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Note

Chromatographic separation of lysine, thialysine and selenalysine

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Thialysine is a well known analogue of lysine, synthesized in 1955 by Cavallini *et al.*[1], who also first reported some data on its metabolism *in vivo*[2]. Thialysine has been shown to be an antimetabolite of lysine[3,4] and various chemical[5,6] and enzymatic reactions[7–11] that it may undergo have been studied.

Recently, we have synthesized selenalysine[12], a lysine analogue that has the γ-methylene group substituted by a selenium atom, with the aim of studying its biological properties in comparison with those of the sulphur analogue.

$H_2N \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$	Lysine
$H_2N \cdot CH_2 \cdot CH_2 \cdot S \cdot CH_2 \cdot CH(NH_2) \cdot COOH$	Thialysine
$H_2N \cdot CH_2 \cdot CH_2 \cdot Se \cdot CH_2 \cdot CH(NH_2) \cdot COOH$	Selenalysine

In this paper, we report the results obtained with paper and ion-exchange chromatographic methods for the separation of the two lysine analogues from each other and from lysine itself, and for their identification. In particular, it has been shown that thialysine and selenalysine can be easily differentiated, and this result may be of some interest if one considers the possibility of exploiting the aminoethylation of proteins for the detection of selenocysteine.

MATERIALS AND METHODS

Thialysine was synthesized according to the method of Rothfus and Crow[13], and selenalysine as recently described[12]. All other compounds and reagents were analytical-reagent grade products from E. Merck (Darmstadt, G.F.R.).

Paper chromatography was performed on Whatman No. 1 paper sheets. A standard amount of 30 μg of the compounds under study was used in all of the chromatographic tests.

Paper electrophoresis was performed on Whatman No. 3MM paper at 25 V/cm.

Ion-exchange chromatography was performed on a Bio-Cal 200 amino acid analyzer. The short column, (12 × 0.9 cm) was filled with Aminex A-5 resin (Bio-Rad Labs., Richmond, Calif., U.S.A.) of particle size 13.5 ± 2 μm; the long column (54 × 0.9 cm) was filled with Aminex A-6 resin of the same particle size.

RESULTS

On paper chromatography, lysine, thialysine and selenalysine showed, in the various solvent systems tested and reported in Table I, the same R_F values. All three compounds showed only one well-defined spot that was reactive to ninhydrin. Nevertheless, they can be differentiated from each other by specific colour reactions. Thialysine and selenalysine gave a positive reaction to iodoplatinate[14], while lysine did not.

TABLE I

R_F VALUES

Lysine, thialysine and selenalysine show the same R_F values and, even when co-chromatographed, they did not show distinguishable spots.

Solvent system	R_F
Phenol (water-saturated; ammonia vapour)	0.85
Collidine-lutidine (1:1, water-saturated)	0.22
n-Butanol-ethanol-water (4:1:5, upper phase)	0.05
n-Butanol-acetic acid-water (4:1:5, upper phase)	0.15
n-Butanol saturated with 3% ammonia	0.00
n-Butanol-pyridine-water (1:1:1)	0.20
n-Butanol-isopropanol-pyridine-water (4:5:3:6)	0.26
n-Butanol-formic acid (95:5, water-saturated)	0.03
Methanol-pyridine-water (1:1:1)	0.50
n-Propanol (water-saturated)	0.66

Both thialysine and selenalysine gave a positive reaction to the test suggested by Rothfus[15] for the identification of thialysine: when the chromatograms were sprayed with a 0.2% solution of dimethylaminobenzaldehyde in 50% dimethylformamide and then dried in an oven at 110° for 30 min, lysine appeared as a yellow spot, while thialysine and selenalysine gave an orange colour.

Selenalysine can be differentiated from thialysine by the test suggested by Scala and Williams[16] for the identification of the selenoamino acids. The reaction takes advantage of the easier oxidizability of the seleno-compounds compared with the sulpho-compounds, and makes it possible to identify the former by means of reactions that are characteristic of selenoxides. The reaction was exploited as follows. After chromatography, the paper sheet was allowed to stand for 2 min over a large petri dish at about 1 cm from a 15% solution of hydrogen peroxide and then sprayed

TABLE II

SPECIFIC REACTIONS

Reagent	*Lysine*	*Thialysine*	*Selenalysine*
Ninhydrin	+	+	+
Iodoplatinate	—	+	+
Dimethylaminobenzaldehyde (orange colour)	—	+	+
H_2O_2-KI in HCl	—	—	+

with a 0.5% solution of potassium iodide in 2 *N* hydrochloric acid[17]. Lysine and thialysine were not affected by standing over hydrogen peroxide and did not give any reaction; selenalysine was oxidized and then liberated iodine from the acidic potassium iodide, giving a brown spot of iodine after a few seconds.

On paper electrophoresis performed in 1 *M* formic acid, in 0.2 *M* pyridine–acetate buffer, pH 3.5 (ref. 8), or in formic acid–acetic acid–water (1:3:16) at pH 1.9 (ref. 18), lysine, thialysine and selenalysine showed the same cathodic mobility. They can be differentiated by the above specific reactions.

On the short column of the amino acid analyzer, lysine, thialysine and selenalysine may be well separated from each other and also from histidine and ammonia. Fig. 1 shows the elution profile obtained when the compounds were loaded on the short column equilibrated with 0.35 *M* sodium citrate buffer, pH 5.28, and then eluted with the same buffer at a flow-rate of 80 ml/h at 50°. By decreasing the flow-rate of the buffer to 50 ml/h and the temperature to 30°, an even better separation of selenalysine from histidine is obtained, as reported in Fig. 2. In this instance, however, histidine overlapped with the ammonia peak.

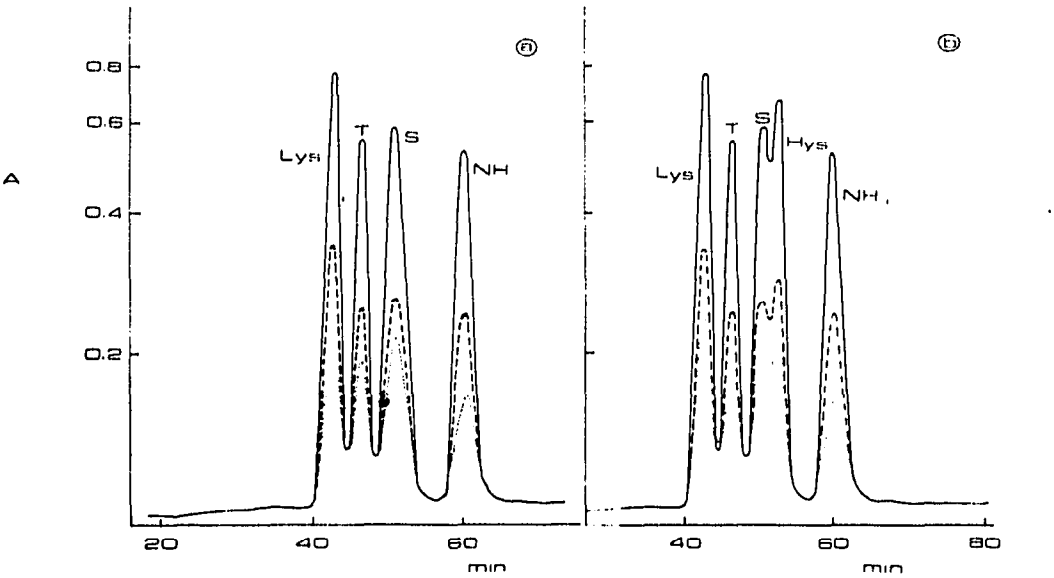

Fig. 1. Elution profile from the short column at 50° C and a flow-rate of buffer of 80 ml/h. Elution buffer: 0.35 *M* sodium citrate, pH 5.28. a, 0.5 μmole of lysine (Lys), thialysine (T), selenalysine (S) and ammonia (NH_3); b, same + 0.5 μmole of hystidine (Hys).

On the long column of the amino acid analyzer, lysine, thialysine and selenalysine can be also separated by means of one of the elution schedules followed for the separation of the acidic, neutral and basic amino acids in the single-column methods. Fig. 3 shows the elution profile obtained by using the two-buffer elution scheme, i.e., 0.2 *M* sodium citrate, pH 3.25, for the first 100 min, followed by 0.2 *M* sodium citrate, pH 4.25, that was 0.6 *M* in sodium chloride. In Table III are reported the elution

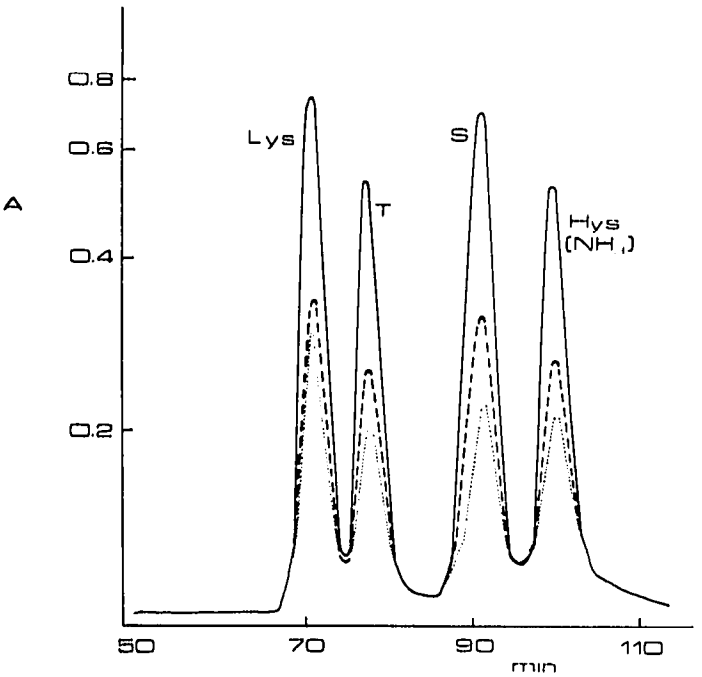

Fig. 2. Elution profile from the short column at 30° and a flow-rate of buffer of 50 ml/h. Elution buffer: 0.35 M sodium citrate, pH 5.28. Peaks as in Fig. 1.

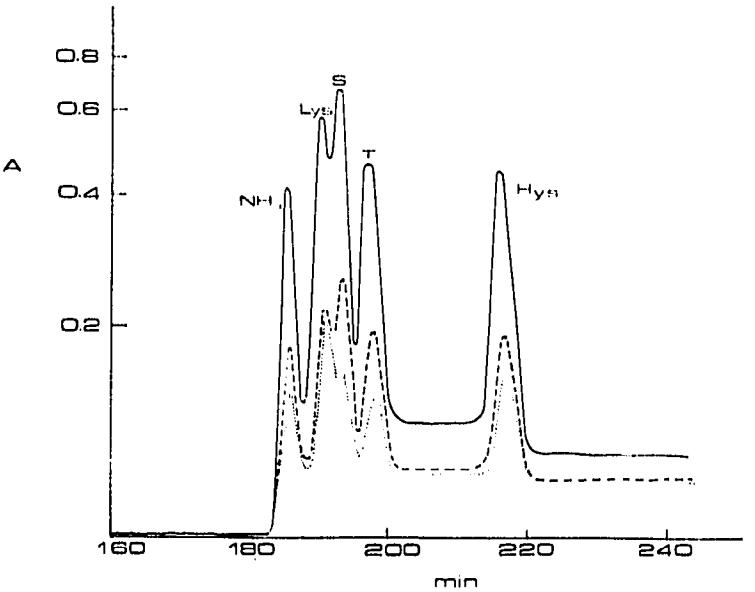

Fig. 3. Elution profile from the long column at 55° and a flow-rate of buffer of 80 ml/h. Elution buffers: 0.2 *M* sodium citrate, pH 3.25, for the first 100 min, then 0.2 *M* sodium citrate, pH 4.25, 0.6 *M* in sodium chloride. Peaks as in Fig. 1.

TABLE III

ELUTION TIMES, COLOUR YIELDS (C_{HW}) AND $A_{440}:A_{560}$ RATIOS

Compound	*Elution times (min)*			C_{HW}	$A_{440}:A_{560}$*
	Short column, pH 5.28, 50°, 80 ml/h	*Short column, pH 5.28, 30°, 50 ml/h*	*Long column, pH 3.25–4.25, 55°, 80 ml/h*		
Lysine	43	71	191	26	0.45
Thialysine	46	77	198	20	0.30
Selenalysine	51	90	194	22	0.33
Histidine	53	98	217	23.4	0.40
Ammonia	60	98	186	—	—

* Calculated from the data for the short column at 50° and a flow-rate of buffer of 80 ml/h.

times, the colour yields and the $A_{440}:A_{560}$ absorbance ratios for the compounds studied.

Finally, the stability of selenalysine under the conditions usually used for the acid hydrolysis of proteins was tested. A 5-mg amount of selenalysine was dissolved in 10 ml of 6 *N* hydrochloric acid and refluxed for 24 h. On the amino acid analyzer, 93 % of the initial selenalysine was recovered and no other compounds were identified. In another experiment, 2 mg of selenalysine were dissolved in 1 ml of 6 *N* hydrochloric acid, sealed in a test-tube under nitrogen and left at 110° for 24 h; in this instance the recovery was complete.

DISCUSSION

The results show that lysine, thialysine and selenalysine can be differentiated on paper chromatograms by specific reactions, and can be separated from each other by ion-exchange chromatography.

It may be envisaged that, as already exploited for the determination of cysteine as thialysine[13,19], the aminoethylation of proteins may be a useful tool for the detection of selenocysteine in proteins as selenalysine. The fact that selenalysine can be easily identified and may be well differentiated from thialysine on the amino acid analyzer, and the fact that, contrary to selenocystine[20], it is stable to acid hydrolysis, are essential factors.

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