, determined for the human near-neutral transferase in the present study. Similarities between these two isoenzymes have earlier been described (1). The finding that the neutral methyl ester of leukotriene $A_4$ gives lower $K_m$ values than does the negatively charged leukotriene $A_4$ (Table 1) is probably a reflection of the hydrophobic nature of the active site of the transferases (1,6).

The present report shows that the human glutathione transferases are active in the conjugation of leukotriene $A_4$, and in view of the high intracellular concentration of the enzymes, their capacity is high *in vivo*. However, further studies are required to elucidate whether the reactions involving leukotriene $A_4$ catalyzed by cytosolic glutathione transferases merely reflect their general capacity to catalyze the conjugation of epoxides (6,22) or whether they have a more specific physiological significance.

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