Effect of Dietary Selenium Level on ^{75}Se Binding to Rat Plasma Proteins[1] (37399)

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Although selenium is known to be an essential element for at least two species (1, 2), knowledge concerning its metabolism is far from complete. Its occurrence in plasma proteins has long been recognized, but identification of specific selenium-containing proteins has been prevented by the use of supraphysiological doses of selenium in ^{75}Se injections and lack of suitable protein separation methods.

More recently Millar (3) has applied gel filtration to the study of selenium-containing plasma proteins from rats fed a diet containing 0.07 ppm selenium and has shown ^{75}Se binding in a single peak. The present study also utilized gel filtration and demonstrates differences in ^{75}Se binding to plasma proteins related to dietary selenium level.

Materials and Methods. The torula yeast diet used in this work contained adequate vitamin E but in its basal form only 0.024 ppm selenium (4). The supplemented diet contained 0.5 ppm selenium as Na_2SeO_3 added in sucrose. Male Holtzman rats [3] were fed the experimental diets *ad libitum* from weaning (wt 50–60 g) and were given tap water. When studied they weighed 200 to 300 g. Blood was taken from the aortic bifurcation under ether anesthesia, and EDTA (2 mg/ml) was added to prevent coagulation.

^{75}Se was injected intraperitoneally in 1 ml of 0.9% NaCl in the form of $H_2{}^{75}SeO_3$ (New England Nuclear, Boston, MA). ^{75}Se determinations were carried out with an automatic gamma-counting system using the 0.40 MeV ^{75}Se photopeak.

Gel filtration was done on Sephadex G200 in a 2.5 cm diameter column. Void volume was 75.5 ml and total bed volume was 196 ml. Sample size was 1.5 ml. Buffer was 0.1 *M* Tris–HCl, pH 7.6. Samples in 20% sucrose were layered on top of a sample applicator and constant downward flow was maintained with a pump. Polyacrylamide gel electrophoresis was performed according to the manufacturer's instructions with an Ortec 4200 electrophoresis system (Ortec, Inc., Oak Ridge, TN) on plasma and samples from peaks A and B collected 24 hr after injection of 100 μCi of $^{75}SeO_3{}^{2-}$ (dose 1.44 μg selenium/kg) into a rat fed the 0.5 ppm selenium diet.

Dialysis was carried out in stirred 4 liter baths at 4° using dialysis tubing with a pore radius permeability of 24 Å. Sample size was 0.5 ml and an undialyzed sample was used for calculating ^{75}Se loss. After dialysis the ^{75}Se content of samples was determined while they were still in the dialysis bags.

Results. Figure 1, which is typical of over 20 experiments, shows gel filtration chromatograms of plasma obtained from rats 72 hr after injection of 40 μCi of $^{75}SeO_3{}^{2-}$

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[3] In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

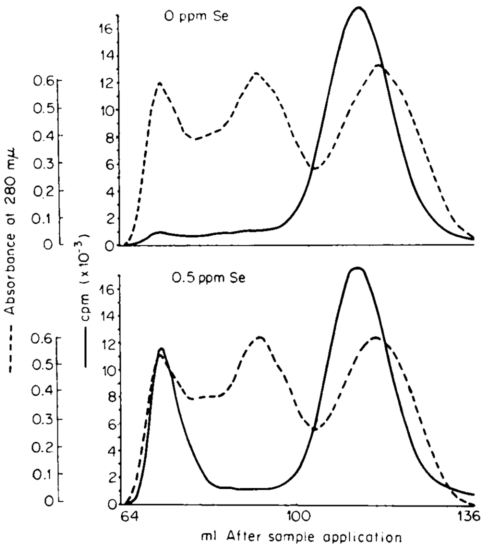


FIG. 1. Sephadex G200 chromatograms of plasma collected 3 days after a submicrogram dose of $^{75}SeO_3^{2-}$. The cpm are graphed per 2.3 ml fraction. Peak B (^{75}Se) was eluted at 112 ml in both experiments while peak A (^{75}Se) was seen at 72 ml only in the experiment using plasma from the animal fed 0.5 ppm selenium.

(dose 0.69 μg selenium/kg) when the elution buffer contained no NaCl. It demonstrates that both the animal fed the basal diet (0 ppm Se) and the one fed the supplemented diet (0.5 ppm Se) had a ^{75}Se peak (peak B) just before the last protein peak but that only the supplemented animal had a ^{75}Se peak at the void volume (peak A). When these samples were subjected to gel filtration using the same buffer except for the addition of 0.1 *M* NaCl (not shown) no change was noted in the chromatogram from the animal fed the basal diet. However, peak A disappeared from the chromatogram of the plasma from the supplemented animal and peak B was proportionately larger.

The eluate fractions comprising each ^{75}Se peak in Fig. 1 were combined and dialyzed against several baths and the results are shown in Table I. Very little ^{75}Se could be removed by dialysis against 0.60 *M* NaCl or 0.5 *M* mercaptoethanol. The alkaline bath, however, removed nearly all the ^{75}Se and did it faster and more completely from peak A than from peak B. No effect of dietary selenium was noted on the dialysis results of peak B.

Electrophoresis of rat plasma collected 24 hr after injection of $^{75}SeO_3^{2-}$ and its peaks A and B are shown in Fig. 2. Three peaks of radioactivity are noted in the plasma and two in each of the pooled peaks.

Discussion. This work confirms the observation by Millar (3) that ^{75}Se is found in a single peak (peak B) on gel filtration under some column conditions. However, it also shows that the peak separates into two components when NaCl is eliminated from the elution buffer if the rat has not been fed a selenium-deficient diet. Thus, the existence of peak A seems to indicate that the animal has been receiving selenium in the diet and may indicate absence of deficiency.

Other studies (5) showed that peak B appeared in the plasma about 30 min after injection and peak A appeared between 6 and 24 hr after injection. Also, neither peak could be produced by *in vitro* incubation of $^{75}SeO_3^{2-}$ with plasma or whole blood (5) suggesting that the selenium was incorporated during protein synthesis or that it had to undergo chemical transformation before it would bind in either peak. These observations all point to incorporation of selenium

TABLE I. Dialysis of Plasma ^{75}Se Peaks from Fig. 1.

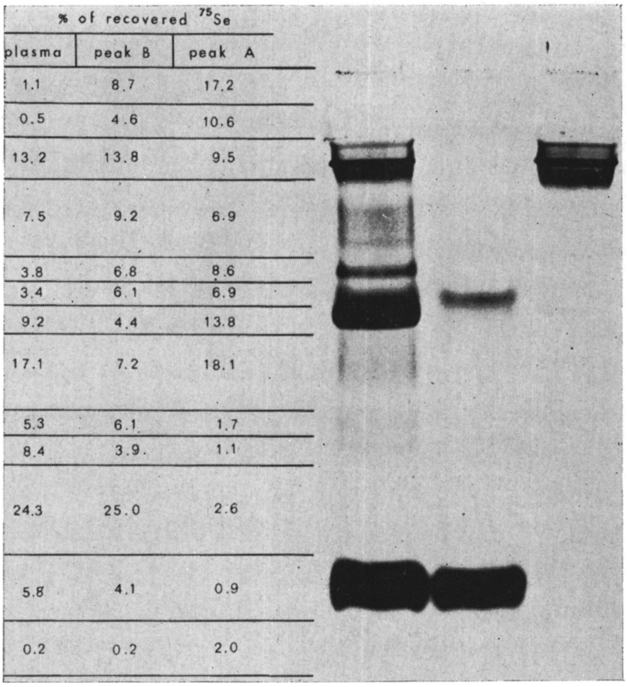
Sample	Dialysis time (hr)	Percentage of ^{75}Se lost during dialysis[a]; dialysis baths: NaCl[b]	ME[c]	Alkaline[d]
0 Se peak B	3	2	7	53
	24	3	4	84
0.5 Se peak B	3	5	6	51
	24	4	4	87
0.5 Se peak A	3	3	8	71
	24	10	9	98

[a] Each value is a single determination rounded to the nearest percent.

[b] 0.6 *M* NaCl, measured pH 6.25.

[c] 0.5 *M* mercaptoethanol and 0.6 *M* NaCl, measured pH 5.56.

[d] 0.5 *M* NaOH and 0.15 *M* NaCl, measured pH 11.82.



% of recovered ^{75}Se		
plasma	peak B	peak A
1.1	8.7	17.2
0.5	4.6	10.6
13.2	13.8	9.5
7.5	9.2	6.9
3.8	6.8	8.6
3.4	6.1	6.9
9.2	4.4	13.8
17.1	7.2	18.1
5.3	6.1	1.7
8.4	3.9	1.1
24.3	25.0	2.6
5.8	4.1	0.9
0.2	0.2	2.0

FIG. 2. Polyacrylamide gel electrophoresis of plasma, peak B, and peak A collected 24 hr after submicrogram ^{75}Se injection. The gel slab was stained with amido swartz and sliced manually with a razor blade. Percentage of recovered counts in each gel slice is indicated on the left. Approximately 150 μg of protein was applied in the plasma sample. The cpm applied to the gel in this experiment were: plasma 1932 cpm, peak B 1005 cpm, peak A 402 cpm. Counting time was 40 min for each gel slice. Recoveries of ^{75}Se applied were 66.0% for plasma, 54.0% for peak B, and 86.6% for peak A.

into a few specific proteins.

The electrophoresis data suggest that peaks A and B may each be composed of two or more components and this is in agreement with Millar's findings (3). However, 13.4 to 46% of the ^{75}Se applied to the gel slab was not recovered. Other experiments have shown similar poor recoveries with ion exchange chromatography (5). All these findings indicate that the selenium–protein bond may be broken or that selenium-containing protein may be altered under some experimental conditions.

There have been many other studies of selenium binding by plasma proteins (3, 6–10), but most of them have used supraphysiological selenium doses and most also separated proteins by paper electrophoresis so that comparison of the present work with them is difficult except in the case of the study by Millar. In general, however, those studies indicated that selenium was localized to certain protein fractions and that it was associated with the albumin fraction only under conditions of selenium excess.

The nature of the selenium–protein bond in peaks A and B remains obscure. ^{75}Se does not appear to be in the form of selenotrisulfides because reduction with mercaptoethanol does not release it. However, alkaline dialysis quite effectively releases it. Jenkins (11) found selenotrisulfides in chick plasma 4 hr after a massive dose of selenium, but in another study (5) none were noted at 3 hr in plasma from rats given a submicrogram selenium dose. This suggests that selenotrisulfide formation may occur only in the presence of excess selenium and raises doubts as to whether the selenotrisulfide is a physiological form of selenium in proteins.

Summary. Two peaks of protein-bound ^{75}Se are seen on the gel filtration chromato-

gram of rat plasma when the rat has been fed a 0.5 ppm selenium diet and no NaCl is added to the Tris–HCl elution buffer. One of those peaks is absent from the plasma of rats fed a very low selenium diet. Polyacrylamide gel electrophoresis of the ^{75}Se peaks suggests that each may have more than one protein component. Dialysis studies indicate that the ^{75}Se is not in the form of selenotrisulfides.

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