Selenium induces changes in the selenocysteine tRNA\[^{[\text{Ser}]}\text{Sec}\] population in mammalian cells

Dolph Hatfield*, Byeong J. Lee, Lori Hampton and Alan M. Diamond

Laboratory of Experimental Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892 and Department of Radiation and Cellular Oncology, University of Chicago, Chicago, IL 60639, USA

Received August 16, 1990; Revised and Accepted January 22, 1991

ABSTRACT

Two isoacceptors of selenocysteine tRNA\[^{[\text{Ser}]}\text{Sec}\] are present in higher vertebrates which are responsible for donating selenocysteine to protein. One such selenocysteine containing protein, glutathione peroxidase, requires selenium for its translation and transcription. Since tRNA\[^{[\text{Ser}]}\text{Sec}\] is a critical component of the glutathione peroxidase translational machinery, the levels and distributions of its isoacceptors were examined from both human and rat cells grown in chemically defined media with and without selenium. Not only did the level of the selenocysteine tRNA\[^{[\text{Ser}]}\text{Sec}\] population increase approximately 20% in cells grown in the presence of selenium, but the distributions of the two isoacceptors also changed relative to each other.

INTRODUCTION

A selenocysteine tRNA was identified recently in bacterial (1) and mammalian cells (2). This tRNA has a dual function in that it serves as a carrier molecule for the biosynthesis of selenocysteine and as a donor of selenocysteine to protein in response to specific UGA codons (1,2). Since this tRNA is initially aminoacylated with serine and the serine moiety is subsequently converted to selenocysteine, it has been designated selenocysteine tRNA\[^{[\text{Ser}]}\text{Sec}\] (3). Two isoacceptors of tRNA\[^{[\text{Ser}]}\text{Sec}\] have been identified in higher vertebrates (4) and both species have been sequenced only from bovine liver (5,6). These tRNAs differ from each other by several pyrimidine transitions which occur in the 5' half of the molecules. Recently, the bovine gene for these tRNAs has been isolated and sequence analysis has revealed that it is the only locus from which both tRNAs could be transcribed (7). It has therefore been suggested that editing of a primary tRNA transcript must be responsible for the generation of the two tRNA species. In addition, other mammals, chickens and Xenopus have two minor tRNAs which recognize UGA in a ribosome binding assay and these isoacceptors have similar chromatographic properties as the bovine tRNAs (4). Also, analogous to the bovine system, human DNA has one functional selenocysteine tRNA gene which has a primary sequence identical to the corresponding rabbit (4) and bovine genes (7). This data is consistent with tRNA editing which is responsible for the generation of two distinct selenocysteine isoacceptors in vertebrate cells.

The gene for mammalian glutathione peroxidase (GPx) contains a TGA codon which corresponds to a selenocysteine moiety in the corresponding protein (8,9). Expression of GPx has been shown to be dependent on the presence of selenium in some mammalian cells (10-12). We therefore evaluated whether selenium would influence the relative abundance of the two selenocysteine tRNAs observed in mammalian cells. Human myeloid leukemia (HL-60) cells and rat mammary tumor (RMT) cells were selected for study as both can be grown in defined media in the presence or absence of selenium. The results show that the amounts of these two tRNAs are in fact influenced by the presence of selenium in the culture media.

MATERIALS AND METHODS

Growth of cells

HL-60 cells, obtained from Pharmacia Diagnostics, Inc., Rockville, MD, USA, were grown on chemically defined media (RPMI 1640) supplemented with insulin (5 μg/ml) and transferrin (5 μg/ml) (12) for two weeks in the absence of selenium, the culture split and half of the cells grown in the presence (10 ng/ml) and half in the absence of sodium selenite for one week before harvesting. HL-60 cells were also grown in the same media except that 10% fetal calf serum was added and the cells grown in the presence (5 μg/ml) and half in the absence of sodium selenite for one week before harvesting. RMT-60 cells were also grown in the same media except that 10% fetal calf serum was added and the cells grown in the presence (5 μg/ml) or absence of sodium selenite. Cells were collected and stored at −80°C until ready for use. RMT cells (TMT-081-MS), obtained from Dr. U. Kim, Roswell Park Memorial Institute, Buffalo, NY, USA, were grown on chemically defined media (Ham's F12/DMEM with 10 mM HEPES, pH 7.4) supplemented with insulin (5 μg/ml), transferrin (5 μg/ml) and triiodothyronine (6.5 ng/ml) for one week in the absence of selenium, the culture split and half the cells grown in the presence (8.65 ng/ml) and half in the absence of sodium selenite for 18 days before harvesting. The cells were collected and stored at −80°C until ready for use.

* To whom correspondence should be addressed
Isolation, aminocacylation and chromatography of tRNA

Total tRNA was isolated from approximately 1 g of frozen cells, aminocacylated with \(^{32}P\)serine (Amersham Corp., specific activity, 30 Ci/mmol) or with \(^{14}C\)serine (Amersham Corp., specific activity, 141 mCi/mmol) and prepared for chromatography as described (13,14). \(^{75}Se\)Selenocysteyl-tRNA\(^{[\text{Ser}]Sec}\) was prepared by administering \(^{75}Se\)O\(_3\) (Spec. Act. 175 Ci/mmol of H\(_2\)SeO\(_3\); obtained from Dr. Kurt Zinn of the University of Missouri Research Reactor) to HL-60 cells in culture, the resulting \(^{75}Se\)selenocysteyl-tRNA\(^{[\text{Ser}]Sec}\) isolated and purified exactly as given (2). Total acceptance of serine in bulk tRNA (i.e., pmol/A\(_{260}\) unit) was determined with \(^{14}C\)serine. Labeled tRNAs were chromatographed on a RPC-5 column (15) initially in a linear gradient at pH 4.5 containing 10 mM NaAc, 10 mM Mg(Ac)_2, 1 mM EDTA and 0.525 M (initial gradient buffer) or 0.675 M NaCl (terminal gradient buffer) (13) and in the same buffer except Mg\(^{++}\) was omitted and the initial eluting buffer contained 0.6 M NaCl and the terminal buffer 0.8 M NaCl.

Northern hybridization assays

One half of the RPC-5 column fractions (1 ml of fractions #54 to 77 which contained a total of 2.04 and 1.18 pmol of \(^{32}P\)-labeled probe (1.75 x 10\(^6\) cpm) encoding the human selenocysteine tRNA gene (16) and an autoradiogram prepared (16 hr exposure). RNA was prepared from HL-60 cells and RMT cells grown in the presence and in the absence of selenium, electrophoresed on an agarose gel and transferred to a nitrocellulose filter as described (17). The filter was hybridized with a \(^{32}P\)-labeled probe (1.5 x 10\(^7\) cpm) encoding the human GPx gene (9) and an autoradiogram prepared (40 hr exposure).

RESULTS

Selenocysteine is biosynthesized on tRNA by aminocacylation of tRNA\(^{[\text{Ser}]Sec}\) with serine, conversion of the serine moiety to an intermediate which in turn is converted to selenocysteine (1,2,18). In mammalian cells, the observation that these tRNAs form phosphoseryl-tRNA (2,6,19) suggests that phosphoserine may be the intermediate. In E. coli, the products of the selD and selA genes function in the biosynthesis of selenocysteine from

---

### Table 1. Distributions of selenocysteine tRNA\(^{[\text{Ser}]Sec}\) in HL-60 and RMT cells.

<table>
<thead>
<tr>
<th>Expt.</th>
<th># Cells</th>
<th>Growth Condition(^1)</th>
<th>% of total seryl-tRNA population(^2)</th>
<th>% Distribution(^2) tRNA (\text{I(NCA)})</th>
<th>% Distribution(^2) tRNA (\text{II(CmCA)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HL-60</td>
<td>+Se, CDM</td>
<td>9.6</td>
<td>3.7</td>
<td>5.9</td>
</tr>
<tr>
<td>2</td>
<td>HL-60</td>
<td>−Se, CDM</td>
<td>7.5</td>
<td>4.6</td>
<td>2.9</td>
</tr>
<tr>
<td>3</td>
<td>RMT</td>
<td>+Se, CDM</td>
<td>9.4</td>
<td>5.2</td>
<td>4.2</td>
</tr>
<tr>
<td>3</td>
<td>RMT</td>
<td>−Se, CDM</td>
<td>7.4</td>
<td>5.7</td>
<td>1.7</td>
</tr>
<tr>
<td>4</td>
<td>HL-60</td>
<td>+(^{75}Se), FCS</td>
<td>1.7</td>
<td>0.2</td>
<td>1.5</td>
</tr>
<tr>
<td>4</td>
<td>RMT</td>
<td>+(^{75}Se), CDM</td>
<td>1.4</td>
<td>0.5</td>
<td>0.9</td>
</tr>
</tbody>
</table>

\(^1\)HL-60 cells were grown in chemically defined media (CDM) with or without selenium (Expt. 1) or in the same media supplemented with 10% fetal calf serum (FCS) with or without selenium (Expt. 2) and RMT cells were grown in chemically defined media with and without selenium (Expt. 3) as given in Materials and Methods.

\(^2\)The amount of seryl-tRNA\(^{[\text{Ser}]Sec}\) within the total seryl-tRNA population from HL-60 cells (Expt. 1 and 2) was determined by dividing the number of counts present in the seryl-tRNA\(^{[\text{Ser}]Sec}\) isoacceptors by the total number of counts eluted from the first chromatographic column in the presence of Mg\(^{++}\) (see figure 2). The distributions of the first eluting seryl-tRNA\(^{[\text{Ser}]Sec}\) isoacceptor (anticodon NCA) and second eluting seryl-tRNA\(^{[\text{Ser}]Sec}\) isoacceptor (anticodon CmCA) were determined by dividing the number of counts present in one of the isoacceptors by the total number of counts. Since greater than 90% of the counts observed in the two late eluting peaks from HL-60 cells were seryl-tRNA\(^{[\text{Ser}]Sec}\) isoacceptors, their amounts within the seryl-tRNA population and their distributions were determined from the first chromatographic column. However, only about 20% of the counts in the late eluting peaks from rat mammary tumor cells were the seryl-tRNA\(^{[\text{Ser}]Sec}\) isoacceptors and their amount within the total seryl-tRNA population and their distributions were therefore determined from the second column run which was carried out in the absence Mg\(^{++}\). Transfer RNA from HL-60 cells grown in the presence and absence of selenium (Expt. 1) contained 117.4 and 121.8 pmol/A\(_{260}\) unit, respectively, while that from RMT cells grown in the presence and absence of selenium (Expt. 3) contained 98.9 and 96.1 pmol/A\(_{260}\) unit, respectively.

\(^3\)Approximately 1 g (wet weight) of HL-60 cells were labeled with 330 μCi of \(^{75}Se\)\(^2\), and the resulting \(^{75}Se\)Selenocysteyl-tRNA\(^{[\text{Ser}]Sec}\) isoacceptors isolated and resolved by RPC-5 chromatography (see Materials and Methods and ref. 2). 7.4 x 10\(^6\) total cpm of \(^{75}Se\)Selenocysteyl-tRNA\(^{[\text{Ser}]Sec}\) were isolated and the distributions of both isoacceptors determined as given above. NA denotes not assayed.
serine where SELA forms a stable complex with seryl-tRNA\[^{[\text{Ser|Sec}]}\] and catalyses it to an unidentified intermediate (which is not phosphoserine) and then SELD functions in donating reduced selenium to form selenocysteyl-tRNA \[^{[\text{Ser|Sec}]}\]. In either case, the relative amounts of the selenocysteine isoacceptors within the seryl-tRNA population can be measured as labeled seryl-tRNA\[^{[\text{Ser|Sec}]}\] which is the means by which the selenocysteine tRNA population was analyzed in the present study. As experiments described in this manuscript have employed human cells, it was appropriate to demonstrate that HL-60 cells also form selenocysteyl-tRNA\[^{[\text{Ser|Sec}]}\]. As shown in table 1 (see exp. 4), both human isoacceptors can indeed form selenocysteyl-tRNA following a 4 hr labeling by the same means as previously used with RMT cells (2).

Cochromatography of labeled seryl-tRNA from HL-60 cells grown in the presence or absence of selenium is shown in Figure 1. Although the relative abundances of the major serine isoacceptors remain superimposable under the two conditions, the amount of the last eluting isoacceptor is increased relative to the major seryl-tRNAs in cells grown in the presence of selenium. In the bovine system, the earlier eluting tRNA has the anticodon NCA while the latter has the anticodon CmCA (5,6). As other mammals also contain these two UGA-responsive selenocysteine tRNAs with similar chromatographic properties (4), it may be appropriate to designate them as tRNA\[^{\text{NCA}}\] and tRNA\[^{\text{CmCA}}\] in each mammalian source. However, since these isoacceptors have not been sequenced in HL-60 and RMT cells, we refer to them herein as tRNA\[^{\text{NCA}}\] and tRNA\[^{\text{CmCA}}\]. The relative amounts of the earlier eluting isoacceptor, seryl-tRNA\[^{\text{NCA}}\] do not appear to be changed in the two tRNA populations. To insure that these results do not reflect an artifact due to the use of different isotopes in the aminoacylation reactions, total tRNA from HL-60 cells grown in the presence or absence of selenium was aminoacylated with \[^{3}\text{H}]\text{serine and chromatographed independently on the RPC-5 column. The resulting elution profiles (Figure 2) were very similar as the corresponding profiles shown in Figure 1. In order to better resolve the selenocysteine tRNAs in the absence of the major seryl-tRNAs, column fractions which included the two selenocysteine tRNAs were pooled and rerun separately on a RPC-5 column in the absence of Mg\(^{2+}\) (see insets in Figure 2). Under these conditions, the major serine isoacceptors become extremely hydrophobic and are retained on the column (2) (data not shown). The relative distributions of the two selenocysteine isoacceptors within the total seryl-tRNA population (see below) fractionated by the latter procedures were similar to that observed in Figure 1.

Similar studies were performed on RMT cells. Labeled seryl-tRNA from RMT cells grown in defined medium with or without...
Figure 3. Chromatography of selenocysteine tRNA^{[Ser]Sec} isoacceptors from RMT cells. [\textsuperscript{3}H]Ser-tRNA from RMT cells grown in the presence or absence of selenium was prepared and chromatographed on a RPC-5 column as given in Materials and Methods (data not shown) and one half (i.e., 1 ml) of the fractions containing the seryl-tRNA^{[Ser]Sec} isoacceptors were pooled and rechromatographed (as given in the legend to figure 2) in the absence of Mg\textsuperscript{++} as shown in A (upper graph, -selenium; and lower graph, +selenium). The remaining half of the column fractions from the first chromatographic run in the presence of Mg\textsuperscript{++} (fractions # 54 to 77) were used for binding to a nitrocellulose filter and the filter was then hybridized with a probe as given in Materials and Methods; the autoradiogram from this study is shown in B.

Selenium was chromatographed independently on a RPC-5 column in the presence of Mg\textsuperscript{++} (data not shown). The column fractions containing the seryl-tRNA^{[Ser]Sec} isoacceptors were rechromatographed in the absence of Mg\textsuperscript{++} (Figure 3A). The profiles show, as in the case with the HL-60 cells, that growth in the presence of selenium results in an increase in the relative amounts of selenocysteine tRNA^{[Ser]Sec} (Figure 3A). The resulting autoradiogram is shown in Figure 3B. The intensities of the positive bands are consistent with the relative distributions of the elution profiles shown in the same figure.

The relative steady state amounts of both selenocysteine isoacceptors within the total seryl-tRNA population were determined as shown in Table 1 (Experiments 1-3). The seryl-tRNA^{[Ser]Sec} population increased approximately 20% in response to selenium in both HL-60 and RMT cells. In addition, the relative amounts of the individual tRNA^{[Ser]Sec} isoacceptors varied under the two sets of growth conditions where tRNA^{[NCA]Sec} decreased and tRNA^{[CmCA]Sec} increased in amounts in cells grown in the presence of selenium as compared to the corresponding levels of isoacceptors from cells grown in the absence of selenium.

We also examined the effect of exogenous selenium on the selenocysteine tRNA^{[Ser]Sec} population in HL-60 cells grown in media containing serum (see experiment 2 in Table 1). HL-60 cells grown with 10% fetal calf serum also showed a slight decrease in tRNA_{[NCA]Sec} and an increase in tRNA_{[CmCA]Sec} in cells grown in the presence of exogenous selenium as compared to the corresponding levels observed in tRNA isolated from cells grown in the absence of exogenous selenium.

Chada \textit{et al.} (12) have shown that the levels of GPx mRNA are the same in HL-60 cells grown in the presence or absence of selenium, but that the gene product is expressed only in cells grown with selenium. As shown in Figure 4, we also find that the levels of GPx mRNA in extracts from HL-60 cells grown in defined media with and without selenium are very similar (see lanes 1 and 2, respectively). However, a higher level of GPx mRNA was observed in extracts from RMT cells grown in the presence than in the absence of selenium (see lanes 3 and 4, respectively).
DISCUSSION

The experiments described in this manuscript were designed to investigate whether the relative levels of the selenocysteine tRNA<sub>[Ser][Sec]</sub> isoacceptors would reflect the presence of selenium in the culture media. This was anticipated as the levels of the selenocysteine containing protein, GPx, have been shown to be controlled translationally (12), as well as transcriptionally (10,11), by selenium levels. Although the cell lines used in this study are derived from two different organisms and cell types, both exhibit a better than 20% increase in the amount of tRNA<sub>[Ser][Sec]</sub> isoacceptors relative to the amount of seryl-tRNAs when cells are shifted to media containing selenium. Similarly, HL-60 cells grown in media containing fetal calf serum also exhibit about a 20% increase in the tRNA<sub>[Ser][Sec]</sub> population when the media is supplemented with selenium. This was surprising as fetal calf serum is known to contain selenium (see 12 and references therein). It may therefore be likely that the selenium present in fetal calf serum is bound to serum proteins and not readily available to the cell. It remains unknown whether the observed increases in the tRNA<sub>[Ser][Sec]</sub> population reflect an increase in transcription rate or tRNA stability.

Differences in the relative amounts of tRNA<sub>[Ser][Sec]</sub> isoacceptors are also observed between experiments 1 and 2 in Table 1. While the reason for these differences are not clear, we note that cells grow considerably slower in chemically defined media than in serum and thus it may not be appropriate to compare directly the levels of the isoacceptors in these two experiments. Importantly, however, as further discussed below, the levels of the total selenocysteine tRNA population increase and shifts in the distribution of isoacceptors occur, in both experiments 1 and 2.

In addition to the increase in the amount of the selenocysteine tRNA<sub>[Ser][Sec]</sub> population, there is also a clear shift in the distribution from the earlier eluting isoacceptor to the latter when cells are shifted to selenium containing media. As summarized in Table 1, there is about a 20% decline in the earlier eluting peak (tRNA<sub>[NCA][Ser][Sec]</sub>) with more than a 100% increase in the latter peak (tRNA<sub>[CmCA][Ser][Sec]</sub>) in HL-60 cells. In RMT cells, tRNA<sub>[NCA][Ser][Sec]</sub> decreases by about 60% and tRNA<sub>[CmCA][Ser][Sec]</sub> increases by about 65%. Other differences between these two cell types are noteworthy. The relative amounts of the tRNA<sub>[Ser][Sec]</sub> population are lower in RMT cells and, as shown in Figure 4, the levels of GPx mRNA are much more responsive to selenium in RMT as compared to HL-60 cells. These differences are likely to represent the differences between hematopoietic (HL-60) and epithe-lial (RMT) cells. Interestingly, the shift in distribution of tRNA<sub>[NCA][Ser][Sec]</sub> to tRNA<sub>[CmCA][Ser][Sec]</sub> in response to selenium that occurs in both cell types accompanies an increase in the expression of GPx in HL-60 cells (12) and an increase in the levels of GPx mRNA (Figure 4) and presumably an increase in GPx in RMT cells. Whether the tRNA<sub>[CmCA][Ser][Sec]</sub> isoacceptor has a preferential role in donating its selenocysteine to the growing polypeptide of GPx remains to be established.

Shifts in the relative amounts of these two tRNA species are particularly intriguing in light of our previous observations that only a single gene encodes both tRNAs (3,4,7). While current efforts are directed towards determining whether the earlier eluting selenocysteine tRNA is a precursor to the later eluting species or whether these two tRNAs are independently generated by editing from a common primary transcript, the data presented herein clearly indicate that this process can be influenced by selenium. Regardless of which pathway operates in a particular cell type, these experiments remain the first example of the relative abundance of two related tRNAs being influenced by a trace element.

REFERENCES