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Note

Separation of S-sulphocysteine and related compounds by anion-exchange chromatography and electrophoresis

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Sulphite is an intermediate in the sulphur metabolism of cysteine in animals. It has been reported that "cystine disulphoxide" reacts quantitatively with sulphite to produce S-sulphocysteine (2-amino-2-carboxyethylsulphosulphane)[1]. It has also been found that "cystine disulphoxide" reacts with thiosulphate to form S-sulphothiocysteine (2-amino-2-carboxyethylsulphodisulphane). Attempts have been made to separate these S-sulpho derivatives of cysteine by Dowex 1 column chromatography

EXPERIMENTAL

S-Sulphocysteine[2] and S-sulphothiocysteine[3,4] were prepared according to reported methods. These amino acids were also synthesized from "cystine disulphoxide" and sulphite or thiosulphate, respectively. in high yields. The details of the methods will be reported elsewhere. S-Sulphoglutathione was prepared according to Eriksson and Rundfeldt[5]. L-Cysteic acid and taurine were obtained from Sigma (St. Louis, Mo., U.S.A.).

Dowex 1-X8 (Cl^-) (200–400 mesh) was packed in a 550 × 10 mm glass tube and washed with water until the washings were neutral. A 1-ml volume of 1 *M* acetic acid containing L-cysteic acid (3 μmol), taurine (3 μmol), S-sulphocysteine (5 μmol) and S-sulphothiocysteine (7 μmol) was placed on the top of the column. Elution was carried out at room temperature with a linear gradient of sodium chloride in 1 *M* acetic acid prepared from 150 ml of 1 *M* acetic acid placed in a mixing chamber and 150 ml of 1 *M* acetic acid containing 2 *M* sodium chloride placed in a reservoir. The flow-rate was regulated at 41 ml/h with a peristaltic pump and 2-ml fractions were collected. Amino acids in the fractions were determined with ninhydrin reagent[6], and with Gaitonde's acidic ninhydrin reagent 2 after treatment with dithiothreitol[7].

High-voltage paper electrophoresis was performed in pyridine–acetic acid–water (0.5:10.0:79.5, pH 3.1)[8] for 45 min. Amino acids were detected with 1% ninhydrin–2% pyridine in acetone.

RESULTS AND DISCUSSION

Fig. 1 shows the elution profile of taurine, cysteic acid, S-sulphocysteine and S-sulphothiocysteine with the Dowex 1-X8 column. These four amino acids were

clearly separated. Purdie *et al.*[9] reported the chromatography of sulphonic and sulphinic acids on a long Dowex 1-X8 (chloroacetate) column, in which S-sulphocysteine and S-sulphothiocysteine were eluted as broad peaks. By using the present method these amino acids were eluted as sharp peaks in a shorter time, and their quantitative determination became possible.

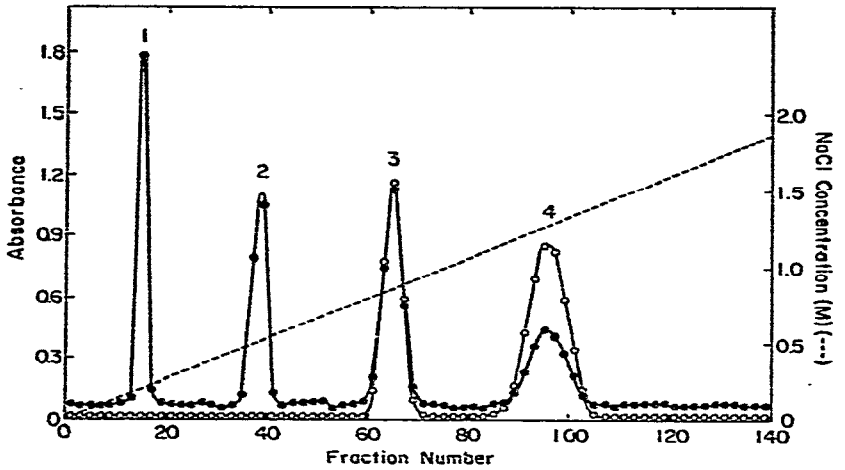

Fig. 1. Chromatography of S-sulphocysteine and related compounds on Dowex 1-X8 column (550 × 10 mm). Elution was carried out with a linear gradient of sodium chloride in 1 *M* acetic acid; flow-rate, 41 ml/h. Fractions of 2 ml were collected. Eluates were checked with ninhydrin reagent (absorbance at 570 nm, ●) and acidic ninhydrin reagent 2 (absorbance at 560 nm, ○). Peaks: 1 = taurine; 2 = cysteic acid; 3 = S-sulphocysteine; 4 = S-sulphothiocysteine.

The colour in the ninhydrin reaction of S-sulphothiocysteine is brownish and its colour value is low, as shown in Table I. However, this was improved by use of Gaitonde's acidic ninhydrin reagent 2, which is equally sensitive to cysteine and cysteine-producing amino acids following treatment with dithiothreitol.

TABLE I

NINHYDRIN COLOUR VALUES OF S-SULPHOCYSTEINE (SSC) AND S-SULPHOTHIOCYSTEINE (SSTC)

Amino acid (0.2 μmol) in 0.5 ml of 1 *M* acetic acid containing 1 *M* NaCl was reacted with 0.5 ml of ninhydrin reagent for the manual method[6] or with 0.5 ml of Gaitonde's acidic ninhydrin reagent 2 after dithiothreitol treatment[7] in standard procedures. The absorbance at 570 nm of the usual ninhydrin reaction of leucine was taken as 100%.

Reaction	*Leucine*	*Cysteine*	*SSC*	*SSTC*
Usual ninhydrin reaction	100	23	80	47
Acidic ninhydrin reaction	0	104	89	91

Fig. 2 shows the paper electrophoretic separation of cysteic acid, S-sulphocysteine, S-sulphothiocysteine, S-sulphoglutathione, aspartic acid and taurine. Although S-sulphocysteine and S-sulphoglutathione were not separated by the present column chromatographic method, they could be separated by electrophoresis.

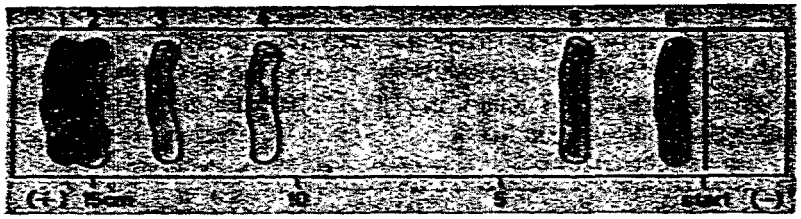

Fig. 2. Paper electrophoresis of S-sulphocysteine and related compounds. Electrophoresis was performed in pyridine–acetic acid–water (0.5:10.0:79.5, pH 3.1) at 85 V/cm for 45 min. Bands: 1 = cysteic acid; 2 = S-sulphocysteine; 3 = S-sulphothiocysteine; 4 = S-sulphoglutathione; 5 = aspartic acid; 6 = taurine.

The present method will be useful for the study of the metabolism of sulphur-containing amino acids.

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