ABSENCE OF GLUCOSE IN THE CYTOSINE NUCLEOSIDE OF PBS 2 DNA

T.L.V. Ulbricht, G.T. Rogers and I. Takahashi

Twyford Laboratories, London N.W.10., England

and

McMaster University, Hamilton, Canada.

Received December 6, 1965

The DNA from Bacillus subtilis phage PBS 2 has several unusual properties: 1) a higher buoyant density in a caesium chloride gradient and 2) a lower thermal denaturation temperature, than that expected from the chemically determined base composition; 3) it contains 2'-deoxyuridine in place of thymidine (Takahashi and Marmur, 1963a) and 4) the DNA also appears to contain glucose as an integral part of the molecule. The glucose was found in the cytosine and guanine fractions from the DNA (Takahashi and Marmur, 1963b). Reducing sugar was still associated with cytosine and guanine after dephosphorylation of the nucleotide fractions, followed by removal of 2-deoxyribose by transdeoxyribosidation, suggesting that the glucose was attached directly to cytosine and guanine. 5-Hydroxymethylcytosine, to which glucose is attached in the DNA of T-even phages (Ulbricht, 1965) is absent (Takahashi and Marmur, 1963b).

The Ultra-violet spectra of the glucosylated cytosine nucleotide fraction have been found to be as follows: in 0.1 N HCl, $\lambda_{max.}$ 280, $\lambda_{min.}$ 240; in 0.1 N NaOH, $\lambda_{max.}$ 272, $\lambda_{min.}$ 251. These values are virtually identical with those of 2'-deoxy-5'-cytidylic acid itself (Cohn, 1955). After removal of phosphate and deoxyribose, the spectra of the glucosylated cytosine were: in 0.1 N HCl, $\lambda_{max.}$ 275, $\lambda_{min.}$ 237; in 0.1 N NaOH, $\lambda_{max.}$ 281, $\lambda_{min.}$ 252, values which are virtually identical with those of cytosine.

These results made it difficult to suggest a structure for the cytosine nucleoside in which glucose was directly attached to the cytosine, since any possible substitution (at C_4, C_5, exocyclic $-NH_2$ or N_1) leads to a shift in the U.V. spectra of at least a few mμ. Consequently it was decided to investigate the cytosine fraction further.

PBS 2 DNA, prepared as previously described (Takahashi and Marmur, 1963b) was heat-denatured, hydrolysed with Snake Venom phosphodiesterase, and chromatographed on Dowex-1-formate (Cohn and Bollum, 1961). The deoxycytidylic acid fraction was concentrated by evaporation in vacuo at 40°C and then treated with E. coli alkaline phosphatase (200 μg./ml.) in 0.25 M Tris buffer pH 8.0 at 37°C for 18 hr. The reaction mixture was passed through a small column of Dowex-1 x 8 (200-400 mesh, chloride form) to remove unreacted nucleotides. The resultant solution (9.8 O.D. units at 270 mμ) was absorbed on a small charcoal column at pH 5, washed free of salt, and eluted with aq. ethanolic ammonia (quantitative recovery). The solution was chromatographe in the following systems: System 1, saturated aq. ammonium sulphate - isopropanol - 0.1 M phosphate buffer pH 7.2 (79:2:19) on Whatman No. 1 paper, descending; System 2, thin layer chromatography on Kieselgel G (E. Merck) with 75% ethanol. Over 80% of the material absorbing at 270 mμ had R_F values identical with those of authentic 2'-deoxycytidine run concurrently (R_F system 1 0.24; System 2, 0.91) and the U.V. spectra were identical ($\lambda_{max.}$ at pH 1, 280; pH 7, 271; pH 13, 272 mμ). In addition, some coloured material remained at the origin, and there was a weakly absorbing spot of lower R_F (System 1, 0.08; System 2, 0.63). Five O.D. units of the solution were run in System 2, and the material with R_F 0.63 extracted with water. It had no definite peak in the U.V. (end-absorption only). Hydrolysis with 1 N HCl at 100° for 1 hour - conditions which liberated glucose from the DNA - and subsequent chromatography indicated that cytosine, 2'-deoxycytidine and reducing sugar were absent.

These results show that there is no glucose in the cytosine nucleoside fraction from PBS 2 DNA, and that more than 80% of this fraction

actually consists of 2'-deoxycytidine. Independent investigations by Kahan have shown that rigorously purified PBS 2 DNA does not contain glucose; the original material may have been contaminated with teichoic acids (Kahan, 1965).

One of us (I.T.) received support from the Medical Research Council, Canada (MA-1669).

References

Cohn, W.E., quoted by Beaven, G.H., Holiday, E.R., and Johnson, E.A., in Nucleic Acids, ed. by Chargaff, E., and Davidson, J.N., Vol. 1, Academic Press (1955).

Cohn, W.E., and Bollum, F.J., Biochim. Biophys. Acta, 48, 588 (1961).

Kahan, F., private communication to I.T., (1965).

Takahashi, I., and Marmur, J., Nature, 197,794 (1963a).

Takahashi, I., and Marmur, J., Biochem. Biophys. Res. Comm., 10, 289 (1963b).

Ulbricht, T.L.V., in Progress in Nucleic Acid Research and Molecular Biology, ed. by Davidson, J.N., and Cohn, W.E., Vol. 4, Academic Press (1965).