Glutathione S-transferase Yc cDNA from Syrian hamster kidney

Frideriki MAGGOUTA*, Sara A. LI†, Jonathan J. LI† and James S. NORRIS*‡

*Division of Rheumatology and Immunology, Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29425 and †Division of Etiology and Prevention of Hormonal Cancers, University of Kansas Cancer Center, University of Kansas Medical Center, Kansas City, KS 66160-7312, U.S.A.

A cDNA encoding alpha-class glutathione S-transferase Yc (GSTYc) has been isolated from a Syrian hamster kidney library, and its nucleotide sequence (968 bp) has been determined. Analysis of the deduced amino acid sequence revealed a high level of identity between Syrian hamster GSTYc, rat GST Yc1 and Yc2 and mouse GSTYc. Northern-blot experiments demonstrated that Syrian hamster GSTYc expression is tissue-specific. A GSTYc mRNA of approx. 1 kb is expressed in liver, kidney, vas deferens and epididymis. Expression of the GSTYc transcript was not detected in testis or uterus.

INTRODUCTION

The glutathione S-transferases (GSTs) are a multigene family of enzymes predominantly active in cellular detoxification pathways by catalysing conjugation of glutathione to a variety of endogenous and xenobiotic electrophiles [1,2]. The cytosolic GSTs can be divided into five classes, termed alpha, mu, pi, theta and sigma [3–5], and each class consists of various dimeric isoforms formed by closely related subunits of the same class.

Several alpha-class isoforms have been purified and characterized from a number of species, including rat and mouse. In the rat at least five subunits (Ya1, Ya2, Yc1, Yc2, Yk) have been found to form the alpha-class GSTs. The sequences of the rat GST Ya subunit [6] and the GST Yc2 subunit [7] have been characterized at the gene level. cDNAs coding for the two different subunits, Ya1 [8] and Ya2 [9], have been described. Furthermore, three cDNAs coding for subunits Yc1 [10,11], Yc2 [11,12] and Yk [13] have been published. In the protein coding region, the Ya and Yc subunit cDNAs are approx. 75 % identical, whereas the 5′ and 3′ untranslated regions of the mRNAs are very divergent [10].

In the mouse, the structure and sequence of the alpha GST Ya subunit gene have been presented by Daniel et al. [14]. So far, two cDNAs encoding subunits Ya [15] and Yc [16] have been isolated and their sequences determined.

The expression of GSTs has been reported to be tissue specific. For example, expression of the rat alpha-class Ya and Yc subunit mRNAs does not occur in heart, seminal vesicles, lung and spleen, but is detected in kidney, testis and liver [17].

Although Syrian hamsters are frequently used as experimental animals, little is known about hamster alpha-class GSTs. Only a few subunits, namely SG1, SG2, A1 and A2, purified from Syrian hamster liver, have been described, but none of the corresponding nucleotide sequences have been determined so far [18,19]. In this paper we report on the cDNA cloning, sequence and expression pattern of an alpha-class GST, designated GSTYc, from Syrian hamster kidney. GSTYc is closely related to the genes encoding rat and mouse alpha-class GSTs and represents the first cDNA clone complementary to an mRNA specific for an alpha-class subunit of Syrian hamster GSTs.

MATERIALS AND METHODS

Cloning and sequencing

Clones from a Syrian hamster cDNA kidney library (Stratagene) were randomly chosen and isolated in a survey of expressed sequence-tagged sites (ETSs) to serve as landmarks in the physical mapping of the Syrian hamster genome.

Partial sequences of the cDNA clones were determined by automated fluorescent sequencing using the Prism Ready Reaction DyeDeoxy Terminator Sequencing Kit (Perkin Elmer) and an ABI Model 373 DNA Sequencer. Sequenced cDNAs were analysed for similarity to sequences in the GenBank nucleic acid database. One cDNA clone was found to match with DNA sequences of alpha-class GST subunit Yc and was termed GSTYc. Sequencing of the complete GSTYc cDNA clone was performed as described above.

RNA preparation, Northern blot and hybridization analysis

RNA was prepared from vas deferens, epididymis, testis, kidney, uterus and liver from control and diethylstilboestrol (DES)-treated Syrian hamsters using guanidinium isothiocyanate/phenol/chloroform extraction [20].

Each RNA (10 mg) was denatured with formaldehyde and electrophoresed on a 1.2 % agarose gel in 1 × Mops buffer (pH 7.0), before vacuum transfer onto a nylon filter in 10 × SSPE (1 × SSPE = 0.15 M NaCl/0.01 M sodium phosphate, pH 7.4/1 mM EDTA). Nylon filters were prehybridized for 4 h and hybridized overnight at 65 °C using a [α-^{32}P]UTP GSTYc antisense RNA probe. The RNA probe was generated from a 228 bp *Hin*cII fragment of the GSTYc cDNA (nucleotide positions: 702 to 930). The filter was washed at 65 °C to a final stringency of

Abbreviations used: GST, glutathione S-transferase; ETS, expressed sequence-tagged site; DES, diethylstilboestrol; GADPH, glyceraldehyde-3-phosphate dehydrogenase.

‡ To whom correspondence should be addressed.

The Syrian hamster GSTYc cDNA sequence data reported have been submitted to the EMBL/GenBank/DDBJ Nucleotide Sequence Databases under the accession number Y09083.

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                -38    GGCACGAGTTTTAACAAGAAAACCAAGTGGTTGCTGCC

ATG GCG GGG AAG CCA GTC CTT CAC TAC TTC GAT GGC CGG GGA AGA   45
 M   A   G   K   P   V   L   H   Y   F   D   G   R   G   R   15

ATG GAG CCT GTC CGG TGG CTC TTG GCT GCA GCT GGA GTG GAG TTT   90
 M   E   P   V   R   W   L   L   A   A   A   G   V   E   F   30

GAA GAG AAA TTT CTG AAA ACT CGG GAT GAC TTG GGA AGG TTA AGA  135
 E   E   K   F   L   K   T   R   D   D   L   G   R   L   R   45

AAT GAT GGG AGT TTG GTG TTC CAG CAA GTG CCC ATG GTG GAG ATT  180
 N   D   G   S   L   V   F   Q   Q   V   P   M   V   E   I   60

GAC GGG ATG AAG CTG GTG CAG ACC AGA GCC ATT CTC AAC TAC ATT  225
 D   G   M   K   L   V   Q   T   R   A   I   L   N   Y   I   75

GCC TCC AAA TAC AAC CTC TAT GGG AAG GAC ATG AAG GAG AGA GCC  270
 A   S   K   Y   N   L   Y   G   K   D   M   K   E   R   A   90

CTC ATT GAC ATG TAT GCA GAA GGT ATA GCG GAT CTG GAT GAA ATC  315
 L   I   D   M   Y   A   E   G   I   A   D   L   D   E   I  105

GTT CTC CAT CAA CCT TAT GTT CCC CGA GAG GAG AAA GAG GCA AAC  360
 V   L   H   Q   P   Y   V   P   R   E   E   K   E   A   N  120

CTT GCC AAG ATC AAG GAT AAA GCA AGG AAC CGT TAC TTC CCT GCC  405
 L   A   K   I   K   D   K   A   R   N   R   Y   F   P   A  135

TAT GAG AAG GTG TTG AAG AGC CAC GGA CAA GAT TAT CTC GTT GGG  450
 Y   E   K   V   L   K   S   H   G   Q   D   Y   L   V   G  150

AAC AGG CTG AGC AGG GCT GAT GTT TAC CTG GTT GAA CTT CTC TAC  495
 N   R   L   S   R   A   D   V   Y   L   V   E   L   L   Y  165

CAT GTG GAA GAG CTG GAT CCC AGC GTT TTG GCC AAC TTC CCT CTG  540
 H   V   E   E   L   D   P   S   V   L   A   N   F   P   L  180

CTG AAG GCA CTG AGA ACC AGA GTC AGC AAC CTC CCA ACA GTG AAG  585
 L   K   A   L   R   T   R   V   S   N   L   P   T   V   K  195

AAG TTT CTT CAG CCT GGC AGC CAG AGG AAA CCT TAC GAG GAC GAG  630
 K   F   L   Q   P   G   S   Q   R   K   P   Y   E   D   E  210

AAA CGT GTA GAA TCA GTA ATG AAG ATT TTC AGT TAAATCAGGCACAGA  678
 K   R   V   E   S   V   M   K   I   F   S                  221

TAGATGGATAGCAGCCACCAGGTCAACCTTCTTAAGCGTCGCAACAATGAAGTGTTTTG  737
ATTAAATGTTGATCCTGCTTATTGTGAGGCTAACATGTTTTCTAAGCTTTGAGCATTAA  796
TTCAAGTAGTCATGACCTGTGCAGAATTGCTAGGATGCTATTGTAGTCCAAATTGGAAT  855
CATGATCACTTCCTAGGATGTTCCCTTGAATTTCAATAAAATAGAACAAGCTTCTTAGA  914
AAAAAAAAAAAAAAAA                                             930
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Figure 1 Nucleotide sequence of the Syrian hamster GST Yc cDNA, with the deduced amino acid sequence in single-letter code underneath

Position +1 is the first base in the ATG initiation code.

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hYc   MAGKPVLHYFDGRGRMEPVRWLLAAAGVEFEEKFLKTRDDLGRLRNDGSL  50
rYc1  MPGKPVLHYFDGRGRMEPIRWLLAAAGVEFEEQFLKTRDDLARLRNDGSL  50
rYc2  MAGKPVLHYFDGRGRMEPIRWLLAAAGVEFEENFLKTRDDLARLRSDGSL  50
mYc   MAGKPVLHYFDGRGRMEPIRWLLAAAGVEFEEKFLKTRDDLARLRSDGSL  50
      *.****************.*************.*********.***.****

      VFQQVPMVEIDGMKLVQTRAILNYIASKYNLYGKDMKERALIDMYAEGIA 100
      MFQQVPMVEIDGMKLVQTRAILNYIATKYNLYGKDMKERALIDMYAEGVA 100
      MFEQVPMVEIDGMKLVQTRAILNYIATKYNLYGKDMKERALIDMYAEGVA 100
      MFQQVPMVEIDGMKLVQTKAILNYIASKYNLYGKDMKERAIIDMYTEGVA 100
      .*.***************.*******.*************.****.**.*

      DLDEIVLHQPYVPREEKEANLAKIKDKARNRYFPAYEKVLKSHGQDYLVG 150
      DLDEIVLHYPYIPPGEKEASLAKIKDKARNRYFPAFEKVLKSHGQDYLVG 150
      DLELMVLYYPYMPPGEKEASLAKIKDKARNRYFPAYEKVLKSHGQDYLVG 150
      DLEIMILYYPHMPPEEKEASLAKIKEQTRNRYFPAFEKVLKSHGQDYLVG 150
      **. ..*. *..*..****.*****...*******.**************

      NRLSRADVYLVELLYHVEELDPSVLANFPLLKALRTRVSNLPTVKKFLQP 200
      NRLSRADVYLVQVLYHVEELDPSALANFPLLKAKRTRVSNLPTVKKFLQP 200
      NKLSRADVSLVELLYHVEEMDPGIVDNFPLLKAKRTRVSNLPTVKKFLQP 200
      NRLSRADIALVELLYHVEELDPGVVDNFPLLKALRSRVSNLPTVKKFLQP 200
      *.*****. **..******.**. ..******* *.**************

                                        % identity to hYc
      GSQRKPYEDEKRVESVMKIFS 221
      GSQRKPLEDEKCVESAVKIFS 221                 91
      GSQRKPFDDEKCVESAKKIFS 221                 87
      GSQRKPFDDAKCVESAKKIFS 221                 85
      ****** .*.* ***. ****
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Figure 2 Comparison of the deduced amino acid sequences of alpha-class GSTs from Syrian hamster (hYc), rat (rYc1 and rYc2) and mouse (mYc)

The amino acid sequence is designated by the single-letter code. Residues indicated with an asterisk show identity between all four sequences. Conservative exchanges are marked by dots.

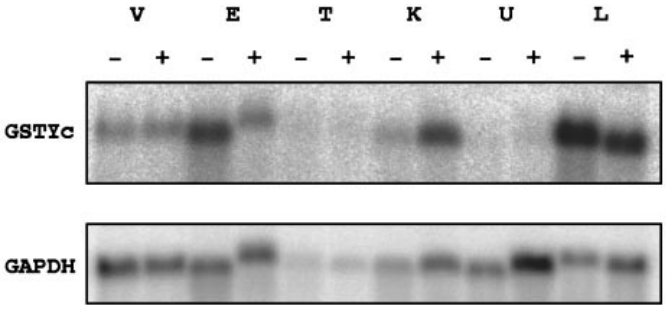

Figure 3 Expression of GSTYc in Syrian hamster tissues

Total RNA was isolated from vas deferens (V), epididymis (E), testis (T), kidney (K), uterus (U) and liver (L) of untreated (–) and DES-treated (+) Syrian hamsters. Total RNA (10 mg) was denatured, electrophoresed, transferred to a nylon membrane and hybridized with an [α-^{32}P]UTP-labelled GSTYc RNA probe. The same blot was rehybridized with GAPDH, to quantify the RNA amount loaded (bottom).

0.5 × SSPE and 0.1 % SDS. To normalize the expression levels to variable amounts of RNA loaded, the same blot was hybridized with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe. Northern-blot analysis was performed using a Phosphorimaging system (Molecular Dynamics).

RESULTS

A Syrian hamster cDNA clone encoding an alpha-class GST isoform, Yc, was isolated from a commercially available cDNA kidney library. DNA sequencing of the 968 nucleotide cDNA clone showed that it includes the presumed complete coding region of 663 nucleotides. The open reading frame is preceded by 38 nucleotides of 5′ non-coding sequence and followed by 250 nucleotides of 3′ non-coding sequence and a poly(A) tail of 17 nucleotides. The putative polyadenylation signal is located between nucleotides 890 and 896. The complete nucleotide sequence and the deduced amino acid sequence indicating a protein of 221 amino acids are shown in Figure 1.

Sequence comparison of the GSTYc cDNA with other alpha-class GSTs revealed an 86 % identity at the nucleotide level with rat Yc1 and Yc2 and mouse Yc subunit cDNAs, and only a 76 % identity with rat Ya1 and Ya2, and mouse Ya subunit cDNAs respectively.

The deduced amino acid sequence from the Syrian hamster cDNA was found to have a 91 % identity with the GSTYc1 subunit and an 87 % identity with the GSTYc2 subunit of the rat. A high identity, 85 %, also exists with the mouse GSTYc. After alignment, there is a 79 % identity between all four sequences (Figure 2). In contrast, only 69 % sequence similarity was observed at the amino acid level between the Syrian hamster GSTYc described here and either of the rat alpha-class subunits GSTYa1 and GSTYa2.

Northern blots of 10 mg of total RNA from vas deferens, epididymis, testis, kidney, uterus and liver of control and DES-treated Syrian hamsters were hybridized with an [α-^{32}P]UTP-labelled RNA probe generated from a 228 bp *Hin*cII fragment of the GSTYc cDNA. A specific GSTYc mRNA of approx. 1000 nucleotides was detected in vas deferens, epididymis, kidney and liver (Figure 3). The size of the detected mRNA corresponds well with the size of the GSTYc cDNA (968 bp). No GSTYc

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     17                                  50
hYc  EPVRWLLAAAGVEFEEKFLKTRDDLGRLRNDGSL
A1
A2   EFI  LLAAAGVEF

     51                                                  100
hYc  VFQQVPMVEIDGMKLVQTRAILNYIASKYNLYGKDMKERALIDMYAEGIADLDE
A1    FQQVP VEIDG KLVQTRAILNYIASKYNLYGKD
A2          VEIDGTTLVQTRAILNYIAT                    YAEGIADLNE
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Figure 4 Syrian hamster alpha-class GSTs

Amino acid sequences deduced from a partial region (positions 17 to 104) of the GSTYc cDNA compared with amino acid sequences of N-terminal and CNBr-derived peptides from the A1 and A2 subunits. The numbering of the residues starts with the initiator methionine.

expression was observed in testis and uterus. Furthermore, no significant differences were found in the GSTYc mRNA levels between control and DES-treated Syrian hamsters.

DISCUSSION

The cDNA clone (GSTYc) isolated from a Syrian hamster kidney library has substantial identity (86%) with cDNA sequences of two rat alpha-class GST subunits (Yc1, Yc2), and a mouse alpha-class GST subunit (Yc). Comparison of the deduced amino acid sequence of the coding region shows that the Syrian hamster GSTYc is more closely related to rat GSTYc1 (91%) than to rat GSTYc2 (87%) and to the mouse GSTYc (85%). Interestingly, the first 140 amino acids corresponding to the N-terminal sequence of all four polypeptides are more conserved than the amino acids corresponding to the central or C-terminal sequence of the proteins. The highly conserved regions indicate common properties shared by all four polypeptides.

Two Syrian hamster alpha-class GSTs subunits, termed A1 and A2, have been purified and partial amino acid sequences of amino-terminal and CNBr-derived fragments of A1 and A2 have been determined by Bogaards et al. [19]. As can be seen in Figure 4, the amino acid sequence deduced from GSTYc cDNA shows a perfect match to the partial sequenced amino acid region (position 51 to 84) of the A1 subunit, indicating that the GSTYc cDNA described in the present paper may represent the A1 subunit. Further investigation is necessary to confirm this. Matching amino acid residues were also found between the amino acid sequences derived from the GSTYc cDNA and the A2 subunit with only four amino acid differences over a compared sequence region of 41 amino acids.

RNA blot analysis with the GSTYc cDNA indicate a tissue-specific expression of GSTYc. It is expressed in vas deferens, epididymis, kidney and liver. There was no hybridization signal with transcripts isolated from testis and uterus. The presence of the GSTYc transcript in liver and kidney and the lack of GSTYc expression in the testis is consistent with the fact that the A1 subunit is a major subunit in liver and kidney, but is not detectable in testis [19]. Tissue-specific expression of GSTYc subunits has also been observed in other species. For example, the rat GSTYc subunit is expressed at a reduced level in kidney and testis as compared with liver and it is not expressed in heart, lung, seminal vesicals and spleen [17]. In mouse, the GSTYc was found to be highly expressed in liver and lower levels of the transcript were detected in lung. In contrast the GSTYc transcript was undetectable in kidney and intestine [16].

Administration of DES to Syrian hamster had no significant effect on GSTYc expression levels in the analysed tissues. In rat, amongst the alpha-class GSTs, expression of subunits Yc and Ya have been found to be affected by hormones. Ovariectomy followed by DES administration resulted in a decrease in the alpha subunit Yc mRNA in rat pituitary gland [21]. The transcript of the rat GST Ya subunit was greatly reduced in the liver following adrenalectomy [22].

Isolation of the Syrian hamster alpha-class GSTYc cDNA is an important step towards a better understanding of function and regulation of alpha-class GSTs.

Received 29 August 1996/15 November 1996; accepted 18 November 1996

This work was supported by grants NIH CA49949, CA52085 (J.S.N.) and CA58030 (J.J.L.).

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