Structural evidence for three different types of glutathione transferase in human tissues

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Cytosolic glutathione transferase was purified from human placenta and human liver. Three different forms of the enzyme were obtained, the acidic (π), the near-neutral (μ), and the basic (α-ε) forms; two had free α-amino groups (π, μ) and one had a blocked α-amino group (α-ε). N-terminal sequence analyses and total compositions gave clearly different results for each form, although transferases π and μ showed 35% sequence homology in the N-terminal regions, with a 1-residue shift in starting position. Consequently, the proteins are concluded to be products of three discrete but related genes.

Glutathione transferase *Amino acid sequence analysis* *Human isoenzyme* *N-terminal processing differences* *Sequence alignment*

1. INTRODUCTION

Glutathione transferases (EC 2.5.1.18) are a family of enzymes that catalyze the conjugation of glutathione with compounds that carry an electrophilic center. This ability makes them important in the detoxication of endogenous and exogenous substances [1,2]. Multiple forms of glutathione transferase exist in many species [2,3]. The cytosol fraction of mammalian liver contains isoenzymes with distinctly different properties. In addition, the microsomes have a separate type of the enzyme [4]. Furthermore, the distribution of isoenzymes varies considerably between tissues [3,5]. Cytosolic glutathione transferases are dimeric proteins with subunit sizes of approx. M_r 25000 [2].

In human tissues, the cytosolic isoenzymes have been divided into 3 major groups on the basis of their isoelectric points [6], the basic, the near-neutral and the acidic forms. The 3 groups of enzyme show overlapping specificities, but certain substrates can be used for discrimination between the isoenzymes. Thus, the basic group is highly active with organic hydroperoxides, the near-neutral group with certain epoxides, and the acidic group with ethacrynic acid [6]. Structural data have recently become available for cytosolic glutathione transferase from human placenta [7]. However, lack of information on the other human enzyme forms has prevented structural comparisons. Here, the 3 cytosolic human glutathione transferases are analyzed and structurally distinguished.

2. MATERIALS AND METHODS

Acidic glutathione transferase (π) was prepared from human placenta [8]; near-neutral (μ) and basic (α-ε) transferases from human liver [9]. All enzyme preparations were homogeneous as judged by SDS-polyacrylamide slab gel electrophoresis. The proteins were reduced with dithioerythritol (100% molar excess over protein cysteine/half-cystine residues) and carboxymethylated with ^{14}C-labelled iodoacetate (20% molar excess over total reagent and protein SH/SS). Deblocking of the N-terminus of the basic type of transferase was attempted by treatment with 6 M HCl for 1 min at 100°C under N_2, followed by immediate cooling and lyophilization. Acid treatment has been found

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in some cases selectively to remove *N*-acyl groups from serine residues [10].

Amino acid analysis was carried out on a Beckman 121M analyzer after hydrolysis for 24 h at 110°C in evacuated tubes with 6 M HCl, 0.5% phenol.

Liquid phase sequencer degradations were performed in Beckman 890D and modified [11,12] 890C instruments. Phenylthiohydantoin derivatives were identified by reverse-phase high-performance liquid chromatography [13]. ^{14}C-carboxymethylcysteine derivatives were also monitored by liquid scintillation counting.

3. RESULTS

Table 1 shows amino acid compositions of the 3 types of human glutathione transferase. The analyses demonstrate significant differences. In particular, none of the 3 forms has an amino acid composition consistent with the possibility that the proteins could be derived from each other by limited proteolysis. For example, Val is most abundant in the acidic type, Phe in the near-neutral, and Ile in the basic type. Consequently, the experimental findings suggest that the primary structures of the 3 types of enzyme are clearly different, excluding precursor-product relationships.

Table 1

Results of amino acid analysis of three human glutathione transferases

Amino acid	Enzyme form		
	Acidic (π)	Near-neutral (μ)	Basic (α-ϵ)
Cys	4.0	4.6	0.8
Asx	22.1	25.3	18.8
Thr	9.5	7.2	4.8
Ser	13.4	16.7	14.1
Glx	25.7	27.2	26.5
Pro	13.4	15.6	12.1
Gly	21.5	22.2	15.6
Ala	16.6	11.9	14.4
Val	13.9	5.6	9.4
Met	3.3	5.3	5.2
Ile	7.5	9.7	13.1
Leu	32.4	26.4	26.3
Tyr	12.2	12.5	9.4
Phe	7.4	12.2	9.1
Lys	13.0	22.8	20.3
His	2.7	6.9	3.0
Arg	8.3	12.2	10.6

The values listed are molar ratios estimated after acid hydrolysis for 24 h. Cysteine was analyzed after carboxymethylation (π and α-ϵ) or taken from [6] (μ). The calculations were based on assumed subunit sizes of M_r 25000 for the acidic, M_r 26300 for the near-neutral, and M_r 23500 for the basic transferase

Table 2

N-terminal amino acid sequences of the two human glutathione transferases with free α-amino groups

Position	Enzyme form	
	Acidic (π)	Near-neutral (μ)
1	Pro (11)	Pro (7)
2	Pro (12)	Met (6)
3	Tyr (13)	Ile (7)
4	Thr (10)	Leu (6)
5	Val (14)	Gly (7)
6	Val (14)	Tyr (6)
7	Tyr (11)	Trp (5)
8	Phe (11)	Asp (4)
9	Pro (10)	Ile (6)
10	Val (10)	Arg (4)
11	Arg (6)	Gly (3)
12	Gly (6)	Leu (4)
13	Arg (4)	Ala (6)
14	Cys (5)	His (+)
15	Ala (8)	Ala (6)
16	Ala (9)	Ile (4)
17	Leu (7)	Arg (3)
18	Arg (4)	Leu (5)
19	Met (6)	Leu (4)
20	Leu (7)	Leu (5)
21	Leu (9)	Glu (2)
22	Ala (7)	Tyr (2)
23	Asp (6)	Thr (+)
Repetitive yield:	96%	96%

Values within parentheses show nmol recovered, as estimated by high-performance liquid chromatography. The proteins were carboxymethylated and Cys in position 14 of π was recovered as Cys(Cm). The dashed lines indicate a structural relationship in an alignment that shows 8 positions (35%) with residue identities and suggests a 1-residue shift in N-terminal starting position

The N-terminal amino acid sequences of the acidic (π) and the near-neutral (μ) glutathione transferases were determined by liquid phase sequencer degradations (table 2). The basic type of transferase did not yield any phenylthiohydantoin derivative, suggesting the presence of a blocked N-terminal amino group. An attempt to deblock the peptide by limited hydrolysis with 6 M HCl at 100°C for 1 min liberated a structure with an N-terminal serine. Further steps of sequence degradation were difficult to interpret since the acid treatment also caused partial hydrolysis of the polypeptide chain, giving a high background, but lysine was tentatively identified in at least one of the following two positions.

4. DISCUSSION

Structural data for the different classes of human glutathione transferase have been lacking, although an N-terminal amino acid sequence of placental glutathione transferase was recently reported [7]. The amino acid sequence of the N-terminal region of the acidic transferase (π) given in table 2 is in full agreement with that report. The present investigation also characterizes the same region of an additional form of human glutathione transferase (μ), and, most significantly, shows that the two sequences are clearly different. Nevertheless, they are still homologous, exhibiting 35% positional identity when shifted by one position, as shown in table 2. The N-terminal serine residue found after deblocking of the basic form is likely to represent a naturally acylated serine structure. Such structures are common in proteins [14]. Consequently, the basic form represents a third isoenzyme with an amino acid sequence distinct from those of the two others. It also demonstrates differences in N-terminal processing of the isoenzymes.

The present results give direct support to the suggestion that 3 different types of the enzyme exist in human tissues. The previous suggestion came from physical properties, such as apparent M_r, isoelectric point, and circular dichroism spectra, as well as from substrate specificities and reactions with antibodies specific for the different transferases [6]. The sequence information and the amino acid compositions lead to the conclusion that the 3 types of enzyme are products of 3 discrete genes. Recent genetic studies of human glutathione transferases [15,16] support this conclusion.

The demonstration of different structures of the human transferases is significant in view of individual differences in isoenzyme occurrence [15–18]. For example, the near-neutral type (transferase μ) is expressed only in 60% of the individuals examined [6]. It has been suggested that the absence of transferase μ may lead to increased sensitivity to certain mutagenic and carcinogenic epoxides in view of the high activity of this isoenzyme with such substrates [6]. Further, fetal liver differs from the adult tissue in the presence of a major acidic isoenzyme [19]. These differences in isoenzyme compositions should have significant implications for protection against toxic chemical substances.

The N-terminal sequence of the human near-neutral transferase (μ), exhibits strict homology with those of mouse transferases GT-8.7 and GT-9.3 [20], of a bovine transferase [21], and of rat transferases 3-3 ('A') and 4-4 ('X') [3,22] (see [23] for new nomenclature). The amino acid sequence of rat glutathione transferase 1-1, deduced from the structure of cloned cDNA [24–26], has the N-terminal structure Met-Ser-Gly-Lys. Excluding the methionine residue, this structure is similar to that of the deblocked human basic transferase determined in this investigation. Consequently, this isoenzyme is apparently processed by removal of the initiator methionine and by subsequent N-terminal acylation. In conclusion, the present results characterize 3 types of cytosolic human glutathione transferase, show similarities between two forms of the enzyme (π and μ), and reveal that the multiple forms have acquired considerable differences during evolution.

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