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## Short Communication

# Liquid chromatographic–mass spectrometric analysis for screening of patients with cystinuria, and identification of cystine stone

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ABSTRACT

Analyses of amino acids in the urine of a normal human and of patients with heterozygous and homozygous cystinuria have been carried out, using liquid chromatography–mass spectrometry with an atmospheric pressure ionization interface system. A kidney cystine stone was also analysed by this system. Very intense quasi-molecular ions ($[M + H]^+$) of standard cystine, arginine, lysine and ornithine were observed on mass chromatograms as base peaks. Mass chromatograms of the urine samples from a normal human and from patients with heterozygous and homozygous cystinuria were easily distinguishable. The retention times in the mass chromatogram and mass spectrum of kidney stone cystine was almost the same as that of authentic cystine.

## INTRODUCTION

Cystinuria is an autosomal recessive hereditary disorder, and phenotypical homozygotes excrete large amounts of cystine, arginine, lysine and ornithine in the urine. Cystine is precipitated in urine because of its relative insolubility and, clinically, urolithiasis is the most important problem for cystinuric patients. The early detection of the cystinuric state is meaningful because cystine bladder calculi occur almost exclusively in children. The cyanide-nitroprusside test has been

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widely applied as a chemical screening procedure [1,2]. The reaction permits easy detection of homozygous stone-formers, who excrete usually more than 250 mg of cystine per gram of creatinine [3,4]. Some, but not all, of these heterozygotes with increased urinary cystine may be detected by this procedure. A positive cyanide-nitroprusside test should be supported by the identification of urinary amino acids by a method such as high-performance liquid chromatography (HPLC). However, HPLC is unsuitable for mass screening of cystinuria, because it takes 2 h for the analysis of a single sample.

We tried to established a new and convenient diagnostic procedure for cystinuria using liquid chromatography combined with atmospheric pressure ionization mass spectrometry (LC–API-MS) [5–7]. The aim of present experiment was to demonstrate the usefulness of this approach in qualitative analyses of cystine and the dibasic amino acids in biological samples.

## EXPERIMENTAL

### *Reagents*

Cystine, arginine, lysine and ornithine were purchased from Sigma. All other chemicals used were of analytical grade.

### *Isolation of amino acids from urine*

Urine samples were obtained from normal subjects and from homozygous and heterozygous cystinuria patients previously diagnosed from the results of HPLC analysis of urinary amino acids. Each 3-ml urine sample was adjusted to pH 3–5 with 2 *M* acetic acid and then transferred to a column containing 10 ml of Diaion SK-1 ($H^+$ form, 100 mesh, Mitsubishi Kasei, Tokyo, Japan), washed with 30 ml of water and eluted with 2 *M* ammonia. The eluate was evaporated to dryness under reduced pressure. The residue was dissolved 1.5 ml of water and subjected to LC–API-MS.

### *Treatment of cystine stone*

The kidney cystine stone was surgically extirpated from a homozygous cystinuric patient diagnosed by HPLC. The stone was identified by infrared spectroscopy as pure cystine. The stone was crushed, dissolved in 0.2 *M* HCl and subjected to LC–API-MS.

### *Instrumentation*

The apparatus used was a Hitachi L-6200 instrument, equipped with a 5-$\mu$m Inertsil ODS 2-packed column (150 mm × 4.6 mm I.D.) from Gasukuro Kogyo (Tokyo, Japan), connected to a Hitachi M-80B mass spectrometer computer system, through the API interface. The nebulizer and vaporizer temperatures were 280 and 390°C, respectively, and the drift voltage was 180 V.

Elution of amino acids was carried out with a mobile phase of acetonitrile–100

m*M* ammonium acetate adjusted to pH 4.0 with 2 *M* acetic acid (10:90, v/v) at a flow-rate of 0.9 ml/min.

## RESULTS AND DISCUSSION

The standards Cys, Arg, Lys and Orn were analysed by the LC–API-MS, and their mass chromatograms and mass spectra were obtained. In the LC–API-MS

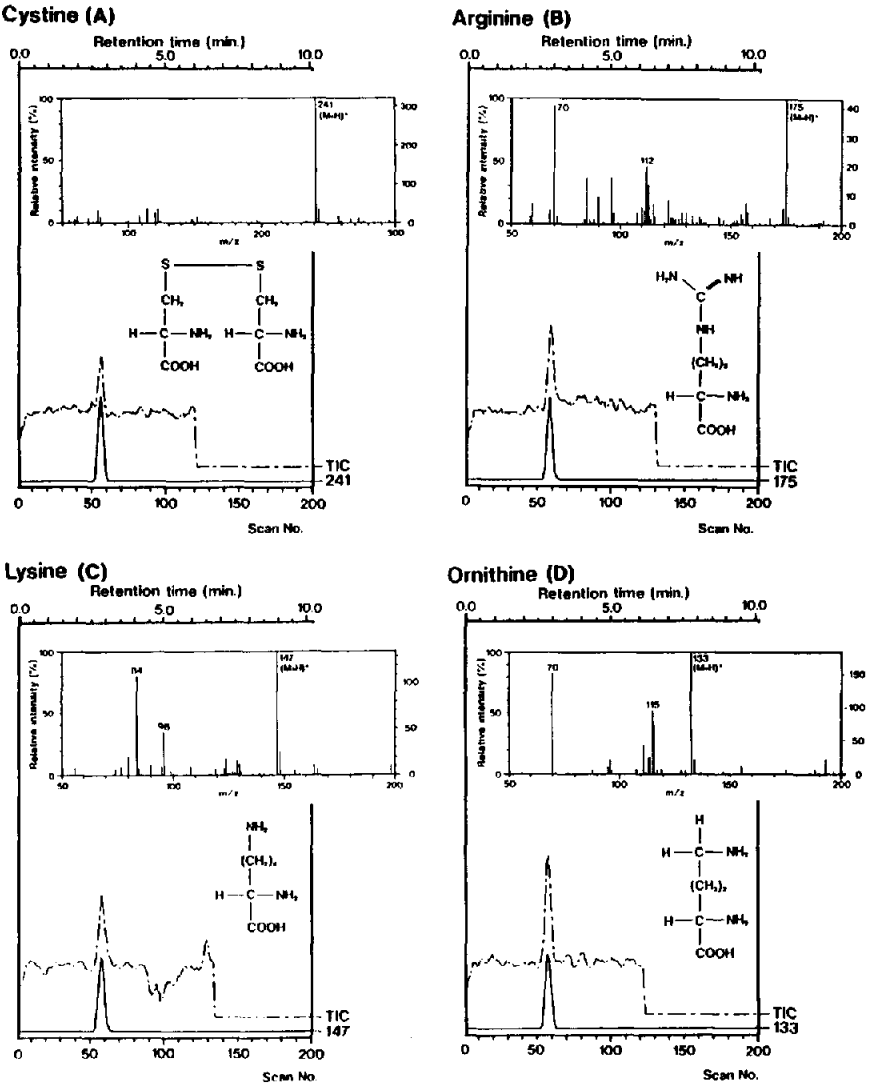


Fig. 1. Mass chromatograms and mass spectra of standard amino acids: (A) cystine (*m/z* 241); (B) arginine (*m/z* 175); (C) lysine (*m/z* 147); (D) ornithine (*m/z* 133).

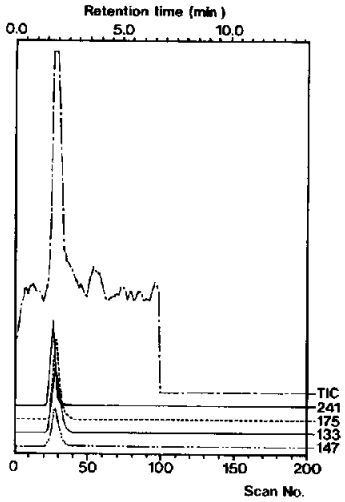


Fig. 2. Mass chromatograms of the mixture of cystine, arginine, lysine and ornithine.

system, quasi-molecular ions $[M+H]^+$ of each amino acid were observed as base peaks (Fig. 1). Mass chromatograms of the mixture of these amino acids are shown in Fig. 2. Peaks of the quasi-molecular ions of each amino acid were clearly distinguished. Mass chromatograms of the urine samples from a normal human and from patients with heterozygous and homozygous cystinuria are shown in Fig. 3. Peaks of the quasi-molecular ions of Cys (*m/z* 241), Arg (*m/z*

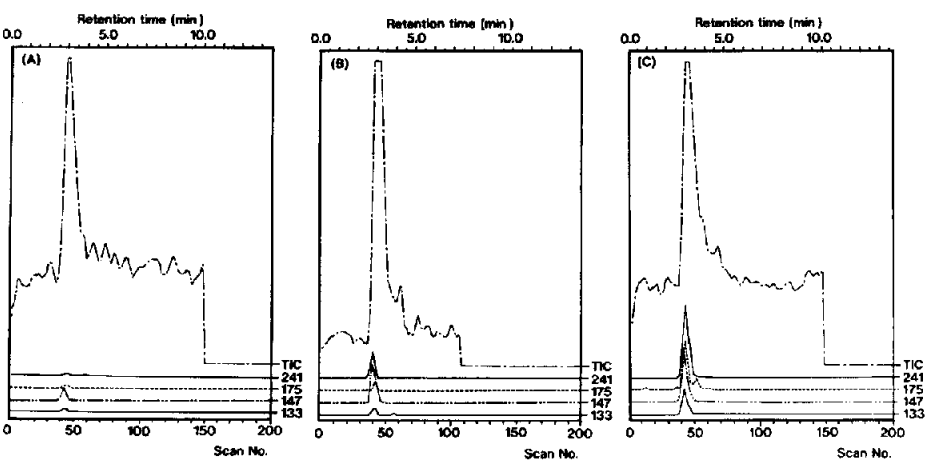


Fig. 3. Mass chromatograms of cystine, arginine, lysine and ornithine in the urine of (A) a normal human, (B) a heterozygote with cystinuria and (C) a homozygote with cystinuria.

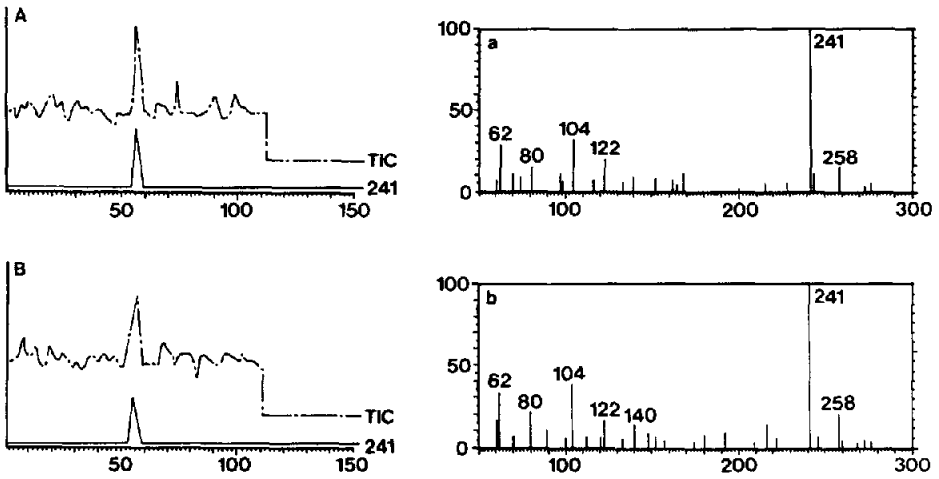


Fig. 4. Mass chromatograms and mass spectra of (A) standard cystine and (B) cystine stone obtained from a homozygote with cystinuria.

175), Lys (*m/z* 147) and Orn (*m/z* 133) appeared in the mass chromatograms obtained from the urine sample from the homozygote (Fig. 3C). The retention times of the *m/z* 241, 175, 147 and 133 ions were the same as those of authentic Cys, Arg, Lys and Orn, so that the patient was identified as a homozygous cystinuric subject. On the mass chromatograms of the urine sample from the heterozygote, peaks at *m/z* 241, 175, 147 and 133 were also observed, but the peaks of Cys and Arg were relatively low compared with those in the mass chromatograms obtained from the urine sample from the homozygote (Fig. 3B). In contrast, although low peaks of the quasi-molecular ions of Lys and Orn were observed, the peaks of Cys and Arg could not be detected in the mass chromatograms obtained from a normal human urine (Fig. 3A). As a result, we could easily distinguish between normal control, heterozygous and homozygous cystinuria.

Mass chromatograms and mass spectra of authentic cystine and kidney stone cystine obtained from a patient with homozygous cystinuria are shown in Fig. 4. A peak of *m/z* 241 was observed in the mass chromatogram of the sample of cystine stone, and also in the mass spectrum as the base peak. The mass spectrum was almost the same as that of authentic cystine, so we could identify cystine stone quickly using the LC–API-MS system.

The results suggest that this method is very useful for the screening of patients with homozygous and heterozygous cystinuria and for the identification of cystine stone.

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