THE DESOXYPENTOSE NUCLEIC ACIDS OF THREE STRAINS OF *ESCHERICHIA COLI**

by

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Studies on the composition and structure of nucleic acids in which this laboratory has been engaged[1-3] served to direct early attention to the unusual composition of several desoxypentose nucleic acids (DNA) of microbial origin[4-7]. Whereas, regardless of individual differences, all DNA preparations of animal and plant origin examined so far belonged to the "AT type"[3,7], three examples of the existence of a "GC type" of DNA, in which guanine and cytosine were the major nitrogenous constituents, were provided by DNA samples derived from microorganisms[6,7]. Other microbial species have, however, been shown to yield DNA of the "AT type"[6,8]; and no rationalization of the meaning of these differences is as yet possible. The present paper describes the isolation and composition of DNA of three different strains of *E. coli*; it provides evidence of the existence of a DNA type intermediate between the two main types mentioned before; it presents a good example of the remarkable constancy of composition of DNA contained in what may be considered as three strains of the same species. Previous results on *E. coli* DNA[9,10] will be discussed below.

EXPERIMENTAL

Material

Three strains of *Escherichia coli* were used: (1) Strain K-12[11], obtained from the Department of Microbiology, Yale University; (2) Strain UQ[12], kindly supplied by Dr M. U. DIANZANI of the University of Genoa; (3) the thymine-requiring mutant No. 11117 (American Type Culture Collection), produced by the irradiation of *E. coli* No. 9723 (American Type Culture Collection)[13,14]. A strain isolated here from cultures of this mutant, obtained through the courtesy of Dr R. R. ROEPKE of the American Cyanamid Company, Stamford, Conn., served for the experiments and will be referred to as "thymineless".

The organisms were cultivated at 37° on 2% "Bacto Nutrient Agar" (Difco) in Roux bottles (150 ml of agar per flask). In the case of the thymineless strain, this medium was supplemented by 10 γ of thymine per ml of agar. In a typical experiment, a 24 hr culture in 86 bottles was rinsed off the surface with 2 liters of 0.1 *M* sodium citrate (pH 7.3); the cells were recovered by centrifugation at 1900 × *g* for one hour, washed three to five times with 100 ml portions of the same buffer, and collected by centrifugation at 18,000 × *g* for 30 minutes. The sediment (25 g wet weight) was stored in the frozen state at −15° for no longer than 48 hours before being processed.

Preparation

All operations were carried out in the cold. One experiment will be described. The frozen cells were ground in a mortar, in 5 to 12 g portions, for 30 minutes with equal weights of washed pyrex powder (diameter 3 μ). The crushed material was suspended in 10 ml of 0.1 *M* sodium citrate buffer of pH 7.3 and the mixture centrifuged at 18,000 × *g* for one-half hour. The white threads, produced

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by the injection of the supernatant into 3 volumes of 90% ethanol, were washed with 90% and stored in 95% alcohol. The sediment produced by the centrifugation was re-extracted for 24 to 48 hours with 30 ml of 10% aqueous sodium chloride, when more fibers precipitable with ethanol were obtained. Six more extractions with decreasing volumes of sodium chloride solution were performed. The solution of the combined fibrous precipitates in 30 ml of 10% aqueous NaCl was freed of protein by ten treatments with chloroform-pentanol[15]. The crude fibrous DNA, produced by precipitation with ethanol, was dissolved in 24 ml of 0.14 *M* aqueous sodium chloride and freed of contaminating pentose nucleic acid by treatment with Norit in the recently published arrangement[16]. Dialysis of the solution against running tap water and ice-cold distilled water, each for 24 hours, and lyophilization yielded the sodium salt of DNA, 17 mg of a white fluff readily soluble in water to give a clear, very viscous solution.

Results

The microprocedures for the estimation in the final DNA preparations of total DNA, pentose nucleic acid, and protein were adaptations of previously used methods which will be described on a later occasion. Hydrolysis was carried out in concentrated formic acid[17]. Separation and analysis of individual purines and pyrimidines were performed in the arrangement described recently[18], and the computations were based on the quantities of phosphorus in the hydrolysates[19,20]. Preparation K-12 contained 6.5% of P, 75% of DNA, when compared with a standard preparation[17], and less than 1% of pentose nucleic acid. The corresponding figures for Preparation UQ were 7.4, 85, 1.7%; for Preparation "Thymineless", 7.3, 85, less than 1%. The protein content of all preparations lay below 2.5%. The composition of the DNA preparations with respect to the distribution of individual purines and pyrimidines is summarized in Table I. Each figure represents the average of three independent hydrolysis experiments and of 24 to 36 single determinations. The conventions adopted for the presentation, in the same table, of the characteristic molar relationships have been discussed in previous publications[17,18,21].

TABLE I

DNA OF *E. coli*; MOLAR PROPORTIONS AND RELATIONSHIPS

	Preparation		
	K-12	*UQ*	*Thymineless*
Moles per mole P			
Adenine	0.247	0.240	0.227
Guanine	0.236	0.234	0.215
Cytosine	0.239	0.239	0.229
Thymine	0.227	0.224	0.221
P accounted for, % P in hydrolysate	94.9	93.7	89.2
Molar ratio			
Adenine to guanine	1.05	1.03	1.05
Thymine to cytosine	0.95	0.94	0.97
Adenine to thymine	1.09	1.07	1.03
Guanine to cytosine	0.99	0.98	0.94
Adenine to cytosine	1.03	1.00	0.99
Purines to pyrimidines	1.04	1.02	0.98
Amino groups to enolic hydroxyls	1.56	1.56	1.54

DISCUSSION

The quality of the DNA preparations examined here was satisfactory; the most troublesome contaminant, pentose nucleic acid, was reduced to insignificant amounts*. In the recent past, *E. coli* DNA appears to have been analyzed twice. WYATT[9] reported figures that indicated a composition differing considerably from the one indicated by the present study. The distribution of purines and pyrimidines (in moles per 100 moles P) was: adenine 23, guanine 20.3, cytosine 26.5, thymine 30. Shortly after the preliminary presentation of our results[3], however, an independent study of SMITH AND WYATT[10] arrived at figures for the DNA of *E. coli*, mutant B/r, which are in good agreement

* Recently published figures[22] on the purine and pyrimidine content of *E. coli* cells cannot be utilized for a consideration of DNA composition, since three of the nitrogenous constituents are contributed by both types of nucleic acid.

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with the results submitted here. One must conclude that DNA of *E. coli* possesses unusual features: the four nitrogenous constituents, adenine, guanine, cytosine, and thymine, are present in nearly equimolar amounts. This is in contrast to the composition of the DNA's found until now in other species. The specimens from *E. coli* represent, in fact, the first instance where the formulation of a "statistical tetranucleotide", though devoid of any meaning when applied to a high polymer, would find some justification, at least on analytical grounds.

It is noteworthy that three different strains of the same species, varying as to origin and biochemical characteristics, yielded DNA preparations that resembled each other so closely with respect to their composition. It is remarkable that the constancy of DNA composition held true even of the strain unable to synthesize thymine, which was cultivated in the presence of an about 50-fold excess of thymine as compared with the quantity necessary for optimal growth.

SUMMARY

The isolation and composition of desoxypentose nucleic acid preparations from three different strains of *E. coli* are described. All specimens resembled each other very closely with respect to the relative amounts of adenine, guanine, cytosine, and thymine. The purine and pyrimidine composition was unusual and distinguished these substances from all other DNA's encountered so far: all nitrogenous constituents were found in nearly equimolar amounts.

RÉSUMÉ

Les auteurs décrivent le mode d'obtention et la composition de préparations d'acide désoxypentosenucléique de trois souches différentes de *E. coli*. Toutes les préparations contenaient environ les mêmes quantités relatives d'adénine, de guanine, de cytosine et de thymine. Les substances en question se distinguent de tous les acides désoxypentosenucléiques précédemment décrits par la répartition peu commune de purine et de pyrimidine: en effet, tous les constituants azotés s'y trouvent en quantités approximativement équimoléculaires.

ZUSAMMENFASSUNG

Die Herstellung und Zusammensetzung von Präparaten von Desoxypentosenukleinsäure aus drei verschiedenen Stämmen von *E. coli* werden beschrieben. Alle Präparate enthielten fast dieselben relativen Mengen von Adenin, Guanin, Cytosin und Thymin. Die ungewöhnliche Purin- und Pyrimidinverteilung unterschied die hier besprochenen Substanzen von allen anderen bisher beschriebenen Desoxypentosenukleinsäuren: alle N-haltigen Bestandteile fanden sich in fast äquimolekularen Mengen.

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