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Characteristics of the Deoxyribonucleic Acid of Tφ3, a Bacteriophage for *Bacillus stearothermophilus*

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It is shown that the individual strands of bacteriophage Tφ3 DNA are intact and that heat-denatured Tφ3 DNA forms a bimodal distribution in a neutral CsCl density gradient.

Recent reports have indicated that the denatured deoxyribonucleic acid (DNA) of several bacteriophages for *Bacillus stearothermophilus* forms two bands in a CsCl density gradient (2, 13, 15). The DNA in the two bands formed by TP-84 DNA was shown to be complementary, but the polynucleotide chains were not intact (13). The material in the two bands formed by the denatured DNA of phage ST_1 was shown to be complementary by annealing experiments (2). In this paper, the denatured DNA of bacteriophage Tφ3 is reported to form a bimodal distribution in a neutral CsCl density gradient, and the individual polynucleotide chains of the DNA of Tφ3 are shown to be intact.

Phage Tφ3 infects *B. stearothermophilus* ATCC 8005 at 60 C. The head of bacteriophage Tφ3 is 57 nm long and the tail is 125 nm long and 10 nm wide (4).

Tφ3 DNA was prepared from purified Tφ3 (4) by the phenol extraction method of Mandell and Hershey (10) by the procedure of Thomas and Abelson (17). The thermal denaturation profile of Tφ3 DNA in standard saline citrate was typical of double-stranded DNA with a melting temperature (T_m) of 87.5 C. This value corresponds to a guanine plus cytosine (G + C) content of 44.4% (11). A G + C content of 43% was obtained from the buoyant density of Tφ3 DNA [ρ (Tφ3 DNA) = 1.702, assuming a density for *Escherichia coli* DNA of 1.710 and a density for *Micrococcus lysodeikticus* DNA of 1.731] in a CsCl density gradient when the procedures of Schildkraut, Marmur, and Doty (14) were used. The results of a chromatographic base analysis of Tφ3 DNA are presented in Table 1.

Electron microscopy of Tφ3 DNA was performed by the procedures of Kleinschmidt et al. (7). The length of individual Tφ3 DNA molecules was determined by tracing enlargements of developed negatives with a map measure. The molecular weight of the Na^+ salt of each molecule was calculated assuming 1.92×10^6 daltons per μm for DNA (3, 8, 9). Measurements of the length of individual Tφ3 DNA molecules which were prepared either by osmotic shock of the phage or from purified DNA gave similar results. The average length of 33 molecules was 12.1 ± 0.7 μm corresponding to a molecular weight of 23.2×10^6 daltons. An electron micrograph of Tφ3 DNA is presented in Fig. 1.

TABLE 1. *Base composition of Tφ3 DNA*[a]

Base	Mole per cent[b]	Molar ratio
A	29.0 ± 0.6	1.00
G[c]	19.4 ± 0.2	0.69
T	30.8 ± 0.8	1.06
C[c]	20.8 ± 0.3	0.72
	100.0	

[a] Whole phage or phage DNA was hydrolized in formic acid (22) and the resulting bases were separated by paper chromatography with solvent I of Kirby (6). The ultraviolet-absorbing spots were cut out and the bases were quantitatively eluted with 0.1 N HCl. The quantity of each base present was determined from the ultraviolet absorption spectra by utilizing the extinction coefficients given by Bendich (1).

[b] Values are averages of three determinations.

[c] Note that total G + C = 40.2%.

Band sedimentation velocity experiments were performed by the procedures of Studier (16) with a type 2 band forming centerpiece (20) and an initial DNA concentration of 8 μg/ml. Densitometer tracings of the ultracentrifuge films are presented in Fig. 2. The sedimentation coefficients, $S_{20,w}$, for native Tφ3 DNA, alkaline denatured Tφ3 DNA, and neutral denatured Tφ3 DNA were 33.6 ± 0.9 S(4 determinations),

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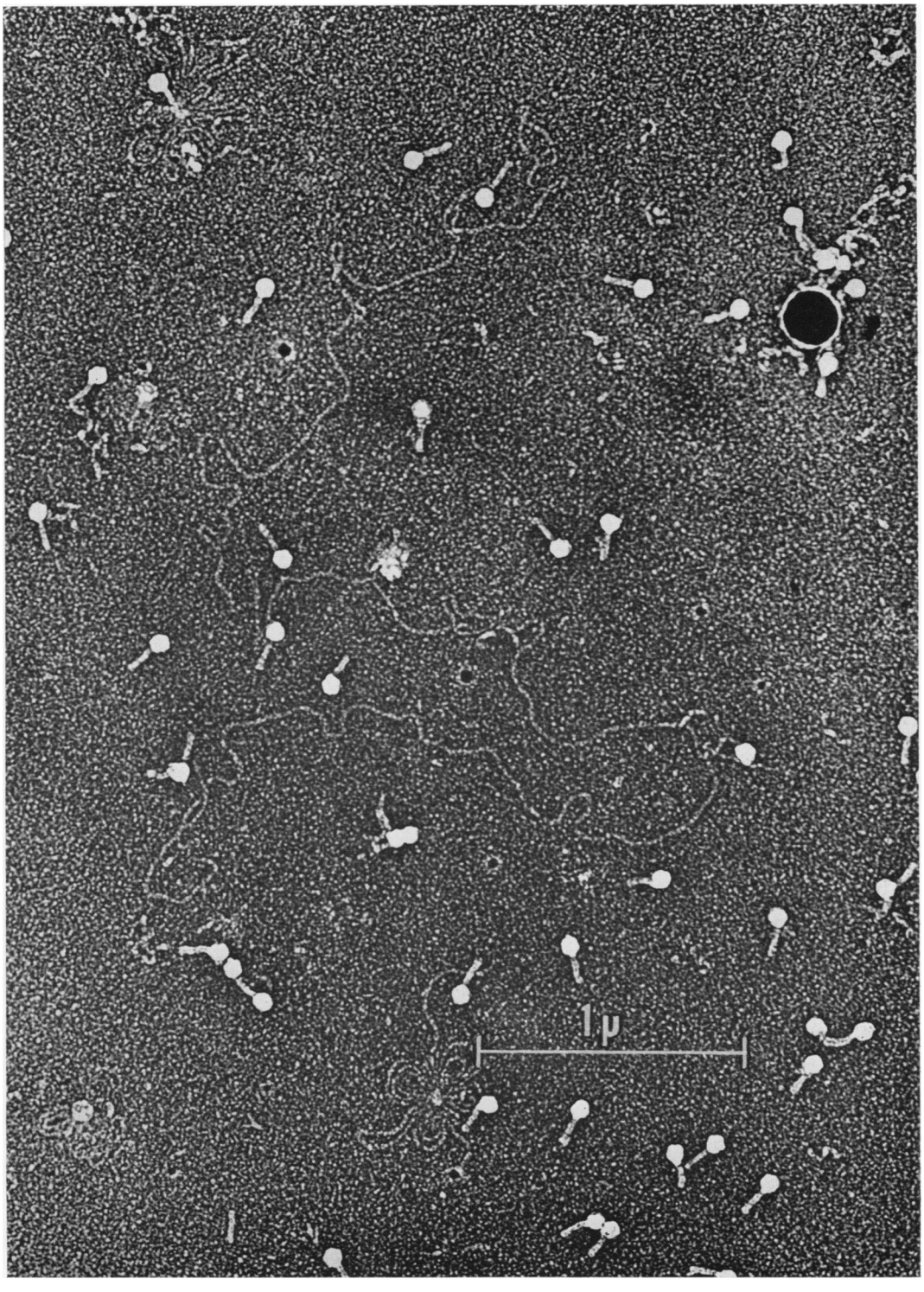

FIG. 1. *Electron micrograph of Tφ3 DNA.*

37.6 ± 0.9S (10 determinations) and 86.4 ± 1.5S (5 determinations), respectively. These sedimentation coefficients correspond (16) to a molecular weight of 28.7 × 10^6 daltons for native Tφ3 DNA, and 13.5 × 10^6 daltons for either alkaline denatured Tφ3 DNA or neutral denatured Tφ3 DNA. The individual polynucleotide chains of Tφ3 DNA are therefore intact.

Band sedimentation velocity experiments that were performed with an initial concentration of 133 μg of freshly isolated Tφ3 DNA per ml (to produce rear sharpening of the band) indicated that less than 5 to 10% of the DNA sedimented as trailing material behind the main band.

The buoyant density of Tφ3 DNA in a CsCl density gradient was determined by the procedure of Vinograd and Hearst (18) assuming the density of *M. lysodeikticus* DNA to be 1.726 (19). Native Tφ3 DNA formed a single band in a neutral CsCl density gradient at a buoyant density of 1.695 g/ml. Heat-denatured Tφ3 DNA formed bands at densities of 1.707 and 1.716 (Fig. 3). After the separated bands of denatured DNA were mixed and annealed in the centrifuge cell, the

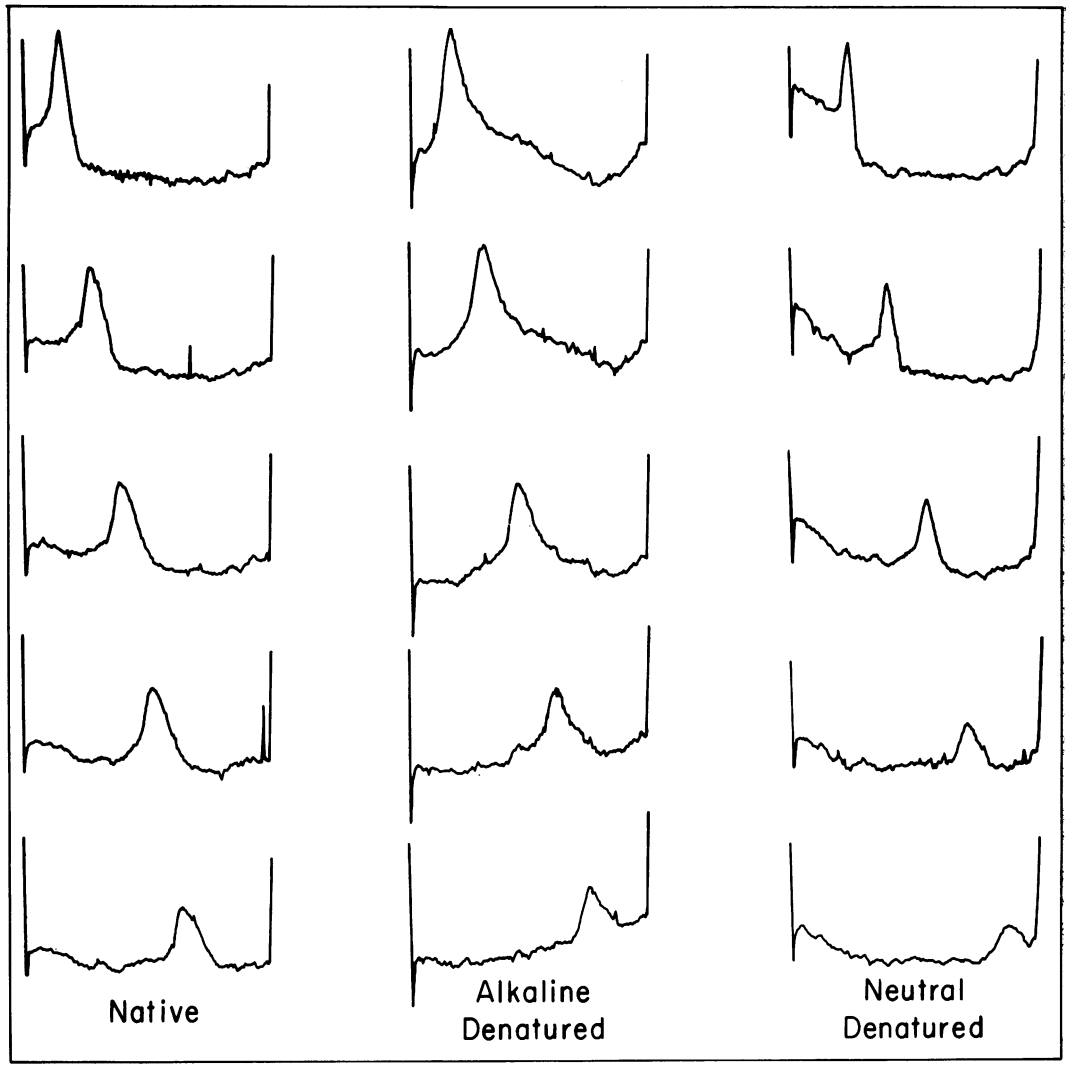

FIG. 2. *Analytical band sedimentation patterns of Tφ3 DNA. The interval between patterns is 16 min for native DNA and alkaline denatured DNA and 8 min for neutral denatured DNA. The sedimentation solvent for native and neutral denatured DNA was 0.1 M NaCl and 0.01 M tris(hydroxymethyl)aminomethane-hydrochloride buffer (pH 7.7 at 25 C); for alkaline denatured DNA it was 0.9 M NaCl and 0.1 M NaOH. The centrifuge was operated at 25,980 rev/min.*

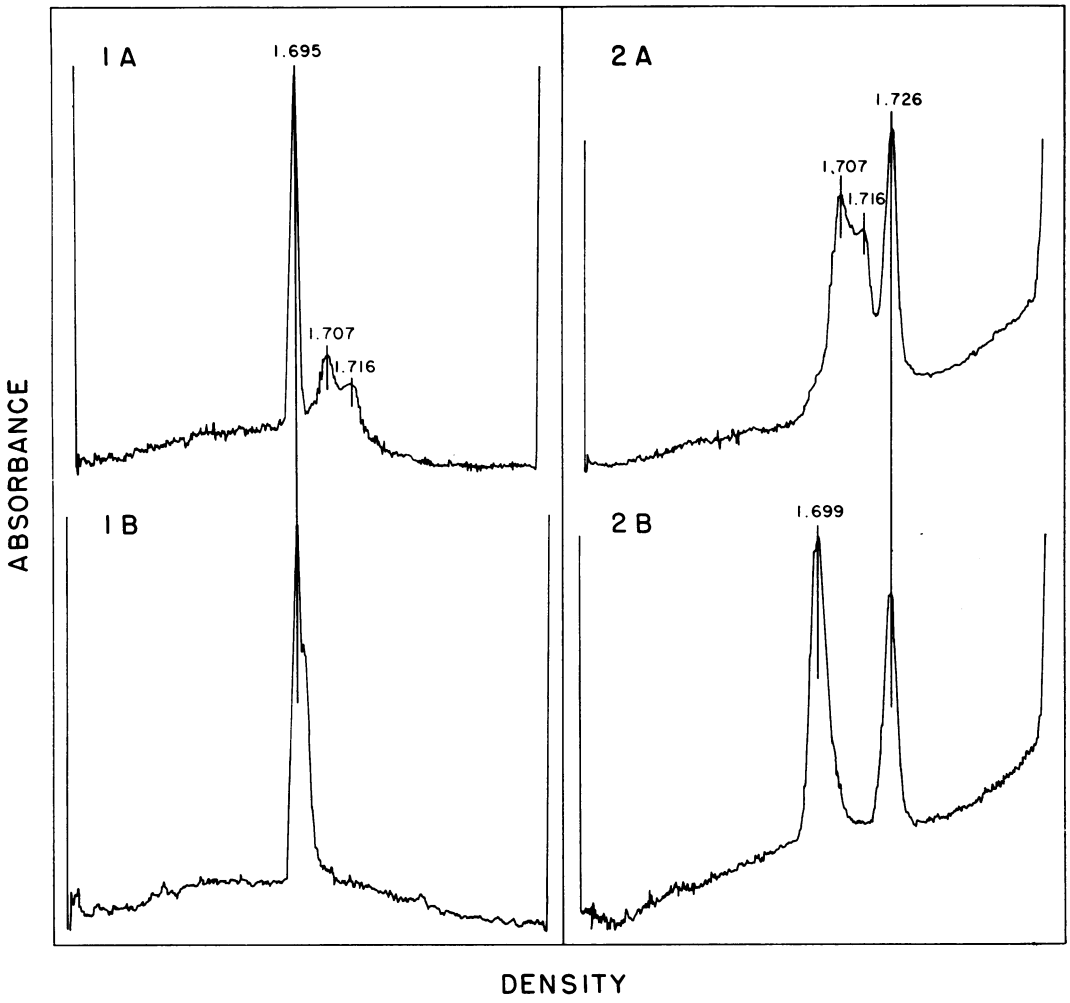

FIG. 3. *Densitometer tracings of CsCl density gradients of heat-denatured Tφ3 DNA: experiment 1A, 0.8 μg of native Tφ3 DNA and 0.8 μg of heat-denatured Tφ3 DNA; experiment 1B, the DNA of experiment 1A was annealed and recentrifuged; experiment 2A, 2.0 μg of heat-denatured Tφ3 DNA and 0.5 μg of native M. lysodeikticus DNA (ρ = 1.726; 19); experiment 2B, the DNA of experiment 2A was annealed and recentrifuged. A 12-mm centerpiece was used in experiment 1, and a 30-mm centerpiece was used in experiment 2. To anneal the DNA for the B part of each experiment, the centrifuge cell was shaken and then incubated at 60 C for 1 hr. The temperature was then reduced to 25 C in 2 hr.*

renatured Tφ3 DNA formed a single band at a density of 1.699 g/ml. The existence of two bands in density gradient experiments on denatured DNA has generally been regarded as evidence that the two strands of DNA have different buoyant densities. The bands formed by heat-denatured Tφ3 DNA are too broad to represent the intact polynucleotide chains. The thermal degradation rate of single-stranded DNA at 100 C (5) is adequate to account for the lack of integrity of the DNA in the heat-denatured bands.

Density gradient studies under alkaline conditions (19) indicated that Tφ3 DNA formed a single band at a density of 1.757 in an alkaline CsCl density gradient.

A comparison of bacteriophage Tφ3 with other bacteriophages for *B. stearothermophilus* indicates several differences. Morphologically, bacteriophage TP84 (12) and Tφ3 (4) are similar. However, the DNA of these two phages differ in buoyant density (13), in the difference in buoyant density of their two strands in a neutral CsCl gradient (13), and in their buoyant behavior in an alkaline CsCl density gradient [TP84 DNA forms two bands (13), whereas Tφ3 DNA forms a single band].

Tϕ3 (4) differs morphologically from phage ST_1 (2) and the sedimentation coefficient of ST_1 DNA (2) is much less than that of Tϕ3 DNA. The buoyant density of the native DNA and the difference in density of the individual strands of the denatured DNA of phage $\phi\mu$4 (15) are different than the corresponding values for Tϕ3 DNA. Bacteriophage TP1 (21) is morphologically distinct from Tϕ3 (4).

I conclude that the two strands of Tϕ3 DNA have different buoyant densities in a neutral CsCl density gradient and that the two strands are intact in the native, alkaline denatured, and neutral denatured forms of Tϕ3 DNA.

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LITERATURE CITED

1. Bendich, A. 1957. Methods for characterization of nucleic acids by base composition, p. 715–723. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. 3. Academic Press, Inc., New York.
2. Carnevali, F., and G. Donelli. 1968. Some properties of a thermophilic phage DNA. Arch. Biochem. Biophys. **125**: 376–377.
3. Caro, L. G. 1965. The molecular weight of lambda DNA. Virology **25**:226–236.
4. Egbert, L. N., and H. K. Mitchell. 1967. Characteristics of Tϕ3, a bacteriophage for *Bacillus stearothermophilus*. J. Virol. **1**:610–616.
5. Eigner, J., H. Boedtker, and G. Michaels. 1961. The thermal degradation of nucleic acids. Biochim. Biophys. Acta **51**: 165–168.
6. Kirby, K. S. 1955. Some new solvent systems for the paper chromatography of nucleic acid degradation products. Biochim. Biophys. Acta. **18**:575–576.
7. Kleinschmidt, A. K., D. Lang, D. Jacherts, and R. K. Zahn. 1962. Darstellung und Langemessungen des gesamten Desoxyribonucleinsaureinhaltes von T_2-bakteriophagen. Biochim. Biophys. Acta. **61**:857–864.
8. MacHattie, L. A., K. I. Berns, and C. A. Thomas, Jr. 1965. Electron microscopy of DNA from *Hemophilus influenzae*. J. Mol. Biol. **11**:648–649.
9. MacHattie, L. A., and C. A. Thomas, Jr. 1964. DNA from bacteriophage lambda: molecular length and conformation. Science **144**:1142–1144.
10. Mandell, J. D., and A. D. Hershey. 1960. A fractionating column for analysis of nucleic acids. Anal. Biochem. **1**:66–77.
11. Marmur, J., and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. Mol. Biol. **5**:109–118.
12. Saunders, G. F., and L. L. Campbell. 1966. Characterization of a thermophilic bacteriophage for *Bacillus stearothermophilus*. J. Bacteriol. **91**:340–348.
13. Saunders, G. F., and L. L. Campbell. 1965. Properties of the deoxyribonucleic acid of the thermophilic bacteriophage TP-84. Biochemistry **4**:2836–2844.
14. Schildkraut, C. L., J. Marmur, and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its buoyant density in CsCl. J. Mol. Biol. **4**:430–443.
15. Sheldrick, P., and W. Szybalski. 1967. Distribution of pyrimidine "clusters" between the complementary DNA strands of certain *Bacillus* bacteriophages. J. Mol. Biol. **29**:217–228.
16. Studier, F. W. 1965. Sedimentation studies on the size and shape of DNA. J. Mol. Biol. **11**:373–390.
17. Thomas, C. A., and J. Abelson. 1966. The isolation and characterization of DNA from bacteriophage, p. 553–561, *In* G. L. Cantoni and D. R. Davies (ed.) Procedures in nucleic acid research, Harper & Row, Publishers, New York.
18. Vinograd, J., and J. E. Hearst. 1962. Equilibrium sedimentation of macromolecules and viruses in a density gradient. Fortschr. Chem. Org. Naturst. **20**:372–422.
19. Vinograd, J., J. Morris, N. Davidson, and W. F. Dove, Jr. 1963. The buoyant behavior of viral and bacterial DNA in alkaline CsCl. Proc. Nat. Acad. Sci. U.S.A. **49**:12–17.
20. Vinograd, J., R. Radloff, and R. Bruner. 1965. Band-forming centerpieces for the analytical ultracentrifuge. Biopolymers **3**:481–489.
21. Welker, N. E., and L. L. Campbell. 1965. Induction and properties of a temperate bacteriophage from *Bacillus stearothermophilus*. J. Bacteriol. **89**:175–184.
22. Wyatt, G. R., and S. S. Cohen. 1953. The bases of the nucleic acids of some bacterial and animal viruses: the occurrence of 5-hydroxymethylcytosine. Biochem. J. **55**:774–782.