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Note

Isotachophoretic analysis of some sulfur-containing amino acids in human urine

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S-(1,2-Dicarboxyethyl)cysteine (DCEC) [1], S-(carboxymethyl)cysteine (CMC) [2] and S-(2-methyl-2-carboxyethyl)cysteine (isobuteine) [3] have been found in normal human urine. They are known to be present in several animal tissues, but their biosynthesis and physiological role are as yet not entirely understood.

So far we have used an automatic amino acid analyzer for the determination of these sulfur-containing amino acids, after preliminary fractionation of fairly large volumes of urine on different ion-exchange resins [4]. The isotachophoretic analysis [5—8] presented here has made possible the rapid determination of these compounds.

METHOD

A 50-ml sample of urine was hydrolyzed in 6 *N* HCl for 20 h on a sandbath. The hydrolyzate was evaporated to dryness under reduced pressure to remove excess HCl. The residue was applied on a column containing 100 ml of Diaion SK-1 (sulfonated cation exchanger (H^+, 100 mesh) produced by Mitsubishi Kasei Co., Tokyo, Japan), washed with deionized water and eluted with 2 *N* ammonia solution. The eluate was evaporated under reduced pressure. The residue was dissolved in about 10 ml of 0.2 *M* acetic acid and filtered. The filtrate was transferred to a column containing 100 ml of Amberlite IRA-68 (CH_3COO^-, 50 mesh) and fractionated with 800 ml of 2 *M* acetic acid and 2 *M* HCl. Each fraction was evaporated to dryness under reduced pressure.

The residues of each fraction were fractionated on a column containing 100 ml of Diaion SK-1 with 0.2 *M* HCl, and each 100 ml of eluate was dried and tested by high-voltage paper electrophoresis [9].

The fractions containing DCEC, CMC and isobuteine were analyzed by isotachophoretic analyzer and amino acid analyzer.

INSTRUMENTATION

The capillary apparatus was a Shimadzu IP-1B isotachophoretic analyzer [10,11] (Shimadzu Seisakusho, Kyoto, Japan). The separations were carried out in a capillary tube, 20 cm × 0.5 mm I.D., which was maintained at a constant temperature of 20°C. The detector cell had an I.D. of 0.5 mm and length of 0.05 mm. The migration current was 50 μA. The leading electrolyte consisted of 0.01 *M* hydrochloric acid and β-alanine (pH 3.1). The terminal electrolyte was 0.01 *M* caproic acid.

The amino acid analyzer used was an Hitachi Model 835 liquid chromatograph. The chemicals used were analytical grade. Authentic DCEC, CMC and isobuteine were synthesized in our laboratory.

RESULTS AND DISCUSSION

It was possible to detect 1 nmol amounts of sulfur-containing amino acids by partial separation of a urine sample of 50—100 ml. The fraction containing DCEC was almost pure (Fig. 1B). The fractions containing CMC and isobuteine contained a couple of other amino acids, but it was possible to detect CMC and isobuteine in each fraction.

The standard curves prepared by plotting zone length against concentration for standard CMC, DCEC, isobuteine and cysteic acid were linear from 1 to 80 nmol.

Isotachophoretic analysis of acidic amino acids is shown in Fig. 1A. When DCEC, CMC, aspartic acid and isovalthine were run separately under only 100 μA, good resolution was obtained, but a mixture of these compounds did not give good separation in the same migration current. Eventually, we obtained good results, as shown in Fig. 1A, by changing the migration current from 100 to 50 μA.

Determination of DCEC, CMC and isobuteine in human urine by isotachophoresis and by amino acid analyzer is compared in Table I.

The recovery of these sulfur-containing amino acids during these column chromatographic procedures was approximately 95—100%. The pretreatment for determination of the amino acids in the isotachophoretic analyzer was more

TABLE I

COMPARISON OF THE DCEC, CMC AND ISOBUTEINE CONTENT DETERMINED FROM THE SAME SAMPLE BY THE ISOTACHOPHORETIC ANALYZER AND THE AMINO ACID ANALYZER

Each value represents the mean ± S.D. obtained from three separate determinations.

	Isotachophoresis (nmol/ml)	Amino acid analyzer (nmol/ml)
DCEC	9.84 ± 1.7	10.32 ± 1.2
CMC	0.56 ± 0.21	0.52 ± 0.12
Isobuteine	5.58 ± 1.5	5.39 ± 0.9

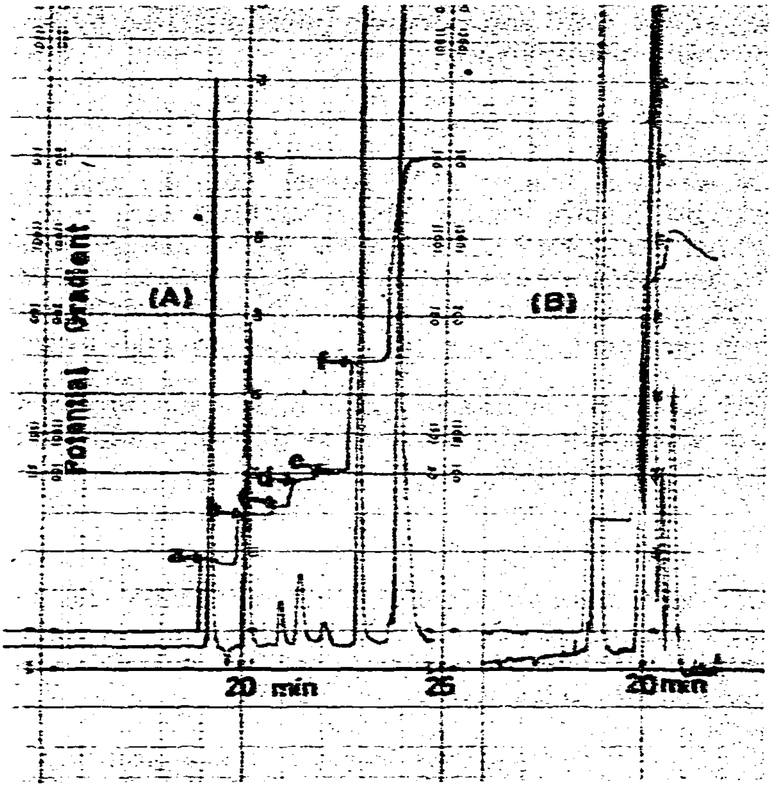

Fig. 1. Isotachophoretic runs of authentic samples (A) and the DCEC fraction (B). The leading electrolyte was 0.01 *M* HCl and β-alanine (pH 3.1) and the terminator was 0.01 *M* caproic acid. Migration current, 50 μA; chart speed, 10 mm/min; temperature of electrolyte, 20°C. Synthetic samples: (a) cysteic acid, (b) DCEC; (c) CMC, (d) isovalthine, (e) aspartic acid, (f) isobuteine.

simple than for the amino acid analyzer as described previously [1], and the method was very useful for detecting metabolites such as those described above.

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