THE NUCLEOTIDE COMPOSITION OF PENTOSE NUCLEIC ACIDS IN DIFFERENT CELLULAR FRACTIONS*

by

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We submit here observations made in the continuation of our efforts to discover whether there exists a correlation between the nucleotide composition, and therefore the nucleotide sequence, of pentose nucleic acids (PNA) and the structure or function of the cell. We have reported previously that the massive metabolic and morphological changes during early embryogenesis in the sea urchin are not accompanied by detectable changes in the composition of the PNA of the whole embryo[1]. The present investigations, portions of which have formed the subject of preliminary communications[2,3], comprise a study of PNA composition in cellular fractions: nuclei, isolated from rat liver and from beef liver and kidney; and centrifugally isolated cytoplasmic fractions from rat and frog liver and kidney.

EXPERIMENTAL

*Isolation of nuclei***

Rat liver nuclei. Two preparations were made from tissue, which had been frozen immediately after removal, by the method of DOUNCE[4], citric acid at pH 4 being employed. For each preparation, the organs from six rats were used.

Ox nuclei. Liver and kidney were frozen in dry ice within one-half hour after their removal and stored at −15°. One batch of liver nuclei was prepared according to DOUNCE AND LITT[5] in 1% aqueous gum arabic kept at pH 4 by the addition of citric acid. Two attempts to prepare similar nuclei from kidney failed.

Single specimens of liver and kidney nuclei were prepared by a modification[6,7] of the technique described by BEHRENS[8]. About 50 g of thinly sliced frozen tissue was lyophilized and ground for 48 hours in a ball mill at 110 r.p.m. in 200 ml of ligroin with about 150 pebbles (2–4 cm diameter). From the suspension, strained through gauze (mesh width 0.5 mm), the nuclei were isolated by repeated centrifugation and re-suspension in benzene-CCl_4 mixtures of increasing density ranging from 1.195 to 1.360. One or two centrifugations runs at a density of 1.4 were interspersed, in order to remove, by sedimentation, material heavier than the nuclei. The last suspension was strained through 140-, 200- and 325-mesh screens. The nuclei were collected by centrifugation, dried in a vacuum and stored at −15°.

Appearance. Microscopic examination of stained preparations accompanied the isolation steps. The nuclei prepared in citric acid were unaltered in appearance; they dispersed evenly in wet smears and stained well, showing prominent nucleoli. No sign of wrinkling or collapse of the nuclear membrane was seen, and only a few nuclei in a hundred showed a break in the membrane or carried bits of extra-nuclear material. Fibrous debris, virtually absent from one rat liver preparation, consti-

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** All preparative operations were carried out at 0 to 5°.

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tuted 10 to 20% of the other, but had no apparent effect on the PNA composition (see the results in Table I). The nuclei prepared in non-aqueous solvents were shriveled and quite contaminated with both fibrous and non-fibrous material.

Preparation of cytoplasmic fractions

The procedure followed that of SCHNEIDER AND HOGEBOOM[9,10]. All operations were performed at 0 to 5° in 0.25 *M* sucrose. The freshly removed, chilled organs were minced with scissors and comminuted in an ice-cooled glass tissue grinder[11]. The pulp suspension was strained through fine cheese cloth, forced through a 20-gauge hypodermic needle and centrifuged twice for 10 minutes at 600–700 × *g*, the pellets being discarded. The supernatant fluid was then subjected to two 10-minute centrifugations, once each at 5,000 and 20,000 × *g*, yielding two brown, granular pellets. The turbid supernatant fluid was centrifuged either at 20,000 × *g* for 3 to 4 hours or at 78,000 × *g* for 1 hour; in either case the sediment was a pink translucent gel and the supernatant solution was perfectly clear. The 5,000 × *g* pellet, the mitochondrial fraction, could not be analyzed satisfactorily (see below). All other sediments gave similar analyses and have been grouped together under the designation of "small particles". These and the final supernatants are the two types of cytoplasmic fraction listed in Tables II and III. Organs from 1 to 4 animals served for each preparation from rat tissues; organs from 15 animals were pooled for each frog specimen.

PNA analysis

The PNA of all preparations was analyzed by a procedure fully described elsewhere[1,12]. In this method no attempt is made to purify the PNA. By means of a modified procedure according to SCHMIDT AND THANNHAUSER[13] the PNA is extracted *in toto* under conditions permitting its quantitative hydrolysis to mononucleotides. These are separated and quantitatively estimated by means of paper chromatography and ultraviolet spectrophotometry.

Satisfactory chromatograms could not be obtained when large amounts of inorganic salt were present in the solutions subjected to separation. NaCl is formed during the hydrolysis with NaOH and the subsequent acidification with HCl; all hydrolysates contained about the same concentration of NaCl. With one exception, all cell fractions yielded excellent chromatograms when portions of the hydrolysate of 0.01 to 0.04 ml were chromatographed. The exception was the cytoplasmic fraction sedimented at 5,000 × *g*, *i.e.*, the mitochondrial fraction, which is known to be very poor in PNA[14]. These hydrolysates required amounts of 0.08 to 0.15 ml (applied in successive portions of 0.01 ml[12]), and the chromatograms were blurred and insufficiently resolved. No analyses of the mitochondrial preparations are, for this reason, included in this paper.

Since the method employed did not permit reduction of the NaCl concentration below the critical level, attempts were made to overcome this difficulty by other means. When KOH and $HClO_4$ were substituted for NaOH and HCl, chromatographic resolution of large portions was good. However, controlled experiments showed that over 20% of the hydrolyzed PNA was lost, apparently through adsorption on the precipitated $KClO_4$, and that the proportion lost was not the same for the different nucleotides. Attempts to neutralize the alkaline hydrolysate with an acidic resin (Dowex-50, H-form, in batch) also resulted in incomplete recovery of nucleotides.

RESULTS AND DISCUSSION

The analytical results are assembled in Tables I and II. As has been mentioned before, one type of cytoplasmic fraction, namely, that sedimenting at 5000 × *g*, could not be analyzed satisfactorily. The other cytoplasmic particle fractions, those brought down at 20,000 and 78,000 × *g*, were very similar with regard to the composition of their PNA and are here grouped under the designation "small particles". These particles (microsomes) and the non-sedimenting fractions remaining in the supernatant fluids are, therefore, the two cytoplasmic constituents considered here. A few of these preparations had been dealt with in previous brief communications[2,3]; for the present paper, all values have been computed again with the use of more recent and more accurate spectral data[1]. The PNA composition is reported as mole % of nucleotide; in addition, several molar ratios are listed that are of interest for the comparison of different nucleic acids.

This comparison forms the substance of Table III which also provides a statistical evaluation (by means of the *t* test[15]) of the significance of the composition differences found by us. It is apparent that the pentose nucleic acids of the preparations of rat and

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TABLE I

ISOLATED NUCLEI, RAT AND OX: PNA COMPOSITION*

Source	Isolation medium**	Moles per 100 moles of nucleotide				Molar ratios				
		A	G	C	U	G/A	G/U	C/A	Pu/Py	$\frac{A+U}{G+C}$
Rat liver	I	20.2	25.7	29.5	24.6	1.27	1.04	1.46	0.85	0.81
Rat liver	I	20.2	26.3	30.2	23.3	1.30	1.13	1.50	0.87	0.77
Ox liver	II	18.9	26.2	32.6	22.3	1.39	1.17	1.72	0.82	0.70
Ox liver	III	20.4	25.3	27.6	26.7	1.24	0.95	1.35	0.84	0.89
Ox kidney	III	22.0	26.2	29.7	22.2	1.19	1.18	1.35	0.93	0.79

* Abbreviations used: A, adenylic acid; G, guanylic acid; C, cytidylic acid; U, uridylic acid; Pu, purines; Py, pyrimidines.

** Conditions of isolation: I, citric acid, pH 4[4]; II, citric acid, gum arabic, pH 4[5]; III, sedimentation in non-aqueous solvents (benzene-CCl_4)[6–8]. For details, see text.

TABLE II

CYTOPLASMIC FRACTIONS, RAT AND FROG: PNA COMPOSITION*

Source	Cell fraction	Number of preparations	Moles per 100 moles of nucleotide				Molar ratios				
			A	G	C	U	G/A	G/U	C/A	Pu/Py	$\frac{A+U}{G+C}$
Rat liver	Small particles	3	16.37	32.07	30.50	21.00	1.967	1.543	1.873	0.943	0.600
Rat liver	Supernatant	4	18.90	31.40	30.18	19.53	1.663	1.633	1.598	1.015	0.628
Rat kidney	Small particles	4	18.45	32.40	29.58	19.55	1.778	1.663	1.613	1.035	0.615
Rat kidney	Supernatant	3	17.77	30.53	30.03	21.63	1.720	1.463	1.690	0.940	0.653
Frog liver	Small particles	3	20.17	26.80	33.17	19.87	1.337	1.350	1.650	0.887	0.670
Frog liver	Supernatant	1	20.5	24.6	31.5	23.4	1.20	1.05	1.53	0.82	0.78
Frog kidney	Small particles	1	19.9	31.2	29.4	19.5	1.57	1.61	1.48	1.05	0.65

* See Table I for abbreviations.

TABLE III

COMPARISON OF SPECIES, ORGANS AND CELL FRACTIONS: PNA COMPOSITION*

Specimens under comparison		Number of preparations	Moles per 100 moles of nucleotide				Molar ratios				
			A	G	C	U	G/A	G/U	C/A	Pu/Py	$\frac{A+U}{G+C}$
Nuclei	Rat	2	20.20	26.00	29.85	23.95	1.285	1.085	1.475	0.860	0.790
	Ox	3	20.43	25.90	29.97	23.73	1.273	1.100	1.477	0.863	0.793
	Prob.**		g	g	g	g	g	g	g	g	g
Cytoplasm, all fractions	Rat	14	17.99	31.64	30.04	20.30	1.773	1.586	1.682	0.989	0.624
	Frog	5	20.18	27.24	32.08	20.50	1.356	1.342	1.592	0.906	0.688
	Prob.**		**c**	**d**	e	g	**b**	f	f	f	e
Rat cytoplasm	Small particles	7	17.56	32.26	29.97	20.17	1.859	1.612	1.724	0.996	0.609
	Supernatants	7	18.42	31.03	30.12	20.43	1.687	1.560	1.639	0.983	0.639
	Prob.**		f	f	g	g	f	g	f	g	f
Rat cytoplasm	Liver	7	17.82	31.69	30.32	20.16	1.793	1.594	1.716	0.984	0.616
	Kidney	7	18.16	31.60	29.77	20.44	1.753	1.577	1.647	0.994	0.631
	Prob.**		g	g	f	g	g	g	f	g	g
Rat	Cytoplasm	14	17.99	31.64	30.04	20.30	1.773	1.586	1.682	0.989	0.624
	Nuclei	2	20.20	26.00	29.85	23.95	1.285	1.085	1.475	0.860	0.790
	Prob.**		**a**	**d**	g	f	**a**	**d**	**d**	**d**	e

* See Table I for abbreviations.

** Prob. = probability (t test) that the means are different. **a**, > 0.999; **b**, 0.999–0.990; **c**, 0.99–0.98; **d**, 0.98–0.95; e, 0.95–0.90; f, 0.90–0.50; g, < 0.50. Bold face type is used when the probability of difference exceeds 95%.

ox nuclei were identical, at least by the criteria applied here, and that rat cytoplasm displayed no significant composition differences between organs (liver, kidney) or cell fractions (small particles, supernatant fractions). A species difference between rat and frog cytoplasmic fractions is indicated; and there certainly exists a difference between the PNA of rat nuclei and that of rat cytoplasm, as analyzed here.

The present investigation had as its main purpose the search for the existence of striking differences in the nucleotide composition of pentose nucleic acids from different species, organs and cell fractions. For the establishment of small differences, let alone of mere divergences in nucleotide sequence, a much more extensive investigation would be required. It should be noted that the calculations of the *t* test take into account the number of data available. When, as in the present case, this number is low, the effect is to diminish not the reliability of the probability calculations, but the likelihood that significant differences will be found. It is thus highly probable that a real composition difference did exist between the pentose nucleic acids of the samples of rat and frog cytoplasmic fractions listed in Tables II and III. It is, however, hardly permissible to infer the discovery of a species difference, in view of the previous finding of a similar difference between two series of sea urchin embryos of the same species[1]. For an actual species difference, if such exist, to be established, a larger number of organs and species will have to be examined in detail.

The composition difference between the pentose nucleic acids of rat nucleus and cytoplasm appears to be more firmly established (Table III). Since the initial announcements of such nucleo-cytoplasmic differences in rat[2] and calf[16], similar findings have been made with respect to the nucleic acids of rat and rabbit liver[17–19] and the starfish oöcyte[20]. OLMSTED AND VILLEE have reported finding three types of PNA in human liver, one each in the nucleus, cytoplasmic particles and cytoplasmic supernatant, but only a single type in all fractions of the rat liver cell[21]. Our results, indicating the presence of two different pentose nucleic acids in rat liver, one in the nucleus and one in all the cytoplasmic fractions investigated, are in close agreement with those of DAVIDSON and his co-workers[17–19]. In both laboratories the average values found for the ratios G/A, G/U, and C/A are, respectively, nuclei: 1.2–1.3, 1.0–1.1, 1.3–1.5; cytoplasmic fractions: 1.7–1.8, 1.6, 1.5–1.7. Similar values were found in nuclei from ox liver and kidney (Table III) and rabbit liver[17], but not calf thymus[17]; and in cytoplasmic fractions from rat and frog kidney (Tables II, III) and rabbit liver[19], but not frog liver (Table II). The inference may be drawn, though very tentatively, that the compositon of PNA is more characteristic of the intracellular location of the PNA (nucleus in contrast to cytoplasm) than of the species or organ. The available evidence is, however, meager and somewhat contradictory.

In this connection, mention may be made of a report that the nuclear pentose nucleic acids of various calf organs contain approximately 40 to 50 % more adenine than do the corresponding cytoplasmic substances[16]. It has, in fact, been suggested that the cytoplasmic PNA may be derived from the PNA of the nucleus, and that this process may be accompanied by the liberation of adenine nucleotides which, by the way of ATP, could serve as an energy source for the synthesis of cytoplasmic proteins[16, 22]. Recent evidence pointing to a more complex series of events has been discussed by BRACHET[23]. It should be pointed out that our data on the composition of the nuclear PNA of ox differ from those of MARSHAK[16]. Where we have compared nuclear and cytoplasmic preparations from the same source (rat liver), we have also found the cytoplasmic PNA to contain

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less adenine than does that of the nucleus; but the difference is less striking, about 10%. As this cytoplasmic PNA, moreover, also contained about 15% less uracil, a selective release of adenylic acid is not obvious from our data. It is altogether apparent that, in view of multiple recent evidence that adenine nucleotides may not be the only widely distributed nucleotides capable of high energy phosphate transfer[24,25], the hypotheses regarding the origin and the uniqueness of ATP and, especially, the functional and metabolic relationship between the various intracellular pentose nucleic acids will have to be scrutinized anew.

SUMMARY

The nucleotide composition of pentose nucleic acids of nuclei from rat liver and ox liver and kidney as well as of cytoplasmic fractions from rat and frog liver and kidney has been studied. While no significant differences either between organs or, with one doubtful exception, between species were found, the hepatic nuclear and cytoplasmic pentose nucleic acids of the rat did differ in composition.

RÉSUMÉ

La composition en nucléotides des acides pentosenucléiques des noyaux du foie du rat, et du foie et du rein du boeuf, ainsi que celle des fractions cytoplasmiques du foie et du rein du rat et de la grenouille a été étudiée. Tandis qu'aucune différence significative, soit entre les organes, soit, à une exception douteuse près, entre les espèces, n'a été trouvée, dans le foie du rat, les acides pentosenucléiques nucléaires ont une composition différente de celle des acides pentosenucléiques cytoplasmiques.

ZUSAMMENFASSUNG

Die Nukleotidzusammensetzung einer Reihe von Pentosenukleinsäuren wurde untersucht. Das Untersuchungsmaterial umfasste Zellkerne aus Rattenleber und Rindsleber und -niere und Cytoplasmafraktionen aus der Leber und Niere von Ratten und Fröschen. Weder zwischen Organen noch, mit einer fraglichen Ausnahme, zwischen Spezies wurden deutliche Unterschiede aufgefunden. Hingegen zeigte der Vergleich von Pentosenukleinsäuren aus dem Kern und solchen aus dem Cytoplasma von Rattenleber, dass es sich um verschieden zusammengesetzte Substanzen handelte.

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