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Osmotic compaction of supercoiled DNA into a bacterial nucleoid

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Abstract

A theory is presented of the phase separation of supercoiled DNA into a nucleoid in a bacterial cell. The suspension consists of DNA interacting with globular proteins in excess salt. A cross virial between DNA and a protein is computed as well as the DNA self-energy arising from excluded volume. The cellular parameters of *Escherichia coli* would appear to be compatible with the thermodynamic equilibrium derived theoretically. The state of superhelical DNA in the nucleoid could be liquid crystalline and rippled.

Keywords: Supercoiled DNA; Bacterial cell; Phase separation; Liquid crystal; Nucleoid; Proteins

1. Introduction

Despite many decades of research, the elucidation of nucleoid structure within bacterial cells is progressing slowly. The problem is that fixation and electron microscopy are generally invasive techniques [1–4]. The structure of the nucleoid is thought to be compact for bacteria in passive states when they are not growing. The nucleoid is not enveloped by a membrane so it must be confined by other forces. Negative supercoiling induced by gyrase has been proposed as one agent promoting DNA compaction, the (dynamic) binding of histone-like proteins to the DNA supercoil another [5]. What is often termed macromolecular 'crowding' [6] in the biophysical literature has also been invoked in hypothesizing phase separation within the cell [7]. It is known that cytoplasm does induce the collapse of linear DNA [8] but the experimental problem of the osmotic compaction of superhelical DNA is still unanswered. No concrete theory has yet been put forward of DNA compaction in bacteria. Our purpose here is to formulate a semiquantitative

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analysis of the depletion collapse of DNA and the resulting nucleoid structure within the admitted limitations of the model employed.

In physical chemistry and physics, the term 'depletion' [9] connotes more than the term 'crowding' for entropy is also involved in the effective interaction among particles. Confinement leads to a diminishing in the number of available degrees of freedom. The concomitant decrease in the number of realizations implies a lower entropy and hence repulsive or attractive forces between two test particles depending on the circumstances. The combination of excluded volume, entropy and bare forces may give rise to effective interactions that are intricate. For a recent example — a globular protein interacting with a semidilute polymer — see [10]. In this work, we consider the opposite limit: the majority of proteins in a bacterial cell [1] are much smaller than the persistence length of the chromosomal DNA.

The bacterial chromosome is supercoiled [11] although the supercoiling is not, strictly speaking, an equilibrium quantity. The degree of supercoiling is in the main determined by a steady-state balance of inducement of superhelicity by gyrase vs. superhelical relaxation by topoisomerase I. Supercoiling itself may, in addition, regulate the gene expression for DNA gyrase which renders DNA supercoiling homeostatically controlled [12]. Moreover, the hydrolysis of adenosine triphosphate delivers the free energy needed for gyrase to supercoil DNA [13]. On the whole, it is possible that this scheme may be subtly perturbed when the DNA supercoil is influenced by other interactions. This sort of perturbation is here disregarded completely. The linking difference will be considered as an equilibrium control parameter that is fixed independently of the forces focused on in this paper. Our reasoning is based solely on equilibrium statistical physics.

The bacterium, *Escherichia coli* for instance, will be viewed as a spherical compartment enclosing an aqueous suspension consisting of monodisperse globular proteins, one DNA superhelix and excess 1–1 electrolyte. A free energy balance is given to show that phase separation could occur because the interaction between the supercoil and the proteins is relatively large. The size of the nucleoid is determined by the equality of the respective osmotic pressures and protein chemical potentials in the nucleoid and in the surrounding cytoplasm. The nucleoidal DNA is rather densely packed so that we investigate the possibility of liquid-crystalline order and an attendant rippling transition [14].

In the Discussion, the implications for real *E. coli* bacteria are considered. In view of the uncertainties in most of the quantities, both theoretical and experimental, a predictive analysis is out of the question for now. Rather, this preliminary study should be viewed as one motivating an elaborate physico-chemical theory in the future.

2. Free energy terms within a cell

2.1. Proteins

The spherical cell has a volume V and contains m monodisperse globular proteins of radius a (diameter $b = 2a$). The proteins are presumed to bear a negative surface charge (see below). The aqueous suspension is assumed to have a uniform permittivity ϵ. If the cytoplasm contains excess salt of concentration n_s, the usual Debye length κ^{-1} for screened electrostatic interactions is given by $\kappa^2 = 8\pi Q n_s$. $Q = q^2/\epsilon k_B T$, the Bjerrum length, where q = the elementary charge, T = the temperature and k_B = Boltzmann's constant.

The free energy F_p of the proteins may be approximated by

$$F_P/k_B T = m \ln \frac{m}{V} - m + mg(\bar{v}) \qquad (1)$$

There is an ideal term and one denoting the interactions between the proteins. In the latter, the bare diameter would have to be renormalized into an effective $\bar{b} = \bar{b}(b,\kappa)$ in view of the electrostatic repulsion between the proteins. The dimensionless function g depending on an effective volume fraction $\bar{v} \equiv \pi \bar{b}^3 m/6V$ will not be specified here. Ultimately, we will balance only lead terms in the analysis.

2.2. Protein–DNA interactions

We have to present a fairly accurate assessment of the electrostatic repulsion between the DNA superhelix and the protein spheres. It is assumed that the supercoil is plectonemic in accordance with the structure seen experimentally under dilute conditions [15]. Recently, it has been shown that the experimental dimensions of long plectonemic DNA [15] may be understood in a so-called 'semiclassical' limit [16]. The free energy of the coil is split into a 'classical' energy consisting of bending and twist contributions and all other perturbative influences like entropy, electrostatics, various external forces, etc. The relationship between the supercoil diameter D and the superhelical pitch angle δ may be derived [16] which turns out to be independent of the perturbations provided they are not too strong.

For the semiquantitative purposes of this paper, this relation reduces to

$$D \simeq h_o/2\pi|\sigma| \tag{2}$$

for the dependence on γ is so weak that it need not be displayed. The DNA helical repeat is denoted by $h_o(\simeq 3.4$ nm) and σ is the specific linking difference.

For the chromosomal DNA of *Escherichia coli*, it has been estimated that $\sigma = -0.025$. Although the supercoiled DNA would have a bare value of $\sigma \simeq -0.06$, the binding of certain proteins to the DNA is believed to reduce the linking difference effectively [17,18]. Therefore Eq. (2) predicts that the supercoil diameter is substantially greater than the diameter of a typical protein in the cytoplasm. Furthermore, the radius of curvature of the DNA helix within the supercoil should on average also be greater than the protein size. To a zero-order approximation, the superhelix–protein interaction is basically that between a straight charged rod (i.e. a straight DNA helix) and a charged sphere.

Now, in view of Boltzmann weighting in the statistical thermodynamics, we merely need to consider the Debye–Huckel-like tails of the interactions between the highly charged particles in solution. The following asymptotic interactions are well known for particles bearing charges of the same sign (taken to be positive for convenience)

Two elementary point charges at distance r

$$u_{ii}/k_BT = \frac{Q}{r}e^{-\kappa r} \tag{3}$$

An elementary point charge and a DNA cylinder [19]; the distance between the point charge and the DNA centerline is r.

$$u_{ic}/k_BT = \left(\frac{2\pi}{\kappa r}\right)^{1/2}\xi_{eff}e^{-\kappa r} \tag{4}$$

An elementary point charge and a sphere [10]; the distance between the point charge and the center of the sphere is r.

$$u_{is}/k_BT = \frac{Z_{eff}Q}{(l+\kappa a)r}e^{-\kappa(r-a)} \tag{5}$$

Here, Z_{eff} is an effective charge on the surface of the sphere and $V_{\text{eff}} \equiv \xi_{\text{eff}}/Q$ is the effective linear charge density along the DNA helical axis. Both parameters may be computed with the help of the Poisson–Boltzmann equation. In order to derive the interaction U_{cs} between a protein and the DNA cylinder, we have no need to solve the Poisson–Boltzmann equation anew. In the outer double layers of the two particles, simple superposition holds so from Eq. (4) and Eq. (5), we have

$$U_{cs}/k_BT = \Lambda\frac{e^{-\kappa r}}{(\kappa r)^{1/2}} \tag{6}$$

$$\Lambda \equiv \frac{(2\pi)^{1/2}Z_{\text{eff}}\xi_{\text{eff}}e^{\kappa a}}{(1+\kappa a)} \tag{7}$$

Next, the statistical interaction between the protein and the DNA of helical contour length L and bare diameter d is expressed by a cross virial coefficient

$$B_c \equiv \pi LE^2 = \int d\vec{r}(1-e^{-U_{cs}^{(\vec{r})}/kBT}) \tag{8}$$

$$E^2 \equiv 2\int_0^\infty drr(1-e^{-U_{cs}(r)/k_BT}) \tag{9}$$

The depletion radius E is defined in such a way that it reduces to the correct value $a + 1/2d$ in the hypothetical absence of charges on both particles. The integral we have to compute from Eq. (6) and Eq. (9) is

$$I \equiv \int_0^\infty duu(1 - e^{-\Lambda e^{-u}/u^{1/2}}) \simeq \tfrac{1}{2}\ln^2\Lambda \qquad (\Lambda \gg 1) \tag{10}$$

The substitution $e^{-u} = p$ and one integration by parts yield an integral containing a logarithmic singularity. A full asymptotic expansion for large Λ is possible but here we retain the lead term only

$$E = \kappa^{-1}\ln\Lambda \tag{11}$$

Note that it is always true that $E > a + 1/2d$ since there is a factor $\exp(\kappa d/2)$ implicit in ξ_{eff} when one solves the Poisson–Boltzmann equation around a charged cylinder. The free energy of m proteins interacting with the DNA supercoil inside the cell is

$$F_c \simeq \frac{mB_c k_B T}{V} \tag{12}$$

2.3. DNA self-interaction

Finally, the interaction of the DNA superhelix with itself is important. We may neglect the translational entropy for a single supercoil whether it is constrained within the entire cell or in the nucleoid. The plectoneme has a contour length $l = 1/2L\sin\delta$. If it were a random coil, it would consist of l/A_s Kuhn segments of Kuhn length A_s. But there is a strong excluded-volume effect between the Kuhn segments, for the DNA is packed in a rather tiny cell. Let us assume the superhelix acts as a spring of hard-core diameter D [14]. The excluded volume between two Kuhn segments [20] is estimated as $\pi A_s^2 D/2$. There are basically $l_2/2A_s^2$ pairs of segments interacting with one another so the total excluded volume pertaining to the DNA supercoil is

$$B_s = \frac{\pi}{4}l^2 D \tag{13}$$

Within the cell, the DNA concentration is so high that the suspension of Kuhn segments is semidilute i.e. the average distance between the segments is less than A_s.

Then, the free energy of the interacting segments [20] or what may be termed the self-energy of the supercoil is given by

$$F_s = \frac{B_s k_B T}{V} \tag{14}$$

3. Phase separation

If the supercoil were dispersed throughout the entire cell, the total free energy would be the sum of Eqs. (1), (12) and (14)

$$F_{\text{tot}}/k_B T = m\ln\frac{m}{V} - m + mg(\bar{v}) + \frac{mBc}{V} + \frac{B_s}{V} \tag{15}$$

When phase separation does occur, the DNA is confined within the nucleoid of volume V_n together with m_n proteins. The general form Eq. (15) is now used to impose thermodynamic equilibrium between the nucleoid and cytoplasm phases (denoted by the indices n and c, respectively). The equality of the osmotic pressure in the respective compartments yields

$$v_c[1 + \bar{v}_c g'(\bar{v}_c)] = v_n[1 + \bar{v}_n g'(\bar{v}_n)] + \frac{B_c v_n}{V_n} + \frac{\pi b^3 B_s}{6V_n^2} \tag{16}$$

The chemical potential of the protein must be uniform throughout the cell

$$\ln v_c + g(\bar{v}_c) + \bar{v}_c g'(\bar{v}_c) = \ln v_n + g(\bar{v}_n) + \bar{v}_n g'(\bar{v}_n) + \frac{B_c}{V_n} \tag{17}$$

4. Liquid-crystalline state within the nucleoid

The packing of supercoiled DNA within the nucleoid may be high enough to cause the DNA segments to order as a liquid crystal. It is assumed that the cholesteric organization induced by the superhelical chirality is weak [14]. As usual in lyotropic systems [20], it is useful to suppose the orientation θ of some superhelical Kuhn segment is Gaussian with respect to the director — the average alignment of the segments

$$G \sim \exp -\tfrac{1}{2}\alpha\theta^2 \quad (\alpha \gg 1) \tag{19}$$

Because of segment alignment, the excluded-volume effect decreases whereas the free energy of confinement is enhanced as first discussed for simple wormlike chains by Khokhlov and Semenov ([21]; for a review see [20]; the application to supercoils was given in [14]). Here, we have for plectonemic DNA

$$F_{lc} \simeq \frac{B_s}{V_n \alpha^{1/2}} + \frac{l\alpha}{A_s} \tag{20}$$

As pointed out recently, the superhelical pitch angle δ is remarkably robust against perturbations [16]. The superhelical contour length l is virtually independent of the ordering parameter α [14]. Minimizing Eq. (20) with respect to α, we get

$$\alpha^{3/2} \simeq \frac{DLA_s}{V_n} \tag{21}$$

However, the nematic field may become so intense that the classical structure of the plectoneme breaks down altogether. This happens when $D \simeq P/\alpha$ where P is the persistence length of the DNA helix (not that of the superhelix). A transition to a rippled state is then predicted to occur [14]. The strong nematic field will cause the DNA chain to be deflected back and forth toward the nematic director i.e. it is rippled on the scale of the so-called deflection length [14]. In the rippled state, the supercoiling energy is merely a weak perturbation compared with the nematic energy. Of course, there is still a topological excluded-volume effect because the supercoil is not able to form knots with itself. The diameter of an end loop is no longer D but rather [14]

$$D_{\mathrm{rip}} \simeq P/\alpha \qquad (\alpha \gtrsim P/D) \tag{22}$$

beyond the rippling transition. The writhing of the DNA helix within the supercoil does not disappear. The superhelix may thus be roughly viewed as a semiflexible cylinder of length $L/2$ and D_{rip} diameter. Hence, Eq. (21) must be rescaled as follows

$$\alpha^{5/2} \simeq \frac{LP^2}{V_n} \qquad (\alpha \gtrsim P/D) \tag{23}$$

We leave unaddressed the folding problems associated with the confinement of the supercoil within the nucleoid of mesoscopic dimensions.

5. Discussion

We apply our computations to an average type of *Escherichia coli* cell as discussed by Woldringh and Nanninga [1]. (For a reassessment of these data, see [22]). For bacteria cultured in alanine [1,22], the available volume is typically approx. $V \simeq 370 \times 10^6$ nm^3 and the volume of the nucleoid is $V_n \simeq 80 \times 10^6$ nm^3. The contour length of the DNA helix $L = 1.6$ mm. The globular proteins have a radius $a \simeq 2.3$ nm; large ribosomes are present in the cell but they exert a negligible influence on the energy balance focused on here. The protein volume fractions are $v_c = 0.166$ in the cytoplasm and $v_n = 0.06$ within the nucleoid [22]. At a physiological ionic strength of 0.2 M, the Debye radius κ^{-1} is 0.67 nm, for the Bjerrum length $Q = 0.71$ nm.

We first calculate the depletion radius E. The Poisson–Boltzmann equation may be solved for DNA by standard methods which leads to $\xi_{\mathrm{eff}} = 6.32$ (DNA helix diameter is: $d = 2$ nm). The charging state of the proteins is unknown but $Z_{\mathrm{eff}} \simeq 10$ seems a realistic estimate for an unbound protein at normal pH. Eq. (7) and Eq. (11) yield $E = 4.7$ nm for the depletion radius which is entirely reasonable. The superhelical diameter D is approx. 22 nm using Eq. (2). It is indeed much

greater than E so Eq. (8) is applicable within the approximations stated: $B_c \simeq 1.11 \times 10^8$ nm^3. By contrast, the self-excluded volume of the DNA supercoil from Eq. (13) is approx. $B_s \simeq 7.1 \times 10^{12}$ nm^3 if we simply set $l \simeq 0.4\ L$ [15].

Since the measured protein volume fractions are only of the order 0.1, it is correct to set $g \equiv 0$ in a theory correct to the leading order. Hence, we numerically solve Eqs. (17) and (18) in order to compute the unknown theoretical variables v_c, v_n and V_n. We also need another relation which is connected with the total amount of protein in the accessible volume V: $v_c(V - V_n) + v_n\ V_n$ is set equal to the equivalent quantity determined microscopically [1,22]. The predicted coexistence is $v_c = 0.162$, $v_n = 0.032$ and $V_n = 68 \times 10^6$ nm^3. The theoretical nucleoid volume is in agreement with the experimental value ($V_n = 80 \times 10^6$ nm^3 [1,22]) within the margin of error. The amount of protein within the nucleoid is underestimated a bit but the number of free, unassociated proteins is not known precisely as has been discussed in Section 2.

Of considerable interest is the theoretical stability of the nucleoid. When does it cease to exist, i.e. when is the supercoil dispersed throughout the accessible volume of the cell? The coexistence Eq. (17) and Equation 18 reduce to

$$1 - \frac{\pi b^3 B_s}{6 v_c V_n^2} = \left(1 + \frac{B_c}{V_n}\right) \exp^{-B_c / V_n} \tag{24}$$

It is straightforward to show that Eq. (24) possesses a solution implying the existence of the nucleoid, provided

$$v_c \gtrsim \frac{\pi b^3 B_s}{3 B_c^2} \tag{25}$$

The numerical coefficient in Eq. (25) has to be estimated by a numerical investigation of Eq. (24). For *E. coli* [1,22], the right-hand side equals 0.059. It is remarkable that the actual bacterial cell with a cytoplasmic protein volume fraction $v_c = 0.166$ [1,22] is so close to instability. Any fairly slight perturbation in the cellular conditions would destroy the nucleoid, at least according to the present depletion theory.

The Kuhn length A_s of the supercoil is estimated to be approx. 100 nm [14]. The ordering parameter α for unrippled DNA in the nucleoid would be approx. 14 from Eq. (21). This high value implies a definite possibility for a rippling transition for $D > P/\alpha \simeq 3$ nm. We should therefore use Eq. (23) to predict a much weaker degree of ordering in the rippled state: $\alpha \simeq 3$. Fortunately, such weak liquid-crystalline order would not perturb the osmotic pressure balance significantly so our conclusion of the previous paragraph remains intact. Experimental work by Merchant and Rill [23,24] on long linear DNA has proved that such a solution becomes liquid-crystalline at a DNA concentration of only 13 g/l in 0.1 M NaCl. Estimates for the concentration within *E. coli* nucleoids have been consistently higher [25]. Surmising a nucleoid mesophase would appear to be reasonable without any appeal to theory though here a tentative theory has been given for superhelical DNA.

In summary, the formation of the nucleoid in *E. coli* may be interpreted as the phase separation of superhelical DNA in a suspension of proteins. The cross viral coefficient has a high value so the interaction between the supercoil and the proteins overwhelms the self-energy of the DNA. The present computations are semiquantitative so a definitive conclusion may have to await an exhaustive analysis of the terms introduced here. The state of the DNA could be liquid-crystalline (i.e. cholesteric); the supercoil may be rippled as well. Unequivocal establishment of these features by experiment is probably difficult because of the mesoscopic size of the nucleoid. Experiments like those performed recently on isolated spermidine nucleoids with regard to osmotic compaction [26] may help in establishing the validity of the depletion theory formulated here.

There is one effect of potential importance in inducing polymer compaction that we have neglected here. There are fluctuations in the protein concentration and these may cause attractive forces between the segments of a polymer immersed in the suspension [27,28]. In particular,

van der Schoot [28] has shown that such a fluctuation-induced attraction may well rationalize computer simulations for a linear flexible chain in a fairly concentrated protein solution [29]. However, it is stressed that the topological excluded volume focused on here, is connected with the relatively large supercoil diameter D and this effect simply overwhelms any induced attractive force acting on much smaller scales. The significance of fluctuation-mediated attraction may need to be investigated in the case of linear DNA.

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