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# Influencing the B-Z switch in supercoiled DNA

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The effect of Z-binding ligands on the supercoiling threshold in the supercoil-induced B-Z transition has been examined from the point of view of a two-state model. Expressions have been derived for the determination of the shift in critical supercoil density in terms of the physical parameters of the DNA-ligand system. Representative calculations indicate that the stabilizing action of Z-binding ligands on the Z conformation in closed circular DNA depends largely on the binding characteristics of the ligand. Application of the theoretical data has been demonstrated using the experimental results reported by Lafer et al. (J. Biol. Chem. 261 (1986) 6438).

## 1. Introduction

In circular plasmids, negative supercoiling has been shown to facilitate B-Z transition in sequences having defined periodicity [1]. A recent study has demonstrated the production and stabilization of short stretches of Z-DNA in vivo by supercoiling generated by biological processes [2], thereby contributing evidence to support the hypothesis that such transitions have biological implications. Theoretical studies have made it possible to calculate the supercoil densities at which such B-Z transitions (which are, as a rule, highly cooperative) would take place quite accurately by considering the detailed energetics of the transition process [3–7]. This enables us to estimate the variations of supercoiling density levels in plasmids in vivo. However, to the author's knowledge, no calculations of the effects of Z-binding ligands on the B-Z equilibrium have been carried out.

The aim of this work is to study the B-Z transition in the presence of Z-binding ligands by taking into consideration the energetics of ligand binding. The types of ligands which are known to bind Z-DNA include anti-Z antibodies [8], and other Z-binding proteins [9], recl and recA proteins [10,11], cations which stabilize the Z-form (like the divalent cation hexaaminecobalt chloride (HCC) and polyamines such as spermine [12]). The relevance of this study rests on the fact that in living cells such ligands are likely to be present and thus influence the value of the critical supercoil density needed to induce the B-to-Z transition as reported in recent in vitro studies [13,14]. It is also of importance to examine whether the use of Z-specific ligands (e.g., anti-Z antibodies) to determine the occurrence of a B-Z transition under superhelical stress significantly perturbs the B-Z equilibrium itself, thereby distorting the results obtained [13].

To this end we have exploited the observed cooperative nature of the B-Z transition to construct a formalism which yields analytical expressions for quantitative calculations of the transition points in terms of the physical parameters of the DNA-ligand system. The relations obtained in section 2 have been applied to the analysis of experimental observations of the shifts in B-Z

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transition points in a plasmid in the presence of anti-Z antibodies [13]. The treatment of the present problem is conceptually similar to the solution of the problem of denaturation transition of macromolecules in the presence of ligands binding differentially to the native and denatured states [15,16]. However, there are some differences between the two systems. In the latter, the energy of the junctions between the states does not occur explicitly as long as it is high enough. The system effectively eliminates the junction by adopting either of the two possible states as a whole. Evidently, in the supercoil-induced B-Z transition in short stretches of DNA, junctions are present whenever there is a transition from the B to Z form. Moreover, the separation between the two junctions, i.e., the number of base-pairs transformed at the critical point, depends on the energy of the junction. Ligand binding, in general, alters these quantities. The consequences of these differences are discussed below.

## 2. Theory

Let us consider that in a circular plasmid of $N$ base-pairs and supercoil density $\sigma$, $N_0$ base-pairs form a potential Z-stretch. Our basic assumption is that the B-to-Z transition in a short potential Z-stretch takes place in an all-or-none manner at a particular value of the supercoil density $\sigma$ if $N_0$ is not larger than a critical length $N_c$ determined by the base sequence and other environmental factors. The magnitude of the supercoil density (which is equal to the ratio of extra linking in the supercoiled molecule to the number of linking in the relaxed state) at which such flipping of base-pairs occurs is referred to as the critical supercoil density $\sigma_c$ which depends among other things on $N_0$. If $N_0$ happens to be longer than $N_c$, the minimum cooperative unit, the B-to-Z transition, proceeds smoothly with increasing negative supercoil after the first cooperative transition of $N_c$ base-pairs at a reduced value of the critical supercoil density. Thermodynamic and statistical-mechanical studies of the transition reveal that the above assumption is quite close to actuality because of the high energy costs of the intermediate species having shorter or multiple stretches of Z-transformed regions [3–7].

In the absence of any ligand, we denote the B-DNA species as $B_0$ and the molecules in which Z form is present as $Z_0$. Then, we can always visualise an equilibrium of the type

$$B_0 \overset{L_0}{\rightleftharpoons} Z_0$$

with an equilibrium constant $L_0$. The equilibrium constant is given by the free energy change of the transition $\Delta G$ when $N_Z$ base-pairs transform from the B to the Z form

$$L_0 = \exp[-\Delta G/RT] \tag{1}$$

where

$$\Delta G = \Delta G_n + N_Z \Delta G_{BZ} + \Delta G_s \tag{2}$$

the respective terms in the sum correspond to the nucleation energy $\Delta G_n$ arising from the deformation at the two junctions separating the Z-stretch from the rest of the B form, the change in intrinsic free energy per base-pair $\Delta G_{BZ}$ resulting from the structural changes involved in going from the B to Z form, and the change in the supercoiling energy $\Delta G_s$. On the basis of the elastic model of DNA, Sen and Majumdar [7] have derived an expression for the change in supercoiling energy when $2m$ base-pairs undergo the B-Z transition in a closed circular molecule of $N$ base-pairs. Substituting the value of $N_Z$ for $2m$ into this expression, we obtain

$$\Delta G_s = \Gamma \phi N_Z \left( \frac{2\sigma}{A_B} + \frac{N_Z}{N} \phi \right) \tag{3}$$

where $\Gamma = 2\pi^2 bc/a(b+c)$ is a term involving the lattice parameter $a$ of the B-DNA double helix, the bending stiffness $b$ of the DNA central axis and the torsional rigidity $c$ for twisting about that axis, and $\phi = (A_B + A_Z)/A_B A_Z$ is a term involving $A_B$, the number of base-pairs per turn in B-DNA and $A_Z$ the corresponding parameter for Z-DNA. For the above case, the critical number of base-pairs $N_c$ undergoing cooperative transition is given by

$$N_c = (\Delta G_n/\Gamma)^{1/2}/\phi \tag{4}$$

which can be determined from the minima of the $\Delta G = 0$ curve [7].

In the presence of a ligand X with differential affinity for B and Z form, additional equilibria will be attained:

$$B_0 + X \overset{\beta_1}{\rightleftharpoons} B_1 \quad Z_0 + X \overset{\zeta_1}{\rightleftharpoons} Z_1$$
$$B_1 + X \overset{\beta_2}{\rightleftharpoons} B_2 \quad Z_1 + X \overset{\zeta_2}{\rightleftharpoons} Z_2$$
$$\vdots \qquad\qquad \vdots$$

Using $(X)$ to denote the activity of ligand X and with $\beta_1, \beta_2, \ldots$ and $\zeta_1, \zeta_2, \ldots$ representing the corresponding equilibrium constants for the series of binding reactions shown above, the binding polynomials for the B and Z form can be written as

$$P_B(x) = 1 + \beta_1(X) + \beta_1\beta_2(X)^2 + \ldots \tag{5}$$

$$P_Z(x) = 1 + \zeta_1(X) + \zeta_1\zeta_2(X)^2 + \ldots \tag{6}$$

where the summations continue up to the terms at which saturation with ligands occurs. By using the binding polynomials the average fraction of Z-transformed species

$$\bar{z} = \{(Z_0) + (Z_1) + (Z_2) + \ldots\}\{(B_0) + (B_1) + (B_2) + \ldots + (Z_0) + (Z_1) + (Z_2) + \ldots\}^{-1} \tag{7}$$

can be expressed

$$\bar{z} = \frac{L_0[P_Z(x)/P_B(x)]}{1 + L_0[P_Z(x)/P_B(x)]} = \frac{L}{1+L} \tag{8}$$

where $L = L_0[P_Z(x)/P_B(x)]$.

We define the critical point as the point where $\bar{z} = 1/2$. This yields the condition $L = 1$, or

$$L_0 = \frac{P_B(x)}{P_Z(x)} \tag{9}$$

Another useful relation which readily transpires from the definition of the binding polynomial is [15,16]

$$\frac{\partial \ln L}{\partial \ln x} = n_Z - n_B \tag{10}$$

where the term on the right-hand side corresponds to the average number of ligands that dissociate or bind to the macromolecule when it undergoes the said transition.

In the absence of ligands, the binding polynomials are equal to unity and hence the condition $L = 1$ or $\Delta G = 0$ gives

$$\sigma_c = -\frac{A_B}{2\Gamma\phi N_Z}[\Delta G_n + N_Z \Delta G_{BZ}] - \frac{A_B N_Z \phi}{2N} \tag{11}$$

with the condition that $N_Z = N_0$ if $N_0 \leq N_c$ and $N_Z = N_c$ if $N_0 > N_c$.

From eq. 11, one finds that the point of transition, $\sigma_c$, depends on the magnitudes of $\Delta G_n$ and $\Delta G_{BZ}$. One can well imagine a situation in which $\Delta G_{BZ}$ decreases in value and becomes negative (say, in high ionic strength solutions) to make the transition possible in the case of the relaxed state ($\sigma_c = 0$) or of positive supercoils ($\sigma_c > 0$).

In the presence of ligands, calculation of the fraction of molecules in the Z form is in general dependent on the detailed structure of the binding polynomials. In a manner similar to that described above, the transition point is obtained for the condition $L = 1$, or

$$\Delta G_n + N_Z \Delta G_{BZ} + \Delta G_s + RT \ln[P_B(x)/P_Z(x)] = 0 \tag{12}$$

which yields

$$\sigma_c' = -\frac{A_B}{2\Gamma\phi N_Z}[\Delta G_n + N_Z \Delta G_{BZ}] - \frac{A_B N_Z \phi}{2N} + \frac{A_B}{2\Gamma\phi N_Z} RT \ln\left(\frac{P_Z(x)}{P_B(x)}\right) \tag{13}$$

with the condition that $N_Z = N_0$ if $N_0 \leq N_c'$ and $N_Z = N_c'$ if $N_0 > N_c'$. $N_c'$ is determined in the same way as that for eq. 4 above. The value of the critical length in the presence of ligands may in general differ from the previous value due to the presence of the additional term involving the binding polynomials in the free energy expression. In the case of ligands exhibiting greater affinity towards the Z form as compared to the B form, the effect of ligand binding can only reduce $N_c$. Therefore, values of $N_0$ which were slightly smaller than $N_c$ in the absence of ligands may turn out to be larger than the critical length in the presence of ligands, with the result that complication of the

calculations occurs. However, situations are also possible where the critical length would remain unchanged after the addition of ligands. Such will be the case when the ligand binding term can be expressed as a linear function of $N_Z$, i.e., as $N_Z \Delta G_L$ where $\Delta G_L$ does not involve $N_Z$. To avoid unnecessary complications in the relations derived, we shall henceforth assume that the change in $N_c$ with the introduction of ligand is small and can be neglected.

A general expression for the shift in the point of transition in terms of the binding polynomials is given as

$$\Delta\sigma_c = \sigma_c' - \sigma_c = \frac{A_B}{2\Gamma\phi N_Z} RT \ln\left(\frac{P_Z(x)}{P_B(x)}\right) \tag{14}$$

We can also derive an alternative relation to eq. 10 for evaluation of the change in the average number of bound ligands occurring at the B-Z transition

$$\frac{\partial\Delta\sigma_c}{\partial \ln x} = \frac{RTA_B}{2\Gamma\phi N_Z}(n_Z - n_B) \tag{15}$$

Eqs 10 and 15 are likely to be useful for the experimental characterization of Z-specific ligand binding.

## 3. Discussion

To illustrate the applicability of the above relations in extracting information from experimental results, we analyse below recently reported data on the effects of anti-Z antibodies on the B-Z equilibrium [13]. The phenomenon studied is the stabilization of Z-DNA by antibody binding in supercoiled plasmids pDPL6 (a 2.2 kb pBR322 derivative) with and without an insert $((dC\text{-}dA)\cdot(dG\text{-}dT))_{60}$ (called $(CA)_{60}$ or (CA)). Filter binding studies with low-affinity antibodies at a concentration of 6.4 nM and at a 10-fold higher concentration demonstrated shifts of the midpoints of the transition from $-0.050$ to $-0.042$ for pDPL6 containing $(CA)_{60}$ and from $-0.075$ to $-0.060$ for pDPL6 without an insert. With a high-affinity antibody preparation the midpoints shifted from $-0.042$ to $-0.02$ for the $(CA)_{60}$ plasmid and from $-0.060$ to $-0.055$ for the parent vector on changing the concentration from 6.4 to 64 nM.

For such a low concentration of ligands we use an approximate form of eq. 15 [15]:

$$\frac{\partial\Delta\sigma_c}{\partial \ln x} = \frac{(\Delta\sigma_c)_2 - (\Delta\sigma_c)_1}{\ln(x_2/x_1)} = \frac{RTA_B}{2\Gamma\phi N_Z}(n_Z - n_B). \tag{16}$$

We further assume that $N_Z \approx 30$ base-pairs for pDPL6 with an insert for the given experimental conditions [7], and $n_B \approx 0$ since antibody is Z-specific. Since we do not know $N_Z$ for pDPL6 without an insert, calculation has not been carried out for data obtained from this particular system. The following typical values for other parameters have been taken: $A_B = 10.4$, $A_Z = 12$, $a = 3.4$ Å, $b = 1.26 \times 10^5$ erg cm rad$^{-2}$ mol$^{-1}$, $c = 1.8 \times 10^5$ erg cm rad$^{-2}$ mol$^{-1}$ [7]. For the plasmid with an insert, one can readily determine the value of $n_Z$ at physiological temperature from eq. 16, namely, $n_Z \approx 6$ (for the low-affinity antibody) and $n_Z \approx 17$ (for the high-affinity antibody).

In spite of the approximate nature of the calculations, the above results provide an indication of the average number of antibody molecules bound per DNA molecule at the transition point.

One can also perform an estimation of the order of magnitude of the shifts in the transition points if the explicit forms of the binding polynomials are known. For example, if the ligands are sizeable, we can assume that, at most, one ligand can bind to the potential Z-stretch in both the B and the Z form. The ligands we consider are also assumed to have negligible affinity for the B form as compared to that for the Z-form, i.e., $\beta_1 \ll \zeta_1$. In such a situation, the fraction of molecules in the Z state is determined by inserting the following values into the expressions for binding polynomials: $\beta_i = 0$ for all $i$ and $\zeta_i(X) = \alpha$, $\zeta_1 = 0$ for $i = 2, 3, \ldots$ The required shift in supercoil density is obtained from eq. 14 as:

$$\Delta\sigma_c = \frac{A_B}{2\Gamma\phi N_Z} RT \ln(1+\alpha) \tag{17}$$

At $\alpha = 0$ we obtain our previous result that $\sigma_c' = \sigma_c$. At $\alpha > 0$ the shift is in the direction of positive

supercoils. For $\alpha$ varying from 0.1 to 10 the value of $\Delta\sigma_c$ varies from $5.3 \times 10^{-5}$ to $1.3 \times 10^{-3}$. Thus, we find that for the given values of concentrations the shifts are quite insignificant compared to the value of $\sigma_c$ in the absence of ligands.

The situation is different when the ligands are of relatively small size and bind to the stretch in considerable numbers. Consider the case, as in the above example, where the ligands bind only to the Z form and the maximum number of ligands binding to the Z-stretch is $n$. For this situation, one can obtain an estimate of the order of magnitude of the shift in the transition point by performing the following simple calculation. If the ligand binds to the Z form non-cooperatively, the binding polynomial for the Z-form $P_Z(x) = (1 + \alpha)^n$. Since $P_B(x) = 1$

$$\Delta\sigma_c = \frac{A_B}{2\Gamma\phi N_Z} RT \ln(1 + \alpha)^n. \quad (18)$$

For $n = N_Z$, i.e., when each base-pair binds a ligand, we have calculated the values of $\Delta\sigma_c$ for the same conditions as given above and found that they vary from $1.6 \times 10^{-3}$ to 0.039 for $\alpha$ varying from 0.1 to 10. It is apparent that in this case the shifts quite rapidly become comparable in order of magnitude with $\sigma_c$ for increasirg ligand concentration. One can clearly envisage a situation where the B-to-Z transition would be stabilized in the relaxed state or even for the case of positive supercoils.

Finally, it should be mentioned that for binding of extended ligands like large proteins to DNA, a more appropriate description of binding is provided by the method of McGhee and Von Hippel [17]. In such cases, the binding energy term involving the binding polynomials should be replaced by the binding energy term calculated from the appropriate McGhee-Von Hippel isotherms.

## 4. Conclusion

From the simple treatment presented above we conclude that the presence of Z-binding ligands can considerably perturb the B-Z equilibrium in supercoiled DNA. The magnitude of this perturbation depends on the binding characteristics or, more specifically, on the binding polynomials. The shift is insignificant for large molecules which bind to the stretch in small numbers even if they possess very strong affinity for the Z form. The shift becomes considerable only when the number of ligands which can bind to the stretch is large. These results may have importance in considerations about the stability of the Z form in natural sequences in vivo. Moreover, the above results can be used to estimate the error in detecting the B-Z transition when Z-binding ligands are used for that purpose. The variation of the shift with ligand concentration gives the change in the number of bound ligands occurring as a result of the transition. This may serve as useful information in characterising the binding mode of the ligand.

It should also be noted that a similar formalism should be valid for investigations regarding cruciform extrusion under negative superhelical stress in plasmids containing palindromic sequences where the transition for short stretches is also strongly cooperative.

Expressions such as those described above are also applicable for cases where the ligands alter the net supercoiling of the DNA molecule by causing winding or unwinding of the DNA during the process of binding. For such cases the equilibrium constants of the ligand binding reactions would contain extra factors involving the change in supercoiling energy due to ligand binding to the molecule for that state [18,19].

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