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Short Communication

Cellular polyamine profile of the phyla *Dinophyta*, *Apicomplexa*, *Ciliophora*, *Euglenozoa*, *Cercozoa* and *Heterokonta*

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Spermidine, spermine and their biosynthetic precursor putrescine are widely distributed in both prokaryotes and eukaryotes. These polyamines have been implicated in a wide variety of cellular reactions, including protein and nucleic acid syntheses. Analysis of cellular polyamine components in eukaryotic algae has been studied to elucidate usefulness for their chemotaxonomy (Hamana and Matsuzaki, 1982, 1985; Hamana et al., 1988, 1990, 2004; Hegewald and Kneifel, 1981, 1982, 1983; Kneifel and Hegewald, 1980). Norspermidine and norspermine were detected as a major polyamine in various photosynthetic algae belonging to the phyla *Euglenozoa*, *Rhodophyta*, *Heterokonta*, *Chlorophyta* and *Charophyta*, and have not been found in the algae located in the phylum *Glaucophyta*.

The phylum *Dinophyta* consisting of unicellular dinoflagellates is phylogenetically located in the parvkingdom *Alveolata* and divergent from the above algal taxa (phyla) (Baldauf et al., 2000; Bhattacharya and Medline, 1995; Cavalier-Smith, 1993, 1998; NCBI home page, 2004; Van de Peer and De Wachter, 1997). Dinoflagellates are characterized by highly condensed nuclear chromatin and about half of their species contain plastids and grow photosynthetically (Dodge, 1973; Taylor, 1987). Polyamines of some photosynthetic dinoflagellates and the members of other two non-photoautotrophic phyla *Apicomplexa* and *Ciliophora* within *Alveolata* were analyzed in the present study. The polyamines detected within the three phyla *Euglenozoa*, *Cercozoa* and *Heterokonta* are shown in order to consider the phylogenetic significance of cellular polyamine distributions in the early evolution of eukaryotes. These six phyla evolved after the loss of primary endosymbiotic plastids and include secondary or tertiary endosymbiotic phototrophs (Falkowski et al., 2004; Ishida and Green, 2002; Nozaki et al., 2003; Yoon et al., 2002).

Two marine dinoflagellates, *Prorocentrum micans* and *Amphidinium carterae*, and two freshwater dinoflagellates, *Peridinium willei* and *Glenodiniopsis uliginosa,* were axenically, phototrophically cultivated in synthetic IMK-SP medium for marine microalgae (IMK medium mixed with artificial seawater) (Wako Chemicals, Tokyo, Japan) and AF-6 medium for freshwater microalgae (NIES Media List, 2000), respectively, at 25°C under the light. The dinoflagellates exponentially growing and at stationary phase were harvested. Cultures of other reference eukaryotes are briefly

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described in Table 1. Packed cells were homogenized in an equal volume of cold 1 M perchloric acid ($HClO_4$) (Hamana and Matsuzaki, 1982, 1985). Polyamines of the whole $HClO_4$ extract were analyzed by high-performance liquid chromatography (HPLC) on a column of cation-exchange resin in a Hitachi L6000 high-speed liquid chromatograph (Hamana et al., 1995). Gas chromatography (GC) was performed on a Shimadzu GC-9A gas chromatograph after the heptafluorobutylation of the concentrated polyamine samples (Niitsu et al., 1993). Polyamines were identified by gas chromatography-mass spectrometry (GC-mass) using a JEOL JMS-DX300 GC-mass spectrometer (Niitsu et al., 1993).

Norspermidine and norspermine (norspermidine/norspermine-type) were identified in the four photosynthetic dinoflagellates by HPLC and GC analyses (Figs. 1 and 2). This is the first report on dinoflagellata polyamines. Norspermine relatively increased at stationary phase. Cadaverine was predominant in the two marine dinoflagellates (Table 1). Diaminopropane was detected as a minor component. The cellular polyamines were not secreted into the culture supernatant. Putrescine and spermidine appeared in the two dinoflagellates grown in the synthetic medium supplemented with putrescine (Fig. 1 and Table 1). These results show the intracellular synthesis of spermidine from the uptaken putrescine. Cadaverine and norspermine levels decreased in this condition (Fig. 1). When L-ornithine was supplemented into the medium, putrescine was not produced. The lack of ornithine decarboxylase activity to produce putrescine from L-ornithine and the occurrence of spermidine-synthesizing ability from putrescine were demonstrated in these dinoflagellates.

The occurrence of putrescine, spermidine and spermine in parasitic *Plasmodium, Eimeria* and *Cryptosporidium* species belonging to the phylum *Apicomplexa* has been estimated (Assaraf et al., 1984; Moon et al., 1982; Nunez et al., 1987). The major polyamine in the isolated schizontes of *Cryptosporidium parvum* was spermidine, the first such report on the clear polyamine analysis of isolated apicomplexa. Spermine detected in the previous reports might be derived from the contamination or incorporation from host tissues.

All the ciliates tested in the phylum *Ciliophora* contained putrescine and spermidine, as found in the additional analyses for *Tetrahymena* (Hamana and Matsuzaki, 1988,1989) and the present analyses for *Didinium, Blepharisma* and *Paramecium*. Polyamine profiles of *Tetrahymena pyriformis* (Fig. 1) could not be duplicated for different growth stages and culture media (Table 1). When L-2,4-diaminobutyric acid was added into the medium, diaminopropane was not produced and the supplement of diaminopropane caused no production of norspermidine. Norspermidine was detected in *Paramecium bursaria* (green paramecium) carring symbiotic *Chlorella* species, suggesting that the *Chlorella* species belonging to the phylum *Chlorophyta* contain norspermidine.

The phylum *Euglenozoa* comprising the subphyla *Diplonemida, Euglenida* and *Kinetoplastida* includes both phototrophs and heterotrophs. Photosynthetic euglenoids, previously (Hamana and Matsuzaki, 1982, 1985) and newly analyzed, ubiquitously contained norspermidine and norspermine in addition to putrescine and spermidine (Figs. 1 and 2) under the different media and growth stages in the light or dark (Table 1). Spermine was incorporated from the media containing spermine; however, this tetra-amine was not detected by cultivation in the polyamine-free 199 (Nissui Pharmaceutical Co., Tokyo, Japan), MEM (Nissui), AF-6 or IMK-SP media. In non-photosynthetic kinetoplasts, it has been reported that *Trypanosoma cruzi, Trypanosoma granulosum* and a mutant of *Leishmania donovani* lack ornithine decarboxylase to produce putrescine (Carrillo et al., 200b; Mastri et al., 2001). Spermidine is produced from the putrescine uptaken from media, and spermine from spermidine in the cells. In the present study, spermine was detected in the all strains of *T. cruzi* cultured in the spermine-free Liver Infusion/Tryptone (LIT) medium (DIFCO Laboratories, USA) (Fig. 1 and Table 1). Cadaverine and agmatine were incorporated from the media containing traces of the two amines. When L-ornithine and L-2,4-diaminobutyric acid were supplemented into LIT medium, putrescine and diaminopropane did not appear in *T. cruzi* MAR 6 (Table 1). Norspermidine was not produced by the supplement of diaminopropane (Table 1). Other trypanosomatids, *Trypanosoma mega, Crithidia faciculata, Leptomonas* sp., *Phytomonas* sp. and *Leishmania mexicana* (Bacchi et al., 1977; Carrillo et al., 2000a), as well as *Leishmania major* grown in Grace's Insect Cell Culture (GICC) medium (Gibco, USA) and *Trypanosoma brucei* in the present study, had putrescine and spermidine and lacked norspermidine, norspermine and spermine.

Photosynthetic *Chlorarachnion reptans* is located in

Table 1. Cellular polyamine concentrations in the phyla *Dynophyta*, *Apicomplexa*, *Ciliophora*, *Euglenozoa*, *Cercozoa* and *Heterokonta*.

Organism	(Medium)		Polyamines (μmol/g wet weight)								
			Dap	Put	Cad	NSpd	Spd	HSpd	NSpm	Spm	Agm
Phylum *Dinophyta*											
Prorocentrum micans NIES-12	(IMK-SP)	Exp.	0.03	—	0.55	0.65	—	—	0.05	—	—
		Sta.	0.02	—	0.25	0.28	—	—	0.35	—	—
Sta. +1 mM Put			0.09	0.12	0.10	0.22	0.33	—	0.20	—	—
Amphidinium carterae NIES-331	(IMK-SP)	Exp.	0.02	—	0.41	0.55	—	—	0.20	—	—
Exp. +1 mM Put			—	0.80	—	0.42	0.41	—	0.01	—	—
Exp. +1 mM Orn			—	—	0.22	0.35	—	—	0.15	—	—
		Sta.	0.02	—	0.20	0.80	—	—	1.10	—	—
Peridinium willei NIES-304	(AF-6)	Exp.	0.02	—	0.06	0.80	—	—	0.20	—	—
Glenodoniopsis uliginosa NIES-463	(AF-6)	Exp.	0.01	—	0.05	0.70	—	—	0.45	—	—
Phylum *Apicomplexa*											
Cryptosporidium parvum NH schizont			—	—	—	—	0.20	—	—	—	—
Phylum *Ciliophora*											
Tetrahymena pyriformis GL	(PYG)	Exp.	—	0.40	—	—	0.45	—	—	—	—
Exp. +1 mM Dap			0.80	0.30	—	—	0.43	—	—	—	—
Exp. +1 mM DABA			—	0.50	—	—	0.32	—	—	—	—
	(PYG)	Sta.	—	0.27	—	—	0.19	—	—	—	—
	(199)	Sta.	—	0.50	—	—	0.25	—	—	—	—
Tetrahymena thermophila V1-M4	(PYG)	(c)	—	0.37	—	—	0.13	—	—	—	—
Didinium sp.	(LE)	Sta.	—	0.30	—	—	0.30	—	—	—	—
Blepharisma sp.	(LE)	Sta.	—	0.70	—	—	1.07	—	—	—	—
Paramecium multimicronucleatum	(LE)	Sta.	0.15	0.32	—	—	0.77	—	—	—	—
Paramecium bursaria	(AF-6)	Sta.	0.05	0.27	—	0.06	1.56	—	—	—	—
Phylum *Euglenozoa*											
Subphylum *Euglenoida*											
Euglena gracilis IAM E-6	(PBY)	Exp.	0.01	0.14	0.18	0.57	0.93	—	0.02	0.03	—
		Sta.	0.01	0.02	0.18	0.15	1.16	—	0.04	0.26	—
	(MEM)	Sta.	—	0.26	—	0.34	0.33	—	0.01	—	—
	(199)	Sta.	—	0.18	—	0.33	0.33	—	0.02	—	—
(PYA) in the light		(a)	0.01	1.01	—	0.32	1.27	0.05	0.81	0.11	—
(PYA) in the dark		(a)	0.14	0.30	—	0.20	0.63	0.06	0.48	0.07	—
Euglena gracilis IAM E-3	(PBY)		0.03	0.10	0.10	0.20	0.35	0.03	0.03	0.08	—
	(AF-6)		0.16	0.15	—	0.20	0.60	—	0.01	—	—
	(199)		—	0.10	—	0.22	0.10	—	0.01	—	—
Euglena gracilis IAM E-10	(PBY)		0.14	0.03	0.10	0.72	0.72	—	0.07	0.10	—
Euglena viridis IAM E-11	(S11)	(b)	0.00	1.11	0.31	0.17	0.85	—	0.01	0.06	—
Euglena mutabilis NIES-286	(AF-6)		0.04	0.42	—	0.12	0.20	—	0.01	—	—
Eutreptiella gymnastica NIES-381	(IMK-SP)		—	0.03	—	0.15	0.05	—	0.04	—	—
Phacus agilis NIES-387	(AF-6)		0.50	1.05	—	0.20	0.39	—	0.04	—	—
Trachelomonas sp. Gunma		(a)	0.23	0.27	—	0.17	0.27	—	0.40	—	—
Subphylum *Kinetoplasta*											
Trypanosoma cruzi MAR 6	(LIT)		—	—	0.01	—	0.67	—	—	0.16	0.02
trypomastigote											
+1 mM Dap			0.80	—	—	—	0.90	—	—	0.04	—
+1 mM Orn			—	—	—	—	0.60	—	—	0.18	—
+1 mM DABA			—	—	—	—	0.65	—	—	0.20	—

Table 1. (Continued.)

Organism	(Medium)		Polyamines (μmol/g wet weight)								
			Dap	Put	Cad	NSpd	Spd	HSpd	NSpm	Spm	Agm
Trypanosoma cruzi Colombia	(LIT)										
trypomastigote			—	—	0.05	—	1.24	—	—	0.30	0.04
epimastigote			—	—	—	—	0.50	—	—	0.10	—
Trypanosoma cruzi CL	(LIT)										
epimastigote			—	—	—	—	0.60	—	—	0.80	—
Trypanosoma cruzi Y	(LIT)										
epimastigote			—	—	—	—	0.60	—	—	0.25	—
Trypanosoma cruzi Tulahuen	(LIT)										
epimastigote			—	—	—	—	0.70	—	—	0.16	—
Trypanosoma brucei brucei GUTat3.1	(LIT)										
trypomastigote			—	0.02	—	—	0.75	—	—	—	0.02
Leishmania major MHOM	(GICC)										
promastigote			—	0.10	—	—	1.30	—	—	—	—
Phylum *Cercozoa*											
Class *Chlorarachniophyceae*											
Chlorarachnion reptans NIES-624	(ESM)		—	0.40	—	0.30	0.35	—	0.05	0.15	—
Phylum *Heterokonta*											
Class *Chrysophyceae*											
Ochromonas danica IAM CS-4	(A-8S)	(b)	—	0.97	0.96	—	0.42	—	—	0.02	—
Ochromonas minuta IAM CS-5	(A-8S)	(b)	—	0.28	1.43	—	0.63	—	—	0.04	—
Poterioochromonas malhamensis IAM CS-1	(A-8S)	(b)	—	0.54	0.20	—	0.10	—	—	—	—
Class *Bacillariophyceae*											
Phaeodactylum tricornutum IAM B-14	(Asp-Try)	(b)	—	0.40	0.05	0.60	0.35	—	0.20	—	—
Nitzschia palea IAM B-18	(P49Si)	(b)	0.04	1.08	0.10	0.01	0.08	—	—	—	—
Nitzschia closterium IAM B-16	(A-25)		—	0.15	—	0.03	0.80	—	—	—	—
Synedra acus Gunma		(a)	—	1.60	—	0.02	0.56	0.04	—	—	—
Class *Phaeophyceae*											
Sargassum thungergii Gunma		(a)	0.10	0.37	0.18	0.03	0.01	—	—	—	—
Sargassum fulvellum Gunma		(a)	—	1.40	0.34	0.01	0.01	—	—	0.01	0.01
Class *Eustigmatophyceae*											
Nannochloropsis oculata IAM ST-4	(A-42)		—	0.65	—	—	1.32	—	—	—	—
IAM ST-6	(A-42)		—	0.47	—	—	1.10	—	—	—	—
Vischeria punctata IAM X-4	(MBM-Pro)	(b)	0.02	0.26	0.62	0.13	0.95	—	—	0.03	—
Vischeria stellata IAM X-5	(MBM-Pro)	(b)	—	0.44	0.45	0.13	0.88	—	—	—	—

Dap, diaminopropane; Put, putrescine; Cad, cadaverine; NSpd, norspermidine; Spd, spermidine; HSpd, homospermidine; NSpm, norspermine; Spm, spermine; Agm, agmatine; Orn, L-ornithine; DABA, L-2,4-diaminobutyric acid; IAM, IAM Culture Collection, Institute of Cellular Biosciences, The University of Tokyo, Japan; NIES, National Institute for Environmental Studies, Tsukuba, Japan; Gunma, collected in Gunma Prefecture; —, not detected (<0.005); Exp., exponentially growing phase; Sta., stationary phase. PYG, peptone/yeast extract/glucose; PBY, peptone/sodium butyrate/yeast extract; PYA, peptone/yeast extract/sodium acetate. S11, A-8S, Asp-Try, P49Si, A-25, A-42 and MBM-Pro are listed in IAM List of Media. *Tetrahymena* strains have been maintained by Hamana of Gunma University. *Trypanosoma* and *Leishmania* strains have been maintained by Nishina of Saitama Medical University and were harvested at trypomastigote, epimastigote and/or promastigote stages. *Didinium, Blepharisma* and *Paramecium* species were purchased from Kyoto Kagaku Co., Kyoto, Japan and cultured in AF-6 medium or lettuce extract medium (LE). *Cryptosporidium parvum* NH was supplied from Saitama Institute of Health, Saitama Prefecture, Japan. (a) Cited from Hamana and Matsuzaki (1982); (b) Hamana and Matsuzaki (1985); (c) Hamana and Matsuzaki (1989).

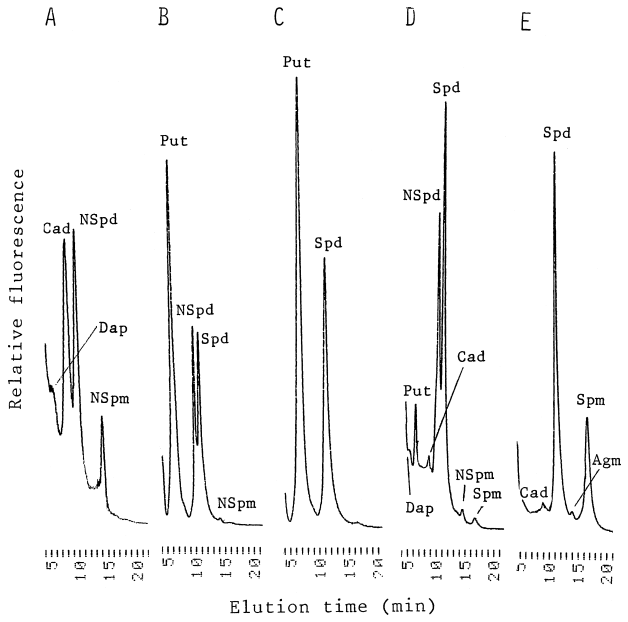

Fig. 1. HPLC of cellular polyamines of *Amphidinium carterae* NIES-331 (Exp.) (A), Exp. in the presence of 1 mM putrescine (B), *Tetrahymena pyriformis* GL (199, Sta.) (C), *Euglena gracilis* IAM E-6 (PBY, Exp.) (D) and *Trypanosoma cruzi* MAR 6 (trypomastigote) (E).

Abbreviations for polyamines are shown in Table 1.

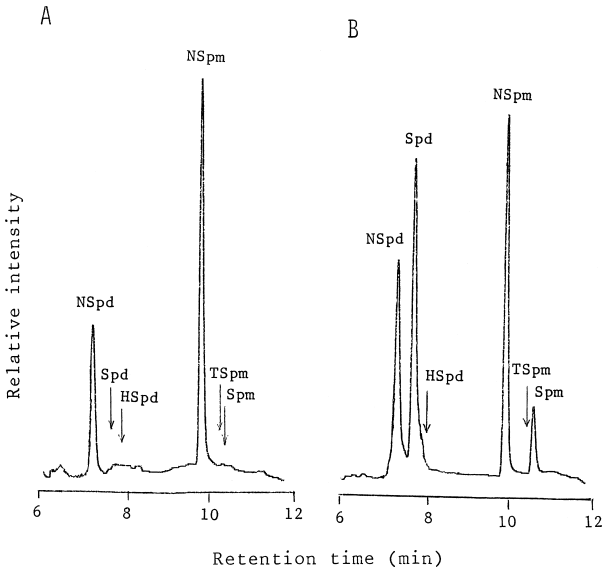

Fig. 2. GC of the concentrated polyamine fraction from *Amphidinium carterae* NIES-331 (A) and *Euglena gracilis* IAM E-6 (B).

Arrows indicate the retention positions of other standard polyamines. TSpm, thermospermine. Abbreviations for other polyamines are shown in Table 1.

the phylum *Cercozoa.* This alga, cultivated phototrophically in ESM medium (NIES Media List) in the light, contained norspermidine and norspermine in addition to putrescine, spermidine and spermine (Table 1). This polyamine profile, as a first report on cercozoa polyamine, is closely similar to those of photosynthetic euglenoids.

Polyamines of some algae belonging to the class *Chrysophyceae*, *Bacillariophyceae, Phaeophyceae* and *Eustigmatophyceae* of the phylum *Heterokonta*, previously reported (Hamana and Matsuzaki, 1982, 1985) and newly analyzed, are shown in Table 1. Although more species should be analyzed, putrescine and spermidine were distributed in the heterokonts. Norspermidine and cadaverine were found in limited species.

A photosynthetic prokaryote, cyanobacterum, is the origin of plastids of photosynthetic glaucophytes, green algae and red algae by its primary endosymbiosis (Baldauf et al., 2000; Moreira et al., 2000). Secondary symbionts of green alga gave rise to euglenoids and chlorarachniophytes, and secondary symbionts of red alga gave rise to cryptophytes, haptophytes and heterokonts (Falkowski et al., 2004). Secondary endosymbiosis of a unicellular red alga or green alga, or the tertiary endosymbiosis of a unicellular cryptophyte or haptophyte (secondary red alga symbiont), into a heterotrophically (phagotrophically) growing non-photosynthetic host cell, brought about three types of photosynthetic dinoflagellates (Falkowski et al., 2004; Ishida and Green, 2002; Yoon et al., 2002). Secondary or tertiary endosymbiotic plastids were found also in parasitic apicomplexans (Nozaki et al., 2003).

Spermidine or homospermidine was the major polyamine in cyanobacteria (Hamana et al., 1983, 1988). Putrescine/spermidine-type alone was found in glaucophytes (*Glaucophyta*) (Hamana and Matsuzaki, 1985). Putrescine/spermidine-type and putrescine/spermidine/norspermidine-type were prevalent in unicellular and multicellular green algae (*Chlorophyta* and *Charophyta*), and putrescine/spermidine/spermine-type and putrescine/spermidine/spermine/norspermidine/norspermine-type were found in unicellular and multicellular red algae (*Rhodophyta*) (Hamana and Matsuzaki, 1982, 1985; Hamana et al., 1990, 2004). In alveolates, norspermidine and norspermine have not been found in non-photosynthetic ciliates (*Ciliophora*) lacking plastids and parasitic apicomplexans (*Apicomplexa*) having plastids, whereas they contain putrescine and spermidine. Non-photosynthetic kineto-

plasts lacking plastids in *Euglenozoa* also lacked norspermidine and norspermine. The occurrence of norspermidine and norspermine and the absence of putrescine and spermidine, as found in the red-algal endosymbiotic, peridinin-containing dinoflagellates, was a unique polyamine profile within *Alveolata*, and furthermore, was distinguished from the profiles found in other photosynthetic algal taxa, the phyla *Glaucophyta, Chlorophyta, Charophyta, Rhodophyta, Euglenozoa* (the subphylum *Euglenida*), *Cercozoa* (the class *Chlorarachniophyceae*) and *Heterokonta*. The polyamine-synthesizing pathway might be related to their evolutional process by endosymbioses. Simple and poor polyamine profiles were found in heterotrophic (parasitic) organisms within lower unicellular eukaryotes. Further polyamine analyses of two other endosymbiotic groups in *Dinophyta*, and non-photosynthetic (osmotrophic, phagotrophic or parasitic) dinoflagellates, euglenoids and cercomonads, as well as two other secondary plastid endosymbiotic algal groups, the phyla *Haptophyta* and *Cryptophyta*, may serve to throw light on early algal evolution, and are being planning in our laboratory.

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