COMPOSITION OF THE DESOXYPENTOSE NUCLEIC ACIDS OF FOUR GENERA OF SEA-URCHIN*

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The present paper continues the attempts of this laboratory to gain an insight into the differences and the regularities exhibited by highly polymerized desoxypentose nucleic acids (DNA) of different cellular origin. The problems investigated, the results achieved, and the provisional conclusions reached have been reviewed (1–3). In its analytical techniques, the present study follows closely one published recently, dealing with the DNA of salmon sperm (4), and reference should be made to this publication for the details.

A comparative study of the composition of DNA from four different genera of the same family, as submitted here, is not without some interest. It will permit one to evaluate the contribution that the quantitative determination of the nitrogenous constituents of DNA can make toward the distinction between DNA specimens from closely related sources; it will furnish material for a future decision on the relationship between chemical composition and biological function of nucleic acids. The availability of a variety of sperm preparations from sea-urchins made the study of their DNA particularly attractive, since the echinoids have formed the subject of many comprehensive investigations of the mechanisms of fertilization and development. (For recent surveys, see Tyler (5) and Runnström (6).) Spermatozoa are particularly suitable as the source of DNA, since the laborious removal of contaminating pentose nucleic acid is not necessary in this case.

The species investigated were *Psammechinus miliaris*, *Paracentrotus lividus*, *Echinocardium cordatum*, and *Arbacia lixula*. With the exception of the first species, at least two sperm preparations from the same species were available, collected at different times and sometimes in different lo-

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calities, so that two or more independently prepared DNA specimens could be compared.

All preparations of sea-urchin DNA examined had several features in common. Their sugar components were indistinguishable chromatographically from 2-desoxyribose, released by identical procedures from calf thymus DNA. They had similar absorption characteristics in the ultraviolet. They all belonged to the "AT type" of DNA (3, 7), representing rather extreme varieties of this class; in three of the genera, the molar concentrations of adenine and of thymine surpassed those of guanine and cytosine by about 85 per cent. In *Arbacia* DNA the corresponding excess amounted to 60 per cent.[1] The differences in the composition of the DNA preparations from *Echinocardium*, *Paracentrotus*, and *Psammechinus* were not sufficiently large to permit a distinction between these sources; but *Arbacia* DNA was different. When the molar ratios of adenine to guanine (A:G) and of thymine to cytosine (T:C) were computed individually for each hydrolysis experiment, the following mean ratios and standard errors were found: in *Arbacia lixula*, A:G 1.62 ± 0.01, T:C 1.58 ± 0.03; in *Echinocardium cordatum*, A:G 1.88 ± 0.03, T:C 1.79 ± 0.03; in *Paracentrotus lividus*, A:G 1.84 ± 0.03, T:C 1.86 ± 0.01; in *Psammechinus miliaris*, A:G 1.78 ± 0.02, T:C 1.80 ± 0.03. The statistical interpretation shows the differences between *Arbacia* DNA and the other three DNA varieties to be highly significant.

Other regularities stressed in previous publications (2–4), namely the equality in the molar concentrations of adenine and thymine, of guanine and cytosine, and of purines and pyrimidines, were exhibited clearly by all preparations. One more uniformity should be added; the ratio of total amino groups to total enolic hydroxyls was remarkably constant, *viz.* 1.4 in all sea-urchin DNA preparations studied here. (See the discussion in a recent survey (3).) In one preparation from *Paracentrotus* a search for the presence of 5-methylcytosine was made; traces of this pyrimidine, corresponding to 6.6 per cent of the cytosine content, were found. Different DNA preparations from the sperm of the same species yielded analytical results that agreed closely, even if the methods of their isolation varied considerably.

EXPERIMENTAL

Material

All sperm preparations, after collection, were freshly dried in the frozen state in a vacuum and stored in sealed tubes at a low temperature. The

[1] A similar composition has recently been found by Wyatt (8) for the DNA of *Echinus esculentus*. The purine and pyrimidine composition of total *Arbacia* sperm has also been reported (9).

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preparations from *Paracentrotus lividus* had been collected in Roscoff, France, and in Naples respectively; the corresponding DNA specimens isolated from them are designated as Preparations Pa-1 and Pa-2. Two preparations of sperm of *Echinocardium cordatum* were gathered on the west coast of Sweden; the DNA specimens are listed as Preparations E-1 and E-2. *Psammechinus miliaris* sperm came from Roscoff; its DNA is designated as Ps. Two sperm preparations from *Arbacia lixula* were obtained, at different times, in Naples (DNA Preparations A-1 and A-2).

Preparation

The isolation of the DNA Preparation A-1 from *Arbacia* sperm typifies the procedures applied in the majority of experiments.[2] The sperm powder (7.47 gm.) was washed thoroughly with 75 cc. of the 0.146 M salt solution described previously ((4), foot-note 2), and the suspension was centrifuged at 1900 $\times$ g. The washings were free of material reacting with diphenylamine and were discarded.[3] The sediment was distributed in 75 cc. of 3 M aqueous sodium chloride for 3 minutes in a high speed mixer and the mixture kept overnight. It was diluted by the addition of the same volume of M aqueous NaCl and centrifuged at 17,000 $\times$ g for 1 hour. The sediment was reextracted 5 times with 100 cc. portions of 3 M NaCl. The combined extracts were clarified once more by centrifugation at 17,000 $\times$ g. The fibers produced by the addition of 2 volumes of 95 per cent ethanol were collected by spooling, squeezed, and washed with 75 and 100 per cent ethanol. The fibers were distributed in 60 cc. of 10 per cent aqueous NaCl and freed of protein by vigorous agitation in a high speed mixer with 30 cc. of chloroform-octanol (9:1). Six to eight such treatments, each for 3 to 5 minutes, followed by centrifugation, were required.[4] The injection of the resulting solution, clarified by centrifugation at 17,000 $\times$ g for 1 hour, into 2 volumes of ethanol (cooled to $-10°$) produced white threads that were lifted, drained, washed with alcohol, and dissolved in 50 cc. of 0.14 M NaCl solution. The DNA, recovered from the frozen solution by evaporation in a vacuum, after thorough dialysis (48 hours against running tap water, 96 hours against distilled water) weighed 611 mg. and formed a white fiber felt.

The isolation of Preparation Pa-2 from *Paracentrotus* sperm was carried

[2] All operations were carried out in the cold.

[3] In the case of *Echinocardium* extracts a strong reaction for desoxy sugars was observed at this stage. This made it necessary either to omit the preliminary washing or to rework the DNA extracted by the 0.146 M salt solution in a manner similar to that described previously for the DNA of yeast (10).

[4] The yields are improved if the protein gels, collected after the first three deproteinization treatments, are washed with salt solution and the washings united with the main solution before the treatment with chloroform-octanol is continued.

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TABLE I

DNA Preparations from Spermatozoa of Different Sea-Urchin Genera

Source	Preparation	Yield	N	P	Desoxypentose nucleic acid	Pentose nucleic acid	Ultraviolet absorption			
							Maximum		Minimum	
		per cent of sperm	*per cent*	*per cent*	*per cent*	*per cent*	*mμ*	*ε(P)*	*mμ*	*ε(P)*
Psammechinus miliaris	Ps	7	14.7	8.59	104	3	260	7300	230	3000
Paracentrotus lividus	Pa-1	3	13.6	7.85	98	2				
	Pa-2	11	15.1	8.84	105	1	260	7400	230	3100
Echinocardium cordatum	E-1	11	14.5	8.82	105	2	260	7350	230	3000
	E-2	11	14.3	8.57	100	3				
Arbacia lixula	A-1	8	15.2	8.90	101	2	260	7300	230	3200
	A-2	3	15.1	8.77	100	3				

TABLE II

DNA of Different Sea-Urchin Genera

Proportions in moles of nitrogenous constituent per mole of P in hydrolysate.

Source	No. of preparations	Constituent	Formic acid hydrolysis*			All analyses†		
			No. of hydrolyses‡	Mean proportion	Standard error	No. of hydrolyses‡	Mean proportion	Standard error
Psammechinus miliaris	1	Adenine	2	0.306	0.004	5	0.290	0.007
		Guanine	2	0.167	0.002	5	0.163	0.003
		Cytosine	2	0.167	0.002	2	0.167	0.002
		Thymine	2	0.300	0.001	2	0.300	0.001
Paracentrotus lividus	2	Adenine	5	0.311	0.003	9	0.303	0.006
		Guanine	5	0.168	0.003	9	0.162	0.003
		Cytosine	5	0.164	0.001	6	0.159	0.005
		Thymine	5	0.304	0.003	6	0.299	0.005
Echinocardium cordatum	2	Adenine	6	0.317	0.006	11	0.300	0.007
		Guanine	6	0.164	0.003	11	0.159	0.002
		Cytosine	6	0.173	0.002	6	0.173	0.002
		Thymine	6	0.310	0.006	6	0.310	0.006
Arbacia lixula	2	Adenine	5	0.294	0.003	9	0.282	0.005
		Guanine	5	0.180	0.002	9	0.175	0.002
		Cytosine	5	0.181	0.003	5	0.181	0.003
		Thymine	5	0.287	0.004	5	0.287	0.004

* Described as hydrolysis Procedure 2 in a previous paper (4).

† This column combines the results obtained with hydrolysis Procedures 1 and 2 (4).

‡ The results are based on a total of thirty-five hydrolysis experiments. In each hydrolysis between eighteen and twenty-four determinations of individual nitrogenous constituent were performed.

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out in the presence of Duponol (11).[5] This detergent was, however, in this case insufficient for the complete removal of protein. It was necessary to subject the crude DNA to five treatments with chloroform-amyl alcohol (4:1). In other respects the procedures outlined in the preceding paragraph were followed.

The weights of the sperm specimens serving as the starting materials varied from 5 to 8 gm. Analytical and spectroscopic data for the various DNA preparations are assembled in Table I.[6]

TABLE III

*DNA of Different Sea-Urchin Genera; Molar Relationships**

	Source			
	Psammechinus miliaris	*Paracentrotus lividus*	*Echinocardium cordatum*	*Arbacia lixula*
Molar ratio				
Adenine to guanine	1.83	1.85	1.93	1.64
Thymine to cytosine	1.80	1.85	1.79	1.58
Adenine to thymine	1.02	1.02	1.02	1.02
Guanine to cytosine	1.00	1.02	0.95	1.00
Purines to pyrimidines	1.01	1.02	1.00	1.01
Amino groups to enolic hydroxyls	1.37	1.36	1.38	1.40
P accounted for, % P in hydrolysate	94.0	94.7	96.4	94.2
Average, gm. atoms N per mole constituent	3.5	3.5	3.5	3.5
Atomic N:P ratio in nucleic acid preparations	3.8	3.8	3.7	3.8

* The computations are based on the mean proportions of each nitrogenous constituent found by formic acid hydrolysis (Table II).

Composition

The hydrolysis methods (Procedures 1 and 2 in Table II) and the chromatographic arrangements have been discussed in a previous publication (4). The same paper should be consulted for a discussion of the manner in which the analytical results are presented. The values for purines and pyrimidines, found in individual hydrolysis experiments, of which thirty-five were performed, are not listed separately, in order to conserve space. The mean proportions of nitrogenous constituents found in the seven DNA preparations studied here are presented in Table II. The molar relationships that serve to bring out the regularities and differences mentioned before are assembled in Table III.

[5] We should like to thank Dr. A. L. Dounce for acquainting us with the details of his procedure.

[6] We are indebted to Miss R. Rother for the microanalyses.

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Sugar

The DNA Preparations Ps, Pa-1, E-1, and A-1 were subjected to a chromatographic study of their sugar components by the methods described previously (12). A standard preparation of calf thymus DNA was examined simultaneously. The usual control tests and reference chromatograms of ribose also were carried out. All preparations of sea-urchin DNA yielded one sugar component which, in three different solvent systems, occupied the same position on the chromatograms, also relative to the position of ribose, as the sugar liberated from calf thymus DNA.

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SUMMARY

The desoxypentose nucleic acids of four different sea-urchin genera were isolated from sperm material. The contents in individual purines and pyrimidines of seven nucleic acid preparations were determined and compared. Certain structural similarities and differences were pointed out. Their sugar constituents were shown to be indistinguishable chromatographically from 2-desoxyribose.

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