Differential regulation of glutathione peroxidase by selenomethionine and hyperoxia in endothelial cells

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We have studied the effect of selenomethionine (SeMet) and hyperoxia on the expression of glutathione peroxidase (GP) in human umbilical vein endothelial cells. Incubation of HUVEC with 1×10^{-6} M SeMet for 24 h and 48 h caused a 65 % and 86 % increase in GP activity respectively. The same treatment did not result in significant changes in GP gene transcription and mRNA levels. Pactamycin, a specific inhibitor of the initiation step of translation, prevented the rise in GP activity induced by SeMet and caused an increase in GP mRNA in both cells grown in normal and SeMet-supplemented medium. Interestingly, SeMet supplementation stimulated the recruitment of GP mRNA from an untranslatable pool on to polyribosomes, so that the concentration of GP mRNA in polyribosomal translatable pools was 50 % higher in cells grown in SeMet-supplemented medium than in cells grown in normal medium. On the other hand, cells exposed to 95 % O_2 for 3 days in normal medium showed a 60 %, 394 % and 81 % increase in GP gene transcription rate, mRNA levels and activity respectively. Hyperoxia also stabilized GP mRNA. Hyperoxic cells grown in SeMet-supplemented medium did not show any change in GP gene transcription and mRNA levels, but expressed an 81 % and 100 % increase in GP activity and amount of GP mRNA associated with polyribosomes respectively, when compared with hyperoxic cells maintained in normal medium. Thus, GP appeared to be regulated post-transcriptionally, most probably co-translationally, in response to selenium availability, and transcriptionally and post-transcriptionally in response to oxygen.

INTRODUCTION

Glutathione peroxidase (GP) is a soluble selenoprotein present in most tissues. It catalyses the reduction of H_2O_2 and organic hydroperoxides to the corresponding alcohols with a specific requirement for glutathione. The protein is a tetramer containing four identical spherical subunits, each of them having a molecular mass of approximately 19 kDa and containing one selenium atom present as selenocysteine [1]. Mammalian GP genes encode the selenocysteine residue at amino acid 47 by the opal terminator codon TGA (UGA in the mRNA transcript). Following a co-translational model for insertion of selenocysteine into GP, a UGA-recognizing tRNA is charged with serine or phosphoserine, which is then enzymically altered to generate a selenocysteine-charged tRNA, which incorporates selenocysteine directly at the UGA codon.

The relationship between selenium supply and GP enzyme activity has received extensive investigation, and it is now clear that GP activity in tissues and cells depends on the availability of selenium. Selenium deficiency results in a decrease in GP protein detectable by immunoprecipitation [2–5], and in enzyme activity [2,4–7]. On the other hand, tissue GP activity and protein can be increased in individuals with low selenium intake by dietary supplementation with certain selenium-containing compounds [2,4,6]. Similarly, selenium-deficient cells transferred to selenium-replete medium showed an increase in GP activity and immunoreactive protein [2,5]. However, the underlying molecular mechanisms of the regulation of GP activity by selenium remain largely controversial. Thus, upon examining different species and different tissues, investigators have suggested transcriptional [7,8], mRNA stabilization [9,10], translational [5,11,12] and co-translational [5,10,13] mechanisms for the control of selenium over GP activity.

We previously reported that in cultured endothelial cells selenomethionine (SeMet) and hyperoxia augmented GP activity in an additive manner [14]. Since this finding is suggestive of different regulatory events in GP activation by selenium and oxygen, and in view of the rather contradictory reports mentioned above, we sought to analyse in detail the molecular events that lead to the observed increase in GP activity brought about by SeMet and oxygen. In this study we report evidence for a co-translational control mechanism for selenium, and a transcriptional and post-transcriptional regulation by oxygen.

EXPERIMENTAL

Chemicals and reagents

All chemicals were of analytical grade and came from Sigma Chemical Co. (St. Louis, MO, U.S.A.) or Fluka (Buchs, Switzerland). DL-Selenomethionine was obtained from Serva (Heidelberg, Germany). RPMI 1640 medium was from Biological Industries (Beth Haemek, Israel). Fetal-calf serum (FCS) was from Seromed (Berlin, Germany) and endothelial cell growth supplement was from UBI (Lake Placid, NY, U.S.A.). Pactamycin was a gift from Upjohn Company (Kalamazoo, MI, U.S.A.) [α-^{32}P]UTP (800 Ci/mmol) and [^{35}S]methionine were obtained from Amersham (Amersham, Bucks, U.K.), and Biodyne membrane was from Pall (Glen Cove, NY, U.S.A.).

Experimental conditions

Endothelial cells, obtained from umbilical cord veins using 0.1 % collagenase, were grown to confluency on gelatin-coated (0.1 %) plastic culture dishes (60 mm-diam.) in RPMI 1640 medium supplemented with 25 mM Hepes, 50 units/ml penicillin, 50 μg/ml streptomycin, 10 % (v/v) FCS, 15 μg/ml endothelial cell growth supplement, and 90 μg/ml heparin in a humidified 5 % CO_2 atmosphere at 37 °C. All experiments were performed with confluent cells at passages 2 and 3. At confluency, the medium was replaced with fresh complete growth medium with

Abbreviations used: SeMet, selenomethionine; GP, glutathione peroxidase; FCS, fetal-calf serum; SOD, superoxide dismutase.

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or without 1×10^{-6} M SeMet, and the culture was maintained for various length of times as indicated in the Figure legends. The selenium concentration present in FCS was 200 nM, and thus the 20 nM selenium in 10% (v/v) FCS present in cell growth medium was much below the normal range found in serum (1–2 μM). Cells grown in this medium can be considered as selenium-deficient, and cells grown in SeMet-supplemented medium can be considered as selenium-replete. In another series of experiments, cells were plated and grown in normal or SeMet-supplemented medium. At confluency, they were exposed to 21% or 95% O_2 for 3 days in normal or SeMet-supplemented medium.

Enzyme activity and Northern-blot analysis

For these experiments, about $(2–5) \times 10^6$ cells were used. Measurement of GP activity, and Northern-blot analysis for GP mRNA and CuZn-superoxide dismutase (SOD) mRNA were performed as previously described [15] except that we used in this study storage phosphor screens (Molecular Dynamics, CA, U.S.A.) for autoradiography. The density of the hybridization signal was quantified using a PhosphorImager (Molecular Dynamics, CA, U.S.A.). As a loading control, the density of the 28S and 18S ribosomal RNAs was quantified by scanning the negatives of the prints of the ethidium bromide-stained gel. All the quantitative data were presented as the ratio of the hybridization signal versus the 28S + 18S ribosomal RNAs intensity.

Nuclear transcription assays

In vitro transcription assays using isolated nuclei (approximately 2×10^7 nuclei per reaction) were performed as previously described [16]. The purified [α-^{32}P]RNA transcripts were resuspended in 2 ml of the hybridization buffer and equal amounts of incorporated counts were hybridized to 5 μg of unlabelled cDNA probes immobilized on Biodyne membranes by dot blotting. Probes included glyceraldehyde-3-phosphate dehydrogenase, $\beta\gamma$ actin, GP and pSPT19, the plasmid vector into which GP cDNA was cloned.

GP mRNA stability after oxygen treatment

At the end of the O_2 exposure period, actinomycin D was added to the culture medium at the final concentration of 5 μg/ml to block RNA transcription. The cells were lysed 3 h, 6 h and 9 h after administration of the drug, and total RNA was extracted and analysed by Northern-blot analysis as described above. Results were expressed as the percentage of the mRNA values obtained before addition of the drug (time 0).

Polyribosome isolation and distribution of mRNA

Approximately 1.5×10^7 cells were used for the isolation of polyribosomes and monoribosomes. Polyribosomes were analysed as described previously [17]. For isolation of RNA, 10 μl of 10 mg/ml proteinase K was added to each fraction gradient and the fractions were incubated at 37 °C for 10 min. They were extracted once with 1 ml of phenol/chloroform (1:1, v/v), and RNA was precipitated with 1 ml of ethanol in the presence of 0.2 M potassium acetate. GP and CuZnSOD mRNA from each fraction were assayed by Northern-blot hybridizations as described above. The amount of GP and CuZnSOD mRNA in each fraction was quantified by scanning the phosphor screens, and the amount of GP mRNA associated with polyribosomes calculated.

Measurement of protein synthesis

To assess overall protein synthesis activity, confluent cells in 35 mm-diam. dishes were labelled with [^{35}S]methionine (5 μCi/ml) for the last 2 h of the treatment period. At the end of the labelling period, cells were washed, scraped in phosphate buffer (50 mM, pH 7.4) and disrupted by sonication. Aliquots of the sonicates were removed for determination of incorporation of labelled methionine into trichloroacetic acid (10%)-precipitated proteins and protein content as already described [17].

Statistical analysis

The Friedman test was first done, then a non-parametric test was performed using the Wilcoxon matched-pair test. Results were expressed as means ± S.E.M.

RESULTS

Effects of SeMet

SeMet increases GP activity, but does not increase the steady-state level of GP mRNA and the transcription rate of the GP gene

To investigate the effects of SeMet on GP activity, we first performed a detailed time-course experiment. At confluence, cells were transferred to medium supplemented with 1×10^{-6} M SeMet, and GP activity was measured at different time intervals. As shown in Figure 1, there was: (a) no change in GP activity 3 h and 6 h after the addition of SeMet in the culture medium; and (b) a 45%, 65% and 86% increase in GP activity 12 h, 24 h and 48 h after supplementation with SeMet respectively.

To gain insight into the mechanisms by which GP activity was

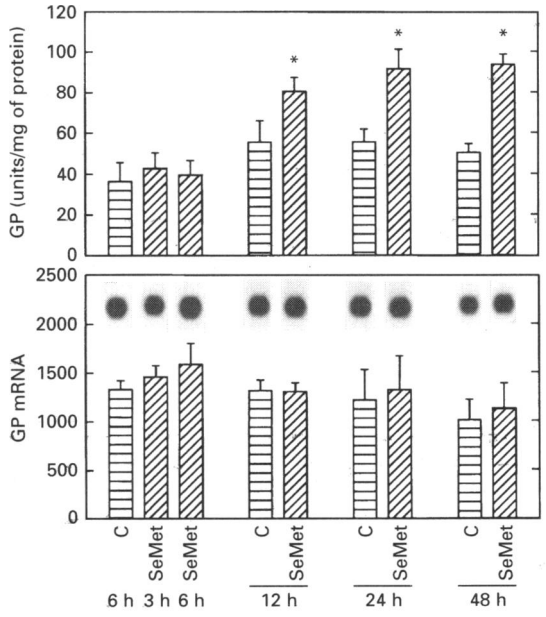

Figure 1 Effects of SeMet on GP activity and mRNA levels

At confluence, the medium was replaced with fresh medium with (SeMet) or without (C) 1×10^{-6} M SeMet, and the culture was continued for 3 h, 6 h, 12 h, 24 h and 48 h. At each time point, the cells were lysed, and GP activity and mRNA levels determined. GP activity are expressed as units/mg of protein, and GP mRNA levels are expressed as arbitrary units. Data are means ± S.E.M. ($n = 6$). *Significantly different from cells maintained in normal medium (C) for the same period.

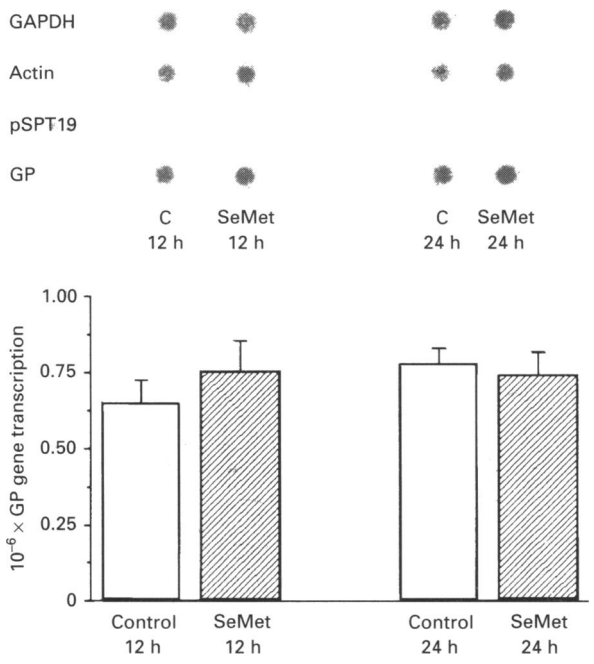

Figure 2 Effects of SeMet on GP gene transcription

At confluence, the medium was replaced with fresh medium with (SeMet) or without (C) 1×10^{-6} M SeMet, and the culture was continued for 12 h and 24 h. Nuclei were then isolated and newly synthesized RNA was labelled by incubation with [α-^{32}P]UTP. RNA was isolated and hybridized to cDNA for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), $\beta\gamma$-actin, GP, and to plasmid DNA (pSPT19). Values are means ± S.E.M. (arbitrary units, $n = 5$).

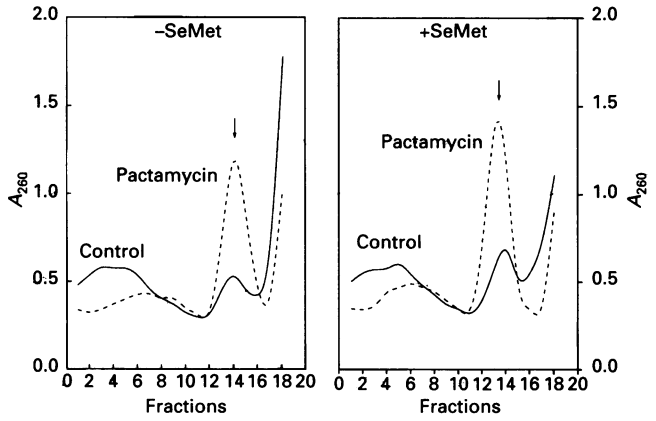

Figure 3 Effects of a 24 h treatment with 1×10^{-7} M pactamycin, in the absence or presence of 1×10^{-6} M SeMet, on the breakdown of polyribosomes

Direction of sedimentation is from right to left and position of the 80 S monomers is indicated by an arrow. Solid line, control cells; broken line, cells treated with pactamycin.

increased by SeMet, we examined the steady-state mRNA levels of GP and analysed the transcription rates of GP gene. At confluence, cells were maintained in medium containing 1×10^{-6} M SeMet for 3 h, 6 h, 12 h, 24 h and 48 h, and the total RNA were extracted to detect GP mRNA levels by Northern blotting. As shown in Figure 1, during the whole time course, the cellular level of GP mRNA did not change significantly in cells

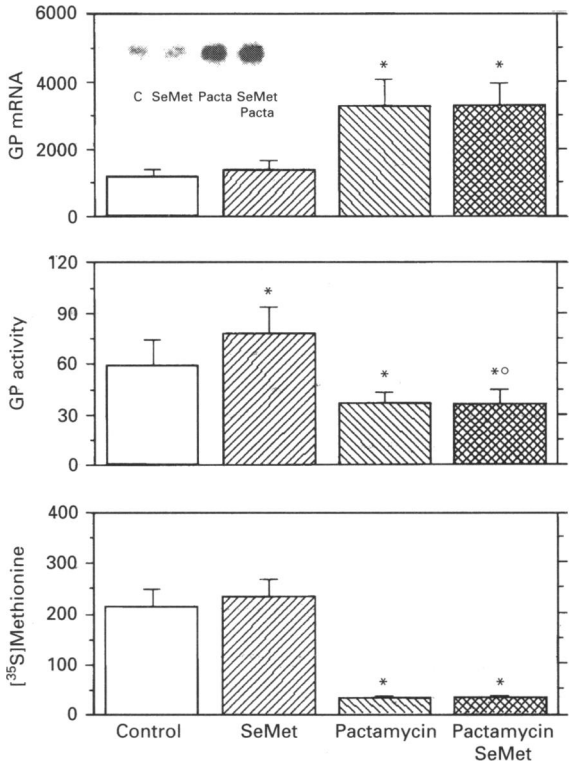

Figure 4 Effects of a 24 h treatment with 1×10^{-6} M SeMet in the absence and presence of 1×10^{-7} M pactamycin on GP mRNA levels, GP activity, and total protein synthesis

Results are expressed as arbitrary units, units/mg of protein and c.p.m. of [^{35}S]methionine incorporated per mg of protein for GP mRNA levels, GP activity and protein synthesis. Values shown are means ± S.E.M. ($n = 5$–6). *Significantly different from cells maintained in normal medium (control). °Significantly different from cells maintained in SeMet-supplemented medium.

grown in SeMet-supplemented medium as compared with that of cells grown in normal medium. Furthermore, *in vitro* transcription assays performed with nuclei isolated from cells maintained in normal and SeMet-supplemented medium showed that treatment with 1×10^{-6} M SeMet for 12 h and 24 h did not increase the transcriptional rate of the GP gene (Figure 2). Transcriptional activity of the genes for glyceraldehyde-3-phosphate dehydrogenase and $\beta\gamma$ actin, which were examined simultaneously, was also unchanged (data not shown).

De novo protein synthesis is required for the induction of GP activity by SeMet

To determine whether *de novo* protein synthesis was required for the SeMet-induced increase in GP activity, cells were exposed to 1×10^{-6} M SeMet for 24 h in the presence or absence of 1×10^{-7} M pactamycin. As shown in Figure 3, a 24 h treatment with 1×10^{-7} M pactamycin caused a conversion of polyribosomes into monoribosomes, in cells grown in normal or SeMet-supplemented medium, thus indicating a specific block in initiation of translation. The same treatment produced an 86% decrease in total protein synthesis, as assessed by [^{35}S]methionine incorporation into trichloroacetic acid-precipitable material, a 30% decrease in GP activity in cells grown in normal medium, and prevented the rise in GP activity caused by SeMet sup-

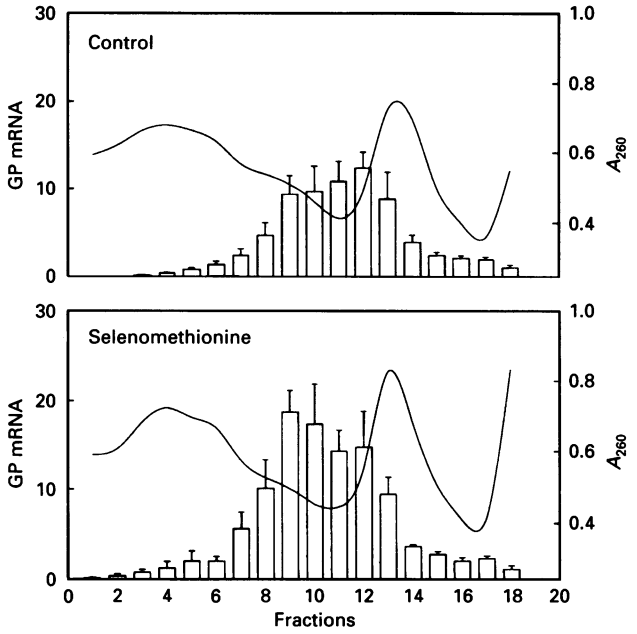

Figure 5 Polyribosomal distribution of GP mRNA in human umbilical vein endothelial cells cultured in normal medium and medium supplemented with 1×10^{-6} M SeMet for 12 h

The histograms display the relative amounts of GP mRNA in each fraction. Values are means ± S.E.M. of five experiments. The solid line of each panel represents a typical polyribosome profile monitored by u.v. absorption at 260 nm. Similar profiles were obtained from each experiment.

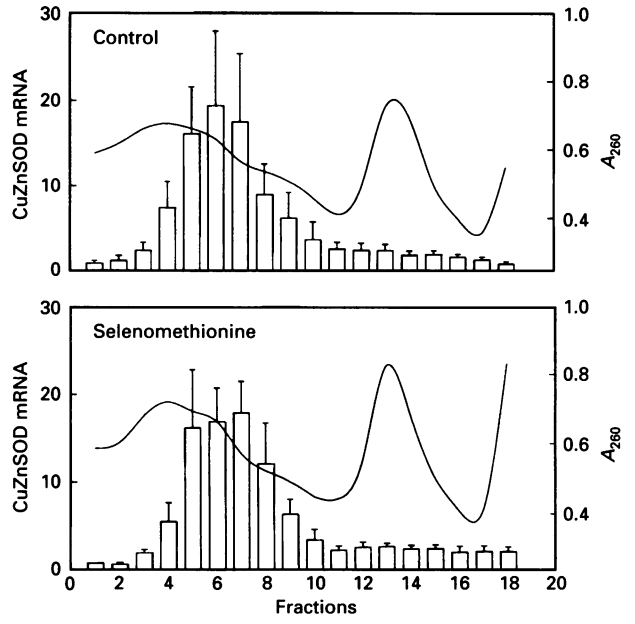

Figure 6 Polyribosomal distribution of CuZnSOD mRNA in human umbilical vein endothelial cells cultured in normal medium and in medium supplemented with 1×10^{-6} M SeMet for 12 h

The blots from the experiments shown in Figure 5 were stripped and reprobed with CuZnSOD cDNA.

plementation (Figure 4). Inhibition of translation resulted in a 120% and 142% increase in the steady-state GP mRNA levels in both cells grown in normal and SeMet-supplemented medium respectively.

SeMet stimulates the recruitment of GP mRNA on to polyribosomes

To investigate further the mechanism of translational control of GP expression, we examined the distribution profile of GP mRNA in polyribosome gradient in cells treated with 1×10^{-6} M SeMet for 12 h. As shown in Figure 5, the polyribosomal profiles of cells grown in normal medium and in SeMet-supplemented medium, as monitored by u.v. absorption at 260 nm of the gradient fractions, were identical. SeMet caused a 50% increase in the proportion of GP mRNA associated with light polyribosomes, but no shift in the GP mRNA pattern to heavier polysomes. The sum of GP mRNA associated to polyribosomes from fractions 7–14 amounted to 5728 ± 889 for cells grown in normal medium, and 8680 ± 983 for cells grown in SeMet-supplemented medium (arbitrary units, means ± S.E.M. of five independent experiments; $P < 0.05$). This effect was specific for GP since the distribution of CuZnSOD mRNA on polyribosomes did not change significantly in SeMet-supplemented cells when compared with that of control cells (Figure 6). The sum of CuZnSOD mRNA associated to polyribosomes from fractions 4–9 was 8219 ± 3193 and 7997 ± 1800 for cells grown in the absence and presence of SeMet respectively. It is interesting to note that CuZnSOD messages were localized to larger polyribosomes than GP mRNA, suggesting different rates of initiation and/or elongation of these two messages.

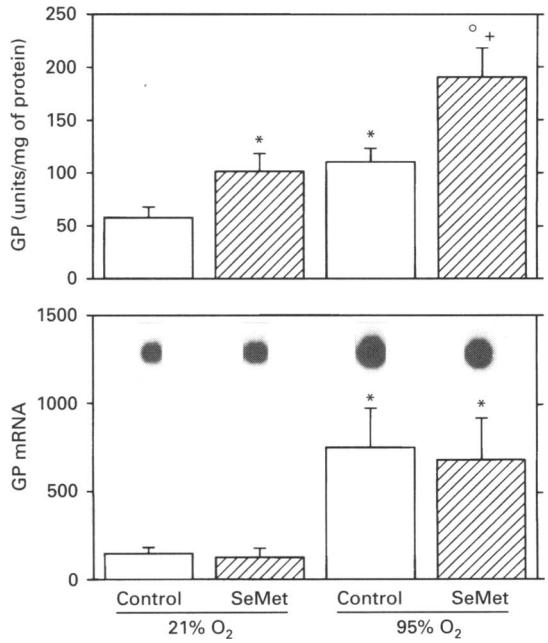

Figure 7 Effects of SeMet, O_2 and SeMet in combination with O_2 on GP activity and mRNA levels

Cells were exposed to 21% O_2 or 95% O_2 for 3 days in normal medium or in medium supplemented with 1×10^{-6} M SeMet. Values are means ± S.E.M. ($n = 6$). *Significantly different from cells maintained in normal medium under 21% O_2; °significantly different from cells maintained in normal medium under 95% O_2; +significantly different from cells maintained in SeMet-supplemented medium under 21% O_2.

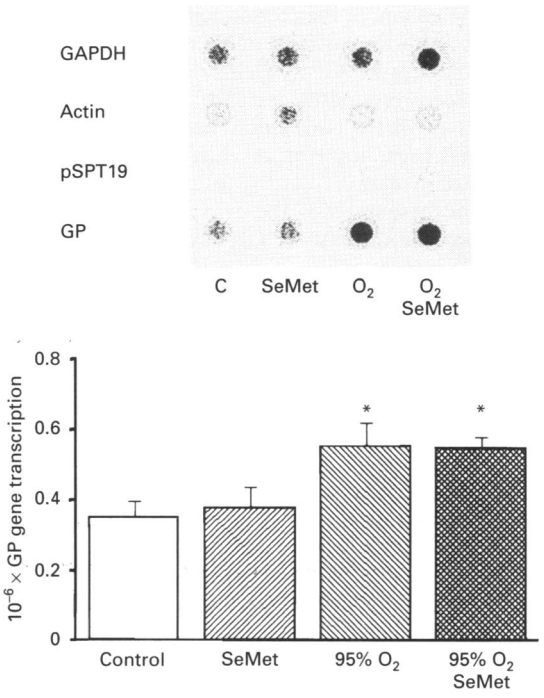

Figure 8 Effects of SeMet, O_2 and SeMet in combination with O_2 on GP gene transcription

Cells were exposed to 21% O_2 or 95% O_2 for 3 days in normal medium or in medium supplemented with 1×10^{-6} M SeMet. Values are means ± S.E.M. (arbitrary units; $n = 5$). *Significantly different from cells maintained in normal medium (control cells) and from cells maintained in SeMet-supplemented medium (SeMet).

Effects of oxygen, and oxygen in combination with SeMet

Hyperoxia increases GP activity and mRNA levels

The results for the effects of hyperoxia on GP activity, mRNA levels and gene transcription are shown in Figure 7. In cells exposed to 95% O_2 for 3 days in normal medium, there was an 81% increase in GP activity and a 394% increase in GP mRNA levels.

Hyperoxia increases GP gene transcription and mRNA stability

To determine whether the above effects were due to O_2 effects on gene transcription, cells were exposed to 95% O_2 for 3 days and nuclear run-on was performed. As shown in Figure 8, hyperoxia induced a 60% increase in GP gene transcription rate. No alterations in the transcriptional activity of the genes for glyceraldehyde-3-phosphate dehydrogenase and $\beta\gamma$ actin were observed after oxygen exposure (densitometric data not shown). Since the increase in GP mRNA level after O_2 treatment was considerably higher than that observed for GP gene transcriptional activity, the question arises as to whether there was also any effects of O_2 on GP mRNA stability. We therefore compared the GP mRNA half-lives in normal and O_2-treated cells. As shown in Figure 9, normal cells showed a 25% decrease in GP mRNA levels 9 h after addition of actinomycin D, when compared with the values obtained before addition of the drug. There was no change in the steady-state GP mRNA levels in hyperoxic cells at this time point.

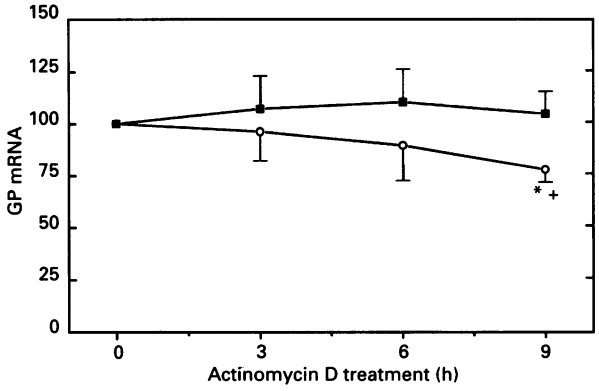

Figure 9 Effects of O_2 on GP mRNA stability

At confluence, the cells were cultured under 21% or 95% O_2 atmosphere for 3 days. Actinomycin D was then added (5 μg/ml, final concentration) and the culture was continued for 3 h, 6 h and 9 h. At each time point, the cells were lysed and GP mRNA levels determined by Northern-blot analysis.. Results (means ± S.E.M.; $n = 6$ or 7) are expressed as the percentage of the mRNA values obtained before addition of the inhibitor (time t = 0). *Significantly different from values from control cells at time = 0; +significantly different from values from hyperoxic cells at time 9 h. ○, normoxic cells; ■, hyperoxic cells.

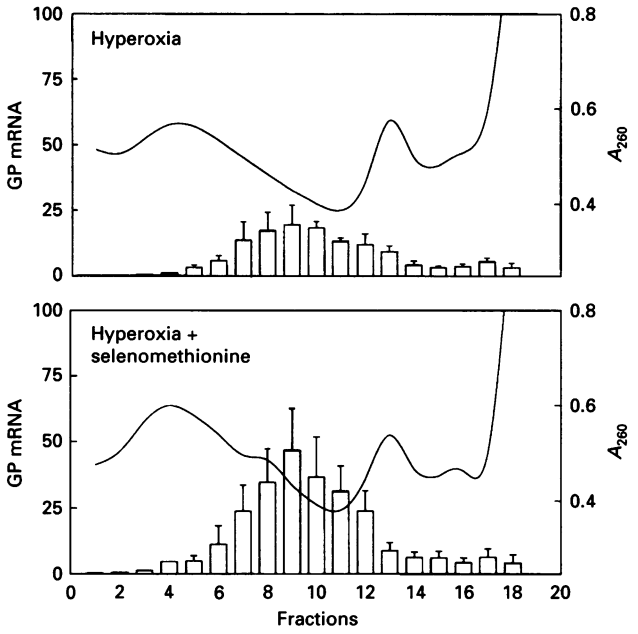

Figure 10 Polyribosomal distribution of GP mRNA in human umbilical vein endothelial cells cultured in normal medium or medium supplemented with 1×10^{-6} M SeMet under 21% O_2 or 95% O_2 for 3 days

The histograms display the relative amounts of GP mRNA in each fraction. Values are means ± S.E.M. of four experiments. The solid line of each panel represents a typical polyribosome profile.

The effects of oxygen and SeMet are additive on GP activity but not on GP gene expression

Addition of 1×10^{-6} M SeMet in the culture medium throughout the O_2 exposure period led to a further increase in GP activity by 80%, but no significant change in either GP mRNA level (Figure 7) or GP gene transcriptional activity (Figure 8). As already observed with normoxic cells, SeMet also induced an increase in

GP mRNA associated with polyribosomes in hyperoxic cells: the amounts of GP mRNA associated with polyribosomal fractions 6–14 were 2241±818 versus 1098±286 for cells in SeMet-supplemented and normal medium respectively (arbitrary units; mean±S.E.M. of four experiments; $P < 0.05$, Figure 10). Of note, the polyribosomal profile of hyperoxic cells differed from that of normoxic cells. Exposure to hyperoxia resulted in a substantial decrease in the amount of 80S monoribosomes relative to that of polyribosomes, consistent with inhibition of protein synthesis at the elongation level as previously reported [17].

DISCUSSION

We examined the expression of GP, in terms of activity, steady-state mRNA levels, gene transcription rate and the distribution pattern of GP mRNA on polyribosomes, in order to determine which step in the process of GP expression was regulated by SeMet and oxygen. We found an ~ 50% increase in GP enzymic activity in cells grown in SeMet-supplemented medium. However, there was no direct relationship between the extent of enzyme activation, the steady-state GP mRNA levels and GP gene transcription. Pactamycin, which specifically inhibits initiation of translation in eukaryotes by interfering with appropriate functional attachment of initiator tRNA to the initiation complex [18], prevented the rise in GP activity induced by SeMet. In fact, inhibition of the translation process led to a decrease in GP activity and a corresponding increase in GP mRNA levels, which were similar in magnitude in both cells grown in normal and SeMet-supplemented medium. This observation eliminates the possibility of a post-translational activation of a selenium-free apoprotein by selenium, in a fashion similar to CuZnSOD by copper [19], or stabilization of a labile pre-existing enzyme by selenium. Interestingly, inhibition of protein synthesis promoted the accumulation of GP mRNA in control cell cultures. This suggests a protein component with a short half-life is involved in the turnover of this message. In accordance with this view, stabilization of diverse mRNAs has been shown to occur in the presence of cycloheximide or of pactamycin, two drugs which inhibit protein synthesis by different mechanisms [20,21].

Most noteworthy in the study described here was the observation that SeMet stimulated the recruitment of GP mRNA on to polyribosomes, so that the concentration of GP mRNA in polyribosomal translatable pools was 50% higher in cells grown in SeMet-supplemented medium than in cells grown in normal medium. This increase can be correlated with the corresponding increase in enzymic activity found after SeMet supplementation. Taken altogether, our data imply that selenium primarily regulates the expression of GP at the post-transcriptional level, and are most consistent with the co-translational model of insertion of selenocysteine from tRNA into the active site of the nascent GP polypeptide chain. Selenium supplementation would lead to an increase in the selenocysteinyl-tRNA population, thus allowing ribosome binding to these tRNAs, translation of pre-existing GP mRNA and synthesis of GP to proceed to completion. Selenium-containing tRNAs have been identified [22–24], and the amounts of these tRNAs have been shown to be influenced by the presence of selenium in either the culture media of mammalian cells [25] or the diets of rats [26]. It is noteworthy that tissues from selenium-deficient rats contain translatable mRNA for the synthesis of GP protein, but are unable to translate GP mRNA into protein. There are precedents in the literature for regulation of gene expression by altered translatability of the messages. For example, iron loading induces a 2-fold increase in polyribosomal mRNA for ferritin in rats [27], and asparagine enhances the recruitment of ornithine decarboxylase mRNA from the untranslatable pool [28].

The antibodies against GP available to us did not enable us to demonstrate a corresponding increase in immunoreactive GP protein levels. A direct correlation between GP activity and protein level has been, however, repeatedly reported by different investigators using immunoblotting technique and e.l.i.s.a. [2,4]. On the basis of these findings and of our data described above, we suggest that the increase in GP activity brought about by SeMet can be attributed to enhanced GP synthesis through stimulation of GP mRNA translation. This is most consistent with previous work demonstrating that the rates of transcription of GP genes were unaffected by the selenium supply in the culture medium and diet [5,10,11], and that selenium-induced increment in GP activity was abolished by cycloheximide treatment [5,10].

Our results obtained with hyperoxic cells further support the co-translational mechanism involved in the regulation of GP by SeMet. SeMet treatment did not cause any significant change in GP mRNA concentration and GP gene transcription rate, which were both increased following a 3 day exposure to hyperoxia. As expected, the effects of SeMet and hyperoxia on GP activity were additive. These findings are suggestive of distinct mechanisms involved in the regulation of GP activity by O_2 and SeMet. The O_2-induced increase in GP activity was related to increased GP mRNA concentration which was due to an increase in GP transcription rate and mRNA stability, while the enhancing effect of SeMet on the O_2-induced activity of GP resulted from a co-translational process, as was the case with normoxic cells.

In conclusion, our results reveal that the activation of glutathione peroxidase is regulated by selenium and oxygen in a strikingly different way. The increase in GP activity associated with selenium is an oxygen-independent process that involves enhanced co-translational insertion of selenium into the nascent polypeptide chain resulting probably from an increase in the amount of the selenocysteinyl-tRNA population. The critical question, then, is how selenium increases the selenocysteine isoacceptors (increase in transcription rate, tRNA stability and/or charging rate). In contrast, the activation of GP by oxygen can be regarded as an oxidative stress response that might involve redox regulation of transcription factors and/or specific *cis*-acting regulatory elements that are located upstream of the protein coding region of the GP gene. A final answer to these questions must await the complete analysis of the 5′ flanking region of the GP gene using point mutations and transfection experiments as well as gel-retardation assays.

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REFERENCES

1 Tappel, A. L. (1984) Curr. Topics Cell. Regul. **24**, 87–97
2 Takahashi, K., Newburger, P. E. and Cohen, H. J. (1986) J. Clin. Invest. **77**, 1402–1404
3 Li, N., Reddy, P. S., Thyagaraju, K., Reddy, A. P., Hsu, B. L., Scholz, R. W., Tu, C. D. and Reddy, C. C. (1990) J. Biol. Chem. **265**, 108–113
4 Knight, S. A. B. and Sunde, R. A. (1987) J. Nutr. **117**, 732–738
5 Chada, S., Whitney, C. and Newburger, P. E. (1989) Blood **74**, 2535–2541
6 Toyoda, H., Himeno, S. and Imura, N. (1989) Biochim. Biophys. Acta **1008**, 301–308
7 Yoshimura, S., Takekoshi, S., Watanabe, K. and Fijii-Kuriyama, Y. (1988) Biochem. Biophys. Res. Commun. **154**, 1024–1028

8 Saedi, M. S., Smith, C. G., Frampton, J., Chambers, I., Harrison, P. R. and Sunde, R. A. (1988) Biochem. Biophys. Res. Commun. **153**, 855–861
9 Christensen, M. J. and Burgener, K. W. (1992) J. Nutr. **122**, 1620–1626
10 Baker, R. D., Baker, S. S., LaRosa, K., Whitney, C. and Newburger, P. E. (1993) Arch. Biochem. Biophys. **304**, 53–57
11 Toyoda, H., Himeno, S. and Imura, N. (1990) Biochim. Biophys. Acta **1049**, 213–215
12 Chang, M. and Reddy, C. C. (1991) Biochem. Biophys. Res. Commun. **81**, 1431–1436
13 Reddy, A. P., Hsu, B. L., Reddy, P. S., Li, N., Thyagaraju, K., Reddy, C. C., Tam, M. F. and Tu, C. D. (1988) Nucleic Acids Res. **16**, 5557–5568
14 Housset, B., Ody, C., Rubin, B., Elemer, G. and Junod, A. F. (1983) J. Appl. Physiol. **55**, 343–352
15 Jornot, L. and Junod, A. F. (1992) Am. J. Respir. Cell. Mol. Biol. **6**, 107–115
16 Jornot, L., Mirault, M. E. and Junod, A. F. (1991) Am. J. Respir. Cell. Mol. Biol. **5**, 265–275
17 Jornot, L., Mirault, M. E. and Junod, A. F. (1987) J. Appl. Physiol. **63**, 457–464
18 Goldberg, I. H., Stewart, M. L., Ayuso, M. and Kappen, L. S. (1973) Fed. Proc. Fed. Assoc. Soc. Exp. Biol. **32**, 1688–1697
19 Steinkuhler, C., Sapora, O., Carri, M. T., Nagel, W., Marcocci, L., Ciriolo, M. R., Weser, U. and Rotilio, G. (1991) J. Biol. Chem. **266**, 24580–24587
20 Schull, S., Heintz, N. H., Periasamy, M., Manohar, M., Janssen, Y. M., Marsh, J. P. and Mossman, B. T. (1991) J. Biol. Chem. **266**, 24398–24403
21 Altus, M. S. and Nagamine, Y. (1991) J. Biol. Chem. **266**, 21290–21296
22 Leinfelder, W., Zehelein, E., Mandrand-Berthelot, M. A. and Bock, A. (1988) Nature (London) **331**, 723–725
23 Lee, B. J., Worland, P. J., Davis, J. N., Stadtman, T. C. and Hatfield, D. L. (1989) J. Biol. Chem. **264**, 9724–9727
24 Leinfelder, W., Stadtman, T. C. and Bock, A. (1989) J. Biol. Chem. **264**, 9720–9723
25 Hatfield, D., Lee, B. J., Hampton, L. and Diamond, A. M. (1991) Nucleic Acids Res. **19**, 939–943
26 Diamond, A. M., Choi, I. S., Crain, P. F., Hashizume, T., Pomerantz, S. C., Cruz, R., Steer, C. J., Hill, K. E., Burk, R. F., McCloskey, J. A. and Hatfield, D. L. (1993) J. Biol. Chem. **268**, 14215–14223
27 Zahringer, J., Liga, B. S. and Munro, H. N. (1976) Proc. Natl. Acad. Sci. U.S.A. **73**, 857–861
28 Kanamoto, R., Nishiyama, M., Matsufuji, S. and Hayashi, S. (1991) Arch. Biochem. Biophys. **291**, 247–254

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