Journal of Chromatography, 579 (1992) 318–323
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6441

Short Communication

Determination of cystathionine and perhydro-1,4-thiazepine-3,5-dicarboxylic acid in the urine of a patient with cystathioninuria using column liquid chromatography–mass spectrometry

Kazunori Sugahara

Department of Chemistry, Kochi Medical School, Kohasu, Oko-cho, Nankoku-shi, Kochi 783 (Japan)

Jun Ohta

Department of Biochemistry, Medical School, Okayama University, Okayama 700 (Japan)

Megumi Takemura

Department of Ophthalmology, Kochi Medical School, Kohasu, Oko-cho, Nankoku-shi, Kochi 783 (Japan)

Hiroyuki Kodama

Department of Chemistry, Kochi Medical School, Kohasu, Oko-cho, Nankoku-shi, Kochi 783 (Japan)

(First received March 16th, 1992; revised manuscript received May 4th, 1992)

ABSTRACT

A method for the measurement of cystathionine and perhydro-1,4-thiazepine-3,5-dicarboxylic acid in the urine of a patient with cystathioninuria has been developed, using column liquid chromatography–mass spectrometry. Cystathionine and perhydro-1,4-thiazepine-3,5-dicarboxylic acid were determined by scanning the $[M + H]^+$ ions of each compound. The recoveries were 80–92.4% for cystathionine and 80–100% for perhydro-1,4-thiazepine-3,5-dicarboxylic acid after ion-exchange treatment. The results agreed well with those obtained using an amino acid analyser. The concentrations found for cystathionine and perhydro-1,4-thiazepine-3,5-dicarboxylic acid were 1.289 ± 0.099 mg/ml and 0.310 ± 0.0067 mg/ml, respectively.

Correspondence to: Professor Hiroyuki Kodama, Department of Chemistry, Kochi Medical School, Kohasu, Oko-cho, Nankoku-shi, Kochi 783, Japan.

0378-4347/92/$05.00

INTRODUCTION

It has been reported previously [1–4] that the following unusual sulphur-containing amino acids are excreted in the urine of a cystathioninuric patient: S-(3-hydroxy-3-carboxy-*n*-propyl)-cysteine, S-(β-carboxymethyl)homocysteine, S-(2-hydroxy-2-carboxymethyl)homocysteine, N-acetylcystathionine, cystathionine sulphoxide and perhydro-1,4-thiazepine-3,5-dicarboxylic acid (PHTZDC). All of these, except perhydro-1,4-thiazepine-3,5-dicarboxylic acid, can be easily determined with an imino acid analyser. The absorptivity of perhydro-1,4-thiazepine-3,5-dicarboxylic acid in the ninhydrin reaction, because it has only an amino group in its structure, is too low to be determined by an amino acid analyser. Recently, the analysis of mixtures of non-volatile compounds has become increasingly important, and column liquid chromatography combined with atmospheric pressure ionization mass spectrometry (LC–API-MS) shows promise as a new analytical method [5–7]. We scanned the quasi molecular ions $[M + H]^+$ of synthetic cystathionine and perhydro-1,4-thiazepine-3,5-dicarboxylic acid using this technique. The aim was to demonstrate the utility of this approach in the quantitative analysis of sulphur-containing amino acids in biological samples.

EXPERIMENTAL

Materials

Synthetic cystathionine (cysta) was obtained from Sigma (St. Louis, MO, USA). Perhydro-1,4-thiazepine-3,5-dicarboxylic acid (PHTZDC) was prepared according to ref. 8. All other chemicals used were of analytical grade.

Urine samples and preparation

Normal human urine samples were obtained from laboratory personnel. The urine sample of the patient with cystathioninuria was obtained from elder sisters as reported in a previous paper [1]. It was stored at −20°C.

Sulphur-containing amino acids were isolated as follows: 1-ml urine samples were applied to a column containing 5 ml of Diaion SK-1 (H form of sulphonated cation exchanger, 100 mesh; Mitsubishi, Tokyo, Japan), washed with 25 ml of water, and eluted with 30 ml of 2 *M* NH_4OH. The washed effluent, wash water and the 2 *M* NH_4OH eluate were collected separately. The 2 *M* NH_4OH eluate was evaporated under reduced pressure. The effluent plus wash water was applied to a column containing 10 ml of Diaion SA-100 (HCOOH form of anion exchanger, 100 mesh. Mitsubishi), washed with water and eluted with 20 ml of 15% formic acid. The eluate was evaporated to dryness under reduced pressure. The residues of the 2 *M* NH_4OH and 15% formic acid eluates were separately dissolved in 1 ml of water, then analysed by an automatic amino acid analyser (Hitachi Model 835 liquid chromatograph) and LC–API-MS.

Instrumentation

The apparatus used was a Hitachi L-6200 HPLC instrument, equipped with a 5 -μm Inertsil ODS-2 packed column (150 × 4.6 mm I.D.) from Gasukuro Kogyo (Tokyo, Japan), connected to a Hitachi M80B mass spectrometer–computer system, through the API interface [6,7]. The nebulizer and vaporizer temperatures were 255°C and 380°C, respectively. Synthetic and urinary sulphur-containing amino acids were separated with a mobile phase of 100 m*M* CH_3COONH_4 titrated with 2 *M* CH_3COOH (pH 4.0)–CH_3CN (90:10, v/v) at a flow-rate of 0.9 ml/min.

RESULTS AND DISCUSSION

Mass chromatograms and mass spectra of synthetic perhydro-1,4-thiazepine-3,5-dicarboxylic acid and cystathionine, obtained using the LC–AP-MS system, are shown in Fig. 1. In the LC–API-MS system, the quasi molecular ions $[M + H]^+$ of these sulphur amino acids were observed as base peaks, at *m/z* 223 for cystathionine and *m/z* 206 for PHTZDC. Additional ions, $M - CO_2$, $M - 2CO_2$ and $[M + NH_4]^+$, were also detected. The standard curves for different concentrations of cystathionine and PHTZDC were

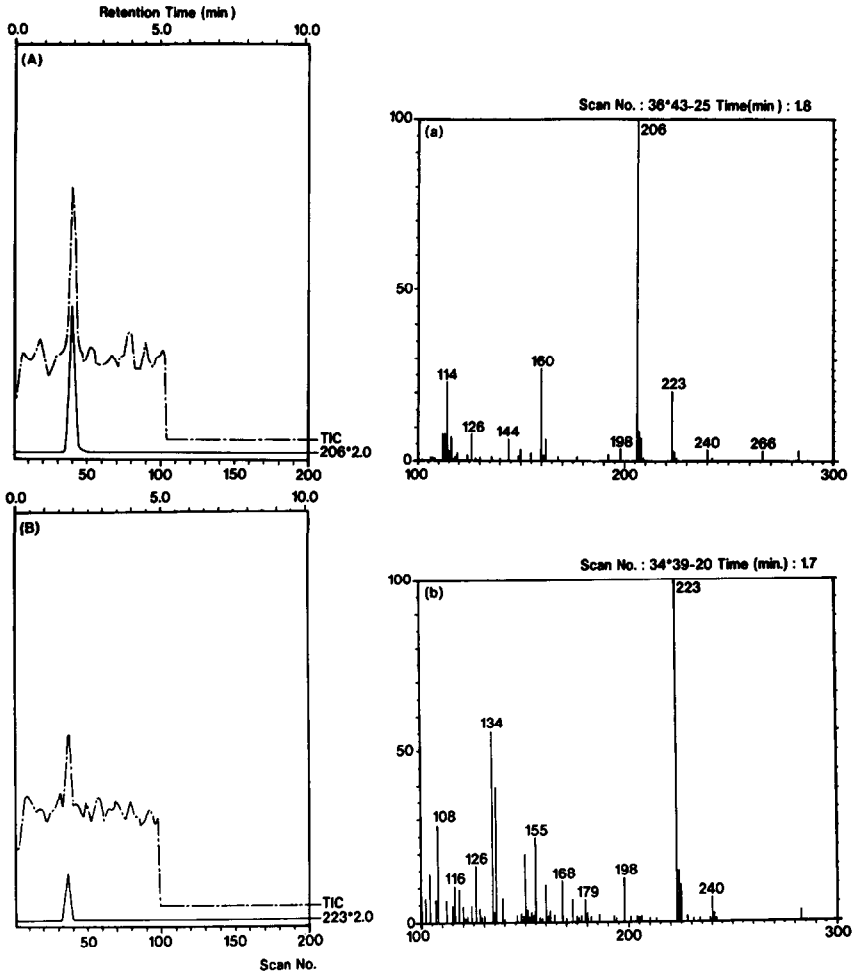

Fig. 1. Mass chromatograms of (A) synthetic perhydro-1,4-thiazepine-3,5-dicarboxylic acid (*m/z* 206) and (B) cystathionine (*m/z* 223), and mass spectra of (a) PHTZDC and (b) cystathionine as scanned at the peak tops of the mass chromatograms in A and B. The chromatographic conditions were: mobile phase, 100 m*M* CH_3COONH_4 titrated with 2 *M* CH_3COOH (pH 4.0)–CH_3CN (90:10, v/v); flow-rate, 0.9 ml/min. The mass spectrometer was scanned from *m/z* 100 to 300 at a rate of 4 s per scan.

linear over the concentration ranges from 100 to 500 or 600 ng, respectively.

The mixed solution of cystathionine and PHTZDC was treated by ion-exchange resins (Diaion SK-I and SA-100) as described in Experimental. The 2 *M* NH_4OH eluate from Diaion SK-I and the 15% formic acid eluate from Diaion SA-100 were analysed during LC–API-MS. Cystathionine (*m/z* 223) was detected in the NH_4OH eluate and PHTZDC (*m/z* 206) in the formic acid eluate, as shown in Fig. 2A and B, respectively. The retention time of cystathionine was *ca.* 1.8 min, and that of PHTZDC *ca.* 2.1 min.

The recoveries of synthetic cystathionine (400 ng/10 μl) and PHTZDC (400 ng/10 μl) after treatment with ion-exchange resins were determined seven times. The recovery of cystathionine was *ca.* 87% (80.4–100.4%). The recovery of PHTZDC was *ca.* 90% (80.4–93.9%). These recoveries indicate that this method is reliable for the measurement of both substances.

Mass chromatograms of the fraction containing cystathionine or PHTZDC from the urine

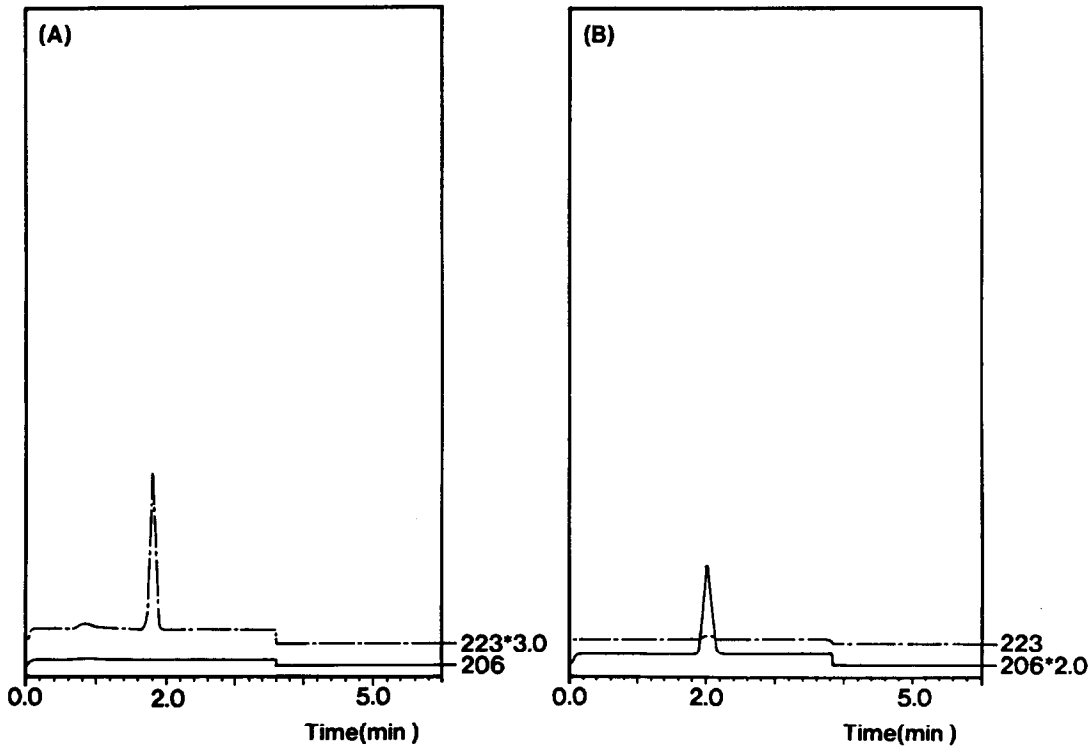

Fig. 2. Mass chromatograms of the eluates of 2 *M* NH_4OH (A) and 15% formic acid (B). A mixture of cystathionine and PHTZDC was applied to Diaion SK-1, washed with water and eluted with 2 *M* NH_4OH. The 2 *M* NH_4OH eluate was evaporated under reduced pressure. The effluent and wash water were combined, applied to Diaion SA-100, washed with water and eluted with 30 ml of 15% formic acid. This eluate was evaporated under reduced pressure. The NH_4OH eluate (A) and the formic acid eluate (B) were analysed by LC–API-MS.

samples of a normal person and from a patient with cystathioninuria are shown in Fig. 3. Neither cystathionine nor PHTZDC was detected in the normal human urine, but both compounds were detected in the urine of the patient with cystathioninuria. The values for cystathionine in the urine obtained using LC–API-MS coincided well with those obtained using an amino acid analyser (Table I). The contents of cystathionine and cystathionine metabolites are presented in Table II.

The other sulphur-containing amino acids, except for PHTZDC, among cystathionine metabolites, were determined by an amino acid analyser following the method reported previously. PHTZDC was excreted at higher levels than other cystathionine metabolites into the urine of the patient with cystathioninuria. These results suggest that the formation of PHTZDC from cystathionine is one of the main pathways of cystathionine degradation in this patient.

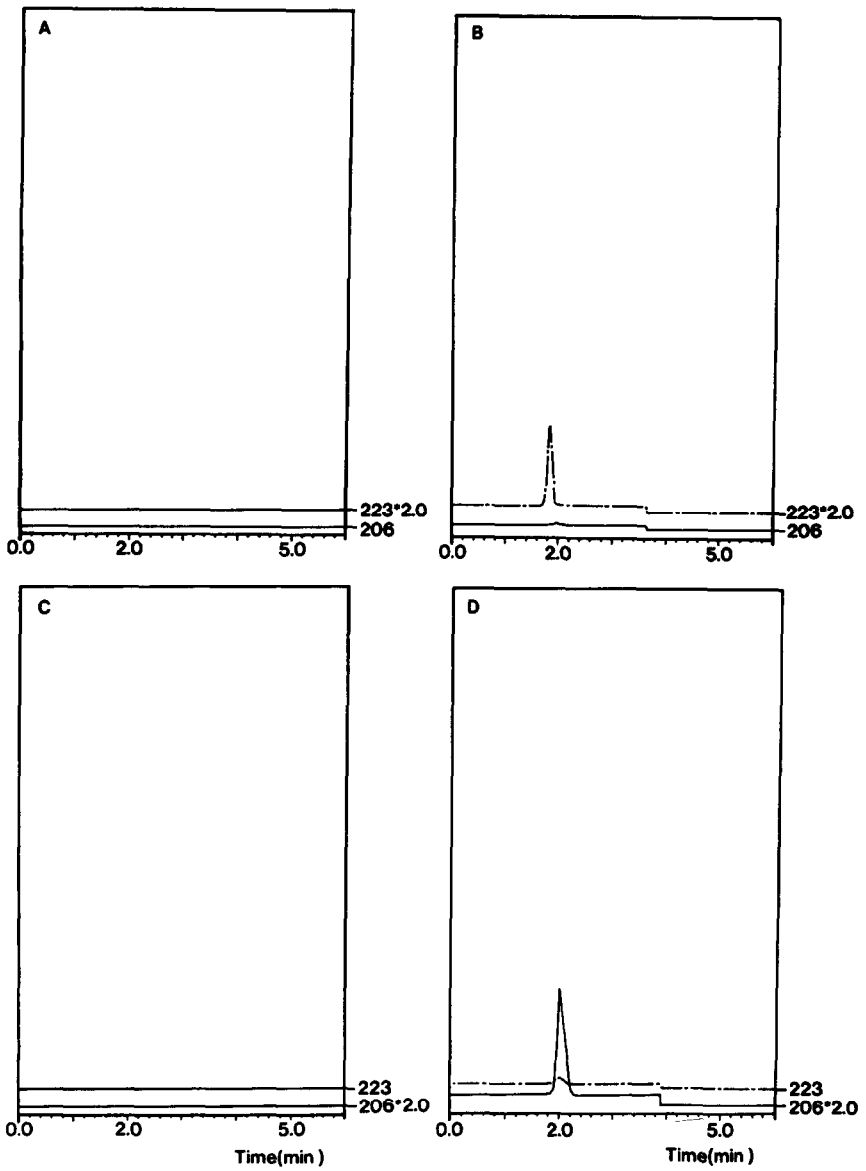

Fig. 3. Mass chromatograms of the 2 *M* NH_4OH eluate (A and B) and the 15% formic acid eluate (C and D) from the urine samples of a normal person (A and C) and a patient with cystathioninuria (B and D). The urine samples were prepared as described in Fig. 3.

TABLE I

CYSTATHIONINE AND PERHYDRO-1,4-THIAZEPINE-3,5-DICARBOXYLIC ACID LEVELS IN THE URINE OF A PATIENT WITH CYSTATHIONINURIA

	Cystathionine (mg/ml)		PHTZDC (mg/ml)
	LC–MS	AAA[a]	LC–MS
1	$116.2 \cdot 10^{-2}$	$122.7 \cdot 10^{-2}$	$31.0 \cdot 10^{-2}$
2	$125.8 \cdot 10^{-2}$	$130.3 \cdot 10^{-2}$	$31.9 \cdot 10^{-2}$
3	$125.9 \cdot 10^{-2}$	$136.3 \cdot 10^{-2}$	$30.2 \cdot 10^{-2}$
4	$146.6 \cdot 10^{-2}$	$146.6 \cdot 10^{-2}$	$30.3 \cdot 10^{-2}$
5	$103.2 \cdot 10^{-2}$	$141.8 \cdot 10^{-2}$	$31.5 \cdot 10^{-2}$
Mean ± S.D.	128.9 ± 9.9	135.5 ± 8.4	31.0 ± 0.67

[a] Values obtained using an amino acid analyzer.

TABLE II

CONCENTRATIONS OF SULPHUR-CONTAINING AMINO ACIDS IN THE URINE OF A CYSTATHIONINURIC PATIENT

Compound	Concentration (mg/ml)
Cystathionine	$119.9 \cdot 10^{-2}$
NAc-cystathionine	$7.4 \cdot 10^{-2}$
Perhydro-1,4-thiazepine-3,5,-dicarboxylic acid (PHTZDC)	$30.9 \cdot 10^{-2}$
S-(3-Hydroxy-3-carboxy-*n*-propyl)cysteine (HCPC)	$2.1 \cdot 10^{-2}$
S-(Carboxymethyl)homocysteine (CMHC)	$4.5 \cdot 10^{-2}$
S-(2-Carboxyethyl)cysteine (β-CEC)	$0.14 \cdot 10^{-2}$
S-(2-Hydroxy-2-carboxyethyl)homocysteine (HCEHC)	$0.08 \cdot 10^{-2}$
S-(3-Hydroxy-3-carboxy-*n*-propyl)-N-acetylcysteine (NAc-HCPC)	$0.5 \cdot 10^{-2}$
S-(2-Carboxyethyl)-N-acetylcysteine (NAc-β-CEC)	$0.05 \cdot 10^{-2}$
Cystathionine sulphoxide	+

REFERENCES

1 H. Kodama, K. Yao, K. Kobayashi, K. Hirayama, Y. Fujii, S. Mizuhara, H. Haraguchi and M. Hirosawa, *Physiol. Chem. Phys.*, 1 (1969) 72.
2 H. Kodama, S. Ohmori, M. Suzuki and S. Mizuhara, *Physiol. Chem. Phys.*, 2 (1970) 287.
3 H. Kodama, Y. Ishimoto, M. Shimomura, T. Hirota and S. Ohmori, *Physiol. Chem. Phys.*, 7 (1975) 147.
4 T. L. Perry, S. Hansen, D. Love and C. A. Finch, *Nature (London)*, 219 (1968) 178.
5 E. C. Horning, D. I. Carroll, I. Dzidic, K. D. Haefele, M. G. Horning and R. N. Stillwell, *J. Chromatogr.*, 99 (1974) 13.
6 M. Sakairi and H. Kambara, *Anal. Chem.*, 60 (1988) 774.
7 H. Kodama, H. Nakamura, K. Sugahara and Y. Numajiri, *J. Chromatogr.*, 527 (1990) 279.
8 G. Ricci, L. Santoro, M. Achilli, R. M. Matarese, M. Nardini and D. Cavallini, *J. Biol. Chem.*, 258 (1983) 10511.
9 H. Kodama, H. Mikasa, K. Sasaki, S. Awata and K. Nakayama, *Arch. Biochem. Biophys.*, 225 (1983) 25.