Hexagonally Packed DNA within Bacteriophage T7 Stabilized by Curvature Stress

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ABSTRACT A continuum computation is proposed for the bending stress stabilizing DNA that is hexagonally packed within bacteriophage T7. Because the inner radius of the DNA spool is rather small, the stress of the curved DNA genome is strong enough to balance its electrostatic self-repulsion so as to form a stable hexagonal phase. The theory is in accord with the microscopically determined structure of bacteriophage T7 filled with DNA within the experimental margin of error.

INTRODUCTION

An important topic in biophysical theory is the explanation of how very long DNA can be packed into the tiny confines of biological compartments. Although the mode of packaging may vary from one type of cell to the next (prokaryotic, eukaryotic, phages, plant and animal viruses), the theoretical ideas may be generic in the sense that we must come to terms with the marked elastic stiffness of the DNA helix, very powerful interhelix interactions, and the considerable reduction of entropy involved in inserting a DNA genome into a compact space. In general, the a priori problem of confining a DNA genome is very difficult: a DNA persistence segment is highly anisometric, so the self-interaction of close-packed DNA is not pairwise additive at all. For this reason, precise quantitative information is needed as input to be able to formulate tractable analytical theories. Here, significant progress in the elucidation of the conformation of encapsidated DNA within bacteriophage T7 (Cerritelli et al., 1997) allows us to develop a physical picture of the way curvature stress competes with electrostatic forces in establishing the hexagonal packing of the DNA.

The modeling of DNA within phages has a long history (Murialdo and Becker, 1978). Preliminary evidence for hexagonal packing of the genome within bacteriophages T2 and T7 was already adduced four decades ago (North and Rich, 1961). Further x-ray and cryoelectron microscopy studies led to a variety of proposals for the arrangement of the DNA chain within phage heads (Earnshaw and Harrison, 1977; Adrian et al., 1984; Lepault et al., 1987). The DNA genome has been envisaged as being wound into a spool (Earnshaw and Harrison, 1977; Riemer and Bloomfield, 1978; Hendrix, 1978; Harrison, 1983; Garashvili et al., 1991), a ball (Richards et al., 1973; Earnshaw and Harrison, 1977), a liquid crystal with hairpins or folds (Earnshaw and Harrison, 1977; Black et al., 1985; Serwer, 1986; Sun and Serwer, 1997; Serwer et al., 1997), and a liquid crystal with defects (Lepault et al., 1987). Tests have been devised for discriminating among the various models (Widom and Baldwin, 1983; Haas et al., 1982; Liu et al., 1981; Mendelson et al., 1992). Of course, models for bacteriophages need not be universal.

A lot of attention has been devoted to determining the structure of bacteriophage T7. Its proteinaceous morphology is well established (Steven and Trus, 1986). Attempts have been made to ascertain the nature of the DNA packing: by x-ray scattering (Stroud et al., 1981; Ronto et al., 1988), by circular dichroism (Karasev and Dobrov, 1988), by the binding kinetics of ethidium (Griess et al., 1986), by DNA-capsid cross-linking studies (Serwer et al., 1992), and by negative staining experiments (Serwer et al., 1997). The interpretation of these experiments was never conclusive, but a new study of well-aligned T7 phages does appear to give a concrete picture of the genome organization (Cerritelli et al., 1997). The alignment is kept under control, because the tailless mutant has heads that are precisely aligned in thin ice films. Cryoelectron micrographs show unambiguously that the DNA coil is wrapped into a coaxial spool; the concentric DNA rings are perpendicular to the phage axis. The DNA spacing was measured accurately from the optical diffraction patterns as a function of the DNA contour length (Cerritelli et al., 1997). We are now in a position to formulate and check a concrete theory of the hexagonal packing.

In previous theoretical work on bacteriophages (Riemer and Bloomfield, 1978; Garashvili et al., 1991), the bending energy of encapsulated DNA was computed numerically by summing over all DNA winds. Here, I perform the summation analytically in a continuum approximation, as introduced earlier in analyses of close-packed toroidal condensates (Ubbink and Odijk, 1995, 1996). The nature of the curvature stress now comes to the fore explicitly in the case of bacteriophage T7: there is a tightly wound inner region in the DNA spool whose energy turns out to be large enough to compete with the self-repulsion of the coiled genome. The DNA coil acts like a broken spring, piled up against the outer rim of its compartment within a watch. In DNA condensates, the bending energy generally competes with

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the surface energy to define the particle shape (Ubbink and Odijk, 1996); the DNA spacing is virtually unperturbed by the curvature stress. The packing problem for phages differs markedly from this case, for the phage coat restrains the global shape and so the bending stress is able to confer stability on the DNA spool within the bacteriophage. Possible mechanisms involved in the energetics of DNA packaging have been discussed by Black (1989). Elastic expansion of the procapsid during the insertion of DNA is thought to play a minor role. Furthermore, bacteriophage T7 is devoid of polyamines (Steven and Trus, 1986). Here, I focus solely on balancing curvature stress against the DNA self-energy that is of electrostatic origin. If we enlarge the cylindrical hole in the middle of the DNA spool, we enhance the packing of the DNA. The self-energy will increase because the DNA helices are packed closer together, whereas the DNA curvature energy decreases, because the average curvature of the DNA winds becomes smaller. Hence, we wish to minimize the total energy with respect to the interaxial spacing of the DNA within the spool.

In developing biophysical theories involving DNA, we recall that it is impossible to model the system at hand with an accuracy better than ~10%. It then makes sense to employ continuum approximations, even for biosystems on a mesoscale (Ubbink and Odijk, 1995, 1996). I show that the curvature stress of hexagonally coiled DNA within bacteriophage T7 is directly related to the osmotic pressure of the DNA with respect to that of the buffer surrounding the phage. Although the connector may be vital to the dynamics of packaging the DNA into the phage (Valpuesta and Carrascosa, 1994), once the DNA is inside, it is supposed that stability of the hexagonal lattice arises from physical forces alone. Next, the osmotic pressure is related to that derived on the basis of the cell model in the Poisson-Boltzmann approximation (Oosawa, 1971). The curvature stress does happen to balance the electrostatic pressure for plausible values of the phage dimensions, so this mechanism is argued to be a likely candidate for the stability of the DNA spool.

DNA CURVATURE ENERGY

Within bacteriophage T7, the genome is wound into a spool in the manner shown in Fig. 1. The principal enclosure is approximated by a sphere of radius $R = 27.5$ nm. Within, there is a cylindrical proteinaceous core of length R and radius $B = 10.5$ nm, which is affiliated with the connector and from which DNA is excluded in the equilibrium packaged state. Bacteriophage T7 is not built like this exactly (Serwer, 1976; Steven and Trus, 1986), but our simplified model is precise enough to illustrate the physical principle we focus on. Within the phage, the DNA is hexagonally packed down to an inner radius E with a uniform interaxial spacing H between adjacent windings; the rings are perpendicular to the phage axis of symmetry. We write the total

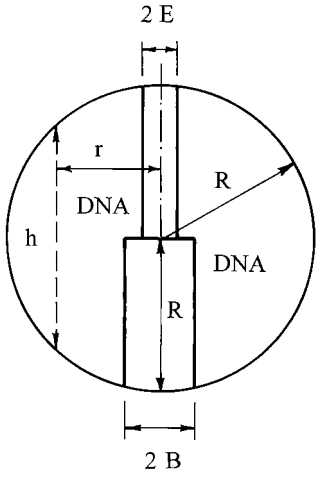

FIGURE 1 Head of bacteriophage T7. The phage coat is assumed to be a sphere of radius R. DNA is excluded from the cylindrical core of radius B and length R. The inner radius of the DNA spool is E. The axis of cylindrical symmetry is in the plane of the paper, and the DNA winds in the spool are perpendicular to the axis.

free energy of the DNA coil as

$$F_{tot} = F_{tot}(H) = F_s + Lf_{int} + U_b \tag{1}$$

The surface term F_s denotes the interaction of the negatively charged DNA adhering to the protein coat and protein cylinder, which are positively charged. The second term is a free energy of interaction, which is extensive, i.e., proportional to the contour length L of the linear genome. It represents the self-interaction of the DNA helix as if it were aligned in a perfectly straight, hexagonal lattice. It is assumed that there are no polyamines enclosed within the lattice. The bending energy

$$U_b = \tfrac{1}{2} Pk_BT \int_o^L \mathrm{d}s\, R_c^{-2}(s) \tag{2}$$

is powerful enough to compete with the repulsive interaction f_{int} per unit length, provided the inner radius E is small enough. In Eq. 2, P is the DNA persistence length, k_B is Boltzmann's constant, T is the temperature, and $R_c(s)$ is the DNA radius of curvature at contour position s. There is another condition on E ($E \gg H$): the decomposition described by Eq. 1 is valid only when the inner radius of curvature (which is close to E) is not too small; the effect of undulatory entropy will be discussed below. Our purpose is to minimize F_{tot} with respect to the spacing H to establish the stability of the hexagonal DNA configuration. It will be assumed that R is a negligible function of H (Black, 1989). We first compute the DNA curvature energy as a function of the spacing.

For a hexagonal lattice it is known that the area of the unit cell is $S = 3^{1/2}H^2/2$. Hence, for a DNA spool within the configuration displayed in Fig. 1, Eq. 2 is rewritten in a

continuum approximation over all winds:

$$U_b(H) = \frac{\pi P k_B T}{S}\int_B^R dr\,\frac{h(r)}{r} + \frac{\pi P k_B T}{2S}\int_E^B dr\,\frac{h(r)}{r} \quad (3)$$

The phage and spool are axisymmetrical, the radial coordinate is r, and the spool height is $h(r) = 2\,(R^2 - r^2)^{1/2}$ (see Fig. 1). We also need E as a function of the spacing H. This is given by a volume constraint,

$$\pi R E^2(H) = \frac{4\pi R^3}{3} - \pi R B^2 - SL \quad (4)$$

This expression is not exact, for the two cylinders in Fig. 1 have rounded ends. The small terms neglected are, however, only of order B^4/R and E^4/R, and may be disregarded within the limitations of a mesoscopic theory as explained in the Introduction. The integrals occurring in Eq. 3 can be evaluated explicitly (Gradshteyn and Ryzhik, 1980):

$$I(E, B) \equiv \int_E^B dr\,\frac{(R^2 - r^2)^{1/2}}{r}$$

$$= \tfrac{1}{2} R \ln\frac{[R - (R^2 - B^2)^{1/2}][R + (R^2 - E^2)^{1/2}]}{[R - (R^2 - E^2)^{1/2}][R + (R^2 - B^2)^{1/2}]} + (R^2 - B^2)^{1/2} - (R^2 - E^2)^{1/2} \quad (5)$$

OSMOTIC PRESSURE WITHIN THE BACTERIOPHAGE

We now obtain the spacing H by minimizing Eq. 1 with respect to H:

$$\frac{df_{int}}{dH} \simeq -\frac{1}{L}\frac{dU_b}{dH} \quad (6)$$

In a treatment correct to the leading order, the surface term F_s may be neglected for a bacteriophage of mesoscopic dimensions, in line with the argumentation put forward for DNA condensates (Ubbink and Odijk, 1995). A discussion of the neglect of various other higher order terms is also given by Ubbink and Odijk. The left-hand side of Eq. 6 simply represents the force acting on the hypothetically straightened hexagonal lattice. But this, in turn, is connected with the osmotic pressure π_{os} of a straight DNA lattice with respect to an ionic buffer:

$$\frac{df_{int}}{dH} = -3^{1/2}\pi_{os}H \quad (7)$$

Note that f_{int}, the free energy per unit length, is three times the interaction of a pair of DNA rods (each rod has six neighbors, but one has to divide this by 2 to avoid double counting). Equations 6 and 7 imply that the curvature stress should be a simple measure of the osmotic pressure if our picture of the phage packing is in accord with reality:

$$\frac{dU_b}{dH} \simeq 3^{1/2}\pi_{os}HL \quad (8)$$

The derivative of the bending energy is now obtained from Eqs. 3 and 4:

$$\frac{dU_b}{dH} = -\frac{2U_b}{H} + \frac{PLh(E)k_BT}{2RHE^2} \quad (9)$$

At this stage, it is useful to investigate the magnitudes of various terms. We know that $E \ll R$ (Cerritelli et al., 1997), but also $B^2 \ll R^2$ from the dimensions of the phage quoted above. Accordingly, the integral $I(E, B)$ in Eq. 5 reduces to $R\ln(B/E)$ to the leading order, and the other integral $I(B, R)$ in Eq. 3 reduces to $R\ln(R/B)$, likewise, to the leading order. Hence the bending energy (Eq. 3) is $U_b = \mathbb{O}(PRk_BT/H^2)$ to within logarithmic terms. But the second term on the right-hand side of Eq. 9 is of magnitude $k_BT\,PR^3/H^3E^2$, so it overwhelms the first (the inequality $E \ll R$ implies $h(E) \simeq 2R$ and $SL = \mathbb{O}(R^3)$ from Eq. 4). Finally, the osmotic pressure is given in terms of the inner radius of the DNA spool,

$$\pi_{os} \simeq \frac{Pk_BT}{3^{1/2}H^2E^2} \quad (10)$$

This result is not exact, in view of the neglect of a variety of very small higher order terms. Nevertheless, it may be viewed as a reliable expression within the context of a mesoscopic model.

The analysis above has shown that Eq. 9, which is a measure of the bending stress, is dominated by the curvature in a thin annular region surrounding the inner hole of radius E. The tightly wound DNA herein pushes against the bulk of the DNA spool and stabilizes the entire hexagonal structure. Thus the approximation of a constant H throughout the DNA spool is not so bad, because the curvature stress is nonuniform only in a thin cylindrical sheath.

CELL MODEL FOR DNA

If the self-interaction of DNA within bacteriophage T7 is indeed that between uniformly charged rods in an aqueous suspension of counterions and added electrolyte, we may conveniently compute the osmotic pressure in terms of a cell model. Owing to thermal motion, there are inevitable undulations of the DNA helix about its reference configuration in the hexagonal lattice. Nevertheless, in bacteriophage T7, the interaxial spacing H is quite small (Cerritelli et al., 1997). In that case, the entropic contribution to f_{int} happens to be negligible according to a recent undulation theory (Odijk, 1993). A test section within the hexagonal lattice is replaced by one surrounded by an appropriate cylindrical sheath on which the electric field is zero. Similarity arguments can be used to solve the Poisson-Boltzmann equation and to determine the osmotic pressure

(Oosawa, 1968, 1971):

$$\pi_{os} \simeq \frac{Ac_p}{2Q}[(1 + w^2)^{1/2} - w]k_BT$$
$$w \equiv 4Qc_s/Ac_p \tag{11}$$

Here, $Q = q^2/\epsilon k_BT$ is the Bjerrum length, q is the elementary charge, ϵ is the permittivity of water, A is the linear spacing of elementary charges along the DNA axis, c_p is the equivalent concentration of counterions arising from the DNA ($c_p = 2/3^{1/2}H^2A$ for a hexagonal lattice), and c_s is the concentration of simple salt in the outside buffer.

A prediction for the inner radius of the DNA spool is then

$$\frac{PQ}{E^2} = (1 + w^2)^{1/2} - w \tag{12}$$
$$w = 3^{1/2}H^2Qc_s$$

On the other hand, E is also given by Eq. 4. Equations 4 and 12 involve experimentally accessible quantities only.

DISCUSSION

We now compare the theory in two ways with the data for bacteriophage T7 in an NaCl buffer of 0.1 M (Cerritelli et al., 1997). Cerritelli et al. measured the spacing H as a function of the DNA contour length L. In Table 1, we present the DNA volumes $SL = 3^{1/2}H^2L/2$ and three values of the inner spool radius E. One route to E is via the phage structure, i.e., Eq. 4 with $R = 27.5$ nm and $B = 10.5$ nm. The other route is via the balance of curvature stress versus electriostatic repulsion, i.e., Eq. 12 with a persistence length $P = 50$ nm and a Bjerrum length $Q = 0.71$ nm for water at room temperature. The predicted inner radii are then about one and a half times larger than the ones determined via the microscopic data. However, this discrepancy is misleading: the structural radii are extremely sensitive to the value of the bacteriophage radius R. If it is set only a bit larger ($R = 27.9$ nm) than that estimated from the micrographs (Cerritelli et al., 1997), the agreement between theory and experiment would be essentially quantitative (see Table 1). Note also that the slight increment in E with decreasing DNA length L is also predicted fairly accurately in that case.

TABLE 1 Inner radii of the DNA spool within bacteriophage T7

H* (nm)	L* (μm)	SL# (10^4 nm^3)	E§ (nm) ($R = 27.5$ nm)	E§ (nm) ($R = 27.9$ nm)	E¶ (nm)
2.54	13.6	7.60	4.3	7.8	7.5
2.64	12.5	7.54	5.0	8.2	7.6
2.75	11.5	7.53	5.2	8.3	7.8

*Data taken from Cerritelli et al. (1997).
#Volume of the DNA spool.
§Inner radii calculated from the bacteriophage structure (Eq. 4).
¶Inner radii predicted theoretically (Eq. 12).

TABLE 2 Predicted spacings of DNA within bacteriophage T7 from Eqs. 4 and 12 for two values of the outer radius R

$R = 27.5$ nm			
L (μm)	13.6	12.5	11.5
H (nm)	2.49	2.59	2.70
$R = 27.9$ nm			
L (μm)	13.6	12.5	11.5
H (nm)	2.55	2.65	2.76

A second comparison of theory with experiment can be carried out in terms of the spacing H. The inner radius E is eliminated from Eqs. 4 and 12. Table 2 shows the resulting spacings, which, for $R = 27.9$ nm, are in quantitative agreement with the experimental values (Cerritelli et al., 1997) quoted in Table 1. Nevertheless, such a comparison is a bit misleading, for the spacings are considerably less sensitive to the change in R than are the inner radii; the latter are, of course, measurable in principle, and their future determination may be a more stringent test of the simple ideas presented here.

It is concluded that the structure of DNA within bacteriophage T7 can be explained simply by balancing electrostatic forces against curvature stress within the DNA spool. An interesting check of the theory could be to monitor the change in interaxial spacing as a function of the ionic strength of the outside buffer. Note that we have found no evidence for attractive forces speculated on at length in recent theoretical work (Ray and Manning, 1994; Odijk, 1994; Rouzina and Bloomfield, 1996; Gronbech-Jensen et al., 1997). This could be due simply to the fact that we are in the tight packing limit.

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