Isolation of *S*-[2-carboxy-1-(1*H*-imidazol-4-yl)ethyl]cysteine from human urine

Masahiro KINUTA,* Toshihiko UBUKA, Kenzaburoh YAO, Shinya FUTANI, Masayuki FUJIWARA and Yoshiatsu KUROZUMI

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

S-[2-Carboxy-1-(1*H*-imidazol-4-yl)ethyl]cysteine (I), a proposed precursor of 3-[(carboxymethyl)thio]-3-(1*H*-imidazol-4-yl)propanoic acid [Kinuta, Yao, Masuoka, Ohta, Teraoka & Ubuka (1991) *Biochem. J.* 275, 617–721], was isolated from healthy human urine by using ion-exchange column chromatography. Identification of the isolated compound with compound (I) was performed by physicochemical analyses involving i.r., m.s. and n.m.r. spectrometries as well as high-voltage paper electrophoresis, t.l.c. and paper chromatography. Compound (I) was synthesized in 80% yield by incubation of a reaction mixture containing *trans*-urocanic acid and 3-fold excess of cysteine at 70–75 °C. From these results we suggest that natural thiol compounds such as cysteine and GSH participate in the metabolism of urocanic acid, a key metabolite of L-histidine.

INTRODUCTION

S-[2-Carboxy-1-(1*H*-imidazol-4-yl)ethyl]cysteine {3-[2-amino-2-carboxyethyl]thio}-3-(1*H*-imidazol-4-yl)propanoic acid (I)} was previously synthesized as a proposed precursor of 3-[(carboxymethyl)thio]-3-(1*H*-imidazol-4-yl)propanoic acid (II), a novel imidazole derivative having a sulphur-containing side chain isolated from human urine [1]. However, we had no direct evidence showing the existence of compound (I) as a natural product, although enzymic degradation of the synthesized compound (I) with rat liver homogenate in a phosphate buffer formed compound (II) as previously described [1]. Further studies on the origin of compound (II) succeeded in finding an unidentified urinary compound that had the same electrophoretic mobility and the same R_F value as those of compound (I). The urinary compound has now been purified, and identified with compound (I). The present paper describes the isolation and identification of the urinary compound, suggesting that compound (I) is one of the physiological precursors of compound (II) and that natural thiol compounds such as cysteine and GSH participate in the metabolism of urocanic acid.

MATERIALS AND METHODS

Chemicals

Ion exchangers and chemicals used in the present work were as described previously [1]. For the detection of imidazole compounds, sulphur-containing compounds and amino acid derivatives, Pauly's reagent [2,3], chloroplatinate reagent [4] and ninhydrin reagent were used as described previously [1].

Paper electrophoresis

High-voltage paper electrophoreses were performed on Whatman no. 1 paper (Whatman, Maidstone, Kent, U.K.) in pyridine/acetic acid/water buffer (1:20:179, by vol.; pH 3.1) [5] as described previously [1], and relative mobilities of compounds on the paper electrophoretogram, termed the mR_{ILA} values, were determined.

T.l.c. and paper chromatography

T.l.c. was carried out on silica gel 60 t.l.c. plates (E. Merck, Darmstadt, Germany) in two solvent systems: solvent A, butan-

1-ol/acetic acid/water (12:3:5, by vol.); solvent B, propan-2-ol/85% (v/v) formic acid/water (16:1:4, by vol.). Ascending paper chromatography was performed on Whatman 3MM paper in solvent A.

Spectroscopy

Fast-atom-bombardment m.s. (f.a.b.-m.s.) and chemical-ionization m.s. (c.i.-m.s.) with a direct-inlet system were carried out on a Shimadzu 9020-DF gas chromatograph-mass spectrometer equipped with a Shimadzu SCAP 1123 data system. In f.a.b.-m.s. the target surface was bombarded by a beam of energetic argon atoms at 5 keV; glycerol was used as a solvent. In c.i.-m.s. 2-methylpropane was used as a reacting gas at an ionization voltage of 150 eV, ion-source temperature of 300 °C, direct-inlet temperature of 98 °C and accelerating voltage of 3 kV. ^1H -n.m.r. and ^{13}C -n.m.r. (400 MHz) spectra were recorded with a JEOL JNM-GX 400 n.m.r. spectrometer in $^2\text{H}_2\text{O}$ with sodium 3-trimethylsilyl(2,2,3,3- $^2\text{H}_4$)propionate as an internal standard. I.r. spectra (in Nujol) were recorded with a Japan Spectroscopic A-102 i.r. spectrophotometer.

RESULTS AND DISCUSSION

Isolation of compound (I) from human urine

The behaviour of compound (I) on ion-exchange chromatography was similar to that of compound (II), although the mR_{ILA} and R_F values of the two compounds were different as

Fig. 1. Structural formulae of *S*-[2-carboxy-1-(1*H*-imidazol-4-yl)ethyl]cysteine (I) and 3-[(carboxymethyl)thio]-3-(1*H*-imidazol-4-yl)propanoic acid (II)

Abbreviations used: f.a.b.-m.s., fast-atom-bombardment mass spectrometry; c.i.-m.s., chemical-ionization mass spectrometry.

* To whom correspondence and requests for reprints should be sent.

Fig. 2. F.a.b. mass spectra of (a) compound (I) and (b) the isolated urinary compound

previously described [1]. Therefore, as checked by paper electrophoresis and t.l.c., the desired urinary compound was purified with the successive ion-exchange columns Diaion SK no. 1, Diaion SA no. 100, Dowex 50 (H^+ form), Dowex 1 (acetate form) and Dowex 1 (OH^- form) essentially according to the previous method [1] used for isolation of compound (II). Further purification of the desired compound was carried out by paper chromatography. After chromatography, the desired compound, the R_f value of which was 0.18, was extracted from the paper with 0.02 M-acetic acid, and the extracts were combined. Finally, the extracts were chromatographed on Dowex 1 (acetate form) and Amberlite CG-50 (H^+ form) columns, and approx. 5 mg of crystals of the desired compound was obtained from a total of 120 litres of healthy human urine. The purity of the isolated compound was established by high-voltage paper electrophoresis, t.l.c. and paper chromatography. All the isolation procedures described above were performed in the dark-room to avoid an isomerization of *trans*-urocanic acid in urine to the *cis* form by u.v. light as previously described [1].

Chemical synthesis of compound (I)

Reaction of *trans*-urocanic acid with L-cysteine under nearly physiological conditions formed compound (I) (Fig. 1) in approx. 20% yield as previously described [1]. The yield of compound (I) was dependent on the reaction temperature and the initial concentration of L-cysteine in the reaction mixture. When the reaction of *trans*-urocanic acid (15 mmol) with a 3-fold excess of L-cysteine (45 mmol) in 20 ml of 2.25 M- Na_2CO_3 was performed at 70–75 °C, the yield was increased markedly to approx. 80%.

In the f.a.b. mass spectrum (Fig. 2a) signals were assigned as

follows: m/z 260 [base, MH^+ (the molecular ion plus proton)], 214 [$MH^+ - (CO_2H + H)$], 172 [$MH^+ - [CH_2-CH(NH_2)-CO_2H]$], 140 [$MH^+ - [S-CH_2-CH(NH_2)-CO_2H]$], 122 ($140 - H_2O$) and 95 ($140 - CO_2H$). In contrast, no signal of MH^+ was found on the c.i. mass spectrum; instead, signals of $MH^+ - H_2O$ and $(C_3H_3N_2-C_2H_2S)H^+$ were given at m/z 242 (71%) and 126 (base) respectively. Signals on the ^{13}C -n.m.r. spectrum were assigned as follows: δ (p.p.m.) (2H_2O) 3.2 ($-S-CH_2-$), 8.5 ($-S-CH<$), 14.0 ($-CH_2-CO_2H$), 25.9 [$-CH(NH_2)-CO_2H$], 89.2 (imidazole 2-C), 105.3 (imidazole 4-C), 107.6 (imidazole 5-C), 144.7 [$-CH(NH_2)-CO_2H$] and 149.5 ($-CH_2-CO_2H$). Present data obtained from the 1H -n.m.r. (400 MHz) spectrum (not shown) supported the previous assignment for the signals on the 1H -n.m.r. (100 MHz) spectrum [1] of compound (I).

Identification of the urinary compound with compound (I)

The compound isolated from urine gave positive reactions with Pauly's reagent, the chloroplatinate reagent and the ninhydrin reagent. It decomposed at 154–155 °C, unchanged by admixture with the authentic compound (I). The urinary compound had mR_{ILA} 1.15 and R_f values 0.20 and 0.14 on t.l.c. in solvents A and B respectively. These values coincide with those of compound (I). The f.a.b. mass spectrum (Fig. 2b) of the urinary compound was identical with that of compound (I). Fig. 2(b) shows no peak of contaminating material is found and this indicates the high purity of the isolated compound. The i.r. spectrum of the urinary compound was also identical with that of compound (I).

From these results, the urinary compound was determined to be compound (I) and it is one of the physiological precursors of compound (II). Judged from the chemical structure of compound (I), we postulate that cysteine and/or GSH and urocanic acid are its precursors. When GSH was added to urocanic acid, *S*-[2-carboxy-1-(1*H*-imidazol-4-yl)ethyl]-L-glutathione (III) was formed as previously described [1]. If compound (III) is metabolized like GSH, it may form compound (I). *trans*-Urocanic acid, a key metabolite of L-histidine, is formed by histidase (histidine ammonia-lyase, EC 4.3.1.3), and it accumulates in epidermis, since urocanase (urocanate hydratase, EC 4.2.1.49) is absent from the skin [6]. The formation of compound (I) may participate in part in the accumulation and the elimination of the epidermal urocanic acid under conditions of sunlight irradiation as previously discussed [1].

We are grateful to Professor Dr. K. Kurahashi (Research Equipment Centre, Kagawa Medical School, Kagawa, Japan) for measurements of the n.m.r. spectra. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1. Kinuta, M., Yao, K., Masuoka, N., Ohta, J., Teraoka, T. & Ubuka, T. (1991) *Biochem. J.* **275**, 617–621
2. Pauly, H. (1904) Hoppe-Seyler's *Z. Physiol. Chem.* **42**, 508–518
3. Macpherson, H. T. (1946) *Biochem. J.* **40**, 470–481
4. Toennis, G. & Kolb, J. J. (1951) *Anal. Chem.* **23**, 823–826
5. Ubuka, T. (1962) *J. Biochem. (Tokyo)* **52**, 440–443
6. Baden, H. P. & Pathak, M. A. (1967) *J. Invest. Dermatol.* **48**, 11–17