ON THE DESOXYPENTOSE NUCLEIC ACIDS FROM SEVERAL MICROORGANISMS*

by

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The present study continues the attempts of this laboratory to gain an insight into the differences in composition, and therefore, presumably, in nucleotide sequence, distinguishing the desoxypentose nucleic acids (DNA's) derived from different species. As has been pointed out on a previous occasion[2], the investigation of microbial DNA's is of particular interest in this connection, not only because of the range of cellular morphology and physiology, much wider than in higher organisms, that may thus be covered, but also because of the role currently assigned to certain microbial DNA's in the phenomena of bacterial transformation[3]. One of the DNA preparations examined here, namely that of *Hemophilus influenzae*, was, in fact, endowed with transforming activity[4].

EXPERIMENTAL

Sources

Representatives of three different bacterial families served for the isolation of DNA. They were: (1) *Serratia marcescens*, mutant *VII*-1-2 (BUNTING), obtained from strain Hy[5] by ultraviolet irradiation. (2) *Bacillus Schatz*, a facultatively autotrophic, Gram-positive hydrogen organism, kindly given us by Drs C. B. VAN NIEL AND A. SCHATZ, Hopkins Marine Station, Pacific Grove, California. (3) *Hemophilus influenzae*, type c.

Preparations

DNA of Serratia marcescens. The organisms were cultivated in Roux bottles at room temperature for 48 hours on Difco Nutrient Agar. The suspension of the cells in physiol. saline exhibited desoxyribonuclease activity; it contained approximately 70 units[6] per g of wet cells. When the organisms were ground in a mortar with Pyrex powder and then extracted with physiol. saline (1.6 ml per g of cells), the extract contained about 25 units per g of bacteria. The assays were carried out in the arrangement described previously[7]. This relatively strong depolymerase, which could have precluded the isolation of highly polymerized DNA, was retarded, but not inhibited, by 0.1 *M* sodium citrate. Its inhibition was, however, almost complete in 3.5 *M* sodium chloride solution. For the isolation of DNA, the 48 hours old organisms were removed from each bottle by washing with 30 ml of 0.1 *M* sodium citrate buffer (pH 7.3), recovered by centrifugation at 18,000 × *g* for 30 minutes and washed three times by suspension in 5 volumes of 3.5 *M* aqueous sodium chloride. The cells deposited by centrifugation, were frozen immediately and ground for 30 minutes in a chilled mortar with one part (by weight) of washed Pyrex powder (diameter 3 μ) and 0.18 part of solid sodium chloride. After extraction with one volume of 3.5 *M* aqueous sodium chloride and centrifugation at 18,000 × *g*

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for 30 minutes, the supernatant was injected into three volumes of ice-cold 90% ethanol. The precipitated threads were washed with 90 and stored in 95% ethanol. The extraction residue was re-extracted 10 to 15 times with decreasing quantities of salt solution, and fibers were collected from each extract. The combined threads were dissolved in 10% aqueous sodium chloride (about 0.5 mg DNA per ml) and freed of protein and purified, as described before[8]. The final yield of DNA was 0.08% of the wet cell weight. It contained 8.99% P, less than 2% pentose nucleic acid, and assayed for 95% of DNA, when compared with a calf thymus DNA standard[2].

DNA of Bacillus Schatz. The organisms were grown in Roux bottles at 30° for 48 hours on Difco Yeast Beef Agar. The yield was about 1 g of packed cells (wet weight) per bottle. With a few exceptions, the procedure for the isolation of DNA followed that described in the preceding paragraph. Owing to the absence of detectable desoxyribonuclease activity 0.1 *M* aqueous sodium citrate (pH 7.1) could be used for the washing of the cells and 10% aqueous sodium chloride for the extraction of the crushed organisms. The precipitation of pentose nucleic acid as an insoluble calcium salt[8] failed to give satisfactory results in this case. For this reason, recourse was had to the removal of contaminating pentose nucleic acid by its conversion to dialyzable pentose nucleotides*. A solution of the crude DNA fibers, collected after the deproteinization, in aqueous sodium hydroxide of pH 13.5 (1.6 mg DNA per ml) was dialyzed with rocking at 30° for 18 hours against the 250-fold volume of the same solvent, for 24 hours against running tap water, and for the same period against ice-cold distilled water. The DNA, recovered by lyophilization of the dialyzed solution, amounted to 0.1% of the cells (wet weight). It contained 8.83% P, less than 2% pentose nucleic acid, and assayed for 94% of DNA.

DNA of Hemophilus influenzae, type c. The isolation of this preparation has been discussed in a separate paper[10].

Procedures

The analytical methods for the estimation of purines and pyrimidines and the determination of total DNA and pentose nucleic acid and of phosphorus have, with one exception, been outlined in detail in a recent publication[11]. An additional procedure, which proved useful in the present and in other work, is based on the unexpected finding that the purines adenine and guanine may be separated from the pyrimidines by what can be considered as real adsorption chromatography on filter paper, with water as the sole solvent**. The DNA hydrolysate was prepared in the usual manner with conc. formic acid[11] and applied to the top of a strip of filter paper (Schleicher and Schuell, No. 597), with the longer edge (at least 60 cm) paralleling the water-marks of the paper. The lower portion of the strip was wound into a roll held together by paper clips, so that the paper fitted into the chromatography assembly normally employed[13]. After neutralization of the dispensed hydrolysate with gaseous ammonia, chromatography was carried out with water or with 0.01 *M* phosphate buffer of pH 7.1 as the solvent. A sufficient separation was achieved within about 165 minutes at room temperature. With a mixture of adenine, guanine, cytosine, and thymine, the pyrimidine zone was found to travel about twice as fast as the purine zone. Following the location of the separated areas by means of a suitable ultraviolet lamp, the zone containing the purines was cut off, the remaining strip was unrolled and the pyrimidines separated from each other by chromatography in the usual fashion[13].

Composition

Table I presents the analytical results on the contents of individual purines and pyrimidines in the DNA preparations examined here. The figures are averages based on between 3 and 10 independent hydrolysis experiments and on a large number of individual determinations. The manner of presentation of the data follows that adopted previously[11,12,14].

DISCUSSION

The quality of the DNA preparations was fairly satisfactory, with the exception of the DNA of the hydrogen organism *Bacillus Schatz*, which was not analyzed as the highly polymerized product. Work in this laboratory, as yet unpublished, has, however, yielded no indications that the depolymerization of a DNA by alkali to non-dialyzable

* The recently published procedure for ridding DNA of pentose nucleic acid[9] was not yet available at the time this work was carried out.

** This method has been found of value for the chromatographic detection of trace components and has, for instance, been utilized recently for the estimation of 5-methylcytosine in the DNA of *Paracentrotus lividus*[12]. It also is useful, when total DNA hydrolysates are to be tested for the presence of uracil, which otherwise is overshadowed by adenine.

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TABLE I

DNA'S OF SEVERAL MICROORGANISMS; MOLAR PROPORTIONS AND RELATIONSHIPS

	Source of preparation		
	Serratia marcescens	*Bacillus Schatz*	*Hemophilus Influenzae, Type c*
Moles per mole P			
Adenine	0.180	0.173	0.296
Guanine	0.237	0.253	0.169
Cytosine	0.278	0.281	0.182
Thymine	0.175	0.162	0.280
P accounted for, % P in hydrolysate	87.0	86.9	92.7
Molar ratio			
Adenine to guanine	0.76	0.68	1.75
Thymine to cytosine	0.63	0.58	1.54
Adenine to thymine	1.03	1.07	1.06
Guanine to cytosine	0.85	0.90	0.93
Purines to pyrimidines	0.92	0.96	1.01
Amino groups to enolic hydroxyls	1.69	1.70	1.44

fragments is attended by appreciable changes in the proportions of the constituents. The figures reported here may be considered as representative of the composition of the DNA's under examination, the more so, since contamination with pentose nucleic acid had been reduced to insignificant levels.

The three DNA specimens are good examples of two of the main types of DNA encountered so far[1,15]: the infrequent "GC type" (represented by the DNA's of *Serratia* and the hydrogen organism) and the much more prevalent "AT type" (here represented by the *Hemophilus* DNA). Quite apart from differences in the proportions of individual nitrogenous constituents, which appear to be characteristic of the species furnishing the particular DNA[16], and from regularities governing the composition of all DNA's analyzed[15], the majority seems to belong to a class in which adenine and thymine exceed guanine and cytosine by 35 to 90%. In the case of microbial DNA's, this "AT type" is represented by the *Hemophilus* DNA described here, by the DNA of yeast[2] and that of *Pneumococcus*, Type III[17]. An intermediate type, containing all bases in nearly equimolar quantities, has been found in several strains of *E. coli*[14,15,18]. The "GC type", containing larger amounts of guanine and cytosine than of adenine and thymine, is exemplified by the DNA of different strains of tubercle bacilli[2,18] and by two of the DNA preparations discussed in the present communication. It is worthy of mention that until now only microorganisms have yielded representatives of all three types. It is, however, clear that the correlation between the composition and the biological functions of a DNA and the taxonomy and phylogeny of the species from which it is derived will not be possible without supplementary methods of investigation, whether they aim at distinction by physical means (compare[19]) or by analysis of the nucleotide sequence.

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SUMMARY

The isolation of the desoxypentose nucleic acids of three microorganisms, viz. *Serratia marcescens*, a facultatively autotrophic hydrogen organism *Bacillus Schatz*, and *Hemophilus influenzae*, type c, is described. The composition of these substances with respect to the contents of adenine, guanine, cytosine, and thymine was determined; this led to their classification in regard to the DNA types to which they belong. A procedure for the separation of the purines adenine and guanine from the pyrimidines cytosine and thymine by adsorption chromatography on filter paper with water as the solvent has been developed.

RÉSUMÉ

Les auteurs décrivent le mode d'obtention des acides désoxypentosenucléiques de trois microorganismes, *Serratia marcescens*, *Bacillus Schatz* (organisme facultativement autotrophe) et *Haemophilus influenzae*, type c. La détermination de la teneur de ces substances en adénine, guanine, cytosine et thymine permet leur groupement par rapport au type d'acide nucléique auquel elles appartiennent. Le procédé de séparation des purines (adénine et guanine) des pyrimidines (cytosine et thymine) en solution aqueuse par adsorption chromatographique sur papier filtre est décrit.

ZUSAMMENFASSUNG

Die Bereitung der Desoxypentosenukleinsäuren aus drei Mikrobenstämmen, nämlich *Serratia marcescens*, *Bacillus Schatz* (einem fakultativ autotrophen Wasserstofforganismus) und *Haemophilus influenzae*, Typus c, wird geschildert. Die quantitative Bestimmung des Gehaltes dieser Verbindungen an Adenin, Guanin, Cytosin und Thymin ermöglichte ihre Gruppierung in Bezug auf die Nukleinsäuretypen, denen sie angehörten. Die Trennung der Purine Adenin und Guanin von den Pyrimidinen Cytosin und Thymin in rein wässeriger Lösung mittels Adsorptionschromatographie auf Filtrierpapier wird beschrieben.

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