POLYAMINE DISTRIBUTION IN EUKARYOTES: OCCURRENCE OF SYM-NOR-SPERMIDINE AND SYM-NOR-SPERMINE IN ARTHROPODS

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Received 3 August 1978

1. Introduction

New polyamines, sym-nor-spermidine (1,7-diamino-4-azaheptane) and sym-nor-spermine (1,11-diamino-4,8-diazaundecane) have been reported in thermophilic bacteria [1,2] besides the well-known polyamines, spermidine and spermine. The proposed role(s) of the new polyamines in thermophiles has been related to the stabilization of the double helical arrangements of DNA at elevated temperatures [3]. Further, it has been reported that sym-nor-spermine significantly increases the melting temperature of calf thymus DNA [4].

More recently the two new polyamines have also been found in the multicellular marine organism, *Penaeus setiferus* [5]. In order to investigate polyamine distribution among the evolutive phyla, two analytical methods have been developed.

2. Materials and methods

2.1. *Chemicals*

Putrescine, cadaverine, spermidine, spermine, 1,7-diaminoheptane, 1,7-diamino-4-azaheptane and 1,11-diamino-4,8-diazaundecane were purchased from Eastman Organic Chemicals, Rochester, NY; [1,4-^{14}C]spermidine from NEN Chemicals; Dowex 50 W resin from Bio Rad Labs. Pyrex glass beads 100–120 mesh and Carbowax 20-M from Carlo Erba, Milano, resin cromo 'beads' type A from Technicon, NY. All other chemicals were the purest available grades from standard commercial sources.

2.2. *Biological sources*

All marine organisms, kindly supplied by the Zoological Station of Naples, were collected in the bay of Naples. *Saccharomyces cerevisiae* type I was furnished as dried yeast by Sigma Chemical Co., St Louis, MO.

Unless otherwise stated (see table 1), the analyses were performed on intact organisms.

2.3. *Polyamine extraction*

Freshly excised organs and tissues (about 30 g) were homogenized in 4 vol. (v/w) 1 N $HClO_4$ with a Waring Blendor. Each supernatant obtained after centrifugation was directly chromatographed on Dowex 50 (H^+ form) column (12 × 2 cm) pre-equilibrated with 1 N HCl. Basic amino acids and other tissue components were eluted with 500 ml 2 N HCl, while polyamines were eluted with 100 ml 6 N HCl. After evaporation of the strongly acidic eluates under reduced pressure, the dry residues were dissolved in 1.5 ml distilled water and submitted to quantitative analysis. In order to calculate the recovery during the extraction procedure, [1,4-^{14}C]spermidine was added to the mixture as internal standard before homogenization. The obtained recovery rates were of 75–80%.

2.4. *Automated ion-exchange chromatography*

Aliquots (50–300 μl) of the samples were applied on Technicon cromo 'beads' type A columns, pre-equilibrated with 0.1 M sodium citrate buffer, pH 6.1, containing 1 M NaCl (Technicon Auto-analyzer). The columns (90 × 6 mm) were washed for 10 min with the equilibrating buffer. The elu-

tion was then automatically switched to a 7-vessel exponential gradient (see 'Technicon Instruction Manual' T-69-129, Aug. 1969) at 60°C. The 7 flasks were connected in series. Flasks 1 and 2 contained 40 ml equilibration buffer; other flasks contained 100 ml 0.1 M sodium citrate buffer, pH 6.1, containing 3.5 M NaCl. The buffer flow was 31 ml/h and the ninhydrin flow was 17 ml/h. Following each analysis the column was regenerated with a solution of 0.2 M NaOH and then equilibrated.

Calibration curves of a standard solution containing 100 nmol of each polyamine in 50 μl 0.1 N HCl are given in fig.1.

2.5. *Gas-liquid chromatography (GLC)*

The polyamines (500 μl sample) were extracted with 250 μl *n*-butanol made strongly basic with solid NaOH. The recovery rate, calculated using 1,7-diaminoheptane as internal standard, was about 90%.

The analysis was performed using a Varian 3700 gas chromatograph with hydrogen flame ionization detector. The pyrex glass column (1.8 m × 3 mm i.d.) was packed with pyrex glass beads 100–120 mesh coated with 1% KOH and 0.4% Carbowax 20-M [6]. The nitrogen flow rate was 20 ml/min; detector and injector temperature was 230°C and temperature program was 10°C/min, from 80–210°C, with 5 min initial isotherme. The molar response, relative to an arbitrary value of 1.00 assigned to 1,7-diaminoheptane, were:

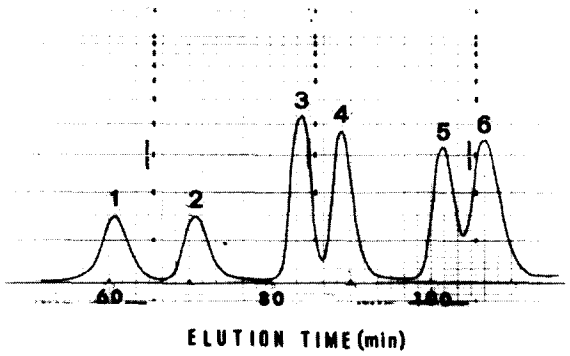

Fig.1. Elution profile of polyamines separated by automated ion-exchange chromatography. (1) Putrescine; (2) cadaverine; (3) sym-nor-spermidine; (4) spermidine; (5) sym-nor-spermine; (6) spermine.

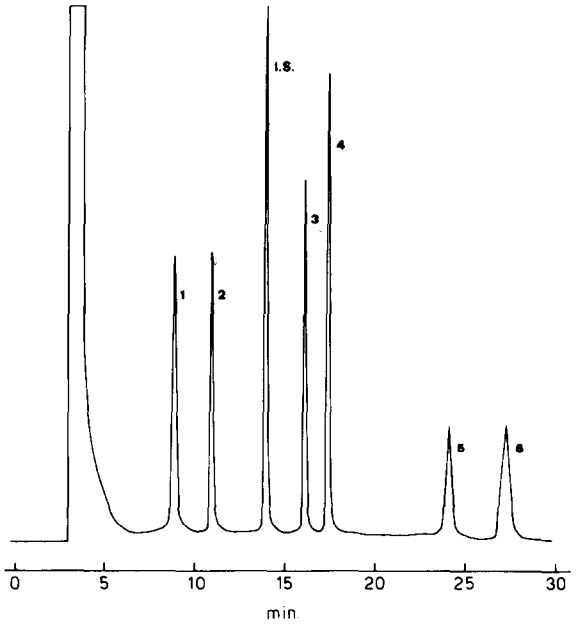

Fig.2. Elution profile of polyamines separated by GLC. (1) Putrescine; (2) cadaverine; (3) sym-nor-spermidine; (4) spermidine; (5) sym-nor-spermine; (6) spermine; (I.S.) internal standard (1,7-diaminoheptane).

0.50, 0.68, 0.68, 0.88, 0.39, 0.56, respectively, for putrescine, cadaverine, sym-nor-spermidine, spermidine, sym-nor-spermine, spermine. A typical gas chromatogram of a standard mixture of free-base polyamines (12.5 μg each) is shown in fig.2.

3. Results and discussion

Despite reports indicating the presence of novel polyamines in biological samples [1,2,5,7], no systematic study has been performed on polyamine pattern and distribution along the evolutive phyla. The reported elution schedule of automated ion–exchange chromatography is particularly useful in this respect, since it permits a rapid quantification of these molecules in biological samples by a single analysis. The method was integrated with a newly developed GLC procedure [6], which gave superimposable results.

The polyamine content in several species representative of various phyla is reported in table 1. The analyses have been performed with both mentioned

Table 1
Polyamine pattern in evolutive phyla

Organism	Sym-nor-spermidine (nmol/g wet wt)	Spermidine	Sym-nor-spermine	Spermine
Phylum: Fungi				
Class: Ascomycetes				
Saccharomyces cerevisiae	n.d.	366	n.d.	380
Phylum: Porifera				
Class: Demospongiae				
Axinella verrucosa	n.d.	n.d.	n.d.	14
Phylum: Cnidaria				
Class: Anthozoa				
Anemonia sulcata	n.d.	5	7	69
Phylum: Ctenophora				
Class: Nuda				
Beroe ovata	n.d.	2	n.d.	31
Phylum: Mollusca				
Class: Gastropoda				
Patella coerulea[a]	10	27	n.d.	592
Class: Bivalvia				
Mytilis galloprovincialis	12	132	n.d.	246
Pinna nobilis[a]	5	47	9	206
Class: Cephalopoda				
Octopus vulgaris[b]	n.d.	19	118	559
Phylum: Anellida				
Class: Clitellata				
Lumbricus terrestris	n.d.	250	n.d.	9
Phylum: Arthropoda				
Class: Crustacea				
Carcinus mediterraneus	6	5	75	20
Eriphia spinifrons[c]	n.d.	35	331	256
Squilla mantis	n.d.	13	235	22
Penaeus kerathurus	21	28	104	n.d.
Class: Insecta				
Tenebrio molitor[d]	n.d.	281	205	160
Ceratidis capitata[e]	n.d.	786	195	29
Phylum: Echinodermata				
Class: Stelleroidea				
Marthasterias glacialis	4	45	n.d.	75
Class: Echinoidea				
Paracentratus lividus[f]	n.d.	15	n.d.	389
Class: Holothuroidea				
Holothuria tubulosa	2	1	8	19

Table 1 (continued)

Organism	Sym-nor-spermidine (nmol/g wet wt)	Spermidine	Sym-nor-spermine	Spermine
Phylum: Tunicata				
Class: Ascidiacea				
Ciona intestinalis	n.d.	6	n.d.	239
Class: Thaliacea				
Salpa maxima	4	n.d.	n.d.	8
Phylum: Vertebrata				
Class: Elasmobranca				
Scyliorhinus canicula[g]	n.d.	81	n.d.	130
Class: Teleostoma				
Lophius piscatorius[g]	n.d.	449	n.d.	236
Class: Reptilia				
Pseudoemys cripta elegans	n.d.	89	n.d.	15
Class: Aves				
Gallus gallus[g]	n.d.	32	n.d.	605
Class: Mammalia				
Equus caballus[g]	n.d.	104	n.d.	1964

[a] without shell
[b] hepatopancreas
[c] without esoskeleton
[d] larvae
[e] pupae
[f] ovary
[g] liver

n.d., not detectable (below 0.5 nmol/g)

Each figure represents the mean value of two separate experiments performed in duplicate with the two procedures described in the text

procedures. Spermidine and spermine are distributed ubiquitously in all the species investigated and the relative concentrations are rather fluctuating, horse liver representing the tissue with the highest content in spermine.

Cadaverine has not been detected in any of the species surveyed while putrescine is present in rather large amounts only in the *Beroe ovata* (260 nmol/g) and in the shrimp *Penaeus kerathurus* (61 nmol/g). The concentrations of spermine, spermidine and putrescine in marin invertebrates are significantly lower if compared to the data in [8].

Sym-nor-spermine and sym-nor-spermidine, reported only in thermophilic microorganisms [1,2] and in the white shrimp *Penaeus setiferus* [5], are present in the arthropods and in some mollusks. The occurrence of the new symmetrical polyamines in arthropods is indicative for a biosynthetic pathway similar to that proposed for *Caldariella acidophila* [1], whereas in mollusks it could be related to their alimentary habits.

It is difficult to recognize any well-defined phylogenetic significance related to the occurrence of the new polyamines in phyla which are in the middle of the evolutionary scale; their distribution could be probably related to the ecology of the organisms. These results together with the data indicating a stabilizing effect of sym-nor-spermine on calf thymus DNA [4], open new questions on the physiological roles of symmetrical polyamines.

Acknowledgments

The authors wish to thank Dr Annamaria De Paris, Mr Enrico Esposito for the technical assistance and Mr Antonio Crispino for GLC analysis.

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