Toxicon 57 (2011) 730–738

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Distinguishing the cyanobacterial neurotoxin β-*N*-methylamino-L-alanine (BMAA) from other diamino acids

S.A. Banack [a], J.S. Metcalf [a,b], Z. Spáčil [c], T.G. Downing [d], S. Downing [d], A. Long [a], P.B. Nunn [e], P.A. Cox [a,*]

[a] *Institute for Ethnomedicine, Box 3464, Jackson, WY 83001, USA*
[b] *National Center for Natural Products Research, School of Pharmacy, University of Mississippi, Oxford, MS 38677, USA*
[c] *Department of Chemistry, University of Washington, Seattle, WA, USA*
[d] *Department of Biochemistry & Microbiology, Nelson Mandela Metropolitan University, P.O. Box 7700, Port Elizabeth 6031, South Africa*
[e] *School of Pharmacy and Biomedical Sciences, University of Portsmouth, Portsmouth, UK*

ARTICLE INFO

Article history:
Received 23 November 2010
Accepted 8 February 2011
Available online 15 February 2011

Keywords:
Amino acid
Mass spectrometry
UHPLC
Neurodegenerative disease
Environmental toxin
Diaminopimelic acid

ABSTRACT

β-*N*-methylamino-L-alanine (BMAA) is produced by diverse taxa of cyanobacteria, and has been detected by many investigators who have searched for it in cyanobacterial blooms, cultures and collections. Although BMAA is distinguishable from proteinogenic amino acids and its isomer 2,4-DAB using standard chromatographic and mass spectroscopy techniques routinely used for the analysis of amino acids, we studied whether BMAA could be reliably distinguished from other diamino acids, particularly 2,6-diaminopimelic acid which has been isolated from the cell walls of many bacterial species. We used HPLC-FD, UHPLC-UV, UHPLC-MS, and triple quadrupole tandem mass spectrometry (UHPLC-MS/MS) to differentiate BMAA from the diamino acids 2,6-diaminopimelic acid, N-2(amino)ethylglycine, lysine, ornithine, 2,4-diaminosuccinic acid, homocystine, cystine, tryptophan, as well as other amino acids including asparagine, glutamine, and methionine methylsulfonium.

1. Introduction

The possible role of the cyanobacterial toxin β-*N*-methylamino-L-alanine (BMAA) in triggering neurodegenerative diseases such as amyotrophic lateral sclerosis, Alzheimer's disease, and Parkinson's disease in vulnerable individuals has resulted in an increased interest in this unusual amino acid (Bradley and Mash, 2009). BMAA is present in environmental samples and human tissues both as a free amino acid, and as a protein-bound amino acid (Murch et al., 2004a, b). It has been hypothesized that its misincorporation into polypeptide sequences results in protein misfolding and collapse. It has also been hypothesized that misincorporation of BMAA triggers protein collapse which is linked to various tangle diseases characterized by irreversible progressive neurodegeneration (Murch et al., 2004a, b; Bradley and Mash, 2009).

Studies of laboratory animals have shown that even a low rate of misincorporation of standard protein amino acids can trigger neurodegenerative disease (Lee et al., 2006).

Misincorporation of the non-protein amino acid L-canavanine results in anomalous proteins and toxicity in herbivores which forage on *Canavalia ensiformis* [Fabaceae] (Allende and Allende, 1964; Rosenthal, 1977). Proteins with misincorporated L-Dopa have been shown to be more resistant to proteolysis and prone to cross-linking and aggregation (Rogers and Shiozawa, 2008).

BMAA is specifically toxic to motor neurons (Rao et al., 2006) as an agonist at NMDA and AMPA receptors, induces oxidative stress, and promotes accumulation of extracellular

* Corresponding author. Tel.: +1 801 375 6214; fax: +1 801 373 3593.
E-mail address: paul@ethnomedicine.org (P.A. Cox).
URL: http://www.ethnomedicine.org

0041-0101/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2011.02.005

glutamate through its action on the cystine/glutamate antiporter (system X_c^-) (Liu et al., 2009). Furthermore, BMAA potentiates neuronal damage from other neurotoxic insults (Lobner et al., 2007). It has been hypothesized that vulnerable individuals may be at increased risk of progressive neurodegenerative illness if exposed to BMAA (Cox et al., 2003; Banack and Cox, 2003a). Given the relative ubiquity of cyanobacteria, which have successfully colonized habitats ranging from Antarctica to the deserts of the Middle East, and which often are present as blooms, mats, or scums in freshwater, estuarine, and marine waterbodies, there is a possibility of exposure to BMAA in disparate parts of the globe (Cox et al., 2005, 2009; Metcalf et al., 2008).

Due to the possible clinical implications of BMAA exposure and the wide array of compounds produced by cyanobacteria, it is important to distinguish BMAA from other similar molecules. To date BMAA has been routinely distinguished from other proteinogenic amino acids as well as its neurotoxic isomer 2,4-diaminobutyric acid (2,4-DAB) by reversed-phase HPLC methods (Banack et al., 2007, 2010) and with the most selectivity by UHPLC with triple quadrupole tandem mass spectrometry (UHPLC-MS/MS) using distinct retention times, diagnostic product ions and characteristic ratios between selective reaction monitoring transitions (Banack et al., 2010; Spáčil et al., 2010).

Another BMAA isomer, N-2(amino)ethylglycine, was analyzed along with other diamino acids and amides, which could potentially interfere with BMAA analysis. We focused on compounds that might occur in complex physiological matrices, such as 2,6-diaminopimelic acid, found in the cell walls of many bacterial species (Work and Dewey, 1953).

2. Materials and methods

2.1. Chemicals

L-BMAA was synthesized by Dr. Peter Nunn and compared to Sigma B-107 (St. Louis, MO). 2,6-diaminopimelic acid (synonym: 2,6-diaminoheptanedioic acid) from Sigma (D1377) was compared to Research Organics # 2045D (Cleveland, OH). N-2(amino)ethylglycine was from TCI America (Portland, OR); Part # A1153. L-lysine, L-ornithine monohydrochloride, L-asparagine, L-glutamine, L-cystine, L-tryptophan, L-2,4-diaminobutyric acid dihydrochloride, methanol and acetonitrile (LC-MS CHROMASOLV®) were purchased from Sigma–Aldrich (St. Louis, MO) as Part #'s L5501, O2375, A0884, G8540, C7602, T0254, 32830, 34966, and 34967 respectively. Ammonium formate (NC9841158) was from Fisher Scientific. 2,4-diaminosuccinic acid [synonym meso-A,A-diaminosuccinic acid] was purchased from Research Organics #0255D (Cleveland, OH). L-homocystine was obtained from MP Biomedicals (Solon, OH); Part # 521061580. Formic acid was purchased from Acros Organics (Geel, Belgium, distributed by Fisher Scientific, NJ, # 270480025). Proprietary compositions of UHPLC eluent A: part #186003838, UHPLC eluent B: part # 186003839, and HPLC eluent A part # 052890 were obtained from Waters Corp. (Milford, MA). Water was purchased from Fisher W6-4 Optima LC/MS for use with the LC/MS/MS or purified in-house (Direct-Q uv3, Millipore Ltd, Bedford, MA) to 18.2 MΩ quality for all other purposes. For comparison purposes, DL-methionine methylsulfonium chloride (Sigma # 64382) was analyzed.

2.2. Standard preparation

All compounds were reconstituted in 20 mM HCl except for L-homocystine and 2,4-diaminosuccinic acid which were initially dissolved in 0.5 M NaOH before being diluted with water or 20 mM HCl for analysis. Each compound was analyzed both as an acid hydrolysate prepared in a final concentration of 6.0 M HCl for 16 h at 110 °C and without hydrolysis. Samples were derivatized with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC Waters AccQTag reagent, PN WAT052880) or with propyl chloroformate (EzFaast™, LC/MS Physiological (Free) Amino Acid Kit KH0-7337) following standardized, validated protocols (Banack and Cox, 2003a; Esterhuizen and Downing, 2008). Standards and blanks were derivatized and analyzed separately and in combinations to evaluate chromatographic separation.

2.3. Ultra high pressure liquid chromatography-Tandem mass spectrometry (UHPLC-MS/MS)

AQC derivatives were analyzed using a triple quadrupole instrument (Thermo Scientific Finnigan TSQ Quantum UltraAM, San Jose, CA) after separation by a Waters Acquity-UHPLC system with a Binary Solvent Manager, Sample Manager and a Waters AccQTag Ultra column (part# 186003837, 2.1 × 100 mm) at 55 °C. Separation was achieved using gradient elution at 0.65 ml/min in aqueous 0.1% (v/v) formic acid (Eluent A) and 0.1% (v/v) formic acid in acetonitrile (Eluent B): 0.0 min = 99.1% A; 0.5 min = 99.1% A curve 6; 2 min = 95% A curve 6; 3 min = 95% A curve 6; 5.5 min = 90% A curve 8; 6 min = 15% A curve 6; 6.5 min = 15% A curve 6; 6.6 min = 99.1% A curve 6; 8 min = 99.1% A curve 6. Nitrogen gas was supplied to the heated electrospray ionization (H-ESI) probe with a nebulizing pressure of 40 psi and a vaporizer temperature of 400 °C. The mass spectrometer was operated under the following conditions: the capillary temperature was set at 270 °C, capillary offset of 35, tube lens offset of 110, auxiliary gas pressure of 35, spray voltage 3500 V, source collision energy of 0 eV, and multiplier voltage of −1719 V. A divert valve was used except during the selected ion monitoring (SIM) scans. The second quadrupole was pressurized to 1.0 m Torr with argon. Ion *m/z* 459 was isolated in the first quadrupole filter as the precursor ion and subjected to collision induced dissociation (CID). Second step mass filtering was performed using selective reaction monitoring (SRM) of BMAA after CID in the collision cell. The following *m/z* transitions were monitored: 459–119, CE 21 eV; 459–289 CE 17 eV; 459–171 CE 38 eV. The resultant three product ions originating from derivatized BMAA (*m/z* 119, 289, 171) were detected, after entering the third quadrupole and their relative abundances were quantified. Compound specific *m/z* transitions were added to our standard method, 459–258 CE 21 eV (BMAA), 459–188 CE 38 eV (DAB) and 459–214 CE 35 eV (AEG) to increase selectivity. A new SRM method was established to detect