A new selenoprotein from human lung adenocarcinoma cells: Purification, properties, and thioredoxin reductase activity

(selenocysteine/selenium-75/flavoprotein/selenoenzyme)

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ABSTRACT We report the isolation and characterization of a new selenoprotein from a human lung adenocarcinoma cell line, NCI-H441. Cells were grown in RPMI 1640 medium containing 10% (vol/vol) fetal bovine serum and 0.1 μM [75Se]selenite. A 75Se-labeled protein was isolated from sonic extracts of the cells by chromatography on DE-23, phenyl-Sepharose, heparin-agarose, and butyl-Sepharose. The protein, a homodimer of 57-kDa subunits, was shown to contain selenium in the form of selenocysteine; hydrolysis of the protein alkylated with either iodoacetate or 3-bromopropiono-nate yielded Se-carboxymethyl-selenocysteine or Se-carboxyethyl-selenocysteine, respectively. The selenoprotein showed two isoelectric points at pH 5.2 and pH 5.3. It was distinguished from selenoprotein P by N-glycosidase assay and by the periodate–dansyl hydrazine test, which indicated no detectable amounts of glycosyl groups on the protein. The selenoprotein contains FAD as a prosthetic group and catalyzes NADPH-dependent reduction of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), and reduction of insulin in the presence of thioredoxin (Trx). The specific activity was determined to be 31 units/mg by DTNB assay. Apparent Kₘ values for DTNB, Escherichia coli Trx, and rat Trx were 116, 34, and 3.7 μM, respectively. DTNB reduction was inhibited by 0.2 mM arsenite. Although the subunit composition and catalytic properties are similar to those of mammalian thioredoxin reductase (TR), the human lung selenoprotein failed to react with anti-rat liver TR polyclonal antibody in immunoblot assays. The selenocysteine-containing TR from the adenocarcinoma cells may be a variant form distinct from rat liver TR.

Selenium is an essential trace element in mammals and birds (1), and its remarkable biological effects in eukaryotes may be related to unique functions of various selenoproteins (2). Well-examined cases are glutathione peroxidase in antioxidant systems (3) and type I tetraiodothyronine 5'-deiodinase in thyroid hormone biosynthesis (4). White muscle disease in animals, observed in selenium deficiency, may be related to selenoprotein W (5). Discovery of a selenoprotein may provide significant insight into the undiscovered biological functions of selenium in human health. The present study was initiated with an aim to isolate and characterize a putative selenoprotein produced by nonsense mutants of a cytochrome P-450. Human cytochrome P-450 IIIB1 (CYP2B6) has a calculated molecular weight of 56,286. A related P-450, CYP2B7, is expressed in lung and has a nonsense mutation (6). If this nonsense mutant could be expressed by decoding its in-frame opal codon with selenocysteine (Sec), the corresponding P-450 protein should be produced as a selenoprotein of 56–58 kDa. Interestingly, a 57-kDa 75Se-labeled protein was produced by a 75Se-enriched human lung adenocarcinoma cell, NCI-H441, which has mRNAs for the CYP2B7 (F. J. Gonzalez, personal communication). We purified this 75Se-labeled protein and chemically identified its Sec residue by dual alkylation experiments. However, its subunit composition, spectroscopic properties, and the presence of an FAD prosthetic group were contradictory to the expected properties of a cytochrome P-450 protein. We also found that the selenoprotein shows thioredoxin reductase (TR) activity with the kinetic parameters and substrate specificity compatible to those of mammalian TRs. Based on the isoelectric points and immunochromatographic properties, we suggest that this selenoenzyme is distinct from rat liver TR.

MATERIALS AND METHODS

Materials. [75Se]Selenite was purchased from the Research Reactor Facility (University of Missouri, Columbia), and mixed with sodium selenite to the final specific activity of 3.67 Ci/mmol (1 Ci = 37 GBq). Heat-inactivated fetal bovine serum, antibiotic–antimycotic solution (×100), trypsin–EDTA solution (×10), and RPMI 1640 medium were purchased from Mediatech (Herndon, VA). N-Glycosidase F was purchased from Boehringer Mannheim. EDTA-Mg,K (MgTritriplex) was from Merck. Precast Gels (12% polyacrylamide) were purchased from NOVEX (San Diego). Escherichia coli thioredoxin (Trx), rat liver Trx, and rabbit antiserum raised against purified rat liver TR were from H.-J. Kim (National Institutes of Health) and H. Z. Chac (National Institutes of Health). Antiserum against human selenoprotein P raised in rabbit was from R. F. Burk (Vanderbilt University, Nashville, TN). Rabbit anti-goat IgG (heavy and light chains) and goat anti-rabbit IgG (heavy and light chains) were purchased from Kirkegaard & Perry Laboratories. Goat anti-rat CYP2B1 serum and phenobarbital-treated rat liver microsomes were purchased from Dai-ichi Pure Chemicals (Tokyo).

Purification of 75Se-Labeled Protein. Three buffers were used in the following steps: buffer A, 20 mM Tris-HCl (pH 8.3); buffer B, 100 mM sodium phosphate (pH 7.3); buffer C, 10 mM sodium phosphate (pH 7.6). All these buffers contained 2 mM dithiothreitol and 1 mM MgTritriplex. Protein elution profiles were monitored by measurement of absorbance at 280 nm. The radioactivity of fractions was determined with a Beckman model 5500 γ counter.

Cell Growth and Preparation of Sonic Extract. The NCI-H441 cell line was obtained from the American Type Tissue Collection. The cells were grown in 44 ml of RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum and 1× antibiotic–antimycotic solution, in a 162-cm² cell culture flask (Costar) under a 90% relative humidity and 5%
with trypsin-EDTA solution, air atmosphere. For preparation of $^{75}$Se-labeled cells, 0.1 µM $^{75}$Se-selenite (3.67 Ci/mmol) was added to the medium, and the cells were grown under the same conditions. Cells were washed with 10 ml of phosphate-buffered saline (15 mM NaCl/100 mM potassium phosphate, pH 7.0), treated with trypsin–EDTA solution, and collected by low-speed centrifugation. The pellet was washed three times with 50 ml of the phosphate-buffered saline and frozen at $-20^\circ$C until used. The cells were thawed in ice water, suspended in an equal volume of buffer A, mixed with $^{75}$Se-labeled cells, and disrupted by sonication. The pellet obtained after centrifugation for 30 min at 8000 x g was again suspended in the same buffer and sonicated. To the combined supernatant solutions, ammonium sulfate was added to 80% of saturation. The precipitate was resuspended in 40 ml of buffer A and dialyzed three times against the same buffer. After insoluble materials were removed by centrifugation for 60 min at 8000 x g, the solution was used as the crude extract.

**DE-23 Column Chromatography.** The crude extract (460 mg of protein) was applied to a DE-23 column (2.5 x 8 cm) equilibrated with buffer A. The column was thoroughly washed with buffer A containing 0.05, 0.1, and 0.15 M NaCl, the $^{75}$Se-labeled protein was eluted with buffer A containing 0.3 M NaCl. Radioactive fractions were pooled and concentrated to 10 ml by ultrafiltration. Ammonium sulfate was added to the final concentration of 0.8 M.

**Phenyl-Sepharose CL-4B Column Chromatography.** The above protein solution (48 mg of protein) was applied to a phenyl-Sepharose CL-4B column (2.5 x 8 cm) equilibrated with buffer B containing 0.8 M ammonium sulfate. After the column was thoroughly washed with buffer B containing 0.8, 0.6, 0.4, and 0.32 M ammonium sulfate, $^{75}$Se-labeled protein was eluted with buffer B containing 0.2 M ammonium sulfate. The radioactive fractions were pooled, concentrated to 5 ml by ultrafiltration, and dialyzed three times against 1 liter of buffer C.

**Heparin-Agarose Column Chromatography.** The protein solution from the phenyl-Sepharose step (14.5 mg of protein) was applied to a heparin-agarose column (1.4 x 12.5 cm) equilibrated with buffer C. The protein was eluted off at a stepwise gradient of 0, 50, and 100 mM NaCl (each at 150 ml) in buffer C. The radioactive fractions were pooled and concentrated by ultrafiltration. Ammonium sulfate was added to the final concentration of 1.2 M.

**Butyl-Sepharose 4B Column Chromatography.** The protein solution from the heparin column (1.15 mg of protein) was applied to a butyl-Sepharose 4B column (1.0 x 8.5 cm) equilibrated with buffer B containing 1.2 M ammonium sulfate. The column was thoroughly washed with buffer B containing 0.8 M ammonium sulfate, and $^{75}$Se-labeled protein was eluted with buffer B containing 0.6 M ammonium sulfate. The radioactive fractions were pooled, concentrated by ultrafiltration, and dialyzed against buffer C. The preparation was used as a purified protein.

**Protein Assay.** Protein was determined by the Coomassie brilliant blue G-250 dye-binding method (7) using Bio-Rad dye reagent. Bovine serum albumin was used as standard.

**Molecular Weight Determination.** Molecular weight was determined by HPLC with TSK-GEL G3000 SW (7.5 mm x 600 mm) at a flow rate of 0.5 ml/min and an elution buffer consisting of 0.1 M sodium phosphate (pH 7.0) containing 0.1 M Na$_2$SO$_4$, 2 mM dithiothreitol, and 1 mM EDTA. A calibration curve was made with the following standard proteins: thyroglobulin ($M_r$, 670,000), gamma globulin ($M_r$, 158,000), ovalbumin ($M_r$, 44,000), and myoglobin ($M_r$, 17,000). The molecular weight of the subunit was estimated by SDS/PAGE with the low molecular weight protein standards from Pharmacia as reference.

**Isoelectric Focusing Electrophoresis.** Isoelectric points were determined by using a NOVEX IEF kit at pH 3-7.

**Identification of Sec.** Purified $^{75}$Se-labeled protein (40 µg) was washed three times with distilled water and concentrated to 40 µl by using a Centricon-10 microconcentrator (Amicon). The solution was mixed with 60 µl of 100 mM NaBH$_4$ in 20 mM NaOH aqueous solution and incubated under argon at room temperature for 30 min. Five micromoles of sodium iodoacetate (or sodium 3-bromopropionate) was added to the mixture and incubated under argon at room temperature for 40 min. Then, 2-mercaptoethanol was added (10 µmol) to quench the alkylation reaction. The protein was washed three times with distilled water by ultrafiltration, taken to dryness, and then hydrolyzed in 6 M HCl at 155°C under argon. The hydrolysate was dried, treated with NaBH$_4$, mixed with authentic Se-carboxymethyl-selenocysteine (CM-Se) and Se-carboxyethyl-selenocysteine (CE-Se) (each at 1.3 µmol), and chromatographed on an amino acid analyzer. The eluate from the analyzer column was collected in 1-min fractions, and the radioactivity contained in these fractions was determined with a Beckman model 5500 γ counter.

**Spectroscopic Properties.** The absorption spectra were recorded on a U-2000 spectrophotometer (Hitachi Instruments, Japan). Bleaching measurements were made in a microcell closed with a rubber seal. Enzyme samples (0.4 ml) were made anaerobic in the cell by flushing with argon through needles passing through the seal. NADPH or NADH was added to the final concentration of 2 mM by a syringe through the seal, and the spectra were taken immediately after the sample was mixed. Fluorescent spectra were taken on a luminescence system LS-100 (Photon Technology International, Princeton, NJ). The CO-binding spectrum of the selenoprotein was compared with that of recombinant human CYP3A4 expressed in SE9 insect cells (8).

**Flavin Cofactor Analysis.** Chromatography of the flavin cofactor was performed as described (9). Flavin cofactor was released from the purified protein by treatment with acidic ammonium sulfate (80% saturation, pH 3.3).

**Amino Acid Analysis.** The protein was dialyzed against distilled water and hydrolyzed in 6 M HCl at 155°C under argon for 45 min. After removal of HCl in vacuo, amino acids were determined by precolumn derivitization with phenyl isothiocyanate, and the amino acid derivatives were determined as described (10). An amino acid standard solution (Pierce, IL) was used for calibration.

**N-Glycosyl Group Assay.** Purified $^{75}$Se-labeled protein (8 µg) was used for deglycosylation tests with N-glycosidase as described for selenoprotein P (11). Periodate oxidation and the dansyl hydrazine staining assay were carried out on SDS/PAGE gels as described (12). For both of the tests, transferrin was used as a positive control.

**TR Assay.** Two separate assays were conducted to examine TR activity. The first assay involves the NADPH-dependent reduction of 5,5'-dithiobis(2-nitrobenzoate) (DTNB) (method 1). The assay mixture (1 ml) contained 500 mM potassium phosphate (pH 7.0), 50 mM KCl, 10 mM EDTA, 0.24 mM NADPH, bovine serum albumin (0.2 mg/ml), and 2.5 mM DTNB (50 µl of a 50 mM solution in absolute ethanol). An aliquot (0.5–3 µl) of purified selenoprotein was added to the assay mixture, and the change in optical density at 412 nm was monitored over 2 min at 30°C. Activity is defined as micromoles of NADPH oxidized per min by ΔA$_{412}$/13.6 x 2), since 1 mol of NADPH yields 2 mol of thionitrobenzoate. When other substrates replaced DTNB, the enzyme reaction was monitored by the change in absorbance at 340 nm due to the oxidation of NADPH. The second assay monitored the change in absorbance at 340 nm due to the oxidation of NADPH in the presence of Trx and insulin (13) (method 2). The assay mixture contained 50 mM potassium phosphate (pH 7.0), 20 mM EDTA, 85 µM insulin, 0.24 mM NADPH, with Trx and enzyme in a total volume of 0.5 ml. The activity is expressed...
as μmol of NADPH oxidized per min at 30°C. Assay mixtures lacking Trx served as controls.

**Immunoblot Analysis.** Purified selenoprotein (5, 10, 20, and 40 ng) was subjected to SDS/PAGE on 12% gels (14) and then transferred to poly(vinylidene difluoride) membranes as described by Towbin et al. (15). Rabbit anti-human selenoprotein P polyclonal antibody, goat anti-rat liver CYP2B1 polyclonal antibody, and rabbit anti-rat liver TR polyclonal antibody were separately used at a 1:1000 dilution. The alkaline phosphatase-conjugated rabbit anti-goat IgG or goat anti-rabbit IgG was used at a 1:1000 dilution and assayed using BCIP/NBT phosphatase substrate system (Kirkegaard & Perry Laboratories).

**RESULTS**

**Purification of 75Se-Labeled Protein.** 75Se-labeled protein was purified 128-fold from sonic extracts of NCI-H441 tumor cells, with an overall yield of 10.9% (Table 1). SDS/PAGE analysis of the protein fractions is shown in Fig. 1. A single protein band of 57 kDa was detected in the fraction from butyl-Sepharose.

**Molecular Weight and Subunit Structure.** The protein was found to have a molecular weight of about 110,000 by gel filtration with TSK-GEL G3000SW in the presence of 2 mM dithiothreitol. The molecular weight of the subunit was estimated as 57,000 by SDS/PAGE. These results indicate that the protein is composed of two subunits apparently identical in molecular weight. The isoelectric points of the purified protein were determined to be 5.2 and 5.3.

**Identification of Sec.** On the amino acid analyzer, CM-Sec and CE-Sec are eluted at 19 and 29 min, respectively. When the labeled protein was alkylated with iodoacetate, the radioactive elution profile of the hydrolysate coincided exactly with CM-Sec. When 3-bromopropionionate was used for the alkylation, 75Se in the hydrolysate coincided with that of CE-Sec (Fig. 2). Throughout the procedure of alkylation, hydrolysis, and chromatography, the recovery of 75Se was 47% and 65% for CM-Sec and CE-Sec formation, respectively. 75Se-labeled selenomethionine was not detected in either experiment. The results clearly show that the selenium moiety of the 75Se-labeled protein is Sec.

**Spectroscopic Properties.** The absorption spectrum of the selenoprotein shows maxima at 275, 340, and 450 nm in a ratio of 1, 0.1, and 0.09, respectively (Fig. 3). Upon reduction with NADPH, the spectrum changed in the region of 400–600 nm. Addition of NADH did not change the spectrum in this region. This indicates that the selenoprotein binds NADPH and acts as an NADPH-acceptor reductase. CO difference spectra of

![Table 1. Summary of purification](image)

<table>
<thead>
<tr>
<th>Protein, mg</th>
<th>75Se, cpm × 10^-6</th>
<th>Specific activity, cpm × 10^-6/mg</th>
<th>Yield, %</th>
<th>Fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>460.0</td>
<td>12.0*</td>
<td>0.026</td>
<td>100</td>
</tr>
<tr>
<td>DE-23</td>
<td>48.0</td>
<td>5.3†</td>
<td>0.110</td>
<td>44.2</td>
</tr>
<tr>
<td>Phenyl-Sepharose</td>
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</tr>
<tr>
<td>Heparin-agarose</td>
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<td>2.6</td>
<td>2.26</td>
<td>21.7</td>
</tr>
<tr>
<td>Butyl-Sepharose</td>
<td>0.39</td>
<td>1.31</td>
<td>3.36</td>
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</tr>
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</table>

*Radioactivity, expressed as cpm × 10^-6, includes 75Se-labeled 57-, 27-, and 23-kDa proteins. The 57-kDa protein accounted for about 70% of the total radioactivity.

†After DE-23, only the 57-kDa protein is labeled with 75Se.

![Fig. 1. SDS/PAGE analysis of 57-kDa selenoprotein during purification. Coomassie blue G-250-stained 12% polyacrylamide gels are shown. Lanes: 1, SeeBlue pre-stained standards (Novex) (myosin, 250 kDa; bovine serum albumin, 98 kDa; glutamic dehydrogenase, 64 kDa; alcohol dehydrogenase, 50 kDa; carbonic anhydrase, 36 kDa; myoglobin, 30 kDa; lysozyme, 16 kDa; aprotonin, 6 kDa; 2, 12.2 μg of crude cell extract; 3, 7.4 μg of DE-23 fraction; 4, 6.4 μg of phenyl-Sepharose fraction; 5, 4.1 μg of heparin-agarose fraction; 6 and 7, butyl-Sepharose fraction, 2.5 and 5.0 μg; 8, low molecular weight standards (Pharmacia) (phosphorylase b, 94 kDa; albumin, 67 kDa; ovalbumin, 43 kDa; carbonic anhydrase, 30 kDa; trypsin inhibitor, 20 kDa). Standards in lane 1 and samples in lanes 2–5 were electrophoresed on one gel and samples in lanes 6 and 7 and standards in 8 were on another gel.](image)

![Fig. 2. Amino acid analyzer chromatograms of 75Se compounds from acid hydrolysates of carboxymethylated (A) and carboxymethylated (B) selenoprotein. The hydrolysates were mixed with CM-Sec (I) and CE-Sec (II) before the chromatography. Solid lines represent amino acid elution and broken lines represent 75Se radioactivity.](image)
the selenoprotein and CYP3A4 were compared (Fig. 4). The 
\(^{75}\text{Se}\)-labeled protein showed an absorbance peak at 490 nm, which was distinctive from that of the cytochrome P-450 protein.

**Flavin Cofactor.** Fluorescence spectra of the selenoprotein showed excitation maxima at 370 and 450 nm and emission maxima at 420 and 530 nm. The excitation maxima at 370 and 450 nm and the emission maximum at 530 nm are characteristic of a flavoprotein. The flavin cofactor released from the selenoprotein was identified as FAD by cochromatography with authentic FAD and UV absorbance profiles obtained using a diode-array detector.

**Glycosyl Group Assay.** Mobility of the selenoprotein on SDS/PAGE was not altered by treatment with N-glycosidase, and the protein band was not stained by the periodate–dansylhydrazine staining test.

**Amino Acid Composition.** The amino acid composition of the purified enzyme is given in Table 2. Glutamate, glycine, and leucine were predominant in the protein, and histidine, serine, and methionine were found in small amounts. Cysteine, as half cystine, and selenocystine were not detected in the profile. Tryptophan was not measured.

**Amino Acid Sequence Analysis.** Carboxymethylated protein (70 pmol) was submitted to amino acid sequencer analysis, but no interpretable data were obtained with this material. Based on the extremely low signals observed, we concluded that most of the protein was N-terminal blocked.

**TR Activity.** The selenoprotein catalyzed NADPH-dependent reduction of disulfide bonds (Table 3). Specific activity was determined to be 31 units/mg by the DTNB assay. Addition of a molar equivalent of FAD to the enzyme did not increase catalytic activity. Sodium arsenite decreased the activity to 63% at 0.02 mM, 13% at 0.2 mM, and to 4.5% at 2 mM. The selenoprotein was able to reduce rat Trx and *E. coli* Trx in the insulin reduction assay (Table 4). The apparent \( K_m \) values for rat Trx and *E. coli* Trx were 3.7 \( \mu \)M and 34 \( \mu \)M, respectively. The relative specificity (\( k_{cat}/K_m \)) indicates that rat Trx is a better substrate than *E. coli* Trx or DTNB.

**Immunoblot Analysis.** Positive control experiments were performed on the same SDS/PAGE gel and poly(vinylidene difluoride) membrane by using rabbit serum for selenoprotein P, phenobarbital-treated rat liver microsomes for CYP2B1, and TR purified from rat liver. The selenoprotein did not show any reactivity in the immunoblot assays with anti-human selenoprotein P, anti-rat CYP2B1, or anti-rat liver TR polyclonal antibodies.

### DISCUSSION

In several reports it has been noted that proteins of 56–58 kDa are highly labeled with \(^{75}\text{Se}\), when \(^{75}\text{Se}\)-enriched homogenates of mammalian tissues are analyzed by SDS/PAGE and autoradiography (16–20). Although SDS/PAGE and autoradiography analysis is a convenient and sensitive method for detecting proteins that have \(^{75}\text{Se}\)-labeled Sec residues (21), some proteins can bind elemental \(^{76}\text{Se}\) or \(^{75}\text{Se}\) selenenite with high affinity, and the resulting radioactive proteins may be confused with selenoproteins (16, 22). Therefore, identification of \(^{75}\text{Se}\)-labeled Sec is the critical evidence for characterization of a specific selenoprotein (11, 23). In the present study, dual alkylation experiments clearly showed that the selenium moiety of the \(^{75}\text{Se}\)-labeled 57-kDa lung protein was in the form of Sec. Selenocysteine-containing proteins should be clearly distinguished from nonspecific selenium-binding proteins or selenomethionine-containing proteins by such a simple chemical test.

Selenoprotein P is the only identified mammalian Sec-containing protein that has the apparent native molecular size of around 60 kDa (24). It is a monomeric glycoprotein that is stained by periodate–hydrazine staining test, and its apparent

<table>
<thead>
<tr>
<th>Residue</th>
<th>No. residues/100 residues</th>
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<tbody>
<tr>
<td>Asx</td>
<td>7.7</td>
</tr>
<tr>
<td>His</td>
<td>2.6</td>
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<td>Leu</td>
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<tr>
<td>Lys</td>
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Table 2. Amino acid composition

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<th>Substrate</th>
<th>Concentration, mM</th>
<th>Turnover, min(^{-1})</th>
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<tr>
<td>DTNB</td>
<td>2.5</td>
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<tr>
<td>DL-Lipoate</td>
<td>2.5</td>
<td>10</td>
</tr>
<tr>
<td>Menadione</td>
<td>0.2</td>
<td>17</td>
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<tr>
<td>Insulin</td>
<td>0.2</td>
<td>ND</td>
</tr>
<tr>
<td>Oxidized glutathione</td>
<td>2.5</td>
<td>ND</td>
</tr>
</tbody>
</table>

Substrate specificity was determined by method I. ND, not detected.
molecular size is altered to 43 kDa by treatment with N-glycosidase (11). Selenoprotein P may play an important role as an antioxidative defense (25), but its catalytic function is unknown. We characterized the lung adenocarcinoma selenoprotein as a dimer of identical subunits whose FAD cofactor is specifically reduced by NADPH. The N-glycosidase assay and periodate–dansylhydrazine staining test indicated no detectable amounts of glycosyl groups on the selenoprotein. Rabbit anti-human selenoprotein P polyclonal antibody did not cross-react with this FAD containing selenoprotein. Thus, the lung adenocarcinoma selenoprotein is not a selenoprotein P.

By the CO binding assay, the selenoprotein did not show the Soret absorption spectrum characteristic of cytochrome P-450 proteins. Anti-rat CYP2B1 antibody did not cross-react with the selenoprotein, whose dimeric subunit composition, FAD content, and TR activity are already contradictory to the expected characteristics of the protein products of the nonsense mutants, h1B2 and h1B3. Read-through of a nonsense codon is an erratic decoding by certain tRNAs in competition with a release factor, while biosynthesis of specific UGA-encoded selenoproteins occurs by insertion of Sec from Sec-tRNA in positions directed by UGA codons and cotranslational factors (26, 27). These nonsense mutants may not be expressed as Sec-containing proteins in amounts sufficient for detection in the lung adenocarcinoma cells we used.

TR has been purified and characterized from E. coli (28), yeast (29), rat liver (30, 31), bovine liver (13, 32), human placenta (33), rat Novikoff tumor (34), and cultured HeLa cells (35). In contrast to the E. coli enzyme, a 68-kDa flavoprotein composed of 34-kDa subunits, the mammalian enzymes are larger proteins with a dimer structure of 58-kDa subunits. The [75Se]selenoprotein, a dimeric flavoprotein of 57-kDa subunits, is closer to the mammalian TRs in subunit structure and molecular mass. Mammalian TRs show UV absorption spectra characteristic of a flavoprotein, and the ratio \( \frac{A_{275}}{A_{460}} \) was reported to be 9 for calf liver TR (32), and \( \frac{A_{290}}{A_{460}} \) is 8.0 for rat liver TR (31). The [75Se]selenoprotein showed the corresponding ratio \( \frac{A_{275}}{A_{450}} \) of 11.

The selenoprotein catalyzed NADPH-dependent reduction of DTNB, and its specific activity was determined as 31.0 units/mg. This closely corresponds to 35.0 units/mg for rat liver TR (31) and to 33.8 units/mg for human placental TR (33). The selenoprotein reduced both E. coli Trx and rat liver Trx in the insulin reduction assay, and this broad specificity is typical of mammalian TRs (36). The selenoprotein was as sensitive to arsenite as bovine liver and rat liver TRs (13, 31).

Despite such similarities in the subunit composition and size, substrate specificity, and kinetic parameters, the selenoprotein failed to cross-react with anti-rat TR polyclonal antibody. This result suggests that the Sec-containing TR from the lung adenocarcinoma is different from the rat liver enzyme. In fact the presence of Sec in the rat enzyme has not been demonstrated. Chen et al. (30) indicated that Novikoff ascites tumor contains a variant form of TR different from the rat liver enzyme; the Novikoff tumor TR and rat liver TR showed different affinities to the anti-Novikoff TR antibody in complement fixation assays. The Novikoff tumor TR shows an isoelectric point at pH 5.1, while rat liver TR shows an isoelectric point at pH 4.9 (30). Human placenta TR has a pI of 4.85 (33). The selenoprotein showed two isoelectric points at pH 5.2 and pH 5.3. At present, we cannot ascertain whether both forms were present in the lung adenocarcinoma cell or if one could have arisen by modification of the other during the purification process, but it is interesting to note that these isoelectric points are closer to that of the Novikoff tumor TR, pH 5.1.

To our knowledge, this is the first report of the biochemical relevance between selenium and TR in human lung adenocarcinoma cells. Based on the fold purification and recovery, this Sec-containing TR seems to account for about 0.5% of the soluble proteins. Further investigation of the biological distribution and physiological catalytic activity of this selenoprotein is required for the better understanding of the relationship between selenium and various TR-dependent cellular regulation mechanisms (36).

We thank Dr. Frank J. Gonzalez, National Cancer Institute, National Institutes of Health, for providing us with interesting information on the [75Se]-labeled 57-kDa protein produced in NCI-H441 cells.