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Expression of glutathione peroxidase I gene in selenium-deficient rats

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ABSTRACT

We have characterized a cDNA pGPX1211 encoding rat glutathione peroxidase I. The selenocysteine in the protein corresponded to a TGA codon in the coding region of the cDNA, similar to earlier findings in mouse and human genes, and a gene encoding the formate dehydrogenase from E. coli, another selenoenzyme. The rat GSH peroxidase I has a calculated subunit molecular weight of 22,155 daltons and shares 95% and 86% sequence homology with the mouse and human subunits, respectively. The 3'-noncoding sequence (>930 bp) in pGPX1211 is much longer than that of the human sequences. We found that glutathione peroxidase I mRNA, but not the polypeptide, was expressed under nutritional stress of selenium deficiency where no glutathione peroxidase I activity can be detected. The failure of detecting any apoprotein for the glutathione peroxidase I under selenium deficiency and results published from other laboratories supports the proposal that selenium may be incorporated into the glutathione peroxidase I co-translationally.

INTRODUCTION

Glutathione peroxidase (GPX I, GSH:H_2O_2 oxidoreductase, EC 1.11.1.9) is the only characterized selenium dependent enzyme in higher animals. This enzyme catalyzes the reduction of H_2O_2 and organic hydroperoxides to the corresponding alcohol with a specific requirement for GSH. The protein is a tetramer containing four identical subunits each with a molecular mass of approximately 19,000 daltons, and each with one selenium atom (for recent reviews, see ref. 1-4). It has been implicated in the protection of cell membranes and possibly DNA by reducing and eliminating highly reactive organic hydroperoxides and H_2O_2 (1-7). Another potentially important role for GPX I is in the metabolism of fatty acid hydroperoxides that are generated by cyclooxygenase and lipoxygenases during the inflammatory responses of leukocytes, platelets and endothelial cells (8,9). X-ray structural analysis at 2.0 Å resolution has been reported by Epp et al. (10) for the bovine GPX I with the suggestion of selenocysteine being located at position 45 out of a 198 amino acid sequence (11). The selenocysteine-containing peptide in rat liver GPX I was determined by Condell and Tappel to be VLLIENVASL[SeCys]GTTTR

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(12). Recently, a mouse genomic DNA sequence for GPX I was reported by Chambers et al (13). The mouse GPX reading frame contains at position 47 of 201 amino acids, a TGA codon that also exists in the corresponding mRNA by sequence analysis using the primer-extension method (13). A human GPX I cDNA clone isolated from a λgt11 cDNA library also contains a TGA codon in a position analogous to the mouse gene's open reading frame (14). The amino acid sequence surrounding the TGA codon in mouse and human genes is highly homologous to the selenocysteine-containing peptide sequence of rat GPX I (12). Therefore, it is suggested that the selenocysteine in GPX I may be encoded by a TGA codon.

The existence of a TGA codon in the middle of otherwise an open reading frame was also reported for the formate dehydrogenase, a selenoprotein from E. coli (15). Using the β-galactosidase gene as a reporter in translational gene fusion experiments, Zinoni et al. have demonstrated that hybrid β-galactosidase activities are dependent upon the presence of selenium in the growth media only when the hybrid genes contain the putative selenocysteine-encoding TGA codon (15). These results suggested that selenocysteine incorporation into the E. coli formate dehydrogenase may follow a co-translational mechanism (15).

As for the GPX I system, Hawkes and Tappel investigated the mechanism of [^{75}Se] selenite incorporation into the GPX I protein using rat liver slices and cell-free extracts (16). Their results indicated that the synthesis of GPX I in rat liver slices was inhibited by cycloheximide or puromycin, and that ^{75}Se was incorporated from [^{75}Se] selenite into free selenocysteine and selenocysteyl tRNA. The interpretation favored a translational mechanism for selenocysteine incorporation (16,17). Using a perfusion system with isolated rat liver, Sunde and Evenson found more ^{14}C incorporation into Se-cysteine of the GPX I protein with [^{14}C]-serine as the precursor rather than with the [^{14}C]-cysteine. Their results demonstrate that serine provides the carbon skeleton for the selenocysteine moiety in GPX I (18). Consequently, these authors favored a co-translational mechanism and suggested that certain suppressor seryl-tRNAs may recognize the TGA codon (18).

These recent developments raised intriguing questions as to how the cell's translational machinery differentiates the function of TGA codons for termination versus selenocysteine incorporation by a translational/co-translational mechanism. Alternatively, the possible presence of an apoprotein for GPX I would be critical in supporting any post-translational mechanism for selenium incorporation into GPX I.

In this report, we present the characterization of rat GPX I cDNA and expression of its mRNA and protein under nutritional perturbation by controlling the diet selenium.

MATERIALS AND METHODS

Nucleotides and enzymes

The dNTPs and ddNTPs were obtained from Pharmacia P. L. Biochemicals (Milwaukee, WI). The four [α-^{32}P] dNTPs, [γ-^{32}P] ATP and [α-^{35}S] dATP were purchased from ICN Pharmaceuticals (Irvine, CA) and New England Nuclear (Boston, MA), respectively. Restriction endonucleases, T4 DNA ligase, DNA polymerase and its Klenow fragment and T4 polynucleotide kinase, were products of New England Biolabs (Beverly, MA) and Boehringer Mannheim (Indianapolis, IN). The rat liver λgt11 cDNA library was purchased from Clontech Laboratories, Inc. (Palo Alto, CA). The 30-mer oligonucleotide, 5' CGTGGTGC-CTCAGAGAGACGCGACATTCTC 3' was chosen as the hybridization probe based on amino acid sequence identity between the partial rat protein sequence and the deduced mouse GPX I (12) sequence (ENVASLSeCGTT, residues 40 to 49 of Fig. 3). It was synthesized by a Pharmacia Gene Assembler. The oligonucleotide was further purified by gel electrophoresis on 20% polyacrylamide/8M urea gels and subsequently labeled at the 5' end with [γ-^{32}P] ATP and T4 polynucleotide kinase (19,20). The tissue cytosolic extracts are the 105,000 g supernatant after homogenization. ^{125}I-protein A was obtained from Amersham Corp. (Arlington Heights, IL).

Screening of cDNA libraries

For the first round screening the rat liver cDNA library was plated on *E. coli* strain Y1090 at a density of 5 X 10^4 phage per 150 mm petri dish (10 plates). The second and third round purifications were carried out at lower dilutions for single-plaque isolation. Nitrocellulose filters with blotted plaque lifts were prehybridized overnight in 6 X SET, 10 X Denhardts, 100 µg/ml of heat-denatured salmon sperm DNA, 0.05 M sodium phosphate, pH 7.0 and 0.5% SDS (20). Hybridization was carried out for 24 hours in the prehybridization buffer containing 0.6-1.0 X 10^6 cpm of kinase-labelled probe per ml. Prehybridization, hybridization and washing were done at 44° and the filters were washed in 2 X SET and 0.5% SDS for 20 minutes and an additional one hour in 1 X SET and 0.1% SDS (20).

DNA purification and sequence analysis

Phage DNAs (λDNA and M13 DNA) were isolated from plate lysates and/or liquid cultures according to the published procedures (20). Plasmid DNAs were isolated by following the alkaline lysis method (21). DNA sequences were

determined by the Sanger's dideoxy chain-termination method (22) using M13 mp18 and mp19 subclones (23) in combination with serial deletions generated by the method of Dale *et al.* (24).

Animals and diet

Post-weanling male Long-Evans Hooded rats (Charles River Laboratory, Wilminton, MA) weighing approximately 50 g were fed for 9 weeks on diets composed of partially-purified ingredients as formulated according to Reddy *et al.* (25). The torula yeast-based diets were formulated to provide adequate amounts of all known nutrients except Se which was present at nearly undetectable levels.

The basic diet was supplemented (+) or deficient (-) with 0.5 mg Se/kg, as sodium selenite to create the following dietary groups: +Se; -Se. Corn oil and lard supplied 36% of the total calories in the diet, reflecting the average calories obtained from fat in human diets in western countries.

After nine weeks on the experimental diets, all animals were sacrificed under anesthesia with sodium pentobarbital (120 mg/kg body weights). Blood was collected via the carotid artery from each rat in a heparinized tube, and stored at -80°C until use in epoxidation assays. A portion of the blood was centrifuged immediately to prepare erythrocyte and plasma sample for vitamin E and GPX I assay, respectively. The levels of vitamin E (as total tocopherols in plasma) and Se (in whole blood) were determined according to Taylor *et al.* (26) and a method of Whetter and Ullrey (27), respectively.

Enzyme assays and other procedures

Rat GPX I protein was purified by a procedure modified from the version published earlier (28). The details will be published elsewhere. Antiserum was raised in female New Zealand white rabbits with SDS gel-purified GPX I peptide. The GPX I enzyme activity was measured by a modification of the coupled assay procedure of Paglia and Valentine (29). The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.2), 1 mM EDTA, 1 mM NaN_3, 0.2 mM NADPH, 1 E.U/ml GSSG reductase, 1 mM GSH, 1.5 mM cumene hydroperoxide or 0.25 mM H_2O_2 in a total volume of 1.2 ml. Absorbance at 340 nm was recorded for 3 minutes and the activity was calculated from the slope of these lines as μmoles NADPH oxidized per minute per mg protein (definition of enzyme activity units). Blank reactions with enzyme source replaced by water were subtracted from each assay. Protein was measured by the method of Lowry *et al.* (30). Poly(A) RNAs were purified from rat livers by published procedures (31,32). Northern blot analysis was carried out as previously described (33). Western immunoblot was performed according to Towbin *et al.* (34).

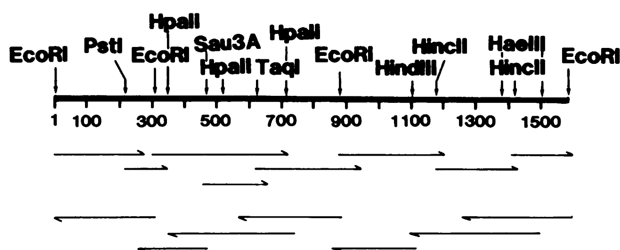

Figure 1. Partial restriction map and sequencing strategy of the rat cDNA clone pGPX1211 encoding GSH peroxidase I. The restriction sites used for subcloning into M13 mp18/mp19 vectors are labelled. Horizontal arrows indicate extents of DNA sequence determined from each subclone.

RESULTS AND DISCUSSION

Screening for the cDNA clones containing the GPX I gene

Six positive plaques against the 30-mer probe were obtained through three rounds of successive plaque purifications. Phage DNAs were purified from plate lysates for each clone and digested with EcoRI. One of them contained three EcoRI fragments for a total of ~1.5 kb and were designated as λGPX1211. The cDNA insert was subcloned together or separately into the EcoRI site of pGEM4 (designated as pGPX1211) (Promega Biotech, Madison, WI) and M13 mp18 and mp19 sequencing vectors. The M13 subclones were further processed for DNA sequence analysis according to the strategy shown in Figure 1.

The complete cDNA sequence of clone pGPX1211 was established from both strands by sequencing overlapping deletion subclones differing in approximately 200 nucleotides each. Figure 2 shows the sequence of pGPX1211 cDNA insert as well as the deduced amino acid sequence for rat GPX I. The cDNA has a 5'-untranslated region of 36 bp, at ~58% homology to the corresponding region of the mouse genomic sequence (13). The 3' noncoding region is at least 930 bp long, much longer than that in the human GPX I cDNA (14). The DNA sequence (nucleotides 166 to 195) matched that of the 30-mer probe except for a single transition at position 180 (Fig. 2). The poly(A) addition signal AATAAA present at nucleotide position 840 was not used in pGPX1211. Our sequence does not contain a series of A residues which a complete cDNA clone should have. Altogether, the results of this study show that the active site selenocysteine residue (SeC) corresponded to the TGA codon at position 47 (Figure 2), as is the case with human and mouse sequences (13,14).

A compilation of the four mammalian GPX I sequence is presented in Figure

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SEPX1211.SEQ
                                                                  10        20        30        40
                                                         GAATTCCGCGCTACAGGCATTTTTGAGTCCAATATCTTCTACAGT
    50         60         70         80         90        100        110        120        130
ATG TCT GCT GCT CGG CTC TCC GCG GTG GCA CAG TCC ACC GTG TAT GCC TTC TCC GCG CGC CCG CTG GCG GGC GGG GAG CCC GTG AGC CTG
MET Ser Ala Ala Arg Leu Ser Ala Val Ala Gln Ser Thr Val Tyr Ala Phe Ser Ala Arg Pro Leu Ala Gly Gly Glu Pro Val Ser Leu
   140        150        160        170        180        190        200        210        220
GGC TCC CTG CGG GGC AAG GTG CTG CTC ATT GAG AAT GTC GCG TCC CTC TGA GGC ACC ACG ACC CGG GAC TAC ACC GAA ATG AAT GAT CTG
Gly Ser Leu Arg Gly Lys Val Leu Leu Ile Glu Asn Val Ala Ser Leu SeC Gly Thr Thr Thr Arg Asp Tyr Thr Glu Met Asn Asp Leu
   230        240        250        260        270        280        290        300        310
CAG AAG CGT CTG GGG CCT CGT GGC CTG GTG GTG CTC GGT TTC CCG TGC AAT CAG TTC GGA CAT CAG GAG AAT GGC AAG AAT GAA GAG ATT
Gln Lys Arg Leu Gly Pro Arg Gly Leu Val Val Leu Gly Phe Pro Cys Asn Gln Phe Gly His Gln Glu Asn Gly Lys Asn Glu Glu Ile
   320        330        340        350        360        370        380        390        400
CTG AAT TCC CTC AAG TAT GTC CGA CCC GGT GGT GGG TTC GAG CCC AAC TTT ACA TTG TTT GAG AAG TGC GAG GTG AAT GGT GAG AAG GCT
Leu Asn Ser Leu Lys Tyr Val Arg Pro Gly Gly Gly Phe Glu Pro Asn Phe Thr Leu Phe Glu Lys Cys Glu Val Asn Gly Glu Lys Ala
   410        420        430        440        450        460        470        480        490
CAC CCG CTC TTT ACC TTC CTG CGG AAT GCC TTG CCA GCA CCC AGT GAC GAT CCC ACT GCG CTC ATG ACC GAC CCC AAG TAC ATC ATT TGG
His Pro Leu Phe Thr Phe Leu Arg Asn Ala Leu Pro Ala Pro Ser Asp Asp Pro Thr Ala Leu Met Thr Asp Pro Lys Tyr Ile Ile Trp
   500        510        520        530        540        550        560        570        580
TCC CCG GTG TGC CGC AAC GAC ATT TCC TGG AAC TTT GAG AAG TTC CTG GTA GGT CCA GAC GGT GTT CCA GTG CGC AGA TAC AGC AGG CGC
Ser Pro Val Cys Arg Asn Asp Ile Ser Trp Asn Phe Glu Lys Phe Leu Val Gly Pro Asp Gly Val Pro Val Arg Arg Tyr Ser Arg Arg
   590        600        610        620        630        640        650        660        670
TTT CGC ACC ATC GAC ATC GAA CCC GAT ATA GAA GCC CTG CTG TCC AAG CAG CCT AGC AAC CCC TAA GGC ATT CCT GGT ATC TGG GCT TGG
Phe Arg Thr Ile Asp Ile Glu Pro Asp Ile Glu Ala Leu Leu Ser Lys Gln Pro Ser Asn Pro  *
   680        690        700        710        720        730        740        750        760
TGA TGG CTG GCT GCC CTC CGG GGG GAG GTT TTT CCA TGA CGG TGT TTC CTC TAA ATT TAC ATG GAG AAA CAC CTG ATT TCC AGA AAA ATC
   770        780        790        800        810        820        830        840        850
CCC TCA GAT GGG CGC TGG TCT CGT CCA TTC CCG ATG CCT TTA CGC CTA AAG AAA GGC GGT TTC ACC ACT AAG AAT AAA GTG CTG CGG AAT
   860        870        880        890        900        910        920        930        940
TCC GTG TCT TCC TGG GCA CGT TTT TGT ATG AAT ATT CAA GAA GGC ACC CCG ATT ACT CCG TGT CCC TGC TGC TGA GAC TTG CTA AGA AAT
   950        960        970        980        990       1000       1010       1020       1030
ATG AAG CCA CAC TGG AGA AGT GCT GTG CTG AAG GCG ATC CTC CTG CCT GCT ACG GCA CAG TGC TTG CAG AAT TTC ACC TCT TGT AGA AGA
  1040       1050       1060       1070       1080       1090       1100       1110       1120
ACC TAA GAA CTT GGT CAA ACT AAC TGT GAG CTT TAC GAG AAG CTT GGA GAG TAT GGA TTC CAA AAC GCA TTC TGG TCC GAT ACA CCC AGA
  1130       1140       1150       1160       1170       1180       1190       1200       1210
AAG CAC CCT AGG TGT CGA CCC CAA CTC TCG TGG AGG CAG CAA GAA ACC TGG GAA GAG TGG GCA CCA AGT GTT GTA CCC TTC CTG AAG CTC
  1220       1230       1240       1250       1260       1270       1280       1290       1300
AGA GAC TGC CCT GTG TGG AAG ACT ATC TGT CTG CCA TCC TGA ACC GTC TGT GTG TGC TGC ATG AGA AGA CCC CAG TGA GCG AGA AGG CTC
  1310       1320       1330       1340       1350       1360       1370       1380       1390
ACC AAG TGC TGT AGT GGG TCC CTG GTG GAA AGA CGG CCA TGT TTC TCT GCT CTG ACA GTT GAC GAG ACA TAT GTC CCC AAA GAG TTT AAA
  1400       1410       1420       1430       1440       1450       1460       1470       1480
GCT GAG ACC TTC ACC TTC CAC TCT GAT ATC TGC ACA CTC CCA GAC AAG GAG AAG CAG ATA AAG AAG CAA ACG GCT CTC GCT GAG CTG GTG
  1490       1500       1510       1520       1530
AAA CAC AAG CCC AAG GCC ACA GAA GAT CAG CTG AAG ACG GTG ATG GGT GAC TTC
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Figure 2. Nucleotide sequence of the cDNA insert in pGPX1211. The TGA codon in the coding region of the GSH peroxidase I cDNA matches the selenocysteine (SeC) determined by protein sequencing by Condell and Tappel (12). The 30-mer oligonucleotide probe is complementary to nucleotides 166 to 195 of the cDNA sequence with a C-A mismatch at position 180. The first seven nucleotides are from the EcoRI linker during cDNA cloning. The double underlined AATAAA signal (nucleotides 838-843) for poly(A) addition is apparently not used in our cDNA clone. The putative termination codon TAA is marked by an asterisk (*). The GPX I subunit (200 amino acids) has a calculated MW of 22,155.

3. Amino acid substitutions occurred in 38 positions out of the 200 total. Twenty-one of the 38 positions can tolerate amino acid substitutions of different side chain groupings (e.g. small polar vs small nonpolar) as classified by Doolittle (35). Relative to the deduced rat GPX I protein

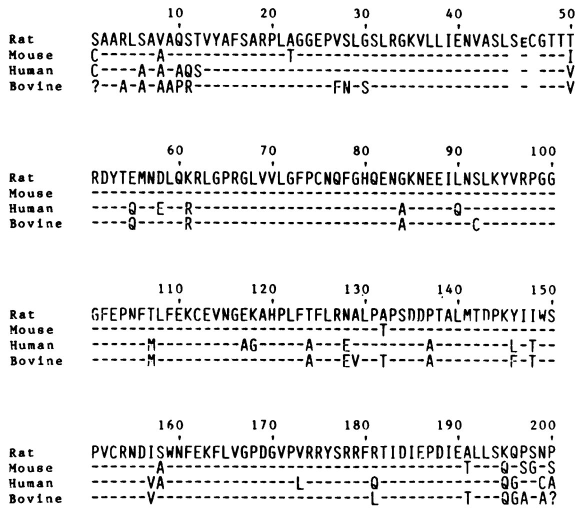

Figure 3. Comparison of GPX I protein sequences from rat, mouse (13), human (14), and bovine (11) origins. Amino acid residues identical to the rat sequence are represented by -'s. Question marks in the bovine sequence represent those missing or nonexistent from the determined protein sequences (11).

sequence, the number of amino acid substitutions in mouse (13), human (14), and bovine (11) GPX I is 11, 28, and 29-31, respectively. Amino acid residues 31 to 49 (including Se-Cys) and 62 to 83 are the two longest stretches of conserved sequences.

Calculation of percentage divergence of nucleotide sequences between rat and human GPX I by Perler's method (36) indicated a divergence of 9.58% for replacement sites. Consequently, these two GPX I genes have diverged from each other more than 108.9 million years ago, comparable to similar calculations between the Y_a (rat) and H_a (human) subunit cDNAs for glutathione S-transferases which have the Se-independent glutathione peroxidase activities (37).

Genomic Southern blot hybridization results in Figure 4 revealed very simple hybridization patterns with EcoRI, HindIII and PstI digested DNA indicating that GPX I is encoded by a single copy or low copy gene.

RNA blotting analysis and protein immunoblotting analysis

This experiment was performed firstly to see the size of the mRNA and secondly to verify the presence of mRNA in the Se-supplemented and the status of mRNA expression in Se-deficient (in diet) rat livers. A hybridization

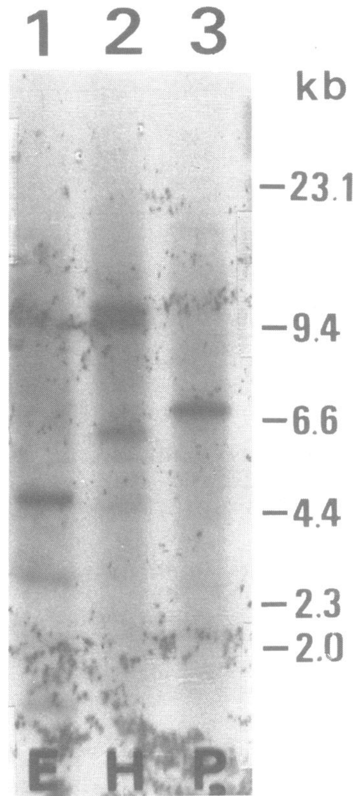

Figure 4. Rat genomic DNA blot hybridization with [^{32}P] cDNA probe pGPX1211. The rat liver genomic DNA (10 μg/lane) was digested with EcoRI (lane 1), HindIII (lane 2), PstI (lane 3), separated by electrophoresis on a 0.6% agarose gel, transferred to nitrocellulose membrane and hybridized with the nick-translated [^{32}P] cDNA insert in the presence of 50% formamide at 42° for 24 h. The membrane was washed in 2 X SSC, 0.5% sodium dodecyl sulfate and 1 X SSC and 0.1% sodium dodecyl sulfate and exposed at -70° with an intensifying screen. Numbers on the side of the panels are the size markers (λ HindIII) in kilobase units.

signal of ~1.5 kb in size was revealed with ^{32}P-labeled pGPX1211 cDNA probe in both of the liver RNA samples (Figure 5). This result suggested that our cDNA clone pGPX1211 is nearly full length.

In the nutritional state of selenium deficiency, the rats do not express GPX I in their livers as assayed by H_2O_2 (0.004 units/mg Se-deficient rat liver cytosol vs 0.19 units/mg Se-supplemented rat liver cytosol). Liver poly(A) RNAs isolated from Se-deficient rats revealed that the GPX I mRNAs are

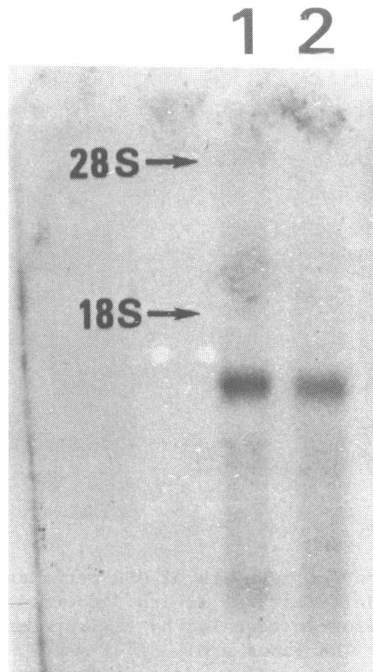

Figure 5. RNA blot hybridization with ^{32}P-labelled cDNA insert of pGPX1211. Rat liver poly(A) RNAs (10 μg/lane) were fractionated by electrophoresis on a 1.2% agarose gel in the presence of formaldehyde and transferred to nitrocellulose membrane according to the published procedures. Lane 1, liver poly(A) RNAs from the selenium supplemented rats and lane 2, liver poly(A) RNAs from the selenium deficient rats. The 28S and 18S are the rRNA markers.

still synthesized and processed to nearly the same extent. Therefore, the lack of GPX I activity (against H_2O_2) resulted either from the lack of protein synthesis, or the synthesis of an apoprotein lacking the active ingredient Se. This latter possibility will be critical in supporting any post-translational mechanism for Se incorporation.

These two possibilities can be distinguished by protein immunoblotting experiments to see if the GPX I peptide (apoprotein) exists in the liver extract from the Se-deficient rats. We have performed such an experiment and the results are shown in Figure 6. It is clear that no apoprotein of GPX I can be detected under Se-deficiency conditions by our antibody preparation. Our experiments did not deal with protein turnover, however. It is possible that the GPX I apoprotein is extremely labile if it exists at all.

Using antibody neutralization of GPX I activity as the method for

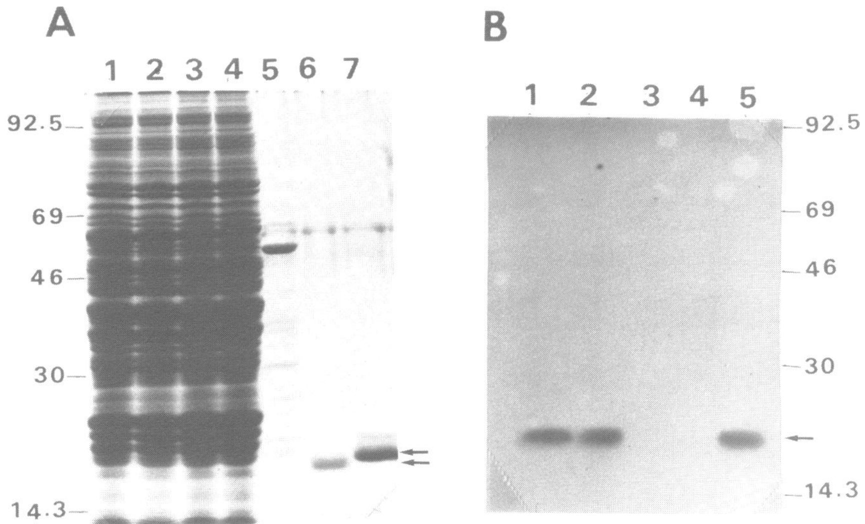

Figure 6. Protein immunoblot analysis of GSH peroxidase I expression in Se-supplemented and Se-deficient rat livers. Coomassiee Blue stained protein patterns (A) and autoradiogram revealed by ^{125}I-protein A after antibody reaction (B) are shown. The lanes are: (A) 1 and 2, Se-supplemented rat liver cytosol (200 μg each); 3 and 4, Se-deficient rat liver cytosol (200 μg each); 5, partially purified rat GPX I (10 μg); 6, our best rat GPX I preparation (6 μg); 7, bovine erythrocyte GPX I (6 μg). Arrows point to the GPX I subunit of rat livers and bovine erythrocytes. Rabbit antiserum was prepared against rat GPX I subunits eluted from SDS gels. (B) 1 and 2 Se-supplemented rat liver cytosol (250 μg each); 3 and 4, Se-deficient rat liver cytosol (250 μg each); 5, same sample as in Lane 6 of Panel A (2 μg). Arrow points to the position of rat GPX I.

detecting rat GPX I, Yoshida et al. found that GPX I level in the Se-deficient rat liver cytosol was approximately 10 fold lower than that in the Se-supplemented rat liver cytosol (38). Using a similar approach, Takahashi et al. observed a direct correlation between GPX I activity and protein content in HL-60 cells and a human volunteer (39). Most recently, Knight and Sunde used immunoblotting technique and enzyme-linked immunoabsorbent assay to demonstrate a direct positive correlation of GPX I activity and protein level with dietary Se content (40).

All these results, however, suggest that GPX I is most probably not synthesized under conditions of selenium-deficiency where GPX I mRNA expression seems to be normal (Figure 4), and thus supporting a co-translational mechanism for Se-cysteine incorporation. The formate dehydrogenase-β-galactosidase gene fusion results by Zinoni et al. are also interpreted in

light of a co-translational mechanism for selenocysteine incorporation. Two related questions for a co-translational mechanism are: 1, the recognition of certain UGA codons for selenocysteine incorporation; and 2, how the selenocysteyl tRNA is generated. Another view of the key issue is whether direct charging of a yet unidentified tRNA with selenocysteine really occurred as suggested by Hawkes et al. (17) or whether a serine charged to a UGA-recognizing tRNA is subsequently modified to form selenocysteyl-tRNA as suggested by Sunde and Evenson (18). The existence in nature of two UGA suppressor serine tRNAs that can carry phosphoserine in mammalian, avian and Xenopus tissues may have provided one important clue (41, for a recent review). The best evidence, however, came from the report by Leinfelder et al. (42). One of the three genes (SelC) essential for selenocysteine incorporation into the E. coli formate dehydrogenase is most likely encoding an unusual seryl tRNA with UCA at the anticodon loop. The question remains as to which UGA codon warrants the incorporation of a selenocysteine residue in vivo, however.

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