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Journal of Chromatography B, 683 (1996) 269–272

JOURNAL OF
CHROMATOGRAPHY B:
BIOMEDICAL APPLICATIONS

Short communication

Aminoethylcysteine ketimine decarboxylated dimer detected in normal human urine by gas–liquid chromatography, selected-ion monitoring and mass spectrometry

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Received 6 December 1995; revised 13 February 1996; accepted 27 February 1996

Abstract

Aminoethylcysteine ketimine is a biochemical product known to be converted spontaneously in the decarboxylated dimer. Since the ketimine has been detected in a mammalian brain, it was assumed that also the dimer could be present in the mammalian body and eventually excreted in the urine. Using human urine as the biological source, an extract was prepared which, submitted to gas–liquid chromatography, selected-ion monitoring and mass spectrometry, indicated the presence of the dimer.

Keywords: Aminoethylcysteine ketimine decarboxylated dimer

1. Introduction

Aminoethylcysteine ketimine decarboxylated dimer (formula in Fig. 3), simply named dimer afterward, is the product of the spontaneous dimerization and decarboxylation of the parent aminoethylcysteine ketimine (see [1] for a general review). The biochemical route leading to the production of the dimer is: (a) enzymatic synthesis of aminoethylcysteine operated by serine sulfhydrase [2,3], (b) α-deamination by a transaminase of wide specificity [4,5] and by L-amino acid oxidase [6,7], (c) spontaneous cyclization to the ketimine [8,9] and (d) spontaneous dimerization and decarboxylation [8,9]. The parent ketimine has been detected in the bovine brain and cerebellum [10] and it was thought that part of this ketimine could also be present in the mammalian body in dimer form and eventually excreted in the urine. This assumption has now been validated by the detection of the dimer in normal human urine as reported in the present note.

2. Experimental

2.1. Chemicals and materials

The authentic dimer was prepared as reported previously [11]. Other chemicals were of the best

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PII S0378-4347(96)00105-3

commercial source. C_{18} Sep-Pak Plus cartridges were purchased from Waters Millipore.

2.2. Urine collection

Morning samples of normal urine from fasting laboratory personnel, male and female, 20–40 years old, with a normal mixed diet, were collected and submitted to the extraction procedure as soon as passed.

2.3. Extraction

Each urine sample (5 ml), after centrifugation at about 1000 *g* for removing suspended particles, was shaken with 3×20 ml chloroform. The collected organic fractions were concentrated in a rotatory evaporator and dried in a desiccator under vacuum. The residue, dissolved in 4 ml water, was loaded in a C_{18} cartridge, previously conditioned with 10 ml methanol and then with 10 ml water. After washing with 10 ml water, the elution was performed with 10 ml methanol at a rate of 5 ml per minute. The eluate was evaporated in a rotatory evaporator and kept in a desiccator under vacuum.

2.4. Gas chromatography

The samples were solubilised in a minimum amount of methanol and injected in the chromatograph without derivatization [9]. Chromatography was carried out on a Perkin-Elmer Sigma 300 chromatograph equipped with a flame photometric detector for sulphur-containing compounds. The Supelco glass column (180 cm×2 mm I.D.) was packed with 3% OV-17 on Chromosorb W HP, 100–120 mesh. Temperatures were: 240°C for the column, 260°C for the injector and 300°C for the detector. Flow-rates were 25 ml/min for N_2 carrier gas, 65 ml/min and 110 ml/min for H_2 and air combustion gases, respectively.

2.5. Gas mass analysis

Gas chromatographic–mass spectrometric analyses were performed on a Hewlett-Packard 5970A MSD system. Chromatographic separations were carried out on a 50 m×0.25 mm I.D. fused-silica capillary column coated with cross-linked methylphenyl silicone, film thickness 0.33 μm, as stationary phase. Injection system: split–splitless at a temperature of 280°C. Column temperature program: 40°C (6 min), then to 280°C at a rate of 15°C/min and held for 20 min. The spectra were obtained in the electron impact mode at 70 eV ionization energy; ion source 280°C; ion source vacuum 10^{-5} Torr; mass range from *m/z* 50 to *m/z* 240. Chromatograms and spectra were collected by a Hewlett-Packard 59970 Chem Station.

3. Results and discussion

The gas-chromatographic profile of the final methanol solution of the human urine extracted as described in Section 2.3 showed repeatedly the presence of a sulphur-containing product co-eluting with samples of the authentic dimer (not shown). This area is disturbed by other unknown sulphur products, and to confirm the presence of the dimer, we made use of the single-ion monitoring technique. When the apparatus was set to register only the molecular ion of the dimer M^+ *m/z* 228, a single

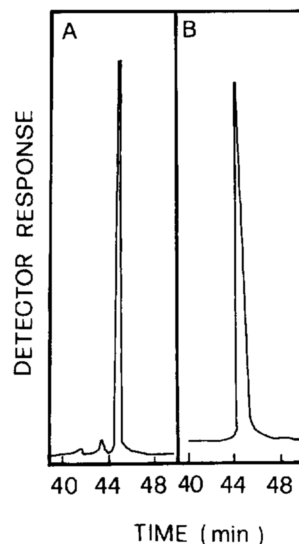

Fig. 1. Selected-ion monitoring profile of (A) extract of human urine and (B) 100 ng of authentic dimer. The chromatograph effluent was injected into the mass spectrometer predisposed to register only the molecular ion M^+ *m/z* 228.

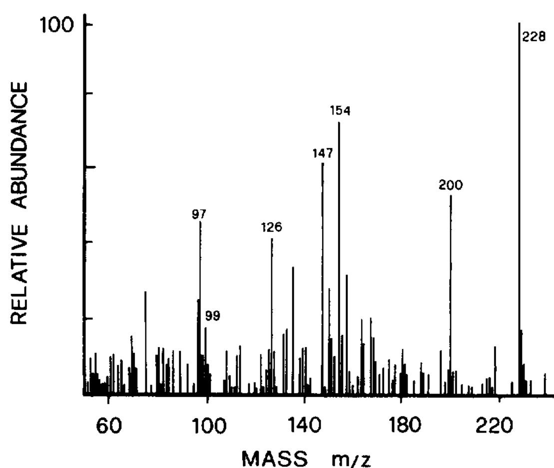

Fig. 2. Mass spectrum of the chromatographic component isolated at retention time 45 min relative to the normal human urine (Fig. 1A).

peak was produced with the urine extract (Fig. 1A) and the same retention time of the peak was obtained with the synthetic dimer (Fig. 1B). The mass spectra of the synthetic and the extracted dimer eluted, as indicated in Fig. 1A and B, are compared respectively in Fig. 2 and Fig. 3. The presence of the molecular ion M^+ m/z 228 as the main component in both patterns is evident, and a typical fragmentation of the authentic dimer is also present in the extracted sample. In the latter spectrum (Fig. 2), additional

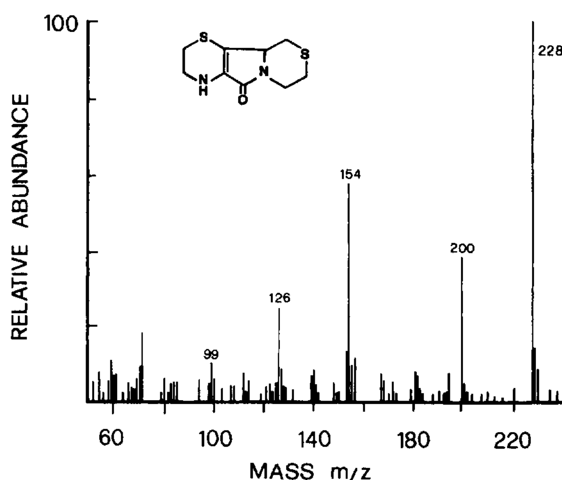

Fig. 3. Mass spectrum of the authentic dimer (Fig. 1B).

ions are seen due to accompanying products not resolved by the chromatographic procedure preceding the spectral analysis. The fragments m/z 200, m/z 154, m/z 126 and m/z 99 are present in both the standard and the sample spectra and have similar relative intensities. The main fragment, m/z 200, can be assigned to the molecular ion m/z 228 minus CO; other fragments are more difficult to interpret. The relevant fragments, m/z 147 and m/z 97, seen only in the sample spectrum (Fig. 2), should be regarded as unresolved impurities.

Although the compound has not been isolated in pure form or submitted to traditional chemical analysis, the results obtained are highly indicative of the occurrence of the dimer in the urine of the twelve subjects analysed. The quantitative determination requires a precise analytical procedure that is now under study and will be reported later.

The dimer has been recognized as a chemical entity and was prepared for the first time by Hermann in 1961 [8]. Since then, very little progress has been made on the understanding of its chemical and biochemical properties. The data available so far can be summarized as follows: (a) the dimer is the product of the spontaneous dimerization and decarboxylation of aminoethylcysteine ketimine, easily prepared by reacting cysteamine with bromopyruvic acid [8,11]; (b) it exhibits absorbancy at 310 nm [8,9], which is useful for its detection and quantization; (c) it can be chromatographed directly by gas–liquid chromatography [9] and by HPLC [11]; (d) it inhibits mitochondrial respiration [12]; (e) it interacts with oxygen-reactive species [11]; (f) protects membranes and lipidic structures from oxidative damage [13]. Since aminoethylcysteine ketimine, i.e. the immediate precursor of the dimer, is also degraded to various products by spontaneous oxidation [14], it is possible that the couple ketimine–dimer could exert a protective function against oxidative damage, a biochemical property that deserves further investigation.

Acknowledgments

This work has been supported in part by grants from MURST and by Sigma Tau Foundation.

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