COLUMN CHROMATOGRAPHY OF SOME SULFUR-CONTAINING AMINO ACIDS

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In previous papers[1,2] we have reported methods for the chromatographic analysis of cysteic acid, cysteinesulfinic acid, taurine and hypotaurine. Some reactions for the determination of oxidation level of sulfur compounds have also been investigated[3,4]. The experience thus gained enabled us to make some improvements to the column chromatographic method of SPACKMAN, STEIN AND MOORE[5], so as to render it applicable to the detection of some sulfur compounds, with the aid of the automatic Amino Acid Analyzers.

In the present paper we report the chromatographic behaviour of the following compounds, whose inter-relationships are evident from their chemical formulae: cysteic acid (CA), cysteinesulfinic acid (CSA), taurine (TAU), hypotaurine (HYP), thiotaurine (TIOT), alanine-thiosulfonic acid (ATS), S-sulfo-cysteine (S-CYS) and S-sulfo-cysteamine (S-CYSA).

CH_2-SO_3H $\vert$ $CHNH_2$ $\vert$ $COOH$	CH_2-SO_2H $\vert$ $CHNH_2$ $\vert$ $COOH$	CH_2-SO_3H $\vert$ CH_2-NH_2	CH_2-SO_2H $\vert$ CH_2-NH_2
CA	CSA	TAU	HYP
CH_2-SO_2-SH $\vert$ $CHNH_2$ $\vert$ $COOH$	CH_2-S-SO_3H $\vert$ $CHNH_2$ $\vert$ $COOH$	CH_2-SO_2-SH $\vert$ CH_2-NH_2	CH_2-S-SO_3H $\vert$ CH_2NH_2
ATS	S-CYS	TIOT	S-CYSA

EXPERIMENTAL

Cysteic acid, cysteinesulfinic acid and taurine were commercial products. Hypotaurine and thiotaurine were prepared according to CAVALLINI *et al.*[6-8]. Alanine-thiosulfonic acid, S-sulfo-cysteine and S-sulfo-cysteamine were prepared according to DE MARCO *et al.*[9,10].

All the other compounds or reagents used were of commercial origin. The chromatographic apparatus used was the Model 120 Beckman-Spinco Amino Acid Analyzer.

The long column (150 cm), filled with ion exchange resin type 150 A of Beckman-Spinco, was equilibrated with a solution made up as follows: 0.1 *M* citric acid, 0.2 *M* NaCl, 0.2% Brij 35 solution (50 g + 100 ml H_2O) and 0.01% caprylic acid. The compounds to be analyzed were dissolved in the same solution. Chromatographic runs were performed at 30°.

All the other conditions (ninhydrin solutions, flow rate, washing of the columns, etc.) were as described for the standard runs with the Amino Acid Analyzer.

RESULTS

Fig. 1 shows that when citric acid is used as eluent a very good resolution of CA, CSA, TAU, urea and HYP is obtained.

This is the major improvement in respect to the standard chromatographic conditions for the Amino Acid Analyzer. In fact, when the pH 3.25 buffer is employed, CA and CSA are eluted together, as are also urea and HYP.

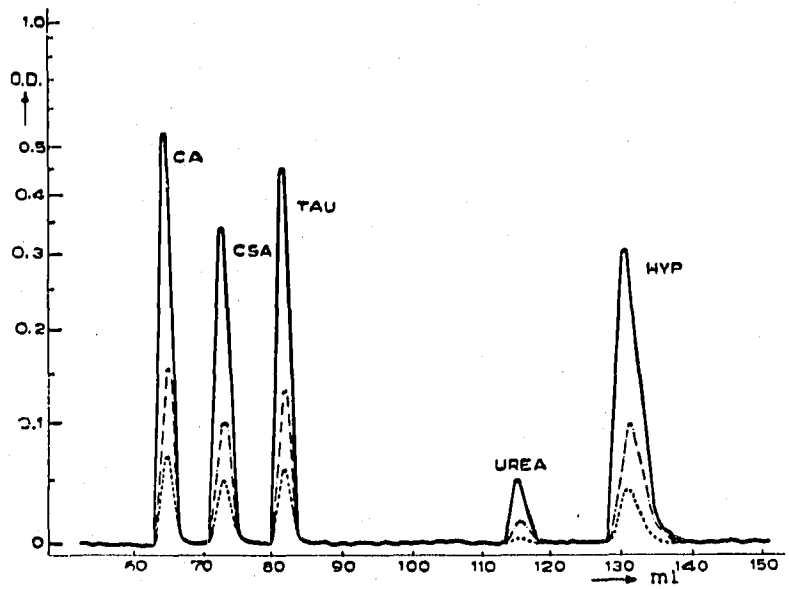

Fig. 1. 0.5 μmoles each of cysteic acid (CA), cysteinesulfinic acid (CSA), taurine (TAU), hypotaurine (HYP) and 1 μmole of urea chromatographed on the long column (150 cm) of the Beckman Spinco Amino Acid Analyzer, using 0.1 *M* citric acid–0.2 *M* NaCl as eluent. Temp. 30°. ——— recording of the unsuppressed 570 mμ photocell, —·—·—· recording of the suppressed 570 mμ photocell, – – – recording of the 440 mμ photocell.

As regards the other compounds studied, S-sulfo-cysteine and ATS are eluted, under our conditions, together with CA; and S-sulfo-cysteamine and thiotaurine together with taurine (Fig. 2).

Nevertheless these compounds may be easily recognized and distinguished from each other by the following procedures.

Detection of S-sulfo-compounds

The presence of the two S-sulfo-compounds may be inferred, in the first instance, by looking at the ratio between the absorbancy at 570 mμ and at 440 mμ. These compounds give a characteristic brown ninhydrin color (clearly evident on

paper chromatograms), which results in a 570:440 mμ ratio of about 2.5 as calculated from the diagrams obtained by repeated chromatographic analyses.

This value is very different from the 570:440 mμ ratios we have calculated for all the other compounds tested under the same experimental conditions, and which vary from 7 to 8.

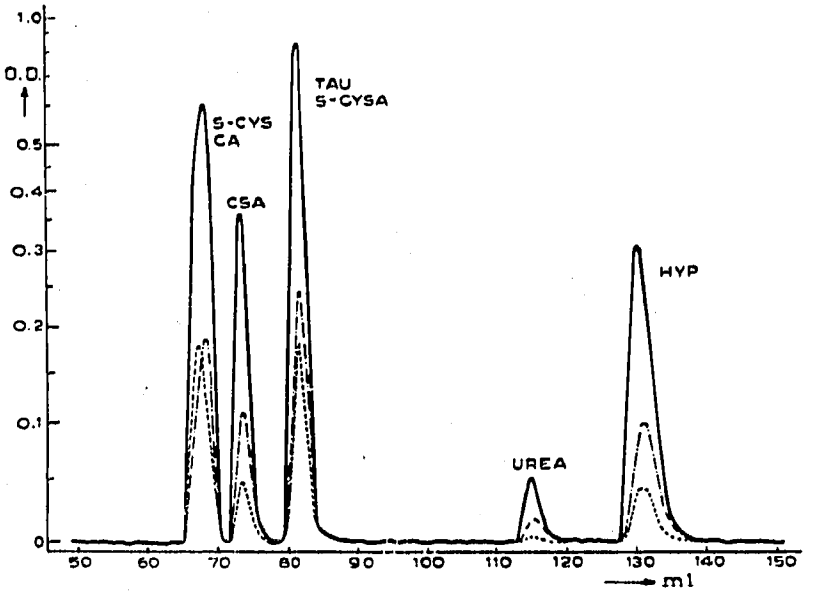

Fig. 2. Chromatography of 0.5 μmoles of the compounds listed in Fig. 1 together with 0.5 μmoles of S-sulfo-cysteine (S-CYS) and S-sulfocysteamine (S-CYSA). All the other conditions as in Fig. 1.

Therefore if S-sulfo-compounds are present together with CA or TAU, the 570:440 mμ ratios of the corresponding peaks are decreased.

Fig. 2 shows the chromatographic pattern of a solution containing S-sulfo-cysteine and S-sulfo-cysteamine besides the other compounds shown in Fig. 1.

With regard to the peak due to CA the asymmetry in the recording of the normal 570 mμ photocell is clearly evident, and there is a correspondingly higher absorption at 440 mμ. This results in a partial separation of the peaks recorded by the 440 mμ and the suppressed 570 mμ photocells. It is clearly evident that we are dealing with two different compounds, the first one, at the left side of the peak, is S-sulfo-cysteine.

As regards the peak due to taurine, the 570:440 mμ ratio calculated from Fig. 2 is 4.55, whereas from the curves in Fig. 1 it is 7.4.

Therefore a low value for the ratio between the absorbancy at 570 and 440 mμ is highly indicative of the presence of S-sulfo-compounds. Further, to ensure that one is dealing with these compounds, they may be destroyed by treatment with an excess of thiol: the S-sulfo-compounds being split into the corresponding sulfide and inorganic sulfite, according to the following reactions[11]:

$$R\text{–}S\text{–}SO_3H + R'\text{–}SH \leftrightharpoons H_2SO_3 + R\text{–}S\text{–}S\text{–}R'$$

$$R\text{–}S\text{–}S\text{–}R' \xrightarrow[R'\text{–}SH]{\text{excess}} R\text{–}SH + R'\text{–}S\text{–}S\text{–}R'$$

The usefulness of this reaction was checked as follows. 2 μmoles of S-sulfo-cysteine and 40 μmoles of cysteamine were dissolved in 1 ml of 1 N acetic acid. After standing for 60 min at room temperature the solution was taken to dryness on a boiling water bath under reduced pressure, to eliminate SO_2, which may interfere in the ninhydrin reaction. Then 2 ml of the citric acid solution used for the column chromatography were added to the test tube and 1 ml of the solution, corresponding to 1 μmole of S-sulfo-cysteine, was put on the column. After 120 ml of citric acid solution had been passed the elution was continued with the pH 4.25 buffer prepared

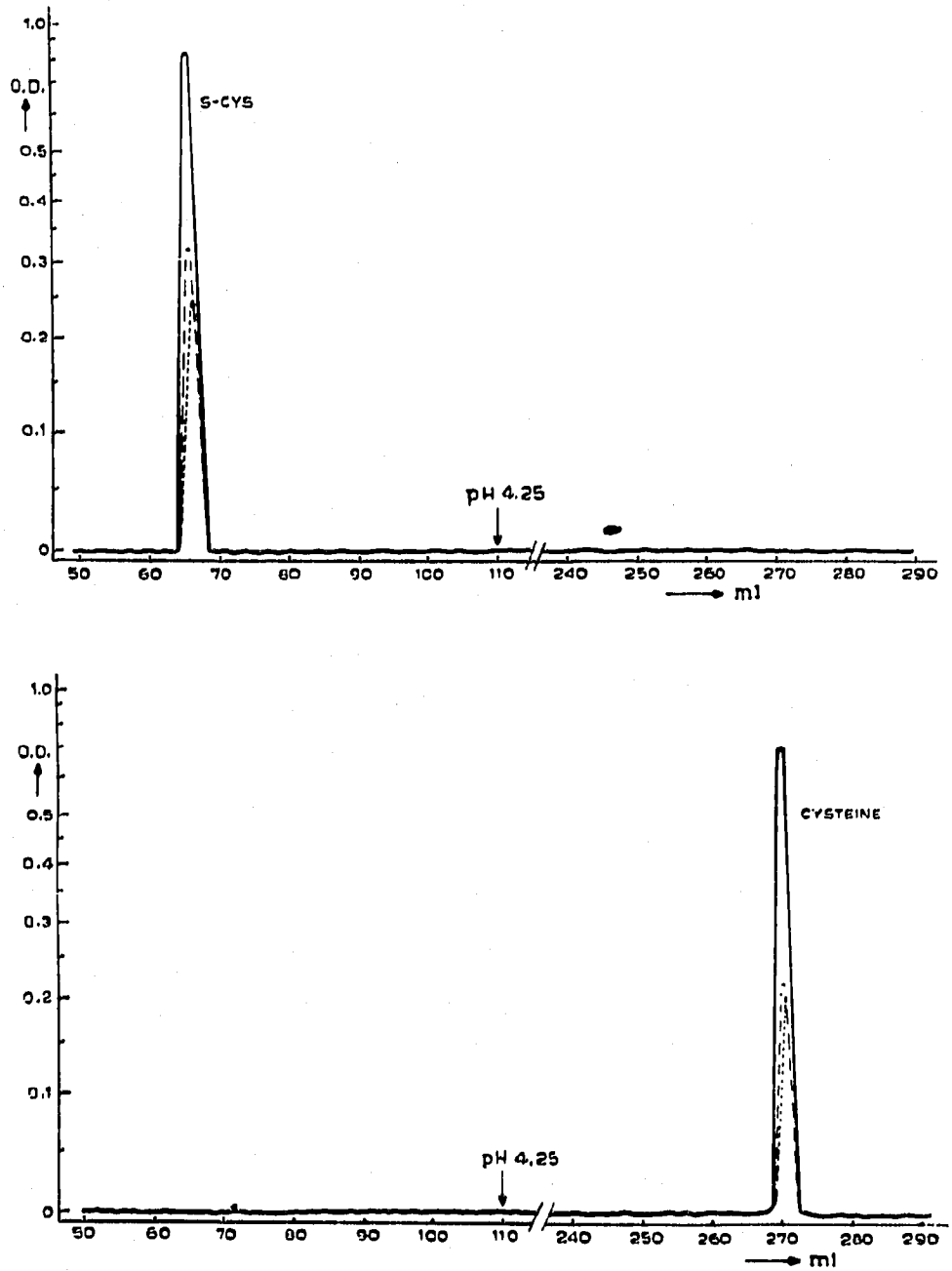

Fig. 3. Chromatography of 1 μmole of S-sulfo-cysteine before (top) and after (bottom) treatment with excess of cysteamine. All the other conditions as in Fig. 1. At the arrow the eluting solution was exchanged for pH 4.25 citrate buffer.

according to SPAKMAN *et al.*[5] to remove cysteine, whose elution pattern had been checked in preliminary experiments performed under the same conditions with the pure compound.

Fig. 3 shows the results obtained: S-sulfo-cysteine has completely disappeared and cysteine is evident (the excess of cysteamine was not eluted from the column, but washed out with NaOH).

S-Sulfo-cysteamine, also, when treated with an excess of cysteamine under the same conditions shows similar behavior, *i.e.* is absent (obviously in this case no cysteine was detected).

Detection of thiosulfonates

A property peculiar to ATS and TIOT is that, unlike the other compounds studied, they are cyanolysable according to the reaction:

$$R\text{–}SO_2SH + HCN \longrightarrow R\text{–}SO_2H + HCNS.$$

Their presence in the solution to be analyzed may then be detected by a positive cyanolysis reaction[12,13]. But this reaction does not permit the identification of the compound with which one is dealing. It is, however, possible by performing column chromatographic analyses before and after the cyanolysis reaction, since ATS is transformed by cyanolysis into CSA and TIOT into HYP.

The validity of this reaction and the experimental conditions have been checked as follows. Two μmoles of ATS were dissolved in 1 ml 1 *N* NH_3, added to 1 ml 0.1 *M* KCN, and left for 30 min in a boiling water bath. Then the solution was acidified with 5 *N* HCl and taken to dryness to remove cyanide (which if present emerges from the column after 90 ml, giving an atypical ninhydrin-positive peak). Two

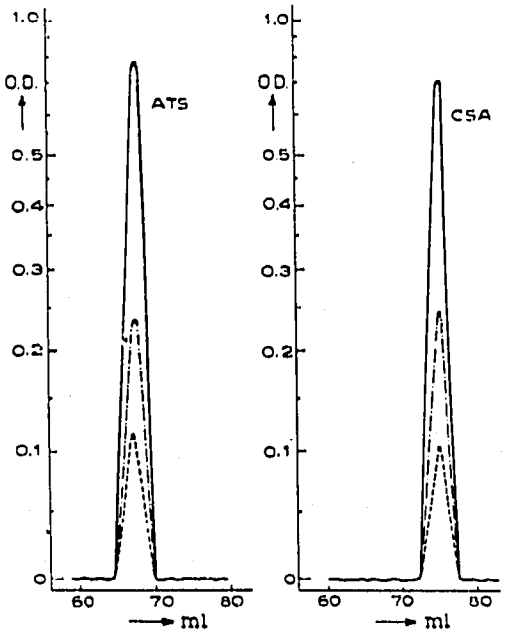

Fig. 4. Chromatography of 1 μmole of alaninethiosulfonic acid (ATS) before (on the left) and after (on the right) the cyanolysis reaction, by which it is transformed into cysteinesulfinic acid (CSA). All the other conditions as in Fig. 1.

milliliters of the citric acid solution were then added to the test tube, and 1 ml was put on the column. Fig. 4 shows the result obtained, *i.e.* the shift of the peak from 65 to 75 ml, indicating the complete transformation of ATS into CSA.

In the same way a solution of thiotaurine, after cyanolysis, shows only the presence of hypotaurine.

Quantitative determinations showed that no destruction of thiosulfonates occurred during cyanolysis, one mole of the latter giving rise to one mole of the corresponding sulfinate.

CONCLUSIONS

By using 0.1 *M* citric acid as the first buffer, good separations of the following compounds, using the long column of the Amino Acid Analyzer, may be obtained: cysteic acid, cysteinesulfinic acid, taurine, urea and hypotaurine.

The S-sulfo-derivatives and the thiosulfonate esters of cysteine and cysteamine, which have chromatographic positions indistinguishable from the corresponding sulfonic acid, may be detected by taking advantage of some of their properties: in the case of the S-sulfo-compounds the higher adsorbancy at 440 mμ after ninhydrin reaction, and the cyanolysis reaction in the case of the thiosulfonate esters.

Methods are described for destroying both types of compounds, thus making possible their quantitative estimation from the difference in the peak areas before and after their elimination.

As a concluding remark the rapidity of the chromatographic method here described is stressed, as it requires only a few hours for a complete screening of the sulfur amino acids here mentioned.

SUMMARY

A method is described for the chromatographic separation in the Automatic Amino Acid Analyzer of the following sulfur-containing amino acids: cysteic acid, cysteinesulfinic acid, taurine, hypotaurine. The chromatographic behavior of S-sulfo-cysteine, S-sulfo-cysteamine, alaninethiosulfonic acid and thiotaurine was also studied, and methods are reported for the identification of these compounds which are not well separated from the corresponding sulfonic acids.

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