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Transport of Selenate and Selenite into Astragalus Roots[1]

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Abstract. **After incubation for 1 hr with ^{75}Se-selenate, excised roots of *Astragalus crotalariae*, a selenium-accumulating species, and *A. lentiginosus*, a nonaccumulator, had absorbed radioactivity to levels well over the external concentration. About 98% of the radioactivity was ethanol-soluble, and when analyzed by column and paper chromatography and by electrophoresis proved to be selenate. This and previous evidence shows an active transport for selenate. Considerably less radioactivity was absorbed when ^{75}Se-selenite was supplied to the excised roots, and levels of the ethanol-soluble radioactivity did not exceed the external concentration. A good deal of the radioactivity was ethanol-insoluble. Analysis of the soluble radioactivity from both species showed appreciable conversion of selenite to other forms.**

Uptake of selenate by excised roots of *Astragalus* species appears to involve an active transport system. The evidence rests primarily on a marked accumulation of radioactivity when roots are incubated with ^{75}Se-labeled selenate, on the effects of respiratory inhibitors such as azide, dinitrophenol, and low temperatures, and on the complete inhibition by sulfate, a competitive antagonist of selenate (15). Roots incubated with radioactive selenite also have given an accumulation ratio greater than one, but evidence for an active transport of this ion is unclear because of some apparent non-biological uptake (15). Since selenate and selenite are both assimilable ions (1, 9, 10, 11, 12, 16, 17), accumulation of selenium by these roots could be either an accumulation of the ions against an electrochemical gradient or an accumulation of seleno-metabolites whose synthesis is energy dependent. The data presented here will show that selenate can accumulate largely unchanged in excised roots of 2 species of *Astragalus;* much of the selenite however, is changed into other selenium compounds or possibly adsorbed.

Materials and Methods

Preparation and Growth of Seedlings. Techniques described earlier for the preparation and growth of seedlings (15) were used in these experiments but modified as follows: after germination in the dark for 2 days (21–22°), the seedlings were laterally illuminated before a bank of fluorescent lights at an intensity of 4300 ft-c for 3 more days (22–25°).

Ion Absorption Procedure. The preparation of root tips and the conditions for the uptake of selenate and selenite were identical with the procedures described earlier (15).

Fractionation of Radioactive Tissues. At the end of the uptake period, roots were blotted on filter paper and weighed rapidly. For measurement of the total radioactivity absorbed, roots were prepared as described earlier (15). For measurement of the ethanol insoluble fraction, the weighed roots were successively extracted with 70% (v/v) ethanol 6 to 8 times until the radioactivity in the ethanol extract was approximately 1% of the radioactivity in the first extraction. The acid digestion mixture was slowly added to the extracted roots to avoid foaming, and the extracted roots were digested as described earlier (15). A precipitate which formed in the digest of the ethanol-extracted roots had no effect on the counts. Samples for total and ethanol-insoluble radioactivity were run in triplicate.

For biochemical analysis of soluble radioactive components, weighed roots from 24 colanders were pooled and extracted with 48 ml of 70% ethanol.

Column Chromatography. The use of Dowex-1-Cl columns for identification of selenite and selenate has been described previously (12).

Paper Chromatography. Whatman No. 1 was used throughout. The following solvent systems were used: No. 1, methanol-pyridine-water (85-4-15, v/v); No. 2, *n*-butanol-glacial acetic water (12-3-5, v/v); No. 3, methanol-pyridine-water (1-1-1, v/v).

Paper Electrophoresis. Electrophoresis was carried out on Whatman No. 1 paper with a Gelman electrophoresis apparatus. Acetate buffer, pH 5.4 was used at a voltage of about 300 volts. The papers were run for varying time periods. After the papers were dried, they were either cut into 1 cm segments and counted, or scanned with a Nuclear Chicago Actigraph III Scanner.

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Counting. Samples were counted in a Packard Autogamma Spectrometer, series 410A at 405 Kev and 1 % window width.

Results

Accumulation of Selenate. The accumulation of selenium in excised root tips of 2 *Astragalus* species after incubation with ^{75}Se-labeled K_2SeO_6 is summarized in table I. Though the conditions for growth of the seedlings in these experiments were somewhat different from those in previous experiments (15), the same pattern of selenate uptake by the 2 species was observed. Almost all of the selenium taken up by both species could be extracted with 70 % ethanol.

The ethanol extracts derived from the 2 species were analyzed directly by Dowex-1-Cl column chromatography. Complete recoveries from columns were obtained. A single major peak, containing 98 % of the recovered radioactivity, emerged with 1.0 N HCl at the position of selenate.

The 1.0 N HCl peak was lyophilized and then analyzed by paper chromatography and H. V. paper electrophoresis. The results are summarized in table II. One component, selenate, was identified.

Uptake of Selenite. Data for the uptake of ^{75}Se-selenite are summarized in table I. Compared to selenate, much less selenite was taken up by the roots of the 2 species. This relationship was observed in earlier experiments, though the total amount of selenite taken up and the accumulation ratios found at that time were considerably higher (15). The reasons for these differences are unknown but are perhaps attributable to the changes in temperature and illumination that were made during the germination and growth of the seedlings.

Ethanol fractionation of the radioactive roots revealed that both species had converted appreciable amounts of selenite into an insoluble form. This conversion was particularly evident in roots of *A. lentiginosus*, the nonaccumulator, in which only 23 % of the total radioactivity that had been absorbed remained soluble. Selenate by contrast, was barely converted into an insoluble form. These findings are in keeping with those reported for excised leaves of these plants (12, 17) and for the duckweed, *Spirodela oligorrhiza* (1).

Table II. *Paper Chromatography and High-Voltage Paper Electrophoresis of Radioactive Peak Eluted From Dowex-1-Cl by 1.0 N HCl*

Species	Paper chromatography Solvent 1	Solvent 2	Solvent 3	High voltage electrophoresis Distance from origin
	R_F values			*cm*
A. lentiginosus				
Standard SeO_4	0.52	0.02	0.66	9.4
1.0 N HCl peak	0.52	0.03	0.66	9.8
A. crotalariae				
Standard SeO_4	0.48	0.04	0.61	6.0
1.0 N HCl peak	0.48	0.05	0.63	5.7

The Dowex-1-Cl elution patterns of the ethanol extracts (Fig. 1) illustrate that large proportions of the soluble radioactivity had been converted to other forms. *A. crotalariae*, the accumulator species, in particular, converted almost all of the soluble radioactivity into a neutral or basic form. Recovery from the column was approximately 93 %. The neutral or basic peak contained 92 % of the recovered radioactivity. Two smaller peaks also appeared. The 0.1 N HCl peak at the position of selenite contained 3.5 % of the recovered radioactivity, and the 1.0 N HCl peak at the position of selenate contained 2.3 %.

The elution pattern from *A. lentiginosus* revealed 3 substantial peaks, the neutral or basic peak with 19 % of the recovered radioactivity, the 0.1 N HCl peak with 51.4 %, and the 1.0 N HCl peak with 22.9 %. Total recovery from the column was 86 %. Low recoveries have been noted before (12); the reason is unknown, but it is not likely to be due to volatilization of selenite, as would occur with sulfite under acid conditions, since selenite is stable in dilute HCl (19, p 332).

Table I. *Uptake of ^{75}-Se-selenate and ^{75}Se-selenite by Excised Root Tips*

Excised root tips of *Astragalus* were incubated with K_2SeO_4 and K_2SeO_3, 0.01 μmole/ml, for 1 hr.

	Selenate		Selenite	
	A. crotalariae	*A. lentiginosus*	*A. crotalariae*	*A. lentiginosus*
Total Se absorbed (μmoles/g fresh wt)	0.0742	0.227	0.0123	0.00741
EtOH-soluble Se (μmoles/g fresh wt)	0.0740	0.223	0.0100	0.00169
% soluble	99.7	98.2	81.3	22.8
Accumulation ratio (soluble Se/external Se)	7.4	22.3	1.0	0.17

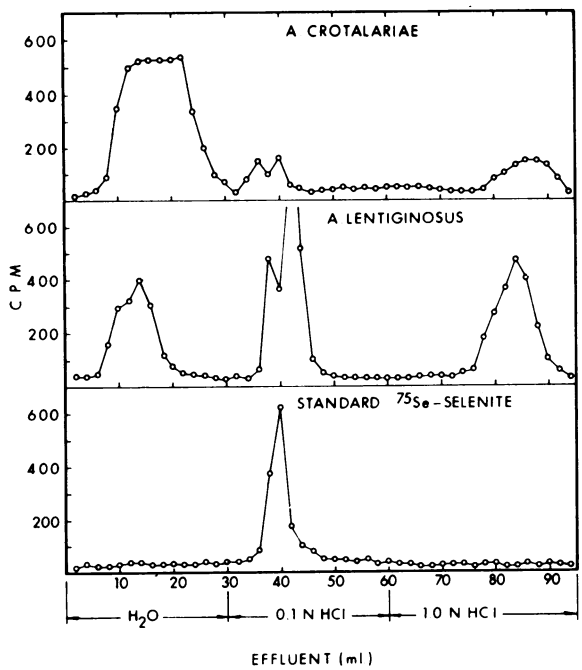

FIG. 1. Dowex-1-Cl elution patterns of 70 % ethanol extracts from excised roots of 2 *Astragalus* species incubated with ^{75}Se-selenite.

Discussion

By all currently accepted criteria, transport of selenate into excised roots of 2 species of *Astragalus* is an active process. The ion accumulates against a concentration gradient, and its entry is sensitive to respiratory and competitive inhibitors. In these many features, accumulation of selenate is comparable to that of sulfate for which active transport has been reported in the roots (2, 7) and leaves (4, 5) of higher plants as well as in algae (6, 18).

The mode of entry of selenite is not as clearcut. Earlier studies showed inhibition by azide, dinitrophenol and low temperature, but the percent inhibition was less than for selenate. Sulfate, which completely eliminated selenate uptake, was partially effective against selenite (15); sulfate, however, is a structural analogue of selenate, and not of selenite, and the reason for its antagonism to selenite is not understood. In these earlier experiments an accumulation ratio greater than one was noted. Under the conditions of the present experiments, however, selenium failed to accumulate at levels higher than the levels in the incubation medium. Uptake of selenite, therefore, may be more dependent on experimental conditions than is uptake of selenate.

Much of the selenite absorbed by the excised roots of both species was changed into other forms. Appreciable selenium appeared in the ethanol-insoluble fraction; of the total absorbed by *A. crotalariae*, 19 % was insoluble; by *A. lentiginosus*, 77 %. These differences between the 2 species may be significant in view of the suggestions recently made by Peterson and Butler to account for the ability of accumulator species of *Astragalus* and other genera to tolerate such high internal levels of selenium (9). According to their interpretation, accumulator species shunt selenium into non-toxic, non-protein seleno-amino acids such as Se-methylselenocysteine and selenocystathionine; non-accumulator plants, however, have not evolved this shunt mechanism, and selenium enters their proteins as selenomethionine and selenocysteine. This would account for the sensitivity of nonaccumulator species to the low concentrations of selenium which are tolerated by accumulators under experimental conditions (14).

The ethanol soluble radioactivity from *A. crotalariae* treated with selenite contained almost no selenite, as evident from the Dowex-1-Cl elution pattern. Almost all of the radioactivity appeared as a neutral or basic form, very likely Se-methylselenocysteine, the amino acid identified as the major component in leaves of this and other accumulator species (12, 13). In *A. lentiginosus*, about 19 % of the radioactivity in the ethanol extract emerged in the neutral or basic fraction and probably contained Se-methylselenomethionine which has been identified in leaves of this nonaccumulator species (17). About 51 % of the radioactivity appeared at the position of selenite, although the components of this peak have not been identified further.

In both *A. lentiginosus* and *A. crotalariae* a peak emerged with 1.0 N HCl at the position of selenate. The peak was particularly large in *A. lentiginosus*, amounting to 23 % of the recovered radioactivity. Though the position in the column eluate is insufficient evidence for a conclusive identification, the existence of this peak raises the possibility that these tissues are able to oxidize selenite to selenate. It must be pointed out, however, that non-metabolic reaction products are also possible, since selenite is a reactive ion and has been shown to react with sulfhydryl-containing metabolites such as cysteine, glutathionine and coenzyme A to give rise to a variety of seleno-derivatives (3).

The results of these studies show that uptake of selenite differs from that of selenate. At this point it cannot be said whether the initial step in entry of selenite is metabolically dependent, or whether it enters continuously by diffusion to be rapidly assimilated into other forms by energy dependent reactions. Uptake of selenate, by contrast, is clearly *via* active transport. Such a mechanism is not restricted to this selenium compound, however, but has also been demonstrated for selenomethionine across the hamster intestine (8). Not only are cells able to enzymically assimilate selenium compounds, but an active transport mechanism which is recognized as the mode of entry for many nutrients operates for several selenium compounds as well.

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