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GLUTATHIONE *S*-TRANSFERASE (TRANSFERASE π) FROM HUMAN PLACENTA IS IDENTICAL OR CLOSELY RELATED TO GLUTATHIONE *S*-TRANSFERASE (TRANSFERASE ρ) FROM ERYTHROCYTES

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Glutathione *S*-transferase (RX: glutathione R-transferase, EC 2.5.1.18) from human placenta has been purified to homogeneity. This enzyme, transferase π, is an acidic protein (isoelectric point at pH 4.8) composed of two subunits. The molecular weights for the dimer and monomer were determined by independent methods as 47 000 and 23 400, respectively. These properties are not significantly different from those of glutathione *S*-transferase ρ from human erythrocytes. Antibodies to transferase π reacted with the enzyme from erythrocytes but not with the basic transferases α–ϵ and the neutral transferase μ isolated from human liver. Antibodies to the latter enzymes did not react with the transferase from placenta. Further similarities between transferases π and ρ appear in amino acid compositions, kinetic constants and substrate specificities. Both the placental and the erythrocyte enzyme have considerably higher activity with ethacrynic acid than any other of the human glutathione *S*-transferases. The glutathione *S*-transferase could be distinguished from two additional acidic glutathione-dependent enzymes, glyoxalase I and selenium-dependent glutathione peroxidase. It is concluded that transferase π from placenta is identical with or very closely related to transferase ρ from erythrocytes.

Introduction

Glutathione *S*-transferases (RX: glutathione R-transferase, EC 2.5.1.18) are assumed to play an important role in conjugation and detoxication of a multitude of endogenous or exogenous electrophiles in the cell [1–3]. In addition to their catalytic function, at least one of these enzymes in rat liver may serve as a binding protein, ligandin [4], and contribute to intracellular binding and transport of steroids, bilirubin, carcinogens and drugs. While present in various organisms, the glutathione *S*-transferases have been characterized most extensively in the rat [1]. Several forms of glutathione *S*-transferase have been isolated in pure form from rat liver [1,5]. There is evidence that the hepatic enzyme forms are present in other organs as well as in the liver, but also that forms of glutathione *S*-transferase existing in other organs are absent from the liver. For example, kidney [6], lung and blood [7] of the rat all appear to have glutathione *S*-transferases distinct from the hepatic enzymes, even if such additional forms have not yet been obtained in pure form and characterized.

In view of the importance of glutathione *S*-transferases in detoxication and their probable role in protection against chemical carcinogenesis, it is important to characterize these enzymes in human tissues. Five basic proteins exhibiting glutathione *S*-transferase activity were first isolated from human liver [8]. The enzymes were referred to as transferases α, β, γ, δ and ϵ, and they were found to have almost identical physical, catalytic and immunological properties [8]. Such transferases with basic isoelectric points were later purified in other laboratories as well [9,10]. Recently, an additional hepatic

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enzyme with a 'neutral' isoelectric point (at pH 6.0–6.5) was detected in some human subjects [11]. This neutral protein has been prepared in pure form and denoted as transferase μ [10]. Finally, small amounts of an acidic transferase have been demonstrated in human liver [12,13]. This form appears to originate from the liver tissue although being very similar to transferase ρ, an enzyme earlier isolated from human erythrocytes [14]. In addition, a pure glutathione *S*-transferase has been isolated from human placenta by Guthenberg et al. [15]. The enzyme from this tissue was also studied in crude form by Polidoro et al. [16]. The placental enzyme is an acidic protein, which will be referred to as transferase π (cf. Ref. 1), in spite of the fact that it is very similar to and may be identical with the erythrocyte enzyme (transferase ρ). While present only in small amounts in adult liver, an acidic transferase in fetal liver, which reacts with antibodies to transferase π, accounts for about half of the activity (with 1-chloro-2,4-dinitrobenzene as electrophilic substrate) in the fetal tissue [17].

Thus, three major groups of glutathione *S*-transferases have been identified in human tissues: basic transferases (α–ϵ), a neutral transferase (μ), and acidic transferases (ρ and π).

In view of the special role of the placenta in the metabolism of xenobiotics as well as of steroids and other endogenous compounds [18], glutathione *S*-transferase has been investigated in the human tissue. The placental transferase may be expected to have a crucial role in the protection of the fetus against electrophiles. As a representative of human transferases, it also has the advantage of being derived from a tissue that can be obtained in large quantities. The present study was undertaken to characterize the human placental enzyme, transferase π, and to clarify its relationship to the acidic transferase from erythrocytes by comparison of their molecular properties.

Materials and Methods

Enzyme preparations. Glutathione *S*-transferase π was purified to homogeneity from full term human placentas as described previously [15,19]. Glutathione *S*-transferase from human erythrocytes (form ρ) was partially purified by chromatography of a stroma-free hemolysate on CM-cellulose. Antibodies to the placental enzyme were raised in rabbits.

Assays of glutathione S-transferase activities. The reaction with glutathione and the following substrates were measured at 30°C using published methods: 1,2-dichloro-4-nitrobenzene [20]; 1-chloro-2,4-dinitrobenzene, ethacrynic acid, and *trans*-4-phenyl-3-buten-2-one [21]; sulfobromophthalein [22]; 1,2-epoxy-3-(*p*-nitrophenoxy)propane [23]; *p*-nitrophenyl acetate [24]; cumene hydroperoxide and H_2O_2 [25]; [^{3}H]benzo(*a*)pyrene-4,5-oxide [26]; [^{3}H]-styrene oxide [27].

Regression analysis. Steady-state kinetic data were analyzed by weighted nonlinear regression. The weights, $w_i \propto v_i^{-\alpha}$ [28] were determined from an analysis of residuals [29], yielding $\alpha = 2.0$.

Materials. All materials were standard commercial products.

Methods. The molecular weight was determined by a combination of gel filtration and ultracentrifugation [30]. The subunit molecular weight was estimated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis [31].

Results

Molecular properties. The molecular weight of glutathione *S*-transferase purified from human placenta (transferase π) was estimated as 47 000 (Table I). In the presence of SDS it dissociated into apparently identical subunits of 23 400 molecular weight, as determined by polyacrylamide gel electrophoresis. The subunits of transferase π clearly had a lower molecular weight than those of the basic transferases α–ϵ (Guthenberg, C., Warholm, M. and Mannervik, B., unpublished data). Likewise, the subunits of glutathione *S*-transferase from erythrocytes (transferase ρ) were shown to have a lower molecular weight than the basic liver enzymes (transferases α–ϵ) [14]. The isoelectric point, determined by isoelectric focusing at 4°C in a gradient covering pH 3.5–10, was at pH 4.8. When the focusing was performed in a pH gradient covering a more narrow interval, the estimate of the isoelectric point was somewhat lower (at about pH 4.6). In all these respects the transferase from placenta is very similar to the transferase from erythrocytes (cf. Table I).

Immunological properties. Antibodies have been

TABLE I

PROPERTIES OF GLUTATHIONE *S*-TRANSFERASE FROM HUMAN PLACENTA (TRANSFERASE π) AND HUMAN ERYTHROCYTES (TRANSFERASE ρ)

n.d., not determined. CDNB, 1-chloro-2,4-dinitrobenzene.

Property	Transferase π [a]	Transferase ρ [b]
Molecular weight	47 000	47 500
Subunit molecular weight	23 400	24 000
Isoelectric point	4.8	4.5
Precipitate with antibodies raised against:		
transferases α–ϵ	–	–
transferase μ	–	n.d.
transferase π	+	+ [a]
Kinetic constants for CDNB and GSH:		
k_{cat} (min^{-1})	19 600 ± 2 300 [c] (at 30°C)	4 450 (at 25°C)
K_m^{CDNB} (mM)	2.1 ± 0.3	1.0
K_m^{GSH} (mM)	0.5 ± 0.1	n.d.

[a] Present study.
[b] Data reported by Marcus et al. [14].
[c] Based on protein determination by a microbiuret method [32].

raised in rabbits to the three major types of human transferases (Guthenberg, C., Warholm, M. and Mannervik, B., unpublished data). Each antiserum gave a precipitin line with its homologous but not heterologous transferases (Table I). Specifically, antibodies to transferase π did not precipitate the basic (α–ϵ) or the neutral (μ) transferases. Neither did antibodies to the latter transferases give precipitates with transferase π. Marcus et al. [14] found that transferase ρ did not react with antibodies to the basic transferases of human liver (α–ϵ), nor did it react with the antibodies to the rat liver transferases A, B and C. The same lack of reactivity of transferase π with antibodies to transferases A, B and C from rat liver was found in the present investigation. However, when antibodies to transferase π were tested in parallel against the placental enzyme and the partially purified transferase ρ from human erythrocytes, transferase ρ gave a precipitin line which merged without spurs with that of transferase π. The continuous precipitate indicated immunological identity between the two transferases.

The IgG fraction of the antiserum raised against transferase π was obtained by biospecific chromatography on protein A-Sepharose CL-4B. The purified antibodies were used for immunotitration of transferase π and the partially purified transferase ρ. Loss of about 98% of the activity of transferase π was found after incubation with the antibodies for 20 h. A similar, but somewhat smaller effect (about 80% inhibition) was obtained when the partially purified transferase from erythrocytes was incubated with the antibodies to transferase π.

Amino acid composition. The amino acid composition of transferase π was determined in two different preparations of the enzyme. Table II shows means of the two determinations, except for tryptophan which was determined in one preparation only. The differences between the two analyses in the figures for the other amino acids correspond to less than one residue per enzyme molecule for all amino acids, with the exception of alanine which had a difference of 2.6. The analyses of transferase ρ [14] are listed for comparison. The largest differences between the enzyme from the two sources were noted for serine, glutamic acid and glycine. Whether these are significant or not is difficult to decide since the analyses have been made in different laboratories. As a quantitative measure of similarity between the amino acid compositions of transferases π and ρ the difference index of Metzger et al. [34] was used. A value of 5 was obtained, which is of the same magnitude as values ob-

TABLE II

AMINO ACID COMPOSITION OF GLUTATHIONE *S*-TRANSFERASE FROM HUMAN PLACENTA (TRANSFERASE π) AND HUMAN ERYTHROCYTES (TRANSFERASE ρ)

n.d., not determined.

Amino acid	No. of residues		
	Transferase π [a]	Transferase ρ [b]	
	HCl	HCl	MSA
Asx	42.1	45.3	47.1
Thr	18.4	19.5	20.1
Ser	20.2	27.1	24.6
Glx	49.2	55.8	53.6
Pro	23.6	26.8	25.8
Gly	37.4	49.6	45.5
Ala	32.0	35.0	34.7
Cys	8.0	n.d.	n.d.
Val	28.1	29.8	25.1
Met	4.8	3.0	3.3
Ile	13.5	8.3	13.1
Leu	62.4	58.7	62.3
Tyr	24.0	19.9	21.7
Phe	13.8	15.1	16.0
His	3.9	4.8	5.6
Lys	24.2	23.9	22.3
Trp	3.4	n.d.	n.d.
Arg	15.9	17.3	16.4

[a] Present study. Two samples from different preparations were hydrolyzed in 6 M HCl. Average of 24 and 72 h values except for Thr and Ser, which were values extrapolated to zero time, and Trp, which was determined separately [33].

[b] Data reported by Marcus et al. [14]. Hydrolysis in 6 M HCl for 48 h or in 4 M methanesulfonic acid for 24 h.

tained when different analyses of the same protein are compared.

Kinetic properties. The steady-state kinetics of the reaction between 1-chloro-2,4-dinitrobenzene and glutathione could be approximated by the rate equation for a simple sequential mechanism. The kinetic constants for transferase π, estimated by weighted nonlinear regression analysis, are given in Table I. The kinetic constants determined for transferase ρ [14] are somewhat lower, but it was not stated whether these values have been obtained at a finite glutathione concentration or by extrapolation to infinite concentration. At 1 mM glutathione the apparent k_{cat} (i.e., $V/[E]_{tot}$) is 7500 min^{-1} for transferase π. In view of this uncertainty, the possible differences in design and analyses of the experiments, and the differences in temperature in the assays (25 vs. 30°C), it cannot be concluded that the two transferases differ significantly in their kinetic constants.

Substrate specificity. Table III shows the results of assays of transferase π with various substrates. Four of the substrates have been investigated with transferase ρ [14] and are included for comparison. It should be noted that, as in the kinetic studies, the temperatures of the assay systems are different. For both enzymes ethacrynic acid is the second-best substrate. This finding is diagnostic, because the specific activity of the basic transferases (α–ϵ) with this substrate is in the range of 0.017–0.044 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ [8]. In general terms, the substrate specificities of transferases π and ρ are very similar to the extent that they have been compared (Table III).

TABLE III

SUBSTRATE SPECIFICITIES OF GLUTATHIONE *S*-TRANSFERASE FROM HUMAN PLACENTA (TRANSFERASE π) AND HUMAN ERYTHROCYTES (TRANSFERASE ρ)

n.d., not determined.

Substrate	Specific activity ($\mu mol \cdot min^{-1} \cdot mg^{-1}$)	
	Transferase π [a]	Transferase ρ [b]
1-Chloro-2,4-dinitrobenzene	105	66
1,2-Dichloro-4-nitrobenzene	0.11	0.025
Sulfobromophthalein	<0.002 [c]	n.d.
Ethacrynic acid	0.86	2.9
trans-4-Phenyl-3-buten-2-one	0.01	n.d.
Styrene-7,8-oxide	0.07	n.d.
1,2-Epoxy-3-(*p*-nitro-phenoxy)-propane	0.37	n.d.
Benzo(*a*)pyrene-4,5-oxide	0.13	n.d.
p-Nitrophenylacetate	0.19	0.20
Cumene hydroperoxide	0.03	n.d.
Hydrogen peroxide	<0.01 [c]	n.d.

[a] Present study. Activities measured at 30°C.

[b] Data from Marcus et al. [14]. Activities measured at 25°C.

[c] The figures given represent the detection limit in the assay used.

Discussion

Several observations indicated extensive similarities between human glutathione *S*-transferase from placenta (transferase π) and erythrocytes (transferase ρ). However, in the first place, it was necessary to evaluate the obvious possibility that the 'placental' enzyme was derived from residual blood in the tissue. Therefore, the absorbance at 410 nm was determined in the crude supernatant fraction of homogenates of placentas in order to obtain an estimate of the amount of contaminating blood. On the assumption that the absorbance was contributed exclusively by the Soret band of hemoglobin an upper limit of the amount of blood present could be calculated. This value in combination with the known concentration of transferase ρ in blood (1.2 mg/100 mg packed erythrocytes [14]) showed that <1% of the glutathione *S*-transferase activity in placenta originated from blood. Only a single form of glutathione *S*-transferase has been detected after isoelectric focusing of crude cytosol fractions of several term placentas [15]. Consequently, the question arises whether or not the enzyme of the placental tissue is identical with the enzyme in erythrocytes.

It can also be concluded that the glutathione *S*-transferase isolated from placenta is distinct from two additional glutathione-dependent enzymes in the same organ. One is glyoxalase I which has similar molecular weight (M_r 46 000) and isoelectric point (at pH 4.8) [35]. This enzyme is removed during purification and its absence established by use of antibodies raised against glyoxalase I. A second enzyme is the selenium-dependent glutathione peroxidase, which is another acidic protein (isoelectric point at pH 4.8) present in human placenta [36]. This enzyme too is absent from the purified glutathione *S*-transferase as evidenced by the lack of activity with H_2O_2 as substrate for the glutathione peroxidase reaction (Table III) [37]. The activity with cumene hydroperoxide (Table III) shows that transferase π is responsible for the low non-selenium-dependent glutathione peroxidase activity of placenta.

The data presented in Tables I–III show clearly extensive similarities between transferase π and transferase ρ. The comparison includes both structural and catalytic properties. Especially noteworthy are the immunological properties and the high catalytic activities with 1-chloro-2,4-dinitrobenzene and ethacrynic acid, which on the one hand show similarities between transferases π and ρ, and on the other hand discriminate these enzyme forms from other glutathione *S*-transferase in human tissues. The minor differences between transferases π and ρ exhibited in Tables I–III are not sufficiently well established to permit the conclusion that the enzymes are non-identical. The properties that would suggest that the enzymes are distinct are the differences in amino acid contents of serine, glutamic acid, and glycine (Table II) and the ratios of the specific activities obtained with different substrates (e.g., 1-chloro-2,4-dinitrobenzene vs. ethacrynic acid). If some glutathione were remaining after the purification of transferase ρ [14] the differences in the amino acid analyses would be partly explained. Nevertheless, the major part of the data would suggest that the enzymes are identical. An additional property showing the similarities appears in the binding of bilirubin. In contrast to the basic transferases (α–ϵ) and transferase μ, which all bind bilirubin tightly, transferase π has a very poor affinity for this ligand (Guthenberg, C., Mannervik, B., Sjöholm, I. and Warholm, M., unpublished data). A poor affinity for bilirubin has also been demonstrated for the enzyme from erythrocytes [14].

The reason for not concluding definitely that transferase π from placenta is identical with transferase ρ from erythrocytes is the finding that in some fetal livers two very similar acidic glutathione *S*-transferases have been separated [17]. Similarly, in adult liver and erythrocytes more than one acidic transferase may occur [12,13]. Likewise, the basic transferases (α–ϵ), although very similar [8], appear to exist as more than one genetically determined form. The widely different activity profiles after isoelectric focusing [11] would be very difficult to rationalize if deamidation [8] were the sole reason for the occurrence of multiple forms.

At the present state of knowledge we can, therefore state that glutathione *S*-transferase π from human placenta is closely related to, and may be identical with, glutathione *S*-transferase ρ from human erythrocytes.

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