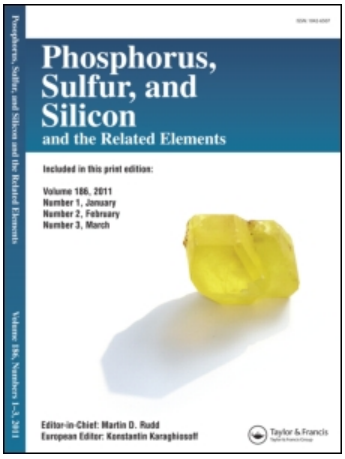

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Primary Structure of Human Plasma Glutathione Peroxidase Deduced From cDNA Sequences

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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 damage[1-2]. This enzyme, found mainly in the cytosol of mammalian cells, contains a

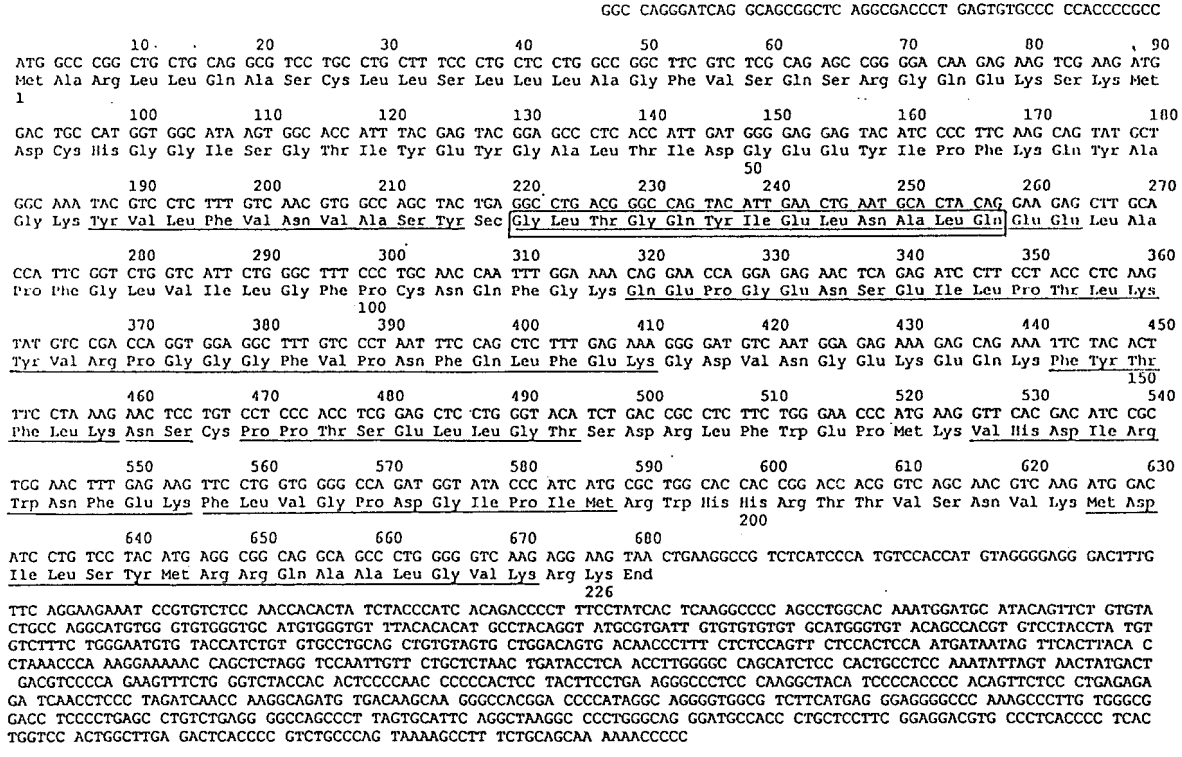

Fig.1 Primary Structure of human plasma GSHPx. The amino acid sequence used as the basis for the synthesis of ologinucleotide probes is enclosed in an open box. Solid underlines show the amino acid residues directly identified for the peptides found in a lysine endopeptidase-digest of the purified GSHPx.

selenocysteine residue in its active site[3] that is encoded by a TGA opal codon[4]. GSHPx activity is also found in plasma. This plasma enzyme is immunologically distinct from the erythrocyte and liver cytosolic enzymes[5-6]. We have isolated and characterized the plasma GSHPx[7], 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}/$_{G}$)TTI AG(^{T}/$_{C}$)TCIAT(^{A}/$_{G}$)TA(^{T}/$_{C}$)TGICCIGTIAGICC-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 enzymes[4]. As the plasma enzyme is a selenoprotein containing one atom of selenium per subunit[7], the in-frame TGA observed at positions 217-219 may be assigned to selenocysteine. The coding sequence corresponded to 226 amino acid

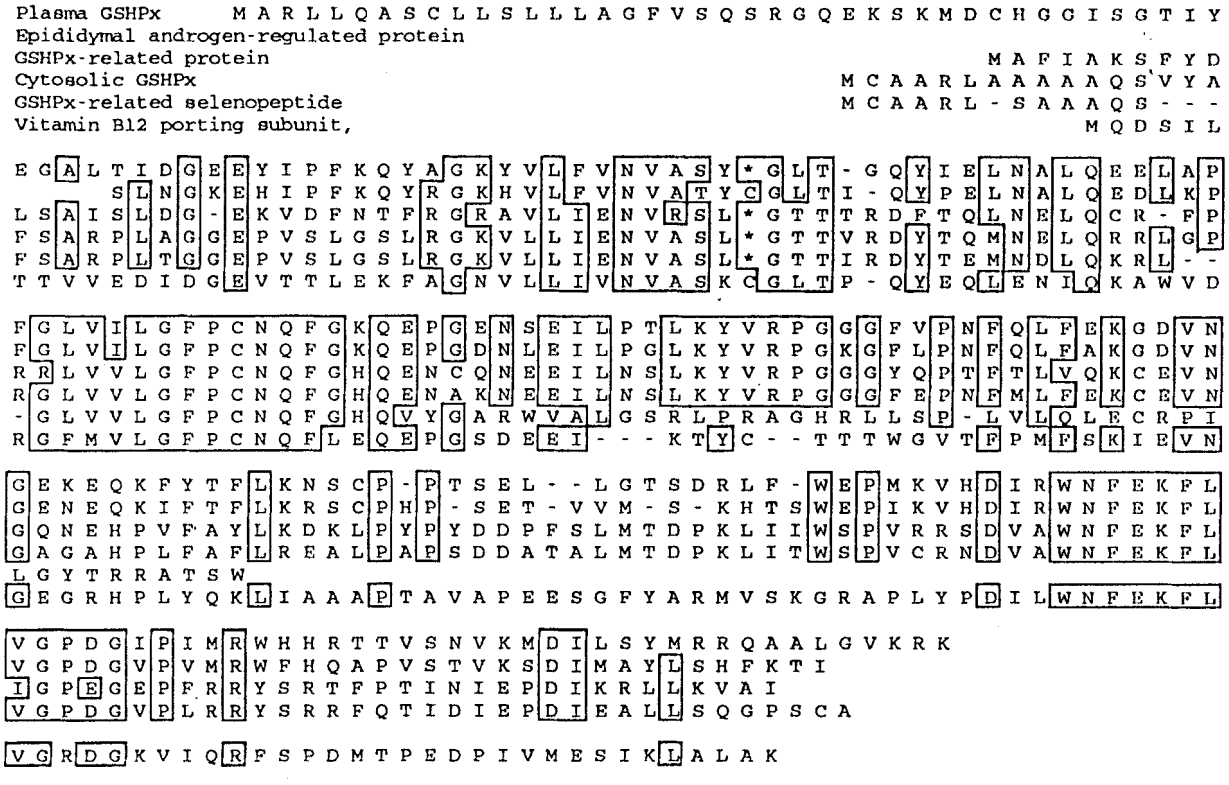

Fig.2 Comparison of the amino acid sequences of plasma GSHPx and five GSHPx-related proteins. Selenocysteine is indicated by an asterisk. Gaps introduced for optimal alignment are shown by dashes, and amino acids identical with those in the human plasma enzyme in four or more of the aligned sequences 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 enzymes[8-9]. 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 ß strand structures, which were proposed to compose the rigid core of the bovine erythrocyte enzyme, as judged on X ray analysis[10] 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 located[10]. The plasma GSHPx exhibits 41, 37, 41 and 71 % homology with vitamin B_{12} transporting subunit[11], GSHPx-related protein[12], GSHPx-related selenopeptide[13], epididymal androgen-regulated protein[14], 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 GSHPx[15]. 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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