# Human glutathione transferase A4-4: an Alpha class enzyme with high catalytic efficiency in the conjugation of 4-hydroxynonenal and other genotoxic products of lipid peroxidation

Ina HUBATSCH, Marianne RIDDERSTRÖM and Bengt MANNERVIK[1]

Department of Biochemistry, Uppsala University, Biomedical Center, Box 576, S-751 23 Uppsala, Sweden

A sequence encoding a novel glutathione transferase, GST A4-4, has been identified in a human fetal brain cDNA library. The protein has been produced in *Escherichia coli* after optimization of the codon usage for high-level heterologous expression. The dimeric protein has a subunit molecular mass of 25 704 Da based on the deduced amino acid composition. Human GST A4-4 is a member of the Alpha class but shows only 53 % amino acid sequence identity with the major liver enzyme GST A1-1. High catalytic efficiency with 4-hydroxyalkenals and other cytotoxic and mutagenic products of radical reactions and lipid peroxidation is a significant feature of GST A4-4. The $k_{cat}/K_m$ values for 4-hydroxynonenal and 4-hydroxydecenal are $> 3 \times 10^6$ $M^{-1} \cdot s^{-1}$, several orders of magnitude higher than the values for conventional GST substrates. 4-Hydroxynonenal and other reactive electrophiles produced by oxidative metabolism have been linked to aging, atherosclerosis, cataract formation, Parkinson's disease and Alzheimer's disease, as well as other degenerative human conditions, suggesting that human GST A4-4 fulfills an important protective role and that variations in its expression may have significant pathophysiological consequences.

## INTRODUCTION

A large number of degenerative diseases are associated with cellular oxidative damage. The tissues of aerobic organisms are constantly being exposed to reactive oxygen species, and their longevity may be dependent on components of the antioxidant system, such as superoxide dismutase and catalase [1]. Oxidative events give rise to organic hydroperoxides, activated alkenes, epoxides and quinones, which are all electrophiles that may cause toxicity, DNA damage and cancer. It is well established that enzyme-catalysed reactions involving glutathione inactivate such products of oxidative metabolism by conjugation or reduction [2]. A recent example is the conjugation of *o*-quinones derived from catecholamines catalysed by glutathione transferases, protective reactions that may counteract the development of Parkinson's disease, schizophrenia and other degenerative disorders [3,4]. Lipid peroxidation gives rise to unsaturated hydrocarbons and reactive aldehydes with 4-hydroxyalkenals as prominent examples of toxic products [5]. 4-Hydroxynonenal (HNE), which is an important product of peroxidative degradation of arachidonic acid, has been widely used as a biomarker for oxidative damage in tissues. For example, HNE was found to be bound to proteins in substantia nigra of patients with Parkinson's disease at a significantly higher concentration than to corresponding proteins of normal subjects [6]. HNE also appears to mediate amyloid $\beta$-peptide-induced apoptotic cell death relevant to Alzheimer's disease [7,8].

An important metabolic route for detoxification and disposition of activated alkenes is conjugation with glutathione (GSH) [9]. The present paper reports the molecular cloning, heterologous expression and functional characterization of a novel human class Alpha GST displaying high catalytic efficiency in the conjugation of HNE and other activated alkenes. The enzyme is named human GST A4-4 in accordance with the guidelines for GST nomenclature [10].

## EXPERIMENTAL

### Materials

Oligonucleotides were custom synthesized by Operon Technologies Inc. (Alameda, CA, U.S.A.). The expression vector pKK223-3 was obtained from Pharmacia Biotech (Uppsala, Sweden). The vector was modified by digestion with *Acc*I to eliminate the second restriction site for *Sal* I and called pKK-D [11]. $\Delta^5$-Androstene-3,17-dione was provided by Professor Paul Talalay, The Johns Hopkins University School of Medicine, Baltimore, MD, U.S.A., 2-cyano-1,3-dimethyl-1-nitrosoguanidine by Professor David Jensen, Jefferson Medical College, Philadelphia, PA, U.S.A., and the 4-hydroxyalkenals by the late Professor Hermann Esterbauer, University of Graz, Austria. Other materials were obtained from commercial suppliers.

### Cloning of cDNA encoding GST A4-4

cDNA from a lambda phage lysate (10 ml, $1.4 \times 10^{11}$ plaque-forming units) of a human fetal brain library (Stratagene, La Jolla, CA, U.S.A.) was purified using LambdaSorb phage adsorbent according to the manufacturer's instructions. A substantia nigra cDNA library from adult human brain was obtained from the same supplier.

Abbreviations used: GST, glutathione transferase; HNE, 4-hydroxy-2,3-*trans*-nonenal; CDNB, 1-chloro-2,4-dinitrobenzene.

[1] To whom correspondence should be addressed.

The nucleotide sequence data reported in this paper appear in the EMBL Nucleotide Sequence Database under the accession number Y13047.

A 5′ primer was designed for optimal expression in *Escherichia coli* [12] by randomizing silent positions in some of the triplets coding for the first 14 amino acids after the initiator methionine. The 5′ and 3′ primers GSTA4START and GSTA4STOP respectively, had the following sequences: 5′-TAA TCT A GAATTC ATG GCD GCD AGR CCN AAR CTB CAY TAY CCN AAY GGH AGR GGH CGB ATG GAG TCC GTG AGA-3′ and 5′-ATA GAATTCTGCAG TTA TTA TGG CCT AAA GAT GTT GTA GAC GGT-3′. The underlined bases define restriction sites for endonucleases *Eco*RI and *Pst*I, used for cloning of the PCR product.

For PCR amplification, 5 $\mu$l of the cDNA library was used in a 100 $\mu$l reaction system consisting of 10 mM Tris/HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 0.2 mM of each dNTP and 0.8 $\mu$M of the 5′ and 3′ primers. After incubation at 95 °C for 10 min, followed by a decrease to 70 °C, 2.5 units of *Taq* DNA polymerase were added. The PCR involved 30 cycles of 95 °C for 1 min, 55 °C for 2 min and 72 °C for 2 min.

The primer GSTA4START is degenerate, and the native 5′ sequence reported (EMBL Nucleotide Sequence Database accession number Y13047) was determined by use of oligonucleotide primers complementary to the upstream 5′ noncoding sequence and an internal sequence.

### Protein expression and purification

The library of variant cDNA sequences resulting from the randomized 5′ primer GSTA4START was ligated into the expression vector pKK-D [11] and used for transformation of *E. coli* JM109 and heterologous expression of the protein. For small-scale expression (100 ml bacterial cultures) different clones of *E. coli* JM109 carrying the expression construct were grown in 2TY medium at 37 °C in the presence of 80 $\mu$g/ml ampicillin. Induction with 0.3 mM isopropyl $\beta$-D-thiogalactoside was made when $A_{600}$ reached about 0.2. The cells were harvested and then lysed by sonication. The supernatants were assayed for 1-chloro-2,4-dinitrobenzene (CDNB) activity [13] and the clone showing the highest activity was chosen for large-scale expression. The enzyme was purified by affinity chromatography on GSH-Sepharose 6B; elution was effected by 5 mM *S*-hexylglutathione. For removal of *S*-hexylglutathione the eluate was subsequently dialysed, concentrated by ultrafiltration and subjected to gel filtration over a PD-10 column (Pharmacia Biotech).

The absence of unwanted mutations in the DNA sequence of the clone pKHA4 chosen for large-scale expression was verified.

### Kinetic measurements

Assays with different substrates commonly employed for characterizing GSTs were performed at 30 °C under standard conditions [13]; for the GSH, conjugation of hexenal and nonenal $\epsilon_{224} = 19.5\ mM^{-1}\cdot cm^{-1}$ and $\epsilon_{225} = 19.2\ mM^{-1}\cdot cm^{-1}$ respectively, were used. The 1,3-bis(2-chloroethyl)-1-nitrosourea denitrosation activity was analysed as described previously [14].

## RESULTS

### Cloning of cDNA encoding human GST A4-4

The amino acid sequences FEEEFLETREQYEKMQKDGHL-LFGQVPL and IPTIKKFL, corresponding to residues 30–57 and 191–198 respectively in the mouse GST A4-4 subunit [15], were used to search for similarities in the GenBank/EMBL Database of expressed sequence tags. The identified nucleotide sequences with similarities to the 5′ and 3′ parts of mouse and rat GST A4 cDNA [15,16] were used for the design of specific PCR primers. [The accession numbers in the GenBank/EMBL Database for the expressed sequence tags covering 3′ or 5′ regions of the isolated GST A4-4 cDNA are N40018, W56000, R66897, R63041 (3′), and H10548, H22595 (5′) respectively.] A human fetal brain cDNA library was analysed and a PCR product of 669 bp was cloned and sequenced (deposited in the EMBL Nucleotide Sequence Database with accession number Y13047). The isolated cDNA encoded a protein subunit (hGSTA4) of 222 amino acids (Figure 1). The calculated subunit molecular mass (including the methionine at position 1) is 25704 Da.

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          1                                                  50
hGSTA4    MAARPKLHYP NGRGRMESVR WVLAAAGVEF DEEFLETKEQ LYKLQDGNHL
mGSTA4    ...K...Y.F ........I. .L........ E......R.. YE.M.KDG..
rGSTA4    .EVK...Y.F Q.......I. .L..T..... E......R.. YE...KDGC.
hGSTA1    ..EK.....F .A......T. .L........ E.K.IKSA.D .D..RNDGY.
                   †*  ****                              †
                         †

          51                                                 100
hGSTA4    LFQQVPMVEI DGMKLVQTRS ILHYIADKHN LFGKNLKERT LIDMYVEGTL
mGSTA4    ..G...L... ...M.T...A ..S.L.A.Y. .Y..D....V R....AD..Q
rGSTA4    ..G...L... ...L.T...A ..S.L.A.Y. .Y..D....V R....AD..Q
hGSTA1    M......... .........A ..N...S.Y. .Y..DI...A .....I..IA
              †            ††

          101                                                150
hGSTA4    DLLELLIMHP FLKPDDQQKE VVNMAQKAII RYFPVFEKIL RGHGQSFLVG
mGSTA4    ..MMMIAVA. .KTPKEKEES YDLILSR.KT .......... KD..EA....
rGSTA4    ..MMMI.GA. .KA.QEKEES LALAVKR.KN .......... KD..EA....
hGSTA1    ..G.MILLL. VCP.EEKDAK LALIKE.IKN ....A...V. KS...DY...
          †  *  ** * *                     †
             †

          151                                                200
hGSTA4    NQLSLADVIL LQTILALEEK IPNILSAFPF LQEYTVKLSN IPTIKRFLEP
mGSTA4    ....W..IQ. .EA..MV..L SAPV..D..L ..AFKTRI.. .....K..Q.
rGSTA4    ....W..IQ. .EA..MV..V SAPV..D..L ..AFKTRI.. .....K..Q.
hGSTA1    .K..R..IH. VELLYYV..L DSSLI.S..L .KALKTRI.. L..V.K..Q.


          201                  222
hGSTA4    GSKKKPPPDE IYVRTVYNIF RP
mGSTA4    ..QR.....G P..EV.RIVL KF
rGSTA4    ..QR.....G H..DV.RTVL KF
hGSTA1    ..PR...M.. KSLEEARK.. .F
                  *     *  *   *  *
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**Figure 1 Comparison of the human GST A4-4 (hGSTA4) amino acid sequence with sequences of other class Alpha GSTs**

Identities are indicated by dots. mGSTA4, mouse GST A4-4 (GST 5.7) [15]; rGSTA4, rat GST A4-4 (GST 8-8) [16]; hGSTA1, human GST A1-1 [13]; amino acids assigned to the G-site (†) or H-site (*) of the human GST A1-1 are labelled.

Using the PCR primers GSTA4START and GSTA4STOP, a corresponding GST sequence was also amplified and sequenced from a substantia nigra cDNA library from adult human brain, demonstrating that the expression of the enzyme is not restricted to fetal tissues.

### Expression of the recombinant protein

In order to optimize the cDNA sequence for expression in *E. coli* [12], the PCR primer for the 5′ region was degenerate and contained silent mutations, such that a library of theoretically about 750000 variant sequences could be obtained. The first 15 codons of the clone pKHA4, chosen for large-scale expression, differ from the wild-type sequence in ten silent positions: ATG GCG GCG AGG CCA AAG CTT CAC TAT CCG AAT GGA AGG GGT CGC (altered bases underlined).

From a 3 litre bacterial culture of the selected expression clone, 40 mg of recombinant human GST A4-4 was recovered in pure form. The isoelectric point was determined as 8.2. The molecular mass estimated from SDS/PAGE and amino acid analysis of the hydrolysed protein were in agreement with the calculated molecular mass and the deduced amino acid sequence.

**Table 1 Specific activities of recombinant human GST A4-4 with different substrates compared with values for other class Alpha GSTs**

Assay conditions are described in the Experimental section

| Substrate (mM) | GSH (mM) | Specific activity ($\mu$mol · min$^{-1}$ · mg$^{-1}$) | | |
|---|---|---|---|---|
| | | Human GST A4-4 | Rat GST A4-4* | Human GST A1-1 |
| 4-Hydroxydecenal (0.1) | 0.5 | 159 ± 10 | | 6.5 |
| 4-Hydroxynonenal (0.1) | 0.5 | 189 ± 9 | 170 | 5.6 |
| Nonenal (0.1) | 0.5 | 205 ± 22 | | |
| 4-Hydroxyhexenal (0.1) | 0.5 | 16.9 ± 0.44 | | 1.0 |
| Hexenal (0.1) | 0.5 | 13.3 ± 0.6 | | |
| 4-Hydroxypentenal (0.1) | 0.5 | 7.4 ± 0.85 | 50 | 0.6 |
| Crotonaldehyde (0.1) | 1 | 7.3 ± 0.1 | 17 | < 0.1† |
| Acrolein (0.1) | 0.5 | 18 ± 2 | 40 | 0.9† |
| CDNB (1.0) | 1 | 7.5 ± 0.5 | 10 | 80† |
| Cumene hydroperoxide (1.5) | 1 | 1 ± 0.09 | 1 | 10† |
| Ethacrynic acid (0.2) | 0.25 | 1.9 ± 0.1 | 9 | 0.2† |
| Phenethyl isothiocyanate (0.1) | 1 | 0.2 ± 0.04 | | |
| 2-Cyano-1,3-dimethyl-1-nitrosoguanidine (1.0) | 1 | 0.1 ± 0.01 | | |
| 1,3-bis(2-Chloroethyl)-1-nitrosourea (2.0) | 5 | < 0.0004 | | |
| $\Delta^5$-Androstene-3,17-dione (0.068) | 0.1 | < 0.004 | 0.06 | 10† |

* Data from [16].
† Data from [13].

### Substrate selectivity and steady-state kinetic parameters

Human GST A4-4 displays the highest specific activities with activated-alkene substrates (Table 1). The values were particularly high with the long-chain 4-hydroxyalkenals. The absolute values are in the range of the highest specific activities obtained with any substrate for the known GSTs [13]. Measurements with nonenal and hexenal demonstrate that the 4-hydroxy group is not important for obtaining high activity (Table 1). GST A4-4 was also assayed with substrates representing other types of chemical reactions, such as aromatic substitution (CDNB), reduction (cumene hydroperoxide) and isomerization ($\Delta^5$-androstene-3,17-dione), as well as reactions with nitroso compounds and an isothiocyanate. The specific activities with these latter substrates (Table 1) were low in comparison with those obtained with other human GSTs [13]. The product of the reaction between GSH and 4-hydroxydecenal was tested as an inhibitor of GST A4-4 using CDNB as the electrophilic substrate under standard assay conditions [13]. At a concentration of 5 $\mu$M the product gave 30 % inhibition, suggesting that product inhibition is of limited physiological significance.

In broad outline, and with reference to the proposed role of the enzyme in the protection of cells against products of lipid peroxidation, the substrate selectivity of human GST A4-4 is similar to that of rat GST A4-4 (previously called GST 8-8 [16]). The substrate selectivity profile of human GST A4-4 is clearly distinct from that of the major isoenzyme in human liver, GST A1-1. In particular, GST A1-1 shows lower specific activities with 4-hydroxyalkenals.

Steady-state kinetic parameters for human GST A4-4 are given in Table 2. The apparent $K_m$ values are lowest and the $k_{cat}$ values highest with the long-chain 4-hydroxyalkenals, resulting in high catalytic efficiencies ($k_{cat}/K_m > 3 \times 10^6$ M$^{-1}$ · s$^{-1}$). The catalytic efficiency with 4-hydroxyhexenal, another product of lipid peroxidation, is only approximately 3 % of the value for HNE. The rate enhancement for the reaction between GSH and HNE is $4 \times 10^6$, as calculated from the quotient of $k_{cat}/K_m$ and the second-order rate constant (0.7 M$^{-1}$ · s$^{-1}$) for the non-enzymic reaction. This value is about 10-fold higher than the rate enhancement for the reaction between GSH and the highly reactive CDNB ($5 \times 10^5$).

**Table 2 Kinetic parameters for recombinant human GST A4-4**

Apparent $K_m$ and $k_{cat}$ values were determined by non-linear regression analysis of the experimental steady-state kinetic data; $k_{cat}/K_m$ values were calculated either from the individual parameters or derived from measurements at low substrate concentrations (*) where $v = (k_{cat}/K_m)[E]_0[S]$, if no saturation curve could be determined; n.d., not determined.

| Substrate | GSH (mM) | $K_m$ (mM) | $k_{cat}$ (s$^{-1}$) | $k_{cat}/K_m$ (mM$^{-1}$ · s$^{-1}$) |
|---|---|---|---|---|
| 4-Hydroxydecenal | 0.5 | 0.022 ± 0.004 | 83 ± 4 | 3800 |
| 4-Hydroxynonenal | 0.5 | 0.037 ± 0.004 | 113 ± 4 | 3100 |
| 4-Hydroxyhexenal | 0.5 | 0.23 ± 0.05 | 23 ± 3 | 100 |
| 4-Hydroxypentenal | 0.5 | 0.18 ± 0.05 | 8.3 ± 2 | 46 |
| Crotonaldehyde | 1 | n.d. | n.d. | 31* |
| CDNB | 5 | 5.8 ± 1.4 | 46 ± 9 | 7.9 |
| Cumene hydroperoxide | 1 | n.d. | n.d. | 0.6* |
| Ethacrynic acid | 1 | 0.08 ± 0.008 | 2.4 ± 0.1 | 30 |
| 4-Nitrocinnamaldehyde | 5 | 0.45 ± 0.05 | 65 ± 5 | 145 |

The $K_m$ value for GSH was 0.1 mM at a fixed concentration (0.1 mM) of HNE. With CDNB at fixed concentration (1 mM), the $K_m$ value for GSH was 1 mM. Thus the kinetic parameters (Table 2) are apparent values, since complete saturation with the second substrate was not achieved. However, the GSH concentrations used are relevant to physiological conditions.

## DISCUSSION

It appears well established that the lipid peroxidation associated with oxidative stress and free-radical pathology is linked to the formation of reactive aldehydes, of which HNE is a major product of peroxidative degradation of ($\omega-6$)-polyunsaturated fatty acids [5]. The HNE concentration, which under normal

physiological conditions is probably $< 1\ \mu$M, may rise significantly and, at least transiently, exceed 100 $\mu$M under conditions of oxidative stress [5]. HNE exerts various cytotoxic effects, including genotoxicity, cytoskeletal modifications, alteration of membrane fluidity, inactivation of enzymes, inhibition of DNA synthesis and induction of cataracts in the lens. In relation to human disease, attention has been drawn to the possible involvement of HNE in atherogenesis [17] neurodegenerative conditions such as Parkinson's disease [6] and Alzheimer's disease [7], as well as aging [18].

In view of the severe toxic effects of HNE and similar electrophiles, it is obvious that efficient protective mechanisms are required for rapid inactivation of the noxious agents. Quantitative metabolic studies suggest that glutathione conjugation is a major pathway of biotransformation of HNE [9]. All available evidence indicates that the conjugation reaction catalysed by GST A4-4 is a detoxification reaction. Transfection experiments with cDNA encoding the mouse enzyme have demonstrated a protective role in mammalian cells [19]. The aldehydic group of the glutathione conjugate forms a ring structure with the 4-hydroxy group and is not freely accessible for reactions with macromolecules [5]. It is also clear that the conjugate of HNE and GSH may serve as a product inhibitor of GST A4-4, as previously demonstrated for rat GSTs [20]. However, the inhibitory effect is minor at concentrations that may occur under physiological conditions and will probably not jeopardize the protective effect of the enzyme.

The present work describes a human enzyme, GST A4-4, with high catalytic efficiency and selectivity for HNE and similar alkene substrates. GSTs with similar activity have previously been cloned from rat [16] and mouse tissues [15]. For human tissues, the isolation of GSTs with HNE-conjugating activity has also been reported, but sequence information is only available for two small peptide fragments and the relationship to the cloned rat and mouse enzymes has not been clearly defined [21].

In spite of their common distinctive substrate profile, the human GST A4-4 protein has $< 60\,\%$ sequence identity with the rat and mouse enzymes (Figure 1), suggesting that, in the molecular evolution of GSTs, accomplishing the detoxication of HNE and other toxic alkenes is not critically dependent on conservation of the primary structure. It is noteworthy that the nucleotide sequences of rat and human GST A4-4 are so divergent (66 % identity) that attempts to use the rat clone as a probe to identify the human sequence in cDNA libraries have failed [22]. GSTs in species other than rodents have been described with apparent similarities to human GST A4-4 [23], but they are incompletely characterized, either structurally or functionally, with respect to HNE and related substrates.

All active-site residues known from the GST A1-1 structure [24] to interact with GSH (the G-site) are conserved, with the exception that Arg-45 is replaced by Gln-45 in the human GST A4-4 sequence. The H-site, responsible for binding the electrophilic substrate, is formed from the side-chains of 15 amino acids in human GST A1-1 [24]. It has been reported that the three-dimensional structure of mouse GST A4-4 is very similar to that of GST A1-1 [25], such that the H-site of GST A4-4 could be regarded as being formed by the corresponding residues. Thus alignment of the sequences shows that only approximately 60 % of the H-site residues are conserved between the human and the rodent structures. In particular, the conspicuous residues 103–105, which are conserved as Met-Met-Met in the rat [16] and the mouse [15] GST A4-4, are Leu-Glu-Leu in the human enzyme. Residue 104 points towards the electrophilic substrate, and mutagenesis of Met-104 in rat GST A4-4 (G. Stenberg and B. Mannervik, unpublished work) and mouse GST A4-4 [25] have indicated its importance for substrate specificity. It is also noteworthy that human GST A1-1 and rat GST A1-1, both with a glutamic acid residue in position 104 [26], have low activity with HNE. In contrast, human GST A4-4 displays high activity with 4-hydroxyalkenals (Tables 1 and 2), in spite of the presence of glutamic acid in position 104, showing that the activity with HNE and similar substrates is not governed by a methionine residue. Nevertheless, an active-site residue common to the human, rat and mouse GST A4-4 is Gly-12, a residue shown to be critically important for the high activity with HNE in the rat enzyme [27].

The high catalytic activity with long-chain 4-hydroxyalkenals is similar to that previously determined for the rodent enzymes (cf. Table 1), and the $k_{cat}/K_m$ values $> 3 \times 10^6\ M^{-1} \cdot s^{-1}$ approach the catalytic efficiencies of the most active enzymes. The catalytic activity is lower with 4-hydroxyalkenals of shorter chain-length, but the related reactive aldehydes acrolein and crotonaldehyde are also relatively good substrates (Table 1). Similar structure–activity relationships among 4-hydroxyalkenals and mammalian GSTs have previously been found [28]. The $K_m$ value for HNE (37 $\mu$M) is compatible with concentrations that may be reached intracellularly under pathophysiological conditions [9]. It is also noteworthy that the catalytic efficiency with HNE is several orders of magnitude higher than that with CDNB (Table 2), the substrate usually giving the highest activity with many other GSTs [13]. Relevant specific activities for human GST A1-1 (Table 1), compared with the data for GST A4-4, show that the substrate-specificity profiles are significantly different. GST A4-4 was also assayed with a series of additional GST substrates, including the naturally occurring phenethyl isothiocyanate and the cytostatic drug 1,3-bis(2-chloroethyl)-1-nitrosourea, but none of the alternative substrates gave particularly high specific activities (Table 1).

GSH has a key role in HNE metabolism and has been shown to attenuate HNE neurotoxicity [7]. Furthermore, transfection of cultured HepG2 cells with mouse GST A4-4 conferred increased resistance to oxidative stress [19]. GSH reacts non-enzymically with HNE, but it has been calculated that the rate of the uncatalysed reaction is negligible in comparison with GST-mediated conjugation [20]. Thus GST A4-4 catalysed GSH conjugation appears to play a significant role in the cellular defense against toxic alkenals produced under pathogenic conditions.

GST A4-4 described in the present work derives from human fetal brain. The tissue distribution of the enzyme in humans is outside the scope of the present investigation, but the corresponding rat and mouse enzymes have been shown to occur in most tissues investigated [29,30]. GST-catalysed conjugation of HNE has been described in adult human tissues, such as liver, heart, cornea and retina [23], suggesting that GST A4-4 is as ubiquitous in human as in rodent tissues. Of particular significance in relation to the role in protection against neurodegenerative processes leading to Parkinson's disease and other disorders suggested here is the demonstration that the GSTA4-4 sequence could be PCR-amplified from a substantia nigra cDNA library from adult human brain.

In conclusion, the present work underscores the significance of GSTs in the protection against genotoxic products of radical reactions and oxidative processes, previously demonstrated with other combinations of enzymes and substrates [3,4,31]. Even though GSTs have overlapping substrate specificities [13,26], and therefore to some degree may fulfil similar functions, the great variations in catalytic efficiencies with individual toxic substrates may have particular importance. For example, it has been proposed that human subjects lacking GST M1-1 would be more

susceptible to the genotoxic effects of carcinogenic epoxides [32] and that GST T1-1 deficiency may alter the biotransformation of alkyl halides [33]. Epidemiological studies suggest that genotypic and phenotypic differences in the expression of these enzymes may influence the risks of contracting cancer and other diseases. The characteristic substrate specificity profile of human GST A4-4 suggests that the genotype and expression of this enzyme may in part determine the extent to which individuals suffer from oxidative processes leading to aging and various degenerative processes.

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