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Melting characteristics of highly supercoiled DNA

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Abstract

The effect of high supercoil densities on the melting characteristics of a supercoiled DNA has been studied. It is found that although the melting temperature increases abruptly on converting a linear DNA merely into the relaxed circular form, it falls back substantially at high supercoil densities. It is further predicted, in such cases, that the number of melted base pairs should be significantly enhanced even at the physiological temperature, which may facilitate the binding of other molecules to the highly supercoiled DNA.

Keywords: DNA melting; Supercoiled DNA; Supercoiling energy

1. Introduction

It is well known that a naturally occurring closed supercoiled DNA (e.g. ϕX174 or polyoma DNA molecule) is marked by its highly elevated melting temperature and a significantly flatter transition curve in comparison with those of its linear form [1,2]. In a recent paper [3] we have derived an expression for the supercoiling energy of the macromolecule in terms of its average elastic parameters, and developed a simple statistical mechanical theory in order to describe the melting of a supercoiled homopolymeric DNA. The results thus obtained are found to be in good agreement with the experimental data in TEA (tetraethyl ammonium bromide) solution, where the DNA molecule actually behaves like a homopolymer. However, it may be noted that these apply to DNA molecules having native supercoil densities $\sigma \approx -0.05$ only. The aim of the present paper is to predict how things stand at much higher degrees of supercoiling as may be achieved with the help of some DNA unwinding agents such as ethidium bromide and the RecA protein [4]. Theoretically we have found earlier [3] that the melting temperature rises abruptly to a high value merely on converting a linear DNA duplex into its relaxed closed form ($\sigma = 0$). For small supercoil densities σ, as in the case of naturally occurring molecules, the melting temperature T_m deviates little from that for $\sigma = 0$. Interestingly, however, on increasing the supercoil density σ to much larger magnitudes, we predict that the melting temperature T_m should decrease continuously and may even approach that of its linear form. Moreover, at such high supercoil densities, we find that the entire melting profile shifts towards the lower temperature region (Fig. 3). This leads to a further prediction that an appreciable number of open base pairs should be present

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even at physiological temperatures. We consider this significant as it is likely to facilitate the binding of other molecules to DNA and thus regulate its biological functions.

2. Theoretical considerations

The supercoiling energy of the closed circular DNA as a function of its elastic parameters has been discussed by several authors [5–9]. However, in our recent papers [3,10] we have used the elastic model to obtain an approximate expression for the supercoiling energy of a partially melted DNA, N base pairs long, in the form

$$G_s(\sigma, \theta) = \frac{CN(\sigma + \theta)^2}{A^2[1 + (\alpha - 1)\theta]} \qquad (1)$$

where $A = 10.4$ represents the number of base pairs per turn of the DNA in its B form, σ is the supercoil density, $\theta = n/N$ is the melted fraction and n is the number of melted base pairs in the molecule. According to ref. [10], $C = 2\pi^2 c_h b_h/(b_h + c_h)$ and $\alpha = b_h c_h (b_c + c_c)/b_c c_c (b_h + c_h)$, where b_h, c_h, and b_c, c_c, are the bending and the torsional stiffness constants respectively, usually expressed in erg/rad^2, for the helical (h) and the coiled (c) regions of the DNA. It may be noted that in deriving this expression, we have explicitly considered the twisting as well as writhing contributions to the supercoiling energy. This is because an appreciable amount of writhing is likely to be present in a highly supercoiled DNA. Clearly, it may now be found from equation (1), by putting $\partial G_s(\sigma, \theta)/\partial\theta = 0$, that for a given value of σ, the supercoiling energy $G_s(\sigma, \theta)$ has its minima at

$$\theta = -\sigma \qquad (2)$$

and

$$\theta = \sigma - 2/(\alpha - 1) \qquad (3)$$

where the eq. (2) may be realised by a negatively supercoiled DNA, while the eq. (3) applies to a positively supercoiled DNA only. The physical reason behind the existence of these minima are easily conceivable. For example, if the molecule is initially negatively supercoiled, the corresponding supercoiling energy is reduced as the melting proceeds, until it reaches zero. This is because the positive turns released by the melted regions, up to this point, gradually neutralise the initial negative supercoiling, effectively "relaxing" the molecule thereby. Beyond this point, however, further melting of the molecule makes the effective supercoiling positive, and consequently its energy increases again, giving a minimum at $\theta = -\sigma$ as given by eq. (2). On the other hand, when the molecule is initially positively supercoiled, its melting makes the effective supercoiling even more positive, so that it appears that the supercoiling energy should always increase. But, at the same time, the rigidity parameters of the melted regions being substantially lower than those of the helical regions ($\alpha \gg 1$), tend to reduce the supercoiling energy of the molecule. Thus, in this case, the competition between these two factors leads to an energy minimum. This is also confirmed by equation (3) which shows that the minimum exists only for $2/(\alpha - 1) < \sigma$ which, in the range of positive values of σ considered, implies $\alpha \gg 1$. The supercoiling energy curves for a few typical values of σ are shown in Fig. 1. It is discussed, at the end of this paper, how the minima of $G_s(\sigma, \theta)$ are important in lowering the

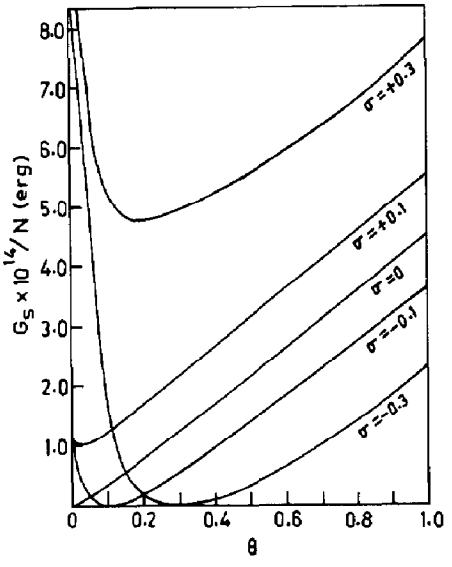

Fig. 1. Variation of supercoiling energy $G_s(\sigma, \theta)$ with the degree of melting θ for different supercoil densities σ.

melting temperature T_m and affecting the melting profile accordingly, for high supercoil densities.

The total free energy of the partially melted supercoiled DNA may be written as

$$F(n, n_j) = n(\varepsilon - T\Delta S) + \tfrac{1}{2}n_j\varepsilon_0 + G_s(\sigma, n) + kT \ln g(n, n_j, N) \quad (4)$$

where $n = \theta N$ is the number of melted base pairs, n_j is the number of helix–coil junctions, ε and ε_0 represent the base pairing and the base stacking energies respectively, and ΔS represents the change in the conformational entropy per base pair due to melting. In eq. (4), the first term represents the free energy arrising from the disruption of n base pairs, and the second term is the energy in creating n_j helix-coil junctions or nucleating $n_j/2$ regions into which n melted base pairs are distributed. $G_s(\sigma, n)$ represents the supercoiling energy given by the eq. (1), and $g(n, n_j, N)$ is the degeneracy factor given by

$$g(n, n_j, N) = \frac{N(N-n-1)!(n-1)!}{\left(N-n-\frac{n_j}{2}\right)!\left(n-\frac{n_j}{2}\right)!\left(\frac{n_j}{2}-1\right)!\left(\frac{nj}{2}\right)!} \quad (5)$$

so that the last term in eq. (4) represents the configurational free energy arising from statistical considerations [3], where k is the Boltzmann constant. Now, minimizing $F(n, n_j)$ with respect to n and n_j, and finally eliminating n_j, we obtain

$$2\eta\theta(1-\theta)(\xi-1) - [\xi(1-\theta)-\theta]\left[1-\{1-4\eta\theta(1-\theta)\}^{1/2}\right] = 0 \quad (6)$$

where

$$\xi = \exp\left[\left(\varepsilon - T\Delta S + \frac{\partial G_s}{\partial n}\right)/kT\right] \quad (7)$$

and

$$\eta = 1 - \exp(\varepsilon_0/kT) \quad (8)$$

When this equation is solved for $\theta = \theta_m = \frac{1}{2}$, where θ_m represents the value of θ at the melting point, we get

$$T_m(\sigma) = T_m^l + \Delta T_m(\sigma) \quad (9)$$

where $T_m^l = \varepsilon/\Delta S$ is the melting temperature of the linear form, and $\Delta T_m(\sigma)$ is given by

$$\Delta T_m(\sigma) = C(1+2\sigma)[(\alpha-1)(1-2\sigma)+4] / A^2(\alpha+1)^2\Delta S \quad (10)$$

This represents the change in melting temperature for the closed circular molecule having supercoil density σ.

3. Results and discussions

From the foregoing considerations, three different cases may arise depending on the values of σ, namely (i) $\sigma = 0$, (ii) $\sigma < 0$ and (iii) $\sigma > 0$. Let us now examine the individual cases as follows.

3.1 Relaxed closed circular DNA ($\sigma = 0$)

Equation (10) shows that there is a substantial rise in the melting temperature, even for $\sigma = 0$, as given by

$$\Delta T_m(0) = C(\alpha+3)/A^2(\alpha+1)^2\Delta S \quad (11)$$

where $\Delta S = 12$ e.u. [11–13]. As the elastic constants b_h, c_h, b_c, and c_c, are not accurately known, we have chosen $C = 11.4 \times 10^{-11}$ erg/rad^2 and $\alpha = 23.4$ to fit the experimentally observed melting curve for ϕX174 DNA ($\sigma = -0.06$) in TEA solution [2] where $\varepsilon = 7.9$ kcal/mol and $\varepsilon_0 = 2.5$ kcal/mol [3]. However, these values of C and α are consistent with the estimated limits for the elastic parameters b_h, c_h, b_c, and c_c [6,14,15], in terms of which they have been expressed after eq. (1). With these values, we finally obtain $\Delta T_m(0) = 28$°C as shown in Fig. 2. For $T_m^l = 58$°C [2], the melting point for the relaxed closed circular DNA ($\sigma = 0$) is clearly elevated to the value $T_m(0) = T_m^l + \Delta T_m(0) = 86$°C. Clearly, this elevation of the melting temperature for the closed circular duplex, even for $\sigma = 0$, is a

natural consequence of the linked topology of its strands, which cannot escape each other, as required by the invariance of the linking number in a closed circular DNA [16,17].

3.2 Negatively supercoiled DNA ($\sigma < 0$)

This case is biologically important as the naturally occurring closed circular DNA molecules are found to be negatively supercoiled. For small negative values of σ (e.g. $\sigma = -0.06$ for ϕX 174), $\Delta T_m(\sigma)$ is close to $\Delta T_m(0)$ as shown in Fig. 2. Also the melting profile for $\sigma = -0.06$, as indicated in Fig. 3, is nearby identical to that of the relaxed circular molecule ($\sigma = 0$) which has not been shown separately in the figure. However, we find from eq. (10) that $\Delta T_m(\sigma)$ is a continuously decreasing function of σ as the molecule becomes more and more negatively supercoiled. Finally an inversion in the sign of $\Delta T_m(\sigma)$ occurs at the point

$$\sigma = \sigma^- = -0.5 \tag{12}$$

Thus, at such high degree of negative supercoiling, the melting point $T_m(\sigma)$, in principle, may even be brought down to that of the corresponding linear molecule (Fig. 2). The experimental situation in this regard is discussed at the end. Further, the melting profile of a closed circular DNA, which shows that the transition is much less cooperative as compared to that of a linear homopolymer, shifts more and more towards the left with increasing negative supercoiling (Fig. 3), thus predicting larger and larger open regions at 37°C.

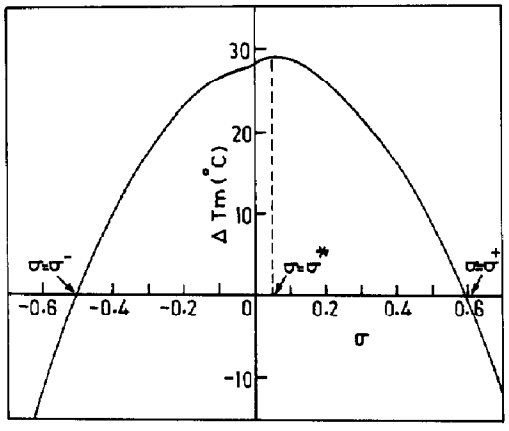

Fig. 2. Variation of $\Delta T_m(\sigma)$ with supercoil density σ, where the melting temperature $T_m = T_m^1 + \Delta T_m$.

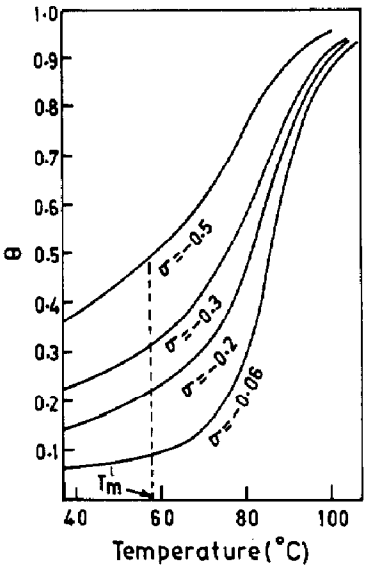

Fig. 3. Melting curves for different supercoil densities σ. As the molecule is more and more negatively supercoiled, the number of open base pairs at temperatures as low as 37°C increases significantly.

3.3 Positively supercoiled DNA ($\sigma > 0$)

From equation (10), it may be seen, by using the condition $d\Delta T_m(\sigma)/d\sigma = 0$, that $\Delta T_m(\sigma)$ has a maximum at $\sigma = \sigma^* = 1/(\alpha - 1)$ which is positive and close to zero for $\alpha \gg 1$. Beyond this point, however, $\Delta T_m(\sigma)$ decreases with σ, and finally there is an inversion in this case also, at the point

$$\sigma = \sigma^+ = (\alpha + 3)/[2(\alpha - 1)] \tag{13}$$

which is normally close to 0.5 as $\alpha \gg 1$ (Fig. 2). Therefore, in this respect, the situation is quite similar to what is expected in the case of a highly negative supercoil density.

Thus, in general, we find that a high degree of supercoiling facilitates melting in a closed circular DNA. Looking into the variation of the supercoiling energy $G_s(\sigma, \theta)$ with θ (Fig. 1), this feature seems to be quite understandable, as for $|\sigma| \ll \theta_m = 1/2$, $G_s(\sigma, \theta)$ has a minimum at a very small value of θ and then increases continu-

ously beyond this point, thus disfavouring the process of melting. This effect has to be neutralised by lowering the entropic free energy part through an elevation of the melting temperature T_m, in order to make the transition feasible. On the other hand, for sufficiently high values of $|\sigma|$ (e.g. $|\sigma| \approx \theta_m = 0.5$), the supercoiling energy G_s decreases upto the melting point or even beyond, so that the melting occurs at lower temperatures mainly at the cost of the supercoiling energy. Finally the melting curves may be computed as in our earlier paper [3]. Figure 3 shows the effect of varying the supercoil density σ on the melting curve of a negatively supercoiled DNA. For higher values of σ, it is found that the degree of melting is substantially enhanced at each given temperature, thus causing a lateral shift of the entire melting curve. This suggests that the presence of sufficiently high supercoil densities may induce significantly large number of open base pairs even at physiological temperatures (≈ 37°C). From the biological point of view, this phenomenon may be considered important as it is likely to facilitate the binding of the other molecules to the DNA.

Regarding the dependability of this theoretical prediction, it may be recalled that a comparison of the melting curve for ϕX174 DNA ($\sigma = -0.06$) with experimental data in our earlier work [3] has indicated very good agreement except for the fact that some discrepancy could be noticed at the low θ region, namely for $\theta < 0.1$. However, it may be seen from Fig. 3. that for higher supercoil densities, the melting curves at 37°C are shifted farther away from the region $\theta < 0.1$, so that the predictions in such cases are expected to be dependable. It may further be pointed out that our conclusions so far apply to homopolymers only. However, we have shown in an earlier paper [10] that the melting profile of a closed circular DNA is rather insensitive to its base sequence. Therefore, we emphasize that the presence of high supercoil densities should have a similar effect in the more realistic case of a closed heteropolymer.

Finally, it may be mentioned that the possibility of such supercoil-induced local denaturation has also been discussed by others [6,18] on the basis of mechanical energy considerations alone. There are also some experimental evidences supporting our results regarding the supercoil-induced enhancement of melting at relatively lower temperatures [19,20]. Regarding the presence of such high degrees of supercoiling, we speculate that it may indeed be present in the nucleosomal DNA due to the following reasons. We know that the binding of each histone octamer to DNA is always associated with two physical turns of the DNA double helix on the octamer [21,22]. These physical turns introduce an equal number of opposite turns in the histone-free regions (linkers) of the closed circular double helix [23], and thus change the effective linking number in the linker regions. Therefore, when a DNA is adequately histone-bound, the number of such turns induced in the linker regions may rise considerably, so that the effective supercoil densities in these regions may reach high values. Furthermore, in a recent paper [4], it has been reported that a high degree of unwinding equivalent to $\sigma \approx -0.5$ is indeed possible to obtain by using RecA proteins. This information suggests that the main features of our predictions on the melting of a highly supercoiled DNA may be amenable to experimental verification.

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