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DISTRIBUTION OF NEUROEXCITATORY AMINO ACIDS IN MARINE ALGAE

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Key Word Index—marine algae; neuroexcitatory amino acids; NMDA; kainic acid; domoic acid; distribution.

Abstract—Contents of three neuroexcitatory amino acids, *N*-methyl-D,L-aspartic acid (NMA), kainic acid (KA) and domoic acid (DA) were investigated in 46 marine algae species. NMA was detected in very small amounts in several marine algae belonging to the Chlorophyceae and Rhodophyceae, but not in algae belonging to the Phaeophyceae. KA was detected in three species and DA was detected in four species all belonging to the family Rhodomelaceae (Ceramiales, Rhodophyceae). Among rhodomelacean algae investigated in this study, KA and DA were detected only in the particular species collected in the south-west Islands of Japan (Tokunoshima Island and Okinawa Main Island). It is noteworthy that the distribution of both KA and DA was strictly restricted some algae of the subtropical area.

INTRODUCTION

N-Methyl-D-aspartic acid (NMDA), kainic acid (KA) and domoic acid (DA) have been previously shown to cause neuronal excitation and neuronal degeneration in higher animals [1–3]. NMDA and KA stimulate gonadotropin-releasing hormone release and induce acute increase in serum luteinizing hormone in mammals [4, 5]. Furthermore, DA has been identified as the toxic agent of amnesic shellfish poisoning that occurred in 1987 in Canada [6]. NMDA was isolated for the first time from the marine bivalve *Scapharca broughtonii* by Sato *et al.* [7]. KA and DA were isolated from the red algae *Digenea simplex* [8] and *Chondria armata* [9] both belong to the family Rhodomelaceae. These three neuroexcitatory amino acids have been isolated from marine organisms, but there is little information on their distribution in marine algae. We have been interested in the distribution and metabolism of these neuroexcitatory amino acids in marine organisms. In this study, we aimed to get the precise information on the distribution of these three neuroexcitatory amino acids in marine algae.

RESULTS AND DISCUSSION

Three neutroexcitatory amino acids, NMDA, KA and DA, could be simultaneously determined by the method shown in Fig. 1. These three amino acids were also clearly separated from the protein amino acids (data not shown). This method could not however separate the amino acid enantiomers; the peak corresponding to NMDA involved both D- and L-form of *N*-methylaspartic acid. The contents of NMDA were thus expressed as the sum of *N*-methyl-D,L-aspartic acid (NMA). The detection limits were 10 pmol for NMA and KA and 5 pmol for DA. The high sensitivity for DA is attributable to its unique absorption at 242 nm [10].

Contents of three neuroexcitatory amino acids, NMA, KA and DA in the 12 species which gave the positive results are shown in Table 1. NMA was detected in very small amounts in one chlorophycean alga, *Bryopsis plumosa*, and several rhodophycean algae, but not in algae belonging to Phaeophyceae. *N*-Methyl-L-aspartic acid (NMLA) has been isolated from the red alga, *Halopytis incurvus* (Rhodomelaceae, Ceramiales), by Sciuto *et al.* [11]. It is noteworthy that NMA occurs not only in the Ceramiales but also in the Cryptonemiales and Gigartinales. Concerning the biological activity of each enantiomer of NMA, NMLA is much less active than NMDA in neuroexcitatory action [2]. The stereochemical structure of the NMA detected in this study is therefore of interest.

KA was detected in only three species *D. simplex*, *Laurencia papillosa* and *Vidalia obtusiloba*, all belonging to the family Rhodomelaceae (Ceramiales, Rhodophyceae). KA has been exceptionally isolated from red alga *Centroceras clavulatum*, (Ceramiaceae) [12]. DA was detected in only four species, *Amansia glomerata*, *C. armata*, *D. simplex* and *V. obtusiloba*, all belonging to Rhodomelaceae (Ceramiales, Rhodophyceae). DA has also been detected in the red

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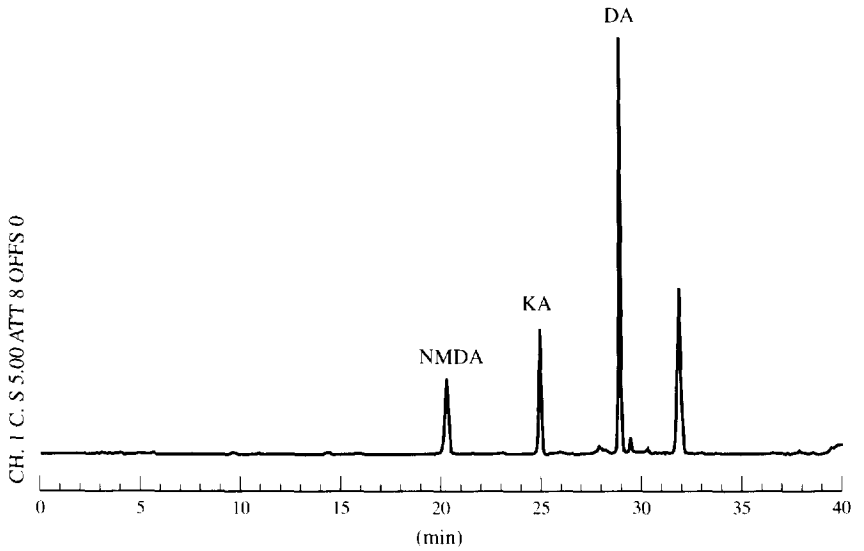

Fig. 1. Chromatography of standard neuroexcitatory amino acids. A 0.5 nmol sample of each PTC-derivative of *N*-methyl-D-aspartic acid (NMDA), kainic acid (KA) and domoic acid (DA) was chromatographed.

alga, *Alsidium corallinum*, (Rhodomelaceae) [12]. The distribution of DA is strictly restricted within the Rhodomelaceae. Among rhodomelacean algae investigated in the study, KA and DA were detected in the particular species collected in the south-west Islands such as Tokunoshima Island (Kagashima Prefecture) or Okinawa Main Island (Okinawa Prefecture), but could not be confirmed from the algae such as *Chondria crassicaulis* and *Neorhodomela larix*, collected in the north-eastern part of Japan, Sanriku (Iwate Prefecture) or Onagawa (Miyagi Prefecture). It is noteworthy that the distribution of both KA and DA in marine algae was restricted to some species from the subtropics.

EXPERIMENTAL

Reagents and materials. NMDA, KA and DA were purchased from Sigma. Phenylisothiocyanate (PITC) and other chemicals of reagent grade were purchased from Wako (Osaka, Japan). The reverse-phase column material TSK-ODS 80T (particle size 5 μm) was obtained from Toso (Tokyo, Japan) and packed into a 250 mm × 4.6 mm i.d. stainless steel column in our laboratory by the conventional slurry-packing technique. High-purity H_2O was prepared by distillation of deionized distilled H_2O with alkaline $KMnO_4$.

Algal samples. Marine algae were collected in Sanriku (Iwate Prefecture), Onagawa (Miyagi Prefecture), Amagi (Tokunoshima Island, Kagoshima Prefecture), and Motobu (Okinawa Main Island, Okinawa Prefecture) in May and June 1994. Algae were washed quickly with H_2O and then freeze-dried, and stored at −20° until analysis.

Prepn of extracts and acidic amino acid fr. Dry alga (about 1 g) was dipped into 10 vol. of 80% EtOH. The alga was cut into small pieces with scissors and then homogenized using a Polytron homogenizer. The homogenate was centrifuged at 10 000 *g* for 30 min. A part of the supernatant of algal extracts was loaded on to a column (4 cm × 1 cm i.d.) of Dowex 1-x8 (acetate form). The column was washed thoroughly with 40 ml H_2O and then elute with 12 ml of 2 M HOAc to recover

Table 1. Neuroexcitatory amino acids contents in marine algae

Family	Species	Sampling		Compound (mg 100 g^{-1} dry wt)		
		Place	Month	NMA	KA	DA
Bryopsidaceae	*Bryopsis plumosa*	Onagawa	June	3.39	0	0
Gloiopeltidaceae	*Gloiopeltis furcata*	Onagawa	June	1.48	0	0
Phacelocarpaceae	*Coelothrix charoides*	Amagi	June	3.06	0	0
Phyllophoraceae	*Ahnfeltia paradoxa*	Sanriku	June	2.74	0	0
	Gymnogongrus flabelliformis	Onagawa	June	0.25	0	0
Gigartinaceae	*Chondrus elatus*	Sanriku	June	1.65	0	0
Rhodomelaceae	*Amansia glomerata*	Motobu	June	1.38	0	0.11
	Chondria armata	Amagi	May	0	0	109
	Digenea simplex	Motobu	June	0	213	2.07
	Digenea simplex	Amagi	June	0	276	2.82
	Laurencia papillosa	Motobu	June	1.04	1.01	0
	Vidalia obtusiloba	Motobu	June	0	0.14	0.07

the acidic amino acid fr. The acidic amino acid fr. was freeze-dried using a centrifuge evaporator and dissolved in small amounts of H_2O.

Equipment. Chromatography was conducted in an HPLC system consisting of the following components: two Jasco 880-PU pumps, a Jasco 801-SC system controller, a Jasco 850-AS autosampler, a Jasco 850-UV–VIS detector, a Jasco 865-CO column oven at 40±0.1°C, a Hitachi D-2500 Chromatointegrater.

HPLC analysis. Phenylthiocarbamate (PTC) derivatives of the acidic amino acid fr. were prepd by the method of ref. [13]. An aliquot of PTC derivative, corresponding 1 to 10 mg of dry algal frond, was used for HPLC analysis. The chromatographic mobile phases were solvent A = 90% 140 mM NaOAc buffer, pH 5 and 10% MeCN, and solvent B = MeCN: H_2O (3:2), with a linear solvent gradient from 0% to 50% B between 0 and 30 min. The flow-rate of the mobile phases was 1 ml min^{-1}. The HPLC eluate was monitored at 254 nm.

No detectable concn of the three neuroexcitatory amino acids could be found in the following species. Ulvaceae: *Enteromorpha intestinalis* (Sanriku; June); *Ulva pertusa* (Sanriku; June); *Ulva pertusa* (Motobu; August). Cladophoraceae: *Chaetomorpha moniligera* (Sanriku; June). Caulerpaceae: *Tydemania expeditionis* (Motobu; August). Dictyotaceae: *Dictyota spinulosa* (Motobu; June); *Dictyopteris latiuscula* (Motobu; June). Chordariaceae: *Analipus japonicus* (Sanriku; June). Desmarestiaceae: *Desmarestia ligulata* (Sanriku; June). Scytosiphonaceae: *Scytosiphon lomentarius* (Sanriku; June); *Colpomenia sinuosa* (Sanriku; June). Laminariaceae: *Costaria costata* (Sanriku; June); *Eisenia bicyclis* (Sanriku; June); *Laminaria japonica* (Sanriku; June); *Laminaria religiosa* (Sanriku; June). Sargassaceae: *Hizikia fusiformis* (Sanriku; June); *Sargassum fulvellum* (Sanriku; June); *Sargassum thunbergii* (Sanriku; June). Bangiaceae: *Porphyra yezoensis* (Sanriku; June). Helminthocladiaceae: *Dermonema pulvinata* (Onagawa; June). Gelidiaceae: *Gelidium pacificum* (Onagawa; June). Dumontiaceae: *Neodilsea yendoana* (Sanriku; June). Corallinaceae: *Calliarthron yessoense* (Sanriku; June); *Corallina pilulifera* (Onagawa; June). Cryptonemiaceae: *Carpopeltis flabellata* (Sanriku; June); *Pachymeniopsis lanceolata* (Sanriku; June). Hypneaceae: *Hypnea* sp. (Amagi; June); *Hypnea saidana* (Amagi; June). Gracilariaceae: *Gracilaria denticulata* (Amagi; June); *Chondrus occellatus* (Sanriku; June); *Rhodoglossum japonicum* (Onagawa; June). Rhodymeniaceae: *Lomentaria hakodatensis* (Onagawa; June); *Rhodymenia palmata* (Sanriku; June); *Chondria crassicaulis* (Onagawa; June); *Neorhodomela larix* (Sanriku; June).

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