CHARACTERIZATION OF THE SELENIUM IN RAT LIVER MITOCHONDRIA AS GLUTATHIONE PEROXIDASE

O. A. Levander, V. C. Morris and D. J. Higgs

Nutrition Institute
Agricultural Research Service
U.S. Department of Agriculture
Beltsville, Maryland 20705

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SUMMARY

A large part of the radioselenium in liver mitochondria prepared from rats given 0.1 ppm Se as $Na_2{}^{75}SeO_3$ in the drinking water can be solubilized by a procedure combining extraction with hypotonic Tris buffer-ethanol and freeze-thawing. The radioselenium in this extract binds to DEAE-Sephadex but can be eluted as a single main radioactive peak with salt solution of relatively low ionic strength. Gel filtration of the radioactive peak fractions from the DEAE-Sephadex column through Sephadex G-150 yielded 2 radioactive peaks, the larger of which was associated with significant glutathione peroxidase activity. These findings are consistent with the concept that most of the selenium in mitochondria exists in the form of glutathione peroxidase.

Selenium has recently been shown to be a constituent of several enzymes. Turner and Stadtman found that their protein A of the clostridial glycine reductase system was a selenoprotein (1) and selenium also apparently plays a role in the formic dehydrogenase of E. coli (2,3,4). Whanger et al. suggested the existence of a selenium-containing cytochrome in ovine muscle (5) and selenium has been demonstrated to occur in the glutathione peroxidase (6,7) of bovine and rat erythrocytes. Levander et al. (8) showed that selenium can markedly accelerate the swelling of rat liver mitochondria induced by certain thiols and postulated that selenium might act to catalyze the transfer of electrons from glutathione to cytochrome c (9). The purpose of the work discussed in this report was to provide a preliminary characterization of the chemical form of selenium in rat liver mitochondria. It has been found that much of the selenium in rat liver mitochondria appears to be associated with glutathione peroxidase.

MATERIALS AND METHODS

Male weanling Fischer 344 rats were fed a selenium-deficient Torula yeast diet (10) supplemented with 100 ppm vitamin E for 4 to 7 weeks. The drinking water contained 0.1 ppm Se added as $Na_2{}^{75}SeO_3$ (0.125 μCi/ml.). Liver mitochondria were prepared in 0.25 M sucrose by standard techniques (8). The mitochondrial pellet was subjected to a sequential extraction procedure similar to that of Druyan et al. (11) except that all the extractions were carried out for 10 minutes at 37°. The mitochondria were suspended in hypotonic Tris buffer-ethanol and were subjected to rapid freezing and thawing before and after extraction with this medium. After 2 extractions with 0.15 M KCl, the mitochondria were further extracted in turn with 1 mM EDTA and 0.8 M NaCl and then were finally solubilized in 3% sodium dodecyl sulfate. The mitochondria were pelleted in the cold after each extraction.

The hypotonic Tris-ethanol extract was dialyzed against the appropriate buffer and concentrated by ultrafiltration. The concentrated extract was then subjected to ion exchange chromatography at 4°C through a DEAE-Sephadex A-50 column (1.2 x 12 cm) equilibrated with 0.05 M Tris-Cl buffer at pH = 8.0. The flow rate was 30 ml/hr and fractions of 3.6 ml were collected. A linear salt gradient (0.0 to 0.5 M NaCl) was used for elution. The fractions were monitored for absorbance at 280 nm and for radioactivity in a deep-well crystal scintillation counter. The peak radioactive fractions from the DEAE column were pooled, dialyzed, and concentrated by ultrafiltration. The concentrated fractions were then submitted to gel filtration at 4°C through a Sephadex G-150 column (2 x 90 cm) equilibrated with 0.05 M Tris-Cl buffer at pH = 8.0. The flow rate was 17 ml/hr and fractions of 3.6 ml were collected. The fractions were monitored for radioactivity and for glutathione peroxidase activity (6).

RESULTS

The results in Table 1 show that a large amount of the mitochondrial ^{75}Se could be solubilized by hypotonic Tris·Cl-ethanol in combination with freeze-thawing. An additional 18% of the mitochondrial ^{75}Se could be removed by 2

TABLE 1

Percentage Distribution of ^{75}Se in Rat Liver Mitochondrial Extracts

Extract	% of Total Radioactivity
0.01 M Tris·Cl-10% ethanol	62.6±1.5
0.15 M KCl (first)	16.5±0.6
0.15 M KCl (second)	1.9±0.1
1 mM EDTA	0.7±0.0
0.8 M NaCl	1.5±0.1
3% sodium dodecyl sulfate	16.3±0.4

Mitochondria from one whole rat liver were sequentially extracted 10 minutes at 37° with 10 ml portions of each of the solutions shown. In addition, the mitochondrial suspension in Tris-ethanol was subjected to rapid freezing and thawing immediately before and after the 37° incubation. Tris-ethanol and EDTA solutions were adjusted to pH = 7.4. Mitochondria were centrifuged in the cold after each extraction. Values represent means ±standard error of 3 animals.

extractions with 0.15 M KCl but further purification of these fractions according to Druyan et al. (11) revealed only negligible amounts of radio-selenium associated with cytochrome c (Levander and Higgs, unpublished observations). Little additional ^{75}Se could be removed from the mitochondria by sequential extraction with 1 mM EDTA or 0.8 M NaCl. Finally, the residual ^{75}Se was solubilized when the remaining mitochondrial precipitate was extracted with 3% sodium dodecyl sulfate.

Ion-exchange chromatography of the concentrated hypotonic Tris buffer-ethanol extract showed that the ^{75}Se bound to DEAE-Sephadex but could be eluted as a single main radioactive peak by relatively low ionic strength salt solution (Fig. 1). Further purification of the radioactive peak from the DEAE column (fractions 28 to 35, Fig. 1) by gel filtration through Sephadex G-150 revealed

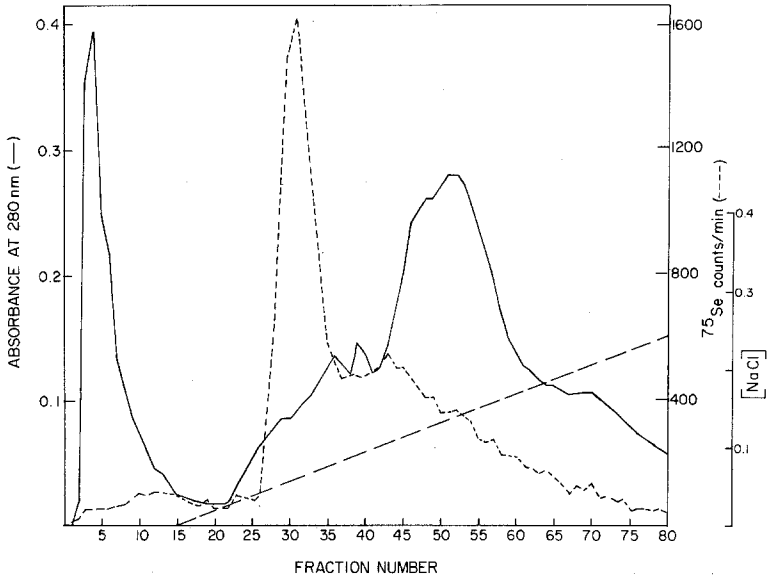

Figure 1. Chromatography of hypotonic Tris buffer-ethanol extract of mitochondrial ^{75}Se on DEAE-Sephadex A-50. See text for details.

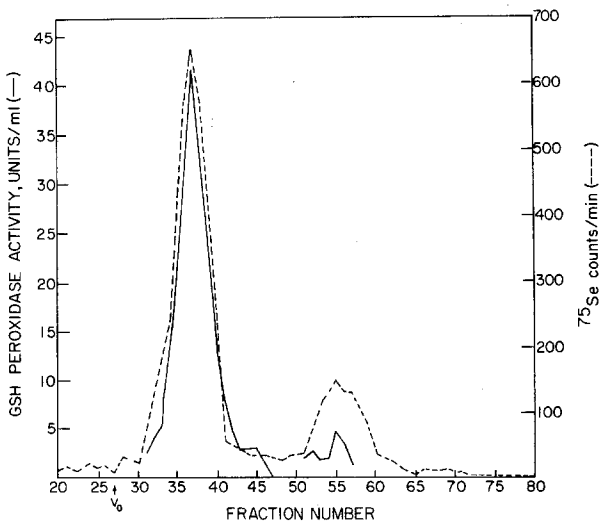

Figure 2. Gel filtration of ^{75}Se peak fractions from DEAE-Sephadex column on Sephadex G-150. See text for details.

the existence of one major and one minor peak of radioactivity (Fig. 2). Calibration of the G-150 column with protein standards of known molecular weight indicated that the major and minor radioactive peaks had approximate

molecular weights of 90,000 and 27,000 daltons, respectively. Glutathione peroxidase activity was mainly associated with the major peak. No absorbance at 280 nm was detectable in either of the 2 peaks.

DISCUSSION

The data presented in this report show that the bulk of the selenium in rat liver mitochondria can be solubilized by a procedure combining 2 freeze-thaw cycles and extraction with hypotonic Tris-ethanol. A 2-step chromatographic purification of such extracts consisting of ion exchange chromatography on DEAE-Sephadex followed by gel filtration through Sephadex G-150 revealed that most of the mitochondrial selenium was associated with glutathione peroxidase activity. The chromatographic behavior of the hepatic mitochondrial selenium associated with glutathione peroxidase seen here is remarkably similar to that of the erythrocytic selenium associated with glutathione peroxidase observed by others (6). The hepatic mitochondrial glutathione peroxidase resembles the erythrocytic glutathione peroxidase also in that the selenium associated with the enzyme is not lost by dialysis against glutathione or EDTA (Levander, Morris, and Higgs, unpublished observations).

If glutathione peroxidase is the sole or major selenoprotein in mitochondria, then the form of selenium in this enzyme apparently is able to accelerate the mitochondrial swelling induced by certain thiols (8). However, about 40% of the mitochondrial selenium is not accounted for by the Tris-ethanol extracts and the possibility of some other forms of selenium in mitochondria responsible for this effect cannot be excluded. It should also be pointed out that the results reported here may not be directly comparable to the earlier work on mitochondrial swelling since the animals used in the previous experiments (8) received more selenium (0.5 ppm in the diet) than the animals used in the experiments reported here (0.1 ppm in the water). Further work is underway in an attempt to clarify these points.

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