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BIOSYNTHESIS OF *Se*-METHYLSELENOCYSTEINE IN LIMA BEANS

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Abstract—It has been shown that maturing seeds of lima beans synthesize *Se*-methylselenocysteine, the selenium analogue of *S*-methylcysteine. The latter amino acid is a natural constituent of these seeds. The leaves of lima beans do not contain detectable amounts of *S*-methylcysteine, and in this tissue *Se*-methionine appears to be the principal product of selenate assimilation.

INTRODUCTION

A NUMBER of investigators have recently studied the uptake and metabolism of inorganic selenium by plants, and have found them capable of synthesizing selenium analogues of such sulphur amino acids as methionine,[1-5] cystine,[1,3,4] *S*-methylcysteine,[2] *S*-propenylcysteine-sulphoxide[6] and cystathionine.[7] The inference from these studies has been that same enzyme systems may be involved in the assimilation of sulphur and selenium.[8] Our investigation of the distribution and biosynthesis of *S*-methylcysteine and *Se*-methylselenocysteine in species of *Astragalus* also point in the same direction.[9-11] Because of these considerations, it appeared likely that selenium non-accumulating plants that synthesize *S*-methylcysteine might also synthesize *Se*-methylselenocysteine if supplied with selenium at suitable concentrations.

In the present communication we wish to report the synthesis of *Se*-methylselenocysteine in lima beans, a plant which is not a selenium accumulator, but which is known to contain *S*-methylcysteine.[12]

RESULTS AND DISCUSSION

Table 1 gives the quantities of *S*-methylcysteine and its glutamyl peptide in the seeds from lima beans supplied with nutrient solution containing no selenium (seeds II), 32 ppm of selenium (seeds III) and 64 ppm of selenium (seeds IV); the selenium being supplied in the form of sodium selenate. The concentration of total *S*-methylcysteine in the seeds declines with increasing selenium concentration, which is in agreement with the earlier

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results of Chow *et al.*[11] with *A. bisulcatus*. Thus, it appears, that in lima beans, as in *A. bisulcatus* selenate 'competes' with sulphate in metabolic processes leading to the synthesis of *S*-methylcysteine. However, in lima bean seeds *Se*-methylselenocysteine did not accumulate in sufficient quantity for detection by either amino acid analysis or PC. Radioactive tracer techniques were used to demonstrate the synthesis of *Se*-methylselenocysteine in the lima beans.

TABLE 1. CONCENTRATION OF *S*-METHYLCYSTEINE AND γ-GLUTAMYL-*S*-METHYLCYSTEINE IN LIMA BEAN SEEDS

Compound	Seeds* I	Seeds† II	Seeds‡ III	Seeds§ IV
		μmol/g dry tissue weight		
S-Me-Cysteine	3·1	3·8	1·7	1·8
γ-Glu-*S*-Me-cysteine	3·6	1·6	1·5	0·9
Total *S*-Me-cysteine	6·7	5·4	3·2	2·7

* Commercial seeds.

† Seeds from plants given nutrient solution containing 64 ppm of sulphur and no selenium.

‡ Seeds from plants given nutrient solution containing 64 ppm of sulphur and 32 ppm of selenium.

§ Seeds from plants given nutrient solution containing 64 ppm of sulphur and 64 ppm of selenium.

Figure 1 shows the distribution of radioactivity and ninhydrin colour in effluent fractions obtained after column chromatography of an aliquot of extract from seeds that had been given 32 ppm of selenium as $Na_2{}^{75}SeO_4$. The first radioactive peak is probably due to inorganic selenate.[2] The second peak coincides exactly with the ninhydrin peak of carrier *Se*-methylselenocysteine. The peak was found to be radiochemically pure by PC, and by

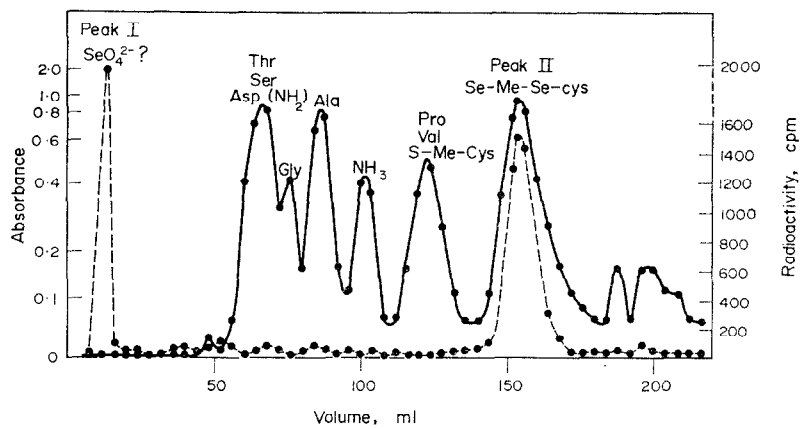

FIG. 1. ION EXCHANGE CHROMATOGRAPHY OF AMINO ACIDS OF LIMA BEAN SEEDS. Extract of 100 mg of seed powder chromatographed on a column of Dowex-50 × 4 (1 × 40 cm H^+ form). The column was eluted with 1 N HCl and 2 ml fractions collected. Entire fractions were counted in a gamma counter and 200 λ aliquots treated with ninhydrin. Solid line indicates ninhydrin colour, and broken line, radioactivity.

amino acid analysis (Fig. 2), and accounted for $1{\cdot}9 \times 10^4$ cpm (0·9%) of the $2{\cdot}32 \times 10^6$ cpm absorbed by the pods. From the amount of radioactivity and selenate ($5{\cdot}36 \times 10^6$ cpm and 1·6 μmol) the concentration of *Se*-methylselenocysteine in the seeds is estimated to be at least 0·03 μmol/g. No radioactivity was found in *Se*-methionine and *Se*-cystine. However, when $^{75}SeO_4^{2-}$ was given to the leaves of lima beans, *Se*-methionine was most highly labeled. It accounted for $1{\cdot}6 \times 10^4$ cpm (0·8%) of the $1{\cdot}88 \times 10^6$ cpm taken up by the leaves. *Se*-methylselenocysteine and *Se*-cystine contained only $2{\cdot}7 \times 10^3$ cpm and $1{\cdot}4 \times 10^3$ cpm respectively of radioactivity taken up by leaves.

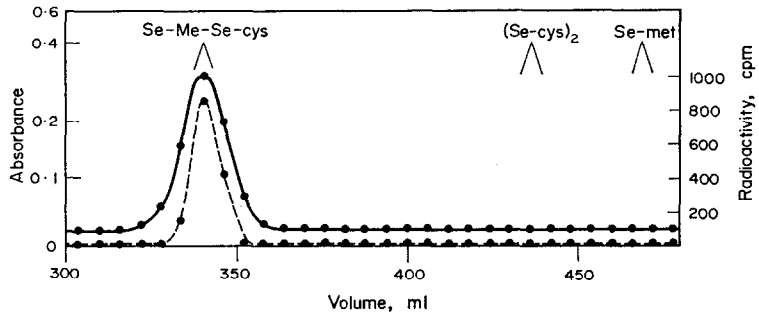

FIG. 2. RADIOCHEMICAL PURITY OF *Se*-METHYLSELENOCYSTEINE.
Se-Methylselenocysteine having 3440 cpm analyzed on the amino acid analyzer as described before.[9] The effluent was collected in 3 ml fractions, and scanned for both radioactivity and ninhydrin colour. About 84% of the radioactivity (2880 cpm) was recovered in the *Se*-methylselenocysteine peak. Solid line indicates ninhydrin colour and broken line, radioactivity.

On the basis of the present work, it is concluded that lima beans are capable of synthesizing the selenium analogues of the sulphur amino acids methionine, cystine and *S*-methylcysteine. On the evidence of the incorporation of $Na_2{}^{75}SeO_4$ into the free amino acids only, it appears that in the leaves selenium is most effectively incorporated into *Se*-methionine, an analogue of protein amino acid methionine, whereas in the seeds, selenium is best incorporated into *Se*-methylselenocysteine, an analogue of the non-protein amino acid *S*-methylcysteine.

EXPERIMENTAL

Materials. Lima bean seeds (thorogreen bush) were purchased from McFayden Seed Co., Brandon, Manitoba. $Na_2{}^{75}SeO_4$ was purchased from Amersham/Searle, Illinois, U.S.A. L-*Se*-Methylselenocysteine was isolated from the foliage of *A. bisulcatus*.[13]

Methods. Lima bean seeds were germinated and grown in pots of soil in the green-house. 2 weeks after germination selenium was given by way of the nutrient solution to two pots at concentrations of 32 and 64 ppm. The Na_2SeO_4 was dissolved in nutrient solution[14] containing 64 ppm of sulphur as sulphate. 100 ml of this solution was given to each pot once every week. The plants that were receiving 64 ppm of selenium grew poorly. However, 1 plant produced a single pod. The growth of the plants receiving 32 ppm of selenium was comparable with plants receiving selenium free nutrient solution (control plants). After the pods had grown to 5–7 cm in length, two pods from the control plants, two pods from the plants receiving 32 ppm of selenium, and the lone pod from the plants receiving 64 ppm of selenium were removed from the plants. The seeds of these were dried and extracted with 30% EtOH.[9] The quantities of *S*-methylcysteine and *Se*-methylselenocysteine in these extracts were determined by amino acid analysis with the Hitachi–Perkin–Elmer amino acid analyser Model KLA3B.[9] The extracts were also subjected to PC in *n*-BuOH–pyridine–H_2O (1:1:1), and the crhomatograms were sprayed with ninhydrin and starch iodide reagents.[15]

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Two ^{75}Se radio tracer experiments were performed as follows: 4 pods from the plants receiving 32 ppm of selenium were given 5·36 × 10^6 cpm of $Na_2{}^{75}SeO_4$ dissolved in 4 ml of nutrient solution containing 32 ppm of selenate selenium for a period of 24 hr through the cut ends of their stalks. The pods were kept in light for the entire period of uptake. At the end of this period the seeds were removed from the pods, dried and (200 mg) were extracted with 30% EtOH. Carrier L-*Se*-methylselenocysteine (2·4 mg) was added to one-half of the extract and radioactive *Se*-methylselenocysteine then isolated by ion-exchange column chromatography on Dowex 50 × 4 column (Fig. 1). The purity of the isolated *Se*-methylselenocysteine was checked by amino acid analysis (Fig. 2). In the second experiment 9·04 × 10^6 cpm of $Na_2{}^{75}SeO_4$ was given to the leaves through the cut ends of their petioles for a period of 6 hr in continuous light, and the leaves extracted as above. The distribution of radioactivity in *Se*-methylselenocysteine, *Se*-methionine and *Se*-cystine in this extract was determined by chromatography on a column of Dowex 50 × 4 as above, and by amino acid analysis with the amino acid analyser of an aliquot after addition of carrier. DL-*Se*-methylselenocysteine, DL-*Se*-methionine and DL-*Se*-cystine. Procedures for column chromatography and amino acid analysis have been described earlier.[9–11]

All radioactivity measurements were carried out with a Nuclear-Chicago sodium-iodide (Thallium) scintillation counter.

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