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Biophysical Chemistry 75 (1998) 229–233

Biophysical Chemistry

An electrostatic model of B-DNA for its stability against unwinding

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Received 31 August 1998; accepted 28 September 1998

Abstract

In single molecule experiments Smith et al. (Science 271 (1996) 795) have unwound the B-DNA helix by stretching it in an aqueous salt solution. They found that the stretching force required for the transition decreases significantly with lowering of the salt concentration. We show that the observed salt effect is consistent with a uniformly charged cylinder model of DNA surrounded by a Poisson–Boltzmann ionic atmosphere. We also derive a simple connection between the sharpness of the center part of the transition and its cooperativity in terms of an average block size of base pairs that unwinds or rewinds.

Keywords: Stretching of duplex DNA; Charged cylinders; Poisson–Boltzmann model; Salt effects; Force-extension data; Cooperative unwinding

1. Introduction

Effects of salts on the stability of B-DNA are well documented. Early work concerned the influence of the ionic environment on the melting temperature of DNA [1–5]. The melting process includes unwinding of the duplex DNA followed by strand separation. A satisfactory comparison of theory and experiment was difficult for at least two reasons. (i) Variation of the melting temperature by salt addition introduced two unknown parameters, the non-ionic contributions to the entropy of melting and to the enthalpy of melting of duplex DNA. (ii) The electrostatic free energy of the double helix (the starting point) and the single stranded DNA coil (the end point) had to be treated on the same footing.

More recently Smith et al. [6] have unwound duplex DNA at constant temperature by stretching single molecules in buffered salt solutions. Fig. 1 gives force-extension data of single molecules of cross-linked λ-DNA in solutions of various ionic strengths. The curves show three parts, corresponding to three different processes. (i) Up to a force of a few pN the coiled B-DNA is essentially being stretched into a straight double

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0301-4622/98/$ - see front matter
PII S0301-4622(98)00211-7

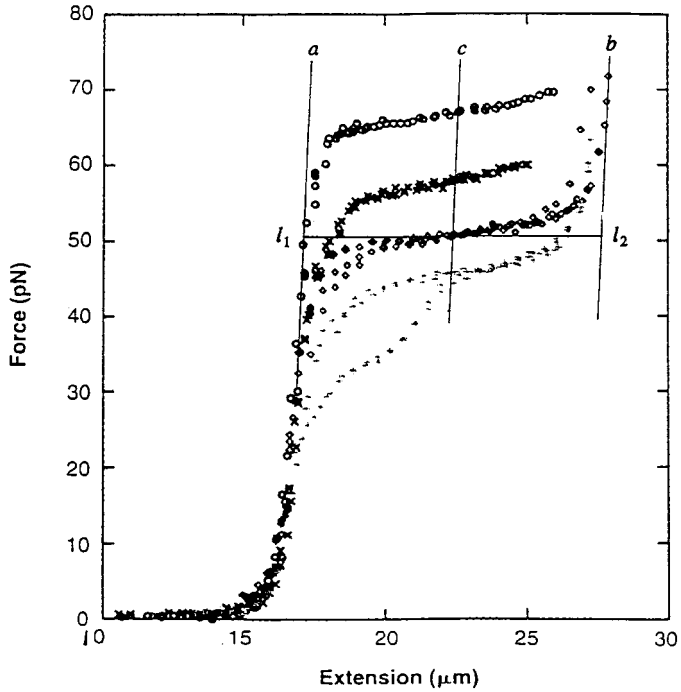

Fig. 1. Force-extension curve of cross-linked λ-phage DNA molecules in solutions of different ionic strength corresponding to, from top to bottom, 0.15 M, 0.005 M, 0.0025 M, and 0.000625 M NaCl. Cross-linking about 5% after exposure to 4,5′,8-trimethyl psoralen. Adapted from Smith et al. [6].

helix with contour length 3.38 Å per bp. (ii) Subjected to forces between about 5 pN and 20 to 65 pN, depending on ionic strength, the DNA extends slowly due to elastic stretch of the double helix. (iii) This is followed by a rapid extension (overstretching) to a contour length of about 5.7 Å/bp. The force required for overstretching increases at higher ionic strength. Parts (i) and (ii) of the stretch curves in Fig. 1 have been well discussed [6,7]. Here we treat the energetics of the unwinding of B-DNA, based on the upper part (iii) of the stretch curves.

Cizeau and Viovy [8] have treated the energetics of overstretching on the basis of a one dimensional Ising model, assuming only nearest neighbor interactions between base pairs (bps), but excluding consideration of the effect of ionic strength. In this communication we deal with the electrostatics of unwinding of DNA. We show that a uniformly charged rod model, surrounded by a Poisson–Boltzmann ionic atmosphere, explains the overstretching of DNA in solutions with different concentrations of monovalent salt. Because the process occurs at constant temperature, only a single unknown parameter, for the non-ionic free energy change, is involved. We also discuss the sharpness of the transition.

2. Overstretching of duplex DNA and electrostatics

For the experiments in Fig. 1 the DNA was cross-linked by 5% intercalation with psoralen to minimize strand separation after unwinding. For the higher salt concentrations reversibility of the stretch curves in Fig. 1 indicates that the double stranded structure remains essentially intact. At the lower salt concentrations in Fig. 1, however, the stretch curve is not completely reversible, but shows hysteresis. This suggests that between cross-links (on average about one per 20 bps) the unwound, double stranded structure is unstable and may melt into single strands that bow out between crosslinks. Upon decreasing the force, a

different path, through incorrectly paired bases, may be required to return to the original double helix, leading to hysteresis in the stretch curve. We assume that in the case of hysteresis the upper data points in Fig. 1, for increasing force, refer to the unwinding transition. We first derive the free energy of the transition from the experimental data.

The free energy change, ΔF, in the unwinding process is given by the work integral

$$\Delta F = \int_{l_1}^{l_2} \tau(l)\mathrm{d}l \qquad (1)$$

where l_1 is the extension at the beginning and l_2 at the end of the transition, and $\tau(l)$ is the stretching force or tension in the DNA. Ignoring hysteresis, we assume that part (iii) of the stretch curves is symmetrical with respect to the horizontal axis. Then we can take the force at the midpoint, $\tau(1/2)$ at line c in Fig. 1, as the average force between l_1 and l_2. We also assume that the values of l_1 are on line a and the values of l_2 on line b in Fig. 1. We discount the slope of lines a and b which is caused by elastic stretch of DNA. The relevant errors in our final results are insignificant. Then Eq. (1) becomes

$$\Delta F = \tau(1/2)(l_2 - l_1) \qquad (2)$$

where we read l_1 and l_2 from Fig. 1. With a contour length at $\tau = 0$ of 16.4 μm, corresponding to 3.38 Å/bp [6], we find the data for l_1 and l_2 in Table 1. Then the conversion 1 pN $\times$ 1 Å $= 10^{-22}$ J, and at room temperature $kT = 4.12 \times 10^{-21}$ J, yields the data for $\Delta F/kT$ per bp in Table 1.

The salt effect on the unwinding is easily understood. The repulsion between the charged phosphate groups promotes unwinding, but this repulsion is shielded by the addition of salt. To make this argument quantitative we model the B-DNA double helix as a cylinder with a uniform surface charge, a radius of 10 Å, and linear charge density $2/l_1$ e/Å. Unwinding does not change the distance between the phosphate charges in a bp. Therefore, we model the unwound double stranded DNA as a rod with the same radius of 10 Å, but now with a linear charge density $2/l_2$ e/Å. The charged cylinders are surrounded by a Poisson–Boltzmann atmosphere of univalent ions in equilibrium with salt concentration M_{NaCl}. The electrical free energy of these models is evaluated as derived earlier by Stigter [9] and by Schellman and Stigter [10]. The results are given in Table 2. The values of ΔF_{el} differ from the experimental ΔF from Eq. (2) by an unknown contribution of the non-ionic free energy of unwinding, ΔF_0 per bp. We assume that this contribution does not depend on the salt concentration and is $\Delta F_0 = 4.94kT$. In Fig. 2 the ΔF values from Eq. (2) are compared with the calculated values of $\Delta F_{el}/kT + 4.94$. The curve for the unwinding transition has the same dependence on the salt concentration as the experimental points. This good agreement of experiment and theory supports the credibility of the unwinding model of the overstretching transition, and of the non-ionic free energy, $\Delta F_0 = 4.94kT$/bp, for the unwinding of B-DNA at 25°C.

Table 1
Experimental free energy of unwinding B-DNA

M_{NaCl}	$\tau(1/2)$ (pN)	l_1 (Å/bp)	l_2 (Å/bp)	ΔF (kT/bp)
0.15	67.1	3.56	5.72	3.50
0.005	58.1	3.55	5.70	3.03
0.0025	50.5	3.53	5.69	2.64
0.000625	45.7	3.51	5.67	2.38

3. Width of unwinding transition

We now elaborate on remarks by Smith et al. [6] on the width of the overstretching transition.

Table 2
Electrical free energy per base pair in units of kT

M_{NaCl}	F_{el} [a]	F_{el} [b]	ΔF_{el} [c]
0.15	3.888	2.658	−1.230
0.005	8.640	6.534	−2.106
0.0025	9.747	7.467	−2.280
0.000625	11.999	9.416	−2.584

[a] Double helical DNA, radius 10 Å, charge density $2/l_1$ e/Å.
[b] Unwound duplex DNA, radius 10 Å, charge density $2/l_2$ e/Å.
[c] $\Delta F_{el}{}^{c} = F_{el}{}^{b} - F_{el}{}^{a}$.

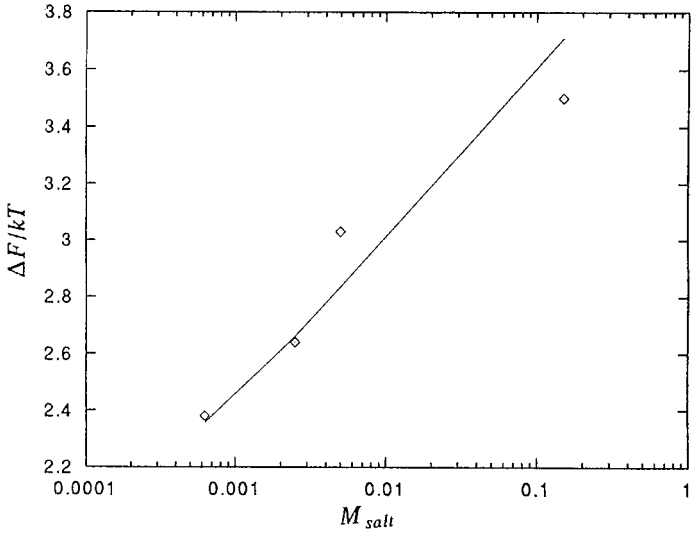

Fig. 2. Free energy changes ΔF per bp in unwinding of DNA. Squares: from experiments in Fig. 1 with Eq. (2) and Table 1. Curve: $\Delta F = \Delta F_{el} + 4.94kT$ with theory for ΔF_{el} from Table 2.

So far we have treated unwinding as an all-or-nothing transition. In fact, unwinding may proceed in short stretches of varying length, as discussed in the theory of the helix-coil transition [11]. It is well known that this kind of cooperativity makes the transition sharper [6,8]. There is interaction between adjacent bps through which their states are correlated. Let us approximate this correlation by assuming that the DNA does not unwind or rewind as single bps, but only as a sequence of n bps. Then the experiments in Fig. 1 yield an estimate of the average bloc size as follows.

Hill [12,13] has given the statistical thermodynamic treatment of the elasticity of homogeneous long chain molecules. Here we describe the unwinding equilibrium by the equality of the chemical potentials of DNA in the helical and unwound state, these states indicated by labels h and u. Consistent with Hill's treatment (Eqs. 7.57 and 7.58 of Ref. [13]) we add the mechanical work terms $\tau n l_h$ and $\tau n l_u$ in the expressions for the chemical potentials of n bps, μ_n^h and μ_n^u. The standard chemical potentials μ_n^{0h} and μ_n^{0u} contain the ionic strength dependent electrostatic free energies discussed above. The helical blocks and the unwound blocks are assumed to be independent. When the molecule has N_n^h helical blocks and N_n^u unwound blocks, with a total length $N_n^h n l_h + N_n^u n l_u$, and is under a tension τ, then the unwinding equilibrium is given by

$$\mu_n^{0h} + kT \ln N_n^h + \tau n l_h = \mu_n^{0u} + kT \ln N_n^u + \tau n l_u \tag{3}$$

We rearrange Eq. (3) as

$$\begin{aligned} \tau &= \frac{\mu_n^{0h} - \mu_n^{0u}}{n(l_u - l_h)} + \frac{kT}{n(l_u - l_h)} \ln \frac{N_n^h}{N_n^u} \\ &= \tau(1/2) + \frac{kT}{n(l_u - l_h)} \ln \frac{1 - f_u}{f_u} \end{aligned} \tag{4}$$

where the tension $\tau(1/2)$ for $N_n^u = N_n^h$ is given in Table 1, and the unwound fraction of the molecule is $f_u = N_n^u/(N_n^h + N_n^u)$.

In Fig. 3 the solid curves are calculated from Eq. (4) for $n = 13$ and the dashed curves for $n = 10$. In the middle region, around $f_u = 0.5$, the slopes of the solid curves are close to those of the various experimental curves in Fig. 1. Near the ends, however, the slope of the theoretical curves is too small. This suggests that near the beginning

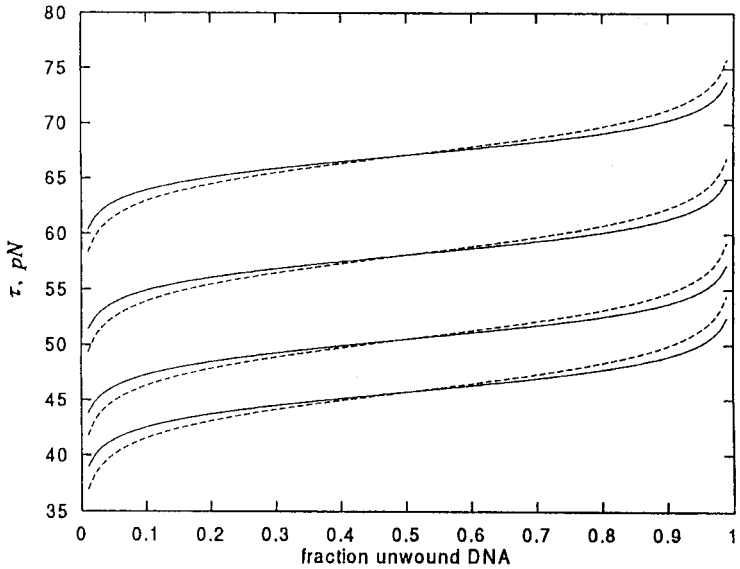

Fig. 3. Force-unwinding curves following Eq. (4). Solid curve: $n = 13$. Dashed curve: $n = 10$.

of the transition unwinding occurs in blocs shorter than $n \sim 13$ bps, and near the end of the transition we have helical blocs shorter than 13 bps.

The repulsion between the charged bps acts over a range of several Debye lengths, $1/\kappa$, which is about 100 Å in 1 mM NaCl. So electrostatic neighbor interactions are significant over a range of the order of 100 bps. The stretch curves of native DNA in Fig. 2 of Smith et al. [6] and in Fig. 1 of Cizeau and Viovy [8] indeed suggest such long range cooperativity, that is, a large average block size of the order of 100 bps. On the other hand, the above analysis of crosslinked DNA shows much shorter blocks, of about 13 bps. Apparently the cooperativity of the unwinding process is broken by the intercalated psoralen of the crosslinks, about one psoralen per 20 bps, as suggested earlier by Smith et al. [6].

References

[1] W.F. Dove, N. Davidson, J. Mol. Biol. 4 (1962) 500.
[2] L. Kotin, J. Mol. Biol. 7 (1963) 309.
[3] C. Schildkraut, S. Lifson, Biopolymers 3 (1965) 195.
[4] G.S. Manning, Biopolymers 11 (1972) 937.
[5] M.T. Record, Biopolymers 14 (1975) 2137.
[6] S.B. Smith, Y. Cui, C. Bustamante, Science 271 (1996) 795.
[7] C. Bustamante, J.F. Marko, E.D. Siggia, S. Smith, Science 265 (1994) 1599.
[8] P. Cizeau, J-L. Viovy, Biopolymers 42 (1997) 383.
[9] D. Stigter, J. Colloid Interface Sci. 53 (1975) 296.
[10] J.A. Schellman, D. Stigter, Biopolymers 16 (1977) 1415.
[11] D. Poland, H.A. Scheraga, Theory of Helix-coil Transitions in Biopolymers, Academic Press, New York, 1970.
[12] T.L. Hill, J. Chem. Phys. 20 (1952) 1259.
[13] T.L. Hill, An Introduction to Statistical Thermodynamics, Addison-Wesley, Reading, MA, 1960.