Subcellular fate of selenium from ^{75}Se-labeled plasma selenoprotein P in selenium-deficient rats

David S. Wilson and Al L. Tappel

Department of Food Science and Technology, University of California, Davis, CA, USA

This study explored the subcellular incorporation of ^{75}Se from ^{75}Se-labeled plasma selenoprotein (PSP) as this might be related to a possible selenium transport function of PSP. PSP was labeled in vivo in rats with ^{75}Se. The ^{75}Se-labeled PSP was then injected into selenium-deficient rats. Groups of rats were killed 3, 12, 18, 24, and 48 hr post injection. Blood, liver, kidney, and testes were analyzed for distribution of ^{75}Se from ^{75}Se-labeled PSP. Liver, kidney, and testes were fractionated to obtain enriched lysosomal, microsomal, and cytosolic fractions. Disappearance of ^{75}Se from plasma paralleled increased accumulation of radioactivity in the tissues. ^{75}Se in the liver rose and then dropped, while its accumulation in kidney remained fairly constant over the 48-hr time period. Total radioactivity in the testes was as much as 15-fold higher than in the other two tissues when calculated on a cpm ^{75}Se/mg protein basis, and the activity continued to increase during the 48-hr period. Radioactivity was highest in the lysosomal and microsomal fractions. These results suggest that PSP delivers selenium to the testes in particular during selenium deficiency.

Keywords: plasma selenoprotein P; selenium transport; lysosomes; microsomes

Introduction

The only mammalian selenoprotein other than glutathione peroxidase[1] and a recently identified deiodinase[2,3] that has been characterized to any extent is the one designated plasma selenoprotein P (PSP), which is found in the plasma of rats and other species.[4,5] The most distinctive feature of the protein is its high selenium content, which averages 7.5 selenium atoms in the deglycosylated, 43 kDa polypeptide.[6,7]

One would expect the function of PSP to derive from its unusual selenium load. A transport function has been hypothesized,[4,7] but this hypothesis would have to account for the fact that the selenium load of the protein must be converted to the selenide form prior to incorporation into selenium-dependent enzymes or other selenoproteins.[8] PSP would be unique as a transport protein for this reason. The transport function would require that the protein bind to target cells that have been shown to require selenium and that the protein be internalized. The selenocysteine residues could then be sequentially degraded to supply selenide for reincorporation into the selenoamino acid and into a selenoprotein such as glutathione peroxidase. Binding of PSP to cell membranes has been investigated,[9,10] but the subcellular fate of selenium delivered in this way has not been systematically explored.

The present study considered the second of these questions, namely, the post-binding fate of selenium contained in PSP. The protein was labeled in vivo with ^{75}Se and was then injected as plasma into rats so that the transported nutrient could be followed in subcellular fractions of tissues known to accumulate selenium. Selenium-deficient rats were used on the assumption that subcellular effects would be magnified in selenium deficiency.

Materials and methods

Preparation of ^{75}Se-labeled PSP in plasma

^{75}Se-labeled PSP in rat plasma was prepared by the method of Motchnik and Tappel.[11] Selenium-depleted male weanling

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Address reprint requests to A.L. Tappel at the Department of Food Science and Technology, 109 FS&T Building, University of California, Davis, CA 95616 USA.

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Sprague-Dawley rats (Bantin and Kingman, Freemont, CA USA) were injected i.p. with 0.3 mCi of ^{75}Se as selenious acid (ICN Radiochemicals, Irvine, CA USA). The specific activity of the selenium used was 26 mCi/mg selenium. Plasma was collected 3 hr after injection of ^{75}Se-labeled selenious acid and stored in 1-ml aliquots for use as tracer. Several studies have shown by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)[11,12] and gel filtration chromatography[9] that 3 hr after injection of ^{75}Se into selenium-deficient rats, radioactivity in plasma is associated only with the 57 kDa plasma selenoprotein P. No other labeled proteins are found in plasma in these conditions. Moreover, 98% of the injected ^{75}Se is recovered in that protein, indicating that PSP would be essentially the sole source of ^{75}Se in the plasma from these rats.[7]

Preparation of animals for injection

Male, weanling Sprague-Dawley rats were depleted of selenium for 10–12 weeks as described by Motchnik and Tappel[11] and weighed 400–450 g at the time of use.

Injection of Triton WR-1339

Four days before the injection of ^{75}Se-labeled PSP, each rat to be used for that time point was injected i.p. with 85 mg Triton WR-1339/100 g body weight (Sigma Chemical Co., St. Louis, MO USA) dissolved in saline at a concentration of 200 mg/mL.[13] Triton WR-1339 has been shown to enhance recovery of lysosomes by decreasing the buoyant density of that fraction.

Injection of plasma selenoprotein P

Five groups of three rats each were depleted of selenium as described above. A 1-mL aliquot of plasma that contained 1.5–2.0 $\times$ 10^6 cpm ^{75}Se-labeled PSP was slowly injected intracardially into each rat of the group. On insertion and at the end of the injection, blood was drawn into the syringe barrel to be certain that the needle was in the heart. Tissues from each group of three rats were subjected to the full fractionation procedure before the next group was used. Tissues (blood, liver, kidney, and testes) were taken at 3, 12, 18, 24, and 48 hr post injection using one group for each time point.

Subcellular fractionation

Protocols for the fractionation procedures were those of Sawant et al.[14] for liver, Shibko and Tappel[15] for kidney, and Ninjoor and Srivastava[16] for testes. The procedures were specifically designed to obtain enriched lysosomal fractions, but microsomal and cytosolic fractions were also collected. Aliquots of tissue homogenate were also saved for counting of ^{75}Se and for enzyme analysis. Rats in each group for a specific time point were lightly anesthetized with ether for collection of blood by cardiac puncture. Liver, kidney, and testes were then excised. For each animal in the group, all tissues were removed, rinsed, and placed on ice before proceeding to the next animal. Pooling of tissues was required to provide sufficient material for fractionation and analysis. Tissues from three rats for each time point were thus pooled before homogenization, recognizing that such a procedure precludes subsequent estimates of variability.

Protein content was determined for each fraction (three tissues and five time points) by the bicinchinonic acid method.[17] Each fraction was counted for ^{75}Se on a Packard model 5210 gamma scintillation counter.

Total blood volume was estimated using published constants.[18] ^{75}Se in urine, feces, and expired volatiles was not measured.

Enzyme assays

Relative activities were determined by standard methods for specific marker enzymes for the fractions isolated: β-N-acetylglucosaminidase (lysosomes), NADP cytochrome *c* reductase (microsomes) and lactate dehydrogenase (cytosol).[19]

Results

Fate of injected PSP

Four fractions (homogenates, lysosomes, microsomes, and cytosol) were investigated in three tissues (liver, kidney, and testes) at five time points. Results typical of the marker enzyme assays are shown in *Figure 1* for the 24-hr group of rats. Activities for each marker enzyme remained relatively constant from the first to the last time period for the three tissues (data not shown). The data of *Figure 1* show that good subcellular fractionation was achieved.

Disappearance of ^{75}Se-labeled PSP from plasma is shown in *Table 1*. At 3-hr post injection 43% of the injected ^{75}Se remained in the blood; at 48 hr, 11% remained. The red blood cell (RBC) level dropped from 3.4 to 1.8% of the injected amount over the 48-hr period. Total retention of the selenium from PSP in the tissues analyzed dropped from 54 to 26% over the experimental time period. Accumulation of ^{75}Se in the tissue homogenates varied according to the specific

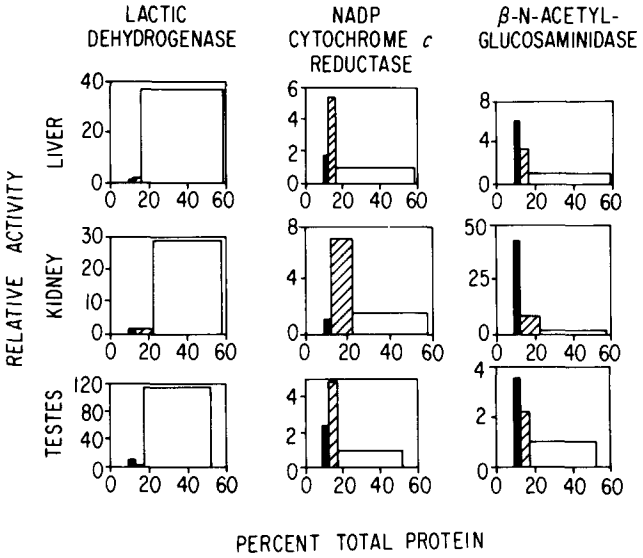

Figure 1 Relative activity of marker enzymes for subcellular fractions. For lysosomes β-N-acetylglucosaminidase was measured; for microsomes, NADP-cytochrome *c* reductase; and for cytosol, lactate dehydrogenase. Activity shown is relative activity, giving the lowest activity of the three enzymes for each tissue a value of one and expressing the other two as multiples of that value. The total protein in each subcellular fraction is also shown as a percentage of that in the total homogenate for that tissue. Bars represent lysosomes (solid), microsomes (diagonal stripe), and cytosol (open).

Table 1 Incorporation of ^{75}Se from injected PSP tissues of selenium-deficient rats

Tissue	Percent of injected ^{75}Se*				
	3 hr	12 hr	18 hr	24 hr	48 hr
Plasma	40.9	23.3	14.4	10.1	9.3
RBC	3.4	3.1	3.7	1.2	1.8
Liver	6.1	9.4	10.7	5.9	5.2
Kidney	2.3	2.5	2.5	2.3	3.0
Testes	1.3	2.4	3.8	4.7	6.8

*About 0.8 μCi ^{75}Se was injected.

tissue. Amounts in the liver rose to a maximum at 18 hr and then declined. Accumulation in the kidney remained quite constant at about 2–3% of the injected dose, but levels in the testes rose continuously over the 48 hr.

^{75}Se from radiolabeled-PSP was measured in homogenates, lysosomes, microsomes, and cytosol. Data for the 18-hr lysosomal fraction were not obtained. Accumulation of ^{75}Se (cpm/mg protein) from the injected PSP in the subcellular fractions varied according to the tissue analyzed *(Table 2)*. Relative total activity (cpm ^{75}Se/mg protein) was much higher in the testes than in liver or kidney. Radioactivity in subcellular fractions in the testes was highest in the lysosomes.

Discussion

It would appear from these results that selenium is transferred from PSP to cells in different tissues. The amount of lysosomal ^{75}Se activity found in the different tissues provides an indication of the metabolic disposition of PSP. Such activity would not be required for Se not associated with proteins. Degradation of endocytosed proteins is thought to be a constitutive function of lysosomes,[20] which would be the site of a requisite first stage in the processing of PSP for the release of its nutrient component.[21] Moreover, glycoproteins are in particular targeted for degradation in the lysosomes,[22] a criterion that PSP appears to fulfill.

Liver and kidney showed very low radioactivity in the lysosomal fraction compared with that in the testes. This indicates either that lysosomal processing of PSP, i.e., degradation of the protein, in liver and kidney occurred outside the time frame of the study or that it did not play a role in these tissues. Turnover times for endocytosed proteins makes the latter a more likely explanation. The same is true of activity in the microsomal fraction, suggesting that the selenium derived from PSP was not involved in protein synthesis in liver and kidneys. The time course of retention of ^{75}Se in the liver showed maximum presence of the label at 18 hr with a subsequent rapid decline over the next 6 hr. It is possible that nonspecifically bound PSP was progressively released from the liver after this time point, contributing to the constant total and specific increase in the amounts of the label found in the testes homogenates.

Only the testes showed substantial activity from transferred ^{75}Se in subcellular fractions. The ^{75}Se cpm/mg protein in all fractions were significantly higher in the testes than in the other tissues. In studies using injected ^{75}Se-selenite, the testes have a priority claim for selenium and retain it longer than either liver or kidney.[23] The pattern of subcellular radioactivity seen in this study is consistent with processing of internalized PSP for use of the transported selenium in cellular metabolism. Evenson and Sunde have also shown a time course for the appearance–disappearance of PSP after injection of ^{75}Se and the appearance of a selenoprotein in the testes that is consistent with the results obtained here.[12]

The rate of accumulation of ^{75}Se in the testes was fastest in the lysosomes and somewhat slower in the microsomes, allowing possibly for the time lapse nec-

Table 2 Accumulation of ^{75}Se from injected PSP in cellular fractions

Tissue/fraction	3 hr	12 hr	18 hr	24 hr	48 hr
Liver					
Homogenate	53	61	108	63	29
Lysosomes	14	38	48	25	17
Microsomes	216	284	191	93	57
Cytosol	68	69	74	75	26
Kidney					
Homogenate	98	133	134	168	110
Lysosomes	196	88	116	70	89
Microsomes	223	303	227	170	150
Cytosol	117	180	149	136	116
Testes					
Homogenate	237	382	990	750	1559
Lysosomes	144	615	n.d.*	1538	1095
Microsomes	312	582	512	1116	1095
Cytosol	173	293	270	299	241

*Not done.
^{75}Se-labeled PSP was injected intracardially and tissues were taken at five time points after injection. Values represent the means of two measurements on fractions from pooled tissues for each group. Values are CPM/mg protein.

essary for transfer of selenium from lysosomes to the protein synthesizing machinery in microsomes. Moreover, radioactivity in microsomes remained high at the 48-hr time point, while that in lysosomes started to decline at 24 hr. Absolute amounts of ^{75}Se present in tissues of larger mass than the testes would no doubt be larger, but the relative processing activity of Se (CPM/mg protein) was much greater in testes. If apparent accumulation of ^{75}Se in the other tissues represents mainly nonspecific binding, such selenium from injected PSP would not quantitatively enter the cell. One would expect such a pattern as the availability of ^{75}Se-labeled PSP from blood decreases, and as the transfer from lysosomes to microsomes progresses.

Such patterns do not preclude specific binding to other tissues. The apparent existence of two isoforms of PSP may explain the differential targeting of the protein to receptors in different tissues.[24] No information is available as to the relative proportions of the two forms.

In conclusion, it appears from these results that selenium is transferred from PSP to cells and that in the selenium-deficient rat the testes are the principal target. Cellular processing of the transferred selenium involves both lysosomal and microsomal fractions.

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