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Amino
Acids

S-[2-Carboxy-1-(1*H*-imidazol-4-yl)ethyl]cysteine in normal human urine

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Summary. A compound, which had the same mobility on a high-voltage paper electrophoretogram and the same R_F value on a thin-layer chromatogram as those of *S*-[2-carboxy-1-(1*H*-imidazol-4-yl)ethyl]cysteine (I), was partially purified from human urine by ion-exchange column chromatography. The compound gave a signal at m/z 260 on its FAB mass spectrum, which was assigned as MH^+ of compound I. These results suggest that the urinary compound is compound I and it is a physiological precursor of 3-[(carboxymethyl)thio]-3-(1*H*-imidazol-4-yl)propanoic acid [Kinuta et al., (1991) Biochem J 275: 617–621].

Keywords: Amino acids – Human urine – Paper electrophoresis – Cysteine – Imidazole compound

Introduction

We previously described that *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)} might be a precursor of 3-[(carboxymethyl)thio]-3-(1*H*-imidazol-4-yl)propanoic acid (II), a compound isolated from human urine (Kinuta et al., 1987, 1991). However, compound I had not been found in any biological samples. Further studies on the origin of compound II resulted in finding an unidentified compound which had the same mobility as that of compound I on a high-voltage paper electrophoretogram sprayed with Pauly's reagent. The unidentified compound has been partially purified from human urine, and it has been identified with compound I by fast atom bombardment mass spectrometry as well as paper electrophoresis and thin-layer chromatography. The present paper describes the partial purification and the identification of the urinary compound with compound I.

Materials and methods

Chemicals

Ion-exchanger Diaion SK # 1 (H^+ form, a sulphonated cation-exchanger) and Diaion SA # 100 (acetate form, a strong anion-exchanger) were purchased from Mitsubishi Kasei Co. (Tokyo, Japan). Dowex 50 W × 8 and Dowex 1 × 8 were from Dow Chemical Co. (Midland, MI, U.S.A.) and Amberlite CG-50 type 1 (H^+ form) was from Organo Co. (Tokyo, Japan). *trans*-Urocanic acid and L-β-imidazolelactic acid [2-hydroxy-3-(imidazol-4-yl)propanoic acid were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Authentic compound I was synthesized by the previous method (Kinuta et al., 1991). Pauly's reagent (Pauly, 1904; Macpherson, 1946), a diazotized sulphanilic acid reagent for the detection of imidazole compounds, and chloroplatinate reagent for the detection of sulphur-containing compounds (Toennies and Kolb, 1951) were prepared before use by the respective standard methods. The ninhydrin reagent used was 1% (w/v) ninhydrin/2% (v/v) pyridine/acetone solution.

Spectroscopy

A fast atom bombardment (FAB) mass spectrum was measured in a Shimadzu 9020-DF gas chromotograph-mass spectrometer equipped with a Shimadzu SCAP 1123 data system; the target surface was bombarded by a beam of energetic argon atoms at 5 keV and 10 mA; glycerine (Nacalai Tesque Co., Kyoto, Japan) was used as a solvent.

Paper electrophoresis

High-voltage paper electrophoresis was performed on Whatman 1Chr paper (Whatman Ltd. Madistone, England) in pyridine/acetic acid/water buffer (1:20:179, by vol.; pH 3.1) at a potential gradient of 100 V/cm for 45 min (Ubuka, 1962). The paper was cooled by immersion in *n*-hexane at 0–5°C. After electrophoresis, the paper was dried with an air-flow drier at 100°C for 30 min. The dried paper was sprayed with Pauly's reagent, chloroplatinate reagent or ninhydrin reagent. Relative mobilities of compounds on the paper electrophoretogram, termed the mRILA value, were determined by comparison of the mobility for L-β-imidazolelactic acid as 1.00.

Thin-layer chromatography

Thin-layer chromatography (TLC) was performed on Silica gel 60 TLC plate (E. Merck Co., Darmstadt, Germany) in two solvent systems: solvent I, *n*-butanol/acetic acid/water (12:3:5, by vol.); solvent II, isopropanol/85% (v/v) formic acid/water (16:1:4, by vol.). The TLC plate was dried by the drier at 80°C for 30 min, and was sprayed with the detecting reagents.

Results and discussion

Purification of the urinary compound

The concentration of the desired compound in human urine was too low for the detection of its reddish band on the paper electrophoretogram sprayed with Pauly's reagent without a preceding concentration step.

Each 10 litres of urine was collected from several adults in bottles containing 10 ml of 6 M HCl and 5 ml of toluene per litre of urine. The collected urine was treated successively with ion-exchange columns Diaion SK # 1, Diaion SA # 100 and Dowex 50 according to the previous method (Kinuta et al., 1991) to obtain the residue desalted. The residues obtained from 40 litres of the collected urine

were combined in 50 ml of water and applied to a Dowex 50 column (NH_4^+ form, 5.5 cm × 20 cm). The desired compound was eluted with 2 litres of 2 M NH_3, and the aq. NH_3 eluate was evaporated to dryness under reduced pressure.

After repetition of the above procedures three times, the residues obtained from 120 litres of the collected urine were combined in 50 ml of water and the solution was applied on a Dowex 1 column (acetate form, 4.4 cm × 42 cm), and on a Dowex 1 column (OH^- form, 1.3 cm × 49 cm) by fractionations with 0.2 M acetic acid. As checked by paper electrophoresis, the eluates containing the desired compound were combined, and dried.

Further purification of the compound was carried out by paper electrophoresis. After electrophoresis, a part of the paper containing the desired compound, the mRILA value of which was 1.15, was cut out with scissors and cut into small pieces. The compound was extracted from the pieces with 0.2 M acetic acid. The extracts were combined and dried to obtain the residue. The residue obtained was used as a sample for the identification as described below.

Identification of the urinary compound with compound I

The urinary compound gave positive reactions with Pauly's reagent, the chloroplatinate reagent and the ninhydrin reagent, suggesting that it is an amino acid derivative having a imidazole group and a sulphur-containing side chain. It had mRILA 1.15 and R_F values of 0.20 and 0.14 on TLC in solvents I and II, respectively. These characteristics are coincidental with those of compound I (Kinuta et al., 1991). In the FAB mass spectrum (Fig. 1) of the urinary compound, a major peak at m/z 260 was found together with minor ones at m/z 115, 207, 282, 299 and 352. The major peak was assigned as $(C_9H_{13}N_3O_4S)H^+$ which is identical with MH^+ of compound I. The minor peaks may be due to several contaminations in the sample. From these results, the compound giving the major peak can be determined as compound I.

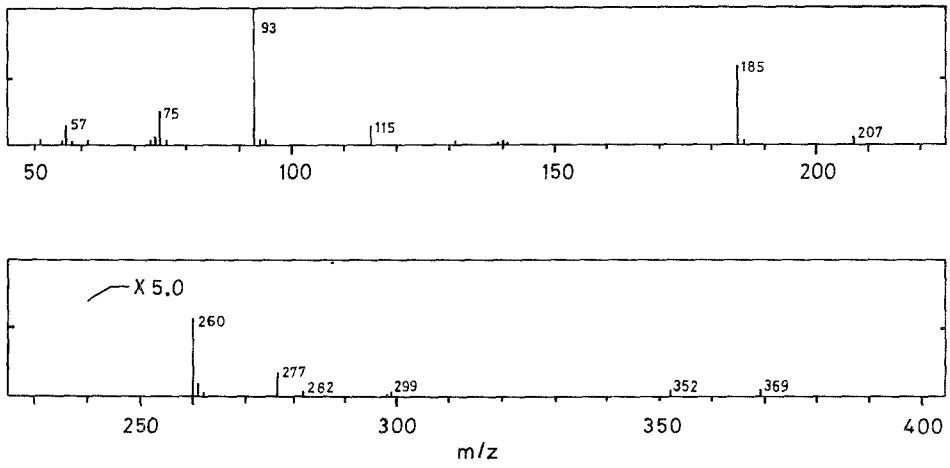

Fig. 1. FAB mass spectrum of the urinary sample. Preparation procedures of the urinary sample are described under 'Results and discussion'. Peaks at m/z 57, 75, 93, 185, 277 and 369 are due to glycerine used as a solvent

Compound I was previously synthesized as one of the proposed precursors of compound II, and the enzymic degradation of compound I was performed with rat liver homogenate in a phosphate buffer to form compound II (Kinuta et al., 1991). However, compound I had not been detected in any biological samples. The present results suggest that compound I is a physiological precursor of compound II.

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