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Ligand binding isotherm for DNA in the presence of supercoil-induced non-B form: a theoretical analysis

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Abstract

A binding isotherm in the form of a modified McGhee–Von Hippel equation is proposed, on the basis of thermodynamical considerations, to include the non-cooperative binding of extended ligands to supercoiled DNA, where a stretch of non-B form may be present under superhelical stress. It is then studied, on the basis of a non-linear Scatchard plot, how the presence of an intercalating ligand can relax the supercoiled molecule and thus destabilise the non-B stretch, which may be recognised by the existence of a significant kink in the Scatchard plot.

Keywords: DNA–ligand interaction; Supercoiled DNA; Conformational transitions; Non-B DNA; Two-state model

1. Introduction

A wide variety of physiological processes reflect the characteristics of ligand interaction with macromolecules involving proteins and nucleic acids. In particular, when extended ligands bind to a linear chain molecule possessing multiple binding sites whose spacing is smaller than the ligand site itself, each binding involves more than one site. Such is the case, for example, when ligands like chloroquine or ethidium bromide bind non-specifically to B DNA. For non-specific binding of proteins to nucleic acids, the number of excluded sites per binding is even much more. It has been shown by McGhee and Von Hippel [1] that such bindings, if non-cooperative, exhibit non-linear Scatchard plots which are convex downward. On the other hand, the binding isotherm for interaction of supercoiled DNA with intercalative dyes was analysed by Bauer and Vinograd [2]. However, under physiological conditions, it would perhaps be more realistic to study the ligand binding characteristics for the supercoiled DNA where structural variations may also exist. Therefore, in the present paper, we propose a modified form of the McGhee–Von Hippel equation, on the basis of thermodynamical considerations, to include the binding of extended ligands to plasmid DNA molecules where non-B forms may be present under appropriate superhelical stress. However, the transition to such non-B forms, in the present model, is assumed to be cooperative, as is the case with supercoil-induced B-to-Z transition for short purine–pyrimidine stretches, the details of which have been published in our earlier papers [3–5]. In the present work we have taken B-to-Z transition as a model for any conforma-

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tional change of the macromolecule into a non-B form.

Let us assume the existence of a short non-B stretch in a negatively supercoiled B DNA molecule. It is well known that the presence of some ligands, which bind to the B form, actually changes the topoisomeric distribution by effectively relaxing the molecule, so that the non-B stretch may be destabilised and finally it may cease to exist beyond a certain critical ligand concentration. We have shown that the resulting flip of the non-B stretch into the usual B form shows up in the form of a significant kink in a non-linear Scatchard plot obtained on the basis of our modified McGhee–Von Hippel equation. Thus the presence of a non-B stretch within the molecule, if any, becomes directly detectable from a Scatchard plot analysis by using known ligands as probes. The role of such alternative structures of DNA in the regulation of gene expression at the transcriptional level is well known [6,7].

2. Partition function

Our theoretical approach involves calculation of the appropriate partition function for a ligand-bound supercoiled DNA where the possibility of transition to a non-B form exists. We find that the modified form of the McGhee–Von Hippel equation follows directly by retaining the largest term in the partition function according to the usual prescription of statistical thermodynamics [8,9].

As a prerequisite to any thermodynamical analysis involving calculation of the partition function, it is first of all necessary to identify the relevant energy factors. Let us consider a negatively supercoiled DNA molecule, N base pairs long, containing a stretch of m_0 base pairs ($m_0 \ll N$) capable of assuming a non-B form under superhelical stress. If the transition to the non-B form actually proceeds with m transformed base pairs (out of m_0) distributed into r regions of the molecule, then the total free energy in the presence of n bound ligands is given by

$$G(\Delta Lk,m,n) = rG_n + mG_t + G_s(\Delta Lk,m,n) \qquad (1)$$

where G_n is the average nucleation energy per region of the molecule, G_t is the average change of intrinsic energy per base pair arising out of the structural changes involved, and G_s is the supercoiling energy due to a linking difference ΔLk of the supercoiled molecule. In reality, the energy parameters G_n and G_t are sequence specific [10], so that here we can only approximate them by their average values.

In the absence of ligands ($n = 0$), the general form of the supercoiling energy term has been extensively studied by us in recent years [9,11,12] where contributions arising from the resulting change of twist as well as the axial writhe were taken into consideration. In particular, for a transition like B-to-Z, it has been shown [3–5] that the supercoiling energy may be approximated by a quadratic form, namely

$$G_s(\Delta Lk,m) = \frac{2\pi^2 C}{N}(\Delta Lk + m\phi_c)^2 \qquad (2)$$

where

$$\phi_c = \left(\frac{1}{A_B} + \frac{1}{A_Z}\right) \qquad (3)$$

In Eq. 2, the constant C is generally a combination of the twisting and bending rigidity parameters, and may be approximated by the twisting parameter alone if the axial writhe of the molecule is ignored. In Eq. 3 $A_B = 10.5$ and $A_Z = 12.0$ represent the number of base pairs per helical turn for the B and the Z forms respectively, so that ϕ_c is the change of twist per base pair transformed. We are considering supercoil densities $\sigma = \Delta Lk/(N/A_B) \ll 1$, for which ϕ_c depends negligibly on ΔLk [13]. In the presence of ligands which bind to the B form, Eq. 2 may be modified to the form

$$G_s(\Delta Lk,m,n) = \frac{2\pi^2 C}{N}(\Delta Lk + m\phi_c + n\phi_l)^2 \qquad (4)$$

where n is the number of bound ligands and ϕ_l is the change of twist per ligand bound. The parameter ϕ_l is characteristic of the ligand concerned and can be estimated from the unwinding of the duplex molecule in the presence of such ligands.

Let K denote the intrinsic binding constant of the ligand and x denote the free ligand concentration. Then the required partition function, in the present case, may be expressed in the form

$$Z = \sum_{m,n} g(m,n)(Kx)^n \exp[-G(\Delta Lk,m,n)/k_B T] \qquad (5)$$

where, as mentioned already, n ligands are bound to the supercoiled molecule having m base pairs in the Z form. In Eq. 5, $g(m,n)$ represents the degeneracy factor and k_B is the Boltzmann constant.

Supercoil-induced B-to-Z transition in the absence of ligands has been extensively studied by us [3–5]. It has been shown that for a sufficiently short Z-potential structural motif (e.g. a purine–pyrimidine stretch, less than about 30 base pairs long) in a typical plasmid DNA molecule, the transition to the Z form under the superhelical stress (at about $\sigma = -0.05$) is fully cooperative or of the all-or-none type. This result is also supported by the available experimental data [10]. Taking this two-state model for transition of a specific stretch into its non-B form in the presence of ligands as well, the above partition function may be approximated as

$$Z = \sum_n (Kx)^n \{ g(0,n) \exp[-G(\Delta Lk,0,n)/k_B T] + g(m_0,n) \exp[-G(\Delta Lk,m_0,n)/k_B T] \} \quad (6)$$

where, as mentioned before, m_0 ($m_0 \ll N$) base pairs is the stretch length capable of assuming the non-B conformation. This short stretch transformed hardly affects the ligand distribution. Therefore, for the degeneracy factors, we may assume

$$g(0,n) = g(m_0,n) = g(n) \quad (7)$$

so that the partition function may be further approximated as

$$Z = \sum_n g(n)(Kx)^n \exp[-G_s(n)/k_B T] \times \{1 + \exp[-\Delta G(n)/k_B T]\} \quad (8)$$

where from Eqs. 1 and 4, $G_s(n) = G(\Delta Lk,0,n)$ actually represents the supercoiling energy of the ligand-bound B DNA alone, and the free energy difference $\Delta G(n)$ is given by

$$\Delta G(n) = G(\Delta Lk,m_0,n) - G(\Delta Lk,0,n) \quad (9)$$

Substituting the values of $G(\Delta Lk,m_0,n)$ and $G(\Delta Lk,0,n)$ by using Eqs. 1 and 4, the partition function may be expressed in the form

$$Z = \sum_n g(n)(Kx)^n \exp[-G_s(n)/k_B T](1 + L_0 c^n) \quad (10)$$

where

$$L_0 = \exp[-\Delta G(0)/k_B T] \quad (11)$$

and

$$c = \exp[-4\pi^2 C m_0 \phi_c \phi_1 / N k_B T] \quad (12)$$

In the above expressions, $\Delta G(0)$ is a part of the free energy change independent of the bound ligand number n, so that L_0 actually represents the equilibrium between the two conformational states of DNA in a ligand-free situation. It has been pointed in our earlier work, that this suggests a formal similarity with the MWC model [14] where L_0 is the analogue of the allosteric constant and the parameter c plays the role of a cooperativity factor [5,15].

3. Modified McGhee–Von Hippel equation

In order to obtain a McGhee–Von Hippel type equation for binding isotherm in the present case, we will actually have to evaluate the partition function Z for which a knowledge of the degeneracy factor $g(n)$ is necessary. A general formulation for solving such combinatorial problems has been proposed [16]. However, in the present case, $g(n)$ represents the number of possible ways in which n extended ligands can bind to N equivalent sites, assuming that each binding excludes l sites. The number of unoccupied sites is, therefore, $(N - ln)$, so that $g(n)$ is approximately given by the following permutation

$$g(n) = \frac{[N - (l-1)n]!}{(N - ln)!\, n!} \quad (13)$$

Since the numbers involved are sufficiently large, the usual procedure for evaluating the partition function Z is to approximate it by its largest term under the summation in Eq. 10. The optimal value of n, which yields the largest term, in the present case, is obtained by putting

$$\frac{\partial}{\partial n} \ln\{ g(n)(Kx)^n \exp[-G_s(n)/k_B T](1 + L_0 c^n) \} = 0 \quad (14)$$

When simplified by using Stirling's approximation in the usual way, Eq. 14 gives

$$\frac{\nu}{x} = K \exp\left[-\frac{1}{k_B T} \frac{\partial G_s}{\partial n} + \frac{L_0 c^n \ln c}{1 + L_0 c^n} \right] (1 - l\nu)$$

$$\times\left[\frac{1-l\nu}{1-(l-1)\nu}\right]^{l-1} \qquad (15)$$

where $\nu = n/N$ is the degree of ligand binding. This can be easily recognised as a modified form of McGhee–Von Hippel equation for binding of extended ligands (effectively l base pairs long and having intrinsic binding constant K) to the supercoiled DNA where the Z form, or more generally a non-B form, may be present under the superhelical stress. Clearly, in the absence of any Z-potential structural motif, $L_0 = 0$, which may be obtained from Eq. 11 by putting $\Delta G(0) = \infty$. Also, in the absence of supercoiling, $G_s = 0$, so that under such conditions, Eq. 15 reduces to the usual McGhee–Von Hippel equation.

4. Results and conclusion

In Fig. 1 is presented a typical Scatchard plot (ν vs. ν/x) on the basis of our modified McGhee–Von Hippel Eq. 15 for binding of the intercalating dye ethidium bromide to a plasmid DNA such as the 2.2 kilobase cloning vector pDPL6 [17]. The Z-potential insert is taken to be 30 base pairs in length. On the basis of the estimated binding parameters in the present case [18], we take $K = 10^4$ M^{-1} and $l = 3$. Also, we take the unwinding angle $\phi_1 = 26°$ for the intercalating ligand ethidium bromide [19]. Finally, on the basis of the estimated energy and elastic parameters for the B-to-Z transition in a ligand-free situation which has been extensively studied by us with reference to the Z-potential insert in pDPL6 [3,4], we take $G_n = 15.4$ kcal mol^{-1} and $G_t - 0.45$ kcal mol^{-1}, $\Delta Lk = -15$ and $2\pi^2 C = 10^3$ kcal mol^{-1}. It is interesting to note from Fig. 1 that the Scatchard plot, as obtained from our theory, shows a significant kink (curve C) indicating the presence of a cooperative B-to-Z transition under superhelical stress. When no such transition is possible, we put $L_0 = 0$, so that the kink is replaced by a continuous line (curve B) similar to that obtained by Bauer and Vinograd [2]. For the sake of comparison, Fig. 1 also shows the usual Scatchard plot for $l = 3$ (concave downward) in the absence of supercoiling, which corresponds to the linear or nicked circular form of the B DNA molecule (curve A). Clearly, the curves B and C suggest that a conformational transition in the short Z-potential stretch within the supercoiled molecule reduces the overall superhelical stress and thus causes the ligand binding affinity of the B DNA to decrease. Curve A, on the other hand, shows that this binding affinity decreases further for the linear B DNA as expected. Finally, we have also checked that the nature of these curves remains the same if the site exclusion parameter l is varied over a wide range.

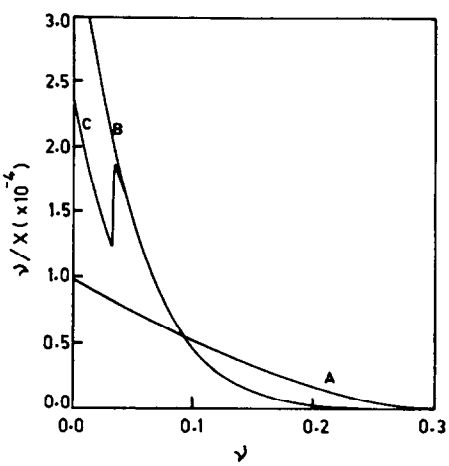

Fig. 1. Scatchard plots as obtained from theory for the binding of an extended ligand with $l = 3$ to (A) linear or nicked circular DNA, (B) supercoiled DNA and (C) supercoiled DNA in the presence of non-B form. The input parameters are for the binding of ethidium bromide to a plasmid DNA molecule (2.2 kilobase cloning vector pDPL6) with a 30 bp Z-potential insert. Clearly, the presence of the non-B form (Z form in the present case) shows up as a kink in the Scatchard plot (curve C).

In conclusion, it may be remarked that our theoretical results suggest the possibility of identifying an unusual DNA structure on the basis of spectroscopic measurements on the binding of ligand to superhelical DNA expressed in the form of a Scatchard plot. A plot similar to the one obtained by us in Fig. 1 (curve C) would clearly indicate the presence of an unusual DNA structure under the superhelical stress.

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