

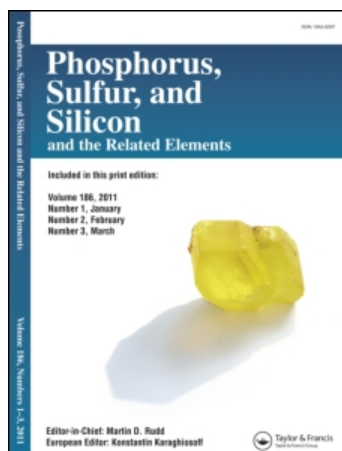
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Primary Structure of Human Plasma Glutathione Peroxidase Deduced From cDNA Sequences

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PRIMARY STRUCTURE OF HUMAN PLASMA GLUTATHIONE PEROXIDASE DEDUCED FROM cDNA SEQUENCES

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Abstract Human plasma glutathione peroxidase (GSHPx) has been shown to be a selenium-containing enzyme immunologically distinct from cellular GSHPx. Oligonucleotide probes, based on a partial amino acid sequence of one peptide in a lysine endopeptidase-digest of the purified enzyme, were used to screen a human placenta cDNA library. Nucleotide sequence analysis of the obtained clones revealed that GSHPx consisted of a 678-base pair open reading frame coding for a 226-amino acid polypeptide. The in-frame TGA codon observed at positions 217-219 was assigned to selenocysteine. The amino acid sequence exhibited only 44% homology with that of human cellular GSHPx. Northern blot analysis revealed a single transcript of 2.2 kilobases in the poly (A)⁺ RNA fractions of human placenta and HepG2 (human hepatic cell line), but not that of human liver. The transcript was also detected in rat kidney, but not in rat liver, lung, heart and brain.

INTRODUCTION

GSHPx (EC 1.11.1.9) catalyzes the reduction of hydrogen peroxide, organic hydroperoxide and lipid peroxides by reduced glutathione and functions in the protection of cells against oxidative damage¹⁻². This enzyme, found mainly in the cytosol of mammalian cells, contains a

GGC CAGGATCATG CGAGCGGCTC AGGCGACCT GAGTGTGCC CCACCGCCG

10. 20 30 40 50 60 70 80 90
 ATG GCC CGG CTG CAG CAG TCC TCG CTG CTT TCC TCG CAG AGC CGG GCA CAA GAG AAC TCG AAG ATG
 Met Ala Arg Leu Leu Gln Ala Ser Cys Leu Leu Ser Leu Leu Ala Gly Phe Val Ser Gln Ser Arg Gly Gln Gln Lys Ser Lys Met
 1

100 110 120 130 140 150 160 170 180
 GAC TGC CAT GGT GGC ATA AGT GGC ACC ATT TAC GAG TAC GGC CTC ACC ATT GAT GGG GAG GAG TAC ACC TTC AAG CAG TAT GCT
 Asp Cys His Gly Gly Ile Ser Gly Thr Ile Tyr Glu Tyr Gly Ala Leu Thr Ile Asp Gly Glu Tyr Ile Pro Phe Lys Gln Tyr Ala
 50

190 200 210 220 230 240 250 260 270
 GGC AAA TAC CTC TTT GTC AAC GTG GCC AGC TAC TGA GGC CTG AGC GAG TAC ATT GAA CTG AAT GCA CTA CAG CAA GAG CTT GCA
 Gly Lys Tyr Val Leu Phe Val Asn Val Ala Ser Tyr Sec
Gly Leu Thr Gly Gln Tyr Ile Glu Leu Asn Ala Leu Gln

280 290 300 310 320 330 340 350 360
 CCA TTC GGT CTG CAG ATT CTG GGC TTT CCC TGC AAC CAA TTT GGA AAA CAG GAG AAC TCA CAG GAG ATC CTT CTT ACC CTC AAG
 Pro Phe Gly Leu Val Ile Leu Gly Phe Pro Cys Asn Gln Phe Gly Lys Gln Glu Pro Gly Gln Asn Ser Glu Ile Leu Pro Thr Leu Lys
 100

370 380 390 400 410 420 430 440 450
 TAT GTC CGA CCA GGT GGA GGC TTT GTC CCT AAT TTC CAG CTC TTT GAG AAA GGG GAT GTC AAT GGA GAG AAA GAG CAG AAA TTC TAC ACT
 Tyr Val Arg Pro Gly Gly Phe Val Pro Asn Phe Gln Leu Phe Gln Lys Gly Asp Val Asn Gly Glu Lys Glu Gln Lys Phe Tyr Thr
 150

460 470 480 490 500 510 520 530 540
 TTC CTA AAG AAC TCC TGT CCT CCC ACC TCG CAG CTC CTG GGT ACA TCT TCT GAG CCG CTC TTC TGG GAA CCC ATG AAG CTT CAC CAC ATC CGC
 Phe Leu Lys Asn Ser Cys Pro Pro Thr Ser Glu Leu Leu Gly Thr Ser Asp Arg Leu Phe Thr Phe Tip Glu Pro Met Lys Val His Asp Ile Arg
 150

550 560 570 580 590 600 610 620 630
 TCG AAC TTT GAG AAG TTC CTG GGG CCA GAT GGT ATA CCC ATC ATG CCG TGG CAC CAG CGG ACC AGC GTC AGC AAC GTC AAG ATG CAC
 Trp Asn Phe Glu Lys Phe Leu Val Gly Pro Asp Gly Ile Pro Ile Met Arg Tip His Arg Thr Thr Val Ser Asn Val Lys Met Asp
 200

640 650 660 670 680
 ATC CTG TCC TAC ATG AGG CGG CAG GCA GCC CTG GGG GTC AAG AGG AAG TAA CTGAGCGCG TCTCATCCCA TGTCACCAT GTAGGGGAGG GACITTTG
 Ile Leu Ser Tyr Met Arg Arg Gln Ala Leu Leu Val Lys Arg Lys End
 226

TTC AGGAGAAAT CCGTCTCC AACACACTA TCTACCATC ACAGACCGCT TTCTATCAC TTAGGCCCC AGCTGGCAC AATGGGATCC ATACAGTTCT GTGTA
 CTCC AGCATGTG GTGGGTGG ATGGGTGT TTACACAT CACTACAGT ATGGTGAT GTGGGTGT GATGGGTG ATGGGTGT ATGGGTGT ATGGGTGT ATGGGTGT
 GTCTTC TGGAAATG TACCATTGT GTCTGGCG GTGGTAGT CTGAGATG AACACCTT CTCTCAGT CTCTCAGT CTCTCAGT CTCTCAGT CTCTCAGT CTCTCAGT
 CTACACCA AAGGAAAC CAGCTTAGG TCCATTGT CTCTTAAC TATACCTA ACCTGGGG CAGCATTC CACTGCTC AATATATG AATATATG AATATATG
 GAGCTCCCA GAAGTTTCTG GTCTACCA CCCCACAC CCCCACAC TACTCTGA AGGCTCCCA TCCCATCC ACAGTTCTC CTGAGAGA
 GA TCACTCCC TAGATCAAC AAGCAGATG TCACAGCA GGGCAGCA CCCCAGCG AGGGTGGG TCTTATAG GAGGAGCC ACAGCTCTG TGGGG
 GACC TCCCTGAG CTGTCTAGG GGCAGGCT TAGTCATC AGGTAGCG CCGTGGCG GAGTGCAC CTGCTCTC GGAGGAGTG CCGTACCCC TCAC
 TGTCTC ACTGGTTGA GACTACCCC GTCTGGCG TAAAGCTT TCTGAGCA AATACCCC

Fig.1 Primary Structure of human plasma GSHPx. The amino acid sequence used as the basis for the synthesis of oligonucleotide probes is enclosed in an open box. Solid underlines show the amino acid residues directly identified for the peptides found in a lysine endopeptidase-digest of the purified GSHPx.

selenocysteine residue in its active site³ that is encoded by a TGA opal codon⁴. GSHPx activity is also found in plasma. This plasma enzyme is immunologically distinct from the erythrocyte and liver cytosolic enzymes⁵⁻⁶. We have isolated and characterized the plasma GSHPx⁷, and some differences in physical and kinetic properties have been found between the plasma and erythrocyte enzymes. In this study, we isolated cDNA clones coding for plasma GSHPx, in order to clarify the amino acid sequence.

CLONING AND SEQUENCING OF cDNA CLONES

For the screening of plasma GSHPx cDNA from a human placenta cDNA library, oligonucleotide probes, 5'-(T/C)TGIAGIGC(A/G)TTIAG(T/C)TCIAT(A/G)TA(T/C)TGICCI GTIAGICC-3', were synthesized on the basis of a partial amino acid sequence (GLTGQY IELNALQ), which had been determined for one of the peptides in a lysine endopeptidase-digest of the purified GSHPx. The oligonucleotides were used to screen the human placenta cDNA library. Two of the 500,000 recombinant clones obtained were found to hybridize with the probe. The larger insert (~1600 bp) was subcloned into vector pUC 118 /119 and sequenced by the dideoxy chain termination technique. The complete nucleotide sequence determined is shown in Fig.1. The cDNA insert consisted of 1603 bp, comprising a 54-bp 5'-noncoding region, a coding region of 678-bp and a 871-bp 3'-noncoding region. The initiator ATG was followed by 675 codons before the termination triplet TAA. An in-frame TGA codon was observed at positions 217-219. Neither a polyadenylation signal sequence nor a poly(A) sequence was observed in the 3'-noncoding region, indicating that this cDNA clone is not of full length. The amino acid sequence of the enzyme predicted from the cDNA sequence is also shown in Fig.1. The genomic code for mouse erythrocyte GSHPx was shown to contain the triplet TGA at the same position where a selenocysteine residue was confirmed in the rat liver and bovine erythrocyte enzymes⁴. As the plasma enzyme is a selenoprotein containing one atom of selenium per subunit⁷, the in-frame TGA observed at positions 217-219 may be assigned to selenocysteine. The coding sequence corresponded to 226 amino acid

Plasma GSHPx	M A R L L Q A S C L L S L L A G F V S Q S R G Q E K S K M D C H G G I S G T I Y	
Epilidymal androgen-regulated protein		
GSHPx-related protein		M A F I A K S F Y D
Cytosolic GSHPx		M C A A R L A A A A A Q S V Y A
GSHPx-related selenopeptide		M C A A R L - S A A A A Q S - -
Vitamin B12 porting subunit,		M Q D S I L
E G A L T I D G E E Y I P F K Q Y A G K Y V F V N V A S Y * G L T T - Q Q Y I E L N A L Q E E L A P		
L S A I S L D G - E K V D F N T F R G R A V L I E N V V R S L * G T T T T R D F T Q L N N E L Q C R - F P P		
F S A R P L A G G E P V S L G S L R G K V L L I E N V A S L * G T T T T R D Y T Q M N N D L Q K R L -		
F S A R P L T G G E P V S L G S L R G K V L L I E N V A S L * G T T T T R D Y T Q M N N D L Q K R L -		
T T V V E D I D G E V T T L E K F A G N V L L I V N V A S K C G L T P - Q Y E Q L E N T Q K A W V D		
F G L V I L G F P C N Q F G K Q E P G E N S E I L P T L K Y V R P G G G F V P N F Q L F E K G D D V N		
F G L V I L G F P C N Q F G K Q E P G E N S E I L P T L K Y V R P G G G F V P N F Q L F E K G D D V N		
R R L V V L G F P C N Q F G H Q E N A K N E E I L N S L K Y V R P G G G F E P N F E M L F E K C E V N		
R G L V V L G F P C N Q F G H Q E N A K N E E I L N S L K Y V R P G G G F E P N F E M L F E K C E V N		
- G L V V L G F P C N Q F G H Q E N A K N E E I L N S L K Y V R P G G G F E P N F E M L F E K C E V N		
R G F M V L G F P C N Q F L E Q E P G S D E E I - - K T Y C - - T T T W G V T F P M F S K I E V N		
G E K E Q K F Y T F F L K N S C P - P T S E L - - L G T S D R L F - W E P M K V H D I R W N F E K F L		
G E N E Q K I F F T F L K R S C P H P P - S E T - V V M - S - K H T S W E P I K V H D I R W N F E K F L		
G Q N E H P V F A Y L K D K L P Y P Y S D D A T A L M T D P K L I T W S P V R R S D V A W N F E K F L		
G A G A H P L F A F L R E A L P A P S D D A T A L M T D P K L I T W S P V R R S D V A W N F E K F L		
L G Y T R R A T S W		
G E G R H P L Y Q K L I A A A P T A V A P E E S G F Y A R M V S K G R A P L Y P D I L W N F E K F L		
V G P D G I P I M R W H H R T T V S N V K M D I L S Y M R R Q A A L G V K R K		
V G P D G V P V M R W F H Q A P V S T V K S D I M A Y L S H F K T I		
I G P G G E P F R R Y S R T F T I N I E P D I K R L L K V A I		
V G P D G V P L R R Y S R R F Q T I D I E P D I E A L L S Q G P S C A		
V G R D G K V I Q R F S P D M T P E D P I V M E S I K L A L A K		

Fig.2 Comparison of the amino acid sequences of plasma GSHPx and five GSHPx-related proteins. Selenocysteine is indicated by an asterisk. Gaps introduced for optimal alignment are shown by dashes, and amino acids identical with those in the human plasma enzyme in four or more of the aligned sequences are boxed.

residues, including the first Met. To confirm this primary structure, about 50 % of the amino acid sequence of the enzyme was determined through automated Edman degradation of the peptides isolated from a lysine endopeptidase-digest. The amino acid sequences of these peptides all confirmed the predicted protein sequence (Fig.1). The initial hydrophobic sequence of the enzyme is likely to be post-translationally processed as a signal peptide, as in the case of other secretory proteins. Sequence analysis of the plasma GSHPx revealed no phenylthiohydantoin-amino acid up to 15 cycles of Edman degradation, indicating that the N-terminus of the protein is blocked.

The amino acid sequence of human plasma GSHPx is compared with those of the cellular GSHPx and four GSHPx-related proteins in Fig.2. The human plasma enzyme exhibits approximately 44% homology with the human cellular enzymes⁸⁻⁹. However, the homology is much higher in the regions of residues 61-74, 93-108 and 119-138. These regions seem to include the first, second and third β strand structures, which were proposed to compose the rigid core of the bovine erythrocyte enzyme, as judged on X ray analysis¹⁰. Homology is also very high in the region of residues 181-192, the second α helix, where presumably the contact regions between the four subunits are located¹⁰. The plasma GSHPx exhibits 41, 37, 41 and 71 % homology with vitamin B₁₂ transporting subunit¹¹, GSHPx-related protein¹², GSHPx-related selenopeptide¹³, epididymal androgen-regulated protein¹⁴, respectively.

NORTHERN BLOT ANALYSIS

To detect plasma and cellular transcripts in human tissues, the PstI fragment of plasma GSHPx cDNA and the EcoRI fragment of liver GSHPx cDNA were used, respectively. In both cases, Northern blot analysis revealed one major band with the poly(A)⁺ RNA extracted from human placenta. The size of the plasma enzyme mRNA was estimated to be 2.2 kb, which was distinct from that of the liver cytosolic enzyme mRNA (1.2 kb). The plasma GSHPx mRNA was more abundant in the placenta than the cytosolic enzyme one. No detectable plasma GSHPx mRNA was found in poly (A)⁺ RNA isolated from human liver or endothelial cells, though transcripts of

cellular GSHPx were observed. HepG2, a human hepatoma cell line, was reported to synthesize both plasma and cellular GSHPxs, but to only secrete the plasma GSHPx¹⁵. The poly (A)⁺ RNA isolated from HepG2 cells expressed both plasma and cellular GSHPx mRNAs. The transcript was also detected in rat kidney, but not in rat liver, lung, heart and brain.

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