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Cloning and expression of a chick liver glutathione *S*-transferase CL 3 subunit with the use of a baculovirus expression system

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Glutathione *S*-transferase CL 3 subunits purified from 1-day-old-chick livers were digested with *Achromobacter* proteinase I and the resulting fragments were isolated for amino acid sequence analysis. An oligonucleotide probe was constructed accordingly for cDNA library screening. A cDNA clone of 1342 bases, pGCL301, encoding a protein of 26209 Da was isolated and sequenced. Including conservative substitutions, this protein has 75–79% sequence similarity to other Alpha family glutathione *S*-transferases. The coding sequence of pGCL301 was inserted into a baculovirus vector for infection of *Spodoptera frugiperda* (SF9) cells. The expressed protein has a high relative activity with ethacrynic acid (47% of the specific activity with 1-chloro-2,4-dinitrobenzene). The enzyme has a subunit molecular mass of 25.2±1.2 kDa (by SDS/PAGE), a pI of 9.45 and an absorption coefficient $A^{1\%}_{1cm}$ of 13.0±0.5 at 280 nm.

INTRODUCTION

Glutathione *S*-transferases (GSTs; EC 2.5.1.18) are a family of multifunctional dimeric proteins catalysing the conjugation of GSH to a wide variety of electrophilic alkylating agents. They are also involved in the metabolism of lipid hydroperoxides, prostaglandins and leukotriene A_4 and in binding of non-substrate hydrophobic ligands such as bile acids, bilirubin, a number of drugs and thyroid hormones (for reviews see refs. [1] and [2]).

Rat and human cytosolic GSTs have been the subject of numerous studies. GSTs exist as isoenzymes that can be distinguished on the basis of their physical, chemical, immunological, enzymic and structural properties [3,4]. Eleven subunits have been identified in rat tissues, and various combinations of these subunits give rise to at least 14 homodimeric or heterodimeric GSTs [1,5]. These isoenzymes are classified into three non-homologous species-independent multigene families, namely Alpha, Mu and Pi.

Avian GSTs comprise a complex isoenzyme system that have received little attention [6,7]. Five groups of GST subunits have been identified in the cytosolic fraction of 1-day-old Leghorn chick livers. These GSTs were designated as CL 1–CL 5 according to their electrophoretic mobility on SDS/PAGE. Subunits from groups CL 2 and CL 3 are the major GSTs expressed in 1-day-old chick livers and they form CL 2–2 and CL 3–3 homodimers as well as CL 1–2 and CL 3–4 heterodimers. CL 2–2 and CL 3–3 were separated into five and six bands respectively on a native analytical isoelectric-focusing polyacrylamide gel [7]. Results suggest that either these homodimers exist in differing oxidative states or there are multiple members in each group. On the basis of immuno-cross-reactivity, substrate-specificity and *N*-terminal sequencing data, subunits CL 2 and CL 3 were proposed to belong to the Mu family and Alpha family respectively [7].

In the present paper we report the cloning and expression of a CL 3 subunit in the baculovirus system. This is the first report of a complete primary sequence of a GST from an avian system. We present evidence suggesting that there is more than one variant in group CL 3.

MATERIALS AND METHODS

Materials

Male white Leghorn chicks were obtained from a local chicken farm and killed 1 day after hatching. The chicken liver cDNA library was obtained from Clontech Co. (Palo Alto, CA, U.S.A.). Substrates for assay of enzyme activity were from Merck (Darmstadt, Germany) or Sigma Chemical Co. (St. Louis, MO, U.S.A.). Primers for sequencing and probes for library screening were synthesized on a Gene Assembler from Pharmacia (Uppsala, Sweden). The Sequenase kit was obtained from United States Biochemical Corp. (Cleveland, OH, U.S.A.). [γ-^{32}P]ATP and [α-[^{35}S]thio]dATP were obtained from New England Nuclear (Wilmington, DE, U.S.A.). *Achromobacter* proteinase I was purchased from Wako Chemicals (Osaka, Japan). Trypsin was obtained from Boehringer Mannheim (Mannheim, Germany). Chemicals used in sequencing were obtained from Applied Biosystems (Foster City, CA, U.S.A.). All other chemicals were reagent grade or better.

Purification of GSTs

Chick liver GSTs were purified as described in ref. [7]. Recombinant GSTs from SF9 cells were isolated essentially as described in ref. [8]. Proteins were eluted from the *S*-hexylglutathione–Sepharose 6B affinity column [9] with 10 mM-Tris/HCl buffer, pH 8.0, containing 5 mM-*S*-hexylglutathione, 0.2 M-NaCl and 6 mM-2-mercaptoethanol and dialysed against 10 mM-potassium phosphate buffer, pH 7.0, containing 1 mM-dithiothreitol and 20% (v/v) glycerol before storage.

Enzyme assay

GST activity was assayed by published methods [10,11] at 25 °C. The protein concentration of crude cell extracts was determined by the Bradford assay [12], with BSA as standard. The concentration of purified GST was determined by measuring the absorbance at 280 nm. The absorption coefficient of the recombinant protein was determined according to the protocol of Gill & von Hippel [13].

Abbreviation used: GST, glutathione *S*-transferase.

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The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number M38219.

Endopeptidase digestion and peptide purification

CL 3 subunits (200 μg) isolated from 1-day-old chick livers were digested with 1.5 units of *Achromobacter* proteinase I (15 h at 37 °C) in 100 μl of 0.1 M-sodium carbonate buffer, pH 9.5. Recombinant CL 3 was digested with trypsin (6 h at 37 °C) in 100 mM-Mops/NaOH buffer, pH 7, containing 100 mM-NaCl at a substrate/enzyme ratio of 20:1 (w/w). The resulting peptides were separated on an Aquapore RP-300 (C_8) reverse-phase column (0.46 cm × 22 cm) and collected for sequence analysis.

Isolation and sequencing of CL 3 cDNA from a λgt11 cDNA library

An oligonucleotide probe (63-mer), 5′-CATCCCCTAAATCTCAACCATTGGCACTTGCTGGAACATCAACACTCCGGCTTGGACTAACTT-3′, was constructed complementary to the coding sequence of a peptide generated by proteinase digestion of CL 3 subunits purified from chick liver. Screening of a chicken liver λgt11 cDNA library was carried out with approx. 250000 plaques under conditions described by Maniatis *et al.* [14]. A positive clone was isolated and designated as λGCL301. The insert was excised from λGCL301 by *Eco*RI digestion, then subcloned into a Bluescript vector for sequence analysis and designated as pGCL301. The DNA sequence was determined by a slight modification of the double-stranded DNA sequencing protocol described in ref. [15] with synthetic oligonucleotides as primers.

Expression of CL 3 cDNA in SF9 cells

The coding sequence of the CL 3 subunit was excised from pGCL301, then inserted into the *Bam*HI site of the baculovirus transfer vector pAcYM1 and designated as pAcYM1/CL3-301 for expression in SF9 cells [8]. The SF9 cells were maintained in culture at 26 °C in TNM-FH medium (GIBCO, Grand Island, NY, U.S.A.) as described by Summers & Smith [16]. Plasmid pAcYM1/CL3-301 was co-transfected into SF9 cells with the wild-type viral DNA AcNPV. Transfer of the CL 3 coding sequence from pAcYM1/CL3-301 to the AcNPV genome was achieved by homologous recombination into the baculovirus polyhedrin gene. The recombinant baculovirus was identified in infected insect-cell monolayers by screening the plaques with rabbit antisera raised against CL 3 subunits. After three cycles of plaque purification, plaques exhibiting no evidence of occlusion bodies (viral polyhedra) under the light microscope were recovered for infection of SF9-cell monolayer cultures for production of recombinant protein.

Protein sequencing and amino acid analysis

Automated cycles of Edman degradation were performed on an Applied Biosystems gas/liquid-phase model 470A/900A sequencer equipped with an on-line model 120A amino acid phenylthiohydantoin analyser according to the procedure of Hewick *et al.* [17]. Proteins were hydrolysed in the gas phase (24 h at 110 °C) with 6 M-HCl containing 1% phenol. After hydrolysis, samples were analysed in a Waters PicoTag amino acid analysis system according to the manufacturer's instructions.

Electrophoresis

Sample preparation and isoelectric focusing of CL 3 on a vertical 7.5% acrylamide gel was performed as described in ref. [7]. SDS/PAGE was performed according to the procedure of Laemmli [18] on a 15% (w/v) acrylamide gel.

Antibody preparation

Antisera against CL 3 subunits from 1-day-old chick livers were prepared by the method of Ramanathan *et al.* [19] on a sample purified on a chromatofocusing column.

RESULTS AND DISCUSSION

Internal sequencing and cloning of GST CL 3

Considerable progress has been made in deducing the amino acid sequences and understanding the molecular mechanisms that regulate the expression of the Alpha family of GSTs [1,20]. Information regarding the structure and function relationship of GSTs in general is, however, still lacking. Apparently homogeneous GST preparations isolated from animals may contain two or more structurally related subunits. Analytical methods such as SDS/PAGE or peptide mapping may not be able to detect this microheterogeneity [21]. We have shown previously that each group of chick liver GSTs isolated by SDS/PAGE can be further resolved into multiple bands on an isoelectric-focusing polyacrylamide gel [7]. In order to obtain a large quantity of homogeneous GSTs for structure and function studies, we resorted to the isolation from chick liver of a GST cDNA clone and the expression of this clone in SF9 cells.

CL 3 subunits are *N*-terminally blocked. Consequently, in order to obtain partial amino acid sequences, CL 3 subunits, purified from 1-day-old chick livers on *S*-hexylglutathione affinity and chromatofocusing columns, were digested with an endopeptidase (*Achromobacter* proteinase I). The resulting fragments were separated by h.p.l.c. on a reverse-phase column (Fig. 1), and collected for *N*-terminal sequence analysis. The amino acid sequence of the peptides (f1–f4) indicated in the chromatogram are YNLYGK, AANRYFPVFEK, LSRADVVLLETILAVVEK and LVQAG(V/F)L(M/L)RQQVPMVEIDGMK.

Peptides f1–f4 show a high degree of sequence similarity to Alpha family GSTs, such as rat subunit 1 (Ya, pGTB38 and pGTR261) [22,23] and subunit 2 (Yc, pGTB42) [24] and human subunits Ha-1 (pGTH1) and Ha-2 (pGTH2) [25,26]. For peptide f4, signals of two amino acid phenylthiohydantoin derivatives at approximately equal intensity were observed for cycles 6 and 8. Probably f4 contains two or more peptides with similar sequences.

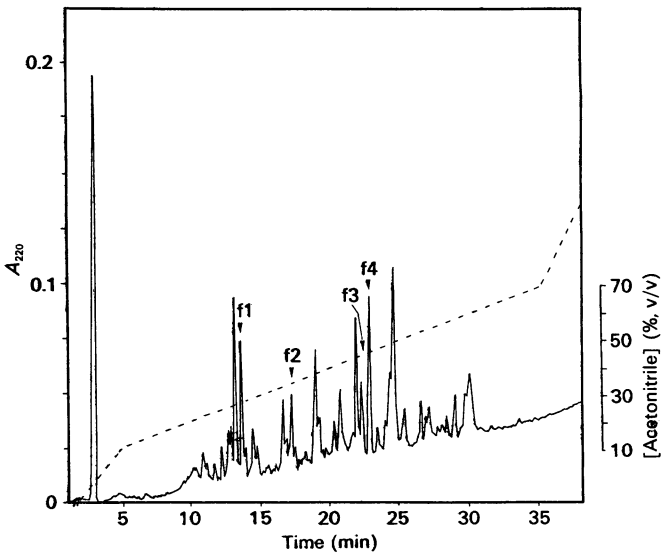

Fig. 1. H.p.l.c. profile of *Achromobacter* proteinase I digests of GST CL 3 subunits isolated from 1-day-old chick liver

Peptides were eluted at a flow rate of 1 ml/min with a linear gradient of 2% (v/v) acetonitrile in 0.08% (v/v) trifluoroacetic acid per min. Fractions isolated for sequence determination are indicated (f1–f4).

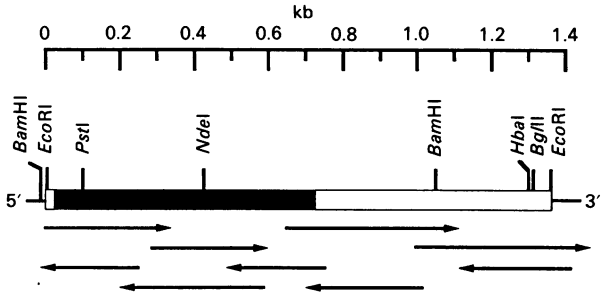

Fig. 2. Partial restriction map and sequencing strategy of pGCL301

Arrows and lines represent the direction and extent of the sequence determinations. The protein coding region is shown by the solid black bar.

Rothkopf *et al.* [27] detected a minimum of five to seven Ya/Yc genes by Southern-blot analysis of rat genomic DNA. The GSTs used in our endopeptidase digestion are obviously heterogeneous, as indicated by isoelectric focusing (see Fig. 5*b*, lane 2). Therefore it is not surprising that fraction f4 consists of at least two peptides with highly similar sequences.

An oligonucleotide probe was constructed according to the f4 peptide sequence for screening a chicken liver λgt11 library. A positive clone was isolated and subsequently inserted into a Bluescript vector and designated as pGCL301 for sequence analysis. A total of nine synthetic primers were used for sequencing both strands of the insert with overlapping regions of at least 60 nucleotide residues. The strategy for sequencing is summarized in Fig. 2.

The nucleotide sequence of pGCL301 and the deduced amino acid sequence are listed in Fig. 3. The insert of pGCL301 is 1342 nucleotide residues long, containing 18 nucleotide residues in the 5′ non-coding region, a 229-codon open reading frame (molecular mass 26209 Da) and a complete (636 nucleotide residues) 3′ non-coding sequence including two poly(A) addition signals. Northern-blotting analysis of mRNA from 1-day-old chick liver with the insert of pGCL301 as probe reveals a single band of approx. 1350 nucleotide residues (results not shown).

The sequence of pGCL301 does not match exactly with peptide fragments f2, f3 and f4 (Fig. 3). The results from peptide sequencing indicated that there are two closely related peptides in fraction f4, neither of which matches the DNA sequence. The data imply that there are multiple CL 3 GSTs.

The deduced amino acid sequence of pGCL301 is listed together with other members of the Alpha family for comparison (Fig. 4). The sequences included are the two variants of rat liver subunit 1 [22,23], subunit 2 [24] and subunit 8 [28], human subunits Ha-1 and Ha-2 [25,26] and mouse Ya subunit [29]. CL 3 encoded by pGCL301 is six to eight residues longer, and has 58% (rat subunit 1 and mouse Ya) to 65% (rat subunit 8) identity of sequence with other members of the Alpha family.

Telakowski-Hopkins *et al.* [30] analysed the structure of a rat liver subunit 1 (Ya) gene and compared it with the subunit 2 (Yc) cDNA. Exon 1 contains the 5′ non-coding region of subunit 1 gene. They pointed out that the amino acid sequences of exons 2 and 4 are highly conserved, whereas exons 3 and 5 encode amino acid residues that are divergent. With all the known sequences for the Alpha family listed in Fig. 4 it is obvious that regions corresponding to subunit 1 exon 2 (residues 1–29), exon 4 (residues 47–91) and the first half of exon 7 (residues 183–205) encode conservative amino acid residues, whereas exon 3 (residues 30–46), exon 5 (residues 92–138) and the second half of exon 7 encode amino acids with dissimilarity. Exon 6 (residues 139–182) has conserved sequences at both ends with a divergent amino acid sequence located in the middle (residues 154–177). The two peptides identified by Hoesch & Boyer [31] as constituting a portion of the active site of GST subunits 1 and 2 are located just at the junction of or outside the conserved regions mentioned above (residues 91–110 and residues 206–218 respectively). It is noteworthy that residues 34–43 are identical in pGCL301 and subunit 8. This similarity is not observed with other members of the Alpha family.

Expression of recombinant CL 3

The CL 3 cDNA clone was expressed in SF9 cells with the use of a baculovirus expression system. A *Bam*HI fragment, containing the coding sequence of CL 3 and approx. 1 kb in length, was excised from pGCL301 and subcloned into pAcYM1. The resulting plasmid (pAcYM1/CL3-301) was co-transfected into SF9 cells with wild-type virus, and the CL 3 gene was integrated into the baculovirus genome through homologous recombination.

SF9 cells infected with recombinant virus were harvested 96 h after infection. Cells were lysed with SDS sample buffer and analysed by SDS/PAGE on a 15% polyacrylamide gel. A protein with a molecular mass of 25.2 ± 1.2 kDa was clearly overexpressed (Fig. 5*a*, lane 3). On the basis of densitometric scanning of the SDS/polyacrylamide gel, this protein band represented 14.7 ± 1.3% of the total soluble proteins.

The recombinant enzyme was purified on an *S*-hexylglutathione–Sepharose 6B column and the recovery is summarized in Table 1. GSH was purposely omitted from the affinity-column elution buffer. The presence of GSH or its oxidized form can cause a rapid decrease in enzyme activity during the course of chromatography. Routinely, 1.4–1.6 mg of purified enzyme can be obtained from 40 ml of cell culture. In an earlier investigation we inserted a GST subunit 3 cDNA with 45 nucleotide residues at the 3′ non-coding region into pAcYM1 for expression and obtained 1.3–1.5 mg of purified proteins per 40 ml of cell culture [8]. In the present construct (pAcYM1/CL3-301), the 3′ non-coding region contains 332 nucleotide residues and a similar amount of expressed proteins was obtained. Therefore the length of 3′ non-coding region (up to 332 nucleotide residues) probably does not play an important role in determining the success or efficiency of expression.

GSTs from rat brain [32], rat liver [33,34], human liver [35,36] and maize [37,38] have been expressed in *Escherichia coli*. The yields of expressed protein obtained by using these techniques are relatively low. The most efficient bacterial expression system for GST was reported by Wang *et al.* [34]. About 50 mg was recovered from 9 litres of *E. coli* culture or about 8 times less than in the baculovirus system. In that particular expression system, approx. 10% of the recombinant protein still retained methionine, probably as a consequence of incomplete processing at the *N*-terminus [34]. In the present study, the recombinant CL 3 was *N*-terminally blocked as is the native protein, even though the nature of the blockage is unknown. Consequently the baculovirus expression system offers the advantages of improved yields and post-translational modifications over other alternatives for producing homogeneous GST.

Preliminary characterization of recombinant CL 3

CL 3 subunits were digested with the proteinase trypsin. The resulting peptides were separated by h.p.l.c. (Fig. 6) and collected for *N*-terminal sequencing. We have confirmed the sequence of 175 amino acid residues in CL 3. The sequences of these peptides are presented in Fig. 3. Peptide T6 is the *C*-terminal fragment and has the sequence MYYDVKPH. Peptide T12 is *N*-terminally

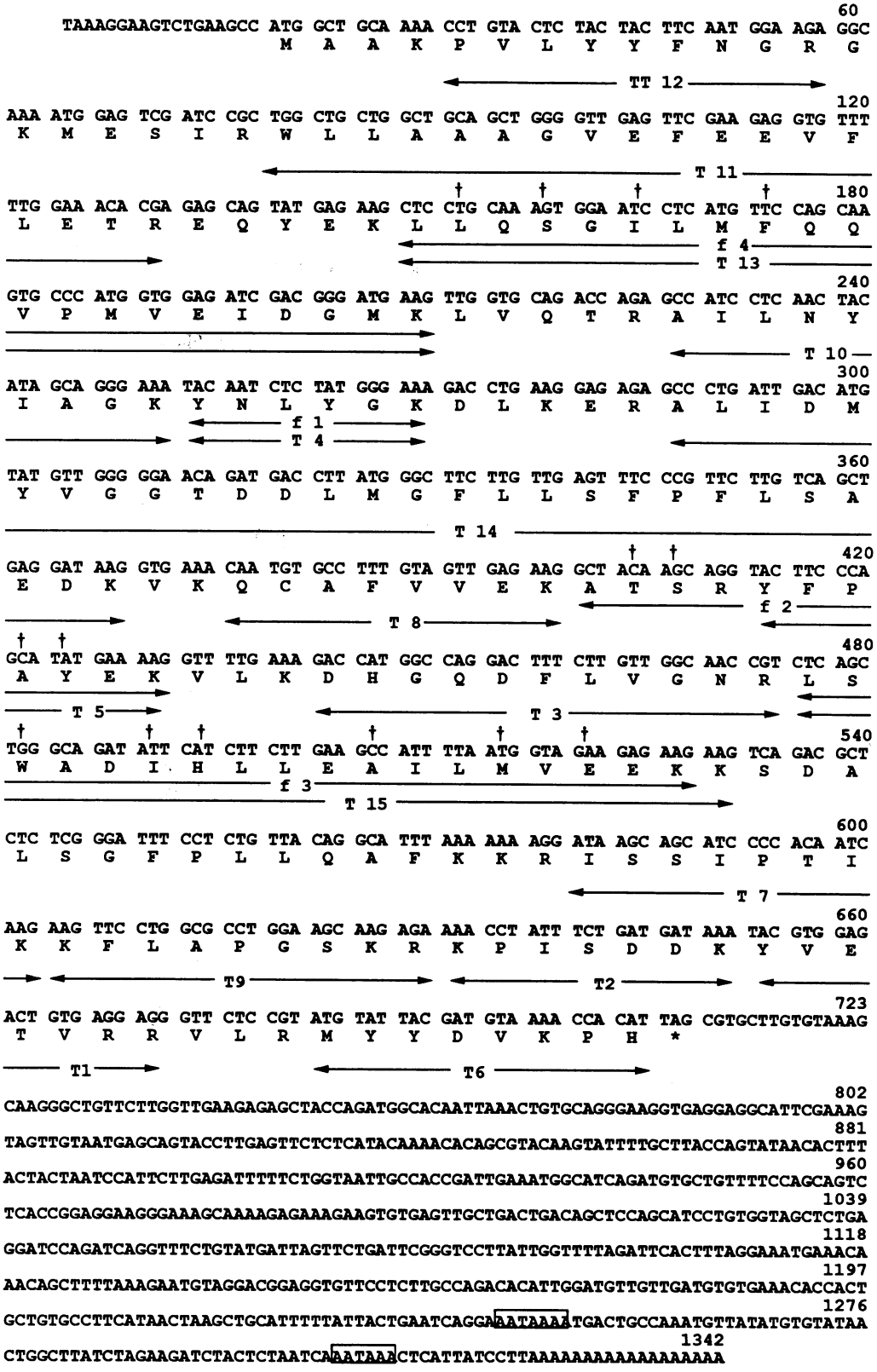

Fig. 3. Nucleotide sequence of pGCL301 and the deduced amino acid sequence

Amino acid sequences of f1–f4 and tryptic-digest fragments of expressed CL 3 subunit (T1–T15) are indicated. † indicates discrepancy between the experimentally determined amino acid sequence of f1–f4 and that predicted from the cDNA sequence. The termination codon is denoted by an asterisk (*) and the polyadenylation signals are boxed.

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CL 3          AAKPVLYYFNGRGKMESIRWLLAAAGVEFEEVFLETREQYEKLLQSGIL  50
Rat 8      AcMEV--K----Q---R--V------T-------E-----------QKDDC-
Rat 1¶     (M)SG----H--AN--R--C--------------KLIQSP-DL---KKD-N-
Rat 1*     (M)SG----H--AN--R--C----------FD--K-IQSP-DL---KKD-N-
Rat 2      (M)PG----H--DG--R--P--------------Q--K--DDLAR-RND-S-
Ha-1       (M)-E--K-H---A--R---T-------------K-IKSA-DLD--RND-Y-
Ha-2       (M)-E--K-H-S-I--R-----------------K-IKSA-DLD--RND-Y-
Mouse Ya   (M)-G----H---A--R--C--------------K-IQSP-DL---KKD-N-

CL 3        MFQQVPMVEIDGMKLVQTRAILNYIAGKYNLYGKDLKERALIDMYVGGTD 100
Rat 8       L-G---L------L-T------S-L-A------------VR----AD--Q
Rat 1¶      ---------------A----------T--D-----M---------SE-IL
Rat 1*      --D------K-----A----------T--D---------------TE-IL
Rat 2       -------------------------T--------M---------AE-VA
Ha-1        -------------------------S--------I---------IE-IA
Ha-2        -------------------------S--------I--K------IE-IA
Mouse Ya    --D------------A----------T--D-----M---------SE-IL

CL 3        DLMGFLLSFPFLSAEDKVKQCAFVVEKATSRYFPAYEKVLKDHGQDFLVG 150
Rat 8       ---MMIIGA--KAPQE-EESL-LA-KR-KN----VF--I-----EA----
Rat 1¶      --TEMIIQLVICPPDQREAKT-LAKDRTKN--L--F-----S----Y---
Rat 1*      --TEMIMQLVICPPDQ-EAKT-LAKDRTKN--L--F-----S----Y---
Rat 2       --DEIV-HY-YIPPGE-EASL-KIKD--RN-----F-----S----Y---
Ha-1        --GEMI-LL-VCPP-E-DAKL-LIK--IKN-----F-----S----Y---
Ha-2        --GEMI-LL-FTQP-EQDAKL-LIQ--IKN-----F-----S----Y---
Mouse Ya    --TEMIGQLVLCPPDQREAKT-LAKDRTKN--L--F-----S----Y---

CL 3        NRLSWADIHLLEAILMVEEKKSDALSGFPLLQAFKKRISSIPTIKKFLAP 200
Rat 8       -Q------Q----------VSAPV--D--------T---N--------Q-
Rat 1¶      ---TRV------LL-Y---FDASL-TS----K---S----L-NV----Q-
Rat 1*      ---TRV------LL-Y---FEASL-TS----K---S----L-NY----Q-
Rat 2       ----R--VY-VQVLYH---LDPS--AN----K-KRT-V-NL--V----Q-
Ha-1        -K--R-----V-LLYY---LD-SLI-S----K-L-T---NL--V----Q-
Ha-2        -K--R-----V-LLYY---LD-SLI-S----K-L-T---NL--V----Q-
Mouse Ya    ---TRV------VLLY---FDASL-TP----K---S----L-NV----Q-

CL 3        GSKRKPISDDKYVETVRRVLRMYYDVKPH                    229
Rat 8       --Q---PP-GHY-DV--T--KF                           222
Rat 1¶      --Q---AM-A-QI-EA-K-FKF                           222
Rat 1*      --Q--LAM-A-QI-EA-KIFKF                           222
Rat 2       --Q---LE-E-C--SAVKIFS                            221
Ha-1        --P---PM-E-SL-EA-KIFRF                           222
Ha-2        --P---PM-E-SL-ES-KIFRF                           222
Mouse Ya    --Q---PM-A-QIQEA-KAFKIQ                          223
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Fig. 4. Comparison of the deduced amino acid sequence of GST CL 3 (pGCL301) and Alpha class GST subunits

Key: Rat 1¶, pGTB38 [22]; Rat 1*, pGTR261 [23]; Rat 2, pGTB 42 [24]; Ha-1, pGTH1 [25]; Ha-2, pGTH2 [26]; Mouse Ya, prYa12 [29]. The primary structure of rat subunit 8 was determined by peptide sequencing [28]. The amino acid sequence is designated by the single-letter code. Dashes (–) indicate identity with amino acid residues of CL 3. Arrowheads (▲) mark the position of introns in subunit 1 and mouse Ya genomic DNA.

blocked. On the basis of amino acid analysis (results not shown) peptide T12 does not contain methionine and the composition of this peptide match residues 2–13 of the sequence deduced from cDNA. Further digestion of peptide T12 with trypsin yields a peptide (TT12) with the sequence PVLYYFNGR. Therefore we have expressed the complete cDNA clone. We have not detected any post-translational modification except the *N*-terminal blockage.

The purified recombinant CL 3 is at least 95% pure on the basis of SDS/PAGE and isoelectric-focusing analysis (Fig. 5). The protein has a pI of 9.45 and an absorption coefficient $A_{1cm}^{1\%}$ of 13.0 ± 0.5 at 280 nm.

The substrate-specificities of CL 3 are listed in Table 2. The activities of rat liver GSTs from the Alpha family [1] and basic 1-day-old chick liver GSTs isolated by chromatofocusing (MonoP 1 and MonoP 2) are also listed for comparison [7]. In general, CL 3 has a lower specific activity than other members of the Alpha family with most of the substrates tested. In particular, CL 3 has no peroxidase activity. A high relative activity with ethacrynic acid (47% of the specific activity with 1-chloro-2,4-

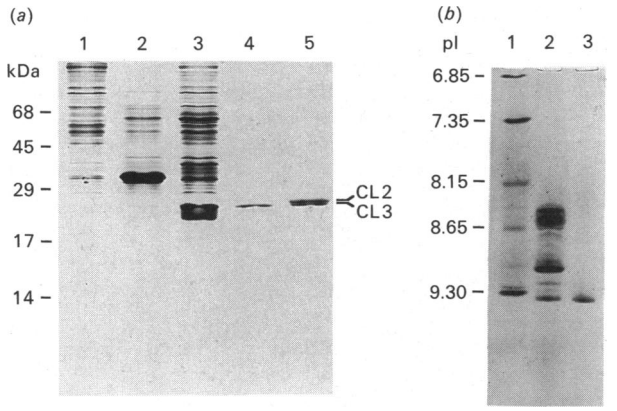

Fig. 5. SDS/PAGE and isoelectric focusing of GST CL 3

(*a*) SDS/PAGE of crude cell extracts from SF9 cells (lane 1), cells infected with wild-type baculovirus (lane 2) and cells infected with recombinant baculovirus containing the CL 3 cDNA (lane 3). Lanes 4 and 5 are purified recombinant protein and GSTs isolated from chick livers on an *S*-hexylglutathione–Sepharose 6B column respectively. (*b*) Isoelectric-focusing analysis of chick liver CL 3 after affinity and chromatofocusing columns (lane 2) and purified recombinant CL 3 (lane 3). Lane 1 was pI markers.

Table 1. Purification of recombinant GST CL 3

Step	Total protein (mg)	Total activity (μmol/min)	Specific activity (μmol/min per mg of protein)	Yield (%)
Crude extract	66±0.1	63±3	0.95±0.05	100
Centrifugation	45±0.1	55±3	1.20±0.07	87
Dialysis	37±0.2	53±4	1.43±0.11	84
S-Hexylglutathione–Sepharose 6B	7±0.1	42±2	6.0±0.3	67

dinitrobenzene) was observed for CL 3. A similar observation was reported for rat GST 8–8, which is an acidic protein with a pI of 6.0 [39].

We have shown previously that CL 3 can be separated into multiple bands with pI values ranging from 8.0 to 9.45 on a native isoelectric-focusing gel [7]. Comparing the CL 3 isozymes isolated from liver tissue and the recombinant protein, it is obvious that the recombinant protein is the most basic and one of the less abundant GSTs of the CL 3 group (Fig. 5*b*). The data further support the notion that there are multiple variants of CL

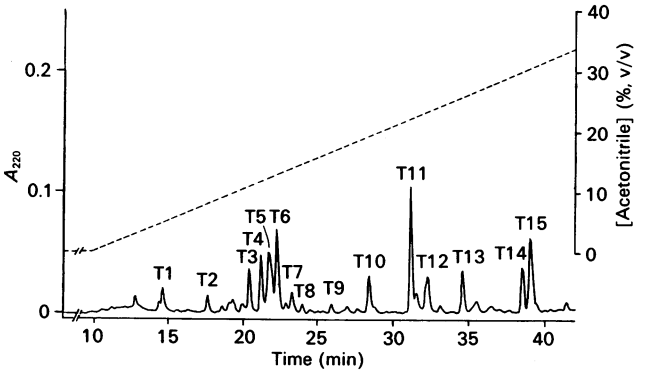

Fig. 6. H.p.l.c. profile of trypsin digests of recombinant GST CL 3

Peptides were eluted at a flow rate of 1 ml/min with a linear gradient of 1% (v/v) acetonitrile in 12 mM-potassium phosphate buffer, pH 6.0, per min. Fractions collected for amino acid sequence analysis are indicated.

3 with distinctive pI values. Nonetheless, we cannot exclude the possibility that they are the same protein with different conformation or post-translational modifications.

In summary, we have isolated and expressed in SF9 cells a cDNA clone from chicken liver. The expressed protein can catalyse the conjugation of GSH and electrophilic alkylating compounds. On the basis of molecular mass (analysed by SDS/PAGE) the expressed protein is a member of the CL 3 group. Amino acid sequence similarity indicates that this protein belongs to the Alpha family of GSTs.

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REFERENCES

1. Mannervik, B. & Danielson, U. H. (1988) CRC Critical Rev. Biochem. **23**, 283–337
2. Coles, B. & Ketterer, B. (1990) CRC Critical Rev. Biochem. **25**, 47–70
3. Tahir, M. K., Guthenberg, C. & Mannervik, B. (1985) FEBS Lett. **181**, 249–252
4. Ålin, P., Mannervik, B. & Jörnvall, H. (1985) FEBS Lett. **182**, 319–322
5. Kispert, A., Meyer, D. J., Lalor, E., Coles, B. & Ketterer, B. (1989) Biochem. J. **260**, 789–793

Table 2. Substrate-specificities of recombinant GST CL 3

Abbreviation: N.D., not detected.

	Specific activity (μmol/min per mg)					
	CL 3	MonoP 1*	MonoP 2*	GST 1–1†	GST 2–2†	GST 8–8†
1-Chloro-2,4-dinitrobenzene	6.0±0.5	23.9	10.8	50	17	10
1,2-Dichloro-4-nitrobenzene	0.023±0.005	N.D.	N.D.	< 0.04	< 0.04	0.12
1,2-Epoxy-3-(*p*-nitrophenoxy)propane	0.45±0.05	0.8	N.D.	< 0.1	< 0.1	N.D.
Androst-5-ene-3,17-dione	N.D.	0.1	0.17	4.2	0.36	N.D.
Ethacrynic acid	2.8±0.2	1.5	1.8	0.08	1.24	7.0
Cumene hydroperoxide	N.D.	2.0	3.0	3.1	7.9	1.1

* Data from Chang *et al.* [7].
† Data from Mannervik & Danielson [1].

6. Yeung, T.-C. & Gidari, A. S. (1980) Arch. Biochem. Biophys. **205**, 404–411
7. Chang, L.-H., Chuang, L.-F., Tsai, C.-P., Tu, C.-P. D. & Tam, M. F. (1990) Biochemistry **29**, 744–750
8. Hsieh, J.-C., Liu, L.-F., Chen, W.-L. & Tam, M. F. (1989) Biochem. Biophys. Res. Commun. **162**, 1147–1154
9. Mannervik, B. & Guthenberg, C. (1981) Methods Enzymol. **77**, 231–235
10. Habig, W. H. & Jakoby, W. B. (1981) Methods Enzymol. **77**, 398–405
11. Lawrence, R. A. & Burke, R. F. (1976) Biochem. Biophys. Res. Commun. **71**, 952–958
12. Bradford, M. M. (1976) Anal. Biochem. **143**, 248–254
13. Gill, S. C. & von Hippel, P. H. (1989) Anal. Biochem. **182**, 319–326
14. Maniatis, T., Fritsch., E. F. & Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor
15. Chi, H. C., Hsieh, J. C., Hui, C. F. & Tam, M. F. (1988) Nucleic Acids Res. **16**, 10382
16. Summers, M. D. & Smith, G. E. (1987) Tex. Agric. Exp. Stn. Bull. no. 1555: A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures
17. Hewick, R. M., Hunkapillar, M. W., Hood, L. E. & Drey, W. J. (1981) J. Biol. Chem. **256**, 7990–7997
18. Laemmli, U. K. (1970) Nature (London) **227**, 680–685
19. Ramanathan, L., Guyer, R. B., Karakawa, W. W., Buss, E. G. & Glagett, C. O. (1979) Mol. Immunol. **16**, 935–941
20. Pickett, C. B. & Lu, A. Y. H. (1989) Annu. Rev. Biochem. **58**, 743–764
21. Pickett, C. B., Telakowski-Hopkins, C. A., Ding, G. J.-F., Wang, R. W. & Lu, A. Y. H. (1985) in Microsomes and Drug Oxidations (Boobis, A. R., Caldwell, J., De Matteis, F. & Elcombe, C. R., eds.), pp. 128–135, Taylor and Francis, London
22. Pickett, C. B., Telakowski-Hopkins, C. A., Ding, G. J.-F., Argenbright, L. & Lu, A. Y. H. (1984) J. Biol. Chem. **259**, 5182–5188
23. Lai, H.-C. J., Li, N., Weiss, M. J., Reddy, C. C. & Tu, C.-P. D. (1984) J. Biol. Chem. **259**, 5536–5542
24. Telakowski-Hopkins, C. A., Rodkey, J. A., Bennett, C. D., Lu, A. Y. H. & Pickett, C. B. (1985) J. Biol. Chem. **260**, 5820–5825
25. Tu, C.-P. D. & Qian, B. (1986) Biochem. Biophys. Res. Commun. **141**, 229–237
26. Rhoads, D. M., Zarlengo, R. P. & Tu, C.-P. D. (1987) Biochem. Biophys. Res. Commun. **145**, 474–481
27. Rothkopf, G. S., Telakowski-Hopkins, C. A., Stotish, R. L. & Pickett, C. B. (1986) Biochemistry **25**, 993–1002
28. Ålin, P., Jensson, H., Cederlund, E., Jörnvall, H. & Mannervik, B. (1989) Biochem. J. **261**, 531–539
29. Daniel, V., Sharon, R., Tichauer, Y. & Sarid, S. (1987) DNA **6**, 317–324
30. Telakowski-Hopkins, C. A., Rothkopf, G. S. & Pickett, C. B. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 9393–9397
31. Hoesch, R. M. & Boyer, T. D. (1989) J. Biol. Chem. **264**, 17712–17717
32. Abramovitz, M., Ishigaki, S., Felix, A. M. & Listowsky, I. (1988) J. Biol. Chem. **263**, 17627–17631
33. Lai, H.-C. J., Qian, B., Grove, G. & Tu, C.-P. D. (1988) J. Biol. Chem. **263**, 11389–11395
34. Wang, R. W., Pickett, C. B. & Lu, A. Y. H. (1989) Arch. Biochem. Biophys. **269**, 536–543
35. Chow, N.-W. I., Whang-Peng, J., Kao-Shan, C.-S., Tam. M. F., Lai, H.-C. J. & Tu, C.-P. D. (1988) J. Biol. Chem. **263**, 12797–12800
36. DeJong, J. L., Chang, C.-M., Whang-Peng, J., Knutsen, T. & Tu, C.-P. D. (1988) Nucleic Acids Res. **16**, 8541–8554
37. Moore, R. E., Davis, M. S., O'Connell, K. M., Harding, E. I., Wiegand, R. C. & Tiemeier, D. C. (1986) Nucleic Acids Res. **14**, 7227–7235
38. Grove, G., Zarlengo, R. P., Timmerman, K. P., Li, N.-Q., Tam, M. F. & Tu, C.-P. D. (1988) Nucleic Acids Res. **16**, 425–438
39. Jensson, H., Guthenberg, C., Ålin, P. & Mannervik, B. (1986) FEBS Lett. **203**, 207–209

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