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Length of DNA from Bacteriophage T2 and its mode of Packing inside the Phage Head

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Osmotic shock was used to extract DNA from the bacteriophage T2 by disruption of the phage head in a single step. The different conformations of the molecules thus released were studied under the electron microscope. The distribution of lengths of the molecules was measured. The most possible mode of packing of DNA inside the phage head has been discussed.

Key Words: Electron microscopy, T2 DNA

Introduction

Kleinschmidt et al. (1962) first used osmotic shock to extract the DNA from phage T2. In this method, later called 'one-step' release method (Kleinschmidt 1968), the phages were first incubated in a high molar salt solution and then released onto water. Osmotic shock was sufficient to disrupt the phage head and release DNA which was instantaneously fixed to a protein film already spread on the water surface. With this method the conformation of DNA immediately after its release from the phage head could be studied. The contour length and hence the molecular weight of DNA of many phages could be determined. The method had the other advantage that the molecules were not exposed to alkali, phenol or other chemicals before electron microscopy.

In the present work, this one-step release method was used to extract DNA from the T2 phage. The pattern of the released DNA was carefully studied in relation to the disrupted phage head. The length distribution of the released DNA was measured. From these observations some information has been obtained about the mode of packing of DNA inside the phage head.

Materials and Methods

DNA from wild type T2 phages was released by the one-step release method of Kleinschmidt (1968). In the spreading solution, the concentrations of phage, ammonium acetate and cytochrome *c* were about 5×10^9/ml, 5M and 0.01%, respectively. The spreading solution was incubated at 4°C for 1 hr prior to spreading. The

hypophase was either double distilled water or 0.25M ammonium acetate. The volume of the spreading solution was 50 μl while that of the hypophase was about 200 ml. Hence the dilution was about 4,000 fold.

Collodion-coated 200-mesh copper grids were used for picking up DNA-protein monolayer complex. DNA bound protein monolayer was stained with uranyl acetate (Davis et al. 1971) which was followed by shadowing with Pt-Pd (80-20%). Electron micrographs were made with a Siemens Elmiskop 101B at 80 KV. Contour lengths were measured on highly magnified optical images of the negatives with a map measurer.

Results and Discussions

In all electron micrographs the T2 phage ghost was visible with its tail connected with the disrupted phage head. There was no evidence of an occasional DNA release through the phage tail as seen in the case of lambda phage (Caro 1965).

The following patterns of DNA release by the osmotic shock were noticeable in this case.

(a) The released DNA formed a rosette-like structure with the phage ghost at the centre of the rosette. Both ends of DNA molecules were free and clearly visible (figure 1).

(b) DNA formed a rosette-like structure with a dense body at the centre. The empty phage ghost was sometimes seen at a distance from the centre of the rosette (figure 2). Occasionally the ghost was not visible in the micrograph.

(c) A stretched DNA molecule with two ends clearly visible, with the phage ghost lying superposed somewhere on the molecule (figure 3). Sometimes the ghost was not present in the micrograph.

Table 1 shows the total number of molecules in different groups observed in water and in NH_4Ac hypophases with the average length in each group.

From table 1 it appeared that groups (b) and (c) occurred more frequently in NH_4Ac hypophase than in water where the majority of the molecules were in group (a). The average length of the molecule in group (b) was greater than that in group (a) and that in group (c) was greater than that in group (b). In water hypophase, only one molecule was observed in group (c). Hence a correct estimate of the average length in this group could not be made.

Table 1 also showed that in any group, the average length in the water hypophase was greater than that in the ammonium acetate hypophase. This observation was consistent with the previous finding that there was a general shrinkage of the DNA molecules lengthwise in going from water to ammonium acetate hypophase (Lang et al. 1967, Inman 1967). The maximum length observed in double distilled water hypophase was 59.5 μ while that in 0.25M ammonium acetate was 52 μ. For water the previously observed maximum length was 58.5 μ (Kleinschmidt et al. 1962), while that for 0.20M ammonium acetate was 52.0 μ (Lang 1970). On the basis of the maximum length for water hypophase, assuming a mass per unit length of 2.06×10^6 daltons/μ (Lang 1970), the molecular weight of T2 DNA was 123×10^6 daltons. The present molecular weight was about 10% less than that deduced from hydrodynamic method (Studier 1965) and from star-counting autoradiography (Leighton & Rubenstein 1969).

How this long DNA is packed inside the phage head merits some consideration. Rich-

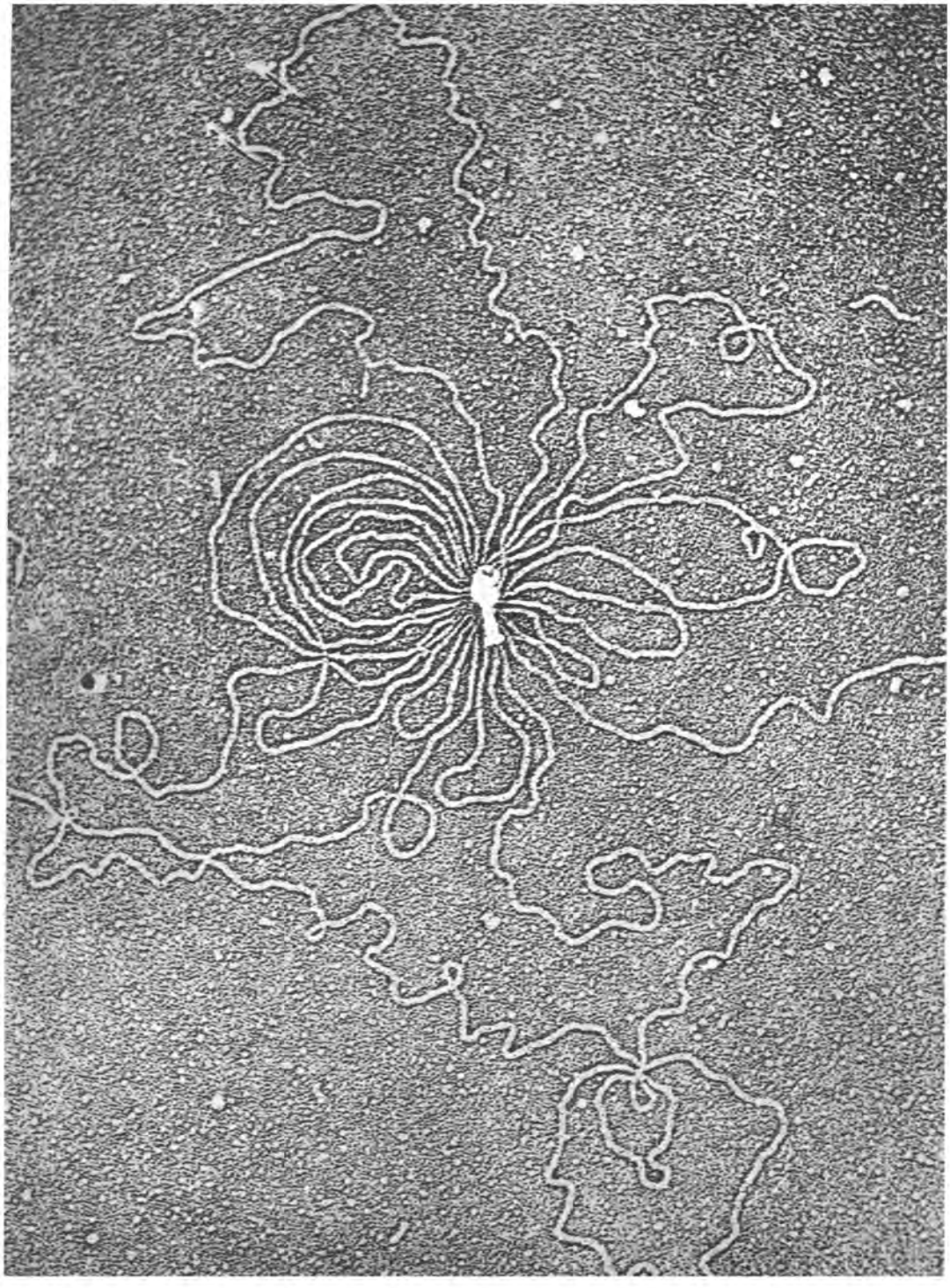

Figure 1 The rosette formed by the DNA released from T2 with the phage ghost at the centre. See group (a), table 1. Specimen stained and shadowed. (× 52,000)

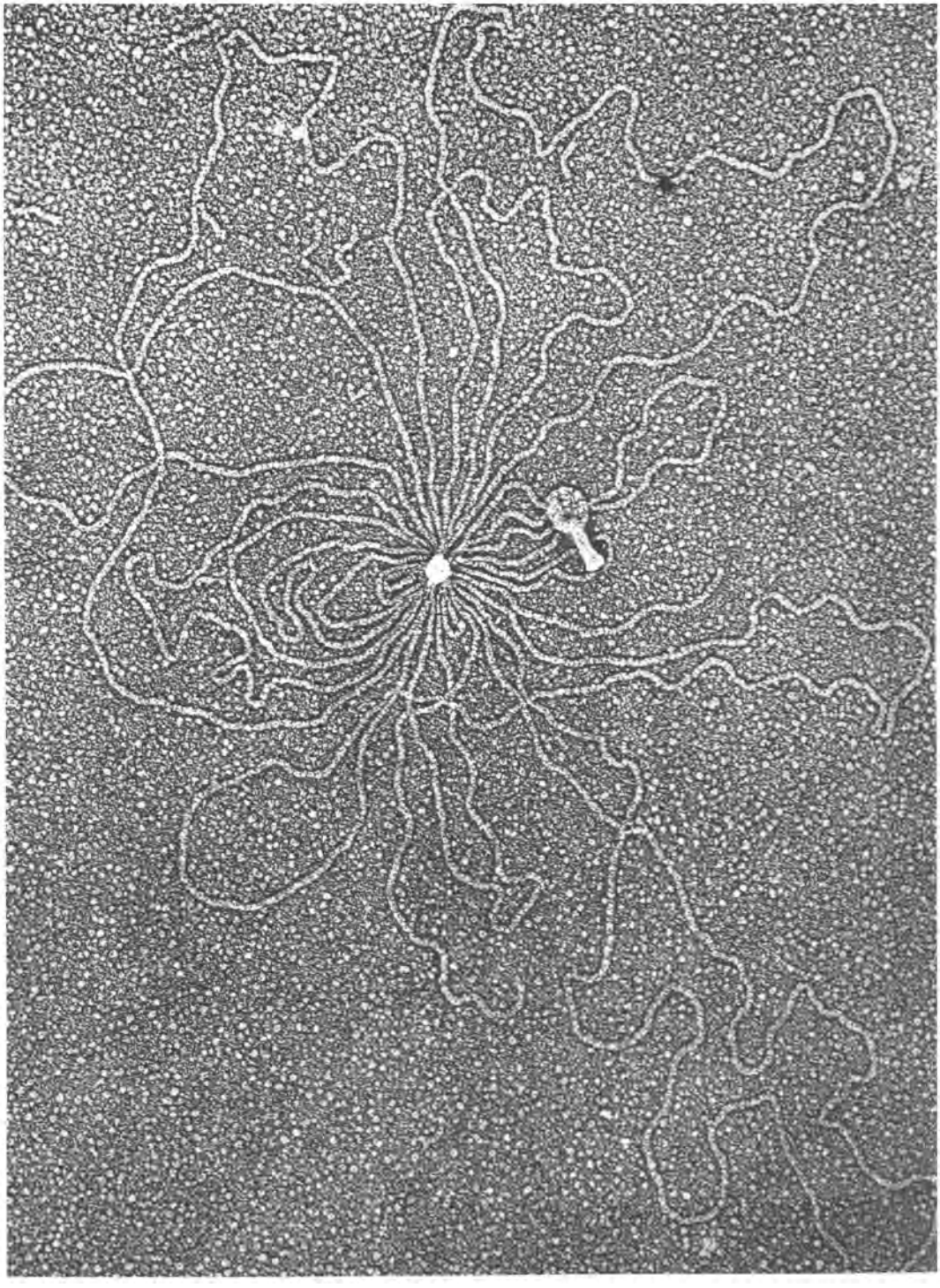

Figure 2 Rosette formed with a dense body at the centre with the phage ghost lying at a side. See group (b), table 1. Specimen stained and shadowed (× 50,000).

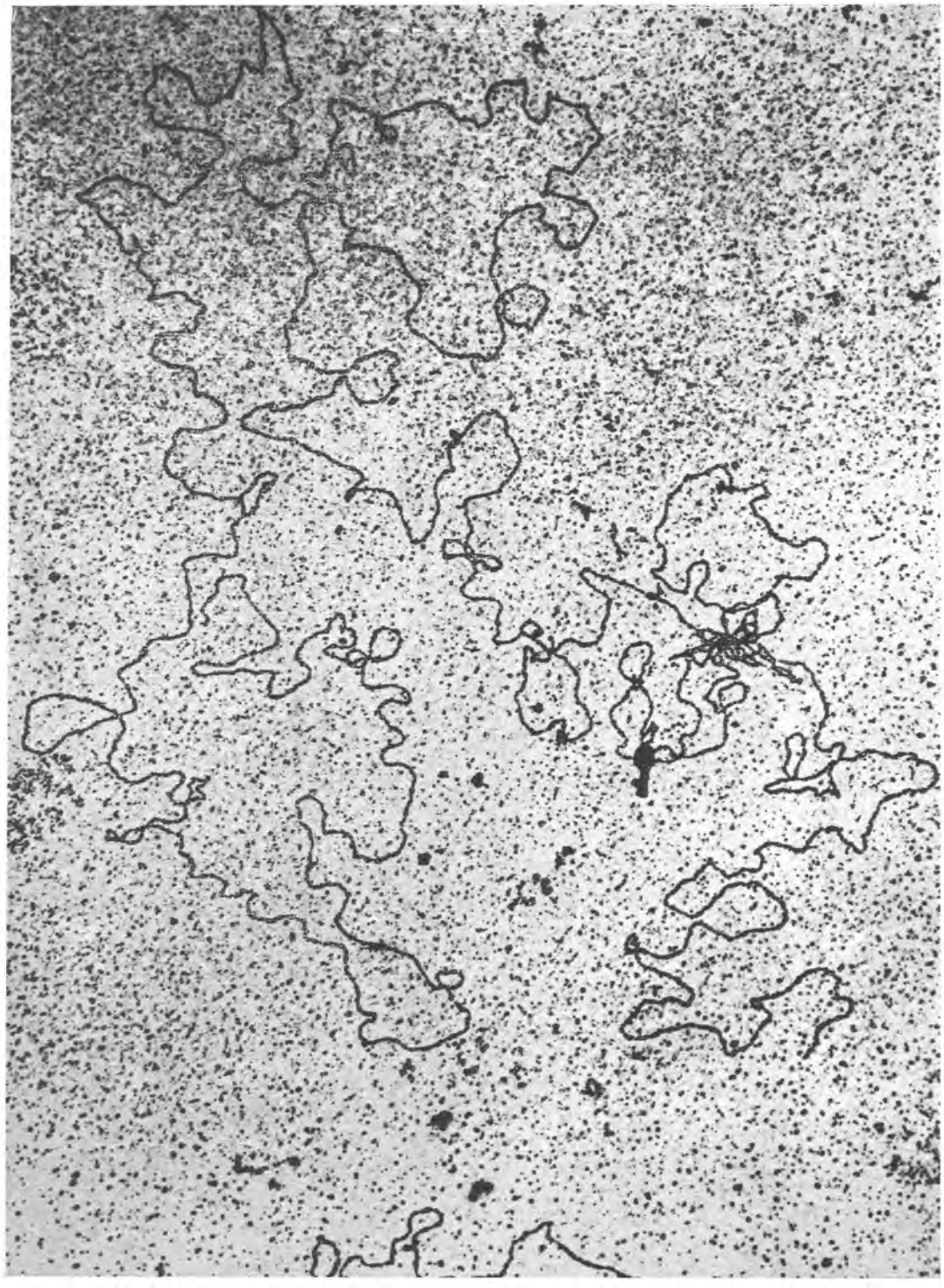

Figure 3 A relaxed DNA molecule with the phage ghost. See group (c), table 1. Stained but unshadowed molecule (× 38,000).

ards et al. (1973) proposed a ball-like arrangement of DNA inside T4 phage with centre-to-centre spacing of DNA about 25 to 30 Å. X-ray data also revealed a spacing of 28 Å (Earnshaw et al. 1976). Assuming a closely packed structure with an inter-helical distance of 25 Å, the minimum volume *(v)* occupied by a 59.5 μ long DNA would be 2.92×10^8 Å^3. The head of T2 is an icosahedron. The dimensions of the length and breadth of the icosahedron as deduced by different workers were as follows : 950 × 650 Å (Williams & Fraser 1953), 1190 × 800 Å (Cummings & Kozloff 1960) and 1000 × 740Å (Branton & Klug 1975). The dimension of the phage head as found by Cummings and Kozloff (1960) and also supported by X-ray data (Klimenko et al. 1967) might be taken as 1190 × 800 Å. From our measurements on negatively stained preparations of empty phage head, the thickness of protein coat was found to be about 35 Å. The internal volume *(V)* of phage head available for the packing of DNA was deduced to be 3.03×10^8 Å^3*. Hence $v/V = 0.96$ or 96% of the available space inside the phage head was filled up with DNA.

From the many symmetrical rosette-like structures observed immediately after rupture of the phage head, it was reasonable to conclude that the molecule was arranged in a rotationally symmetric form inside the phage head. A few turns at the core of such symmetrically packed DNA, might not be able to separate from each other within the short time available between bursting of the phage head and fixation of DNA to the protein film. In such a case, subsequent staining and shadowing would give rise to the dense body at the centre of the rosette (figure 2). When the ghost was present at the centre of the rosette (figure 1), it was natural to expect that a larger number of turns at the core would remain associated with the ghost. It was also noticed that the average length in group (b) was greater than that in group (a) both for water and ammonium acetate hypophases (table 1). This made the above couclusion most probable and also consistent with a ball-like arrangement of DNA inside the phage head.

Table 1 *Analysis of the coli phage T2 DNA contour lenth spread on two different types of hypophases**

	Water hypophase		NH_4Ac hypophase	
	No. of molecules	Average length of DNA (μ)	No. of molecules	Average length of DNA (μ)
a (Fig. 1)	10	46	4	36.3
b (Fig. 2)	5	52.6	13	43.9
c (Fig. 3)	1	51.3	3	50.2

* T2 phage DNA was spread by single-step release technique on either water or 0.25M ammonium acetate hypophase. The technique of single-step release was described in Materials and Methods. The DNA molecules were grouped as *a*, *b* and *c* depending on their pattern of release. The figures in parenthesis refer to the electron micrographs. Figures 1 and 2 were of DNA released on water hypophase. Figure 3 was of DNA released on NH_4Ac hypophase.

A study of the DNA release pattern showed that out of a total of 55 molecules released in a single-step, 80% had two free ends. This could be explained if in the ball- or spool-like structure in which DNA was packed, both ends of the DNA were at the periphery and could easily get detached from the main body.

* This was deduced from the relation $V = 0.718\ b^3l - 0.322\ b^3$ where l and b were the length and width of the icosahedron (Klimenko et al. 1967).

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