

Polyamine Distribution Profiles within the Phyla Nematoda, Platyhelminthes, Annelida and Cnidaria

Koei HAMANA^{1)*}, Azusa SAKAMOTO¹⁾,
Masami NISHINA²⁾ and Masaru NIITSU³⁾

(Received September 30, 2006; Accepted December 11, 2006)

Abstract : Cellular polyamines of nematodes of the phylum Nematoda, planarians, cestodes and trematodes of the phylum Platyhelminthes, earthworms, leeches and sandworms of the phylum Annelida, and hydras of the phylum Cnidaria, were analyzed by HPLC and GC to obtain phylogenetic informations and parasitic peculiarities on polyamine profiles in lower invertebrates. Spermidine and spermine were ubiquitously distributed in four zoo-parasitic nematodes. A plant parasite, *Pratylenchus* and a fungivore, *Bursaphelenchus* and two entomopathogens, *Steinernema* as well as free-living bacteriovores, *Caenorhabditis* and *Dorylaimus*, contained spermidine and lacked spermine. Zoo-parasitic cestodes *Hymenolepis* and *Diphyllobothrium*, and zoo-parasitic trematodes *Paragonimus*, *Fasciola* and *Schistosoma*, belonging to the phylum Platyhelminthes, ubiquitously contained spermidine and spermine, suggesting their dependence on host animals for uptake of spermine. Putrescine, cadaverine, spermidine and spermine were the major polyamines in freshwater, marine and land planarians (the phylum Platyhelminthes), and free-living earthworms, leeches and sandworms (the phylum Annelida). Hydras belonging to the phylum Cnidaria contained putrescine and spermidine alone. Homospermidine was distributed in a zoo-parasitic helminth of the phylum Nematoda and two zoo-parasitic tapeworms of the phylum Platyhelminthes as well as planarians, earthworms and leeches. Norspermidine and/or norspermine were found in two land planarians, a leech and a sandworm. This is the first report on the occurrence of these novel polyamines within lower invertebrates.

Key words : Polyamine; Nematoda; Platyhelminthes; Annelida; Cnidaria; Parasite

INTRODUCTION

Putrescine, spermidine and spermine are known to be widely distributed in vertebrates and higher invertebrates as the major cellular polyamines. The occurrence of uncommon polyamines such as norspermidine and norspermine was restricted in some members of higher invertebrates¹⁻⁴⁾. Another

unusual polyamine, homospermidine, was detected in some arthropods, echinodermus and tunicates within higher invertebrates¹⁻⁴⁾ and some planarians, leeches and earthworms^{5,6)} within lower invertebrates.

Spermidine and spermine were major polyamines in some parasitic helminths belonging to the phylum Nematoda^{5,7-13)}. Two free-living nematodes contained

¹⁾School of Health Sciences, Faculty of Medicine, Gunma University, Maebashi, Gunma 371-8514, Japan

²⁾Department of Immunology, Saitama Medical School, Moroyama, Saitama 350-0495, Japan

³⁾Faculty of Pharmaceutical Sciences, Josai University, Sakado, Saitama 350-0290, Japan

*Reprint address : School of Health Sciences, Faculty of Medicine, Gunma University, Maebashi, Gunma 371-8514, Japan

putrescine and spermidine but lacked spermine⁵. A phylogenetic analysis of nematodes identified five major clades in the phylum, all of which include parasitic species and suggested that vertebrate parasitism and plant parasitism arose independently at least four times and three times, respectively¹⁴. Therefore, polyamines of some additional plant-parasitic, vertebrate-parasitic, fungivore, bacteriovore and entomopathogenic nematodes were analyzed in the present study to consider phylogenetic relations and parasitic peculiarities on their polyamine compositions.

Free-living freshwater planarians belonging to the class Turbellaria of the phylum Platyhelminthes contained putrescine, cadaverine, spermidine, homospermidine and spermine^{5,6}. In addition to new members of freshwater planarians, various land-dwelling planarians and marine planarians were analyzed in the present study. Some parasitic cestodes and trematodes contained putrescine, spermidine and spermine^{7,10,15,16}. In the present study, details on cellular polyamines of additional five vertebrate parasites belonging to the classes Cestoda and Trematoda of this phylum, were obtained.

In our previous studies on cellular polyamines of earthworms belonging to the class Oligochaeta and leeches belonging to the class Hirudinea within the phylum Annelida, the ubiquitous occurrence of putrescine, cadaverine, spermidine, homospermidine and spermine was observed^{5,6}. Polyamines of sandworms (polychaetes) belonging to the class Polychaeta of this phylum and of hydras belonging to the class Hydrozoa of the phylum Cnidaria, were analyzed in the present study to display their polyamine patterns and to obtain phylogenetic information on polyamine distributions within lower invertebrates.

MATERIALS AND METHODS

Nematodes

Trichinella spiralis (ISS 412 strain) was maintained by passages in mice and the larvae were isolated from the mice muscle by pepsin-1% HCl digestion and the adults were collected from the mice small intestines by Dr. M. Sato of Saitama Medical School¹⁷. *Anogiostrongylus cantonensis* (adult) and

Strongyloides venezuelensis (larva) were isolated from rats and *Ancylostoma duodenale* (adult) was clinically collected from human intestines in Saitama Medical School. *Bursaphelenchus xylophilus* (S 6-1 strain) grown on *Botrytis cinerea*-PDA medium and *Pratylenchus vulnus* isolated from the soil surrounded strawberry roots were supplied by Dr. K. Matsuura of Forestry and Forest Prod. Res. Institute, Ibaraki, Japan and Dr. Y. Shishida of Agricultural Station of Gunma Prefecture, Maebashi, Gunma, Japan, respectively. Two *Steinernema* species (third larva) were kindly supplied from Dr. H. Tanabe of SDS Biotech. Co., Ibaraki, Japan.

Platyhelminthes

Adults of parasitic cestodes, *Hymenolepis diminuta* and *Diphyllobothrium nihonkaiense*, and parasitic trematodes, *Paragonimus westermanii*, *Fasciola hepatica* and *Schistosoma japonicum*, were clinically obtained from human in Saitama Medical School. Freshwater planarians, *Phagocata vivida* and *Seidlia auriculata*, were obtained in Agatsuma, Gunma. Other five freshwater planarians and three marine planarians collected in Aomori were supplied by Dr. W. Yoshida of Hirosaki University, Aomori, Japan¹⁸. A land planarian, *Bipalium mobile*, was collected in Shibukawa, Gunma. Two land planarians, *Bipalium kewense* and *Platydemus manokwari*, were collected in Ogasawara Island and supplied by Dr. T. Ono of Ogasawara Subtropical Agricultural Center, Tokyo and Dr. I. Okochi of Forestry and Forest Prod. Res. Institute, Ibaraki¹⁹.

Annelids

Marine sandworms, *Perinereis brevicirris*, *Marphysa sanguinea* and *Lumbrineris japonica*, were purchased from a fishing-bait store in Maebashi, Gunma. The fragmenting potworms (*Enchytraeus japonensis*) were supplied from Dr. M. Myohara of National Institute of Agrobiological Sciences, Tsukuba, Ibaraki²⁰. Other five annelids were collected in Maebashi, Gunma.

Cnidaria

Two freshwater hydras were purchased from Kyoto Kagaku, Kyoto.

Polyamine analysis

Organisms weighing 100-500 mg wet weight were washed with saline and homogenized in equal weights

of cold 1.0 M HClO₄ by a glass-glass homogenizer to extract polyamines. The polyamines extracted in 10-100 μ l of supernatant were analyzed by high-performance liquid chromatography (HPLC) on a L6000 High-Speed Liquid Chromatograph (Hitachi, Tokyo) using a column of cation-exchange resin⁵. The elution was carried out by a linear gradient prepared from two buffers and polyamines eluted were detected by fluorometry after heating with *o*-phthalaldehyde reagent. Acid hydrolysis and alkaline hydrolysis of polyamine samples before HPLC analysis were performed to identify polyamine peaks⁵. Gas chromatography (GC) of the concentrated polyamine fractions from 10 g wet weight of organisms on a GC-9A gas chromatograph (Shimadzu, Kyoto), was carried out after heptafluorobutyrylation²¹. Diamines, spermidine, spermine, histamine and agmatine as the standards were purchased from Sigma (St Louis, USA). Norspermidine and norspermine were purchased from Eastman Kodak Co. (Rochester, USA). Homospermidine was synthesized in our laboratories²¹.

RESULTS AND DISCUSSION

Typical HPLC chromatograms of polyamine samples are shown in Fig. 1. Diaminopropane, putrescine, histamine, cadaverine, norspermidine, spermidine, homospermidine, agmatine, norspermine and spermine were separated. Agmatine disappeared after alkaline hydrolysis, showing further evidence that it is agmatine. Other amine peaks were resistant for alkaline hydrolysis as well as acid hydrolysis. Cellular concentrations of the detected polyamines are shown in Table 1. Our previous data of related organisms are cited in the table.

Spermidine and spermine ubiquitously distributed as the major cellular polyamines in the adults of the four zoo-parasitic nematodes, *T. spiralis*, *A. duodenale*, *A. cantonensis* and *S. venezuelensis* (Fig. 1 and Table 1). The three zoo-parasitic nematodes, *Anisakis simplex*, *Ascaris suum* and *Diriofilaria immitis*, contained spermidine and spermine, as previously reported⁵. Similar cellular polyamine levels were observed between the larva and adult in *T. spiralis*. Two entomopathogenic *Steinernema* species, a plant root parasite, *Pratylenchus vulnus*,

and a free-living fungivore, *Bursaphelenchus xylophilus*, contained spermidine and lacked spermine (Table 1). As reported previously, two free-living bacteriovores, *Caenorhabditis elegans* and *Dorylaimus fodori*, also contained spermidine and lacked spermine, however, they contained putrescine as a major polyamine⁵.

The occurrence of spermidine and spermine has been reported in other nine zoo-parasitic firial nematodes, *Acanthocheilonema viteae*, *Brugia patei*, *B. pahangi*, *Litomosoides carinii*, *Onchocerca volvulus*, *Setaria cervi*, *Ascaridia galli*, *Ancylostoma ceylanicum* and *Nippostrongylus brasiliensis*, roughly analyzed by others⁷⁻¹³. Since cellular spermine level is low in bacteria, fungi and plant roots whereas animal tissues contained significant amounts of spermine, dependence on host animals for uptake of spermine is suggested in zoo-parasitic nematodes. Significant difference on polyamine profile among various classes (as shown in Table 1) of the phylum Nematoda was not observed. The polyamine distribution profiles depend on their growing surroundings rather than their phylogenetic positions.

Putrescine, spermidine and spermine were ubiquitously distributed within the class Turbellaria of the phylum Platyhelminthes. Homospermidine was found in some land planarians and marine planarians as well as freshwater planarians within the class (Table 1). Norspermidine and/or norspermine were detected in the land planarians analyzed in the present study.

Spermidine and spermine were major polyamines and significant amount of putrescine was not detected in the parasitic cestodes and trematodes analyzed in the present study (Table 1). In the class Cestodes, Miyaji *et al.* reported that the larval taeniids of *Taenia crassiceps*, *T. hydatigena*, *T. taeniaeformis* and *Echinococcus multilocularis* contained significant levels of putrescine, spermidine and spermine¹⁵. Their putrescine levels were much higher than these of the adults of *D. nihonkaiense* (Table 1), *H. diminuta* (Table 1), *H. nana*⁷ and *Cotugnia digonopora*¹⁰. In the class Trematodes, spermidine and spermine were the major polyamines in the adults of *P. westermanii*, *F. hepatica* and *S.*

Table 1. Cellular polyamine concentrations in Nematoda, Platyhelminthes, Annelida and Cnidaria

Organism	Polyamines (μ mol/g wet weight)									
	Dap	Put	Cad	NSpd	Spd	HSpd	NSpm	Spm	His	Agm
Phylum Nematoda (nematodes)										
Class Secernentea										
Order Anolostomatida										
<i>Ancylostoma duodenale</i> * *	-	-	0.10	-	0.30	-	-	0.10	-	-
Order Metastrogylida										
<i>Angiostrongylus cantonensis</i> * *	-	0.02	-	-	0.40	-	-	0.10	-	-
Order Rhabditida (Strongyloididae)										
<i>Strongyloides venezuelensis</i> * *	0.02	0.13	-	-	0.56	0.05	-	0.14	-	-
Order Rhabditida (Rhabditoidae)										
<i>Caenorhabditis elegans</i> * * * *	(a)	-	1.00	0.02	-	1.41	-	-	-	-
Order Rhabditida (Steinernematidae)										
<i>Steinernema carpocapsae</i> * * * * *	-	-	-	-	0.75	-	-	-	-	-
<i>Steinernema glaseri</i> * * * * *	-	-	-	-	0.54	-	-	-	-	-
Order Ascaridida										
<i>Anisakis simplex</i> * *	(a)	-	0.08	-	-	0.95	-	-	0.52	-
<i>Ascaris suum</i> * *	(a)	-	0.20	-	-	0.84	-	-	0.19	-
Order Spirurida										
<i>Dirofilaria immitis</i> * *	(a)	-	0.06	0.02	-	0.54	-	-	1.64	-
Order Aphelenchida										
<i>Bursaphelenchus xylophilus</i> * * *	-	0.04	-	-	1.34	-	-	-	-	-
Order Tylenchida										
<i>Pratylenchus vulnus</i> *	-	-	-	-	0.20	-	-	-	-	-
Class Adenophorea										
Order Dorylaimida										
<i>Dorylaimus fodori</i> * * * *	(a)	-	0.45	0.02	-	0.11	-	-	-	0.01
Order Trichocephalida										
<i>Trichinella spiralis</i> * * (larva)	-	-	-	-	1.33	-	-	0.25	-	-
(adult)	-	-	0.01	-	1.47	-	-	0.17	-	-
Phylum Platyhelminthes (platyhelminthes)										

Class Turbellaria

Order Tricladida (triclad)

(freshwater planarians)

<i>Bdellocephala brunnea</i>		0.03	1.40	0.20	-	0.02	-	-	0.20	0.10	0.02
<i>Dendrocoelopsis lactea</i>		0.04	1.20	0.10	-	0.12	-	-	0.16	-	-
<i>Phagacata teshirogii</i>		0.04	1.90	0.72	-	0.04	-	-	0.30	-	-
<i>Phagacata vivida</i>		-	1.00	0.20	-	0.07	-	-	0.24	-	-
<i>Seidtia auriculata</i>		-	1.36	0.02	-	0.09	0.01	-	0.30	-	0.05
<i>Dugesia ryukyuensis</i>		-	1.60	0.70	-	0.90	0.02	-	0.44	0.10	-
<i>Dugesia japonica</i>	(b)	-	1.40	0.04	-	3.14	0.08	-	1.29	0.02	0.02
<i>Girardia tigrina</i>	(b)	-	1.19	0.05	-	0.02	0.17	-	0.46	0.05	0.01

(land planarians)

<i>Bipalium mobile</i>		0.12	0.95	-	0.03	0.30	0.30	0.25	0.86	-	0.15
<i>Bipalium kewense</i>		-	0.55	0.02	0.02	1.40	-	0.10	0.30	-	-
<i>Platydemus manokwari</i>		-	0.44	0.27	-	0.35	0.02	0.04	0.75	-	-

Order Polycladida (polyclads)

(marine planarians)

<i>Pseudostylochus intermedius</i>		-	0.70	0.05	-	0.12	0.01	-	0.15	0.40	-
<i>Thysanozoon brocchii</i>		0.05	0.75	0.10	-	0.04	-	-	0.56	-	-
<i>Planocera multitentaculata</i>		-	0.07	1.02	-	0.14	-	-	0.10	0.16	-
<i>Planocera reticulata</i>		-	0.10	0.65	-	0.34	-	-	0.15	-	-
<i>Notoplana humilis</i>		0.08	0.15	0.20	-	0.40	-	-	0.30	0.04	-

Class Cestoda (tapeworms)(cestodes)

<i>Hymenolepis diminuta</i> * *	(whole)	-	-	0.02	-	0.45	0.02	-	0.87	-	-
	(head)	-	-	-	-	0.10	-	-	0.65	-	-
	(middle)	-	-	-	-	0.17	-	-	0.85	-	-
	(tail)	-	-	-	-	0.25	-	-	0.40	-	-
<i>Diphyllobothrium nihonkaiense</i> * *		-	-	0.30	-	0.95	0.10	-	0.20	-	-

Class Trematoda (trematodes)

<i>Paragonimus westermanii</i> * *		-	-	0.05	-	0.30	-	-	0.20	-	-
<i>Fasciola hepatica</i> * *		-	-	-	-	0.15	-	-	0.13	-	-
<i>Schistosoma japonicum</i> * *		-	-	-	-	0.25	-	-	0.06	-	-

Phylum Annelida (annelids)

Class Oligochaeta (earthworms)										
<i>Limnodrilus socialis</i> (freshwater)	-	0.76	0.08	-	1.36	0.02	-	0.22	-	-
<i>Enchytraeus japonensis</i> (terrestrial)	-	0.50	0.15	-	0.85	0.15	-	0.05	-	-
<i>Eisenia foetida</i> (terrestrial) (a)	-	0.28	0.01	-	1.70	0.02	-	0.74	-	-
<i>Tubifex hattai</i> (terrestrial) (a)	-	0.78	0.04	-	1.62	0.01	-	0.60	-	-
<i>Pheretima communissima</i> (freshwater) (a)	-	0.50	0.01	-	1.42	0.01	-	0.38	-	-
Class Hirudinea (leeches)										
<i>Whitmania pigra</i> (freshwater)	-	0.02	0.02	-	1.20	0.20	-	0.02	-	-
<i>Glossiphonia complanata</i> (freshwater)	-	0.30	0.02	-	1.30	0.25	-	0.10	-	-
<i>Erpobdella lineata</i> (freshwater)	0.02	0.18	0.04	0.04	1.72	0.02	-	0.10	-	-
<i>Mimobdella japonica</i> (freshwater)	-	0.30	-	-	2.04	0.40	-	0.10	-	-
<i>Hirudo nipponia</i> (freshwater) (b)	-	1.48	0.30	-	4.86	0.27	-	0.93	0.04	0.04
<i>Haemadipsa zeylanica</i> (terrestrial) (b)	-	0.26	0.03	-	2.38	0.18	-	0.94	0.04	0.12
Class Polychaeta (sandworms)										
<i>Perinereis brevicirris</i> (marine)	-	0.04	0.47	-	0.29	-	-	0.17	-	0.05
<i>Marphysa sanguinea</i> (marine)	-	0.77	4.21	0.07	0.47	-	-	0.44	-	-
<i>Lunbrineris japonica</i> (marine)	0.01	0.01	0.03	-	0.49	-	-	0.16	0.08	0.01
Phylum Cnidaria (cnidarians)										
Class Hydrozoa										
Order Hydroida										
<i>Hydra vulgaris</i> (freshwater)	-	1.05	-	-	0.05	-	-	-	-	-
<i>Chlorohydra viridisima</i> (freshwater)	-	1.50	0.07	0.22	0.15	-	-	0.06	-	-

Dap, diaminopropane; Put, putrescine; Cad, cadaverine; NSpd, norspermidine; Spd, spermidine; HSpd, homospermidine; NSpm, norspermine; Spm, spermine; His, histamine; Agm, agmatine; -, <0.005. Data were obtained from single samples. a, cited from Hamana et al. (1995). b, cited from Hamana et al. (1997). *, Plant parasite; **, Vertebrate parasite; ***, Fungivore; ****, Bacteriovore; *****, Entomopathogen.

japonicum (Table 1), and *Gastrethylax crumerifer*, *Paramphistomum cervi* and *Calicophoron calicophorum*¹⁰⁾, and *Paragonimus uterobilateralis*¹²⁾.

Ornithine decarboxylase (ODC) catalyses the conversion of ornithine to putrescine, an obligate precursor to spermidine and spermine. Although negligible ODC activity has been detected in some larval and adult parasitic nematodes^{7-9,12,13)} and cestodes^{7,15)}, membrane-associated ODC was

identified in the parasitic nematode *Haemonchus contortus* as well as the free-living nematode *C. elegans*²²⁾. Cellular putrescine and spermidine levels were depleted in an ODC-deficient mutant of *C. elegans*²³⁾. These findings show that the parasites with low putrescine level also contained the synthetic ability of putrescine and spermidine. Uptake of putrescine, spermidine and spermine from the culture medium was reported in some filarial parasitic nematodes^{8,9,11,13)}. Interconversion of spermidine and

spermine into putrescine after the acetylation of the polyamines, was suggested in some parasitic nematodes and taeniids (Cestoda)^{13,15}. Cadaverine, produced by lysine decarboxylase, was sporadically detected within the cestodes and trematodes analyzed in the present study, as well as planarian tubellarias (Fig. 1 and Table 1).

Homospermidine has been found in earthworms and leeches of Annelida and planarians of Platyhelminthes^{5,6}. Two earthworm species and four leech species newly analyzed in the present study, contained homospermidine (Table 1). This unusual triamine was also detected in a parasitic helminth nematode, *S. venezuelensis*, and two parasitic

tapeworms, *H. diminuta* and *D. nihonkaiense*, as shown in Fig.1 and Table 1, suggesting the distribution of homospermidine within the vertebrate parasites belonging to Nematoda and Platyhelminthes. A phylogenetic study proposed that the subphylum Clitellata comprise the three classes Oligochaeta, Hirudinea (Euhirudinea) and Branchiobdellida, and the class Polychaeta is located outside Clitellata in Annelida²⁴. Homospermidine detected in earthworms (Oligochaeta) and leeches (Hirudinea), was not found in the three sandworms belonging to the class Polychaeta, as shown in the present study. Another unusual triamine, norspermidine, was found in two land planarians

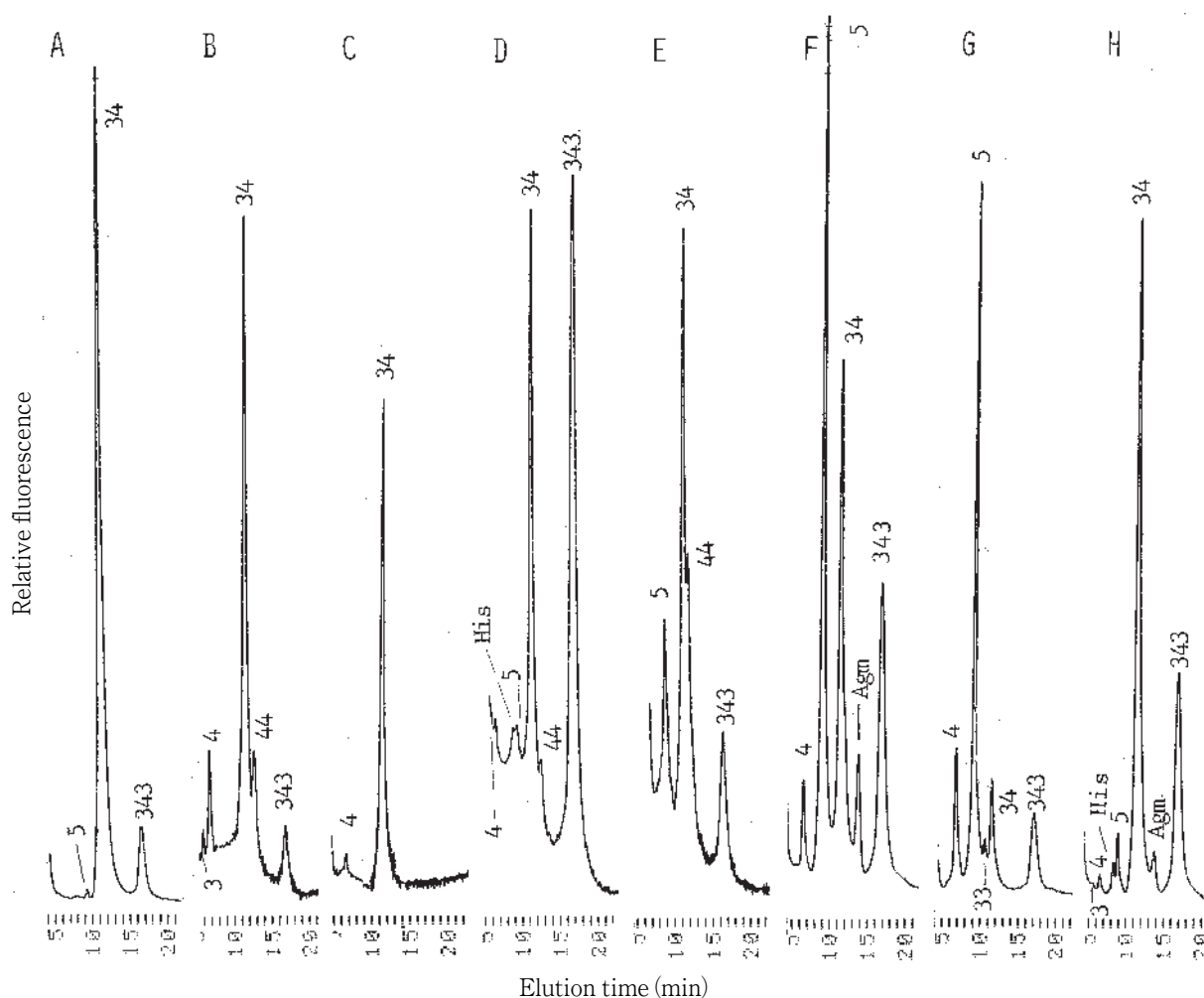


Fig. 1. HPLC analysis of cellular polyamines of the nematodes *Trichinella spiralis* (A), *Strongyloides venezuelensis* (B) and *Bursaphelenchus xylophilus* (C), and the cestodes *Hymenolepis diminuta* (D) and *Diphyllobothrium nihonkaiense* (E), and the annelids *Perinereis brevicirris* (F), *Marphysa sanguinea* (G) and *Lumbrineris japonica* (H). Abbreviations for polyamines: 3, diaminopropane (Dap); 4, putrescine (Put); 5, cadaverine (Cad); 33, norspermidine (NSpd); 34, spermidine (Spd); 44, homospermidine (HSpd); 333, norspermine (NSpm); 343, spermine (Spm); His, histamine; Agm, agmatine.

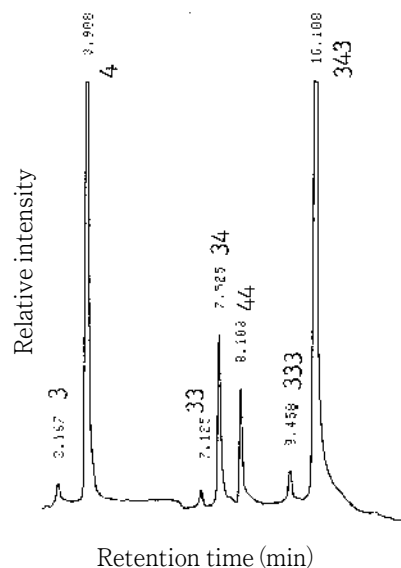


Fig. 2. GC analysis of cellular polyamines of the planarian *Bipalium mobile* after the purification of the polyamine fraction by two ion-exchange chromatographies. Agmatine was destroyed during GC. Abbreviations for polyamines are as in Fig. 1.

within the analyzed platyhelminthes, and a leech and a sandworm within the analyzed annelids (Fig. 1 and Fig. 2, and Table 1). An unusual tetra-amine, norspermine, were detected in the three land planarians. This is the first report on the occurrence of norspermidine and norspermine within lower invertebrates. Distributions of these unusual polyamines serve for a consideration to their evolutionary strategy on polyamine syntheses within lower invertebrates.

Within the phylum Cnidaria, two hydras were available and analyzed in the present study. *Hydra vulgaris* contained putrescine and spermidine. *Chlorohydra viridisima* contained cadaverine, norspermidine and spermine in addition to putrescine and spermidine. The symbiosis of green algae in this green hydra, *C. viridisima*, has been known. It is suggested that cadaverine, norspermidine and spermine found in the green hydra derived from the symbiotic green algae containing these polyamines.

Acknowledgements

We would like to thank Drs M. Sato of Saitama Medical School, Y. Shishida of Agricultural Station of Gunma Prefecture, K. Matsuura and I. Okochi of Forestry and Forest Prod. Res. Institute, T. Ono of Ogasawara Subtropical Agricultural Center, W.

Yoshida of Hirosaki University, M. Myohara of National Institute of Agrobiological Sciences, and H. Tanabe of SDS Biotech Co., for supplying organisms.

REFERENCES

- 1) Hamana K, Matsuzaki S, Inoue K. Changes in polyamine levels in various organs of *Bombyx mori* during its life cycle. *J Biochem* 1984; 95: 1803-1809.
- 2) Hamana K, Suzuki M, Wakabayashi T, Matsuzaki S. Polyamine levels in the gonads, sperm and salivary gland of cricket, cockroach, fly and midge. *Comp Biochem Physiol* 1989; 92B: 691-695.
- 3) Hamana K, Niitsu M, Samejima K, Matsuzaki S. Novel tetraamines, pentaamines and hexaamines in sea urchin, sea cucumber, sea squirt and bivalves. *Comp Biochem Physiol* 1991; 100B: 59-62.
- 4) Hamana K, Niitsu M, Samejima K, Matsuzaki S. Novel polyamines in insects and spiders. *Comp Biochem Physiol* 1991; 100B: 399-402.
- 5) Hamana K, Hamana H, Shinozawa T. Alterations in polyamine levels of nematode, earthworm, leech and planarian during regeneration, temperature and osmotic stresses. *Comp Biochem Physiol* 1995; 111B: 91-97.
- 6) Hamana K, Hamana H, Shinozawa T. Polyamines of planarians and leeches: The presence of

- homospermidine. *Ann Gunma. Health Sci* 1997; 18: 7-9.
- 7) Sharma V, Visen PKS, Katiyar JC, Wittich R-M, Walter RD, Ghatak S, Shukla OP. Polyamine metabolism in *Ancylostoma ceylanicum* and *Nippostrongylus brasiliensis*. *Int J Parasitol* 1989; 19: 191-198.
 - 8) Sharma V, Tekwani BL, Saxena JK, Gupta S, Katiyar JC, Chatterjee RK, Ghatak S, Shukla OP. Polyamine metabolism in some helminth parasites. *Exp Parasitol* 1991; 72: 15-23.
 - 9) Singh RP, Saxena JK, Ghatak S, Shukla OP, Wittich R-M, Walter RD. Polyamine metabolism in *Setaria cervi*, the bovine filarial worm. *Parasitol Res* 1989; 75: 311-315.
 - 10) Srivastava DK, Roy TK, Shukla OP. Polyamines of helminths. *Indian J Parasitol* 1980; 4: 187-189.
 - 11) Tekwani BL, Mishra M, Chatterjee RK. Polyamine uptake by a rodent filariid, *Acanthocheilonema viteae* (Nematoda: Filarioidea). *Int J Biochem Cell Biol* 1995; 27: 851-855.
 - 12) Walter RD. Polyamine metabolism of filaria and allied parasites. *Parasitol Today* 1988; 4: 18-20.
 - 13) Wittich R-M, Kilian H-D, Walter RD. Polyamine metabolism in filarial worms. *Mol Biochem Parasitol* 1987; 24: 155-162.
 - 14) Blaxter ML, De Ley P, Garey JR, Liu LX, Scheldeman P, Vierstraete A, Vanfleteren JR, Mackey LY, Dorris M, Frisse LM, Vida JT, Thomas WK. A molecular evolutionary framework for the phylum Nematoda. *Nature* 1998; 392: 71-75.
 - 15) Miyaji S, Katakura K, Matsufuji S, Murakami Y, Hayashi S, Takami H, Oku Y, Okamoto M, Kamiya M. Polyamine metabolism in taeniid metacestodes. *Jap J Parasitol* 1992; 41: 327-333.
 - 16) Miyaji S, Katakura K, Matsufuji S, Murakami Y, Hayashi S, Oku Y, Okamoto M, Kamiya M. Failure of treatment with alpha-difluoromethylornithine against secondary multilocular echinococcosis in mice. *Parasitol Res* 1993; 79: 75-76.
 - 17) Nishina M, Watanuki T, Kato K. Appetite loss induced by the infection with a parasitic nematode, *Trichinella spiralis*. *J Phys Fit Nutr Immunol* 2001; 11: 3-5.
 - 18) Ishida S, Yoshida W. Data Base of Planarians in Japan. HIROIN (Hiroshima University) 2002; 19: 45-50.
 - 19) Kawakatsu M, Okochi I, Sato H, Ohbayashi T, Kitagawa K, Totani K. A preliminary report on land planarians (Turbellaria, Seriata, Tricladida, Terricola) and land nemertine (Enopla, Hoplonemertea, Monostylifera) from the Ogasawara Islands. *Occ Publ Biol Lab Fuji Woomen's College, Sapporo* 1999; 32: 1-8.
 - 20) Myohara M, Yoshida-Noro C, Kobari F, Tochinai S. Fragmenting oligochaete *Enchytraeus japonensis*: A new material for regeneration study. *Dev Growth Differ* 1999; 41: 549-555.
 - 21) Hamana K, Otsuka E, Eguchi F, Niitsu M. Occurrence of homospermidine and canavalmine as a major polyamine in mushrooms. *Mushroom Sci Biotech* 2005; 13: 95-102.
 - 22) Schaeffer JM, Donatelli MR. Characterization of a high-affinity membrane-associated ornithine decarboxylase from the free-living nematode *Caenorhabditis elegans*. *Biochem J* 1990; 270: 599-604
 - 23) Macrae M, Kramer DL, Coffino P. Developmental effect of polyamine depletion in *Caenorhabditis elegans*. *Biochem J* 1998; 333: 309-315.
 - 24) Martin P, Kaygorodova I, Sherbakov DY, Verheyen E. Rapidly evolving lineages impede the resolution of phylogenetic relationships among Clitellata (Annelida). *Mol Physiol Evol* 2000; 15: 355-368.