Selenium and Amino Acid Composition of Selenoprotein P, the Major Selenoprotein in Rat Serum*

(Received for publication, March 2, 1990)

Robert Read, Terri Bellew, Jian-Guo Yang, Kristina E. Hill, Ivan S. Palmer,§ and Raymond F. Burk§

From the Division of Gastroenterology, Department of Medicine, and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232 and the Department of Chemistry, South Dakota State University, Brookings, South Dakota 57007

Selenoprotein P is the second plasma selenoprotein to be purified. It is a glycoprotein and has been shown to be distinct from plasma glutathione peroxidase. This study characterizes selenoprotein P further. Deglycosylation of the protein shifts its migration on sodium dodecyl sulfate-polyacrylamide gel electrophoresis from $M_1$, 57,000 to $M_2$, 43,000, indicating it has a substantial carbohydrate component. Measurement of selenium indicates a selenium content of 7.5 ± 1.0 atoms/molecule based on a polypeptide weight of 43,000. Ammonium analysis accounts for all the selenium as selenocysteine. The protein is also rich in cysteine (17 residues) and histidine (23 residues). Fragmentation of selenoprotein P by tryptic and by cyanogen bromide produces peptides with varying selenium content. This indicates that selenium-rich regions of the protein exist. The concentration of selenoprotein P determined by radioimmunoassay in serum from control rats was 26.3 ± 4.5 µg/ml and in serum from selenium-deficient rats it is 2.7 ± 0.8 µg/ml. Depletion of selenium P from control serum using an immunoaffinity column indicates that over 60% of serum selenium in the rat is contained in this protein. These results demonstrate that selenoprotein P is the major form of selenium in rat serum. It is the first selenoprotein described which has more than one selenium atom/polypeptide chain.

Selenoprotein P is a rat plasma selenoprotein which has recently been purified by immunoaffinity chromatography (1). It has been distinguished from the selenoenzyme glutathione peroxidase by its immunologic and chromatographic properties. Dietary deficiency of selenium causes a fall both in the concentration of selenoprotein P and in glutathione peroxidase activity (2). However, selenoprotein P appears to take precedence over glutathione peroxidase in the utilization of limiting amounts of selenium (2, 3).

Selenium has a number of metabolic functions which cannot be attributed to glutathione peroxidase (4). The element appears to exert its functions as a constituent of selenoproteins, so there is a need to characterize selenoproteins other than glutathione peroxidase. This paper reports further characterization of selenoprotein P.

MATERIALS AND METHODS

Animals—Weanling male Sprague-Dawley rats were fed a Torula yeast-based selenium-deficient diet or the same diet with 0.5 mg of selenium added/kg as sodium selenate (5). Animals were housed in a facility with 12-h light and dark cycles. Blood was removed from the aorta while rats were anesthetized with pentobarbital (65 mg/kg). Serum was obtained commercially (Biorad Products for Science, Inc., Indianapolis, IN) or was separated by centrifugation after blood had clotted at room temperature for 60 min. Some blood samples were treated with 1 mg of disodium EDTA/ml to prevent coagulation and plasma was separated by centrifugation.

Monoclonal Antibodies—Production of the first monoclonal antibody, 2C5, was reported previously (1). To produce more antibodies, a female BALB/c mouse was inoculated twice with 10 µg of purified selenoprotein P at an interval of 2 weeks and given a final priming injection of 10 µg intraperitoneally after an antibody response was verified by radioimmunoassay. Three days later a fusion was carried out and one clone positive by radioimmunoassay for selenoprotein P was isolated (1). It was designated 8F11.

Hybridomas were grown in pristane-primed mice (6). Antibodies were purified from ascites using Protein G-Sepharose 4 Fast Flow from Pharmacia LKB (Uppsala, Sweden). They were eluted with 0.1 M glycine, pH 2.5, and neutralized with 2 M Tris base. Dialysis was then carried out against 50 mM sodium borate, pH 8. They were isolated by Ouchterlony double immunodiffusion against reagents from Bionetics Laboratory Products (Kensington, MD) and Miles Scientific (Napierville, IL). 8F11 is IgG kappa, and 2C5 is IgG kappa.

The binding of selenoprotein P to monoclonal antibodies attached to polyvinylchloride plates was studied. Scatchard plots of the results indicated that the affinity of 2C5 for selenoprotein P ($K_d$, $3.29 \times 10^{-9}$ M) was greater than that of 8F11 ($K_p$, $1.02 \times 10^{-10}$ M). While 2C5 can theoretically provide greater sensitivity in the radioimmunoassay, it is less hardy than 8F11. Storage of plates with 2C5 bound to them led to loss of selenoprotein P binding capacity. Immunoaffinity columns made with 2C5 exhibited decreased binding after several uses while those made with 8F11 could be used repeatedly. For these reasons 8F11 has been the antibody used in most of this work. 2C5 was used for a tube radioimmunoassay.

Purification of Selenoprotein P—The key to the purification is the use of an immunoaffinity column. Reacti-Gel (6x) was filtered free of acetone and washed with 3–5 volumes of cold deionized water. It was added to a tube containing 12–15 mg of purified monoclonal antibody/g of moist gel. The pH was adjusted to 9.5–10 with sodium hydroxide, and the reaction was allowed to proceed at room temperature for 2–3 h with additions of NaOH to keep the pH constant. The mixture was shaken overnight and then washed free of unbound antibody. Unreacted groups were blocked with ethanolamine as described by the manufacturer.

The purification scheme was modified from the one reported before (1). Serum was used and a small amount of 75Se-labeled selenoprotein P was added to allow identification of peaks by gamma counting. After the Affi-Gel Blue column step, fractions which contained 75Se

---

*This work was supported by National Institutes of Health Grants ES 02497 and ES 00267. It was published in part in abstract form (Read, R., Yang, J.-G., Hill, K. E., and Burk, R. F. (1990) FASEB J. 4, A371). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§To whom correspondence should be addressed: C-2104, Medical Center North, Vanderbilt University Medical Center, Nashville, TN 37232.
were pooled, concentrated, and applied to the immunoaffinity column. The immunoaffinity column (1–2 ml bed volume) was washed with 10–20 volumes of phosphate-buffered saline (137 mM NaCl, 2.6 mM KCl, 10 mM sodium phosphate, pH 7.4) containing 0.5 mM EDTA and 0.02% NaN3. Then the column with selenoprotein P bound to it was washed with 1-bed volume of 1 M NaCl and eluted with 0.15 M glycine, pH 2.5, into tubes containing 2 M Tris base to raise the pH to 7.5. Fractions containing 35Se were pooled, concentrated, and dialyzed against 100 mM ammonium acetate, pH 8.

A one-step purification method was used to obtain selenoprotein P for the trypsin digestion experiment (Fig. 4). Serum (5 ml) was made 1 mM in phenylmethylsulfonyl fluoride and passed through a 0.22-μm filter. The filtrate was passed over a 0.3-ml BF11-Reacti-Gel column. The first 0.7 ml was discarded and 0.7 ml was collected. The filtrate was passed through a 0.22-μm filter. The filtrate was passed over a 0.3-ml BF11-Reacti-Gel column. The first 0.7 ml was discarded and 0.7 ml was collected. The filtrate was then concentrated and applied to the immunoaffinity column.

Selenoprotein P was eluted as described for the immunoaffinity column.

Amino Acid Analysis—Amino acid analysis of purified selenoprotein P was performed to determine the number of cysteine and selenocysteine residues, carboxymethylation of the reduced protein was first carried out as described by Stadtman (7). Purified selenoprotein P at 0.18–0.28 mg/ml was denatured by addition of three volumes of argon-saturated 8 M guanidine HCl, 50 mM Tris-HCl, pH 8. Some samples were then reduced for 5–10 min by mixing with 0.02 volumes each of 0.25 M dithiothreitol and 1 M K3[PO4] under argon. Then 0.42 volumes of NaOH-neutralized 2 M iodoacetic acid was added and allowed to react under argon for 1–1.5 h in the dark. Carboxymethylation was carried out on the same way except 2 M iodoacetamide was added. The reaction was stopped by addition of 0.12 volumes of 2-mercaptoethanol. The mixture was dialyzed against two changes of argon-saturated 100 mM ammonium acetate, pH 8, at 4°C.

Native and carboxymethylated selenoprotein P were hydrolyzed in HCl at 110°C for 24 h using a Waters PICO-TAG™ Workstation, and amino acid composition was determined using the Waters PICO-TAG System which uses precolumn derivatization with phenylisothiocyanate (Millipore Corporation, Bedford, MA). Serine and threonine contents were adjusted from a time course hydrolysis of 20, 48, and 72 h by extrapolating to zero time. Valine, isoleucine, and leucine values were obtained from the 72-h hydrolysis. Cysteine and selenocysteine residues were determined from carboxymethylated preparations. Methionine was determined from a performic acid oxidative hydrolysis and measurement of methionine sulfone (8).

[3H]Carboxymethylselenoprotein P was prepared according to a published method (8). It was mixed in two different chromatographic conditions with carboxymethylcysteine and the internal standard glutamic acid for standardization of the analysis of carboxymethylated amino acids. The amino acid peaks were collected after chromatographic separation, and the carboxymethylated selenoprotein P was unambiguously identified by liquid scintillation counting.

Deglycosylation—Approximately 10 μg of affinity purified selenoprotein P in 100 mM ammonium acetate was dried and resedimented in 50 μl of 0.15% SDS with 0.1 M 2-mercaptoethanol. Samples were boiled for 5 min and then redissolved and dialyzed in 50 μl of water. Nonidet P-40 was added at 6.5 times the amount of SDS and the sample was made 1 mM in l,lO-phenanthroline hydrate to inhibit tryptic digestion. The reaction was allowed to continue overnight. Samples were run in triplicate and standard curves were run with each assay. The standard material used was rat serum which had been calibrated against pure selenoprotein P and stored in aliquots at −80°C. The protein content of the pure selenoprotein P was determined by amino acid analysis and all selenoprotein P values are given in terms of total amino acid content.

A tube radioimmunoassay was performed in V-bottom polystyrene microtiter plates (Dynatech Laboratories Inc., Chantilly, VA) using the monoclonal antibody 8F11 (1). Samples were run in triplicate and standard curves were run with each assay. The standard material used was rat serum which had been calibrated against pure selenoprotein P and stored in aliquots at −80°C. The protein content of the pure selenoprotein P was determined by amino acid analysis and all selenoprotein P values are given in terms of total amino acid content.

A one-step purification method was used to obtain selenoprotein P for the trypsin digestion experiment (Fig. 4). Serum (5 ml) was diluted with an equal volume of phosphate-buffered saline. After passage through a 0.22-μm filter, 4.5 ml of each sample was passed through a 0.4-ml bed volume 8F11-Reacti-Gel column. The first milliliter was discarded and the next 3.5 ml was collected and compared with corresponding untreated sample (Table III).

For experiment II 3.7 ml of filtered undiluted serum was passed over a 0.7-ml bed volume 8F11-Reacti-Gel column. The first 0.7 ml was discarded and the next 3 ml was collected and used for comparison to undiluted serum.

To verify that this treatment removed selenoprotein P, serum was obtained from a rat 4 h after administration of a tracer dose of 35Se. A previous report showed that virtually all the 35Se in such a sample was associated with selenoprotein P (1). The serum sample was divided into 2 aliquots and one of them was passed over the column as described above. Then 35Se was determined and gel filtration chromatography using Sephacryl S-200 HR (Pharmacia LKB, Uppsala, Sweden) was performed on both samples. The depleted serum contained only 5.6% as much 35Se as the control and the 35Se in both depleted and non-depleted serum was in the chromatographic peak which is recognized as selenoprotein P.

The solid-phase immunoassay was performed in V-bottom polystyrene microtiter plates (Dynatech Laboratories Inc., Chantilly, VA) using the monoclonal antibody 8F11 (1). Samples were run in triplicate and standard curves were run with each assay. The standard material used was rat serum which had been calibrated against pure selenoprotein P and stored in aliquots at −80°C. The protein content of the pure selenoprotein P was determined by amino acid analysis and all selenoprotein P values are given in terms of total amino acid content.

A tube radioimmunoassay for selenoprotein P using Reacti-Gel (6X) with the monoclonal antibody 2C5 bound to it was used. Ten μl of control serum or 100 μl of selenoprotein-P depleted serum was mixed with 10 μl of serum with 10 μl of a 10 mM sodium citrate, pH 8.5. Then 35Se was determined and gel filtration chromatography using Sephacryl S-200 HR (Pharmacia LKB, Uppsala, Sweden) was performed on both samples. The depleted serum contained only 5.6% as much 35Se as the control and the 35Se in both depleted and non-depleted serum was in the chromatographic peak which is recognized as selenoprotein P.

The solid-phase immunoassay was performed in V-bottom polystyrene microtiter plates (Dynatech Laboratories Inc., Chantilly, VA) using the monoclonal antibody 8F11 (1). Samples were run in triplicate and standard curves were run with each assay. The standard material used was rat serum which had been calibrated against pure selenoprotein P and stored in aliquots at −80°C. The protein content of the pure selenoprotein P was determined by amino acid analysis and all selenoprotein P values are given in terms of total amino acid content.

A tube radioimmunoassay for selenoprotein P using Reacti-Gel (6X) with the monoclonal antibody 2C5 bound to it was used. Ten μl of control serum or 100 μl of selenoprotein-P depleted serum was mixed with 10 μl of serum with 10 μl of a 10 mM sodium citrate, pH 8.5. Then 35Se was determined and gel filtration chromatography using Sephacryl S-200 HR (Pharmacia LKB, Uppsala, Sweden) was performed on both samples. The depleted serum contained only 5.6% as much 35Se as the control and the 35Se in both depleted and non-depleted serum was in the chromatographic peak which is recognized as selenoprotein P.
Characterization of Selenoprotein P

NADPH oxidized/min. Selenium was measured in duplicate by a fluorometric method (14).

Chemicals—Reacti-Gel (6×) was purchased from Pierce Chemical Co. Affi-Gel Blue, acrylamide, bis-acrylamide, ammonium persulfate, 2-mercaptoethanol, sodium dodecyl sulfate, and protein molecular weight standards were purchased from Bio-Rad. 35Se as H2SeO3 was obtained from Du Pont-New England Nuclear and from the University of Missouri Research Reactor Facility (Columbia, MO). [3H] Iodoacetic acid (64 mCi/mmol) was purchased from Du Pont-New England Nuclear. Selenocystine, S-carboxymethylcysteine, iodoacetamide, dansyl hydrazine, and Nonidet P-40 were purchased from Sigma. Other chemicals were of ACS grade or higher.

RESULTS

Purification and Amino Acid Analysis—A two-step procedure using Affi-Gel Blue chromatography and immunaffinity chromatography was used to purify selenoprotein P for further study. Fig. 1 shows the purified protein in lanes 2 and 6. Lane 6 was overloaded to demonstrate the purity of the preparation.

Amino acid analysis of the native protein was carried out. Thiols and selenols were protected by derivatization before hydrolysis. Carboxymethylation of selenoprotein P reduced its migration on SDS-PAGE (Fig. 1, lane 4) as noticed by others (15). However, carboxymidomethylation had no effect on migration (Fig. 1, lane 5).

The results of the amino acid analysis are shown in Table I and indicate the presence of 7.5 selenocysteines for a polypeptide mass of 45,000 (see below for determination of this polypeptide size). A protein of this size would also contain 23 cysteines and 23 histidines. Amino acid analysis (Fig. 2) yielded a cysteine value of 8.0 if carried out without reduction of disulfides before carboxymethylation. This suggests that over half the cysteines are involved in internal disulfide bonds. The number of selenocysteines was not increased by reduction, suggesting that they exist in the native protein as selenols. Thus, native selenoprotein P contains approximately equal numbers of thiols and selenols.

Carbohydrate Content—Purified selenoprotein P was subjected to digestion with N-glycanase. Fig. 3 shows glycoprotein bands of the amino acid analyses of purified selenoprotein P are shown. The sample shown in panel A had not been carboxymethylated. Panel B shows results from a sample that had been carboxymethylated without prior reduction. Panel C shows results from a sample which had been reduced with dithiothreitol and KBH3 prior to carboxymethylation (see “Materials and Methods”). The identity of the peaks (see labels in panel B) is 1, aspartate + asparagine; 2, glutamine + glutamate; 3, carboxymethylcysteine; 4, carboxymethylselenocysteine; 5, serine; 6, glycine. Peak heights are given in relative units.

Fig. 1. SDS-PAGE of selenoprotein P. The gel is 10% polyacrylamide and the stain is Coomassie Blue. Molecular mass standards are in lane 1. They are phosphorylase B, 92.5 kDa; bovine serum albumin, 66.2 kDa; ovalbumin, 45 kDa; and carbonic anhydrase, 31 kDa. Lanes 2 and 6 contain, respectively, 2 μg and 12 μg of native purified selenoprotein P. Lane 3 contains enzymatically deglycosylated (sequential N-glycanase and O-glycanase digestions) selenoprotein P (3 μg). Lanes 4 and 5 contain, respectively, carboxymethylated (1.5 μg) and carboxymidomethylated (1.5 μg) selenoprotein P.

Table I

<table>
<thead>
<tr>
<th>Amino acid residues of selenoprotein P/43,000 of peptide weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values were calculated from analyses of native (n = 23) and</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Se-cys</td>
</tr>
<tr>
<td>Cys</td>
</tr>
<tr>
<td>Asx</td>
</tr>
<tr>
<td>Glx</td>
</tr>
<tr>
<td>Ser</td>
</tr>
<tr>
<td>Gly</td>
</tr>
<tr>
<td>His</td>
</tr>
<tr>
<td>Arg</td>
</tr>
<tr>
<td>Thr</td>
</tr>
</tbody>
</table>

* The value given was for selenoprotein P subjected to reduction before carboxymethylation. When no reduction was done a value of 8.0 was obtained.

Fig. 2. Effect of reduction on carboxymethylation of selenocysteine and cysteine in selenoprotein P. Partial chromatograms of the amino acid analyses of purified selenoprotein P are shown. The sample shown in panel A had not been carboxymethylated. Panel B shows results from a sample that had been carboxymethylated without prior reduction. Panel C shows results from a sample which had been reduced with dithiothreitol and KBH3 prior to carboxymethylation (see “Materials and Methods”). The identity of the peaks (see labels in panel B) is 1, aspartate + asparagine; 2, glutamine + glutamate; 3, carboxymethylcysteine; 4, carboxymethylselenocysteine; 5, serine; 6, glycine. Peak heights are given in relative units.
Characterization of Selenoprotein P

Fig. 3. Deglycosylation of selenoprotein P. Native and N-deglycosylated selenoprotein P were analyzed by SDS-PAGE. Native protein was loaded in lane 1 (2 μg) and lane 2 (1 μg), and N-deglycosylated protein was loaded in lane 3 (~8 μg) and lane 4 (~4 μg). Panel A shows fluorescence of the gel stained with dansyl protein standards. The kDa positions of the bands are indicated on the side of the figure as calculated from the same gel stained with Coomassie Blue. Molecular mass of the bands in kDa are indicated on the side of the figure as calculated from densitometric analysis which is not shown) reveals that the selenium content of the peptides is shown in the lower part of the figure. During the 60-min digestion, the 75Se accumulated in the 19- and 15-kDa peptides. The other peptides of 40, 34, and 21 kDa contained little or no 75Se.  

Table II

<table>
<thead>
<tr>
<th>Sample</th>
<th>Volume</th>
<th>Protein</th>
<th>Selenium</th>
<th>43,000 Da of peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>10/7/88</td>
<td>50</td>
<td>16.0</td>
<td>206</td>
<td>7.0</td>
</tr>
<tr>
<td>10/25/88-I</td>
<td>50</td>
<td>22.5</td>
<td>475</td>
<td>8.8</td>
</tr>
<tr>
<td>10/25/88-II</td>
<td>50</td>
<td>34.5</td>
<td>485</td>
<td>7.6</td>
</tr>
<tr>
<td>10/25/88-III</td>
<td>30</td>
<td>24.0</td>
<td>366</td>
<td>8.3</td>
</tr>
<tr>
<td>2/6/89</td>
<td>50</td>
<td>63.0</td>
<td>690</td>
<td>6.0</td>
</tr>
<tr>
<td>4/20/89</td>
<td>50</td>
<td>16.3</td>
<td>229</td>
<td>7.6</td>
</tr>
</tbody>
</table>

Mean ± S.D. 7.5 ± 1.0

concentration. All these experiments resulted in two bands as seen in lanes 3 and 4 of Fig. 3. Inspection of the figure (and densitometric analysis which is not shown) reveals that the amount of protein in the native protein band in lane 1 is approximately equivalent to the amount of protein in the upper band (deglycosylated) in lane 4. However, the glycoprotein stain indicates a much lower carbohydrate content in the upper deglycosylated band (lane 4) than in the native protein (lane 1). Comparison of the upper with the lower bands in each of lanes 3 and 4 demonstrates that the lower band contains much less carbohydrate than the upper one in each case. This indicates that carbohydrate in some of the selenoprotein P molecules is resistant to removal by the enzymes used and suggests that there is heterogeneous glycosylation. Based on the most deglycosylated band, the polypeptide molecular mass is estimated to be 43,000.

Selenium Content—Several preparations of the purified protein have been analyzed for selenium. Table II shows the results. Protein concentration was determined as total amino acid content. There is generally good agreement among preparations. These results indicate that selenium is 1.38% of the total amino acid weight. Therefore a polypeptide of 43,000 would contain an average of 7.5 selenium atoms. This agrees very well with the determination of selenocysteine in Table I and indicates that all the selenium in the protein is present as selenocysteine.

Peptide Fragments—Fig. 4 shows the time course of trypsin digestion of selenoprotein P. Five major tryptic peptides (bands 2-6) are demonstrated. At 60 min only the three smallest peptides were detectable. The 75Se content of the peptides is shown in the lower part of the figure. During the 60-min digestion, the 75Se accumulated in the 19- and 15-kDa peptides. The other peptides of 40, 34, and 21 kDa contained little or no 75Se.

Selenoprotein P was also digested with cyanogen bromide to cleave it at methionines. Analysis of the digest using SDS-PAGE demonstrated two peptides of 20 and 40 kDa (not shown). These peptides were carboxymethylated, separated by reversed-phase HPLC, and subjected to amino acid analysis. Fig. 5 shows the chromatograms of the fragments and of the native protein. Selenocysteine constituted 5.5% of the amino acid residues of the 20-kDa peptide (n = 3) and only 1.3% of the residues of the 40-kDa peptide (n = 2). Corresponding values for cysteine residues were 7.9 and 3.2% and for histidine 3.0 and 8.5%. Both these peptide fragmentation experiments indicate that selenium is not distributed uniformly through the polypeptide. Selenoprotein P appears to have one or more selenium-rich regions.

Serum Selenium Composition—Two selenoproteins, glutathione peroxidase and selenoprotein P, are present in serum. Table III shows measures of their content and of selenium in two experiments with rat serum. Immunodepletion of selenoprotein P was carried out to assess its contribution to serum selenium. Over 90% of selenoprotein P was removed from control serum by immunodepletion as was 58–63% of the selenium. Glutathione peroxidase activity was not affected.

Fig. 4. Trypsin digestion of 75Se-containing selenoprotein P. The upper part of the figure shows SDS-PAGE of a selenoprotein P preparation digested with trypsin for different times. A single-step immunoaffinity purification was used to obtain the starting material from rat serum labeled with 75Se in vivo. Digestion times in minutes are given above the lanes. Numbers on the left of the gel indicate bands of interest: 1, 57 kDa; 2, 40 kDa; 3, 34 kDa; 4, 21 kDa; 5, 19 kDa; 6, 15 kDa. The lower part of the figure shows the 75Se detected in the bands of the gel. No 75Se was detected in the minor contaminating bands present in the zero time lane above and below the position of band 3.

Fig. 5. Chromatograms of the fragments and of the native protein. Selenocysteine constituted 5.5% of the amino acid residues of the 20-kDa peptide (n = 3) and only 1.3% of the residues of the 40-kDa peptide (n = 2). Corresponding values for cysteine residues were 7.9 and 3.2% and for histidine 3.0 and 8.5%. Both these peptide fragmentation experiments indicate that selenium is not distributed uniformly through the polypeptide. Selenoprotein P appears to have one or more selenium-rich regions.
This indicates that most of the selenium in serum is associated with selenoprotein P.

Selenium deficiency led to sharp falls in serum glutathione peroxidase activity, selenoprotein P concentration, and selenium concentration (Table III). The rats in experiment I had been fed the experimental diets for 21 weeks and those used in experiment II for 6 weeks. Glutathione peroxidase activity in selenium-deficient rats was 1.1% and 1.7% of control and selenium concentration was 1 and 4% of control, respectively, reflecting the severity of the deficiency. Selenoprotein P concentration, on the other hand, was 12% of control in experiment I and 8% of control in experiment II. Thus, it fell in response to selenium deficiency but appeared to plateau at around 10% of control. This is consistent with an earlier report (2).

In both experiments the serum selenium was too low to account for the selenoprotein P concentration based on a selenium content of 1.38% of peptide weight. The discrepancy was more marked in experiment I in which the deficiency was more severe. Further experiments were carried out to investigate the nature of the selenoprotein P in selenium-deficient serum. One possibility considered was that the radioimmunoassay gave a falsely high value due to interference by immunologically cross-reactive proteins. This was investigated by performing gel filtration of selenium-deficient serum and assaying the fractions eluted for selenoprotein P. There was a sharp peak of selenoprotein P detected with the radioimmunoassay at the expected elution volume of selenoprotein P and none elsewhere (not shown). This result indicates that the immunoreactivity has the same size as selenoprotein P and suggests that selenoprotein P is present in the selenium-deficient serum. Moreover, immunodepletion of selenium-deficient serum (Table III) removed both selenoprotein P and selenium. Quenching of the selenium assay by substances in serum was also considered. Selenium-deficient serum was immunodepleted of selenoprotein P yielding serum with a selenium content of 1.9 ng/ml. Recoveries of 4 and 8 ng of selenium added as selenate to 0.9 ml of immunodepleted serum were 97.5 and 104%, respectively. Thus, no indication of quenching was obtained. These results do not provide a clear explanation for the discrepancy between selenoprotein P and selenium concentrations in selenium-deficient plasma and leave open the possibility that a form of selenoprotein P with diminished selenium content is present.

**DISCUSSION**

Selenoprotein P has a number of distinctive features. Its high selenium content is unprecedented. Glutathione peroxidase and the bacterial selenoproteins which have been characterized contain one selenium atom as selenocysteine/polypeptide chain (16). Selenoprotein P contains approximately eight selenocysteines in its polypeptide chain. There is no evidence for forms of selenium in selenoprotein P other than selenocysteine. The presence of many selenols could explain why attempts to purify selenoprotein P with conventional column chromatography have been unsuccessful (1, 15). Selenols are easily oxidized and exposure to oxidizing conditions during purification might cause changes that would alter the chromatographic characteristics of the protein leading to its elution in multiple peaks.

<table>
<thead>
<tr>
<th>Glutathione peroxidase</th>
<th>Selenoprotein P</th>
<th>Selenium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(units/ml)</td>
<td>(μg/ml)</td>
</tr>
<tr>
<td>Control animals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native</td>
<td>922 ± 145</td>
<td>26.3 ± 7.1</td>
</tr>
<tr>
<td>Immunodepleted</td>
<td>933 ± 105</td>
<td>2.4 ± 0.6</td>
</tr>
<tr>
<td>Amount removed</td>
<td>0</td>
<td>23.9</td>
</tr>
<tr>
<td>Selenium-deficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native</td>
<td>16 ± 3</td>
<td>3.1 ± 0.4</td>
</tr>
<tr>
<td>Immunodepleted</td>
<td>10 ± 4</td>
<td>&lt;0.5</td>
</tr>
</tbody>
</table>

*ND, not done.*
The $M_\text{r}$ of selenoprotein P has been estimated to be 80,000 to 100,000 by gel filtration (3, 15) and 57,000 by SDS-PAGE (Fig. 1). This discrepancy has suggested to some workers that the protein has a subunit structure (15) and that SDS treatment might dissociate a small selenium-poor subunit. The present report does not support this idea and suggests that selenoprotein P consists of a single polypeptide chain because purified selenoprotein P, which elutes at the same position as native selenoprotein P in serum on gel filtration, yields only one band on overloaded gels (Fig. 1, lane 6). Glycoproteins often deviate from predicted migration on chromatography and the differences in $M_\text{r}$ estimation by the two methods might be caused by the substantial carbohydrate complement of selenoprotein P.

The amino acid composition of selenoprotein P has several unusual features. Comparison of it with the amino acid composition of rat liver glutathione peroxidase (17) indicates that, for equivalent peptide mass, selenoprotein P has 3.4 times the cysteines, 4.5 times the selenocysteines, and 7 times the histidines of glutathione peroxidase. Evidence has been presented that regions of the protein are enriched with these amino acids. Cleavage with cyanogen bromide (Fig. 5) yielded a 20-kDa fragment which contained more selenium than the other major fragment of 40 kDa, and cleavage with trypsin yielded 19- and 15-kDa fragments which were selenium rich while larger fragments were almost devoid of the element.

Histidine and cysteine are involved in metal binding in some proteins and might serve such a function in selenoprotein P. Metabolic relationships between selenium and heme (4) and selenium and copper (18) are known. Further experiments will be needed to explore these relationships and to determine whether selenoprotein P binds metals.

Two selenoproteins are known to be present in plasma, glutathione peroxidase, and selenoprotein P. Plasma glutathione peroxidase is immunochemically distinct from tissue glutathione peroxidase (19). Both plasma selenoproteins are glycosylated and are secreted by the liver (1, 3, 20). Other forms of selenium have been reported in plasma under certain circumstances, but no evidence of another specific plasma selenoprotein (containing selenium as selenocysteine) has been presented.

Selenoprotein P accounts for over 60% of the serum selenium (Table III). Plasma content of selenoprotein P is the same as serum content. Based on a value of 4 ml of plasma/100 g of body weight, there is 1.2 $\mu$g of selenium/100 g of rat weight in selenoprotein P. Whole body selenium is about 15 $\mu$g/100 g (calculated from results in 21, 22), so plasma selenoprotein P contains about 8% of whole body selenium. Selenium in glutathione peroxidase, which is found in all tissues, has been estimated to be 33% (23) and 40% (22) of whole body selenium. Thus, selenoprotein P constitutes a major body selenium pool.

A previous study indicated that when dietary selenium was limiting, selenoprotein P content was maintained better than liver and plasma glutathione peroxidase activity (2). This suggests that selenoprotein P takes precedence over glutathione peroxidase in utilizing selenium within the liver. Measurement of selenoprotein P would thus appear to provide an additional index of selenium status. Further studies will be necessary to determine the value of selenoprotein P measurement in assessing selenium nutritional status.

A previous report from this laboratory suggested that selenoprotein P concentration was 60 $\mu$g/ml of plasma (2). The present report indicates that that figure should be revised to 26 $\mu$g/ml (Table III). This lower value is the result of improved accuracy in determining the protein content of purified selenoprotein P which is used to standardize the radioimmunoassay. This report employs total amino acid content whereas the previous report (2) used a colorimetric method standardized to bovine serum albumin for determining protein.

There is a substance in selenium-deficient rat serum which cross-reacts with the monoclonal antibodies to selenoprotein P but which does not contain selenium in the same stoichiometry as selenoprotein P purified from control rats (Table III). This substance elutes from a gel filtration column at the same position as does selenoprotein P and thus, seems likely to be related to selenoprotein P. The nucleic acid codon for selenocysteine in glutathione peroxidase and in bacterial selenoproteins is the opal nonsense codon (24, 25). In the absence of a suppressor tRNA (charged with selenocysteine) which recognizes this codon, it terminates translation. It is conceivable that a truncated peptide or a peptide with another amino acid replacing selenocysteine is produced by this process and is secreted into the plasma in selenium deficiency. Such altered forms of glutathione peroxidase have been sought in rat liver without success, however (26). Clarification of this point will require further research.

The function of selenoprotein P in unknown. The plasma concentration of selenols in selenoprotein P can be calculated to be 3.5–4.0 $\mu$M (Table III). The protein contains approximately equal numbers of thiols and selenols so together they are 7–8 $\mu$M. This compares with the plasma glutathione concentration of 20 $\mu$M and indicates that selenoprotein P contains significant reducing capacity in the plasma. The protein contains a number of histidines and cysteines which are involved in metal binding in some proteins. Thus, selenoprotein P has a number of potential redox-active sites. These properties are consistent with it being a protective agent in the plasma against free radicals or other reactive molecules.

More studies will be needed to test this hypothesis and others of selenoprotein P function.

Acknowledgment—We are indebted to Thressa C. Stadtman for advice and encouragement.

REFERENCES

2 R. Read and R. F. Burk, unpublished observations.
Selenium and amino acid composition of selenoprotein P, the major selenoprotein in rat serum.
R Read, T Bellew, J G Yang, K E Hill, I S Palmer and R F Burk

Access the most updated version of this article at http://www.jbc.org/content/265/29/17899

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/265/29/17899.full.html#ref-list-1