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MODEL AND METHOD OF IONIC EFFECTS ON THE STABILITY OF SUPERHELICAL DNA

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ABSTRACT
The familiar Watson–Crick B–DNA double helix has a linear form. However, DNA from a variety of prokaryotic and eukaryotic sources exists in compact folded forms instead of the linear form. The DNA double helix can wind in space to form a new helix of a higher order, in which case it is said to be supercoiled or superhelical. Supercoiling takes a variety of forms. Many viral DNAs adopt covalently closed circular forms. In chromatin of higher organisms DNA is wound around a core of protein to form a left–handed solenoidal superhelix. Supercoiling can be manifested by any spatially constrained DNA sequence. DNA organized in living organisms is both negatively and positively supercoiled and has roughly one superhelical turn for every twenty turns of the local helix. Supercoiling is an important facet of biological processes that entail local helical winding and unwinding, such as replication, transcription and recombination. Many physical and chemical properties of DNA such as its hydrodynamic behavior, energetic, enzymology, and others are affected by the closed circularity and the deformations associated with supervoiling.
KEY WORDS*:* DNA, organisms, properties

INTRODUTION

(a1) MATHEMATICS
Supercoiling can be characterized mathematically by White's (1969) formula Lk=Tw + Wr, where Lk is the linking number of DNA or the number of times the two strands of DNA are intertwined (total number of revolutions of one strand about the other when the DNA lies in a plane), Tw is the total twist which describes the twisting of the double–helical axis, and Wr is the writhing number which represents the winding of the helix in space. It should be pointed out that the writhing number is not a unique characterization of the DNA tertiary structure since it may be the same for very different spatial trajectories of the DNA double– helical axis (i.e., two superhelical DNA configurations may have the same Wr but different values for the radius of gyration). When the DNA backbone is covalently closed (in the absence of strand breaks) the two strands of DNA are not free to change the number of times they rotate around each other, and therefore Lk is a fixed integer. For DNA found in living organisms, changes of Lk are caused by enzymes known as topoisomerases. On the other hand, Tw and Wr are differential geometrical parameters that can vary continuously with the shape of the duplex (i.e., they do not have to assume integer values as is the case for Lk) as long as the change in Tw is compensated by a change in Wr such that the equation Lk = Tw + Wr holds. Changes in Tw may involve torsional deformations of the DNA duplex and/or local transitions to alternate conformational forms (Wang et al., 1982; Nordheim et al., 1982), while variations in Wr requires some sort of chain bending. The linking number difference ΔLk is defined as the difference between the linking number of the DNA in its naturally occurring closed circular state Lk and the linking number of DNA in the relaxed closed circular state Lk_o. The linking number difference ΔLk is defined as the difference between the linking number of the DNA in its naturally occurring closed circular state Lk and the linking number of DNA in the relaxed closed circular state Lk_o. The linking number difference can be expressed by the equation ΔLk = ΔTw + Wr. The writhe of DNA in the relaxed closed circular form is zero. ΔLk is negative and positive for native DNA, which are said to be negatively and positively supercoiled, respectively.

(a2) EXPERIMENTAL STUDIES
Early work of Venograd et al. (1965) showed that highly supercoiled DNA exhibits compact structures while its relaxed circular form, which contains nicks, is a more open structure. Experimental evidence

(Dean et al., 1982) indicates that DNAs differing in linking number have dramatic differences in their conformation in solution, and in general, the more the linking number of supercoiled DNA differs from Lk_o, the more compact it behaves. The absolute value of ΔLk is what matters. Usually, as the absolute value of the linking number difference increases the absolute value of Wr and the number of superhelical turns increases.

The superhelical conformation of DNA and the topological parameter Lk as well as the differential geometrical parameters Tw and Wr which describe it are influenced by environmental conditions such as temperature, pH, ionic strength and the binding of small molecules or proteins. The different forms of superhelical DNA in solution have been detected by a variety of techniques such as electron microscopy (Vinograd et al., 1965; Espejo et al., 1969), hydrodynamics (Gray, 1967; Bauer & Vinograd, 1968; Wang, 1969), light scattering (Campbell, 1978), small angle X–ray scattering (Brady et al., 1983; Brady et al., 1984, Brady et al., 1987), dynamic light scattering (Langowski, 1987; Langowski & Giesen, 1989), and time–resolved fluorescenece polarization anisotropy (Shibata et al., 1984). All these experimental methods indicate that compact interwound and toroidal structures may predominate depending upon the above mentioned solution conditions. A toroidal trajectory is that of a closed–circular DNA superhelix with the axis of the double helix winding around the surface of an imaginary torus. A hydrodynamic study performed by Gray (1967) indicates that superhelical DNA undergoes a salt dependent transition between an interwound configuration(high salt) and a toroidal form (~0.01 M NaCl). Other experimental studies (Bottger & Kuhn, 1971; Vollenweider et al., 1976; Brady et al., 1983) also suggest that the toroidal conformations are favored at low salt concentrations. However, some experimental studies (Langowski, 1987; Brady et al., 1984) indicate that there is not such salt induced interwound →toroidal conformational change over a broad NaCl range (0.001 M – 3 M). According to a small angle X–ray scattering study done by Brady et al. (1984), the interwound form (which appears in only a few samples) appears to tighten up (i.e., becomes more compact) with increasing salt concentration since the mean interstrand distance decreases. This trend could be due to the enhanced screening of the electrostatic repulsions at higher salt concentrations which permits the phosphates to come closer together and therefore leads to more folded supercoiled structures. More experimental studies are warranted in order to establish unambiguously which conformations are adopted by superhelical DNA over a broad range of salt concentration and therefore to gain a better understanding of the influence of salt on the stability of superhelical DNA.

MODEL AND METHOD

We start out, in Subsection (a), by giving a description of the model/methology employed in our analysis of the relative ionic stability of the interwound and toroidal forms of superhelical DNA at different salt concentrations. In Subsection (b) 23 explain the model/method used in our computer simulations of electrostatic effects on closed circular DNA.

(a) NUMERICAL COUNTERION CONDENSATION ANALYSIS

The reader is referred to it for a detailed presentation. We will consider only the finite and three–dimensional distribution of phosphate groups of representative interwound and toroidal forms of superhelical DNA. Since the coordinates for the phosphates (the phosphate charges are considered to be centered at the phosphorus atoms) for both forms of superhelical DNA are still not available from X–raycrystallographic literatre, it is necessary to obtain them using geometric modeling techniques. To generate the phosphorus coordinates we used a differential geometry procedure (Olson & Cicariello, 1986 Cicariello, 1991) where the three–dimensional modelis based on the deformation of B–DNA around present interwound and toroidal space curves. In naturally occurring DNA there is roughly one superhelical turn for every twenty turns of the local–B–DNA helix (one superhelical turn per 200 base pairs), and the DNA supercoiling is mostly left–handed. Based on this experimental evidence we chose the simplest model possible; both toroidal and interwound forms have one left–handed superhelical turn and a total length of 200 base pairs. For a more detailed presentation of the differential geometric techniques here employed, the reader is referred elsewhere (Cicariello, 1991).

We employ both the constant–dielectric and dielectric saturation models for the solvent. In the constant–dielectric model the dielectric constant is distance independent and set to 78.3, the value for water at room temperature. In the latter solvent model, a distance–dependent function is used to account for the dielectric saturation effect. We assume the B–DNA line model value of the condensation volume for both interwound and toroidal forms of superhelical DNA (v = 635 cm^3/mol–phosphate).

(b) COMPUTER SIMULATIONS OF ELECTROSTATIC EFFECTS ON SUPERHELICAL DNA

Previous theoretical studies have modeled closed circular DNA as an isotropic elastic thin rod. Only recently have excluded volume effects been taken into account. In this paper we will treat closed circular superhelical DNA even more realistically by taking into consideration the polyelectrolyte character of DNA.

The axis of the closed double–helical trajectory is here represented by piecewise cyclic order 4 (cubic) B–spline curves that are approximated by 14 (15 for the leaf rose starting configuration) controlling points. The B–splines are defined by aseries of polynomial expressions which smoothly connect the given sequence of controlling points. These curves are regionally defined functions that are evaluated separately over difference intervals of the chain trajectory with as many continuous derivatives as are needed for the mathematical analyses to be performed. Since in our case only continuous first and second derivatives are required for the calculation of the differential geometric (e.g., writhing number and curvature integral) and the energetic parameters of superhelical DNA we employ order 4 (cubic) B–splines. Furthermore, the cyclic form of the curves satisfies the ring closure constraints of cyclic DNA. These curves provide a description of any complex folded trajectory of the double–helical axis of closed circular DNA for which there are no known geometric expression. The configuration of the chain is modified by simply varying the coordinates of the controlling points. For a more detailed discussion of the B–spline method here used the reader is referred elsewhere (Hao & Olson, 1989; Hao, 1988; Olson & Zhang, 1991). Chains of different contour lengths are obtained by simply re–scaling the controlling points. We consider chains of size 100–175 base pairs. The phosphate groups (considered centered at the phosphorus atoms) are evenly spaced on the double–helical axis (represented by the B–spline curve). The distance (i.e., are length) between the neighbouring phosphates is ~ 1.7 Å.

We assume that the total energy, E, of the closed circular DNA has four terms. The first two terms takes into account the elastic character of superhelical DNA. They are the bending E_B and twisting E_T contributions which are given by classical elasticity theory concerning homogenous isotropic rods. The third contribution $E_{H–S}$ considers excluded volume effects by means of a hard–sphere potential. The last contribution $E_{D–H}$ accounts for the polyelectrolyte character of DNA. The charged phosphate groups interact via a Debye–Huckel potential. The charges are reduced to account for the counterion condensation phenomenon. Therefore, the total energy can be expressed as:

$$E = E_B + E_T + E_{H–S} + E_{D–H} \quad (1)$$

The bending energy of superhelical DNA is taken as the bending energy of a homogeneous isotropic thin rod. It is given by,

$$E_B = \frac{A}{2}\int_0^L k(s)^2 ds \quad (2)$$

Where k is the curvature of the centroid of the rod, s the arc length, L the total contour length of superhelical DNA (prior to scaling), and A the bending stiffness constant. We assume a mean bending angle of 8.5° and use the equation (from classical elasticity theory) $A = 2kTh/\langle A^2 \rangle$ (where, h is the step height of DNA, 3.4Å, <A> is the mean bending angle, in units of radians, k the Boltzmann constant, and T the temperature in Kelvin) to obtain $A = 1.2755\times10^{-11}$ erg– Å. This value of the bending constant corresponds to a persistence length of 310 Å (from the relation $P_t = A/kT$) which is roughly the non–electrostatic contribution to the total persistence length.

The twisting energy of superhelical DNA which behaves as an elastic homogeneous isotropic rod is:

$$E_T = \frac{C}{2}\int_0^L (\omega - \omega_o)^2 ds \qquad (3)$$

where ω is the rate of local twist, ω_o the intrinsic rate of twist, and C the twisting stiffness constant. We assume a mean twisting angle of 6° and use the equation (from classical elasticity theory) $C = kTh/\langle\phi^2\rangle$ (where, h is the step height of DNA, 3.4 Å, $\langle\phi\rangle$ is the mean twisting angle, in units of radians, *k* the Boltzmann constant, and *T* the temperature in Kelving) to obtain $C = 1.2755\times10^{-11}$ erg-Å. In this case, A/C = 1. However, for the A/C = 2 (with $A = 1.2755\times10^{-11}$ erg–Å), $C=0.63775\times10^{-11}$ erg– Å, which corresponds to a mean twisting angle of 8.5°. We consider A/C = 1 and 2.

According to Fuller (1971) and Tanaka & Takahasi (1985) the expression for E_T given in eq. (3) can be rewritten as:

$$E_T = \frac{2\pi^2 C}{L}(\Delta Lk - Wr)^2 \qquad (4)$$

where ΔLk is the imposed linking number difference of superhelical DNA relative to the relaxed (unwrithed) closed circular form and Wr is the writhing number. The Gaussian and Fuller (Fuller, 1978) methods are employed to calculate the writhing number. For a detailed presentation of these methods the reader is referred elsewhere (Hao, 1988; Hao & Olson, 1989). Both eqs. (20 and (3) assume that the rod has a circular cross–section and that the DNA exhibits no preferential directions of bending and twisting. Also, the independence of bending and torsional deformation in the closed DNA from is assumed.

A hard–sphere potential is used to account for excluded volume effects. This energy contribution represents the effect of interaction between segments that are far apart along the DNA chain. Due to the finite volume of each repeating segment, other segments cannot occupy the same space. Therefore, the hard–sphere potential restricts the contacts of chain contour points to distances greater than a cutoff radius D and is given by:

$$E_{H-S} = \begin{cases} \infty & r_{ij} \leq D \\ 0 & r_{ij} \succ D \end{cases} \qquad (5)$$

where r_{ij} is the distance between any two points along the double–helical axis. Ponts i and j are separated by an arc length greater than ~68 Å, which is roughly two helical turns, D is the diameter of DNA (~20 Å).

The electrostatic energy between any phosphate pair along the double–helical axis is given by:

$$E_{D-H} = (q^{|})^2 \sum_{k<j}^{P} \frac{e^{-k r_{kj}}}{\varepsilon r_{kj}} \qquad (6)$$

where P is the total number of phosphate groups, r_{kj} is the distance between the k^{th} and j^{th} phosphate groups, ε is the dielectric constant of water at 25° (i.e., 78.3) and $q^{|}$ is the effective phosphate charge, reduced by $(1-N\theta_N)$ to account for counterion condensation. Since the B–DNA there is 76% neutralization of the phosphate charges by bound monovalent (Na^+) ions, the effective charge $q^{|}$ is given by q×0.24 (since $(1-N\theta_N) = 0.24$), where q is the unit electrical charge, k is the Debye screening parameter which is given by,

$$k = 0.329\sqrt{c_S} \qquad (7)$$

Where c_s is the concentration of NaCl in molarity units. We consider two extreme cases, that is, no added salt (c_s = 0 M) and the high salt region (c_s = 1 M).

The total energy is expressed in reduced form as $\tilde{E}$, a nondimensional quantity, which is obtained by dividing the total energy eq. (1) (with the exception of the hard–sphere potential) by the constant $2\pi^2C/L$.

We obtain DNA superhelical configurations that minimize the total energy while satisfying the constraints of ring closure and chain length. The contour length is preserved by using the following harmonic expression:

$$E_L = K_L (L - L_o)^2 \quad (8)$$

where K_L is the stretching force constant, here taken as 0.3 erg–Å^{-2}, L_o is the given starting contour length, and L is the actual contour length. This pseudo–potential allows the chain to fluctuate to a small degree about its given starting value L_o during the simulation. If the contour length is kept strictly constant few moves can be made, even at high temperatures.

In order to identify the global minimum of the total energy (in reduced energy units) under the constraints of ring closure and chain length we use an algorithm that combines Metropolis Monte Carlo sampling with a simulated annealing procedure. The starting configuration is either a circle (in most cases) or a leaf rose structure. The B–spline controlling points are moved at random with new configurations of lower energy automatically accepted and those of higher energy accepted on the basis of the Boltzmann factor of the increase in energy. The system is allowed to approach an equilibrium distribution at a given starting temperature T_o. The starting temperature T_o is obtained from the expression $kT_o=\sigma E_o$, where k is the Boltzmann constant, E_o is the starting initial energy, and σ is chosen as 0.01. This value of σ guarantees that 30–50 % of the moves among all trials are accepted. The temperature is then reduced by an accelerative cooling procedure where the temperature is lowered by a factor 0.95p at the p^{th} temperature lowering step, and the system is allowed to reach equilibrium once again. At each temperature, the configuration is relaxed by 20,000 Monte Carlo moes. The step size is chosen as 0.8% of the initial contour length of the DNA. The cooling process is allowed to take place 17 times, but ends when the configuration no longer changes (i.e., when the temperature is below the freezing point).

CONCLUSION

In conclusion, we have gained a better understanding of how the polyelectrolyte character of DNA governs the overall folding of the DNA chain. It is of special interest to carry out additional monte Carlo/simulated annealing simulations using the polylectrolyte (ionic) free energy (which includes both an electrostatic and mixing energy) instead of the simple Debye–Huckel energy.

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