Molecular Weight of Bacteriophage PBS2 DNA

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Received for publication 24 November 1975

The molecular weight of bacteriophage PBS2 DNA has been determined by viscoelastic retardation time experiments to be 1.50×10^8 .

The molecular weight of the DNA of bacteriophage PBS1 has been determined by sucrose gradient centrifugation by Hunter et al. (1) to be 1.9×10^8 . In viscoelastic retardation time experiments we have measured the molecular weight of the DNA of PBS2, a clear plaque mutant of PBS1 (5), and found it to be 1.50×10^8 .

For our molecular weight measurements, purified PBS2 was given to us by Steve Clark. (For phage isolation, *Bacillus subtilis* strain 3610 was grown at 32 C in TY broth, infected with PBS2 at a multiplicity of about 2, and shaken for 3 h [Steve Clark, personal communication].) The buffer the phage were in contained, per liter: yeast extract, 1 g; NaCl, 4 g; K_2SO_4 , 5 g; KH_2PO_4 , 1.5 g; Na_2HPO_4 , 3 g; $MgSO_4$, 0.025 g; $CaCl_2$, 0.0011 g; and $FeCl_3$, 0.001 g. The phage titer was 2×10^{12} particles/ml.

Lysates of the phage for retardation time measurements were made in the 0.195 M Na^+ buffers of Klotz and Zimm (4) in the following manner. A 3.8-ml portion of a mixture of equal volumes of glycerol (containing 0.195 M NaCl) and boric acid buffer (4) containing 1% *N*-lauryl-sarcosine was pipetted into a test tube. A 0.1-ml amount of 20 mg of Pronase per ml (Calbiochem) in 0.195 M NaCl was added, and these solutions were mixed on ice. Then, to obtain the desired phage concentration, 0.2 ml (total volume) of PBS2 suspension in phage buffer plus phage buffer without PBS2 was added and mixed into the lysis solution by gently rolling the test tube on ice for 15 min. The mixture containing unlysed phage was pipetted into the chamber, which was at 20 C, and, to lyse the phage, the temperature of the chamber was raised to 50 C for at least 5 h. The temperature of the chamber was lowered to 21.5 C to make readings.

In Fig. 1 is a plot of retardation time τ versus amount of PBS2 suspension used, a quantity which is proportional to DNA concentration. For each experimental point, four measurements were made on a lysate and then averaged. Extrapolation to zero concentration

yields $\tau^0 = 6.4$ s. Adjusting to the standard conditions of 50 C and water as solvent yields $\tau_{w,50}^0 = 0.53$ s. ($\tau_{w,50}^0 = [0.549 T/323 \eta] \tau^0$, where T is the absolute temperature of measurement, η is the viscosity of the solvent at this temperature, and 0.549 is the viscosity of water at 50 C [2].) Using the equation of Klotz and Zimm (3, 4) for the relationship between $\tau_{w,50}^0$ and molecular weight for experiments done in 0.195 M Na^+ : $M = 2.2 \times 10^8 \tau_{w,50}^{0.6}$. We find $M = 1.50 \times 10^8$ for the molecular weight of PBS2 DNA. On Fig. 1 is also plotted τ/η_{rel} versus concentration, in which η_{rel} is the viscosity of the lysis solutions containing the phage relative to that of the lysis solutions without phage. That the τ and τ/η_{rel} plots extrapolate to the same τ^0 value is an additional check on the accuracy of our determination of τ^0 (4).

It should also be noted that the unusual base composition of PBS2 DNA (6) (thymidylic acid is completely replaced by deoxyuridylic acid) should have no effect on the relaxation of the DNA and thus should not affect the determination of the molecular weight by retardation time measurements.

The DNA of the bacteriophage PBS2 is of an ideal size to serve as a standard in molecular weight determinations by viscoelasticity, su-

FIG. 1. Retardation times and retardation times divided by relative viscosity plotted against concentration for lysates of PBS2 in 0.195 M Na^+ at 21.5 C.

crose sedimentation, or electron microscopy. Hence, its true molecular weight is a matter of some interest. In this range of molecular weights (i.e., $\leq 2 \times 10^6$), the viscoelastic technique should give a result accurate to $\pm 10\%$ or less. We thus feel that our measurement of 1.50×10^6 for the molecular weight of PBS2 DNA is a significant improvement over the earlier findings on PBS1 DNA (1), assuming the two DNAs are the same size. However, it is possible that the mutation that yielded PBS2 from PBS1 was a deletion and that the molecular weight of PBS1 DNA is higher than that of PBS2 DNA.

This work was supported by grant GB 34293 from the U.S. National Science Foundation. G. D. L. is a predoctoral fellow of the U.S. National Science Foundation.

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