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Separation of ε-carboxymethyl-lysine from methionine on 60-cm columns of ion-exchange resins

Some peaks of carboxymethyl-(CM-)amino acids overlap the free amino acid peaks in the amino acid analysis performed according to or derived from the method of SPACKMAN *et al.*[1–4]. GOREN *et al.*[4] proposed an alternative procedure to the ninhydrin detection of CM-amino acids of alkylated proteins based on the use of radioactive alkylating agents and the detection of radioactive products with a flow cell for scintillation counting. In this case, the overlap of CM-amino acid peaks with free amino acid peaks is no longer a problem. When, however, this method cannot be used, it is necessary to have procedures for the separation of CM-amino acids from the free amino acids.

CM-histidine can be separated from the other ninhydrin-positive peaks if the pH of the first buffer of amino acid analysis is lowered from 3.25 to 3.19 (ref. 2). ε-CM-lysine is resolved from methionine on a 150-cm column system; however, they overlap on a 60-cm column system[3–5]. It must be pointed out that many amino acid analysers are equipped only with 60-cm columns.

In this note, the conditions for the resolution of ε-CM-lysine from methionine in a 60-cm column system are described. ε-CM-lysine and ε,ε-di-CM-lysine were prepared from poly-L-lysine (mol. wt. 50–100000; Cyclo Chem. Co.) as reported by GUNDLACH *et al.*[5] S-CM-cysteine was purchased from Mann. All the other reagents were of analytical grade. A Beckman Unichrom amino acid analyser, equipped with 60-cm columns filled with Beckman custom-research resin type AA-15, was used. The standard working conditions of this apparatus are those described by BENSON AND PATTERSON[6]. The following operation conditions were used: flow rate of the buffer, 67.5 ml/h; flow rate of the buffer plus ninhydrin, 101.5 ml/h; first elution buffer, 0.2 *N* Na citrate (pH 3.25); second elution buffer, 0.2 *N* Na citrate (pH 3.8 instead of 4.25); buffer change after 120 min. The analysis was started at 42°, and immediately

TABLE I

ELUTION VOLUME OF SOME FREE AND CARBOXYMETHYLATED AMINO ACIDS

These elution volumes apply exactly only to our conditions (see the text). Each elution volume is the average of that in a number of similar chromatograms. The elution volume, calculated from the chromatograms, includes the volumes of the buffer and of the ninhydrin reagent.

Amino acid	*Elution volume (ml)*
ε,ε-Di-CM-lysine	61.5
S-CM-cysteine	73.5
Aspartic acid	80.5
Alanine	166.5
Half-cystine	184.0
Valine	212.0
Methionine	256.0
ε-CM-lysine	273.5
Isoleucine	288.0
Phenylalanine	373.0

after starting the temperature was raised to 60°. This temperature was reached with our apparatus and with a room temperature of 20–22° in about 1 h.

The total analysis time for acidic and neutral amino acids under these operation conditions was 3 h 50 min instead of 3 h 10 min under standard conditions.

In Table I, the elution volume of some peaks of free amino acids and CM-amino acids is reported. Under the working conditions reported above, the ε-CM-lysine peak appears between methionine and isoleucine and is well separated from both these amino acids. ε,ε-Di-CM-lysine, S-CM-cysteine and aspartic acid peaks are also well separated from each other, and half-cystine peak is found between alanine and valine peaks.

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Istituto di Chimica Biologica,
University of Pisa,
Pisa (Italy)

G. RONCA
R. CHITI
A. LUCACCHINI

1 D. H. SPACKMAN, W. H. STEIN AND S. MOORE, *Anal. Chem.*, 30 (1958) 1190.
2 R. G. FRUCHTER AND A. M. CRESTFIELD, *J. Biol. Chem.*, 240 (1965) 376.
3 R. L. HEINRIKSON, *J. Biol. Chem.*, 241 (1966) 1393.
4 H. I. GOREN, D. M. GLICK AND E. A. BARNARD, *Arch. Biochem. Biophys.*, 126 (1968) 607.
5 H. G. GUNDLACH, W. H. STEIN AND S. MOORE, *J. Biol. Chem.*, 234 (1959) 1754.
6 J. V. BENSON AND J. A. PATTERSON, *Anal. Chem.*, 37 (1965) 1108.

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