

Comparison of the mRNA sequences for Pi class glutathione transferases in different hamster species and the corresponding enzyme activities with *anti*-benzo[*a*]pyrene-7,8-dihydrodiol 9,10-epoxide

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Glutathione S-transferase (GST) of class Pi (GST Pi) is known to detoxify the mutagenic and carcinogenic (+)-*anti*-benzo[*a*]pyrene-7,8-dihydrodiol 9,10-epoxide [(+)-*anti*-BPDE] by conjugation with glutathione. Previously, we have shown that Chinese hamster V79 cells contain GST Pi, but seem to lack the capacity to conjugate (+)-*anti*-BPDE, although these cells do conjugate other substrates with GSH [Romert, Dock, Jenssen and Jernström (1989) *Carcinogenesis* **10**, 1701–1707; Swedmark, Romert, Morgenstern and Jenssen (1992) *Carcinogenesis* **13**, 1719–1723; Swedmark and Jenssen (1994) *Gene* **139**, 251–256]. In the present study we have compared four cell lines derived from different hamster species with respect to GST cDNA sequences and capacity to conjugate (+)- or (–)-*anti*-BPDE. The cell lines were V79 and Chinese hamster ovary cells (CHO), Armenian hamster lung (AHL) cells and baby hamster kidney (BHK) cells. The sequencing revealed a complete homology between the V79 and CHO cDNA for GST Pi, whereas the corresponding amino acid sequences predicted from the corresponding AHL and BHK cDNAs differed by six and nine amino acids, respectively, from the predicted V79 sequence. None of these changes alone was

found to influence the xenobiotic substrate-binding site. The cytosolic fractions from BHK and AHL cells were found to catalyse conjugation of (+)-*anti*-BPDE with GSH, whereas the corresponding activity in CHO cells was non-detectable. As shown previously, V79 cells were devoid of activity towards (+)-*anti*-BPDE. All the cell lines studied demonstrated appreciable GST activity towards 1-chloro-2,4-dinitrobenzene, but no activity with (–)-*anti*-BPDE. The latter result suggests that GST Pi is the sole or predominant GST in these cell lines. This was confirmed by HPLC analysis of purified enzymes obtained by affinity chromatography. However, when the catalytic activities of the pure enzymes were determined, all four different GST Pi enzymes were found to be highly capable of conjugating (+)-*anti*-BPDE with GSH. This observation indicates the existence of an intracellular factor that selectively inhibits conjugation of (+)-*anti*-BPDE, but not of 1-chloro-2,4-dinitrobenzene in the V79 and CHO cell lines. This new phenomenon seems to be specific for Chinese hamster, since both these cell lines originate from this species.

INTRODUCTION

The glutathione S-transferases (GSTs) constitute a family of multifunctional enzymes which have the capacity to detoxify electrophilic xenobiotics and xenobiotic metabolites and protect tissues against various types of damage [1,2]. The GSTs catalyse the nucleophilic addition of glutathione (GSH) to electrophilic centres in a great number of compounds, thus rendering them more water-soluble for elimination. These proteins also have a ligand-binding function, which makes it possible to transport numerous hydrophobic and amphipathic compounds *in vivo* [3].

The GST isoenzymes identified so far are divided into five classes: the soluble enzymes of the Alpha, Mu, Pi, and Theta classes, which are mainly present in the cytosol, and a unique form present in the endoplasmic reticulum [4–6]. The cytosolic enzymes are more or less structurally related, whereas the microsomal form shows no obvious sequence homology with other GSTs [4]. Recently, a new class of GST (Sigma) has been found in invertebrates [7]. GST monomers belonging to the same class may form homo- or hetero-dimers with molecular masses of approx. 50 kDa. Each subunit contributes an active site to the dimer which functions independently of the corresponding site in the other subunit [1].

It has been shown previously that the GST Pi class enzymes from human and rat efficiently catalyse the conjugation of *anti*-benzo[*a*]pyrene-7,8-dihydrodiol 9,10-epoxide (*anti*-BPDE) with GSH and with an exclusive preference for the highly mutagenic and carcinogenic (+)-enantiomer [8–10]. In contrast, V79 Chinese hamster cells were found to lack the capacity to conjugate (+)-*anti*-BPDE [10], despite the presence of significant amounts of GST Pi [11]. This lack of activity towards the diol epoxide suggested the presence of an altered GST Pi isoenzyme in these cells.

The mRNA for GST Pi in V79 cells was sequenced and found to be very similar to other known mRNA sequences for GST Pi. The sequence similarity at the amino acid level was at least 84%. No difference that could explain the lack of conjugating activity towards (+)-*anti*-BPDE was found in the active site of the V79 enzyme relative to the corresponding GST Pi enzymes of the rat, mouse and human [12].

In order to determine whether the lack of GSH-conjugating activity towards (+)-*anti*-BPDE was unique for the V79 cell line, three other cell lines derived from hamster, i.e. AHL-1 (lung cells from Armenian hamster), BHK-C13 (kidney cells from Syrian hamster) and CHO-AA8 (ovary cells from Chinese hamster), were investigated with respect to GST Pi. The coding sequences

of their GST Pi mRNAs and their capacities to conjugate 1-chloro-2,4-dinitrobenzene (CDNB) and *anti*-BPDE were investigated.

MATERIALS AND METHODS

Cell cultivation

AHL-1 cells were cultured in minimum essential medium (Eagle) containing non-essential amino acids (100 mM), 90% Earle's balanced salt solution, 10% fetal bovine serum, penicillin (88 units/ml) and streptomycin (8 mM). CHO and V79 cells were cultured as described previously [11] and BHK cells in α -minimal essential medium containing 10% fetal bovine serum, 10% tryptose phosphate broth, penicillin (88 units/ml) and streptomycin (8 mM). All cell lines were incubated at 37 °C. AHL-1 and BHK-C13 cells were obtained from the American Type Culture Collection, U.S.A.; CHO-AA8 cells were a kind gift from Larry Thompson of Livermore, CA, U.S.A.; while the V79 cells were kindly provided by Dr. A. T. Natarajan, Leiden, Holland. Preparation of RNA, synthesis of cDNA, purification and PCR were performed according to the methods of Swedmark and Jenssen [12].

Preparation of cytosol

Cytosolic fractions were prepared according to the method of Swedmark et al. [11], with the following minor modification: 20 mM Tris/HCl, 0.25 M sucrose and 0.5 mM EDTA, pH 7.5, was used rather than the TKE buffer employed previously. The different GST Pi enzymes were purified by affinity chromatography on glutathione-Sepharose 4B (Pharmacia, Sweden) according to the manual except that Triton X-100 was excluded. The proteins were concentrated using spin columns with a cut-off range of 10 or 30 kDa (Millipore, U.S.A.) and passed through NAP10 columns (Pharmacia) equilibrated with 50 mM Tris/HCl, pH 7.5, to remove GSH and phosphate.

The protein concentration was determined according to the method of Peterson [13].

HPLC analysis of the purified GST enzymes

GST isoenzymes purified from the different cell lines were analysed by HPLC as originally described by Östlund Farrants et al. [14]. Briefly, a reversed-phase analytical column (Dynamax C₁₈, 5 μ m, 300 Å, 4.6 mm \times 250 mm) was employed and the samples were eluted with 0.1% trifluoroacetic acid in water (solvent A) and 0.1% trifluoroacetic acid in acetonitrile (solvent B) using a gradient ranging from 35% B to 60% B in A for 40 min, followed by isocratic elution with 60% B in A for 10 min. The flow rate was 1 ml/min and the absorption of the effluent was monitored at 214 nm. Purified recombinant human GST Pi (S. Swedmark, K. Sundberg, B. Jernström, A. J. Townsend, A.-C. Bergman, R. Weinander, R. Morgenstern and D. Jenssen, unpublished work) was used as a chromatographic standard. In the light of the identical number of amino acid residues present in the GST Pi class enzymes in the different cell lines, it was assumed that the absorption coefficients of these isoenzymes at 214 nm were identical or very close to that of recombinant human GST Pi.

PCR: strategies

The PCR primers used were the following:

C: TTGAATTCATGCCGCCATACACCATTGTCTAC;

D: AAAAGCTTTTACTGCTTGCCATTGCCATTAAT;

K: CCTTCCAGCTCTGGCCCTGGT;

L: TTGAATTCGCCCAAGATCAAGGCCCTTCCTGT;

and **M:** AAGCTTCTGTYTCTTCTGTCCATTACTGTTTG.

These primers were supplied by Scandinavian Gene Synthesis, Sweden.

The PCR products were purified using Wizard PCR Preps (Promega, U.S.A.) and cleaved with *Eco*RI (**C** and **L** primers) or *Hind*III (**D** and **M** primers). After cleavage, the PCR products were purified a second time with Wizard PCR Preps and ligated into a cloning vector (Bluescript vector, Stratagene, U.S.A.) using a ligation kit and the instructions from Amersham, U.K. The vector was transformed into competent bacteria (XL1-Blue MRF', Stratagene) according to Maniatis [16] and these were plated on to Luria Broth (LB)-plates containing 50 μ g/ml ampicillin, 100 μ l of 40 mM isopropyl thio- β -D-galactoside and 100 μ l of 2% (w/v) 5-bromo-4-chloro-3-indolyl β -D-galactoside. The plates were incubated overnight and white colonies were isolated and grown in LB-medium. Plasmid DNA was prepared using a QIAprep Spin Plasmid Kit (Qiagen, Germany), cleaved with *Eco*RI and *Hind*III and checked for the expected size of the insert on an agarose gel. Selected clones were sequenced using T3 and T7 sequencing primers, the PRISM Ready Reaction Dye-Deoxy Terminator Cycle Sequencing Kit (Perkin-Elmer, U.S.A.) and an IBI sequencing machine.

GST-catalysed conjugation of CDNB or BPDE with GSH

GST activities towards CDNB in the cytosolic fractions were measured according to the method of Habig and Jacoby [17]. The (+)- and (–)-enantiomers of *anti*-BPDE were obtained through the Cancer Research Program of the NCI, Division of Cancer Cause and Prevention, Bethesda, MD, U.S.A. CDNB was obtained from the Sigma Chemical Co., St. Louis, MO, U.S.A.

The cytosolic activities towards *anti*-BPDE were measured as follows: cytosolic protein (approx. 4 mg/ml) was incubated for 1 min at 37 °C with 40 and 80 μ M (+)- or (–)-*anti*-BPDE (added in DMSO, final concentration 5%) and 5 mM GSH in 50 mM Tris/HCl, pH 7.5 (final volume 100 μ l). The reaction was terminated by addition of 25 μ l of alkaline 2-mercaptoethanol. The non-enzymic reaction between the diol epoxide and GSH was determined by incubating the compound as described above in the absence of enzyme and correction was made for this value.

The activity of purified GST enzymes towards (+)-*anti*-BPDE was determined in essentially the same manner, but using 15–80 μ g of enzyme/ml.

Following precipitation of the protein with perchloric acid (final concentration 5%), the products formed were analysed by HPLC as described previously [10].

RESULTS

Sequencing the cDNA for GST Pi

In order to sequence the cDNA for GST Pi derived from the three hamster cell lines, at least two libraries were constructed to avoid mutations. The PCR primers **C** and **D** could be used for all three cell lines and all gave rise to PCR products of ~650 bp, as judged from an agarose gel, while the total coding region consists of 633 bp including start and stop codons (Figure 1).

In order to identify unknown sequences covered by the **C** and **D** primers, the cDNA libraries were circularized according to the method of Swedmark and Jenssen [12]. Since the levels of PCR products obtained were quite low and only faintly visible on an agarose gel, they were subsequently cloned into the Bluescript vector and expanded in bacteria. Nevertheless, it was found that

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CHO  AUGCCGCCAUACACCAUUGUCUACUUCCAGUUCGAGGACGCUGUGAGGCCAUGC  57
BHK      G                               G
AHL      G

CHO  AUACUGCUGGCUGACCAGGCCAGAGCUGGAAGGAGGAGGUGGUACUGUAGAAACCCUG  117
BHK      A           C G
AHL  C G           A           C G   UU

CHO  AGGAAAGGUUCGCUCAAGUCCACCUGUUUGUAUGGGCAGCUCUCCUAAAUUUGAGGAUGGA  177
AHL  G       C                               A
BHK  GU      C                               A       C

CHO  GACCUCACCCUGUACCAAUCUAAUGCCAUCUUGAGGCACCUGGGCCGAUCCUUGGGGCUU  237
AHL      U           A
BHK      U

CHO  UAUGGGAAAGACCAGAGGGAGGCUGCCCUGGUGGAUAUGGUGAAUGAUGGGGUGGAAGAC  298
AHL
BHK      A                               C

CHO  CUUCGUGCAAUAUCAUACCCUCAUCUACACCAAUAUGAGGAGGCAAGGAUGACUAU  357
AHL      G   G                               C
BHK      G                               C

CHO  GUGAAGGCCUGCCUGGACACCUGAAGCCUUGAGACCCUGCUGUCCAGAACCAAGGG  417
AHL      A
BHK

CHO  GGCAAAGCCUUCUUCGUGGGUGACCAGAUCUCCUUGUGGAUUACAACUUGCUAGAUUCUG  477
AHL      C           C
BHK      CC           U

CHO  CUGCUGAUCCACCAGGUCCUGGCCUUGGACAAUCCUCCUCCUGCUCUCUGCC  537
AHL
BHK      A                               U

CHO  UAUGUGGCACGGCUUAGUGCCCGCCCAAGAUCAAGGCCUUCUGUCCUCCUCCUGACCAU  597
AHL      U   U   C
BHK  C           A                               U

CHO  GUGAACCGUCCCAUUAUGGCAAUGGCAAGCAGUAA  633
AHL      A
BHK  U       C       C       A

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Figure 1 The coding sequences for the GST Pi enzyme in AHL, BHK and CHO cells

These sequences have been assigned the following GenBank nos.: AHL, L46796; BHK, L40382; CHO, L40381.

several of the clones sequenced were incomplete, e.g. they could contain two 5' ends, but no 3' end.

In the case of cDNA from AHL cells, it was necessary to construct the primer M, since no definitive information could be obtained from the circular PCR for two bases in the D primer position. On the other hand, enough information was available from the non-coding region on the 3' side and, consequently, this primer was designed to bind in the border region of the 3' end (Figure 2).

Generally, the circularization strategy was not as efficient as in the case of V79 cDNA [12]. This might be explained by the fact

that the K and L primers were constructed for use with the cDNA from all three cell lines. Since fewer positions in the cDNA sequences from the three different hamster species were complementary to these primers, they were not optimal for all these cell lines. It may also be that the level of the relevant mRNA in these other cell lines is lower than in V79 cells.

When the predicted amino acid sequences were compared (Figure 3), all three cell lines were found to be quite similar, the sequence similarity being higher than 96%. It can be noted that although the CHO and V79 cell lines both originate from Chinese hamster and have been cultured separately for a long

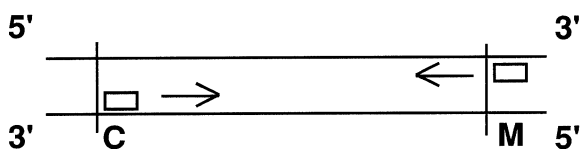


Figure 2 The PCR strategy used for obtaining information about two bases at the 3' end of the coding region

The boxes **C** and **M** indicate the positions of the PCR primers binding to the GST Pi cDNA from AHL cells. Vertical lines indicate the border between coding and non-coding nucleotides.

		+ * *	+ +	*	+
R	PPYTI	VYFPVRGRCEAT	RMLLADQQGSW	KEEVVTI	DVW 38
M	PPYTI	VYFPVRGRCEAM	RMLLADQQGSW	KEEVVTI	DTW
H	PPYT	VYFPVRGRCAAL	RMLLADQQGSW	KEEVVTETW	
AHL	PPYTI	VYFPVRGRCEAMRI	LLADQQGSW	KEEVVTETW	
BHK	PPYTI	VYFPVRGRCEAMRI	LLADQQGSW	KEEVVTETW	
CH	PPYTI	VYFPVRGRCEAMRI	LLADQQGSW	KEEVVTETW	
		+	++		
R	LOGSL	KSTCLYGQLPKF	EDGDLTYQSNAI	LRHLGRSLG	77
M	MOGL	LKPTCLYGQLPKF	EDGDLTYQSNAI	LRHLGRSLG	
H	QEGSL	KASCLYGQLPKF	QDGLTYQSNAI	LRHLGRSLG	
AHL	KKGS	LKSTCLYGQLPKF	EDGDLTYQSNAI	LRHLGRSLG	
BHK	MKGS	LKSTCLYGQLPKF	EDGDLTYQSNAI	LRHLGRSLG	
CH	RKGS	LKSTCLYGQLPKF	EDGDLTYQSNAI	LRHLGRSLG	
		+		*	
R	LYGKD	QEAALVDMVNDG	VEDLRCKYGT	LIYTNYENGK	116
M	LYGKN	QEAAMDMVNDG	VEDLRCKYVTL	LIYTNYENGK	
H	LYGKD	QEAALVDMVNDG	VEDLRCKYSL	LIYTNYEAAGK	
AHL	LYGKD	QEAALVDMVNDG	VEDLRCKYVT	LIYTKYEEGK	
BHK	LYGKD	QEAALVDMVNDG	VEDLRCKYVT	LIYTKYEEGK	
CH	LYGKD	QEAALVDMVNDG	VEDLRCKYVT	LIYTKYEEGK	
		+			
R	DYVKAL	PGHLKPFETLL	SONQGGKAFI	VGDQISFADYNL	155
M	DYVKAL	PGHLKPFETLL	SONQGGKAFI	VGDQISFADYNL	
H	DYVKAL	PGHLKPFETLL	SONQGGKAFI	VGDQISFADYNL	
AHL	DYVKAL	PGHLKPFETLL	SONQGGKAFI	VGDQISFADYNL	
BHK	DYVKAL	PGHLKPFETLL	SONQGGKAFI	VGDQISFADYNL	
CH	DYVKAL	PGHLKPFETLL	SONQGGKAFI	VGDQISFADYNL	
		*			
R	LDLLLV	HQVLAPGCLDNF	PLLSAYVARLSARPKI	KAFLS	194
M	LDLLLI	HQVLAPGCLDNF	PLLSAYVARLSARPKI	KAFLS	
H	LDLLLI	HEVLAPGCLDA	FPLLSAYVGRLSARPKL	KAFLS	
AHL	LDLLLI	HQVLAPGCLDNF	PLLSAYVARLSARPKI	KAFLS	
BHK	LDLLLI	HQVLAPGCLDNF	PLLSAYVARLSARPKI	KAFLS	
CH	LDLLLI	HQVLAPGCLDNF	PLLSAYVARLSARPKI	KAFLS	
		*			
R	SPDHL	NRPI	NGNGKQ	209	
M	SPEHV	NRPI	NGNGKQ	209	
H	SPEYV	NLP	NGNGKQ	209	
AHL	SPDHV	NRPI	NGNGKQ	209	
BHK	SPDHV	NRPI	NGNGKQ	203	
CH	SPDHV	NRPI	NGNGKQ	209	

Figure 3 Alignment of the amino acid sequences of mammalian Pi-class GSTs

A comparison of the predicted amino acid sequences for GST Pi from rat (R), mouse (M), human (H), Armenian hamster lung cells (AHL), Syrian hamster kidney cells (BHK) and Chinese hamster ovary cells (CH) is shown. Shaded areas indicate complete amino acid identity for all species, while amino acid residues that differ for AHL and BHK GST Pi enzymes compared with the Chinese hamster (CH) sequence are boxed. The active sites are indicated with + for the G-site and * for the H-site, according to García-Sáez et al. [21].

period of time, the CHO sequence shows complete homology to the V79 sequence, including portions of the non-coding 5' and 3' ends (results not shown). This demonstrates that these cell lines have not developed any substitutions in the exons of the GST Pi gene.

The GST Pi amino acid sequences for AHL and BHK cells differed in six and nine positions, respectively, from the corresponding sequences for V79 and CHO cells, as summarized in Table 1. At position 19 the BHK sequence contains Leu, whereas the AHL and CHO sequences contain Ile. At position 33 the AHL sequence contains Ile and the BHK and CHO sequences have Val.

In position 35 there is a non-conservative difference. The AHL and BHK sequences have Gly, which is uncharged, while CHO

Table 1 Comparison of the predicted amino acid sequences of the GST Pi enzymes in the AHL, BHK and CHO cell lines

Position of amino acid in the primary sequence of GST Pi	Cell line		
	AHL	BHK	CHO
19	Ile	Leu	Ile
33	Ile	Val	Val
35*	Gly	Gly	Val
36	Glu	Asp	Glu
37	Thr	Ser	Thr
39*	Gly	Val	Arg
61*	Thr	Ile	Thr
93	Val	Ala	Val
104	Val	Val	Ile
135*	Lys	Gln	Gln
151*	Ala	Ala	Val

* Non-conservative differences.

contains a non-polar Val. At positions 36 and 37 the AHL and CHO sequences have Glu and Thr, respectively, whereas BHK cells have Asp and Ser. Another non-conservative difference is found at position 39, where the CHO sequence has Arg, BHK has Val and AHL has Gly, all belonging to different amino acid groups. At position 61 the AHL and CHO sequences have a polar Thr residue, whereas BHK has a non-polar Ile, another non-conservative exchange.

At position 93 BHK has Ala, while AHL and CHO have Val. Two more conservative differences are found at positions 104 and 151, where the AHL and BHK sequences have Val and Ala, respectively, and CHO cells have Ile and Val. Finally, there is a non-conservative difference at position 135, where BHK and CHO have an uncharged Gln, while AHL has a basic Lys. None of these amino acids is in the portions of the sequence proposed by several authors to constitute the active site except amino acid 35 [18–21]. The CHO and BHK GST Pi enzymes have partially been sequenced by Lo et al. [22] and Bogaards et al. [23], respectively. No deviations in the amino acid sequences were found when comparisons were made.

Based on these data for the GST Pi amino acid sequences, it is also possible to discuss the evolutionary relationship between different species of hamster.

Conjugation of BPDE with GSH

Cytosolic fractions from V79, AHL, BHK and CHO cells were incubated with (+)- or (–)-*anti*-BPDE as described in the Materials and methods section and the results obtained are compiled in Table 2. As demonstrated previously [10], the cytosolic fraction from V79 cells lacks the capacity to conjugate (+)-*anti*-BPDE with GSH. Expressed on a protein basis, AHL cells exhibit the highest activity, followed by BHK, CHO and V79 cells, in that order. The specific activities for CDNB conjugation were also determined for these cell lines (Table 2).

None of the cytosolic fractions exhibited activity with (–)-*anti*-BPDE (results not shown). Since both Alpha and Mu class GSTs demonstrate activity towards both *anti*-enantiomers [8], these results strongly suggest that GST Pi is the major or only GST isoenzyme expressed in the cells studied here. This was confirmed by HPLC analysis of enzymes purified by affinity chromatography employing GSH–Sepharose 4B [24].

The HPLC analyses of GSTs purified from AHL and BHK cells demonstrated the presence of single peaks with retention

Table 2 The specific activities for CDNB conjugation by cytosolic fractions and the rates of and k_{cat}/K_m values for (+)-*anti*-BPDE conjugation by GST Pi isoenzymes purified from AHL, BHK, CHO and V79 cells

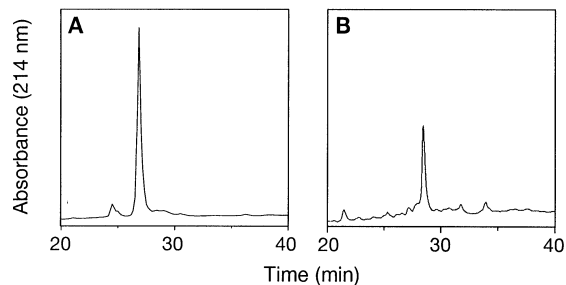
The specific activities for CDNB conjugation and the rates of and catalytic efficiencies for (+)-*anti*-BPDE conjugation were calculated after determination of the amounts of the GST Pi enzymes present by HPLC.

Cell line	Substrate ...CDNB	(+) - <i>anti</i> -BPDE	
	specific activity (μmol of GSH conjugate formed/ min per mg of GST Pi)	Cytosolic specific activity* (nmol of GSH conjugate formed/ min per mg of cytosolic protein)	Isolated enzyme catalytic efficiency† ($\text{mM}^{-1} \cdot \text{s}^{-1}$)
V79	130	nd‡	3.0
CHO	130	nd‡	5.6
AHL	44	4.5	4.4
BHK	33	3.7	4.2

* These activities were obtained using $80 \mu\text{M}$ (+)-*anti*-BPDE.

† Catalytic efficiencies (k_{cat}/K_m) of the purified enzymes were determined from the experiments described in [28].

‡ No activity above the non-enzymic reaction of BPDE with GSH was detectable.

**Figure 4** The HPLC patterns for GST Pi isoenzymes affinity-purified from BHK and V79 cells**Table 3** The HPLC retention times of GST Pi enzymes purified from V79, CHO, AHL and BHK cells and their percentage contributions to the total amounts of proteins eluting as GSTs

Percentage values were of the total protein eluted between 20 and 40 min.

Cell line	Retention time	Percentage of the total GSTs recovered
V79	28	72
CHO	30	79
AHL	26	96
BHK	27	92

times (28 min) similar to that of the human GST Pi isoenzyme (Figure 4). The elution pattern was more complex in the case of V79 and CHO cells (Figure 4). In addition to the major peak eluting as a GST Pi enzyme, some minor peaks were present.

The nature of these peaks is unknown, although the presence of isoenzymes belonging to other classes of GST (e.g. class Mu and Alpha) or decomposition products cannot be excluded. Previous work has shown that GST Pi elutes prior to GST Mu enzymes followed by GST Alpha isoenzymes [25]. All the GSTs are expected to elute with retention times of 20–40 min and the percentages of the total protein eluted in this interval accounted for by GST Pi were calculated. These results are summarized in Table 3 and it is concluded that the GST Pi class isoenzyme is the

only, or greatly predominant, species in all the cell lines investigated here.

The catalytic efficiency values, k_{cat}/K_m , of the purified GST Pi isoenzymes towards (+)-*anti*-BPDE were determined and, surprisingly, all of these were found to be highly active. These values, which are quite similar, are compiled in Table 2.

DISCUSSION

Previous studies have shown that significant amounts of GST Pi are present in Chinese hamster V79 cells [10–12]. However, these cells apparently lack the capacity to lower or prevent mutagenicity caused by exposure to (+)-*anti*-BPDE [10]. This finding was unexpected, since Pi class GSTs from both human and rat efficiently detoxify *anti*-BPDE, and in particular the highly mutagenic (+)-enantiomer, by conjugation with GSH [8,9].

Previously, we found that V79 cells express the mRNA for GST Pi at the same level as rat liver HIE4 cells [11], a cell line that efficiently conjugates *anti*-BPDE with GSH [10]. Immunoprecipitation confirmed these results, showing similar levels of GST Pi protein in these two cell lines [11]. Since V79 cells are commonly used in toxicological studies, we considered it of importance to examine this situation further.

In the present study, the mRNA sequences of GST Pi in three different hamster cell lines were determined and compared with the corresponding sequence for V79 cells (Figure 1). The cell lines studied were AHL, derived from Armenian hamster (*Cricetulus migratorius*) lung cells; BHK, derived from Syrian hamster (*Mesocricetus auratus*) kidney cells; and CHO, Chinese hamster (*Cricetulus griseus*) ovary cells. The enzymic activities of cytosolic preparations from these cells towards CDNB and (+)- and (–)-*anti*-BPDE were also measured.

The mRNA and amino acid predicted sequences obtained for the cell lines studied here differed in only one amino acid residue expected to be part of the H-site (position 35) and, thus, to affect the catalytic activity towards (+)-*anti*-BPDE [18–21]. However, variation in position 35 does not seem to affect GST Pi activity since in V79 and CHO cells and humans position 35 is occupied by the non-polar amino acid valine, in BHK and AHL cells by the uncharged polar amino acid glycine and in the rat by the non-polar amino acid isoleucine.

As shown here (Table 2), cytosolic fractions from AHL and BHK cells were considerably more active in conjugating (+)-*anti*-BPDE with GSH than the corresponding fractions derived

from Chinese hamster (CHO and V79) cells. A low level or lack of activity in the cytosol of CHO cells was expected, considering their relationship to V79 cells. All cytosolic fractions demonstrating activity with *anti*-BPDE were highly selective towards the (+)-enantiomer. In fact, no activity at all was detected with (–)-*anti*-BPDE. These results suggest that the predominant or only GST isoenzyme present is of the Pi class, since both Alpha and Mu class GSTs are less enantioselective with this substrate [8]. This conclusion was confirmed when the affinity-purified enzymes were analysed by HPLC.

However, when the pure isoenzymes were incubated with (+)-*anti*-BPDE, all four were highly efficient in catalysing the conjugation of diol-epoxide with GSH. In fact, their k_{cat}/K_m values are similar to the value recently observed with the human GSTP1-1 (K. Sundberg, M. Widersten, A. Seidel, B. Mannervik and B. Jernström, unpublished work). This discrepancy between cytosols and purified GSTs obtained from V79 and CHO cells with respect to conjugation of (+)-*anti*-BPDE with GSH suggests the existence of an intracellular factor which selectively inhibits conjugation of (+)-*anti*-BPDE, but not of CDNB. This new phenomenon seems to be associated with the Chinese hamster, since V79 and CHO cells both originate from this species.

Another interesting result of this study is the implication of an evolutionary split between the Syrian and Chinese hamster. In agreement with our data O'hUigin and Li [27] have proposed a closer evolutionary relationship between Armenian and Chinese hamsters than between Syrian and Chinese hamsters. These authors suggest a date, 20–29 million years ago, for the divergence of the Syrian hamster from the Chinese-Armenian hamster based on five genes. When the same five genes in the mouse and rat were compared, the same rate of nucleotide substitution was found, suggesting that the Syrian and Chinese hamsters diverged at about the same time as the mouse and rat.

This investigation was supported by the Swedish Cancer Society. Kathrin Sundberg is gratefully acknowledged for her technical assistance.

REFERENCES

- Mannervik, B. and Danielson, U. H. (1988) *Crit. Rev. Biochem.* **23**, 283–337
- Ketterer, B. and Chistodoulides, L. C. (1994) *Adv. Pharmacol.* **27**, 37–69
- Listowsky, I. (1993) in *Structure and Function of Glutathione Transferases* (Tew, K. D., Pickett, T. J., Mantle, T. J., Mannervik, B. and Hayes, J. D., eds.), pp. 199–209, CRC Press, Boca Raton
- Mannervik, B. and Widersten, M. (1995) in *Advances in Drug Metabolism in Man* (Pacifci, G. M. and Francchia, G. M., eds.), pp. 408–459, European Commission
- Meyer, D. J., Coles, B., Pemble, S. E., Gilmore, K. S., Fraser, G. M. and Ketterer, B. (1991) *Biochem. J.* **274**, 409–414
- Morgenstern, R. and De Pierre, J. W. (1988) in *Glutathione Conjugation: Mechanism and Biological Significance* (Sies, H. and Ketterer, B., eds.), pp. 157–174, Academic Press, London
- Ji, X. H., Vonrosenveing, E. C., Johnson, W. W., Tomarev, S. I., Piatigorsky, J., Armstrong, R. N. and Gilliland, G. L. (1995) *Biochemistry* **34**, 5317–5328
- Robertson, I. G. C., Guthenberg, G., Mannervik, B. and Jernström, B. (1986) *Cancer Res.* **46**, 2220–2224
- Robertson, I. G. C., Jensson, H., Mannervik, B. and Jernström, B. (1986) *Carcinogenesis* **7**, 295–299
- Romert, L., Dock, L., Jenssen, D. and Jernström, B. (1989) *Carcinogenesis* **10**, 1701–1707
- Swedmark, S., Romert, L., Morgenstern, R. and Jenssen, D. (1992) *Carcinogenesis* **13**, 1719–1723
- Swedmark, S. and Jenssen, D. (1994) *Gene* **139**, 251–256
- Peterson, G. L. (1977) *Anal. Biochem.* **83**, 346–356
- Östlund Farrants, A.-K., Meyer, D. J., Coles, B., Southan, C., Aitke, A., Johnson, P. J. and Ketterer, B. (1987) *Biochem. J.* **245**, 423–428
- Reference deleted.
- Maniatis, T., Fritsch, E. F. and Sambrook, J. (1989) *Molecular Cloning. A Laboratory Manual*, Cold Spring Harbour Laboratory, Cold Spring, NY
- Habig, W. H. and Jacoby, W. B. (1981) *Methods Enzymol.* **77**, 398–405
- Reinemer, P., Dirr, H. W., Ladenstein, R., Schaeffer, J., Gallay, O. and Huber, R. (1991) *EMBO J.* **10**, 1997–2005
- Reinemer, P., Dirr, H. W., Ladenstein, R., Huber, R., Bello, M. L., Federici, G. and Parker, M. W. (1992) *J. Mol. Biol.* **227**, 214–226
- Dirr, H., Reinemer, P. and Huber, R. (1994) *J. Mol. Biol.* **243**, 72–92
- García-Sáez, I., Párraga, A., Phillips, M. F., Mantle, T. J. and Coll, M. (1994) *J. Mol. Biol.* **237**, 298–314
- Lo, J. F., Wang, H. F., Tam, M. F. and Lee, T. C. (1992) *Biochem. J.* **288**, 977–982
- Bogaards, J. J., van Ommen, B. and van Bladeren, P. J. (1992) *Biochem. J.* **286**, 383–388
- Vander Jagt, D. L., Hunsaker, L. A., Garcia, K. B. and Royer, R. E. (1985) *J. Biol. Chem.* **260**, 11603–11610
- Widersten, M., Pearson, W. R., Engström, Å. and Mannervik, B. (1991) *Biochem. J.* **276**, 519–524
- Reference deleted.
- O'hUigin, C. and Li, W. H. (1992) *J. Mol. Evol.* **35**, 377–384
- Jernström, B., Seidel, A., Funk, M., Oesch, F. and Mannervik, B. (1992) *Carcinogenesis* **13**, 1549–1555