Long-Range Interactions between Ligands Bound to a DNA Molecule Give Rise to Adsorption with the Character of Phase Transition of the First Kind

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Dmitri Y. Lando*
and Vladimir B. Teif

Institute of Bioorganic Chemistry,
Belarus National Academy of Sciences,
Kuprevich Str. 5/2,
220141 Minsk, Belarus

Abstract

Influence of long-range interactions between ligands bound to DNA molecule on the character of their adsorption is studied using computer modeling. For this investigation, two calculation procedures are developed. They are based upon the method of the free energy minimum and on the partition function method. The both procedures demonstrate that in the case of a strong enough attraction between *all* the bound ligands their binding to DNA has the character of phase transition of the first kind. There is a break in the binding curve $c(c_o)$ where c - relative concentration of bound ligands, c_o - molar concentration of free ligands. The break occurs because there is an interval of central degrees of binding (~50% of the maximum c value) that are prohibited for individual DNA molecules. Such a transition might be caused by some types of DNA condensation. Attraction between the *neighboring* ligands only, adjacent or/and separated by double helix regions, does not cause this effect.

Introduction

There are two types of interactions between ligands bound to a DNA molecule. Firstly, there are direct contact interactions or any other kind of short-range interactions between ligands that occupy adjacent base pairs. Influence of such interactions on ligand binding to DNA was theoretically considered by McGhee and von Hippel (1). Another type of short-range interactions was investigated later (2,3). An example of contact interactions is "glue ends" of subunits of lac-repressor that govern their assembling on DNA. Secondly, there are long-range interactions that are occurred due to alteration of DNA structure or/and DNA charge density around bound ligands (4) or of the whole DNA molecule (5,6). Various antibiotics and drugs (7-13), metal ions (14-18) and many other compounds give rise to such interactions. Some types of long-range interactions may give rise to DNA condensation (19,20).

There are two main theoretical methods that allow including of long-range interactions. The first one that was proposed by Zasedatelev and co-authors (4) includes interactions between neighboring ligands only. It can be adjacent ligands that occupy adjacent base pairs or ligands separated by long regions of the double helix. The second approach was proposed by Scatchard (21) and updated by Nechipurenko (5) to make it suitable for studies of DNA-ligand interactions. It allows including of interaction of all the adsorbed ligands. In the Scatchard (21) approach, the total free energy of every bound ligand is assumed to be a linear dependence on relative concentration of all bound ligands. The dependence can be taken arbitrary in the Nechipurenko method. The approaches (5,21) has been successfully used for description of ligand binding to the helical DNA molecules (14-17) and single-stranded polynucleotides (17,18).

To study adsorption with the character of phase transition, our modification of the

*Phone: 375-17-2648263;
Fax: 375-17-2648647;
E-mail: lando@ns.iboch.ac.by

Nechipurenko method (5) is used in this work. As in his approach, it is taken into account that every ligand may cover m DNA units (base pairs) and an arbitrary form for the concentration dependence of the free energy of long-range reciprocal interactions between all the ligands bound to the DNA molecule can be used in the modeling.

Method of Calculation of Binding Curves

Let us consider an ideal solution of ligands and DNA using the method of the free energy minimum, which is equivalent to the method of the most probable distribution (maximum of the entropy). The chemical potential of a free ligand in solution is given by standard Eq.[1]:

$$\mu = \mu_o + R \cdot T \cdot ln(c_o) \qquad [1]$$

where c_o - molar concentration of free ligands; R - universal gas constant per mole; T -temperature (K).

The free energy of a bound ligand includes three constituents:

1) Ψ_o -free energy of direct binding of a ligand to m base pairs that are covered by the ligand (Figure 1).

2) $-G(c^*)$ - the free energy of interaction between a bound ligand with all other bound ligands, where $c^*=k/N$ is the relative concentration of bound ligands (degree of binding), k is the number of bound ligands, and N is the number of base pairs (units) in a DNA chain. As in (5, 21), it is supposed that $G(c^*)$ is independent of ligand distribution along DNA chain. It is obvious that $G(0)=0$.

3) The term corresponding to the entropy of adsorbed ligands (per ligand) is equal to $(-TS/k)$ where $S=R \cdot ln(W_b)$ and W_b is the number of permutations of k ligands along DNA chain of N units. If every ligand covers m base pairs, W_b is given by Eq.[2] (see (22-24)):

$$W_b = \frac{[N/r - (m-1) \cdot k]!}{k! \cdot (N/r - m \cdot k)!} \qquad [2]$$

where N/r is the number of binding centers in a DNA chain.

If $m>1$ and $r=1$, then every ligand covers m base pairs; another ligand cannot bind to the same m base pairs (Figure 1A). If $m>1$ and $r=0.5$, then ligands can interact with different DNA strands independently, and a base pair can bind two ligands simultaneously (Figure 1B). If $m=1$, then r is the number of base pairs per potential binding site; in this case DNA is supposed to be a heterogeneous molecule and only one of its r units (base pairs) contains a site of binding (Figure 1C).
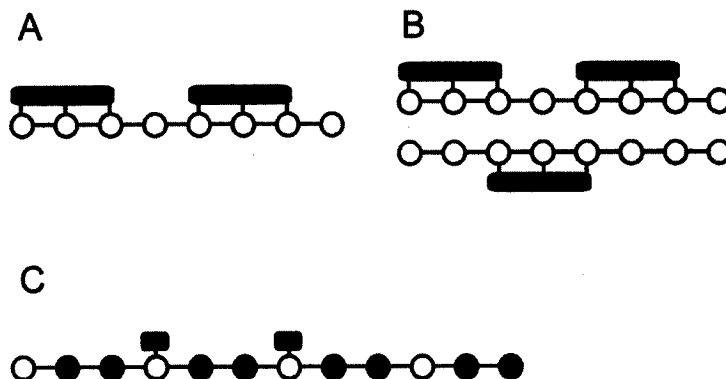

Figure 1: Different types of ligand binding to DNA and corresponding r values.
A - m=3 bp, r=1 bp (every base pair contains one binding center; (O - base pair));
B - m=3 bp, r=0.5 bp (every nucleotide contains one binding center; (O - nucleotide));
C - m=1 bp; r=3 bp (every three base pairs contain one binding center; (O and ● - base pair with and without a binding center respectively)).

It is obvious that the maximum relative concentration of bound ligands ($c_{max}=k_{max}/N$) is equal to $1/(rm)$.

A change in the free energy (ΔF) caused by binding of k ligands to DNA of N units, for given c_o, can be calculated using Eq.[3]:

$$\Delta F(k, c_o) = k\Psi_o - k\cdot G(k/N) - R\cdot T\cdot ln(W_b) - k\cdot[\mu_o + R\cdot T\cdot ln(c_o)] \qquad [3]$$

$\Delta F(k, c_o)$ corresponds to removing of k ligands from solution of molar concentration c_o and their binding to DNA. For any c_o, $\Delta F=0$ if all the ligands are free ($k=0$). Let us find c, the equilibrium value of degree of binding (c^*), using the method of the free energy minimum. The equilibrium (the most probable) c^* value corresponds to the minimum of ΔF if $k,N\rightarrow\infty$. For large N's, one can find c from the equation $\partial(\Delta F)/\partial k=0$ and the Stirling expression $\{ln(n!) = n\cdot[ln(n) - 1]\}$. As the result, Eqs.[4] are obtained for c calculation:

$$K\cdot A = \frac{r\cdot c\cdot[1 - r\cdot(m-1)\cdot c]^{m-1}}{c_o\cdot(1 - r\cdot m\cdot c)^m} \qquad [4]$$

$$K=exp[(\mu_o-\Psi_o)/(RT)]; \quad A=exp\{[G(c) + c\cdot G'(c)]/(RT)\}$$

where K is the binding constant, and A is the constant that characterizes long-range interactions of all bound ligands.

For computer modeling, let us use r, m and $G(c)$ corresponding to divalent metal ions (14-18). It is usually supposed that $m=1$ and $G(c)=w\cdot c$. For this case, one obtains Eq.[5]:

$$K\cdot exp[2wc/(RT)] = \frac{r\cdot c}{c_o\cdot(1 - r\cdot c)} \qquad [5]$$

It is known that the maximum relative concentration (c_{max}) of bound divalent metal ions corresponds to approximately one ion per two nucleotides. In some studies and in our case, c is taken as the relative concentration of bound ligands per base pair and therefore $c_{max}=1/(rm)=1$. In other papers, relative concentration is calculated per nucleotide ($r=2$, $c_{max}=0.5$). To make w and other parameters independent of the definition of a DNA unit, let us transform Eq.[5] into the following form:

$$K\cdot exp[2\cdot W\cdot(c/c_{max})] = \frac{c/c_{max}}{c_o\cdot(1 - c/c_{max})} \qquad [6]$$

$$W=w\cdot c_{max}/(RT)$$

This form is more convenient because in some experimental studies only c/c_{max} can be determined and c is not available. Eq.[6] is especially useful if $c_o>>M$; M - molar concentration of base pairs in solution.

For c calculation, c_o is expressed from one of the Eqs.[4]-[6] in the form $c_o=c_o[r,m,K,G(c),c]$ or $c_o=c_o(r,m,K,W,c)$. Computation of c_o is carried out for a given r, m, K, $G(c)$ (or W, w) and variable c. As a result, for a taken c, c_o is obtained to plot the binding curve $c(c_o)$.

Results of Computer Modeling

Calculations carried out for parameters corresponding to DNA complexes with

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2-valent ions ($m=1$; $r=1$; $c_{max}=1$) demonstrate that the binding curve becomes steeper with increasing W (Figure 2). W determines the type of binding: $W=0$ - noncooperative, $W>0$ - cooperative, $W<0$ - anticooperative. If $W\leq 2$, the binding curve, $c(c_o)$, is monotonous. However for $W>2$, it shows an anomalous S-like form.

Figure 2: Binding curves, $c(c_o)$, calculated using Eq.(6) for $m=r=1$, $K=10$ M^{-1} and $W=0, 1, 2, 4$. W values are shown in the Figure.

To locate all the curves in the same interval of abscissa, c_o is multiplied by $K\cdot A$ (see Eqs.[4]) where A corresponds to $c/c_{max}=0.5$, i.e. $A=exp(W)$.
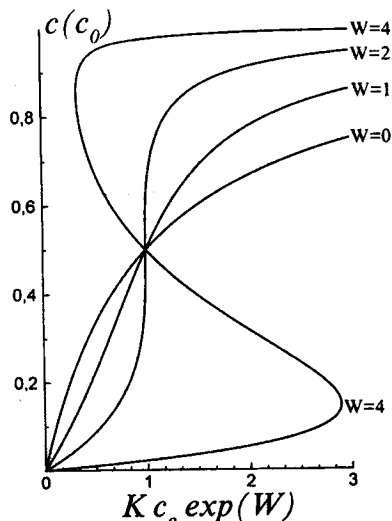
To elucidate the cause of such an anomalous binding behavior let us calculate the "nonequilibrium" free energy per nucleotide in RT units ($\Delta F/(RTN)$) for given c_o and c^* using Eq.[3] and the Stirling expression:

$$\frac{\Delta F(c^*,c_o)}{NRT} = -c^*\cdot ln(A^*\cdot K\cdot c_o) + r^{-1}(1-r\cdot m\cdot c^*)\cdot ln\frac{1-r\cdot m\cdot c^*}{1-r\cdot(m-1)\cdot c^*} + c^*\cdot ln\frac{r\cdot c^*}{1-r\cdot(m-1)\cdot c^*} \quad [7]$$

where $A^*=exp[G(c^*)/(RT)] = exp(W\cdot c^*/c_{max})$. It is necessary to note that $A\neq A^*$ (compare Eqs.[4] and [7]).

For $W\leq 2$, $r=m=1$ and any fixed c_o, the function $\Delta F(c^*,c_o)$ exhibits the only minimum. However for $W>2$, there are two minimum's with a maximum between them (Figure 3). The maximum corresponds to an anomalous (decreasing with rising c_o) central part of the binding curve that has no physical sense but $\partial(\Delta F)/\partial k=0$ for it.

In Figure 4, values of $\Delta F[c(c_o),c_o]/(RTN)$ are shown at corresponding points of the binding curve for which $W=4$. One can see that, for both lower and upper increasing parts of the binding curve, ΔF decreases monotonously from zero for the lower part and from a positive value for the upper part. The decrease is more rapid for the upper
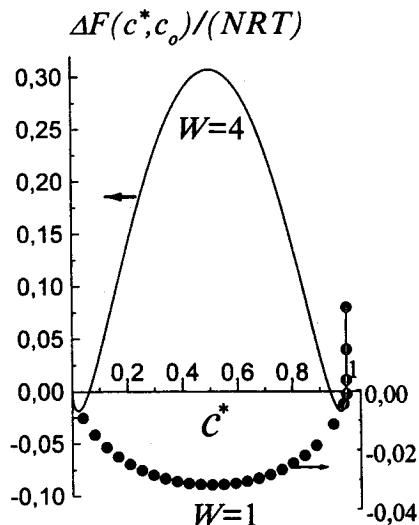

Figure 3: The dependence of "nonequilibrium" free energy per base pair in units of (RT) on the degree of binding (c^*) for $W=1$ and $W=4$. c_o value used in calculation corresponds to $c/c_{max}=0.5$. W values are shown in the Figure.

part. Therefore the value of $c_o=c_{ocr}$ exists for which $\Delta F_{lower}(c_{ocr})=\Delta F_{upper}(c_{ocr})$. For $c_o<c_{ocr}$, the lower part is more probable because $\Delta F_{lower}(c_o)<\Delta F_{upper}(c_o)$. For $c_o>c_{ocr}$, the probability of the upper part is higher $(\Delta F_{lower}(c_o)>\Delta F_{upper}(c_o))$.
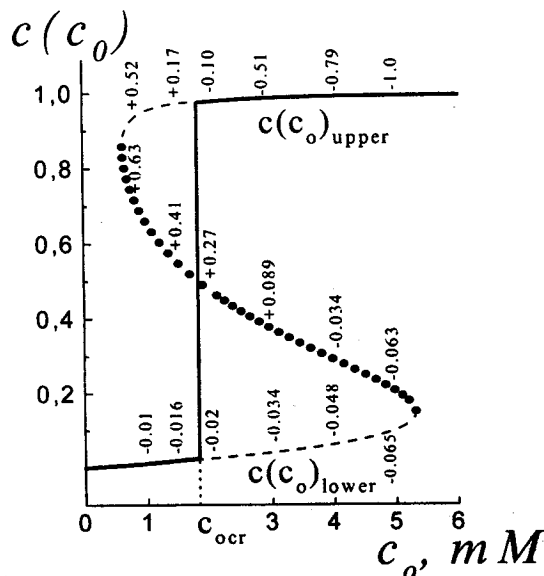
Figure 4: An anomalous binding curve, $c(c_o)$, calculated for $W=4$. The points corresponding to $c(c_{ocr})_{lower}$ and to $c(c_{ocr})_{upper}$ are connected by a solid vertical line. For these two points, $\Delta F_{lower}(c_{ocr})=\Delta F_{upper}(c_{ocr})$. $\Delta F[c(c_o),c_o]/(RTN)$ values calculated using Eq.[7] for some of the points of the binding curve $(c(c_o))$ are shown in the Figure.

Thus the calculated binding curve "jumps" from the lower part to the upper one at $c_o=c_{ocr}$ if the method of the free energy minimum is used for computation. It means that for any c_o, the most probable c from the interval $c_{lower}(c_{ocr})<c<c_{upper}(c_{ocr})$ are prohibited for individual DNA molecules. The whole binding curve can be plotted by connection of points corresponding to $c_{lower}(c_{ocr})$ and $c_{upper}(c_{ocr})$ with vertical straight line (Figure 4). Such a binding has the character of a phase transition from $c(c_{ocr})_{lower}$ to $c(c_{ocr})_{upper}$, which is caused by c_o alteration. Calculation of the "equilibrium" $\Delta F[c(c_o),c_o]$ demonstrates that it is a phase transition of the first kind because a decrease in the free energy is more rapid for the upper part of the binding curve, i.e. $\partial \Delta F[c(c_o),c_o]/\partial c_o$ for different phases are not equal when $c_o=c_{ocr}$.

For all the c_o values except $c_o=c_{ocr}$, there is unique most probable degree of binding for all the DNA molecules. If $c_o=c_{ocr}$, there are two types of molecules of equal probability. However, the fraction of individual molecules of the higher degree of binding $[c(c_{ocr})_{upper}]$ is restricted by total ligand molar concentration (D) and by total molar concentration of base pairs (M). The restriction is given by Eq.[8]:

$$c(c_o)=(D-c_o)/M \qquad [8]$$

It is necessary to note Eq.[8] is fulfilled for all the points of binding curves but we use it for $c_o=c_{ocr}$ only.

For $c_o=c_{ocr}$ the fraction (θ) of individual molecules of the higher degree of binding $[c(c_{ocr})_{upper}]$ monotonously increases with total ligand molar concentration (D) (without a change in $c_o=c_{ocr}$) from zero for $(D-c_o)/M=c(c_{ocr})_{lower}$ to unity for $(D-c_o)/M=c(c_{ocr})_{upper}$. After that, c_o increases with D again. Therefore the total most probable degree of binding $c(c_o)$ can be calculated using Eq.[9]:

$$c(c_o) = \begin{cases} c(c_o)_{lower} & \text{for } c_o<c_{ocr} \\ (D - c_{ocr})/M = (1-\theta)\cdot c(c_{ocr})_{lower} + \theta\cdot c(c_{ocr})_{upper} & \text{for } c_o=c_{ocr} \\ c(c_o)_{upper} & \text{for } c_o>c_{ocr} \end{cases} \qquad [9]$$

where θ is the fraction of individual molecules of the higher degree of binding $c(c_{ocr})_{upper}$, which is given by Eq.[10]:

$$
\theta = \begin{cases} 0 & \text{for } c_o < c_{ocr} \\[2mm] \dfrac{D/M - c_{ocr}/M - c(c_{ocr})_{lower}}{c(c_{ocr})_{upper} - c(c_{ocr})_{lower}} & \text{for } c_o = c_{ocr} \\[2mm] 1 & \text{for } c_o > c_{ocr} \end{cases} \qquad [10]
$$

However using the method of the minimum of the free energy only (Eqs.[1]-[10]), it is impossible to prove that the binding exhibits the character of phase transition in the case of an anomalous form of the binding curve. It is also impossible to determine the minimum N value for every W for which calculations are correct, i.e. the most probable degree of binding (c) is equal to its average value (C) for any c_o. These problems can be solved using the partition function method that directly gives the dependence of the average degree of binding on molar concentration of free ligands, $C(c_o)$. The method also allows computation of $P(c^*,c_o)$, the probability of a given degree of binding c^*. If $c(c_o)$ calculated using Eqs.[6],[10] is equal to $C(c_o)$ computed using the partition function method for large enough N, then the described above procedure (based on the method of the free energy minimum) is suitable for calculation of binding curves when adsorption has the character of phase transition. From the other side, existence of an interval for central $c^*{\sim}0.5 \cdot c^*_{max}$ in which $P(c^*,c_o){\approx}0$ for any arbitrary c_o value, demonstrates that the adsorption has the character of phase transition.

The term of the partition function corresponding to the binding of k ligands is given by Eq.[11], and the whole partition function is calculated using Eq.[12]:

$$
Z(k, c_o) = [K \cdot c_o \cdot exp(W \cdot k/N)]^k \cdot \frac{[N/r - (m-1) \cdot k]\,!}{k!\,(N/r - m \cdot k)!} \qquad [11]
$$

$$
Z(c_o) = \sum_{k=0}^{N/(rm)} Z(k) \qquad [12]
$$

Probability $P(c^*,c_o)$ of a given degree of binding, $c^*=k/N$, for a fixed c_o can be calculated using Eq.[13]:

$$
P(k, c_o) = P(c^*, c_o) = Z(k, c_o)\,/\,Z(c_o) \qquad [13]
$$

The dependence of the average degree of binding on molar concentration of free ligands, $C(c_o)$, one can find using Eq.[14]:

$$
C(c_o) = N^{-1} \cdot \sum_{k=0}^{N/(rm)} k \cdot P(k, c_o) \qquad [14]
$$

A comparison of the results of both methods of calculation shows the following:

1. Calculations for $W=4$ and different N demonstrate that the partition function method gives results independent of N if $N \geq 5000$ bp.

2. The both calculation methods give the same results for $W=4$ if $N \geq 5000$ bp in the partition function method. It means that the most probable c value is equal to its average C. Thus the procedure based upon the method of the minimum of the free energy [Eqs.[1]-[10]] can be used for calculation of binding curves if DNA molecule is long enough. The minimum N value for which the method of the free energy minimum gives correct results increases with absolute value of W.

3. Calculations carried out using the partition function method [Eqs.[10]-[14]] demonstrate a "jump" in the binding curve at $c_o=c_{ocr}$ from $C=c(c_{ocr})_{lower}$ to $C=c(c_{ocr})_{upper}$ if $W=4$ and $N{\geq}5000$ bp (Figure 5A). In this case, the intermediate degrees of binding $(c_{lower}<C<c_{upper})$ exist in a very narrow interval of c_o values (Δc_o); $\Delta c_o/c_o <0.001$ for $N=5000$ bp.
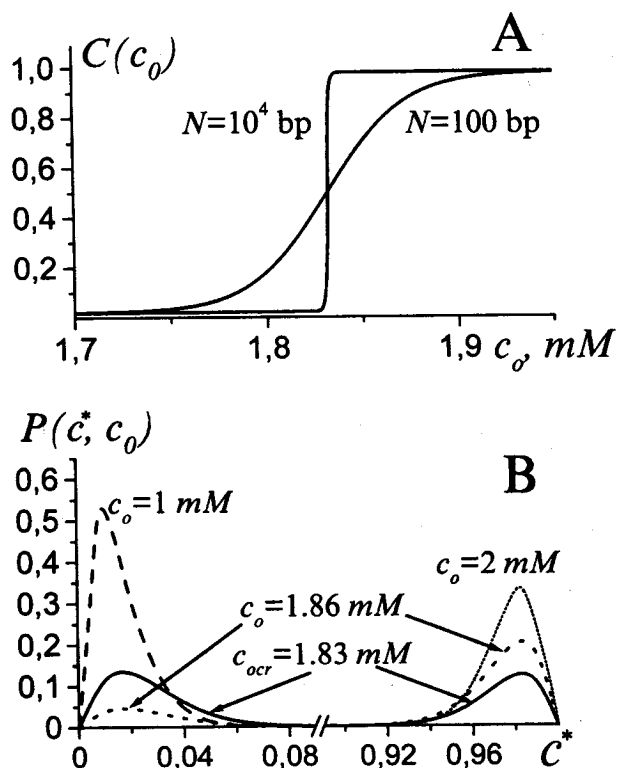

Figure 5: Dependencies $C(c_o)$ and $P(c^*,c_o)$ calculated with the partition function method.
A. The dependence of the average degree of binding, C, on molar concentration of free ligands, c_o. $W=4$; $N=100$ bp and $N=10^4$ bp.
B. The probability, $P(c^*,c_o)$, that the degree of binding is equal to c^*. Calculations are carried out for $N=100$ bp, $W=4$ and various c_o shown in the Figure.

4. The two methods give different results $[c(c_o){\neq}C(c_o)]$ for $N=100$ bp (Figure 5A). The "jump" in the binding curve, $C(c_o)$, is absent. However, as follows from the dependencies $P(c^*,c_o)$ shown in the Figure 5B, there is a large interval of c^* located between $c(c_{ocr})_{lower}$ and $c(c_{ocr})_{upper}$ in which $P(c^*,c_o){\approx}0$ for any c_o. It means that the intermediate degrees of binding from a central part the interval $c_{lower}<c<c_{upper}$ are prohibited for short individual molecules (N~100 bp) as for longer ones $(N{>}5000)$ considered above. Thus for short DNA molecules, the adsorption has the character of phase transition, too. However the degrees of binding corresponding to the lower $(c(c_o)_{lower})$ and upper $(c(c_o)_{upper})$ parts of the binding curve coexist in a wide interval of c_o values around c_{ocr}. Calculations demonstrate that $\Delta c_o/c_o{\sim}0.1$ for $N=100$ bp. Therefore the phase transition character of adsorption is not obvious in the case of short DNA molecules if only the dependence $C(c_o)$ is considered without $P(c^*,c_o)$.

Similar results were received in experimental studies of condensation of individual DNA molecules when the cetyltrimethylammonium bromide (25) or spermidine (26) are used as condensing agents.

The same calculations has been carried out for short-range and long-range interactions that occur only between neighboring ligands. Method of Zasedatelev et al. (4) has been used. It was shown that interactions between neighboring ligands only cannot cause adsorption with the character of phase transition.

Conclusion

1. Strong enough attractive interaction between *all* bound ligands gives rise to adsorption on a DNA molecule which has the character of phase transition. If the free energy of this interaction per ligand (-G) is a linear function of relative con-

centration of bound ligands ($G=W \cdot c$) and $m=r=1$ bp, then the relative most probable concentration (c) and the relative average concentration (C) of bound ligands, takes on any value from interval $0<c<1$ for $W \leq 2$. If $W>2$, central $c\sim0.5 \cdot c_{max}$ are prohibited for individual molecules, i.e. the adsorption has the character of phase transition that is caused by c_o alteration. Therefore for long enough DNA molecules ($N \geq 5000$ bp) and strong enough attractive interaction between all the bound ligands ($W=4$), a "jump" or "break" in binding curve occurs. In this case the two degrees of binding (c_{lower} and c_{upper}) that correspond to various phases coexist in a very narrow interval of molar concentration of free ligands: $\Delta c_o/c_o < 0.001$. If N is not large (~ 100 bp), the binding curve is smooth because $\Delta c_o/c_o \sim 0.1$. Therefore the phase transition is not revealed. However it occurs for individual molecules as it follows from the dependencies $P(c^*, c_o)$.

2. The found phase transition is of the first kind because the derivatives $\partial \Delta F[c(c_o), c_o]/\partial c_o$ for different phases are not equal when $c_o = c_{ocr}$.

3. In theoretical studies of DNA condensation, two-state models are usually used. The condensed and non-condensed states of a DNA molecule are characterized by different values of parameters K, r, m etc. In this study, it is demonstrated that similar phenomena might appear in a DNA system that is described by a single set of parameters that is not changed during condensation. In this case, θ given by Eq.[10] can be considered as degree of condensation.

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