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Chromatographic separation of β-methyldiaminopropionic acid in hydrolysates of modified phosphoproteins in an amino acid analyzer

The study of the structure of proteins and the identification of the functional groups of active centres in enzymes involves the modification of amino acids. One method of achieving such modifications is by conversion of phosphoserine residues to a diaminopropionic acid derivative. As we established earlier, such a process takes place when methylamine interacts with phosphorylated derivatives of serine[1,2] and phosphoproteins[3]. This conversion of phosphoserine, which is acidic, into β-methyldiaminopropionic acid seems to be a general reaction and may be used for studying the structure of phosphorus-containing proteins.

A simple and reliable method had to be developed for the identification and quantitative determination of β-methyldiaminopropionic acid in the presence of all the other basic amino acids and methylamine, as no standard method of amino acid analysis can be used in this instance. The same was true of numerous modifications that are commonly used for separating complex mixtures of amino acids and N-methylated amino acids[4–18].

It was found that when separating all the components of this mixture, a de-

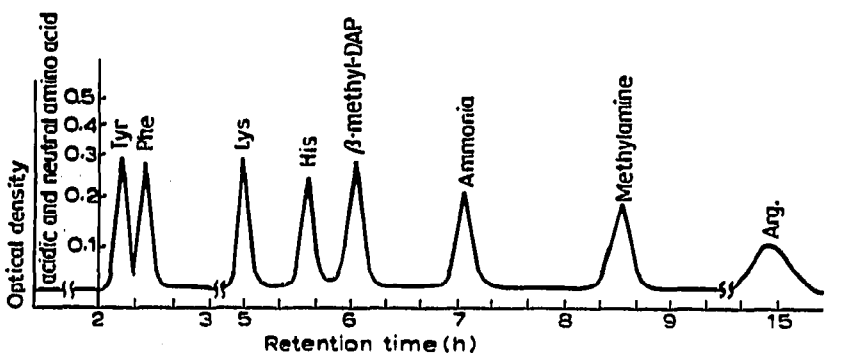

Fig. 1. Chromatography of β-methyldiaminopropionic acid and the standard mixture of amino acids in a 50-cm column packed with 3105 resin. Elution was performed with 0.35 *N* Na^+–citrate buffer, pH 6.08 ± 0.02 at 30 ± 1°. The flow-rate of buffer was 30 ml/h. The mixture contained, in addition to ammonia and methylamine, 0.25 μmole of each component.

TABLE I

REPRODUCIBILITY OF THE RETENTION TIMES AND $H \times W$ VALUES[a]

Compound	*Retention time (min)*	*C.V.[b] of retention time (%)*	$H \times W$[c] *value*	*C.V.[b] of* $H \times W$ *value (%)*
Lysine	300	0.9	47.88	1.1
Histidine	336	1.4	44.60	0.27
β-Methyldiaminopropionic acid	364	1.1	44.46	0.91

[a] Each value is an average of 5 experiments.
[b] Coefficient of variation.
[c] The values for 0.25 μmole were calculated.

creased temperature and a lower rate of elution proved to be very effective. We established that if elution is carried out with 0.35 *N* Na^+–citrate buffer* of pH 6.08 at 30°, the results were excellent. We used a Hitachi (Japan) amino acid analyzer, Model KLA-3b, with a 0.9 × 50 cm column packed with 3105 cation exchanger. Fig. 1 shows the separation of the mixture. Table I gives the retention times of the substances, the areas of the peaks calculated by the $H \times W$ method and the variation coefficients.

The curve is readily reproducible. It should be noted that if the pH of the eluting buffer is changed considerably (± 0.1), the time of recovery of histidine will vary. Arginine is eluted in 15 h. The total duration of the analysis can be considerably reduced if the temperature of elution at the end of the experiment is raised to 55°.

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* The buffer was prepared by adding 10 *N* NaOH to the standard buffer of pH 5.28.