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Note

Isotachophoretic analyses of cystine, homocystine and cystathionine in urines from patients with inborn errors of metabolism

NORIKO MIZOBUCHI, TOMIKO AGETA, KEIKO SASAKI and HIROYUKI KODAMA*

Department of Chemistry, Kochi Medical School, Oko-cho, Nankoku-shi, Kochi 781-51 (Japan)

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Cystinuria [1, 2] is one of the oldest recognized and most common inborn errors of metabolism. Cystine (Cys) and the dibasic amino acids lysine, ornithine and arginine are excreted in large amounts into the urine owing to the impaired absorption by the renal mucosa [3, 4].

In cystathionine β-synthase deficiency (homocystinuria), large amounts of homocystine (Hcys), an amino acid not normally detected in human urine, are excreted [5, 6]. Cystathionine (Cysta) is a key intermediate in the transsulphuration pathway of the methionine metabolism. It is not detectable in the urine from normal subjects but it is excreted in considerable amounts in the urine from patients with cystathioninuria [7].

The determination of sulphur-containing amino acids in the urine from patients with inborn errors of metabolism described above has been achieved mainly using an amino acid analyser. However, this method is time-consuming. Therefore, a new method for the simultaneous determination of urinary sulphur amino acids was devised using an isotachophoretic analyser [8—12].

The isotachophoretic method presented here has several advantages over previously described techniques.

EXPERIMENTAL

Authentic cystathionine, cystine and homocystine were obtained from Sigma (St. Louis, MO, U.S.A.). All other chemicals used were of analytical-reagent grade.

The samples from normal subjects were obtained from laboratory personnel. The samples from patients with inborn errors of the metabolism of sulphur amino acids were obtained from patients reported in previous papers [13—15].

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A 1-ml sample of each urine sample was applied to a column containing 5 ml of Diaion SK-I (H^+ cation exchanger, 100 mesh; Mitsubishi Kasei, Tokyo, Japan), washed with 50 ml of water and eluted with 30 ml of 2 *M* ammonia solution. The eluate was evaporated to dryness under reduced pressure.

An aliquot of the residue was analysed using isotachophoretic and amino acid analysers.

Apparatus

The capillary apparatus used was a Shimadzu IP-1B isotachophoretic analyser (Shimadzu Seisakusho, Kyoto, Japan). The separations were carried out in a capillary tube (20 cm × 0.5 mm I.D.) maintained at a constant temperature of 20°C. The migration current was 75 μA. The detector cell had an I.D. of 0.5 mm and a length of 0.05 mm. The chart speed was 10 mm/min. The leading electrolyte consisted of 0.01 *M* hydrochloric acid and 2-amino-2-methyl-1,3-propanediol (amediol) containing 5% of polyvinyl alcohol (pH 8.9). The terminal electrolyte was 0.01 *M* γ-aminobutyric acid and barium hydroxide (pH 10.9).

RESULTS AND DISCUSSION

Isotachophoretic analyses of a mixture of reference compounds and the urine from a patient with cystinuria are shown in Fig. 1.

We have ascertained that the authentic cystine, homocystine and cystathionine were well separated under the analytical conditions described under

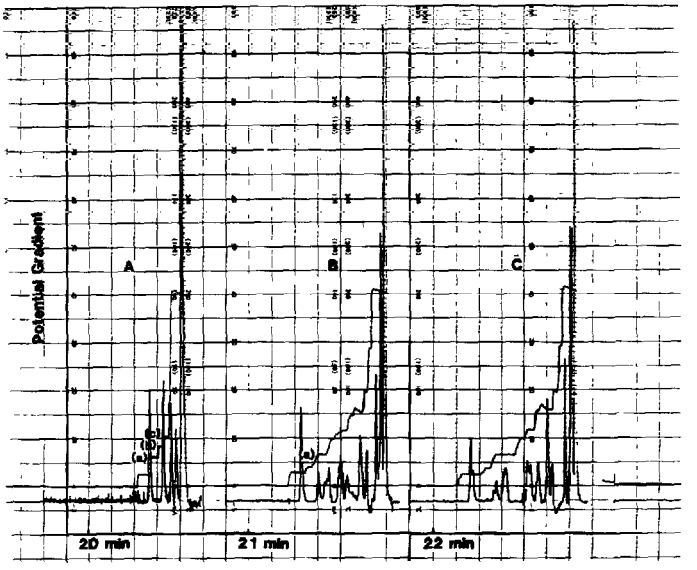

Fig. 1. Isotachophoretic analyses of (A) authentic sulphur amino acids [(a) cystine, (b) cystathionine and (c) homocystine]; (B) the urine from a patient with cystinuria; (C) B + cystine. The leading electrolyte was 0.01 *M* hydrochloric acid and amediol containing 5% of poly(vinyl alcohol) (pH 8.9). The terminal electrolyte was 0.01 *M* γ-aminobutyric acid and barium hydroxide (pH 10.9).

Experimental (Fig. 1A). The slopes of the calibration graphs drawn by plotting the zone length versus the concentrations of cystine, homocystine and cystathionine were linear over the concentration range from 0 to 100 nmol. A zone that had the same potential gradient as authentic cystine was detected in the urine sample from a patient with cystinuria (Fig. 1B). The zone of cystine in Fig. 1B and that of authentic cystine overlapped on addition of authentic cystine to the urine sample, resulting in an elongation of the zone of cystine in the urine sample, as shown in Fig. 1C. The recoveries of authentic cystine, homocystine and cystathionine added to normal urine (20 nmol) analysed using the chromatographic procedure described in this paper were 101 ± 2%, 94 ± 1% and 100 ± 3% (n=5), respectively.

When non-hydrolysed urine from a patient with homocystinuria was analysed by isotachophoresis, a zone with the same potential gradient as authentic homocystine on the isotachopherogram was not detected in the urine from a homocystinuric patient, but it was detected in the hydrolysed urine (Fig. 2A). When authentic homocystine was added to the hydrolysed urine from a homocystinuric patient, the two zones overlapped, resulting in an elongation of the zone of homocystine in the urine of the homocystinuric patient, as shown in Fig. 2B.

The determination of cystathionine in the urine from a cystathioninuric patient has been reported in a previous paper [16]. The zones of cystine,

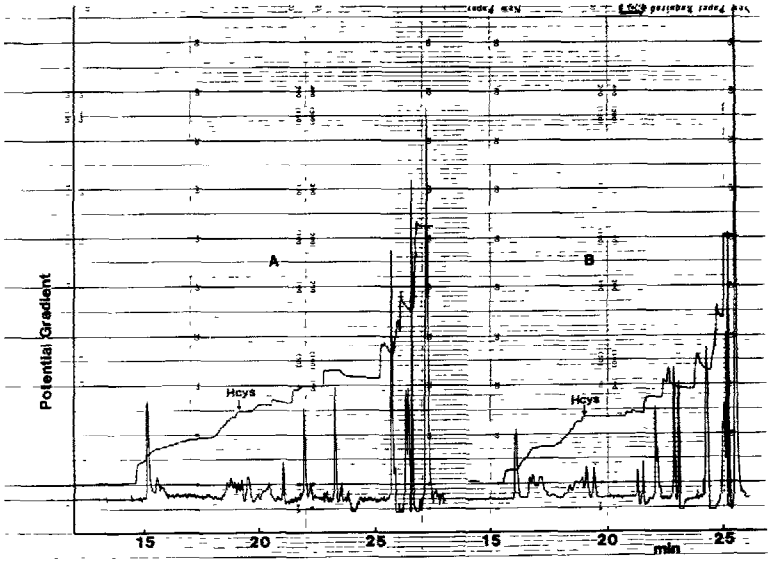

Fig. 2. Isotachophoretic analyses of (A) the hydrolysate of the amino acids fraction from the urine of a patient with homocystinuria; (B) A + homocystine. The urine from a patient with homocystinuria was hydrolysed by adding 2 mmol of sodium hydroxide and boiling at 100°C for 3 min. The hydrolysate was applied to a column containing 5 ml of Diaion SK-I, washed with 50 ml of water and eluted with 30 ml of 2 *M* ammonia solution. The eluate was evaporated to dryness under reduced pressure. An aliquot of the residue was analysed using an isotachophoretic analyser

TABLE I

COMPARISON OF CYSTINE, HOMOCYSTINE AND CYSTATIONINE CONTENTS IN HUMAN URINES DETERMINED USING ISOTACHOPHORETIC AND AMINO ACID ANALYSERS

Values are expressed in nmol/ml

Sample	Isotachophoretic analyser						Amino acid analyser					
	Non-hydrolysed			Hydrolysed			Non-hydrolysed			Hydrolysed		
	Cys	Hcys	Cysta	Cys	Hcys	Cysta	Cys	Hcys	Cysta	Cys	Hcys	Cysta
Normal human	—	—	—	—	—	—	22.9	—	—	20.7	—	—
Cystinuria	640+35*	—	—	560+40*	—	—	559 5	—	—	554.6	—	—
Homocystinuria	—	—	—	—	241.4+6.4*	—	—	—	—	—	210 2+4.1*	—
Cystathioninuria	—	—	2866.7+52*	—	—	2120+60*	—	—	2281.5	—	—	2295.3

*Mean + standard error (n=5).

homocystine and cystathionine in normal human urine were not detected on the isotachopherogram.

The results of the determination of cystine, homocystine and cystathionine in the urines of cystinuric, homocystinuric and cystathioninuric patiens using an isotachophoretic analyser and amino acid analysers are compared in Table I.

The values of cystine and cystathionine in non-hydrolysed urine determined using isotachophoresis were higher than those obtained using an amino acid analyser. Conversely, the values of cystine and cystathionine in the hydrolysed urine agreed well with those obtained with the amino acid analyser. Homocystine was not detected in non-hydrolysed urine from a homocystinuric patient. However, it was detected in the hydrolysed urine, and the value agreed well with those obtained using the amino acid analyser. These results indicate that homocystine in the urine from a homocystinuric patient was present mainly as homocysteine thiolactone.

The mass screening for subjects with cystinuria and homocystinuria was carried out using the cyanide—nitroprusside test, but it was impossible to distinguish between cystinuria and homocystinuria using this test. However, this is possible using the isotachophoretic method presented here. In addition, the analysis time of the isotachophoretic method is shorter than that using an amino acid analyser.

REFERENCES

1 F. Toel, Ann. Chem. Pharm., 96 (1855) 247—258.
2 H.W. Stein, Proc. Soc. Exp Biol., 78 (1951) 705—708.
3 L.H. Yeh, W. Frankl, S.M. Dunn, P. Parker, B. Hughers and P. Gyorgy, Am. J. Med. Sci., 214 (1947) 507—512.
4 E.C. Dent and A.G. Rose, Q. J. Med., 20 (1951) 205—219.
5 A.N. Carson and D. Neill, Arch. Dis. Childh., 37 (1962) 505—513.
6 T. Gervitsen, G.J. Vaughn and A.H. Waisman, Biochem. Biophys. Res. Commun., 9 (1962) 493—496.
7 H.S. Mudd, in J.B. Stanburg, J.B. Wijngaarden and D.S. Fredrickson (Editors), The Metabolic Basis of Inherited Disease, McGraw-Hill, New York, 4th ed., 1978, p. 458.
8 H. Huglund, Sci. Tools, 17 (1970) 2—12.
9 L. Arlinger, Biochim. Biophys. Acta, 393 (1975) 393—403.
10 H. Kodama and S. Uasa, J. Chromatogr., 163 (1979) 300—303.
11 M. Mikasa, T. Ageta, N. Mizobuchi and H. Kodama, Anal. Biochem., 126 (1982) 52—57.
12 H. Kodama, N. Mizobuchi, K. Sasaki and H. Mikasa, Anal. Biochem., 133 (1983) 100—103.
13 H. Kodama, T. Ikegami and T. Araki, Physiol. Chem. Phys., 6 (1974) 87—89.
14 H. Kodama, S. Mizuhara, K. Yao, K. Kobayashi, K. Hirayama, Y. Fujii, H. Haraguchi and M. Hirosawa, Physiol. Chem. Phys., 1 (1969) 72—76.
15 H. Kodama, Physiol. Chem. Phys., 3 (1971) 159—167.
16 H. Kodama, K. Sasaki, H. Mikasa, D. Cavallini and G. Ricci, J. Chromatogr., 311 (1984) 183—188.