Use of Stable Isotopic Selenium As a Tracer to Follow Incorporation of Selenium into Selenoproteins (43949)

JOHN W. FINLEY,[1] RICHARD A. VANDERPOOL, AND EUGENE KORYNTA

United States Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, North Dakota 58202-9034

Abstract. **Stable isotopes of selenium (Se) have been used in human studies to measure Se absorption, retention and excretion. The purpose of this study was to examine whether stable Se could also be used to follow the incorporation of Se into selenoproteins and whether selenoproteins are labeled with stable isotopes the same way they are with radioactive Se. Rats fed either a Se-deficient or a high-Se diet were injected with either a radioactive (^{75}Se) or a stable isotope of Se (^{77}Se), and the liver cytosol was chromatographed on Sephadex G-200. Compared with ^{75}Se, a greater percentage of ^{77}Se was incorporated into cytosol, but the distribution and the effect of dietary Se was similar for both isotopes. New Zealand long-eared rabbits were also injected with either ^{77}Se or ^{75}Se, and the plasma was chromatographed. More of the ^{75}Se was incorporated into the plasma, but again the patterns of incorporation were similar for both isotopes. Plasma from a male subject who ingested 60 μg of ^{77}Se was chromatographed, and the stable Se was detected in column fractions and showed a distribution similar to that observed for rabbit plasma. Finally, a polyacrylamide gel electrophoresis (PAGE) method was developed that allowed loading of sufficient protein to analyze for ^{77}Se in individual protein fractions. The distribution of ^{77}Se and ^{75}Se in rabbit plasma was similar. Human plasma was electrophoresed by a similar method and peaks of 56 and 23 kDa were detected. These data show that stable isotopes of Se can be used for selenoprotein production in the same way as radioactive isotopes. They also show that, when physiological amounts of stable Se are ingested by humans, the isotope can be detected in blood-borne proteins separated by column chromatography and PAGE.** [P.S.E.B.M. 1995, Vol 210]

Since the discovery that selenium (Se) is necessary for the activity of glutathione peroxidase (GSH-Px) (1), considerable research has been directed toward understanding the metabolism of selenoproteins *in vivo*, and understanding the relationship between dietary Se status and selenoprotein synthesis (2). Essential to any study examining the relationship between selenoproteins and Se is the need for an *in vivo* Se tracer. The γ-emitting radioactive isotope, ^{75}Se, has been utilized in animal (3, 4) and cell culture studies (5), and in a few previous human studies (6, 7) (although it has not been used to study the incorporation of Se into selenoproteins). However, due to the long half-life, relative strength of the γ energies, and the tendency of Se to be highly conserved in the body and concentrated in some tissues, ^{75}Se is no longer available for use in humans.

Stable isotopes offer an alternative to radioactive isotopes for use in human studies. There are six stable isotopes of Se, and four are suitable for use as tracers in metabolic studies. The preferred method of analysis for these isotopes has been inductively coupled plasma mass spectrometry (ICP-MS) (8, 9), neutron activation analysis (NAA) (10, 11), or gas chromatography mass spectrometry (GC-MS) (13, 14). Stable isotopes of Se have been employed as markers in previous human Se

[1] To whom requests for reprints should be addressed at USDA, ARS, GFH-NRC, P.O. Box 9034, Grand Forks, ND 58202-9034.

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studies (10–13, 15, 16). These studies have used stable Se isotopes as a tracer to determine balance and bioavailability, but have not used them to study Se incorporation into selenoproteins.

The purpose of the present study was to evaluate the use of stable Se isotopes as tracers for following the incorporation of Se into selenoproteins. Initial studies were conducted in animals, but the ultimate objective was to validate the use of stable isotopes of Se as tracers for following the incorporation of Se into proteins of humans.

Materials and Methods

Animal Studies. Weanling Sprague-Dawley rats (sixteen males) were randomly assigned to Torula yeast-based diets with addition of 0 (basal diet) or 0.5 μg/g added Se as sodium selenite. Animals were fed on the respective diets for 6 weeks. Animals were maintained in a room controlled for temperature (22–23°C), humidity (55%) and light cycle (12 h on and 12 h off). After 6 weeks, animals within each diet group were subdivided into two other groups; one group was injected intraperitoneally (ip) with 10 μg of ^{77}Se prepared as selenite in physiological saline (18). The second group was injected ip with 1.85 mBq of ^{75}Se as sodium selenite (Dupont-New England Nuclear, Danvers, MA) which also contained 10 μg of natural abundance Se as selenite (1 ml total volume for all injections). After injections, animals were returned to their cages for 36 hr (food and water *ad libitum*). Animals then were anaesthetized with sodium pentobarbital, extensively perfused through the portal vein with ice-cold saline, and tissues dissected. Rat livers were homogenized in 0.25 *M* sucrose buffer containing protease inhibitors (0.2 g/l sodium azide, 35 mg/l phenylmethylsufonyl fluoride, 0.6 mg/l leupeptin, and 0.9 mg/l pepstatin) using a Potter-Elvehjem tissue grinder and then centrifuged at 40,000*g* for 30 min. The supernatant was saved and considered to be the cytosolic fraction. Cytosols within a diet × isotope group were pooled for chromatography.

In a second experiment, two New Zealand rabbits (Hazelton Res. Products; Denver, PA) that had consumed rabbit chow for six months were injected (ip) with either 1.85 MBq of ^{75}Se (also containing 50 μg of natural abundance Se) or 50 μg of ^{77}Se. The animals were given food and water *ad libitum* for 36 hr and then blood was withdrawn from the ear vein (0.14% EDTA as anticoagulant) and separated into red cells and plasma.

Human Studies. To examine the chromatographic distribution of stable Se in the human plasma, a 38-year-old male subject, in good health, ingested an oral dose of 60 μg of ^{77}Se as selenite, after an overnight fast. Twenty milliliters of blood were withdrawn (using oxalate as an anticoagulant) 36 hr after ingestion of the isotope, and plasma was prepared by the same procedure as in the animal studies.

In order to study the distribution of stable Se in human plasma subjected to polyacrylamide gel electrophoresis (SDS-PAGE), excess plasma was obtained from a study in which a young man and young woman living in area of low Se intake had consumed an oral dose of 100 μg of ^{74}Se as selenate. Blood was collected, using sodium heparin as an anticoagulant, and the plasma was prepared as before.

Chromatography. Rat cytosol, plasma and human plasma were chromatographed on Sephadex G-200 using a 1.6 × 100 cm column equilibrated with 10 m*M* Tris-HCl buffer (pH 8.6) run at a flow rate of 12.0 ml/hr. The void volume of the column was determined with blue dextran and the total volume of the column was determined with deuterium oxide. The column was calibrated with alcohol dehydrogenase, bovine albumin, carbonic anhydrase, cytochrome C, and aprotonin. Three to four milliliters of sample were loaded; individual fractions were collected for ^{75}Se analysis, and adjacent fractions were combined for analysis of stable isotope. All samples were run at least twice and representative results are shown.

SDS-PAGE. Large amounts of sample needed to be electrophoresed in order to detect stable Se in selenoproteins separated by SDS-PAGE. For plasma, a total of 0.35 ml was the minimum volume that gave good results. In order to run this amount of plasma, it was important to first remove low–molecular weight molecules and ions. Samples were prepared first by chromatography on PDG-6 Biogel (desalt resin with 6000 mol wt exclusion limit; Bio-Rad Scientific, Richmond, CA). To insure complete removal of ions and low–molecular weight materials, this was followed by dialysis for 24 hr (Spectra-Por membrane; 1000 Dalton mol wt cutoff) and then precipitation with 10% ice-cold acetone. Precipitated samples were then boiled in SDS buffer and filtered through a 0.45-μ filter before application to gels.

Polyacrylamide gels (9% acrylamide; 3 mm thick, 16 cm wide × 20 cm long) were loaded with prepared samples in a single lane the width of the gel. Gels were run for 27 hr at 20 mAmps, following which they were sliced into 2.7-mm fractions. Adjacent fractions were combined and analyzed for the presence of stable or radioactive isotopes of Se.

Stable Isotopes. Samples were prepared for stable isotope analysis by weighing aliquots into tall form beakers to which was added 10 ml of 40% $Mg(NO_3)_2$, 2 ml of 6 *M* HCl, and 10 ml of 16 *M* HNO_3. In addition, ^{76}Se was added as an internal spike solution. Samples sizes were adjusted to produce a final natural abundance Se concentration of 5–10 ng/ml and the added

^{76}Se spike was adjusted to 20%–60% of the sample's Se content. Samples were covered with watch glasses and allowed to reflux overnight. Samples were then evaporated to dryness and ashed at 490°C for 12 hr. Ash was then reacted with 4.5 ml of 12 *M* HCl and a sufficient volume of 12 *M* HCl and water to give a final HCl concentration of 3.8 *M* (13).

A natural abundance Se stock solution was prepared from SeO_2. (Æsar, Puratronic, 99.99%) and was used as a primary standard. Enriched ^{77}Se (metal, 76.00 atom%; Liquid Carbonic, Inc., Scarborough, Ontario, Canada) was converted to $K_2{}^{77}SeO_3$, while enriched ^{76}Se (metal, 90.50 atom%; Advanced Materials & Technology, New York, NY) was prepared in a similar manner to give $H_2{}^{76}SeO_3$. The former was used as the *in vivo* tracer and the latter was used as the *in vitro* spike for isotope dilution analysis (8). The stock solutions of ^{77}Se and ^{76}Se were quantified by ICP-MS by spiking each into a natural abundance Se standard solution.

Stable isotopes were detected using an inductively coupled plasma mass spectrometer (ICP-MS, PlasmaQuad 2+; VG Elemental, Winsford Cheshire, United Kingdom) with a custom-built hydride generation sample introduction system (13). The amount of total Se (natural abundance) and the amount of tracer ^{77}Se present in samples were calculated from ICP-MS intensity data using a computer program developed for use with multiple stable isotope tracers (TTMR9 Ver 1.0) (19).

Radioactive Isotopes. Samples containing ^{75}Se were digested using the stable isotope procedure, but without the stable isotope spike. Individual digested tissues, or adjacent chromatography fractions, were combined and counted for γ energies from 60 to 467 keV (Packard 5003 gamma counter; Packard Instrument Co., Downer Grove, IL 60515).

Results

Distribution of Isotopic Se in Rat Liver Cytosol. Figure 1A compares the chromatographic distribution of radioactive ^{75}Se to that of stable isotopic ^{77}Se in the liver cytosol of rats fed the torula yeast-based diet with no added Se (pooled cytosols of four rats for each isotope). The pattern of isotope distribution was similar for the two isotopes, although a greater percentage of the administered dose of ^{77}Se was incorporated into cytosol. Three major protein peaks were observed: A high molecular weight (HMW) peak coming shortly after the void volume contained 47% of radioactive isotope and 48% of the stable isotope; an intermediate molecular weight peak (IMW) contained 40% of both isotopes; and a low molecular weight (LMW) peak eluting shortly before the total volume contained 13 and 12% of the radioactive and stable isotope, respectively.

Rats fed the diet high in Se incorporated less Se isotope into proteins in liver cytosol (Fig. 1B) than rats that were fed the diet deficient in Se. More ^{77}Se was incorporated into liver cytosolic proteins than ^{75}Se. The peak labeled to the greatest extent was the IMW peak, which contained 54% and 51% of the isotope (radioactive and stable, respectively); the HMW peak contained 23% and 24% of the isotopes, and the LMW peak contained 23% of both isotopes.

Distribution of Isotopic Se in Rabbit Plasma Separated by Column Chromatography. Almost all radioactive and stable Se in rabbit plasma was found in an intermediate molecular weight peak (Fig. 2; 94% and 93% of the radioactive and stable isotope, respectively). A very small amount of both isotopes was incorporated into a protein eluting shortly after the column void; this peak was much more apparent when natural abundance Se was measured (data not shown).

Distribution of Isotopic Se in Rabbit Plasma by SDS-PAGE. Figure 3 compares the distribution of radioactive ^{75}Se and stable ^{77}Se in rabbit plasma after SDS-PAGE. Both isotopes were localized in two proteins of approximately 60 and 20 kDa, and the relative distribution of isotope among the two peaks was similar for both isotopes.

Distribution of Stable Isotopic Se in Human Plasma Separated by Column Chromatography. Figure 4 shows the distribution of stable isotopic Se in human plasma. Because ^{75}Se was not used, there was no comparison with another isotope, but the isotopic distribution was compared with natural abundance Se. Three, and possibly four, protein peaks were observed to contain natural abundance Se. The greatest amount of natural abundance Se was found in an IMW peak, and the next greatest amount was in a peak that eluted as a slightly smaller shoulder of the main peak. The third most abundant Se-containing peak was a HMW peak eluting shortly after the column void. Finally, a very small amount of Se was found in a peak eluting shortly ahead of the column total volume.

Stable isotopic Se was incorporated primarily into the main IMW peak. The shoulder of the IMW peak labelled with stable Se appeared much smaller than the shoulder of the IMW peak containing natural abundance Se. No ^{77}Se was found in the HMW peak, and a very small amount was found in the LMW peak which eluted just before the total volume.

Distribution of Stable Isotopic Selenium in Human Plasma after Separation by SDS-PAGE. Figure 5 shows the distribution of ^{77}Se in human plasma after SDS-PAGE. Although there is no radioactive gel comparison, the distribution is similar to that of ^{75}Se in rabbit plasma. Two major proteins were labelled; one was approximately 56 kDa and the other was approximately 23 kDa.

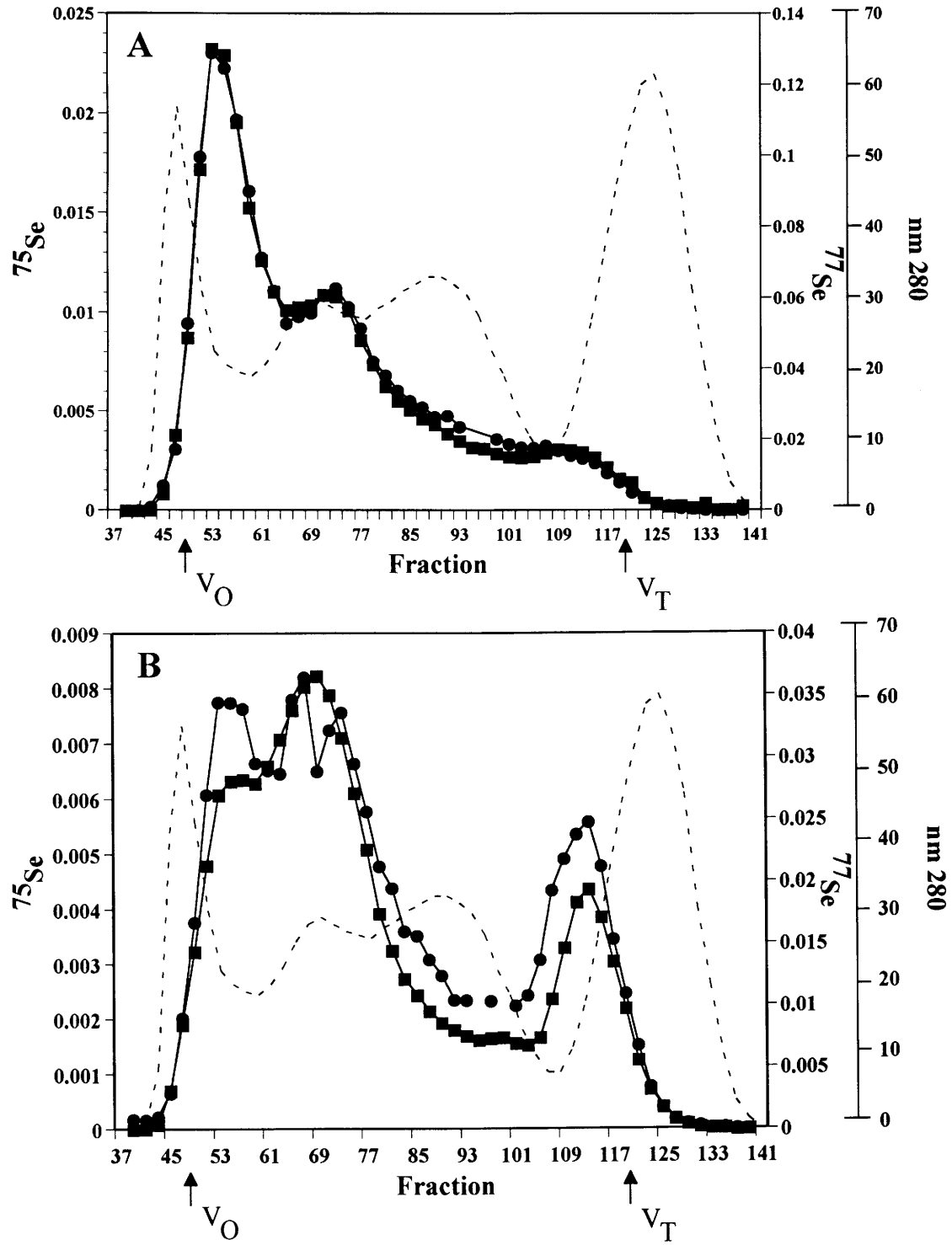

Figure 1. The distribution of tracer Se in the liver cytosol of rats fed diets either deficient or high in Se, and chromatographed on Sephadex G-200. The tracer Se was administered as either radioactive ^{75}Se (—■—), or as stable ^{77}Se (—●—). The values are presented as the percentage of the absorbed dose found in each fraction. Note that the total percentage of ^{77}Se and ^{75}Se incorporated into a peak is different; the *y* axis scale has been presented differently for each isotope to facilitate comparison of the pattern of incorporation of ^{77}Se and ^{75}Se into selenoproteins. The absorption at 280 nm is also shown (- - -). The column void volumn (V_o) and total volumn (V_t) are shown with arrows on the *x* axis. (A) Distribution in cytosol of rats fed a low Se diet. (B) The distribution of Se in the cytosol of rats fed a high Se diet.

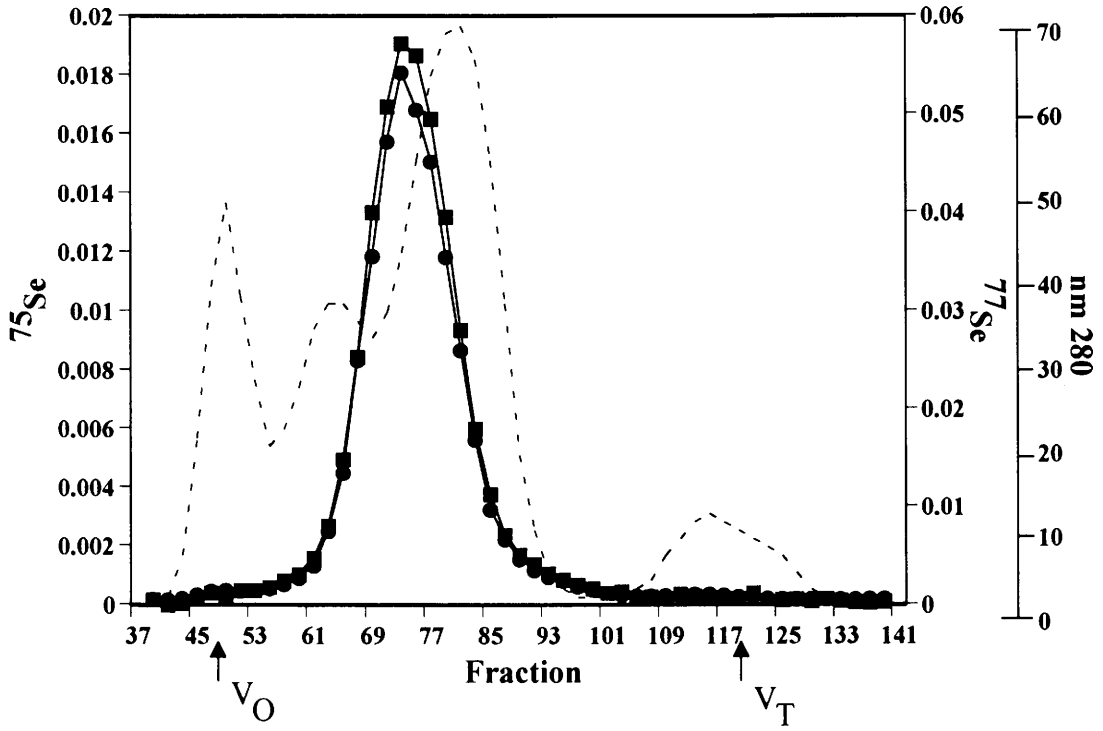

Figure 2. The distribution of radioactive ^{75}Se (—●—) and stable ^{77}Se (—■—) in the plasma of rabbits fed a diet adequate in Se and chromatographed on Sephadex G-200. The absorption at 280 nm is also shown (- - -). Column void (V_o) and total volumn (V_t) shown with arrows on *x* axis. Note that the total percentage of ^{77}Se and ^{75}Se incorporated into a peak is different; the *y* axis scale has been presented differently for each isotope to facilitate comparison of the pattern of incorporation of ^{77}Se and ^{75}Se into selenoproteins.

Discussion

The study of Se metabolism in animals has been greatly aided by studying the incorporation of tracer ^{75}Se into proteins. Such studies have identified many selenium-dependent proteins (2) and have also shown that there is a hierarchy of demand for Se which results in some proteins being labeled preferentially (20, 21). Other studies have shown that the degree to which Se is incorporated into various proteins often depends on the amount of time after Se administration (20).

While much is known about the effects of Se nutriture on domestic animals, relatively little is known about Se metabolism, and the consequences of Se de-

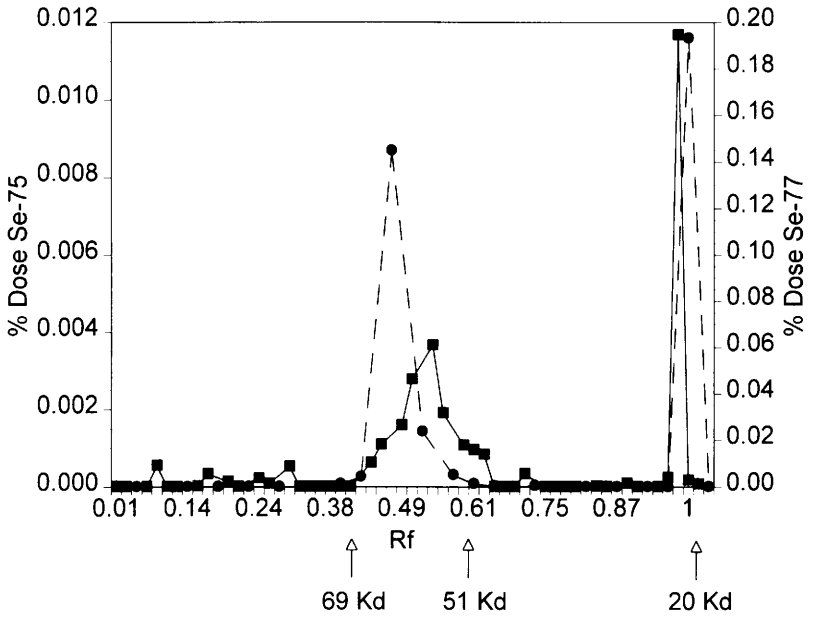

Figure 3. The distribution of isotopic selenium in the plasma of rabbits after separation by SDS-PAGE. Rabbits were injected with radioactive ^{75}Se (—■—) or stable ^{77}Se (—●—).

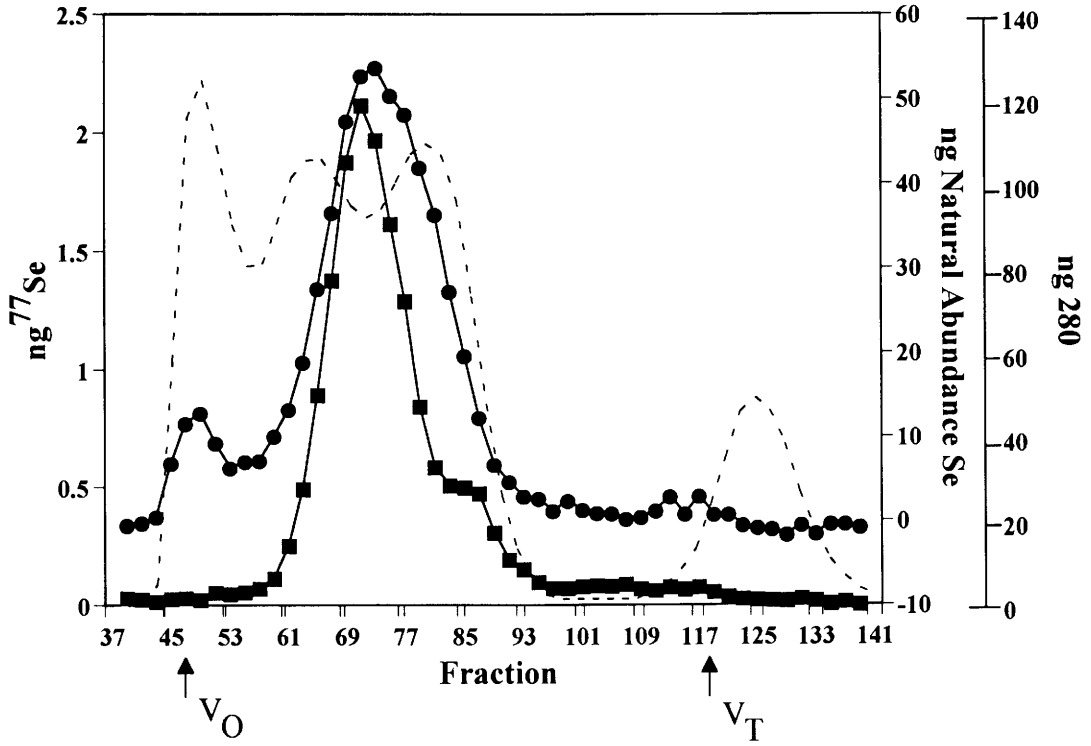

Figure 4. The distribution of naturally occurring Se (—■—) or stable isotopic ^{77}Se (—●—) in human plasma which was chromatographed on Sephadex G-200. The plasma was prepared from a person which was consuming a high Se diet. The absorption at 280 nm is also shown ([0093]-), and the column void volumn (V_o) and total volumn (V_t) are shown with arrows on the *x* axis.

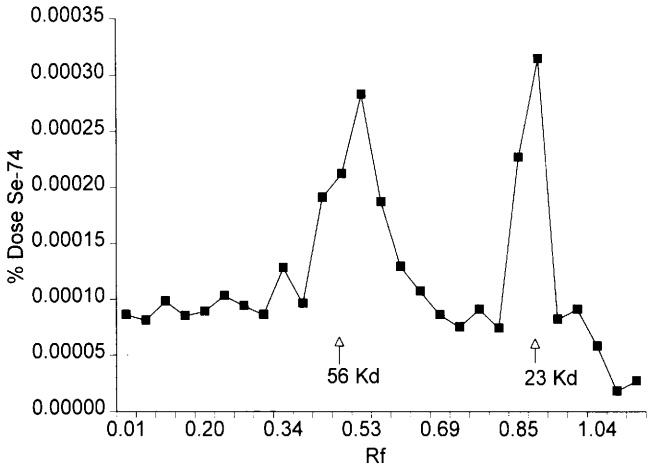

Figure 5. The distribution of stable ^{74}Se among human plasma proteins which were separated by SDS-PAGE. The gels were cut into sections and each section was analyzed by mass spectrometry for total Se and ^{74}Se. The graph shown is a composite of three gels.

ficiency, in humans. The lack of progress in understanding human Se metabolism has been due, in part, to the lack of a suitable isotope that could be used as a tracer in the types of studies previously identified with animals.

The purpose of this study was to validate the use of stable isotopes of Se as tracers of Se incorporation into selenoproteins. Stable isotopes of Se have been used previously in humans to follow the overall movement of Se through the digestive tract or into blood fractions (10, 13–15, 17, 22). This study has shown that a physiologic oral dose of stable Se can be detected in human blood-borne proteins. We have shown that SDS-PAGE can be used to separate the selenoproteins with high resolution, and to estimate molecular weights of those proteins. We have also shown that the pattern of incorporation of stable Se into proteins is similar to the pattern of incorporation of radioactive ^{75}Se; this was true for different species, different tissues, and under conditions of different dietary intakes of Se.

While the pattern of incorporation of stable isotopic Se into proteins was identical to that of radioactive Se, the absolute amount incorporated was different. In rat liver cytosol, a greater amount of ^{77}Se was incorporated, whereas in rabbit plasma, a greater amount of ^{75}Se was incorporated. Since the pattern of incorporation was similar, it does not appear that the isotopes are being metabolized differently. A possible reason for such a difference could be that the redox states of radioactive and stable isotopes differed. Selenite is reactive and may be reduced to Se metal or oxidized to selenate (23). The stable isotope was prepared as selenite from Se metal (18). The radioactive isotope was received as selenite, but nonradioactive sodium selenite was added in order to equalize the total Se dose

between the two groups. Thus, the selenite used in this study came from three different sources and was prepared by three different laboratories. Small differences in techniques and laboratory conditions could have resulted in different amounts of the Se being present as selenite. Also, individual differences in animals could affect the oxidation potential of the gut, which could further alter the amount of Se present as selenite. Another possible reason for the discrepancy is that there is no reliable way to cross calibrate between a mass spectrometer and a γ radiation counter.

In addition to a suitable tracer, metabolic studies examining protein incorporation of Se also need a high resolution method for separating the proteins of interest, and be capable of separating a sufficient amount of protein to allow detection of the isotope by mass spectrometry. Column chromatography yields broad bands with much protein cross-contamination between bands. The semipreparative SDS-PAGE procedure in this report separates proteins into distinct bands and allows for the loading of a sufficient amount of sample so that the gels can be sliced and the stable isotope detected in individual slices. We have found that sample preparation is essential for high resolution; a combination of size exclusion chromatography, dialysis and precipitation was most effective, and removing any of these steps resulted in poor quality and distorted gels.

This study did not attempt to characterize selenoproteins *per se,* but the chromatographic and electrophoretic patterns of selenoproteins observed in this study have been observed previously. A previous study using Sephadex G-150 chromatography showed the same distribution of Se in rat liver cytosol and the same effect of dietary Se status (24). Rat plasma separated by Sephadex G-150 chromatography showed the same Se distribution as rabbit plasma in this study (25).

The use of heparin as an anticoagulant for the human plasma used for SDS-PAGE may be questioned, as heparin binds to selenoprotein-P (26) and can affect its migration on Sephadex gels. However, these samples were used for SDS-PAGE and were prepared by boiling in SDS which should have eliminated the interaction. The molecular weights of the labeled proteins were similar to the molecular weights of selenoproteins reported in the plasma of animals and humans (2). The protein of approximately 60 kDa (rabbit) or 56 kDa (human) is most likely selenoprotein-P (SeP) (27), and the protein of 20 kDa (rabbit) or 23 kDa (human) is most likely plasma GSH-Px (28).

This study has shown that stable isotopes of Se can be used to study molecular aspects of Se metabolism (i.e., they can be used to trace the incorporation of Se into proteins). We have shown that this can be accomplished in humans after feeding a single dose of stable isotope in an amount similar to a normal day's intake. We have also shown that SDS-PAGE can be used to separate proteins in sufficient quantities to measure the stable Se incorporated into the individual protein bands. These techniques will allow a more mechanistic approach to Se metabolism in future human studies.

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1. Rotruck J, Pope A, Ganther A, Swanson A, Hafeman D, Hoekstra W. Selenium: Biochemical role as a component of glutathione peroxidase. Science **179:**588–590, 1973.
2. Sunde RA. Molecular biology of selenoproteins. Annu Rev Nutr **10:**451–474, 1990.
3. Davidson W, Kennedy D. Synthesis of 75Se selenoproteins is greater in selenium-deficient sheep. J Nutr **123:**689–694, 1993.
4. Wilson D, Tappel A. Subcellular fate of selenium from 75-Se-labeled plasma selenoprotein P in selenium deficient rats. J Nutr Biochem **4:**208–211, 1993.
5. Chen C, Whanger P. Effect of vitamin B-12 status on selenium methylation and toxicity in rats: In vivo and in vitro studies. Toxicol Appl Pharmacol **118:**65–72, 1993.
6. Thomson C, Stewart R. The metabolism of 75Se selenite in young women. Br J Nutr **32:**47–57, 1974.
7. Griffiths N, Stewart R, Robinson M. The metabolism of 75-Se selenomethionine in four women. Br J Nutr **35:**373–382, 1976.
8. Buckley WT, Budac JJ, Godfrey DV. Determination of selenium by inductively coupled plasma mass spectrometry utilizing a new hydride generation sample introduction system. Anal Chem **64:**724–729, 1992.
9. Ting BTG, Mooers CS, Janghorbani M. Isotopic determination of selenium in biological materials with inductively coupled plasma mass spectrometry. Analyst **114:**667–674, 1989.
10. Christensen MJ, Janghorbani M, Steinke FH, Istfan N, Young VR. Simultaneous determination of absorption of selenium from poultry meat and selenite in young men: Application of a triple stable isotope method. Br J Nutr **50:**43–50, 1983.
11. Sirichakwal PP, Young VR, Janghorbani M. Absorption and retention of selenium from intrinsically labeled egg and selenite as determined by stable isotope studies in humans. Am J Clin Nutr **41:**264–269, 1985.
12. Swanson C, Reamer D, Veillon C, Levander O. Intrinsic labeling of chicken products with a stable isotope of selenium (^{76}Se) J Nutr **113:**793–799, 1983.
13. Swanson C, Reamer D, Veillon C, King J, Levander O. Quantitative and qualitative aspects of selenium utilization in pregnant and nonpregnant women: An application of stable isotope methodology. Am J Clin Nutr **38:**169–180, 1983.
14. Reamer DC, Veillon C. A double isotope dilution method for using stable selenium isotopes in metabolic tracer studies: Analysis by gas chromatography/mass spectrometry (GC/MS). J Nutr **113:**786–792, 1983.
15. Ducros V, Favier A, Guigues M. Selenium bioavailability as selenite (^{74}Se) and as a selenium drug (^{76}Se) by stable isotope methodology. J Trace Elem Electrolytes Health Dis **5:**145–154, 1991.
16. Janghorbani M, Christensen MJ, Nahapetian A, Young VR. Se-

lenium metabolism in healthy adults: Quantitative aspects using the stable isotope $^{74}SeO_3^2$. Am J Clin Nutr **35**:647–654, 1982.
17. Kasper LJ, Young VR, Janghorbani M. Short-term dietary selenium restriction in young adults: Quantitative studies with the stable isotope $^{74}SeO_3^2$. Br J Nutr **52**:443–455, 1984.
18. Janghorbani M, Christensen M, Steinke F, Young V. Feasibility of intrinsic labelling of poultry meat with stable isotope of selenium (^{74}Se) for use in human metabolic studies. J Nutr **111**:817–822, 1981.
19. Buckley WT, Budac JJ. *TTMR (VER 1.0)*. Agriculture Canada Research Station; P.O. Box 1000, Agassiz, British Columbia, Canada VOM 1A0.
20. Evenson JK, Sunde RA. Selenium incorporation into selenoproteins in the Se-adequate and Se-deficient rat. Proc Soc Exp Biol Med **187**:169–180, 1988.
21. Behne D, Hilmert H, Scheid S, Gessner H, Elger W. Evidence for specific selenium target tissues and new biological important selenoproteins. Biochim Biophys Acta **966**:12–21, 1988.
22. Patterson BH, Zech LA, Swanson CA, Levander OA. Kinetic modeling of selenium in humans using stable isotope tracers. J Trace Elem Electrolytes Health Dis **7**:117–120, 1993.
23. Greenwood NN, Earnshaw A. Chemistry of the Elements. New York: Pergamon, pp882–919, 1984.
24. Sunde R, Hoekstra W. Incorporation of selenium into liver glutathione peroxidase in the Se-adequate and Se-deficient rat. Proc Soc Exp Biol Med **165**:291–297, 1980.
25. Motchnik P, Tappel A. Rat plasma selenoprotein P properties and purification. Biochem Biophys Acta **993**:27–35, 1989.
26. Åkesson B, Mårtensson B. Heparin interacts with a selenoprotein in human plasma. J Inorg Biochem **33**:257–261, 1988.
27. Deagen JT, Butler JA, Zachara BA, Whanger PD. Determination of the distribution of selenium between glutathione peroxidase, selenoprotein P, and albumin in plasma. Anal Biochem **208**:176–181, 1993.
28. Takahashi K, Avissar N, Whitin J, Cohen H. Purification and characterization of human plasma glutathione peroxidase: A selenoglycoprotein distinct from the known cellular enzyme. Arch Biochem Biophys **256**:677–686, 1987.