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Differences in the Chemical Composition of the Phage Nucleic Acids

IT has been known for some time that the nucleic acids of the coli-dysentery phages, T_2, T_4 and T_6, contain hydroxymethylcytosine and that the composition of their purine and pyrimidine bases is essentially identical[1]. More recently it was found in this laboratory that a hexose is present in T_4 phage[2]. This sugar was identified as glucose and was shown to be a constituent of the viral nucleic acid[3]. It was afterwards demonstrated that T_2 and T_6 nucleic acids also contain glucose and that enzymatic hydrolysates of T_2 and T_4 nucleic acids contain small quantities of the glucoside of hydroxymethylcytidylic acid[4,5]. In the present communication it will be shown that the nucleic acids of T_2, T_4 and T_6 phages differ in their content of glucose and that this hexose is attached to the acid-resistant part of nucleic acid, the apurinic acid.

The wild type (r^+) strains of T_2, T_4 and T_6 viruses were used in this study. They were grown on *E. coli B* in synthetic medium and purified by procedures already described[6]. In order to obtain the viral nucleic acids, aqueous suspensions of the phages were repeatedly frozen and thawed and then deproteinized with chloroform and octyl alcohol in presence of 1 *M* sodium chloride. The nucleic acids were precipitated with ethanol, then dialysed and dried from the frozen state.

The materials thus obtained formed viscous solutions and showed adsorption spectra characteristic of nucleic acid. Their atomic extinction coefficients ($\varepsilon(P)$ at 260 mμ) varied between 7020 and 7280. From Table 1 it can be seen that the nucleic acids derived from T_2, T_4 and T_6 phages differed slightly yet significantly in their nitrogen and phosphorus content. Colorimetric and chromatographic analyses revealed that deoxypentose as well as glucose were components of each. The purine-bound deoxypentose was determined quantitatively with diphenylamine using deoxyribose as a standard. Glucose was determined by means of the anthrone reaction.

Table 1. CHEMICAL COMPOSITION OF THE NUCLEIC ACIDS AND THE APURINIC ACIDS OF T_2, T_4, T_6 VIRUSES

	N (per cent)	P (per cent)	Glucose (per cent)	[Glucose]/[P]
T_2 nucleic acid	14·1	8·4	6·7	0·14
T_4 " "	13·8	8·0	8·4	0·18
T_6 " "	12·8	7·4	12·0	0·28
T_2 apurinic acid	5·9	9·8	7·9	0·14
T_4 " "	5·7	9·7	9·9	0·18
T_6 " "	5·2	9·0	14·0	0·27

Since deoxyribose produces a colour with this reagent, absorption was measured at 620 and at 500 mμ and the content of glucose calculated according to the procedure of Knudson, Meloche and Juday[7]. The amount of deoxyribose in the three nucleic acids varied but slightly (18·8–19·8 per cent), whereas differences in glucose content were considerable. The T_2, T_4 and T_6 nucleic acids were found to contain 6·7, 8·4 and 12·0 per cent of glucose respectively. Thus, for each mole of phosphorus there was present 0·14 moles of glucose in the T_2 nucleic acid, 0·18 moles in the T_4 nucleic acid and 0·28 moles in the T_6 nucleic acid. Since each nucleic acid contained approximately 0·17 mole of hydroxymethylcytosine per mole of phosphorus[1] it is evident that the amount of glucose in the nucleic acids derived from T_4 and T_6 exceeds that of the pyrimidine. In the case of T_2 nucleic acid this amount is less.

In order to gain some information concerning the nature of the glucose linkage in the viral nucleic acids, the latter were hydrolysed with dilute sulphuric acid at *p*H 1·6 and at 37° C. After dialysis to remove purine bases, the apurinic acids were recovered by lyophilizing the residues[1].

The three viral apurinic acids showed typical absorption spectra when measured at *p*H 6·8, with maxima at 267–68 mμ and minima at 235–38 mμ; their atomic extinction coefficients ($\varepsilon(P)$ at 260 mμ) varied from 4050 to 4280. Chemical analyses revealed that each contained considerably less nitrogen and more phosphorus than the intact nucleic acids from which they were derived. The low molar ratio of N/P (1·3) indicated that on hydrolysis the bulk of the purines was split off. The content of deoxyribose and glucose was increased to 23·0–23·8 and 7·9–14·0 per cent respectively. The molar ratios of glucose to phosphorus were similar to those found in the viral nucleic acids themselves. This fact indicates that glucose is attached mainly to the acid-resistant part of the nucleic acid molecule.

From the data which have been presented, it is apparent that the nucleic acids of T_2, T_4 and T_6 phage differ from the usual nucleic acids in that they contain not only hydroxymethylcytosine but glucose as well. The amount of this saccharide in the three viral nucleic acids appears to be different. The evidence at hand indicates that glucose is bound to the apurinic part of the molecule, probably in the form of a glucoside of hydroxymethylcytidylic acid. Where more glucose than hydroxymethylcytosine is present, it is apparent that part of the sugar must be linked to the pyrimidine in a different manner, perhaps as a disaccharide. The confirmation of this must await further experimentation.

Since this communication was submitted for publication, a report describing variations in the glucose–deoxyribose ratio in r^+ and r variants of T-even phages has appeared[8].

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