GLUCOSYLATED DNA FROM A TRANSDUCING PHAGE FOR *BACILLUS SUBTILIS*

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Examination of DNA from phage PBS 2, a clear-plaque mutant of phage PBS 1 for *Bacillus subtilis* (Takahashi, 1961; Takahashi, 1963), revealed that the buoyant density of native preparations in a CsCl gradient was 1.722 gm/c.c. This corresponds to a guanine plus cytosine (G + C) content of 62% (Schildkraut *et al.*, 1962 a), although the thermal denaturation temperature (T_m) was 76.5°, corresponding to a composition of 17.5% G + C (Marmur and Doty, 1962). Chemical analysis revealed, in fact, a G + C content of 28%, and also that deoxyuridylic acid replaced the thymidylic acid (Takahashi and Marmur, 1963). When PBS 2 DNA was hydrolysed with perchloric acid and with formic acid, according to the technique of Wyatt and Cohen (1953), very similar base composition values were obtained (Table 1). Ultraviolet absorption spectra of cytosine and guanine obtained from formic acid hydrolysis were identical to those of authentic bases, indicating the absence of an unusual base such as 5-hydroxymethylcytosine. The actual recovery of bases after formic acid hydrolysis was 93% (based on the phosphorus content of hydrolysates). Furthermore, RNA synthesized with the DNA-dependent RNA-polymerase from *Escherichia coli*[1] or

[1]This analysis was performed by Drs. F. M. Kahan and J. Hurwitz, New York University, School of Medicine.

Agrobacterium tumefaciens[2] in which PBS 2 DNA was used as primer, had a G + C base composition of 30%.

TABLE 1

Base Composition (mole %) of PBS 2 DNA[1]

Hydrolysed by	Adenine	Guanine	Cytosine	Uracil
Perchloric acid	35.9	13.4	14.7	35.9
Formic acid	36.4	14.5	13.8	35.4

[1]Average of 4 determinations

However, it was considered unlikely that the anomalous base compositions estimated from buoyant density and T_m value could be attributed solely to the presence of uracil in the DNA molecule in place of thymine. Schildkraut et al., (1962 a; 1962 b) reported that DNA preparations from T-even phages of E. coli possess higher buoyant densities than those expected from their chemically determined base compositions. In this case the anomalous buoyant density was attributable to the presence of 5-hydroxymethylcytosine (Wyatt and Cohen, 1952) and/or glucose (Sinsheimer, 1954; Volkin, 1954; Jesaetis, 1957) in the DNA molecule. These findings prompted a search for a possible sugar constituent in addition to deoxyribose in PBS 2 DNA.

Phage particles were collected and purified by several cycles of centrifugation at 34,800 x g for 60 minutes and at 3,000 x g for 15 minutes. They were then resuspended in a small volume of 0.15 M NaCl plus 0.1 M phosphate buffer, pH 7.0 and treated with lysozyme (100 μg/ml), DNase (10 μg/ml) and RNase (10 μg/ml) at 37° for 30 minutes. The phage particles were collected again by centrifugation and the DNA was extracted by the phenol method of Mandell and Hershey (1960). Purified PBS 2 DNA was hydrolysed with 1 N HCl at

[2]This analysis was performed by Dr. R. M. Hochster and Miss V. M. Chang, Microbiology Research Institute, Canada Department of Agriculture, Ottawa, Canada.

100° for 60 minutes and subjected to paper chromatography using isopropanol-H_2O (160:40) as solvent. Reducing sugars were located by the aniline-diphenylamine reagent (100 ml acetone containing 1 gm diphenylamine and 1 ml aniline, mixed with 10 ml conc. H_3PO_4). A strong spot having an R_f value corresponding to that of glucose was found. This spot disappeared when the neutralized hydrolysate was treated with glucose oxidase prior to chromatography. After HCl hydrolysis, PBS 2 DNA released 0.12 mole of reducing sugar as determined by the method of Park and Johnson (1949) per mole of phosphorus (King, 1932).

Nucleotides from PBS 2 DNA were obtained by enzymatic hydrolysis with deoxyribonuclease and snake venom phosphodiesterase. Mononucleotides were separated by ion-exchange chromatography on Dowex-1 formate and eluted with formic acid and ammonium bicarbonate (Cohn and Bollum, 1961). The four nucleotides (dCMP, dAMP, dUMP and dGMP)[3] thus obtained were hydrolysed with 1 N HCl and the sugar liberated was identified as described above. A reducing sugar showing the same R_f value as glucose was obtained only from dCMP and dGMP fractions.

Free cytosine and guanine were prepared by dephosphorylating the nucleotide fractions with alkaline phosphatase of E. coli and then removing the deoxyribose moiety by use of nucleoside transdeoxyribosidase of Lactobacillus helveticus (McNutt, 1955) in the presence of adenine or cytosine as the deoxyribose receptor. The cytosine and guanine fractions liberated were separated from the other reaction products (adenine deoxyriboside and cytosine deoxyriboside) by paper chromatography with water (pH 10.5, adjusted with 1 N NH_4OH) as solvent. These base fractions released on hydrolysis (in 1 N HCl at 100° for 60 minutes) 0.6 mole and 0.2 mole of reducing sugar per mole of cytosine and guanine, respectively. The sugar, therefore, seems to be attached directly to the cytosine and guanine rings in PBS 2 DNA.

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[3] 5'-phosphates of deoxyribosyl cytosine, adenine, uracil and guanine.

The foregoing results indicate that the DNA from PBS 2 contains a sugar as an integral part of the molecule. The sugar was tentatively identified as glucose from its chromatographic behavior and its sensitivity to glucose oxidase. Although the nature and position of the glucosidic linkage is not clear, the aberrant T_m of the DNA suggests that the sugar may be linked to the cytosine or guanine ring in a manner which would interfere with hydrogen bonding between these bases.

The association of glucose with dCMP is also found (I. Mahler, unpublished results) in the DNA from another B. subtilis phage, SP 8 (Kallen et al., 1962). Determination of glucose showed that approximately 26% of the dCMP fraction was glucosylated. This suggests that the degree of glucosylation may be a characteristic and heritable property of each B. subtilis phage as in the case of T-even phages of E. coli.

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