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Multiple Levels of Regulation of Selenoprotein Biosynthesis Revealed from the Analysis of Human Glioma Cell Lines

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ABSTRACT. To gain a better understanding of the biological consequences of the exposure of tumor cells to selenium, we evaluated the selenium-dependent responses of two selenoproteins (glutathione peroxidase and the recently characterized 15-kDa selenoprotein) in three human glioma cell lines. Protein levels, mRNA levels, and the relative distribution of the two selenocysteine tRNA isoacceptors (designated mcm^5U and mcm^5Um) were determined for standard as well as selenium-supplemented conditions. The human malignant glioma cell lines D54, U251, and U87 were maintained in normal or selenium-supplemented (30 nM sodium selenite) conditions. Northern blot analysis demonstrated only minor increases in steady-state GSHPx-1 mRNA in response to selenium addition. Baseline glutathione peroxidase activity was 10.7 ± 0.7, 7.6 ± 0.7, and 4.3 ± 0.7 nmol NADPH oxidized/min/mg protein for D54, U251, and U87, respectively, as determined by the standard coupled spectrophotometric assay. Glutathione peroxidase activity increased in a cell line-specific manner to 19.7 ± 1.4, 15.6 ± 2.1, and 6.7 ± 0.5 nmol NADPH oxidized/min/mg protein, respectively, as did a proportional increase in cellular resistance to H_2O_2, in response to added selenium. The 15-kDa selenoprotein mRNA levels likewise remained constant despite selenium supplementation. The selenium-dependent change in distribution between the two selenocysteine tRNA isoacceptors also occurred in a cell line-specific manner. The percentage of the methylated isoacceptor, mcm^5Um, changed from 35.5 to 47.2 for D54, from 38.1 to 47.3 for U251, and from 49.0 to 47.6 for U87. These data represent the first time that selenium-dependent changes in selenoprotein mRNA and protein levels, as well as selenocysteine tRNA distribution, were examined in human glioma cell lines. BIOCHEM PHARMACOL **60**;4:489–497, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. glioma; glutathione peroxidase; selenium; selenocysteine; selenoprotein; tRNA

The trace element selenium is an essential human nutrient that has been correlated to a decreased cancer risk for over two decades [1–13]. Selenium is present in eukaryotic protein in the form of the amino acid selenocysteine. Selenocysteine represents the 21st amino acid and is encoded by the UGA triplet in selenoprotein mRNA [14, 15]. Although UGA most often functions as a stop codon, UGA-encoded incorporation of selenocysteine into the growing polypeptide is determined by the presence of a specific stem–loop secondary structure within the 3′ untranslated region of the selenoprotein mRNA [16, 17]. This region is termed the SECIS** element. Mutations of the SECIS element that alter secondary structure result in termination of translation in response to UGA and resultant dramatic decreases in selenoprotein levels. Several selenoproteins have been characterized in mammals. These include glutathione peroxidases, thioredoxin reductases, iodothyronine deiodinases, selenoprotein P, selenoprotein W, and selenophosphate synthetase [18, 19]. Recently, a

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** *Abbreviations*: SECIS, selenocysteine insertion sequence; GSHPx-1, cytosolic glutathione peroxidase; IDI, iodothyronine deiodinase; cDNA, complementary DNA; CHO, Chinese hamster ovary; UTR, untranslated region; $tRNA^{[Ser]Sec}$, selenocysteine tRNA; mcm^5U, methylcarboxymethyl-5′-uridine, designated as the unmethylated selenocysteine isoacceptor; mcm^5Um, methylcarboxymethyl-5′-uridine-2′-O-methylribose, designated as the methylated selenocysteine isoacceptor; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; and RPC, reverse phase chromatography.

15-kDa selenoprotein with unknown function has been isolated from human lymphocytes [20].

The mechanism by which selenium exerts its chemopreventive effect is unknown. One possibility is the resultant modulation of selenoprotein enzyme levels, which, along with the respective mRNAs, can occur in response to available selenium. The known selenoproteins and their respective mRNAs respond independently in a cell line-specific and tissue-specific manner to changes in available selenium [21–24]. For example, protein levels of the GSHPx-1 decline sharply along with GSHPx-1 mRNA in the livers and hearts of rats fed selenium-deficient diets [21]. However, when the normal selenium state is compared with the supplemented state, increases in GSHPx-1 protein and enzyme activity are seen in certain cell lines (Ref. 25, and references therein) and organs [21, 26] without significant increases in steady-state mRNA levels. Type 1 IDI exhibits marked organ specificity in its selenium-dependent regulation. In rats fed selenium-deficient diets, IDI protein and mRNA levels decline in the liver, but both protein and mRNA show marked increases in the thyroid gland. In rats fed selenium-supplemented diets, however, IDI protein and mRNA decline in the thyroid gland [21].

One potential level of selenoprotein regulation by selenium may involve the effect of this element on the levels and distribution of the two major selenocysteine tRNA isoacceptors, which are initially aminoacylated with serine that is then converted to selenocysteine [15]. These tRNAs, therefore, are designated $tRNA^{[Ser]Sec}$, which distinguishes them from serine-specific tRNAs, designated $tRNA^{Ser}$. The two selenocysteine isoacceptors differ by the 2′-*O*-methylation of the nucleotide in the wobble position of the anticodon [27, 28]. Deficient versus adequate dietary selenium has been shown to affect both the total $tRNA^{[Ser]Sec}$ amount and the relative distribution between the methylated (mcm^5Um) and the unmethylated (mcm^5U) isoacceptors in an organ-specific manner [27, 29]. Similar results have been reported for a variety of cells in culture [30].

To examine the relationship between selenium supplementation, selenoprotein expression, and the relative distribution of the two $tRNA^{[Ser]Sec}$ isoacceptors, three human malignant glioma lines were chosen for study due to their variation in baseline GSHPx-1 activity as well as their varied increases in GSHPx-1 activity with selenium supplementation. These lines were compared with regard to the selenium-dependent expression of both GSHPx-1, and the newly described 15-kDa selenoprotein, as well as the potential protection provided by selenium against oxidative stress. In addition, associated alterations in the $tRNA^{[Ser]Sec}$ populations with and without selenium supplementation were determined. Collectively, these data provide insight into the heterogeneous response of related cells to added selenium and represent the first time that selenium-induced changes in selenoprotein levels, steady-state levels of mRNAs, and selenocysteine tRNAs have been examined in multiple cell lines of the same histology.

MATERIALS AND METHODS

Cells and Culture Conditions

Three human malignant glioma cell lines, D-54 MG (D54), U-251 MG (U251), and U87 MG (U87), were obtained from the American Type Culture Collection. D54 and U251 were maintained in Dulbecco's modified Eagle's medium/Ham's F12 nutrient mixture (Gibco), and U87 was maintained in minimal essential medium with Earle's salts (Gibco), 1% non-essential amino acids (Gibco), and 1% sodium pyruvate (Gibco). Both types of media were supplemented with 10% fetal bovine serum (Intergen Co.) and 1% penicillin/streptomycin (Gibco). All cell lines were maintained at 37° in a humidified 5% CO_2 incubator. Sodium selenite (Sigma Chemical Co.) was prepared in water as a 3 μM stock solution and sterilized by filtration. To obtain supplemented conditions, sodium selenite was added to culture media to achieve a final concentration of 30 nM for at least 3 days prior to experiments. This concentration of sodium selenite has been shown previously to result in maximal GSHPx-1 induction after 3 days of incubation in other cell lines (Ref. 31 and unpublished data).

Northern Blot Analysis

Total RNA was isolated using the RNeasy Total RNA Kit (Qiagen) according to the manufacturer's protocol, electrophoresed in 1% (w/v) agarose/formaldehyde gels, and transferred to Gene Screen Plus hybridization membranes as described by the vendor (DuPont). ^{32}P-Labeled probes were generated for hybridization using random oligolabeling [32]. For the GSHPx-1 probe, the HindIII/EcoRI fragment containing the entire cDNA was isolated and labeled [33]. Filters were hybridized overnight at 65° and then washed twice in 0.5x SSC (1 × SSC is 15 mM sodium citrate, 150 mM sodium chloride, pH 7.0) and 1% SDS (w/v) at 65° for 2 hr. Filters were dried, and autoradiographs were obtained by exposure to x-ray film (Dupont) with an intensifying screen at −65°. Bands were quantified using a GS-710 densitometer (Bio-Rad) and normalized to the hybridization signal obtained for the constitutively expressed 7S rRNA [34].

Determination of Glutathione Peroxidase Activity

GSHPx-1 activity was measured using the standard coupled spectrophotometric method as previously described [35]. Briefly, cells were scraped, washed with ice-cold PBS, and resuspended in ice-cold sodium phosphate buffer (0.1 M, pH 7.0). Cells were lysed by sonication, and debris was removed by centrifugation in a microfuge for 3 min. The assay contained 0.25 mM NADPH, 5 mM glutathione, 5 mM EDTA, 1 U glutathione reductase, 5 mM sodium azide, 5–100 μL lysate, and 65 μM hydrogen peroxide in a 1-mL reaction volume. The rate of oxidation of NADPH, corresponding to the change in absorbance at 339 nm, was

measured in a Beckman DU 640B spectrophotometer at 30-sec intervals for 5 min, and the results were expressed as nanomoles of NADPH oxidized per minute per milligram protein. Samples were analyzed in triplicate, and statistical evaluation of the consequences of added selenium on GSHPx-1 activity, as well as the cell survival studies described below, was determined by a two-tailed unpaired *t*-test using InStat biostatistics software.

Cell Survival Analysis

Each of three human glioma cell lines were cultured in microtiter plates and maintained sub-confluent with or without the addition of 30 nM sodium selenite for 3 days. After incubation, the indicated amount of H_2O_2 was added to the wells, and incubation was continued for another 24 hr. Survival was assessed by the MTT assay (Sigma) using standard methods described by the vendor.

Western Blot Analysis

Western blot analyses of the 15-kDa selenoprotein were performed as described previously using the ECL detection system (Amersham) and rabbit polyclonal antibodies raised against the C-terminal peptide of the 15-kDa selenoprotein [20]. An isolated recombinant human 15-kDa selenoprotein (U93C mutant) that was overexpressed in Escherichia coli (Korotkov KV and Gladyshev VN, unpublished data) served as a control.

Isolation and Chromatography of tRNA$^{[Ser]Sec}$

Total tRNA was isolated from approximately 1 g of each cell line, deacylated, aminoacylated with [^{3}H]serine, and chromatographed on a RPC-5 column [36] as described previously [37]. The total [^{3}H]seryl-tRNA population was chromatographed twice on the RPC-5 column, first in the presence of Mg^{2+} and then in the absence of Mg^{2+}, as described [29]. [^{3}H]Seryl-tRNA^{Ser} is less hydrophobic than [^{3}H]seryl-tRNA$^{[Ser]Sec}$ in the presence of Mg^{2+} and thus elutes earlier from the RPC-5 column, and is more hydrophobic in the absence of Mg^{2+} and thus elutes later from the column. These two chromatographic steps ensure that seryl-tRNA$^{[Ser]Sec}$ is resolved completely from seryl-tRNA^{Ser} for determining the levels and distributions of the two selenocysteine isoacceptors [27, 29, 37].

RESULTS

Effect of Selenium on GSHPx-1 Activity

As an initial characterization of the human glioma cell lines, GSHPx-1 activity was determined in cell extracts from cells grown in standard media and compared with that obtained following supplementation of media with 30 nM sodium selenite. Since the glutathione peroxidase assay is conducted under conditions of limiting protein extract, it generally is considered to be a quantitative method for assessing GSHPx-1 protein levels. Because of the limited expression pattern and substrate specificity of the other glutathione peroxidases, the resultant activity presented in Fig. 1 is almost exclusively a representation of GSHPx-1 protein levels. As shown in the figure, the GSHPx-1 activity for D54, U251, and U87 was determined to be 10.7 ± 0.7, 7.6 ± 0.7, and 4.3 ± 0.7 nmol NADPH oxidized/min/mg protein, respectively. With the addition of 30 nM sodium selenite for 3 days to the culture media, GSHPx-1 activity of D54, U251, and U87 increased to 19.7 ± 1.4, 15.6 ± 2.1, and 6.7 ± 0.5 nmol NADPH oxidized/min/mg protein, respectively (Fig. 1). Thus, an increase in GSHPx-1 activity was seen, with a 1.8-fold induction in D54 cells ($P < 0.05$), and a 2.1-fold induction in U251 cells ($P < 0.05$). The increase observed for U87 (4.3 to 6.7), the glioma cell line with the lowest GSHPx-1 activity, was only marginally significant ($P = 0.05$). Due to their heterogeneous baseline levels of GSHPx-1 and their responses to selenium supplementation, these three glioma cell lines were examined further as model systems to study cellular responses to selenium.

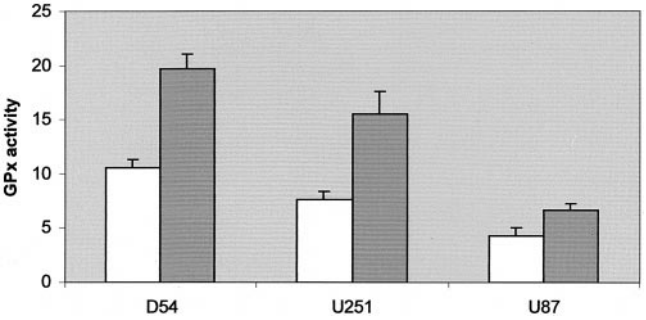

FIG. 1. GSHPx-1 activity of three human glioma cell lines under standard culture conditions (open bars) or following incubation in 30 nM sodium selenite (shaded bars) for 3 days. Data are presented as nanomoles NADPH oxidized per minute per milligram protein and represent the means of triplicate results. Error bars represent the SEM.

Effect of Selenium on the Cellular Resistance to Oxidative Stress

To assess whether selenium could protect the glioma cell lines from the toxic consequences of oxidative stress, survival was determined following exposure to H_2O_2. The cells were exposed to various concentrations of H_2O_2 following 3 days of incubation in either selenium-supplemented or unsupplemented media. Survival was determined by MTT assay and shown graphically in Fig. 2. The protective effect of selenium against H_2O_2-mediated toxicity varied among the cells examined, with the most pronounced effect in U251 and D54 cells ($P < 0.05$). No statistically significant effect of selenium on the protection of U87 cells was observed ($P > 0.5$).

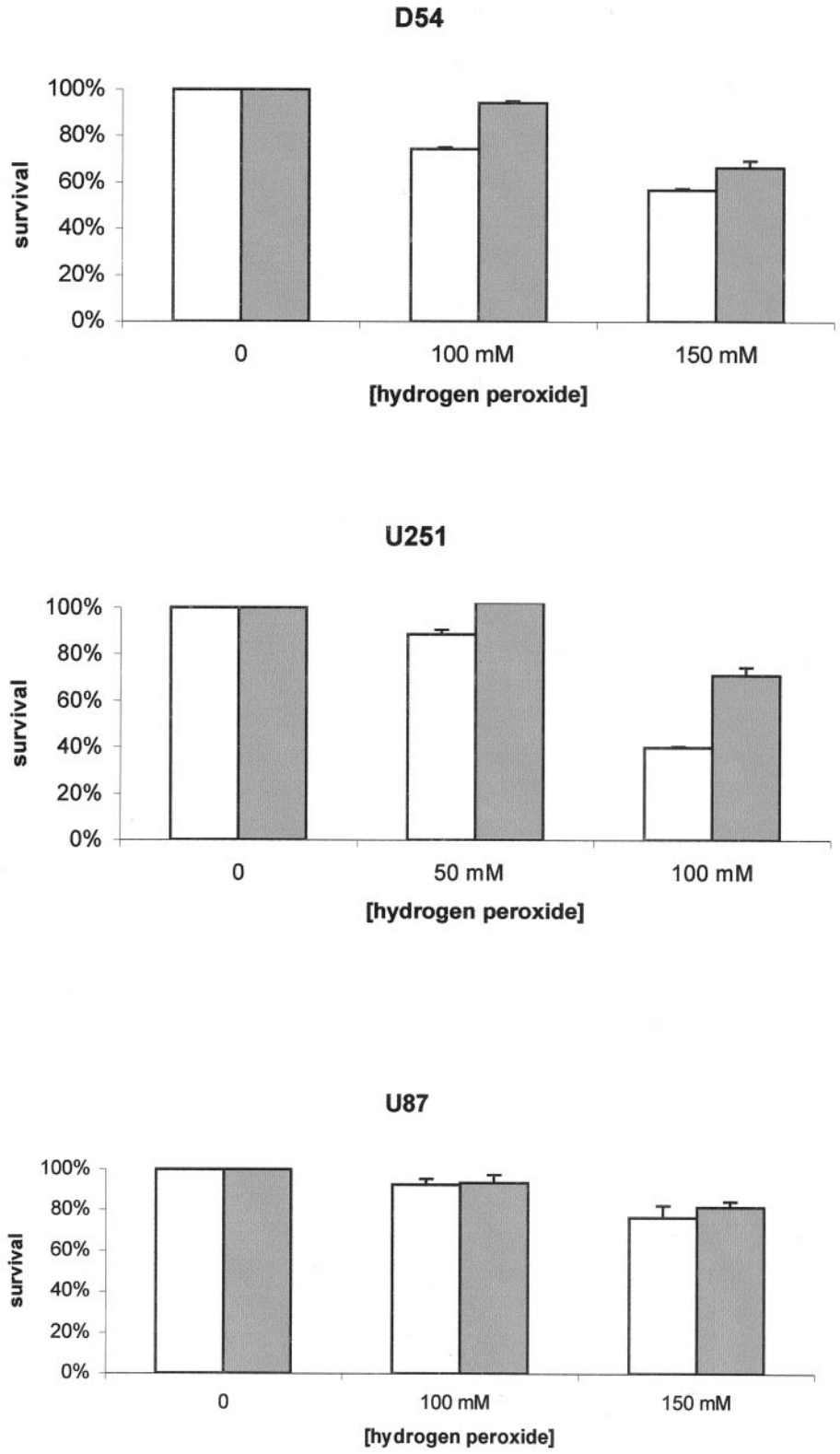

FIG. 2. Effect of selenium supplementation on the survival of human glioma cells following exposure to H_2O_2. The indicated cells (1–2 × 10^4 cells/well) were incubated in microtiter wells either in standard culture media (open bars) or in media supplemented with 30 nM sodium selenite (shaded bars) for 3 days. After the incubation period, the indicated concentration of H_2O_2 was achieved, and the cells were incubated for an additional 24 hr. Cell survival was determined by MTT assay, and the data are presented relative to the growth of the same cells grown in the absence of H_2O_2. Mean levels of relative survival are presented from three experiments, and the error bars indicate the SEM. The differences between the survival of D54 and U251 cells as a function of selenium supplementation were significant at $P < 0.05$. The difference in survival of the U87 cells grown either in standard media or with added selenium following exposure to H_2O_2 was not statistically significant ($P > 0.5$).

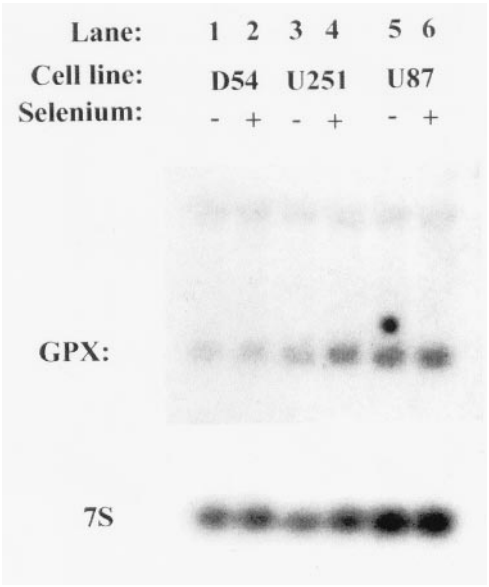

FIG. 3. Northern blot analysis of total RNA from human glioma cell lines probed with ^{32}P-labeled GSHPx-1 cDNA. Cells were maintained in normal media or media supplemented with 30 nM sodium selenite. The intensity of the bands was quantified by densitometry, and the 7S density was used to normalize for differences in loading. The intensities of the GPX bands normalized to the respective 7S intensity were 7.71, 7.04, 7.44, 5.41, 3.31, and 2.87 for lanes 1 through 6, respectively.

Effect of Selenium on GSHPx-1 mRNA Levels

To determine if the selenium-dependent increase in GSHPx-1 activity seen in the glioma cell lines was associated with changes in steady-state mRNA levels, northern blot analysis was performed on RNA obtained from cells incubated with and without the addition of 30 nM selenite (see Fig. 3). The filters were rehybridized to a 7S RNA probe to correct for differences in loading, and densitometry of both GSHPx-1 and 7S signals was used to quantitate and normalize the relative amounts of the GSPX-1 message. The normalized densities of the bands are presented in the legend to Fig. 3 and indicate that the observed increment in GSHPx-1 activity obtained by selenium supplementation was not associated with a comparable increase in the GSHPx-1 transcript.

Selenium Effects on the tRNA$^{[Ser]Sec}$ Population

The data presented above indicated a post-transcriptional stimulation of the GSHPx-1 activity of human glioma cells. One possible mechanism of selenium-induced translational induction would be by altering levels of tRNA$^{[Ser]Sec}$. The tRNA$^{[Ser]Sec}$ levels, as well as a shift in distribution towards the methylated isoacceptor, have been shown to be influenced by selenium in both cultured cells and whole animals [27, 29]. Furthermore, recent data have been presented indicating that increasing the levels of the unmethylated form of tRNA$^{[Ser]Sec}$ in CHO cells does not alter selenoprotein levels, and it has been suggested that the methylated isoacceptor is more relevant in that regard [38]. To evaluate the relationship between selenium supplementation, resultant changes in GSHPx-1 levels, and the relative amounts of the two tRNA$^{[Ser]Sec}$ isoacceptors, we determined both the total tRNA$^{[Ser]Sec}$ content and the changes in the distribution between the two isoacceptors before and after exposure to selenium supplementation. Total tRNA was isolated from glioma cells, either untreated or exposed to 30 nM sodium selenite for 3 days, using conditions identical to those used for GSHPx-1 induction. Total tRNA was aminoacylated with [^{3}H]serine, which labels both serine and selenocysteine tRNAs, and then aminoacyl-tRNA was chromatographed twice by RPC-5 chromatography to separate the tRNA$^{[Ser]Sec}$ from the serine tRNAs to permit quantification of the tRNA$^{[Ser]Sec}$ population. The resulting chromatograms are presented in Fig. 4, and the tRNA$^{[Ser]Sec}$ levels, expressed as a percent of the total serine tRNA in the cells in either normal or selenium-supplemented conditions, are presented in Table 1. The total percent of tRNA$^{[Ser]Sec}$ relative to the tRNA^{Ser} (which is unaffected by selenium supplementation) obtained from glioma cells grown under standard conditions was 1.42, 2.37, and 2.40 for D54, U251, and U87, respectively. Marginal changes were seen with selenium supplementation, with total percent tRNA$^{[Ser]Sec}$ decreasing in D54 to 1.20, but increasing in U251 and U87 to 2.71 and 2.54, respectively. The observed shift in the relative amounts of the isoacceptors towards the methylated, later-eluting species also occurred with selenium supplementation in a cell line-dependent manner. When comparing the levels of tRNA$^{[Ser]Sec}$ in unsupplemented conditions with those in selenium-supplemented conditions, the methylated form increased from 35.3 to 47.2% in D54, and from 38.1 to 47.3% in U251. In U87, however, the methylated form of tRNA$^{[Ser]Sec}$ remained nearly constant after selenium supplementation (49.0 vs 47.6%). Therefore, selenium supplementation resulted in increased tRNA$^{[Ser]Sec}$ levels in two of three lines examined, and also stimulated the shift to the methylated isoacceptor in two of three lines.

15-kDa Selenoprotein

To evaluate whether the increments in GSHPx-1 activity observed with added selenium were a general property of selenoproteins, the mRNA and protein levels of the 15-kDa selenoprotein were determined under similar culture conditions as described above. As in the case of the data obtained with GSHPx-1, the mRNA for the 15-kDa selenoprotein was easily detectable by northern blot analysis (Fig. 5) and, as seen in the figure, was unaffected by selenium supplementation. However, western blot analysis of the 15-kDa selenoprotein using anti-15-kDa selenoprotein specific antisera indicated undetectable protein levels with or without the addition of 30 nM sodium selenite. In contrast, less than 10 ng of bacterially expressed protein, included as a positive control for sensitivity, was readily detectable, as was the 15-kDa protein signal obtained from

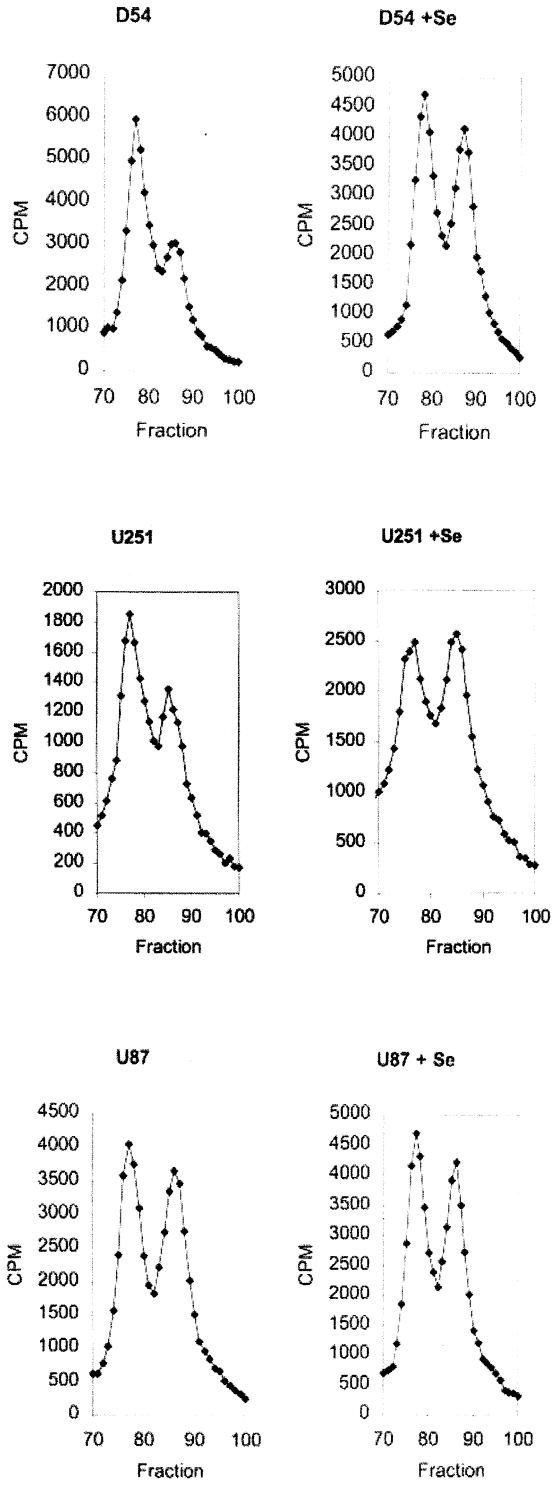

FIG. 4. Relative amounts of the two tRNA$^{[Ser]Sec}$ isoacceptors in three human glioma cell lines determined by RPC-5 chromatography. Cells were maintained in normal conditions or were supplemented with 30 nM sodium selenite (+Se) for 3 days. Chromatography was performed after aminoacylation with [^{3}H]serine, and counts per minute (cpm) were quantified on a scintillation counter. The later eluting peak represents the 2′-O-methylation of the nucleotide in the wobble position of the anticodon.

TABLE 1. Distribution between unmethylated (mcm^{5}U) and methylated (mcm^{5}Um) selenocysteine tRNA isoacceptors among the three glioma cell lines under standard conditions or after supplementation with 30 nM sodium selenite (+Se)

Cell line	% tRNA$^{[Ser]Sec}$	% mcm^{5}U	% mcm^{5}Um
D54	1.42	64.7	35.3
D54 + Se	1.20	52.7	47.2
U251	2.37	61.9	38.1
U251 + Se	2.71	52.7	47.3
U87	2.40	51.0	49.0
U87 + Se	2.54	52.4	47.6

Total selenocysteine tRNA levels are expressed as percent of the total amount of serine tRNA (% tRNA$^{[Ser]Sec}$). The data in this table were obtained from the experiment presented in Fig. 4.

equal amounts of protein obtained from several human prostate cell lines (data not shown).

DISCUSSION

These data represent the first time that selenium-induced changes in selenoprotein levels, steady-state levels of selenoprotein mRNAs, and tRNA$^{[Ser]Sec}$ were examined in multiple cell lines derived from human tumors of the same histology. Despite selecting three cell lines derived from human gliomas for this study, similarities as well as significant differences were observed regarding both baseline and selenium-inducible endpoints. Each of the lines differed with regard to baseline GSHPx-1 activity and GSHPx-1 mRNA levels. Examination of GSHPx-1 transcript levels by northern analysis indicated that there was approximately half as much GSHPx-1 mRNA and GSHPx-1 activity in D54 cells relative to both U87 and U251 cells. In addition, D54 cells also had approximately half of the total tRNA$^{[Ser]Sec}$ of the other two glioma cell lines. However, the data provided by selenium supplementation studies indicated that neither GSHPx-1 mRNA nor tRNA$^{[Ser]Sec}$ levels are limiting for GSHPx-1 activity levels. Adding low levels of selenium to the culture media of these cells resulted in a significant increase in GSHPx-1 activity, 1.8-fold for D54, 2.1-fold for U251, and 1.6-fold for U87, without a corresponding increment in either GSHPx-1 mRNA or tRNA$^{[Ser]Sec}$. Nominal increases in both RNAs were observed following selenium supplementation, but these increases were insufficient to account for the post-transcriptional stimulation observed for this protein. None of the observed differences among the cell lines are likely to be due to differences in selenium availability, as the overwhelming majority of selenium is provided to the media from the added serum, and the media of all three lines contained the same amount and source of serum. These results point to an additional component of the selenoprotein translation machinery that is responsive to low-level selenium supplementation. One possible candidate for such a regulatory factor would be the mammalian homologue of

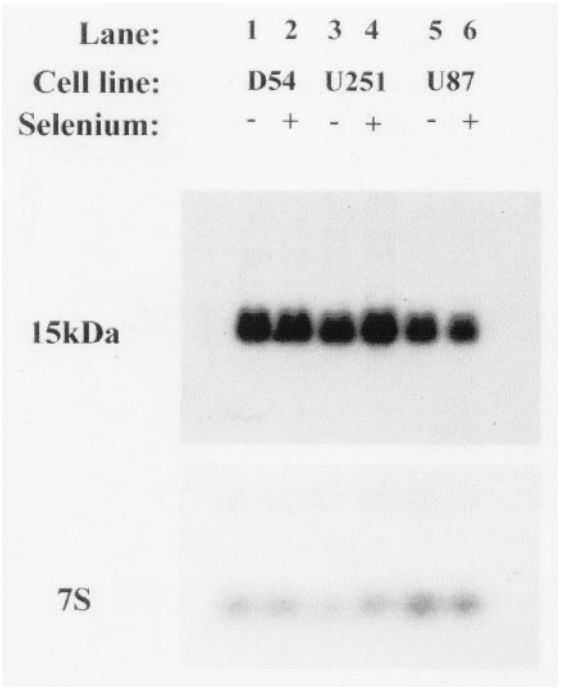

FIG. 5. Northern blot analysis of total RNA from human glioma cell lines probed with ^{32}P-labeled 15-kDa selenoprotein cDNA. Cells were maintained in normal media or media supplemented with 30 nM sodium selenite. The intensity of the bands was quantified by densitometry, and the respective 7S density was used to normalize for differences in loading. The intensities of the 15-kDa selenoprotein RNA bands normalized to respective 7S intensity were 32.9, 28.2, 36.5, 30.3, 10.8, and 9.68 for lanes 1 through 6, respectively.

the selenocysteine-specific translational elongation factor, the product of the *selB* gene of *E. coli* [39].

Examination of the tRNA$^{[Ser]Sec}$ levels and the distributions of both major isoacceptors revealed that tRNA$^{[Ser]Sec}$ from both D54 and U251 underwent a characteristic selenium-induced shift towards the later-eluting species, containing mcm^{5}Um at the wobble position of the anticodon. This is the first time cultured cells of neural origin have been examined for this effect. Previously, the tRNA$^{[Ser]Sec}$ populations of the brains of rats fed either a selenium-deficient or -proficient diet were shown to contain predominately the mcm^{5}Um-containing isoacceptor, and this distribution was insensitive to dietary selenium status [27]. The difference between the response of cultured glioma cells and whole brains may be accounted for by the retention of selenium in the brains of rats fed a selenium-deficient diet [40, 41] or the fact that glioma cells represent only a portion of the cell types in the whole brain. It is interesting to note that regardless of the starting distribution of tRNA$^{[Ser]Sec}$ isoacceptors, the resulting distribution among all three cell lines was approximately 53 and 47% for the mcm^{5}U- and mcm^{5}Um-containing isoacceptors, respectively. This suggests that the upper limit of the levels of the later tRNA$^{[Ser]Sec}$ species may be highly regulated and perhaps is regulated in a cell-type-specific way. In this regard, it is interesting to note that when tRNA$^{[Ser]Sec}$ levels were increased by as much as 10-fold following transfection of the tRNA$^{[Ser]Sec}$ gene into CHO cells, the entire increment was restricted to the unmethylated isoacceptor [38]. The functional differences between these isoacceptors remain to be determined.

The addition of low levels of selenium to the culture media of the three human glioma cell lines increased H_2O_2 resistance for the D54 and U251 lines, but not the U87 cells. U87 underwent both the smallest absolute and relative increase in GSHPx-1 activity following selenium supplementation, arguing that the antioxidant protection offered by selenium was mediated at least in part by its influence on GSHPx-1 levels. Whereas no correlation was observed between total tRNA$^{[Ser]Sec}$ levels and GSHPx-1 activity (compare Fig. 1 and Table 1), there appeared to be a relationship between the effect of selenium on inducing a shift from the unmethylated isoacceptor to the methylated species and the absolute magnitude of the observed stimulation of GSHPx-1 and subsequent protection against H_2O_2. These data are collectively supportive of our previous speculation that GSHPx-1 activity is mediated principally by the methylated isoacceptor [38] and further suggest that the regulation of the distribution of tRNA$^{[Ser]Sec}$ isoacceptors is important in the cellular response to oxidative stress.

Finally, it is noteworthy to contrast the transcriptional and translational response of GSHPx-1 and the 15-kDa selenoprotein to selenium. Whereas a marginal transcriptional stimulation and significant increase in GSHPx-1 enzyme levels were observed, no detectable 15-kDa protein was evident in these cells despite significant levels of its transcript (Fig. 5), which was unaffected by the added selenium. These biological responses to selenium supplementation were observed in all three of the glioma cell lines analyzed. Since both GSHPx-1 and the 15-kDa selenoprotein genes are exposed to the same putative regulatory factors involved in these selenium-dependent effects in the intracellular environment, it is likely that the elements that govern the steady-state selenoprotein levels reside, at least in part, within the genes themselves. Selenium-mediated stimulation of GSHPx-1 translation has also been observed using a cDNA expression construct in which the bulk of the 5′-untranslated region has been replaced by vector sequences (Ref. 33 and unpublished observations). In addition, the 3′-UTR of GSHPx-1 has been implicated previously in both the regulation of mRNA stability and the translational stimulation of GSHPx-1 by selenium [42, 43]. These data collectively argue that the 3′-UTR of mammalian selenoproteins is the source for a multitude of regulatory signals that ultimately determine selenoprotein levels.

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