PAPER CHROMATOGRAPHIC AND ELECTROPHORETIC SYSTEMS FOR THE IDENTIFICATION OF SULPHUR AND SELENIUM AMINO ACIDS

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INTRODUCTION

During investigations into the uptake and metabolism of ^{75}Se-selenite ion by higher plants[1], methods of identification of the selenium analogues of sulphur amino acids were required.

The literature records many chromatographic systems of use in the separation of amino acids[2-8]. In general, two-dimensional systems have been employed, in which phenol–water is usually the first solvent[2,3,8] although it has many disadvantages[9] and its use has led to considerable reduction in recoveries of the individual amino acids present[10,11]. In particular, there is considerable oxidation and loss of the sulphur amino acids[5,8,12]. Selenium analogues of the common sulphur amino acids are more unstable[13], and therefore methods of separation involving use of mild solvent systems must be employed.

This paper reports the systems found most useful for the separation of sulphur and selenium amino acids.

EXPERIMENTAL

Paper chromatography

Whatman 3 MM paper was used throughout for partition chromatography (descending). The systems were:

Solvent 1: *n*-butanol–pyridine–water (1:1:1 v/v)[5].

Solvent 2: *n*-butanol–acetic acid–water (25:6:25 v/v, upper phase).

Solvent 3: *n*-butanol–ethanol–water (2:2:1 v/v)[6].

Solvent 4: *tert.*-butanol–formic acid–water (14:3:3 v/v)[14].

For two-dimensional papers, solvent 1 was followed by solvent 2.

Anion exchange chromatography (descending) was carried out using diethylaminoethylcellulose (DEAE), Whatman DE 20, by modifications of the method of KNIGHT[15]:

(1) The dependence of the separation and resolution on pH was investigated using the following buffers[16]:

0.02 *M* acetate buffer, pH 4.7 and 5.8,

0.02 *M* phosphate buffer, pH 7.5.

(2) The effect of two heavy metal complexing agents on the resolution of amino acids on DEAE paper was tested. These were:

(a) The incorporation of 0.001 *M* ethylenediaminetetra-acetic acid (EDTA) in the buffer solution.

(b) The inclusion of hydrogen cyanide in the vapour phase by the inclusion of a small quantity of potassium cyanide crystals in a dish in the chromatography tank[17].

Paper electrophoresis

Electrophoresis was carried out on Whatman 3 MM paper in the apparatus of MARKHAM AND SMITH[18] at 20 V/cm for 4 h or 8 h. Preliminary separation into neutral, acidic or basic amino acids was carried out in a volatile pyridine–acetic acid buffer, pH 6.0, for 4 h[19]. After elution and reapplication to other electrophoretic strips, further separation was effected in phosphate–citrate buffer at pH 2.7 for 4 h or 8 h[20].

Miscellaneous

By treatment with 3 % (w/v) hydrogen peroxide at room temperature for 5 min, methionine and methylcysteine were oxidised to the respective sulphoxides[5,21]. By addition of 0.02 % (w/v) ammonium molybdate the sulphoxides were oxidised to the sulphones[5,22,23].

Colour development with ninhydrin reagent[24] was carried out at 20° for 24 h. The colours produced on the DEAE paper for the various compounds were very characteristic and more stable than the corresponding compounds on 3 MM paper.

Sulphur amino acids were obtained from California Corporation for Biochemical Research, Los Angeles, U.S.A. Selenium analogues of cystine, methylcysteine and methionine were supplied by Dr. A. A. DI SOMMA, College of Pharmacy, Columbia University and Dr. A. SHRIFT, Kaiser Foundation Research Institute, U.S.A.

RESULTS AND DISCUSSION

On DEAE paper the sulphur amino acids were most clearly separated at pH 4.7. With decreasing acidity, the amino acids tended to group into two bands at approximately R_F 0.25 and 0.50.

The use of either cyanide vapour in the chromatography tank, or the incorporation of EDTA in the solvent when DEAE papers were being run, eliminated the frontal beard to the ninhydrin-positive areas. EDTA was preferred, since cyanide reacts with cystine[25].

In Table I are shown the R_F values for paper partition chromatography of the sulphur amino acids in 4 solvent systems. Their electrophoretic properties and R_F values on DEAE paper at pH 4.7 are shown in Table II.

It was possible on chromatographic behaviour alone to tentatively identify most of the sulphur amino acids present. The additional information on the mobility of these compounds on DEAE paper and electrophoresis for 4 h or 8 h allowed the compounds to be characterised with a high degree of certainty. The ninhydrin colours on DEAE paper gave further information, in that the compound under consideration could readily be placed into a group, *e.g.* purple colour, methionine and its derivatives. The chromatographic and electrophoretic behaviour of methionine and methylcysteine before and after oxidation with peroxide was a useful verification procedure.

TABLE I

R_F VALUES OF SULPHUR AMINO ACIDS ON WHATMAN NO. 3 MM

No.	Amino acid*	R_F			
		Solvent 1	Solvent 2	Solvent 3	Solvent 4
1	Cystine	0.14**	0.08	0.05	0.08
2	Cysteine	0.16**	0.09	0.35	0.07
3	Methylcysteine	0.48	0.41	0.33	0.53
4	Methylcysteine sulphoxide	0.30	0.20	0.17	0.24
5	Methylcysteine sulphone	0.38	0.19	0.15	0.20
6	Ethylcysteine	0.58	0.50	0.43	0.63
7	Butylcysteine	0.73	0.63	0.69	0.75
8	Cysteic acid	0.27	0.13	0.11	0.12
9	Cysteine sulphinic acid	0.28	0.19	0.12	0.22
10	Homocystine	0.27	0.24	0.09	0.24
11	Homocysteic acid	0.30	0.10	0.18	0.16
12	Taurine	0.41	0.14	0.24	0.18
13	Cystathionine	0.17	0.05	0.04	0.13
14	Methionine	0.55	0.49	0.42	0.66
15	Methionine sulphoxide	0.31	0.24	0.20	0.35
16	Methionine sulphone	0.39	0.25	0.19	0.36
17	Methionine sulphoximine	0.26	0.18	0.14	0.21
18	Methylmethionine sulphonium chloride	0.19	0.23	0.12	0.18
19	Djenkolic acid	0.13	0.14	0.04	0.14

* 20 μg of each amino acid applied to the paper.
** Streaks at high amino acid concentration.

TABLE II

ELECTROPHORETIC MIGRATION DISTANCES AND R_F VALUES ON DEAE PAPER OF SULPHUR AMINO ACIDS

No.	Amino acid*	Electrophoresis pH 2.7**	DEAE, pH 4.7	
			R_F	Ninhydrin colour
1	Cystine	— 3.3	0.14	brown
2	Cysteine	— 3.1	0.11	brown
3	Methylcysteine	— 2.1	0.36	brown-grey
4	Methylcysteine sulphoxide	— 1.8	0.25	yellow-brown
5	Methylcysteine sulphone	— 1.7	0.26	yellow-brown
6	Ethylcysteine	— 2.0	0.38	brown-grey
7	Butylcysteine	— 2.1	0.40	brown-grey
8	Cysteic acid	+ 15.5	0.05	grey-blue
9	Cysteine sulphinic acid	+ 14.0	0.10	blue
10	Homocystine	— 4.8	0.15	blue
11	Homocysteic acid	+ 13.0	0.10	blue
12	Taurine	— 1.9	0.33	grey-blue
13	Cystathionine	— 1.2	0.18	blue
14	Methionine	— 4.0	0.43	purple
15	Methionine sulphoxide	— 2.7	0.34	purple
16	Methionine sulphone	— 2.6	0.29	purple
17	Methionine sulphoximine	— 3.0	0.28	purple
18	Methylmethionine sulphonium chloride	— 19.0	0.80	purple
19	Djenkolic acid	— 3.2	0.15	grey

* 20 μg of each amino acid applied to the paper.
** Distance migrated (cm) towards anode or cathode in 4 h.

The chromatographic and electrophoretic behaviour of selenocystine, Se-methylselenocysteine and selenomethionine in all systems was indistinguishable from the corresponding sulphur compounds. A number of ^{75}Se-labelled compounds extracted from plant tissues also behaved identically with various sulphur amino acids in these systems[1]. The only differences noted in the behaviour of the seleno- and sulphur-analogues were for the substances having a high mobility upon electrophoresis at pH 2.7. Thus the relative migration of ^{75}Se selenocysteic acid and cysteic acid over a 4 h period were 14.9 cm and 15.5 cm respectively.

In all the systems used, the ninhydrin-positive spots were compact. However, when ^{75}Se-labelled compounds of high activity from plant extracts were chromatographed in the four solvent systems, solvent 4 caused severe decomposition of the labelled compounds present[1]. This solvent has been used by previous workers[14], but was found most unsuitable under our conditions. When ^{75}Se- and ^{35}S-labelled compounds were chromatographed in solvent 2, rate-meter tracings and radioautographs indicated slight decomposition. There was no evidence of decomposition of radioactive selenium or sulphur amino acids in solvents 1 and 3, but the former was superior in resolution.

By a combination of the above methods, it was possible to separate an 80% aqueous ethanol extract of perennial ryegrass (*Lolium perenne*) grown in ^{75}Se-selenite incorporated in the nutrient solution, into 17 discrete radioactive spots[1].

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SUMMARY

Procedures are described for paper chromatographic and electrophoretic separations of sulphur amino acids and of their selenium analogues, using systems in which very little decomposition occurs. Sulphur and selenium analogues behaved identically except for compounds having high electrophoretic mobility.

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