Short Communication

Cellular polyamines of lower eukaryotes belonging to the phyla Glaucophyta, Rhodophyta, Cryptophyta, Haptophyta and Percolozoa

Koei Hamana^{1,*} and Masaru Niitsu²

¹ Gunma University School of Health Sciences, Gunma 371–8514, Japan

² Faculty of Pharmaceutical Sciences, Josai University, Saitama 350–0290, Japan

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Analysis of cellular polyamine components in the nine lower eukaryotic phyla, the phylum Apicomplexa, Chlorarachniophyta (Cercozoa), Chlorophyta, Ciliophora, Dinophyta, Euglenophyta (Euglenozoa), Glaucophyta, Heterokontophyta (Heterokonta) and Rhodophyta, have been studied in order to consider the phylogenetic significance of cellular polyamine distributions in early evolution of eukaryotes (Hamana and Matsuzaki, 1982, 1985; Hamana et al., 1990, 2004a, b). In the nine phyla, the three phototrophic phyla Glaucophyta, Rhodophyta and Chlorophyta have plastids by the primary endosymbiosis of a phototrophic prokaryote, cyanobacterium, and multicellular species evolved within Rhodophyta and Chlorophyta (Bhattacharya et al., 2004; Cavalier-Smith, 1998; Falkowski et al., 2004; NCBI website, 2006; Rodriguez-Ezpeleta et al., 2005). The other six unicellular phyla include heterotrophs evolved without the endosymbiosis of cyanobacteria, phototrophs by secondary or tertiary endosymbiotic plastids and non-photosynthetic heterotrophs evolved after the loss of primary endosymbiotic plastids (Bhattacharya et al., 2004; Cavalier-Smith, 1998; Falkowski et al., 2004; NCBI website, 2006; Rodriguez-Ezpeleta et al., 2005).

The distribution pattern of three triamines, norspermidine, homospermidine and spermidine, and two tetraamines, norspermine and spermine, was almost phylum-specific among the nine phyla; furthermore, a part of their polyamine components seems to be correlated to their evolutionary endosymbiotic process.

The remaining three taxa (phyla) among total twelve phyla of lower eukaryotes, include the non-photosynthetic phylum Percolozoa without the endosymbiosis of cyanobacteria and the two photosynthetic phyla Cryptophyta and Haptophyta taken secondary endosymbiotic plastids (Bhattacharya et al., 2004; Cavalier-Smith, 2002, 2003; Falkowski et al., 2004). Cellular polyamines in the three phyla were first analyzed in the present study. Additional polyamine catalogues of some primitive phototrophic members belonging to Glaucophyta and Rhodophyta were determined.

Two strains of Glaucophyta, seventeen strains of Rhodophyta, seven strains of Cryptophyta and eight strains of Haptophyta were supplied from IAM, NIES and MBIC, and were cultivated phototrophically in the light using the media designed by the culture collections (IAM Catalogue Strains, 2004; Kasai et al., 2004; MBIC Strain Catalog Algae, 2006). *Cyanidioschyzon* and *Cyamidium* species were grown at 37°C. Other red algae and two glaucophytes were grown at 15–25°C. Seven cryptophytes and eight haptophytes were grown at 10–15°C and 15–20°C, respectively. Five non-photosynthetic species of Percolozoa were

* Address reprint requests to: Dr. Koei Hamana, School of Health Sciences, Faculty of Medicine, Gunma University, Showa-machi, Maebashi, Gunma 371–8514, Japan.

E-mail: khamana@health.gunma-u.ac.jp

Fig. 1. HPLC chromatogram of the whole polyamine extract from *Rhodomonas atrorosea* NIES-699 (A-a), *Porphyridium sordidum* MBIC 10454 (A-b) and *Isochrysis galbana* MBIC 10554 (A-c), and GC chromatogram of the concentrated polyamine fraction from *Rhodomonas atrorosea* NIES-699 (B).

Abbreviations for polyamines are shown in Table 1. Printed number on the peaks is elution time or retention time.

purchased from ATCC and NIES. *Heteramoeba*, *Tetramitus* and *Naegleria* species were cultivated at 25°C in the dark on the ATCC agar plates (ATCC Bacteria and Bacteriophages, 1996) spreading with *Escherichia coli* IAM 12119 grown in polyamine-free synthetic Eagle MEM medium (Nissui Pharmaceutical Co., Tokyo). A species of *Percolomonas* was grown in URO medium supplemented with wheat grains (Kasai et al., 2004) at 15°C in the dark.

Living organisms at early stationary stage were harvested by the centrifugation at 1,500×*g*. In Percolozoa, trophozoites (amoebae) were harvested and washed with Artificial seawater SP (Wako Chemicals, Tokyo) or PBS (Nissui Pharmaceutical Co., Tokyo). Packed cells were homogenized in an equal volume of cold 1 M perchloric acid (HClO₄) (Hamana and Matsuzaki, 1982, 1985). Polyamines were extracted into 0.5 M HClO₄ and 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), as shown in three typical chromatograms (Fig. 1). Gas chromatog-

raphy (GC) was performed on a Shimadzu GC-9A gas chromatography after the heptafluorobutylation of the concentrated polyamine fraction (Niitsu et al., 1993), as shown in a typical chromatogram (Fig. 1). Polyamines were identified by gas chromatography-mass spectrometry (GC-mass) using a JEOL JMS-700 GC-mass spectrometer (Niitsu et al., 1993). Cellular concentrations of polyamines estimated by HPLC are shown in Table 1.

In euglenophytes and chlorophytes, the photosynthetic cultures in the light and heterotrophic cultures in the dark were shown as the same polyamine profiles (Hamana et al., 2004a, b), suggesting that the two growth conditions cannot affect the algal polyamine synthesis in the present study. Since the same polyamine compositions have been observed in the axenic and non-axenic (including collected samples from fields) cultures for algae and cyanobacteria belonging to same genus or species (Hamana and Matsuzaki, 1982; Hamana et al., 2004a, b; Hosoya et al., 2005), non-axenic cultures in the present study were utilized for the determination of major polyamine com-

Table 1. Cellular polyamine concentrations in lower eukaryotes.

Organism			Polyamines (μmol/g wet weight)							
			Dap	Put	Cad	NSpd	Spd	HSpd	NSpm	Spm
Phylum Glaucophyta										
<i>Cyanophora paradoxa</i> NIES-763			—	0.80	—	—	1.10	—	—	—
IAM M-130	(b)		0.02	0.61	0.03	—	0.63	—	—	—
<i>Cyanophora tetracyanea</i> NIES-764			—	0.02	—	—	0.80	—	—	—
<i>Glaucocystis nostochinearum</i> IAM M-124	(b)		—	0.96	—	—	1.42	—	—	—
Phylum Rhodophyta										
Class Bangiophyceae										
Order Cyanidiales										
<i>Cyanidium caldarium</i> (RK-1)IAM R-11*	(c)		—	1.28	—	—	1.24	—	—	0.85
(KS-1) (Nagashima)*	(c)		—	0.85	—	—	1.11	—	—	0.32
<i>Cyanidium</i> sp. MBIC 10236*			—	1.30	—	—	0.77	—	—	0.20
<i>Cyanidioschyzon melorae</i> NIES-1332*			—	1.57	—	—	0.40	—	—	0.13
<i>Galdieria sulphuraria</i> IAM M-8*	(b)		—	1.72	—	0.08	3.09	—	0.59	0.82
Order Porphyridiales										
<i>Dixoniella grisea</i> MBIC 10460*			0.02	0.35	—	0.40	0.04	—	—	0.02
<i>Porphyridium sordidum</i> MBIC 10454*			—	0.55	—	—	0.77	—	—	0.18
<i>Porphyridium purpureum</i> IAM R-1*	(b)		—	0.67	0.02	—	0.53	0.04	—	0.10
IAM R-3*			—	0.60	—	—	0.20	—	—	0.02
<i>Porphyridium</i> sp. NIES-1032*			—	0.08	—	—	0.87	—	—	0.08
<i>Rhodella maculata</i> MBIC 10824*			—	0.02	—	0.02	0.65	—	0.01	0.01
<i>Rhodella</i> sp. NIES-1036*			—	0.20	—	0.07	0.35	—	—	0.19
Order Stylonematales										
<i>Rhodorus</i> sp. MBIC 10854*			—	0.27	—	0.02	0.15	—	0.10	0.30
Order Bagniales										
<i>Porphyra tenera</i> (Yokosuka)*	(a)		0.02	0.01	—	—	0.01	—	—	0.12
Order Erythropeltidales										
<i>Compsopogn coeruleus</i> NIES-1462*			—	0.03	—	—	0.32	0.10	—	0.02
<i>Compsopogonopsis japonica</i> NIES-1463*			—	0.02	—	—	0.30	0.18	—	0.05
Class Florideophyceae										
Order Batrachospermales										
<i>Batrachospermum vagum</i> IAM R-4*			—	0.40	—	—	1.15	0.60	—	0.22
<i>Batrachospermum atrum</i> NIES-1456*			—	0.04	—	0.20	0.75	0.32	—	0.02
<i>Batrachospermum helminthosum</i> NIES-1457*			—	0.12	—	—	0.60	0.44	—	0.08
<i>Thorea gaudichaudii</i> NIES-1473*			—	0.02	—	—	0.37	0.50	—	0.22
<i>Thorea okadae</i> NIES-1516*			—	0.02	—	0.10	0.51	0.45	—	0.22
<i>Nemalionopsis tortuosa</i> NIES-1467*			—	0.02	—	—	0.42	0.30	—	0.40
Phylum Cryptophyta										
<i>Chilomonas paramecium</i> NIES-715			—	0.06	—	0.08	0.12	—	—	—
<i>Chroomonas nordstedtii</i> NIES-706			0.26	0.69	—	0.02	0.10	—	—	—
<i>Cryptomonas ovata</i> NIES-274			—	0.20	—	0.35	—	—	—	—
<i>Cryptomonas platyuris</i> NIES-276			0.02	0.37	—	0.35	—	—	—	—
<i>Cryptomonas rostratiformis</i> NIES-277			—	0.20	—	0.40	—	—	—	—
<i>Cryptomonas tetrapyrenoidosa</i> NIES-282			0.02	0.30	—	0.40	—	—	—	—
<i>Rhodomonas atrosea</i> NIES-699*			—	0.40	—	0.50	0.08	0.03	0.03	—
Phylum Haptophyta										
<i>Chrysochromulina hirta</i> NIES-741*			—	0.09	—	0.05	—	—	—	—
<i>Cricosphaera roscoffensis</i> NIES-8			—	—	—	0.04	0.07	—	—	—
<i>Gephyrocapsa oceanica</i> NIES-353			—	—	—	—	—	—	—	—

Table 1. Continued.

Organism	Polyamines ($\mu\text{mol/g}$ wet weight)							
	Dap	Put	Cad	NSpd	Spd	HSpd	NSpm	Spm
<i>Hymenomonas coronata</i> NIES-1016*	—	—	—	0.10	—	—	0.14	—
<i>Phaeocystis globosa</i> NIES-388*	—	—	—	—	—	—	—	—
<i>Prymnesium parvum</i> NIES-1017*	—	0.04	—	0.05	0.03	—	0.05	—
<i>Isochrysis galbana</i> MBIC 10554*	—	0.06	—	0.62	0.21	—	0.29	—
<i>Pavlova pinguis</i> MBIC 10458*	—	—	—	0.07	0.52	—	0.16	0.02
Phylum Percolozoa								
Class Heterolobosea								
<i>Heteramoeba clara</i> ATCC 30972*	—	0.20	—	—	0.35	—	—	—
<i>Naegleria gruberi</i> ATCC 30887*	—	0.35	0.40	—	0.08	—	—	—
<i>Naegleria morganensis</i> ATCC 50351*	—	0.10	0.05	—	0.17	—	—	—
<i>Tetramitus rostratus</i> ATCC 30216*	—	0.05	0.37	—	0.06	—	—	—
Class Percolatea								
<i>Percolomonas</i> sp. NIES-1441*	—	0.04	0.10	—	0.26	—	—	—
<i>Escherichia coli</i> IAM 12119** (used as foods for Heterolobosea)	0.45	0.32	0.16	—	0.01	—	—	—
Wheat grain*** (used for the medium for Percolatea)	—	—	—	—	—	—	—	—

Dap, diaminopropane; Put, putrescine; Cad, cadaverine; NSpd, norspermidine; Spd, spermidine; HSpd, homospermidine; NSpm, norspermine; Spm, spermine; —, not detected (<0.005); IAM, Institute of Cellular Biosciences, the University of Tokyo, Tokyo, Japan; NIES, National Institute for Environmental Studies, Tsukuba, Japan; ATCC, American Type Culture Collection, Manassas, Virginia, USA; MBIC, the Marine Biotechnology Institute Culture Collection, Kamaishi, Iwate, Japan. (a) Cited from Hamana and Matsuzaki (1982); (b) Hamana and Matsuzaki (1985); (c) Hamana et al. (1990). * Non-axenic unialgal (uniprotocol) strain. Others were axenic unialgal strain. ** Polyamines were extracted by PCA from the packed cells. *** Polyamines were extracted by PCA from 10 ml of the medium supplemented with wheat grains.

position.

Two additional species of *Cyanophora* and *Glaucocystis* belonging to Glaucophyta, available in the present study, as well as other two species previously analyzed (Hamana and Matsuzaki, 1985), contained putrescine and spermidine as the major polyamines. Norspermidine and homospermidine have not been found in this phylum evolved after the endosymbiosis of cyanobacteria. On the other hand, two different triamine-distribution types, spermidine-dominant type and homospermidine-dominant type, were observed in cyanobacteria (Hamana et al., 1983; Hosoya et al., 2005). A certain relationship between glaucophytes and spermidine-dominant cyanobacteria as a symbiont is suggested by their same polyamine profiles.

Polyamines of thirteen species of unicellular red algae belonging to five orders of the class Bangiophyceae (six orders in total) and six multicellular red algae belonging to one order of the class Florideophyceae (22 orders in total) were analyzed. Pu-

trescine, spermidine and spermine were common polyamines in the phylum Rhodophyta. Within various algal phyla, this phylum is characterized by a appreciable amount of spermine. Within unicellular red algae, relatively high concentration of total polyamines and high spermine level were observed in the three primitive, slightly thermophilic genera, *Cyanidioschyzon*, *Cyanidium* and *Galdieria*, belonging to the order Cyanidiales. Norspermidine sporadically distributed in some red algae. Homospermidine was rich in multicellular red algae of the order Batrachospermales.

The widespread occurrence of norspermidine has been observed in unicellular and multicellular green algae (the phyla Chlorophyta) (Hamana et al., 2004a). The absence of norspermidine in cyanobacteria and the distribution of norspermidine within red algae as well as green algae suggest that its synthetic ability is taken in their host cells before or after the endosymbiotic process of cyanobacteria in their evolution.

Photosynthetic cryptophytes and haptophytes were

evolved by the secondary symbiosis of a red alga to a host eukaryotic cell (Bhattacharya et al., 2004; Falkowski et al., 2004; Rodriguez-Ezpeleta et al., 2005). Putrescine and norspermidine were ubiquitously distributed in the seven species tested in the phylum Cryptophyta. Spermidine, homospermidine and norspermine were also detected in a cryptophyte, *Rhodomonas atrovirens*. Within the eight haptophytes analyzed, norspermidine was found in six species and norspermine was detected in four species. No detection of polyamines in *Gephyrocapsa oceanica* and *Phaeocystis globosa*, found in the present study, is a novel result in our polyamine researches within lower eukaryotes. However, there is no doubt that norspermidine and norspermine were also distributed within the phylum Haptophyta.

The four species belonging to the class Heterolobosea or Percolatea of the non-photosynthetic phylum Percolozoa evolved without the symbiosis of cyanobacteria, contained putrescine, cadaverine and spermidine. A species of Heterolobosea contained putrescine and spermidine alone. *E. coli* grown in a synthetic medium and used as a food, contained diamines and a trace amount of spermidine, as shown in Table 1. Heterolobosea polyamine profiles were distinguished from *E. coli* polyamine profile. This polyamine profile of Percolozoa is similar to the profiles found in the phylum Ciliophora (such as *Tetrahymena* and *Paramecium*) and the phylum Apicomplexa (such as *Cryptosporidium* and *Plasmodium*) in which primary endosymbiotic plastids have been lost within the parvkingdom Alveolata (Assaraf et al., 1984; Hamana et al., 2004b), except for the occurrence of cadaverine in the various percolozoa. It is suggested that the loss of endosymbiotic plastids gave rise to lose cellular distribution of norspermidine and norspermine within Alveolata. The photosynthetic phylum Dinophyta of Alveolata contained norspermidine and norspermine (Hamana et al., 2004b). In other non-photosynthetic groups, the phylum Metamonada (such as *Trichomonas* and *Giardia*) contained putrescine and cadaverine, the phyla Acanthamoebidae (such as *Acanthamoeba*), Lobosea (such as *Amoeba*) and Entamoebidae (such as *Entamoeba*) contained diamino-propane, putrescine and spermidine, and the class Kinetoplastea of the phylum Euglenophyta (Euglenozoa) (such as *Trypanosoma* and *Leishmania*) contained putrescine and spermidine (Gillin et al., 1984; Hamana et al., 2004b, 2006). Although the five groups

phylogenetically evolved without the endosymbiosis of cyanobacteria, ubiquitously lacked norspermidine, homospermidine, spermine and norspermine, their polyamine profiles are varied and almost phylum (class)-specific.

Norspermidine was detected in the non-photosynthetic achlorophyllous green algae, the genera *Polytoma*, *Polytomella*, *Prototheca* and *Helicosporidium* (Hamana et al., 2004a). Further polyamine analyses of non-photosynthetic (osmotrophic, phagotrophic or parasitic) heterotrophs of dinoflagellates, euglenoids, cercomonads and heterokonts are in progress in our laboratory.

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