# PROPERTIES OF DNA OF BACTERIOPHAGE N1, A DNA WITH REVERSIBLE CIRCULARITY

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The DNA of N1 bacteriophage (Naylor and Burgi, 1956), like that of its host, Micrococcus lysodeikticus, has a high guanine plus cytosine content (Scaletti and Naylor, 1959). We report here the results of some further physicochemical studies of the properties of this bacteriophage DNA. The most important results are that the DNA, as prepared by lysis of the phage, is linear but cyclizes when suitably annealed, and that the circular molecules revert to a linear form on heating and quenching. Thus, the molecules contain cohesive ends just as does the DNA of bacteriophage λ. The buoyant density indicates a base composition of 64(± 1)% GC. The molecular weight, as estimated from the sedimentation velocity and by electron-microscope length measurements, is $33(\pm 1.5) \times 10^6$. There appear to be no inherent single-strand breaks in the DNA.

A method is described for positive staining with uranyl ion of DNA samples prepared for electron microscopy by the basic protein film technique (Kleinschmidt and Zahn, 1959). This modification makes shadowing unnecessary.

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## EXPERIMENTAL

N1 phage were grown on Micrococcus lysodeikticus by the method of Scaletti and Naylor (1959). T7 phage were grown on E. coli B/5 (Davison and Freifelder, 1962). T4 phage were a gift from Professor W. Dreyer. Phage were purified by differential centrifugation and, in the case of N1 and T7, by banding in CsCl of density 1.50, plus 0.01 F $MgCl_2$, 0.01 F Tris OH-HCl, pH 7.8 (F means formula weights per liter). The standard phage solvent was 0.2 F NaCl, 0.01 F $MgCl_2$, 0.01 F Tris OH-HCl, pH 7.8.

DNA was prepared from bacteriophage by phenol extraction (Mandell and Hershey, 1960). The solution, after ether extraction, was dialyzed against 0.1 F NaCl, 0.01 F EDTA, pH 7.8, and exhaustively against 0.1 F NaCl, 0.01 F Tris-HCl, pH 7.8. DNA solutions were frozen at -20° for storage. Freezing and thawing did not induce single-or double-strand breaks, even in T4 DNA, as judged by band sedimentation experiments.

Band velocity sedimentation experiments (Vinograd, Bruner, Kent, and Weigle, 1963), were performed with 30-50 microliters of $A_{260} = 0.1$ DNA, with a 30 mm light path. Bulk solutions were 3.0 F CsCl ($\rho = 1.37$), 0.01 F Tris-HCl, pH 7.8; or, for analysis of single-stranded DNA, 0.1 F NaOH

A modified basic protein film technique was used for electron microscopy of the DNA's. The grids were made with 3% w/v parlodion in amyl acetate and without additional carbon coating. After the cytochrome c-DNA mixed film was transferred to the grid, the grid was dipped for 30 sec in a 95% ethanol solution of stain and then for 10 sec in isopentane. The stain solution was a 100-fold dilution of a stock solution of 0.005 F uranyl acetate, 0.05 F HCl in 95% ethanol. The stain solution must be kept in a concentrated form. The DNA, when observed, has the same contrast as that achieved by shadowing techniques. Length histograms were obtained by first photographing circular molecules all at the same instrumental magni-

fication (5000) on a Phillips EM 200 electron microscope. Enlargements onto F-5 printing paper were then measured.

## RESULTS AND DISCUSSION

Grids prepared for electron microscopy by the staining technique described above are comparable to well-shadowed preparations in quality (Fig. 1). The technique allows for a considerable saving of time when using the Kleinschmidt technique.

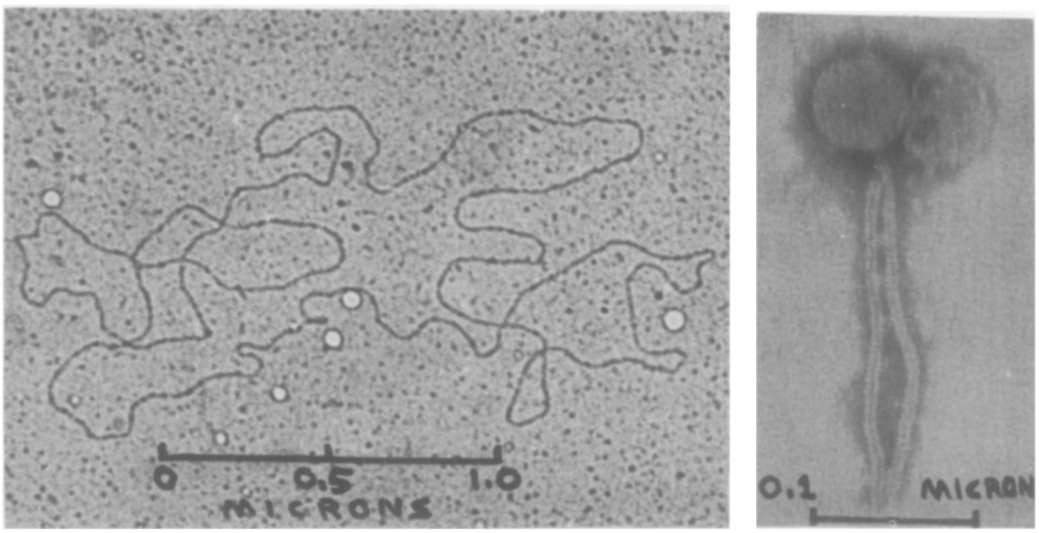


Fig. 1. Electron micrograph of a circular N1 DNA molecule, and of two phage particles (the latter by negative staining with phosphotungstate).

The GC content of N1 DNA has been previously determined to be slightly less than that of M. lysodeikticus DNA (Scaletti and Naylor, 1959). The buoyant densities in CsCl of several markers (Vinograd and Hearst, 1962; Vinograd, Morris, Davidson, and Dove, 1963) and of N1 DNA were measured as: T4 DNA, $\rho = 1.692$; T7, $\rho = 1.703$, 49% GC; N1, $\rho = 1.718$; M. lyso. DNA, $\rho = 1.727$, 72% GC. By interpolation, the GC content of N1 DNA is calculated as $64(\pm 1)\%$.

A sample of N1 DNA was heated in 1.5 M $NH_4Ac$, 0.001 F EDTA, pH 7, to 70° and cooled slowly. By electron microscopy, the sample was seen to

contain more circular than linear molecules (Fig. 1). All further annealing experiments were performed in the salt medium specified plus 0.01 F EDTA, pH 7.8. Failure to add EDTA resulted in 100% irreversible conversion to linear molecules. Band-sedimentation velocity experiments on a sample which by electron microscopy contained both linear and circular molecules showed two bands with relative S values of circular/linear of 1.105. This agrees with the ratio observed for other DNA's (Vinograd, et al., 1965; Hershey, Burgi, and Ingraham, 1963). Thus, band sedimentation may be used to measure the relative amounts of linear and circular molecules.

A sample of N1 DNA at $A_{260}$ = 0.25 had a circular/linear (C/L) ratio of 0.7 with, in addition, a clearly visible dimer peak. After annealing for six hours at 25° in 2.0 F NaCl at $A_{260}$ = 0.1, the sample was almost completely cyclized [(C/L) = 4.0]. Heating for one hour at 80° in 0.1 F NaCl and quenching caused complete conversion of circular molecules to linears. Subsequent annealing at 30° in 2.0 F NaCl for 12 hours results in (C/L) = 3.8, demonstrating the reversibility of the process. Thus, N1 DNA resembles λ DNA (Hershey, Burgi, and Ingraham, 1963) in its ability to reversibly form circles and multimers. The rate of cyclization seems to be faster for the case of N1 than for λ b2b5c (Wang and Davidson, 1966). Preliminary experiments indicate that the melting temperature of the cohesive ends appears to be slightly lower for the N1 case.

The relative sedimentation velocities of linear T7 and N1 DNA's were 1.14 at pH 7.8 and 1.12 at pH 13 after correction for the buoyancy factors. Accepting the value of $25 \times 10^6$ for the molecular weight of T7 DNA, these ratios imply (Crothers and Zimm, 1965; Studier, 1965) a molecular weight of $33 \times 10^6$ for N1 DNA. The runs at pH 13 showed less than 20% single strand breaks in either DNA. Length histograms of λ b2b5c and N1 circular DNA's gave a ratio of lengths of 0.78, which agrees almost exactly (Caro, 1965; MacHattie and Thomas, 1964) with that expected from the sedimentation determination of the molecular weight of N1 DNA.

Phage λ and several closely related temperate phages all have similar or identical cohesive ends. The unrelated temperate coliphage 186 has cohesive ends (Baldwin, et al., 1966). Campbell (1962) has suggested that circularization via the cohesive ends is a first step in lysogenization. The functional significance of the cohesive ends of N1 DNA is not known at present.

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