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Note

Ion-exchange chromatography of S-(carboxymethyl)cysteine and Se-(carboxymethyl)selenocysteine

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Carboxymethylation may be envisaged as a useful tool for detecting either free or protein-bound selenocysteine in biological materials. Since we recently had the opportunity of preparing Se-(carboxymethyl)selenocysteine (CMSeC) in order to

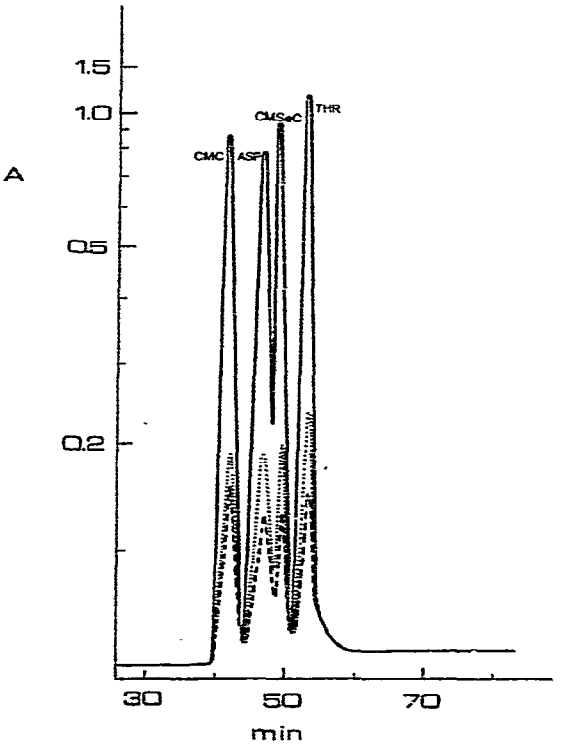

Fig. 1. Elution profile from the long column (54 cm × 9 mm) of the amino acid analyzer at 50° and a buffer flow-rate of 80 ml/h. Elution buffer: 0.2 *M* sodium citrate pH 3.25. The amount of each compound loaded on the column was 0.5 μmol. CMC = S-(carboxymethyl)cysteine; CMSeC = Se-(carboxymethyl)selenocysteine; ASP = aspartic acid; THR = threonine. ——— = Absorbance at 570 nm (3 mm light path); ... = "suppressed" absorbance at 570 nm (1.5 mm light path); – – – = absorbance at 440 nm (3 mm light path).

study its oxidative deamination[1], it seemed interesting to optimise conditions for its separation from S-(carboxymethyl)cysteine (CMC). This is, indeed, essential if carboxymethylation is to be used for the detection of selenocysteine in the presence of cysteine.

CMSeC was prepared from DL-selenocystine (Nutritional Biochemicals, Cleveland, Ohio, U.S.A.), reduced with borohydride and then allowed to react with monochloroacetate[1]. CMC was obtained from Fluka (Buchs, Switzerland). A Bio-Cal 200 amino acid analyzer was used, the long column (54 cm × 9 mm) being filled with Aminex A-6 resin (particle size 13.5 ± 2 μm; Bio-Rad Labs., Richmond, Calif., U.S.A.).

Fig. 1 shows the elution pattern of CMC, CMSeC, aspartic acid and threonine from the long column, with 0.2 *M* sodium citrate buffer of pH 3.25 as eluent (flow-rate 80 ml/h) and a column temperature of 50°. It can be seen that CMSeC is well separated from CMC (elution times 48 and 42 min, respectively) and from aspartic acid (44 min) and threonine (51 min), the only common amino acids eluted in the same time range. CMSeC is retarded with respect to CMC; in this it resembles other seleno-compounds whose separation on sulphonated resins has been studied[2–5], all of which are retarded with respect to the corresponding sulphur compounds.

A colour constant, C_{HW}, (of value 39) has been calculated for CMSeC; the corresponding value for CMC was 30. The ratio between the absorbance at 440 nm

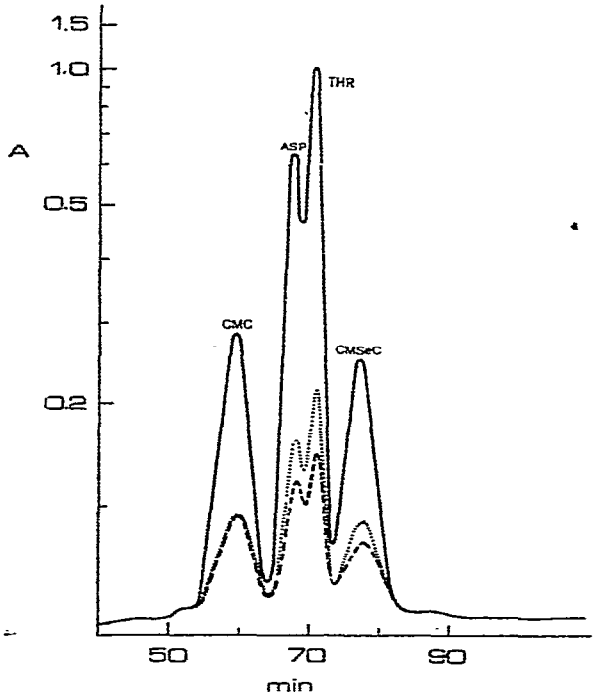

Fig. 2. Elution profile with the column operated at 30° and a buffer flow-rate of 60 ml/h; other conditions as in Fig. 1.

and that at 570 nm was 0.14 for CMSeC and 0.19 for CMC, indicating that both compounds give a typical colour in the ninhydrin reaction.

When the elution was performed under the same conditions of flow-rate and column temperature, but with the buffer containing 4% of ethanol to delay elution of threonine, there was no improvement in resolution of the four compounds examined; indeed, partial overlapping of the peaks of aspartic acid and CMSeC occurred. However, when the column temperature was lowered from 50 to 30° and the buffer flow-rate from 80 to 60 ml/h, the elution pattern changed as shown in Fig. 2, the relative positions of threonine and CMSeC being reversed. This led to better separation of CMC from CMSeC (eluted at 60 and 78 min, respectively).

Thus, CMSeC can be easily identified and well separated from CMC on an amino acid analyzer by using elution schedules commonly followed in analysis for acidic amino acids.

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