

# The Determination of the DNA Base Composition in 19 Species of Adriatic Sponges with High-Pressure Liquid Cation-Exchange Chromatography

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The (adenine+thymine)/(guanine+cytosine) base ratios of 19 species of adriatic sponges have been determined by high-pressure liquid cation-exchange chromatography. The base ratios vary from 1.49 (*Mycale massa*) to 0.63 (*Hippospongia communis*) according to an (A+T) content of 59.7 and 38.6 mol%, respectively.

The DNAs of sponges of the order *Keratosa* showed marked differences in their (A+T) contents (39.5 to 58.8 mol%) whereas those of *Tetractinellida* and *Halichondrina* were nearly identical (39.3 to 40.8 and 49.5 to 49.8 mol%, respectively).

The 5-methylcytosine (5MC) content was determined in 8 sponge DNAs by a semiquantitative method. The values differed from 0.8 to 2.2 mol% of 5MC.

## Introduction

In the course of characterization of the DNAs of lower marine organisms, especially sponges, their base ratios had to be determined. For this purpose we established a highly sensitive method for the determination of the four major deoxyribonucleosides and of deoxyinosine which might in trace amounts occur as a deamination product of dAdo and for 5-methyldeoxycytidine<sup>1, 2</sup>.

The base ratio of a DNA can be a helpful parameter for taxonomic studies as has been shown for many microorganisms<sup>3</sup>.

As a new species of sponges, *Tethya limski*, and one possibly new species, *Geodia limski*, had been discovered in our laboratory at Rovinj<sup>4</sup> it proved to be of some interest whether their DNAs showed similarities in the base composition as compared to other members of their orders, *Hadromerina* and *Tetractinellida*, respectively.

The knowledge of the base ratio of a DNA sample is an important feature, too, if the DNA has to be used in reassociation experiments<sup>5</sup> as the kinetics of reassociation depend on the GC-content.

Furthermore, pollution of the environment may cause severe changes on the ability of a cell to syn-

thesize nucleic acids. As, for example in sponges, there are many types of RNA which differ much from each other in chemical and physico-chemical respects as well as in isolation behaviour this heterogeneous class of the nucleic acids may be used as a measure for the severity of pollution. This means that the total amount of RNA which is found in a cell or an organism needs not strictly be related to the biomass, in contrary to the DNA or thymine amount which becomes not changed by intermittent or even severe pollution<sup>6</sup>. If the thymine amount of a sample is determined at a base ratio of 1 it represents about 10% of the total DNA contained in the sample. At base ratios different from 1 the base ratio must therefore be taken in account in order to calculate correct DNA amounts.

As all DNA samples which were used in this study in the final step of purification were centrifuged through a CsCl density gradient appreciable contamination with bacterial DNA was unlikely, except for the case of similarity in base composition.

## Materials and Methods

A Varian LCS 1000 liquid chromatograph was used for this study equipped with a 254 nm UV-flow cell detector. Stainless steel columns were 1.8 mm I.D. and 40 cm or 60 cm in length, respectively.

Deoxyribonuclease I (EC 3.1.4.5) (1,000 units/ml) and snake venom phosphodiesterase (EC

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3.1.4.1) (100 units/ml) were obtained from Worthington, Freehold N.J. (USA), alkaline phosphatase from calf intestine (highly purified from adenosine deaminase) (EC 3.1.3.1) (1 mg/ml) from Boehringer, Mannheim (West Germany).

The major deoxyribonucleosides (dAdo, dCyd, dGuo, dThd) and deoxyinosine (dIno) were purchased from Papierwerke Waldhof-Aschaffenburg, Mannheim (West Germany), 5-methyldeoxycytidine (5MedCyd) from P·L-Biochemicals, Milwaukee, WI (USA).

CsCl, 25% ammonia, and 96% formic acid were obtained from Merck, Darmstadt (West Germany). All chemicals used were of analytical grade.

Strongly acidic cation exchange resin, type M71, particle diameter 10–12  $\mu\text{m}$ , was from Beckman, München (West Germany).

Sponges were harvested in the adriatic sea near Rovinj (Yugoslavia) and kept for a day in well aerated tanks to decay feeder organisms.

Crude preparations were taken to Germany and the DNA was prepared according to Hönig *et al.*<sup>7</sup> and Hönig and Zahn<sup>8</sup>.

The DNAs were purified from contaminating RNA and protein as well as from denatured DNA by isopycnic CsCl-centrifugation in a Beckman L 265 ultracentrifuge with a type 65 rotor according to Meselson *et al.*<sup>9</sup>.

20 to 80  $\mu\text{g}$  of DNA which were dissolved in 100  $\mu\text{l}$  of water were digested with 50  $\mu\text{l}$  of 50 mM Tris-HCl, pH 8.0, 20  $\mu\text{l}$  of 0.07 M  $\text{MgCl}_2$  solution, 50  $\mu\text{l}$  of deoxyribonuclease I, and 50  $\mu\text{l}$  of snake venom phosphodiesterase at 37 °C for 12 hours.

Then 10  $\mu\text{l}$  of 0.001 N NaOH solution and 20  $\mu\text{l}$  of alkaline phosphatase were added and the mixture incubated at 37 °C for 6 hours. Afterwards, the digests were frozen to –30 °C until use.

The stainless steel tubes were filled with Beckman M71 strongly acidic cation-exchange resin according to the method of Scott and Lee<sup>10</sup>. The columns were eluted with 0.4 M (with respect to the  $\text{NH}_4^+$ -concentration) ammonium formate, pH 4.6, at a column oven temperature of 55 °C and at a flow rate of 13.3  $\text{ml}\cdot\text{h}^{-1}$  and 16.0  $\text{ml}\cdot\text{h}^{-1}$  (flow velocity 8.7  $\text{cm}\cdot\text{min}^{-1}$  and 10.5  $\text{cm}\cdot\text{min}^{-1}$ ) on the 40 cm and 60 cm column, respectively.

Using a 60 cm column for the determination of the 5MC content of the DNAs instead of a 100 cm column as described elsewhere<sup>2</sup> restricted the determination of 5MC to an amount of more than 0.8 mol%.

## Results and Discussion

The base ratio determinations were carried out on the 40 cm column. On this column no separation

was achieved between the two cytosine compounds, deoxycytidine and 5-methyldeoxycytidine. The small amounts of 5MedCyd, however, which were contained in the small volumes of the samples required for the base ratio determinations did not cause inaccuracy to the ratios.

The 60 cm column gave satisfying separations between dCyd and 5MedCyd. To determine, however, the 5MedCyd fraction quantitatively the injected volumes of samples had to be raised by a factor of 10 to 20. So the elution peaks of the major deoxyribonucleosides except dCyd, mostly, showed full scale deflection on the recorder chart.

Figs 1 and 2 show the elution patterns obtained from the 40 cm column and the 60 cm column after

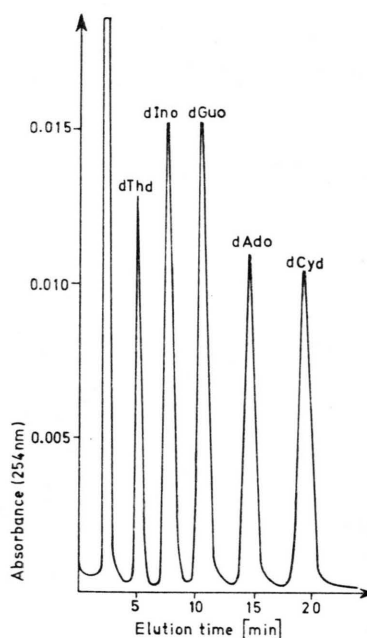


Fig. 1. Elution pattern of the major deoxyribonucleosides and of deoxyinosine as obtained from the 40 cm column (separation conditions as described under Materials and Methods).

the injection of the four major deoxyribonucleosides and of deoxyinosine and of these five compounds and of 5-methyldeoxycytidine, respectively.

Figs 3 and 4 show the elution patterns of a hydrolysate of *Mycale massa* DNA on either the 40 cm or the 60 cm column. The base ratio was hereby determined as 1.49, the 5MC content was found to be 1.9 mol%.

Table I shows the values of the base ratios and of the 5MC content for the other sponges that were ex-

Table I. Classification \* of the examined sponges, the distribution of their purine and pyrimidine bases and the 5 MC content (the values in mol%) and the calculated base ratios.

Class	Order	Family	Genus	Species	Distribution of					Pu Py	Base Ratio
					Purine Bases		Pyrimidine Bases		5 MC		
					Adenine	Guanine	Cytosine	Thymine			
Calcispongiae	Homocoela	—	<i>Clathrina</i>	<i>coreacea</i> (Mont.)	27.9	21.9	20.8	28.0	1.5	1.01	1.26
Demospongiae	Homosclerophorida	Chondrosiidae	<i>Chondrosia</i>	<i>reniformis</i> (Nardo)	23.2	26.7	26.0	23.3	0.8	0.98	0.87
	Tetractinellida	Geodiidae	<i>Geodia</i>	<i>cydonium</i> (Jameson)	19.7 **	29.8 **	30.8 **	19.6 **	n.d. ***	0.98	0.65
			<i>Geodia</i>	<i>limski</i>	20.1 **	29.6 **	30.2 **	20.0 **	n.d.	0.99	0.67
			<i>Ancorina</i>	<i>cerebrum</i> (Schmidt)	20.5	29.7	29.5	20.3	n.d.	1.01	0.69
	Hadromerina	Tethyidae	<i>Tethya</i>	<i>limski</i>	26.0	24.0	22.5	26.2	1.2	1.00	1.09
			<i>Tethya</i>	<i>aurantium</i> (Plall.)	27.1	23.2	23.0	26.7	n.d.	1.01	1.16
			<i>Suberites</i>	<i>domuncula</i> (Olivi)	22.7	27.4	27.2	22.4	n.d.	1.01	0.83
	Halichondrina	Axinellidae	<i>Axinella</i>	<i>polypoides</i> (Schmidt)	25.1	24.9	25.5	24.4	n.d.	1.00	0.98
			<i>Hemimycale</i>	<i>columella</i> (Bowerb.)	24.9	25.0	25.2	24.9	n.d.	1.00	0.99
			<i>Mycale</i>	<i>massa</i> (Schmidt)	30.2	20.0	18.2	29.5	1.9	1.01	1.49
	Poecilosclerina	Mycalidae	<i>Mycale</i>	<i>tunicata</i> (Schmidt)	28.2	21.8	20.2	28.2	1.4	1.00	1.30
			<i>Crellida</i>	<i>rosea</i> (Topsent)	24.3	25.9	23.8	23.8	2.2	1.01	0.93
			Haplosclerina	Haploscleridae	<i>Pellina</i>	<i>semitubulosa</i> (Lieberkühn)	22.4	27.2	27.4	23.0	n.d.
	<i>Petrosia</i>	<i>ficiiformis</i> (Poiret)			20.8	29.6	29.4	20.3	n.d.	1.01	0.70
	<i>Dysidea</i>	<i>avara</i> (Schmidt)			29.4	20.7	18.6	29.4	1.6	1.01	1.44
	Keratosia	Spongiidae	<i>Hippospongia</i>	<i>communis</i> (Lam.)	19.3	30.5	30.9	19.2	n.d.	0.99	0.63
			<i>Cacospongia</i>	<i>scalaris</i> (Schmidt)	25.4	24.5	23.7	25.5	0.8	1.00	1.04
			<i>Verongia</i>	<i>aerophoba</i> (Schmidt)	20.0	29.6	29.9	20.4	n.d.	0.99	0.68

\* For classification see reference <sup>13</sup>. \*\* These values were calculated from 7 determinations, all other values from 4 determinations. \*\*\* n.d., Not detected.

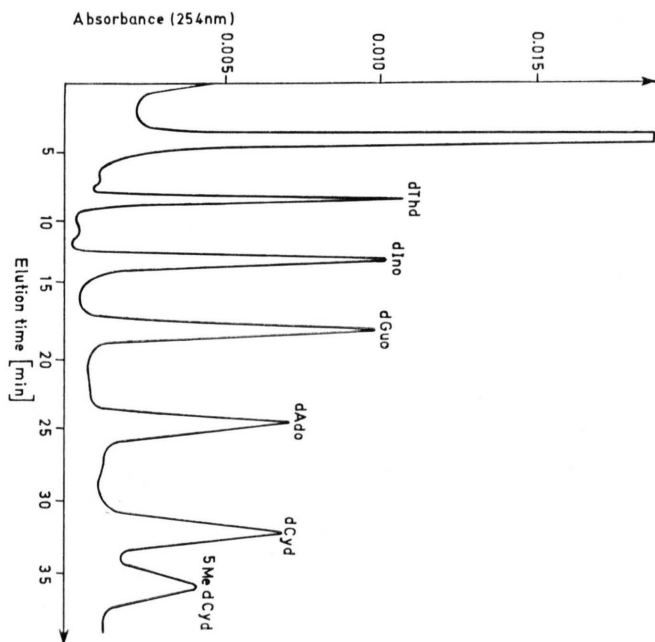


Fig. 2. Elution pattern of the major deoxyribonucleosides and of 5-methyl-2'-deoxycytidine as obtained from the 60 cm column (separation conditions as described under Materials and Methods).

aminated. As can be seen the base ratios differ in a wide range from 1.49 to 0.63, which means an (A+T) content of 59.7 and 38.6 mol%, respectively.

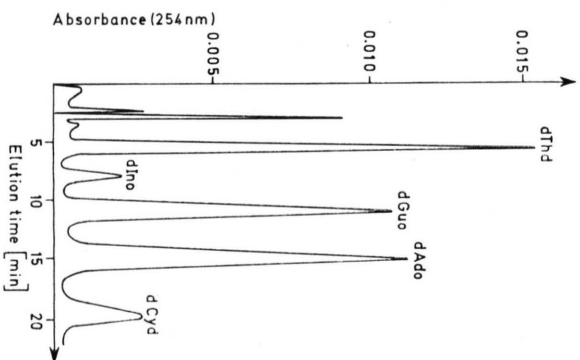


Fig. 3. Elution pattern of 10 µl of a *Mycale massa* DNA hydrolysate as obtained from the 40 cm column (separation conditions as described under Materials and Methods).

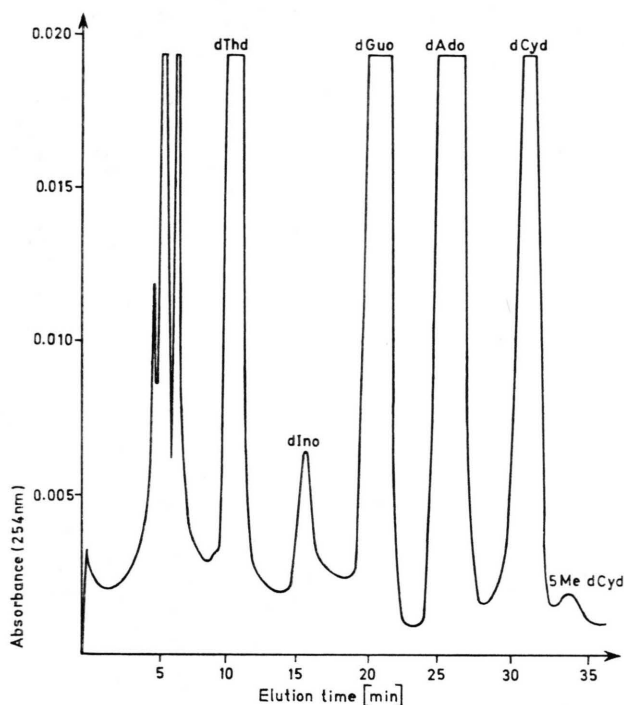


Fig. 4. Elution pattern of 40  $\mu$ l of a *Mycale massa* DNA hydrolysate as obtained from the 60 cm column (separation conditions as described under Materials and Methods).

The sponges of the orders *Tetractinellida* and *Halichondrina* which had been examined showed

nearly identical base ratios, those of the order *Keratosa* exhibited markedly different values.

Furthermore, the DNA of the sponges of the order *Tetractinellida*, *Halichondrina* and *Haplosclerina* showed no detectable 5MC content (less than 0.8 mol%).

A very interesting finding was that in the order *Hadromerina* only *Tethya limsky* contained detectable amounts of 5MC whereas the sponges *Tethya lyncurium* and *Suberites domuncula* showed no detectable amounts of this rare base. Vanyushin *et al.*<sup>11</sup> found 1.3 mol% of 5MC in *Suberites domuncula*. As the (A + T) content of 61.5 mol% which was described by these authors differs markedly from the value obtained in our experiments (45.1 mol%) these differences may be due to species diversity of the animals used.

The order *Poecilosclerina* contains relatively high amounts of 5MC.

The order *Keratosa* shows a 5MC content less than 0.8 mol% if the (A + T) content is low whereas the species with high (A + T) content contain 1.6 and 0.8 mol%, *Dissidea avara* and *Cacospongia scalaris*, respectively.

As we had been interested in the question whether the sponges *Geodia limski* and *Geodia cydonium* might possess markedly different base ratios we thoroughly investigated the composition of their DNAs. No significant difference, however, was found<sup>12</sup>.

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