

Short Communication

Analysis of cellular polyamines of slime molds in comparison to the polyamine profiles of phylogenetically related organisms

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Phototrophic eukaryotic algae, the phyla *Glauco-phyta*, *Rhodophyta* and *Chlorophyta*, contain primary endosymbiotic plastids (Cavalier-Smith, 1998; Dyall et al., 2004; Falkowski et al., 2004; Nozaki et al., 2003). The unicellular phyla *Euglenophyta* (*Euglenozoa*), *Dinophyta*, *Heterokontophyta* (*Heterokonta*), *Apicomplexa*, *Ciliophora*, *Cercozoa*, *Percolozoa*, *Cryptophyta* and *Haptophyta* evolved secondary or tertiary endosymbiosis of phototrophs (Cavalier-Smith, 1998; Dyall et al., 2004; Falkowski et al., 2004; Nozaki et al., 2003). Analysis of the cellular polyamines of the twelve phyla has been carried out in order to consider the phylogenetic significance of cellular polyamine profiles in the early evolution of eukaryotes (Hamana and Matsuzaki, 1982, 1985; Hamana et al., 1990, 2004a, b). Distribution patterns of the two triamines, spermidine and norspermidine, and the two tetraamines, spermine and norspermine, seem to be correlated to their endosymbiotic process (Hamana et al., 2004a, b).

Non-photosynthetic lower eukaryotes which evolved before primary endosymbiosis of oxigenic phototrophic cyanobacteria are distributed within the major six taxa (phyla), *Mycetozoa*, *Microsporidia*, *Acanthamoebidae*,

Lobosea, *Entamoebidae* and *Metamonada* (Cavalier-Smith, 1998; NCBI home page, 2005). Therefore they lack photosynthetic plastids. *Mycetozoa*, *Acanthamoebidae* and *Lobosea* have mitochondria derived from endosymbiosis of an oxigenic alphaproteobacterium (Dyall et al., 2004). Urogenital parasitic *Trichomonas vaginalis* belonging to *Metamonada*, a parasitic degenerated fungus, *Gulgea plecoglossi* belonging to *Microsporidia*, *Entamoeba* of *Entamoebidae*, and *Giardia* of *Metamonada* lost typical mitochondria and have hydrogenosomes or mitochondrial relics (mitosomes) (Arisue et al., 2002; Dyall et al., 2004; Henze and Martin, 2003).

The phylum *Mycetozoa* contains two groups (classes) of slime molds, the class *Myxogastria* (formerly *Myxomycetes* or *Myxomycota*) and the class *Dictyosteliida* (formerly *Dictyostelida*, *Dictyostelia* or *Dictyosteliales*) (Hagiwara, 1997; NCBI, 2005; Yamamoto, 1998). In the present study, cellular polyamines of various slime molds were analyzed in comparison with the polyamine profiles of microsporidia, trichomonas, acanthamoeba and amoeba. This is the first report on the systematic polyamine analyses of plasmodial slime molds and cellular slime molds.

Fruiting bodies (including plasmodiocarp and aethalia) of wild plasmodial slime molds were scraped from wood pieces collected in Kochi Pref., Japan by

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Y. Yamamoto of Sakawa High School. Cellular slime molds collected by H. Hagiwara of the National Science Museum were cultivated at 20°C for 2 weeks on *Escherichia coli* (Type strain, IAM 12119) spread on agar plates, as a food. *E. coli* was previously grown in Nutrient Broth, harvested and washed with 0.8% NaCl. An isolated strain of *Dictyostereum purpureum* was kindly supplied by Dr. Kazunari Takahashi of Okayama-Ichimiya High School, Okayama, Japan. Fruiting bodies (sorocarps) of cellular slime molds were collected from 5–10 petridish plates. Major parts of the harvested fruiting bodies were spores. Fruiting bodies were homogenized in an equal volume of 1 M perchloric acid (HClO₄) (Hamana and Matsuzaki, 1982, 1985). The cysts of *Glugea plecoglossi* were isolated from the infected fish, *Plecoglossus altivelis*, kindly supplied by Dr. Michitaka Yamamoto of the Experimental Station of Fisheries of Shiga Pref., Japan (recommended by Dr. Hajime Arai of the Experimental Station of Fisheries of Gunma Pref., Japan). *Amoeba proteus* was purchased from Kyoto Kagaku Co., Kyoto, Japan and cultivated in NaCl/KCl/CaCl₂ buffer (pH 6.8) containing *Tetrahymena pyriformis* GL. *Trichomonas vaginalis* was purchased from ATCC and cultured in ATCC 361 medium. *G. plecoglossi*, *A. proteus* and *T. vaginalis* were harvested, washed with 0.8% NaCl by centrifugation and homogenized in an equal volume of cold 1 M HClO₄. Polyamines of the whole HClO₄ 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).

Cellular polyamine concentrations of slime molds and four representatives of lower non-phototrophic eukaryotes are shown in Table 1. The common polyamines within 31 species (18 genera) of fruiting bodies of wild plasmodial slime molds (the class *Myxogastria*) were putrescine and spermidine. Diaminopropane, cadaverine and a guanidinoamine, agmatine, were sporadically distributed. It was confirmed that the polyamine profile of various plasmodial slime molds was not altered by the concentration of wood pieces (No.2 in Table 1). Although the occurrence of an unusual triamine, homospermidine, was found as a minor polyamine in the axenically grown diploid plasmodia of *Physarum polycephalum* (Hamana and Matsuzaki, 1984), this triamine was distributed as a major polyamine in the fruiting bodies of various wild plasmodial slime molds (Table 1). For *Physarum flavico-*

lum, the major polyamines were putrescine, cadaverine and spermidine in the cultured haploid myxamoebae and dormant cysts (Zhu and Henney, 1990), whereas fruiting bodies of a wild strain analyzed in the present study lacked cadaverine. The taxonomic relation between polyamine profile and subordinate taxa within the class, and change of polyamine profile during cell differentiation of plasmodial slime molds is not clear.

Diaminopropane, putrescine and spermidine were ubiquitously detected in the fruiting bodies (sorocarps) of 13 cellular slime mold species belonging to 3 genera of the class *Dictyosteliida*, cultivated on *E. coli* IAM 12119 (Table 1). Although *E. coli* IAM 12119 contained putrescine, cadaverine, spermidine and acetylspermidine, cadaverine and acetylspermidine were not detected in the cultures of cellular slime molds (Table 1). These findings indicate that homospermidine found in the two strains, *Dictyostereum purpureum* JKS 275 and *Dictyostereum polycephalum* NYA 35, in addition to diaminopropane, putrescine and spermidine were produced in the cellular slime molds. The putrescine level was low in the fruiting bodies of the cultured *D. discoideum* WS 656 with *E. coli* (Table 1), whereas putrescine was detected as a major polyamine in the myxamoebae of this species ATCC 28363 grown axenically (Hamana and Matsuzaki, 1984). It has been reported that a significant amount of diaminopropane was detected in the myxamoebae of *D. discoideum* and *D. mucoroides* but was absent from the myxamoebae of *D. purpureum*, *Polysphondylium pallidum* and *P. violaceum* (North and Murray, 1980). In the present study, the diaminopropane level was very low in the fruiting bodies of the latter three species, suggesting similar polyamine profile between myxamoeba and spore (fruiting body) in cellular slime molds.

Homospermidine, norspermidine and norspermine were not found in the representatives of the four phyla *Microsporidia*, *Metamonada*, *Acanthamoebidae* and *Lobocea* (Table 1). Within the phylum *Microsporidia*, the polyamine profile of *Glugea plecoglossi*, analyzed first in the present study, was identical to another profile previously found in *Encephalitozoon curiculi*, a microsporidian (Bacchi et al., 2001). The two amitochondrial microsporidia contained spermine as a major polyamine. They are similar to typical yeasts and molds belonging to the phylum *Ascomycota* of the kingdom *Fungi* (Otsuka et al., 2004) in their cellular polyamine components. Amitochondrial *Trichomonas*

Table 1. Cellular polyamines in slime molds, *Glugea*, *Trichomonas*, *Acanthamoeba* and *Amoeba*.

Organism	Polyamines ($\mu\text{mol/g}$ wet weight)								
	Dap	Put	Cad	NSpd	Spd	HSpd	NSpm	Spm	Agm
Phylum Mycetozoa									
Class Myxogastria (plasmodial slime molds, true slime molds)									
<i>Physarum polycephalum</i> ATCC 204388(a) ¹ SD	0.01	0.89	0.27	—	1.57	0.05	—	0.01	—
(Strain M3CVII)(a) ¹ O	0.01	0.79	0.34	—	0.66	0.08	—	0.02	—
(a) ¹ A	0.04	0.33	0.09	—	0.24	0.01	—	—	—
<i>Physarum flavicomum</i> YY-28135	No.1	—	0.12	—	0.18	0.18	—	—	0.20
	No.2	—	0.16	—	0.29	0.50	—	—	0.15
<i>Physarum globuliferum</i> YY-28406		—	0.33	—	0.30	0.14	—	—	0.04
<i>Physarum nucleatum</i> YY-28441		0.02	0.19	0.05	—	0.29	0.35	—	0.04
<i>Physarum stellatum</i> YY-28413		—	0.20	—	0.30	0.15	—	—	—
<i>Arcyria cinerea</i> YY-28158		—	1.04	—	0.77	—	—	—	—
<i>Arcyria denudata</i> YY-28207	No.1	—	0.61	0.05	—	0.36	—	—	0.50
	No.2	—	0.50	—	—	0.40	—	—	0.30
	No.3	0.04	0.55	0.15	—	0.45	0.14	—	0.77
YY-28462		0.02	0.40	0.20	—	0.25	—	—	0.60
YY-28345		—	0.56	—	—	0.38	—	—	—
<i>Arcyria obvelata</i> YY-28147		—	0.20	—	—	0.32	0.02	—	0.02
<i>Ceratiomyxa fruticulosa</i> YY-28391		—	1.20	—	—	0.24	0.04	—	0.02
<i>Cribraria cancellata</i> YY-28184		—	0.63	—	—	1.07	—	—	—
YY-28245		—	0.85	—	—	0.95	—	—	0.02
<i>Cribraria intricata</i> YY-28246	No.1	—	0.18	—	—	0.30	0.15	—	0.03
	No.2	—	0.25	—	—	0.25	0.10	—	—
	No.3	—	0.30	—	—	0.40	0.20	—	0.04
YY-28376		—	0.19	—	—	0.35	0.10	—	—
var. <i>dictyodioides</i> YY-28175		—	0.40	—	—	0.27	—	—	0.04
<i>Collaria arcyrionema</i> YY-28292	No.1	—	0.16	0.10	—	0.20	0.60	—	—
	No.2	0.02	0.40	0.10	—	0.14	0.40	—	0.08
<i>Diderma spumarioides</i> YY-28386		—	0.15	—	—	0.20	—	—	0.10
<i>Didymium squamulosum</i> YY-28150		—	0.77	0.02	—	0.70	0.02	—	0.28
<i>Fuligo aurea</i> YY-28465		—	0.40	—	—	0.40	0.19	—	—
<i>Fuligo candida</i> YY-28297		—	0.35	—	—	0.88	—	—	—
YY-28160		—	0.28	—	—	0.50	—	—	—
<i>Fuligo septica</i> f. <i>flava</i> YY-28438		—	0.46	—	—	0.40	0.20	—	—
<i>Hemitrichia clavata</i> var. <i>calyculata</i> YY-28459		—	0.39	0.10	—	0.24	0.04	—	—
<i>Hemitrichia serpula</i> YY-28362		—	0.50	—	—	0.50	0.12	—	—
<i>Lindbladia cribrarioides</i> YY-28251		—	0.71	—	—	0.63	—	—	—
YY-28268		—	1.20	0.02	—	0.95	—	—	0.03
<i>Lindbladia tubulina</i> YY-28267	No.1	—	0.12	—	—	0.13	1.32	—	—
	No.2	—	0.10	—	—	0.15	1.25	—	—
<i>Lycogala epidendrum</i> YY-28390		—	1.45	—	—	1.12	—	—	—
<i>Perichaena depressa</i> YY-28276		—	1.20	—	—	0.78	0.18	—	—
<i>Reticularia lycoperdon</i> YY-28291		—	1.05	—	—	1.20	—	—	—
<i>Stemonaria longa</i> YY-28293		—	1.50	0.20	—	1.50	0.27	—	—
<i>Stemonitis axifera</i> YY-28427	No.1	0.28	0.15	0.37	—	0.53	—	—	1.07
	No.2	0.15	0.27	0.15	—	0.80	—	—	1.20
<i>Stemonitis fusca</i> YY-28452		—	0.80	0.25	—	0.50	0.05	—	—
<i>Stemonitis splendens</i> YY-28205		—	0.93	—	—	0.60	—	—	0.02
YY-28298		—	0.55	—	—	0.55	—	—	—

Table 1. Continued.

Organism	Polyamines ($\mu\text{mol/g}$ wet weight)								
	Dap	Put	Cad	NSpd	Spd	HSpd	NSpm	Spm	Agm
<i>Stemonitopsis typhina</i> var. <i>similis</i> YY-28454	—	1.00	0.67	—	1.15	0.02	—	—	—
<i>Trichia favoginea</i> var. <i>persimilis</i> YY-28364	—	0.52	—	—	0.37	0.03	—	—	—
<i>Tubifera dimarphotheca</i> YY-28361	—	0.57	0.25	—	1.18	—	—	—	—
<i>Tubifera ferruginosa</i> YY-28372	—	1.20	—	—	0.50	—	—	—	—
Class Dictyosteliida (dictyostelid cellular slime molds, amoeboid slime molds)									
<i>Dictyostelium discoideum</i> ATCC 28363(a) ² HL5	0.62	0.84	0.01	—	0.90	—	—	—	—
(Strain AX-3)(a) ² FM	0.22	0.55	—	—	0.22	—	—	—	—
<i>Dictyostelium discoideum</i> WS 656	0.40	0.01	—	—	0.27	—	—	—	—
<i>Dictyostelium purpureum</i> Takahashi	0.04	0.33	—	—	0.20	—	—	—	—
<i>Dictyostelium purpureum</i> Hagiwara 5 (TNS-C-7)	0.01	0.57	—	—	0.39	—	—	—	—
<i>Dictyostelium purpureum</i> JKS 275	0.02	0.95	—	—	0.55	0.05	—	—	—
<i>Dictyostelium polycephalum</i> NYA 35	0.01	1.05	—	—	0.25	0.12	—	—	—
<i>Dictyostelium aureostipes</i> CUH 1	0.01	0.85	—	—	0.89	—	—	—	—
<i>Dictyostelium delicatum</i> NY 31	0.11	1.18	—	—	1.10	—	—	—	—
<i>Dictyostelium firmibasis</i> Hagiwara 23	0.77	0.32	—	—	0.27	—	—	—	—
<i>Dictyostelium septentrionalis</i> NY 10	0.02	0.31	—	—	1.13	—	—	—	—
<i>Dictyostelium microsporum</i> Hagiwara 143	0.10	0.37	—	—	0.19	—	—	—	—
<i>Dictyostelium minutum</i> TNY 7	0.01	0.27	—	—	0.75	—	—	—	—
<i>Polysphondylium pallidum</i> Hagiwara 226	0.01	0.35	—	—	0.39	—	—	—	—
<i>Polysphondylium tenuissimum</i> Hagiwara 297	0.10	1.24	—	—	1.10	—	—	—	—
<i>Polysphondylium violaceum</i> Hagiwara 33	0.03	0.42	—	—	0.35	—	—	—	—
<i>Acytostelium</i> sp. NY 46	0.04	0.55	—	—	0.20	—	—	—	—
Phylum Microsporidia									
<i>Glugea plecoglossi</i> Shiga Pref.	—	0.20	—	—	0.32	—	—	0.10	—
Phylum Metamonada (superclass Parabasalia)									
<i>Trichomonas vaginalis</i> ATCC 30001 Exp.	—	1.45	0.25	—	0.04	—	—	0.02	—
Sta.	—	1.52	0.82	—	0.09	—	—	0.04	0.03
Phylum Acanthamoebidae									
<i>Acanthamoeba castellanii</i> ATCC 30010 (b)	2.60	0.03	—	—	0.15	—	—	—	—
Phylum Lobosea									
<i>Amoeba proteus</i> Kyoto Kagaku	2.40	0.25	—	—	0.12	—	—	—	—
Foods									
<i>Tetrahymena pyriformis</i> GL (c)	—	0.40	—	—	0.45	—	—	—	(AcSpd)
<i>Escherichia coli</i> IAM 12119	—	0.24	1.28	—	0.20	—	—	—	0.58

Dap, diamino propane; Put, putrescine; Cad, cadaverine; NSpd, norspermidine; Spd, spermidine; HSpd, homospermidine; NSpm, norspermine; Spm, spermine; Agm, agmatine; AcSpd, *N*¹-acetylspermidine; ATCC, American Type Culture Collection, Manassas, Virginia, USA; —, not detected (<0.005); Exp., exponentially growing phase; Sta., stationary stage. No. 1–No. 3, different samples (No. 2 contained some pieces of the slime mold-growing wood). (a) Cited from Hamana and Matsuzaki (1984). ¹ Extracted from plasmodia, ² Extracted from myxamoebae. (b) Hamana and Matsuzaki (1989). (c) Hamana et al. (2004b).

vaginalis cultured in the present study contained putrescine and cadaverine as the major polyamines. The absence of spermidine and spermine syntheses in the trichomonas has been reported (Yarlett et al., 2000). Spermine incorporated from culture media was back-converted to spermidine by polyamine oxidase located

in hydrogenosome of the trichomonas (Yarlett et al., 2000). Therefore, spermidine, spermine and agmatine detected as the minor components in the present study are considered as a component incorporated from the growth medium containing these polyamines.

A diamino propane-rich profile has been reported in

Acanthamoeba species of the phylum *Acanthamoebidae* (Hamana and Matsuzaki, 1989; Kim et al., 1987; Zhu et al., 1989). *Amoeba proteus*, analyzed first in the present study, contained a high concentration of diaminopropane in addition to putrescine and spermidine. *Tetrahymena pyriformis* GL used for the culture of *A. proteus* contained putrescine and spermidine alone (Hamana et al., 2004b) (Table 1), indicating the production of diaminopropane in *A. proteus*. It has been reported that amitochondrial *Entamoeba histolytica* belonging to the phylum *Entamoebidae* and amitochondrial *Giardia intestinalis* (formerly *G. lamblia*) belonging to the phylum *Metamonada* contained putrescine, spermidine and spermine (Gillin et al., 1984). Uptake of spermidine and/or spermine from culture media in the previous study is not excluded. Although detailed chemotaxonomic comparisons of cellular polyamine components among the five phyla *Microsporidia*, *Acanthamoebidae*, *Lobosea*, *Entamoebidae* and *Metamonada* were difficult, their polyamine distributions seem to be phylum-specific and phylogenetically significant. However, the presence or absence of mitochondrial organelles in them do not affect their polyamine profiles.

Norspermidine and norspermine, widespread in phototrophic lower eukaryotic algae containing endosymbiotic plastids as a major cellular polyamine (Hamana et al., 2004a,b), have never been detected in non-photosynthetic lower eukaryotes located in the six phyla *Mycetozoa*, *Microsporidia*, *Acanthamoebidae*, *Lobosea*, *Entamoebidae* and *Metamonada*. The cellular polyamine profiles found in various plasmodial slime molds (the class *Myxogastria*) are similar to the polyamine profiles found in mushrooms belonging to the phylum *Bacidiomycota* of the kingdom *Fungi* (Hamana et al., 2005) in their wide distribution of homospermidine and agmatine.

Cellular slime molds (the class *Dictyosteliida*) contain diaminopropane and lack agmatine, however, similarly to the phyla *Acanthamoebidae* and *Lobosea* in their polyamine patterns. Polyamine distributions found in lower eukaryotes suggest a relationship between their polyamine synthetic abilities and their evolutionary positions.

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