PN 1285

Biosynthesis of selenocystathionine from selenate in *Stanleya pinnata*

Many species of Astragalus, Oonopsis, Xylorrhiza and Stanleya can accumulate selenium from the soil. Selenium has been shown to be essential for several species of Astragalus[1], and presumably plays an important role in the metabolism of the other selenium accumulators. The nature of organic selenium compounds that occur and their biosynthesis in selenium-accumulator plants, deserve greater attention. An understanding of the interrelationship between sulfur and selenium metabolism is also important. HORN AND JONES[2] obtained a crystalline amino acid complex containing sulfur and selenium from air dried plants of *Astragalus pectinatus*. From its empirical formula, the authors inferred that it consisted of a mixture of cystathionine and its selenium analogue. TRELEASE *et al.*[3] demonstrated the presence of Se-methylselenocysteine in an extract from dried leaves of *A. bisulcatus*. Recently, SHRIFT AND VIRUPAKSHA[4] have shown the biosynthesis of Se-methylselenocysteine from selenite in *A. crotalariae* and *Oonopsis condensata*. This report deals with experiments in which [^{75}Se]selenate, supplied to *Stanleya pinnata*, was in part converted to the selenium analogue of cystathionine.

[^{75}Se]selenate was prepared from carrier-free $H_2{}^{75}SeO_3$ (obtained from Oak Ridge National Laboratory) according to a modification of the method of MCCONNELL (personal communication). About 15 ml of conc. HNO_3 were added to $H_2{}^{75}SeO_3$ and evaporated slowly on a H_2SO_4 bath at 130–140°. Digestion with conc. HNO_3 was repeated twice. 5 ml of 30 % H_2O_2 were added to the residue and evaporated slowly. Digestion with 5 ml portions of H_2O_2 was continued until the reaction mixture showed

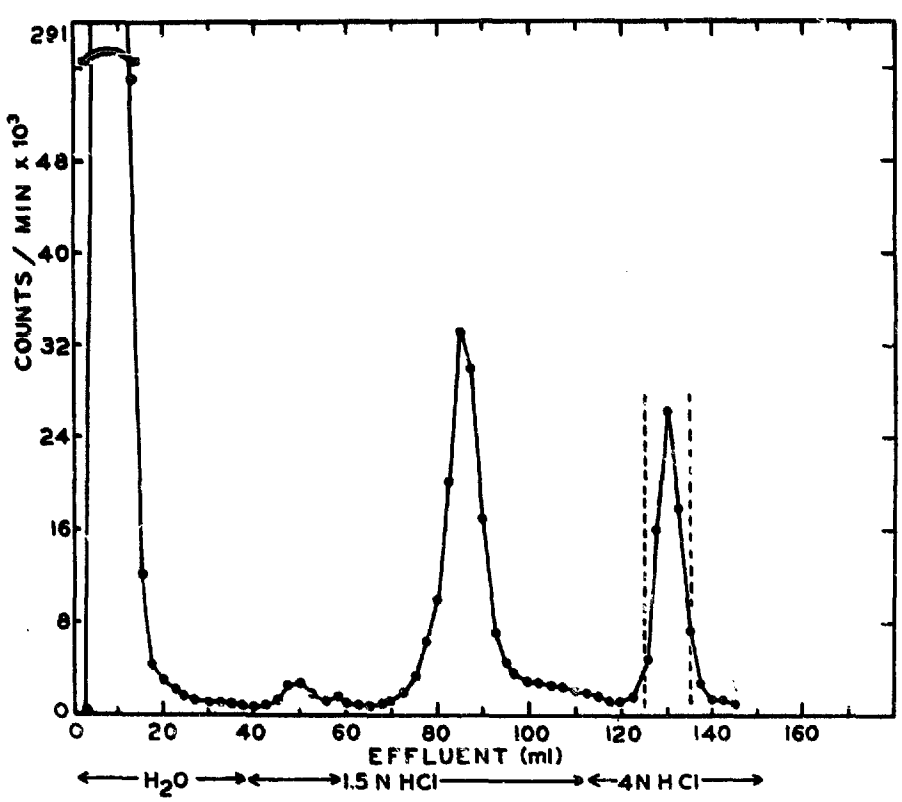

Fig. 1. Column chromatography of trichloroacetic acid extract from *Stanleya pinnata* leaves supplied with [^{75}Se]selenate; on Dowex-50 X8, H^+ form, 200–400 mesh, 1 × 14-cm column; 2.5-ml fractions collected.

only traces of selenite by high-voltage paper electrophoresis at pH 6.4. The [^{75}Se]-selenic acid was dissolved in a small amount of H_2O and aliquots were administered to plants.

Seeds of *Stanleya pinnata* var. *bipinnata* (Cruciferae) were supplied by Professor O. A. BEATH (University of Wyoming). The procedure for the germination of seeds, absorption of radioactive selenium by excised leaves and extraction of the tissues with trichloroacetic acid has been described[4].

An aliquot of the trichloroacetic acid extract was placed on a column of Dowex-50, H^+ form and eluted successively with water, 1.5 N HCl and 4 N HCl. The elution pattern is shown in Fig. 1. Effluent fractions corresponding to a volume of 125–135 ml were pooled and evaporated to dryness in a rotary evaporator at about 35°. The residue was dissolved in a small amount of water and evaporated *in vacuo* over anhydrous $CaCl_2$ and NaOH to remove traces of HCl. A small sample was applied to Whatman No. 1 paper for high-voltage paper electrophoresis at pH 6.4 (pyridine–acetic acid–water; 10:0.4:90, v/v). A potential of 40 V/cm was applied for 1 h. The paper was sprayed with 0.2 % ninhydrin in 95 % ethanol. Radioactivity was detected on the paper by cutting out successive half inch strips which were counted in an autogamma spectrometer as described elsewhere[4]. All the radioactivity was found to be located in a ninhydrin spot which had the mobility of neutral amino acids at pH 6.4.

Since the seleno-amino acid was eluted with basic amino acids during Dowex-50 column chromatography, but behaved like a neutral amino acid on electrophoresis at pH 6.4, it was suspected that the material might be the selenium analogue of cystathionine. When a mixture of cystine, *S*-methylcysteine and cystathionine was chromatographed on a Dowex-50 column, cystathionine was found to be eluted from the columns with 4 N HCl in the same position as that of the presumed seleno-cystathionine.

The seleno-amino acid, eluted from Dowex-50 column, was further purified by high-voltage paper electrophoresis at pH 6.4, and the radioactive band was eluted from the paper with water for 20 h at room temperature and evaporated to a small volume. Aliquots were subjected to the following identification procedures.

1. One-dimensional chromatography: The seleno-amino acid had the same R_F as marker cystathionine on one-dimensional chromatograms in Solvent 1, Solvent 2, or Solvent 3*. Solvent 3 caused some decomposition of the labeled compound, radioactivity appearing as a smear rather than a compact spot on Whatman No. 1 paper chromatograms.

2. Oxidation with H_2O_2: A sample of the seleno-amino acid was treated with 30 % H_2O_2 on Whatman No. 1 paper and chromatographed in Solvent 3 (ref. 5). This procedure caused decomposition of the presumed selenocystathionine; selenate and a ninhydrin spot which had the R_F of marker cysteic acid could be detected on the paper chromatograms. Partial decomposition of synthetic cystathionine to cysteic acid also occurred under these conditions. The presence of selenocysteic acid and selenate among the products of the H_2O_2-treated presumed selenocystathionine was also established by high-voltage paper electrophoresis at pH 6.4.

* Solvents for paper chromatography: Solvent 1, *n*-butanol–acetic acid–water (60:15:25, v/v); Solvent 2, *n*-butanol–pyridine–water (1:1:1, v/v); Solvent 3, *n*-propanol–conc. HCl–water (6:2:1, v/v)[5].

3. Hydrogenolysis with Raney nickel: A sample of the presumed selenocystathionine was dissolved in 3 ml of water and refluxed for 2 h with 30 mg Raney nickel. The Raney nickel was removed by filtration and the filtrate was extracted with an equal volume of 1 % 8-hydroxyquinoline in $CHCl_3$ (w/v) and twice with $CHCl_3$ alone. The aqueous solution was evaporated to a small volume and aliquots were applied to Whatman No. 1 for chromatography in Solvent 1. Two prominent ninhydrin spots of equal intensity appeared on the chromatogram in the positions of alanine and α-aminobutyric acid[6].

These experiments suggest that the compound under investigation is selenocystathionine. Its optical configuration could not be determined because of the small amounts isolated.

Selenocystathionine accounted for about 10% of the radioactivity in the trichloroacetic acid extract of *Stanleya pinnata*. The other organic selenium compounds in the extract are under investigation.

Cystathionine is a key intermediate in the metabolic conversion of methionine to cysteine and also in the synthesis of methionine from cysteine in microorganisms[7]. Whether selenocystathionine has a similar role in the synthesis of seleno-amino acids in *Stanleya pinnata* is not known. It should be pointed out that under our experimental conditions selenocystathionine was not formed in detectable amounts by the selenium accumulators, *Astragalus crotalariae*, *A. bisulcatus*, and *Oonopsis condensata*. These differences among the selenium-accumulators may indicate a special role for selenocystathionine in *Stanleya pinnata*.

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The effect of oligomycin on the phosphorylating respiration of ascites hepatoma cell

After the first experimental use of oligomycin by LARDY, JOHNSON AND MCMURRAY[1], it was found to be useful as a "true inhibitor of oxidative phosphorylation"[2]. The effect of the antibiotic on isolated mitochondria has been studied extensively[1–4]. However, few studies, except those using rat-liver slices[5], have been reported con-