Selenoamino acid identification on paper chromatograms

The selenium analogues of methionine, S-methylcysteine and cystine have been identified in plant and animal protein[1–3]. Radioautography of hydrolysates of proteins from plants, microorganisms and animals grown in the presence of selenium-75 has been used almost exclusively in the identification of these amino acids by paper chromatography[1–3]. Isotope techniques are necessary as the selenium amino acids occur in concentrations so low as to render detection by ninhydrin almost impossible. The problem is further complicated by the similarity of the R_F values of the selenium amino acids to those of the naturally occurring sulfur amino acids.

In order to overcome the above complications, a method has been developed which can be used to identify specifically selenomethionine and selenocystine on paper chromatograms in quantities as low as 0.5 mμ moles. This method is based on the reaction of hydriodic acid with sulfoxides as described by TOENNIS[4]. This reaction of hydriodic acid with sulfoxides was later incorporated into a starch–hydriodic acid spray for paper chromatograms by THOMPSON[5].

The amino acid (protein hydrolysate) is spotted on paper in the normal manner. Selenomethionine and selenocystine are then selectively oxidized prior to chromatography by holding the spot over 15 % hydrogen peroxide for 45 to 60 sec. A petri dish containing enough hydrogen peroxide to allow 1 cm from the peroxide to the paper is excellent for the purpose.

After chromatography, the chromatogram is dried at room temperature. The starch–hydriodic acid spray is made fresh daily as follows: (1) 5 % soluble starch; (2) 0.035 *M* sodium iodide; (3) 1.0 *M* hydrochloric acid. Stock starch solution is prepared and stored; the sodium iodide and hydrochloric acid are added immediately before use. Care must be taken to spray the paper very lightly. Selenomethionine selenoxide and selenocystine diselenoxide react in less that 45 sec to give a purplish color which fades to brown on drying at 50° in a forced draft oven. Selenocystine gives more color per mole than selenomethionine. This is probably due to the formation of the diselenoxide, or possibly two selenic acids, by peroxide oxidation.

Preservation of the chromatograms is accomplished by placing them in a manila envelope in which they are pressed against a paper which has been similarly sprayed and dried. In this manner 0.5 mμmole spots have been clearly visible after two weeks.

The sulfur amino acids are not oxidized by the described conditions and will therefore not interfere with the identification of the selenoamino acids. With this technique 0.5 mμmoles of selenomethionine was detected in the presence of 200 mμmoles of methionine. The two amino acids were mixed, applied to the paper, and then the selenomethionine oxidized as described, prior to placing the spotted paper in the chromatography tank. Similar mixtures of selenocystine and cystine have given analogous results.

The sulfur amino acids can be identified by the ninhydrin reaction. Before spraying with ninhydrin, it is necessary to immerse the chromatogram in an ammonia atmosphere for 1 h. This process decolorizes the paper and clear ninhydrin spots are obtained.

Alternatively, the chromatogram can be immersed in a peroxide atmosphere for 30 min. This oxidizes the sulfur amino acids and they give positive starch hydriodic acid spots. Also, the sulfur amino acids are frequently oxidized when the paper is

dried following development by certain solvents as described by THOMPSON[5]. The rate of color formation for the sulfoxides is markedly slower than for the selenoxides and, therefore, provides a very good method of differentiation.

Table I shows some R_F values obtained for selenomethionine and selenocystine treated as described above. Methionine and cystine were identified by ninhydrin. The

TABLE I

R_F VALUES (× 100) OF OXIDIZED SELENOMETHIONINE, SELENOCYSTINE, METHIONINE AND CYSTINE
The selenoamino acids were oxidized prior to chromatography and were identified by the starch–hydriodic acid spray described in the text. All chromatography was done on Whatman No. 1 paper.

Solvent	R_F × 100			
	Oxidized Se-methionine	*Oxidized Se-cystine*	*Methionine*	*Cystine*
Butanol–acetic acid–H_2O (4:1:1)	22	8	55	4
Ethanol–1 *M* NH_4OH–H_2O (90:5:5)	20	5	38	6
Phenol–H_2O–NH_4OH (100:20:0.03)	95	26	76	21

differences in R_F values between the selenoxides and their unoxidized sulfur analogues illustrate the usefulness of this technique. It is anticipated that selenomethylcysteine can also be identified by this method.

A previous publication from this laboratory described the identification of selenomethionine by radioautography from hydrolysates of protein taken from *E. coli* grown in ^{75}Se-selenite[6]. The method described in this report was applied to similar protein hydrolysates and selenomethionine was clearly observed. Other spots were observed, which correspond to selenocystine, but which have not yet been unequivocally identified[6,7].

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