Spectrophotometric Characteristics of Cholera Phage φ2 DNA

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SUMMARY

Purified preparations of cholera bacteriophage φ2 were treated with cold phenol and the nucleic acid examined for its hydroxyapatite chromatographic pattern, thermal denaturation profile, base composition and mol. wt.

The physical, chemical and physiological properties of cholera bacteriophages including the cholera phage φ2 have been studied in detail (Maiti & Chatterjee, 1971; Chatterjee & Maiti, 1973; Maiti et al. 1973, 1977; Maiti, 1978; Maiti & Chaudhuri, 1979). The φ2 phage is of special interest as it can differentiate Vibrio cholerae classical strains from non-agglutinating Vibrio strains (Mukerjee, 1964). It has also been used for epidemiological studies of cholera. The hitherto unreported physico-chemical properties of φ2 DNA have been investigated and the results are presented in this communication.

Cholera phage φ2 belonging to Mukherjee's group II and the host cell Vibrio cholerae OGAWA 154 were used in this study. High titre phage stocks were prepared by the soft agar layer method (Adams, 1959). Purification of the phage was done by alternate cycles of low and high speed centrifugation (Maiti, 1978) followed by hydroxyapatite chromatography (Das & Ghosh, 1977). The purity of the final phage preparation (2 × 10^12 p.f.u./ml) was assessed by u.v. spectrophotometry using a Zeiss automatic double-beam recording spectrophotometer Specord UV VIS. The u.v. absorption spectrum of the purified phage suspension showed a minimum at 238 nm and two maxima at 240 nm and 260 nm with $A_{260}/A_{230} = 1.13 ± 0.02$ and $A_{260}/A_{280} = 1.51 ± 0.12$; molar extinction coefficient at 260 nm is 6800 mol⁻¹ cm⁻¹ (expressed in terms of phosphorus). The optical cross-section ($A_{260}/p.f.u. × 10^{11}$) is 0.68. All the above values are normal and are in agreement with the corresponding values reported for coliphages (Thomas & Pinkerton, 1962; Schito et al. 1966) indicating a high degree of purity of the phage preparation used in the present investigation.

For extraction of φ2 DNA the purified phage particles (2 × 10^12 p.f.u./ml) were suspended either in SSC (0.15 M-NaCl, 0.015 M-sodium citrate, pH 7.0) or in 0.1 M-PB (0.05 M-Na₂HPO₄, 0.05 M-NaH₂PO₄, pH 6.8) with an equal volume of re-distilled phenol, following the method of Silverstein & Goldberg (1976). The type of nucleic acid present in φ2 phage was determined by the diphenylamine (Burton, 1956) and orcinol (Ashwell, 1957) reactions and it was found that φ2 phage contained only DNA. The purified φ2 DNA exhibited a characteristic u.v. absorption spectrum in the range 200 to 300 nm with two maxima at 206 nm and 260 nm and also a minimum at 230 nm with $A_{280}/A_{260} = 1.20 ± 0.15$ and $A_{280}/A_{230} = 1.85 ± 0.05$. The molar extinction coefficient of φ2 DNA with respect to nucleotide phosphorus was 7450 mol⁻¹ cm⁻¹ at 260 nm in 0.1 M-PB which had a phosphorus content of 10.0 ± 0.5%.

Hydroxyapatite chromatography of purified φ2 DNA gave sharp peaks at 0.25 M and 0.3 M-PB when stepwise elution was used. When elution was carried out with a linear molarity gradient of PB varying from 0.1 M to 0.5 M-PB only one peak at 0.27 M-PB was observed.
The thermal transition profile of φ2 DNA under different conditions was obtained by using a Zeiss Spectrophotometer (VSU 2-P) equipped with a thermostaper cell-holder and mLw type U1 thermostatic bath. To measure the temperature, a previously calibrated thermocouple was inserted in the reference cell. The cuvette was heated at the rate of approx. 1 °C/min to a final temperature of 95 °C. The values of thermal denaturation profiles described by the parameters: (i) total hyperchromicity; (ii) the melting temperature (Tm); (iii) the breadth of thermal transition (ΔT2/3); and (iv) a rough estimate of intra- and inter-chain heterogeneity (σ) are presented in Table 1. When φ2 DNA was heated for 10 min in a boiling water-bath and then chilled quickly, the increase in absorbance was 13%, i.e. about 66% of the bases were still non-specifically hydrogen-bonded. The hyperchromicity of φ2 DNA after treatment with NaOH was 35% and after digestion with DNase (Sigma Chemicals, St. Louis, Mo., U.S.A.) it was 32%. When DNA was heated in 0.1 × SSC for to min at 100 °C in the presence of 1% formaldehyde, rapidly chilled in ice and then equilibrated at room temperature, the hyperchromicity was 42%, while it was only 14% in the absence of formaldehyde under identical conditions. If formaldehyde was absent during heating but was added immediately afterwards, the hyperchromicity was about 22%. When φ2 DNA was incubated at 37 °C for 50 min (instead of heating at 100 °C) in the presence of the same amount of formaldehyde, no significant hyperchromicity could be detected, i.e. the absorbance was the same as that of native DNA. Besides the presence of absorption maxima and minima and also the molar extinction coefficients, the form of hydroxyapatite chromatographic pattern, melting curves, change in u.v. absorption spectrum upon heating and formaldehyde reaction are all indicative of a preparation containing native double-stranded DNA. It was further characterized by derivative melting curves (Li & Bonner, 1971) and probability plots resulting in unimodal and approximately Gaussian distribution of G+C pairs.

The base composition of φ2 DNA determined by different spectrophotometric methods is presented in Table 2. According to Mandel & Marmur (1968), the value of Tm (86.5 °C...
in SSC) corresponds to a G+C content of 42.0 ± 0.1 mol %. The G+C content of φ2 DNA was 41.5 ± 0.1 mol % as determined from the formula of Stuart & Ferretti (1975) in 7.2 M-NaClO₄ + 0.002 M-EDTA, pH 7.5. Using highly purified calf thymus DNA (G+C content 42 mol %), *V. cholerae* DNA (G+C content, 48 mol %), *E. coli* B DNA (G+C content, 50 mol %), *Micrococcus lysodeikticus* DNA (G+C content, 72 mol %) as standards, the G+C content of φ2 DNA was 42.2 ± 0.3 mol % obtained from the measured ratio of absorbance $A_{260}/A_{270}$, $A_{240}/A_{260}$ and $A_{280}/A_{275}$ following the method of Ulitzer (1972) and was also found to be 42.1 ± 0.4 mol % as determined by the method of Fredericq *et al.* (1961), from the absorbance ratio $A_{260}/A_{380}$ in 0.1 M-acetic acid. The G+C content of φ2 DNA, obtained from the $T_m$ value, is in good agreement with those determined by the spectrophotometric methods of Ulitzer (1972) and Fredericq *et al.* (1961). This indicates that the phage DNA does not contain any unusual or modified bases in appreciable quantities. The presence of the usual bases was further confirmed by paper chromatographic analysis (Marshak & Vogel, 1951) of the hydrolysate products of φ2 DNA. Quantification was by u.v. light absorption of the eluate containing each base at the wavelength maximum; a corresponding control eluate was used as a blank.

The reassociation kinetic measurements (Britten *et al.* 1974) were performed by optical hypochromicity at 260 nm as well as by hydroxyapatite chromatography and the results obtained are presented in Table 2. For renaturation studies, purified DNA samples in either SSC or in 0.12 M-PB were sonicated (in an MSE sonicator) with 15 s bursts with 1 min cooling intervals (Shugalii & Fonarev, 1975). DNA fragments in SSC (Na⁺ concentration = 0.195 M) or in 0.12 M-PB (Na⁺ concentration = 0.18 M) were heated in a boiling water-bath for 10 min and immediately transferred to a thermostatically controlled spectrophotometer chamber or hydroxyapatite column (bed dimension: diam. 1 cm, height 1.5 cm) fitted with an mLw type U1 thermostatic bath held at 25 °C below $T_m$. The extent of renaturation was followed by measuring the decrease in absorbance at 260 nm as a function of time. DNA concentration was determined from the initial absorbance at 260 nm (Wetmur & Davidson, 1968). The $C_{ot\frac{1}{2}}$ values were calculated and found to be 0.41 ± 0.04. Under identical experimental conditions, *E. coli* B DNA (mol. wt. 2.2 × 10⁶) formed duplexes with an average $C_{ot\frac{1}{2}}$ value of 8.2 ± 0.5, which is in close agreement with that found by Kohne & Britten, 1971. By comparing its $C_{ot\frac{1}{2}}$ value with that of *E. coli* DNA, the mol. wt. of the φ2 DNA was estimated as 1.11 ± 0.05 × 10⁶. When reassociation kinetics of sonicated φ2 DNA and *E. coli* B DNA were performed with hydroxyapatite chromatography, the average $C_{ot\frac{1}{2}}$ values became 0.27 ± 0.02 and 5.2 ± 0.4, respectively. Using $C_{ot\frac{1}{2}}$ values, the average genome size of φ2 DNA was found to be 1.14 ± 0.035 × 10⁶. The mol. wt. of φ2 DNA as obtained from reassociation kinetics is normal and comparable to those of coliphage T₄ (Freifelder, 1970) and Mycobacteriophages butyricum and pheli (Somogyi *et al.* 1975). The reciprocal plot from renaturation kinetics data presented by φ2 DNA did not show the presence of any repeated sequence.

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