

Short-Range Interactions and Size of Ligands Bound to DNA Strongly Influence Adsorptive Phase Transition Caused by Long-Range Interactions

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Abstract

Long-range interaction between all the ligands bound to DNA molecule may give rise to adsorption with the character of phase transition of the first kind (D. Y. Lando, V. B. Teif, *J. Biomol. Struct & Dynam.* 18, 903-911 (2000)). In this case, the binding curve, $c(c_0)$, is characterized by a sudden change of the relative concentration of bound ligands (c) at a critical concentration of free (unbound) ligands, $c_0=c_{ocr}$, from a low c value to a high one where c_0 is molar concentration of free ligands. Such a transition might be caused by some types of DNA condensation or changes in DNA topology. For the study of the conditions necessary for adsorption with the character of phase transition, a calculation procedure based on the method of the free energy minimum is developed. The ligand size and two types of interactions between ligands adsorbed on DNA molecule are taken into consideration: long-range interaction between *all* the ligands bound to DNA and contact interactions between neighboring ligands. It was found that a) Stronger long-range interaction is required for longer ligands to induce phase transition that is occurred at greater c_{ocr} values; b) Pure contact interaction between neighboring ligands can not itself initiate phase transition. However contact cooperativity strongly decreases the threshold value of energy of long-range interaction necessary to give rise to the transition.

Introduction

DNA involving biological processes are governed by cooperative binding of various molecules (ligands). The cooperativity is a result of different types of long-range and short-range interactions between ligands bound to DNA as well as a change in the structure of the double helix. There are the three main types of interactions between bound ligands:

1. *Contact interactions* may occur between ligands that occupy adjacent base pairs (1, 2). An example of contact interactions is "glue ends" of subunits of lac-repressor that govern their assembling on DNA.
2. *Long-range interactions between neighboring ligands* located at a distance of up to several tens of base pairs influence their binding. A change in DNA charge density around bound ligand or alteration of DNA secondary structure (3) can give rise to such an effect. A theoretical model for computer modeling of influence of such interactions on ligand binding to DNA was first proposed by Zasedatelev and co-authors (1). Adsorption on DNA of various drugs (4-9) is described using this approach.
3. *Long-range interactions between all the ligands bound to DNA* (10-12) may be caused by an alteration of total charge density of a DNA molecule (13), changes in the higher order DNA structure (14-18) as well as in DNA topology (19).

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A theoretical approach that allows including of long-range interaction between all the small ligands bound to a macromolecule was proposed by Scatchard (10). He introduced the free energy of long-range interaction in the form $G(c)^* = w^* \cdot c^2$ per macromolecule that is equivalent to $G(c) = w \cdot c$ per bound ligand, where c is the relative (per base pair) concentration of bound ligands, w (or w^*) is the factor that is dependent on the strength of long-range interactions. Scatchard's formalism have been successfully used for investigation of bivalent metal ion binding to double stranded (20-23) and single-stranded (23, 24) DNA. This approach has been updated by Nechipurenko (11) to make it suitable for studies of DNA-ligand interaction that are characterized by an arbitrary dependence of the free energy of long-range interactions on c .

In his work (10), Scatchard has shown that a threshold value of w might exist for which the slope of $c(c_o)$ curve becomes infinite, where c_o is the molar concentration of free ligands in solution. This conception of adsorption with the character of phase transition is applicable to DNA-ligand systems. In our previous paper (12), it was shown that large enough interaction between all the ligands bound to a DNA molecule may give rise to a sudden change in binding curve $c(c_o)$ and the system undergoes adsorptive phase transition of the first kind. This model might describe abrupt DNA condensation *in vitro* and some peculiarities of chromatin condensation (25-28).

In the present study, we have developed the model of ligand binding proposed earlier (12) to take into account both contact and long-range interactions. Using it, a strong influence of ligand-DNA stoichiometry and contact ligand-ligand interactions on the adsorption with the character of phase transition has been found. It is revealed that phase transition occurs if effective binding constant, K_{eff} , increases rapidly enough with the relative (per base pair) concentration of bound ligands, c . To satisfy this requirement, sufficiently large w that characterizes long-range interaction between bound ligands is required ($G(c) = wc$). The threshold value of w necessary to give rise to a phase transition increases with the ligand length. However, this value is strongly lowered by contact cooperativity of ligand binding to DNA.

Methods

Calculation of binding curves, $c(c_o)$

As was shown in our previous work (12), the method of free energy minimum can be used for study of adsorption with the character of phase transition for sufficiently long DNA molecules (>1000 bp). An ideal solution of ligands and their binding to DNA are considered using this method. Complexes of ligands with DNA are characterized by stoichiometry parameters m and r where m is the number of base pairs covered by a ligand. If $m \geq 1$ and $r = 1$, then every ligand covers m base pairs; another ligand cannot bind to the same base pairs. If $m \geq 1$ and $r = 0.5$, then ligands can interact with different DNA strands independently, and a base pair can bind two ligands simultaneously. In the case $m = 1$, r can be considered as the number of base pairs per potential binding site; i.e. DNA is supposed to be a heterogeneous molecule and only one of its r units contains a site of potential binding for a given type of ligands (see (12, 29, 30) for details).

The chemical potential of a free ligand in solution is given by standard Eq. [1]:

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

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

The free energy of a bound ligand includes four constituents:

1) Ψ_o - free energy of direct binding of a ligand to DNA.

2) $-G(c^*)$ - free energy of long-range interaction between a given bound ligand and all the 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 units (base pairs) in a DNA chain. The maximum relative concentration of bound ligands is $c_{max}=1/(r \cdot m)$. It is supposed that $G(c^*)$ is independent of ligand distribution along DNA chain. It is obvious that $G(0)=0$. The positive and negative $G(c^*)$ values correspond to cooperative and anticooperative binding respectively.

3) The term that corresponds to contact interaction between neighboring ligands that are bound to adjacent DNA base pairs is equal to $b \cdot \varepsilon$, where ε is the free energy corresponding to one contact between ligands and b is the number of contacts that are formed by k ligands bound to DNA. Depending on the ligand distribution for a given state, b value may lie in the interval $0 \leq b \leq k-1$ if $0 < k \leq N/[r(m+1)]$ and $(m+1)k-N-1 \leq b \leq k-1$ if $N/[r(m+1)] < k \leq N/(rm)$.

4) The term that corresponds the entropy of adsorbed ligands (per ligand) is equal to $(-T \cdot S/k)$ where $S=R \cdot \ln(W_b)$ and W_b is the number of permutations of k ligands which form b ligand-ligand contacts along DNA chain of N base pairs. If every ligand covers m base pairs, W_b is determined using slightly changed Eq. [2] of Zasedatelev and co-authors (1):

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

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

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

$\Delta F(k, b)$ corresponds to removing of k ligands from solution of molar concentration c_o and their binding to DNA with formation of b contacts between ligands that occupy adjacent base pairs. Let us denote the degree of binding k/N as c^* and find c , its equilibrium value, using the method of the free energy minimum. For any c_o , $\Delta F=0$ if all the ligands are free ($k=0, b=0$). The equilibrium (the most probable) $c^*=c$ corresponds to the minimum of ΔF for $k, b, N \rightarrow \infty$. For a large N , one can find c from the Stirling expression $\ln(n!) \approx n \cdot [\ln(n) - 1]$ and Eqs. [4]

$$\begin{aligned} \partial(\Delta F)/\partial k &= 0, \\ \partial(\Delta F)/\partial b &= 0. \end{aligned} \quad [4]$$

As a result, one obtains Eqs. [5]-[7] for c calculation:

$$K \cdot A(c) = \frac{(1 - m \cdot r \cdot c)^m}{[1 - (m+1) \cdot r \cdot c + r \cdot z]^{m+1}} \cdot \frac{r \cdot (c - z)^2}{c_o \cdot c} \quad [5]$$

$$A(c) = \exp \{ [G(c) + c \cdot G'(c)] / (R \cdot T) \} \quad [6]$$

$$a = [1 - (m+1) \cdot r \cdot c + r \cdot z] \cdot \frac{z}{r \cdot (c - z)^2} \quad [7]$$

where $K = \exp[(\mu_0 - \Psi_0)/(R \cdot T)]$ is the binding constant, $A(c)$ is the factor of long-range interactions, $a = \exp[-\mathcal{E}/(R \cdot T)]$ is the factor of contact interactions of bound ligands; $z = b/N$ is the relative concentration of ligand-ligand contacts.

z in Eq. [5] can be calculated for a given c using Eqs. [8], [9] obtained from Eq. [7]:

$$z = \begin{cases} c + B - \sqrt{B^2 + D}, & a > 1 \quad \text{cooperative binding} \\ c + B + \sqrt{B^2 + D}, & a < 1 \quad \text{anticooperative binding} \\ \frac{r \cdot c^2}{1 - r \cdot (m-1) \cdot c}, & a = 1 \quad \text{non-cooperative binding} \end{cases} \quad [8]$$

where

$$B = \frac{1 - (m-1) \cdot r \cdot c}{2 \cdot r \cdot (a-1)}, \quad [9]$$

$$D = \frac{c \cdot (1 - m \cdot r \cdot c)}{r \cdot (a-1)}$$

If there is no interactions between ligands that occupy neighboring base pairs ($a=1$, non-cooperative binding) then one obtains Eq. [10] from Eqs. [5], [6], [8]:

$$K \cdot A(c) = \frac{r \cdot c \cdot [1 - r \cdot (m-1) \cdot c]^{m-1}}{c_0 \cdot (1 - r \cdot m \cdot c)^m} \quad [10]$$

We use the linear form of the potential of long-range interactions $G(c) = w \cdot c$ (per bound ligand). It is equivalent to $w^* \cdot c^2$ per macromolecule in the Scatchard studies (10). This linear form of potential has been successfully used for description of binding of bivalent metal ions to DNA (20-23). As follows from Eq. [6],

$$A(c) = \exp(2 \cdot W \cdot c) \quad \text{for} \quad G(c) = RTWc \quad [11]$$

where $W = w/(R \cdot T)$ - parameter of long-range interaction expressed in $R \cdot T$ units.

For calculation of binding curve $c(c_0)$, z is obtained from Eqs. [8], [9] for a given c , and then c_0 is found using Eq. [5] (or Eq. [10] for $a=1$) which gives c_0 in the form $c_0 = c_0[r, m, K, a, G(c), c]$ or $c_0 = c_0(r, m, K, a, W, c)$. Computation of c_0 is carried out for given $r, m, K, a, G(c)$ and variable c . As a result, c_0 is obtained for a taken c to plot the binding curve $c(c_0)$.

The binding curves $c(c_0)$ calculated using Eqs. [5]-[9] for $r=1, m=2, a=10$ and various W values are represented in Figure 1. W determines the type of long-range interaction: $W=0$ - noncooperative, $W>0$ - cooperative, $W<0$ - anticooperative. One can see that binding curve becomes steeper with increasing W . If $W<1.5$, then c monotonously increases with increasing c_0 . However for larger W , a region of the binding curve appears where c decreases with increasing c_0 .

To elucidate the cause of such an anomalous binding curve let us introduce the "nonequilibrium" free energy of the system, $\Delta F(c^*)$, for a given c_0 . It can be found from Eq. [3] and the Stirling expression:

$$\Delta F(c^*)/(RTN) = -c^* \cdot \ln(K \cdot A^* \cdot c_0^* \cdot c^*) - z \cdot \ln(a) - (1/r - m \cdot c^*) \cdot \ln(1/r - m \cdot c^*) + 2 \cdot (c^* - z) \cdot \ln(c^* - z) + [1/r - (m+1) \cdot c^* + z] \cdot \ln[1/r - (m+1) \cdot c^* + z] + z \cdot \ln(z) \quad [12]$$

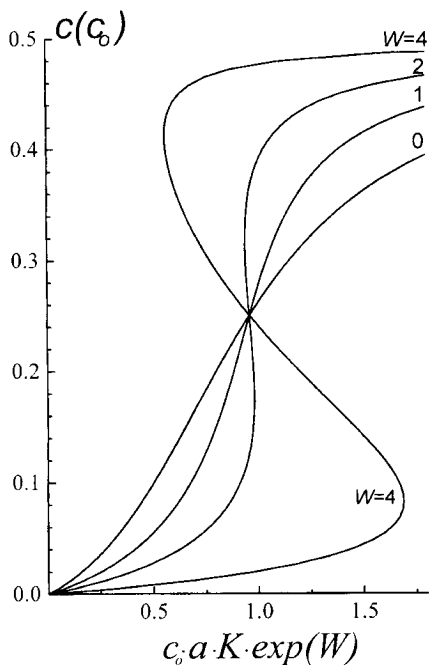


Figure 1: Binding curves, $c(c_0)$, calculated using Eqs. [5]-[9] for $r=1, m=2, a=10, K=10 \text{ M}^{-1}$ and $W=0, 1, 2, 4$. W values are shown in the figure. To locate all the curves in the same concentration interval, c_0 is multiplied by $a \cdot K \cdot \exp(W)$.

where $A^* = \exp[G(c^*)/(R \cdot T)] = \exp(W \cdot c^*)$. $\Delta F[c(c_o)]$ is the extremum value(s) of the free energy (see Eqs. [4]) corresponding to given c_o and $c^* = c(c_o)$ in Eq. [12] where $c(c_o)$ is obtained from Eqs. [5]-[9].

Results and Discussion

The Adsorptive Phase Transition is the Transition of the First Kind

If W in Eq. [11], is lower than its threshold value, W_{cr} , then the relative (per base pair) concentration of bound ligands, c , monotonously increases with the concentration of free ligands, c_o . In this case, the function $\Delta F(c^*)$ given by Eq. [12] has the only extremum (minimum) for any fixed c_o . The minimum corresponds to $c^* = c$, equilibrium c^* value for a given c_o .

If $W > W_{cr}$ the binding curve, $c(c_o)$, calculated using Eqs. [5-9] exhibits S-like form (Figure 1, curve 4). For $W > W_{cr}$, there is an interval of c_o values for which the function $\Delta F(c^*)$ has two minimums and a maximum between them. Therefore Eqs. [5]-[9] has the three solutions. Lower and upper increasing parts of the binding curve correspond to the minimums. The anomalous central part of the binding curve (decreasing with c_o) corresponds the maximum of the function $\Delta F(c^*)$ and has no physical meaning (12).

Binding curve 4 from Figure 1 and corresponding $\Delta F[c(c_o)]$ for its increasing parts are exhibited in Figure 2. For both lower and upper increasing parts of the binding curve $[c(c_o)]$, ΔF decreases monotonously with c_o (and with c). The decrease is more rapid for the upper part. The value of $c_o = c_{ocr}$ exists for which free energies for both lower and upper parts of the binding curve are equal. For all c_o values except $c_o = c_{ocr}$, there is unique most probable degree of binding that corresponds to the minimum of the three $\Delta F[c(c_o)]$ values calculated for a given c_o . For $c_o < c_{ocr}$ the lower part is more probable because $\Delta F_{lower}[c(c_o)] < \Delta F_{upper}[c(c_o)]$. For $c_o > c_{ocr}$ the probability of the upper part is higher: $\Delta F_{lower}[c(c_o)] > \Delta F_{upper}[c(c_o)]$. If $c_o = c_{ocr}$ then $\Delta F_{lower}[c(c_o)] = \Delta F_{upper}[c(c_o)]$. Therefore the binding curve in Figure 2A exhibits a sudden change from a lower to a higher degree of binding. This change is represented by a straight vertical line in Figure 2A. As follows from Figure 2B, the first derivative $[\Delta F(c_o)]'_{c_o}$ for degrees of binding corresponding to the lower and upper parts of the binding curve are not equal at point c_{ocr} (Figure 2B). This indicates that adsorptive phase transition is the transition of the first kind. The lower and upper parts of the binding curve correspond to different phases.

Influence of Ligand Size and Contact Interactions on the Adsorptive Phase Transition

For the linear form of the free energy of long-range interactions per bound ligand $[G(c) = (RTWc)]$, the adsorptive phase transition arises if W reaches the threshold value W_{cr} . Computer simulation carried out using Eqs. [8], [9] demonstrates that $W_{cr} = 2$ in the absence of contact interactions ($a=1$) for small ligands ($m=1, r=1$) (12).

For ligands which cover more than one base pair ($m > 1$), W_{cr} is greater. The influence of ligand size on the character of adsorption is summarized in Figure 3 where the dependence $W_{cr}(m)$ in the absence of contact cooperativity ($a=1$) is shown. One can see that adsorption with the character of phase transition is more favorable for small m values. W_{cr} increases linearly with an increase in m . Thus for big ligands, stronger interactions are required for appearance of phase transition. Besides W_{cr} stoichiometry parameters also affect the concentration of phase transition, c_{ocr} . In Figure 4, the dependence of $c_{ocr}(m)$ is plotted for different W values. One can see that c_{ocr} increases with the ligand length (m). These results are consistent with experimental data suggesting that small ligands of the same charge and

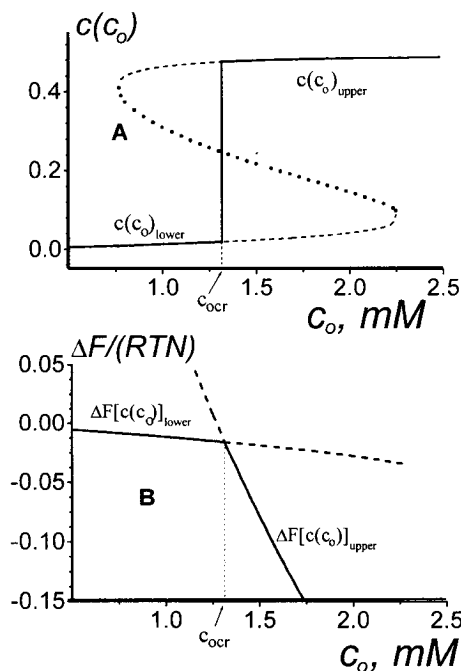


Figure 2: Illustration of the adsorptive phase transition. The binding curve $c(c_o)$ and the free energy $\Delta F[c(c_o)]$ calculated for $r=1, m=2, a=10, K=10 \text{ M}^{-1}, W=4$. Parts of both functions corresponding to the minimum (equilibrium) free energy for a given c_o are plotted with a solid line. See ref. (12) for details.

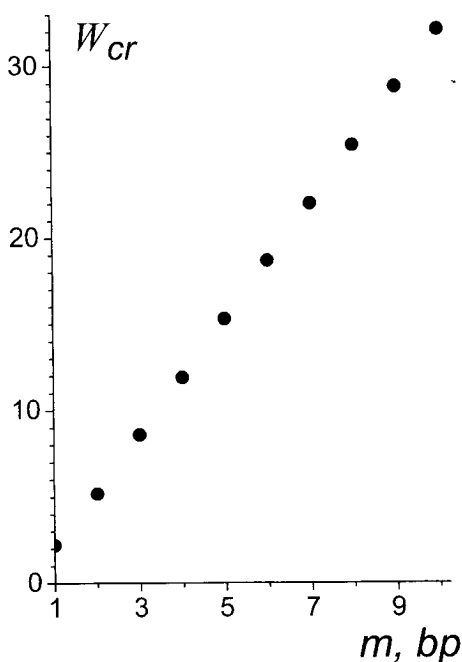


Figure 3: Dependence of the threshold value of W that gives rise to a phase transition (W_{cr}) on the ligand length

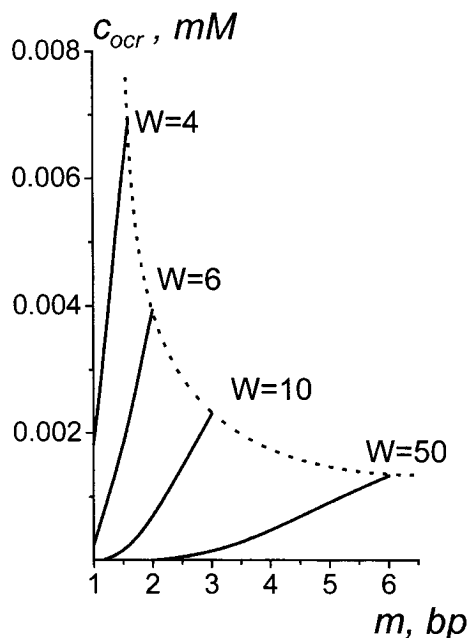


Figure 4: Dependence on the ligand length (m) of free ligand molar concentration ($c_o=c_{ocr}$) at which the phase transition occurs. $r=1$, $K=10 \text{ M}^{-1}$, $a=1$; $W=4, 6, 10, 50$.

Calculation of binding curves demonstrates that only contact interaction itself cannot give rise to adsorption with the character of phase transition. However it changes the threshold value W_{cr} and thus substantially influence phase transition caused by long-range interaction. It is well seen from Figure 5 that represents the influence of contact cooperativity on the value of W_{cr} . As follows from dependence $W_{cr}(a)$, positive contact cooperativity ($a>1$) substantially lowers the threshold W_{cr} value. In the case of strong contact cooperativity, very small $W_{cr}>0$ is sufficient to cause adsorptive phase transition. $W_{cr}\rightarrow 0$ for $a\rightarrow\infty$. Anticooperative contact interaction ($a<1$) increases W_{cr} . Infinite anticooperativity ($a\rightarrow 0$) prohibits binding to the free base pair adjacent to bound ligand but cannot prohibit adsorptive phase transition if W is high enough. Infinite anticooperativity is equivalent to increase in m value by one unit. It is well seen from comparison of Figure 3 and Figure 5, which give $W_{cr}=5.2$ for $m=2$, $a=1$, and for $m=1$, $a\rightarrow 0$.

Thus, large ligands covering more than one base pair ($m>1$), like polyamines, histones or regulatory proteins, require sufficiently larger long-range interaction, than small ligands like metal ions ($m=1$, see Figure 3). However the strength of long-range interactions required for occurrence of phase transition is decreased if contact cooperativity is strong enough ($a>>1$, Figure 5).

To study the role of contact and long-range interactions in the adsorptive phase transition, let us use the effective binding constant. In our case, the effective binding constant, K_{eff} , equals to the binding constant calculated for given c and c_o in the frame of a simplified model where all long-range and contact interactions between bound ligands are neglected ($A(c)\equiv 1$ or w , $W=0$ and $a=1$). It should be noted that, in contrary to K , K_{eff} is not a constant value and depends on c . Values of c and c_o may be taken from experimental data or calculated for more complex models that take into account ligand-ligand interaction. For a taken simplified model, K_{eff} is determined by Eq. [13] that is obtained from Eqs. [5]-[9] or Eq. [10] for $A(c)\equiv 1$ and $a=1$:

$$K_{eff}(c) = \frac{r \cdot c \cdot [1 - r \cdot (m-1) \cdot c]^{m-1}}{c_o \cdot (1 - r \cdot m \cdot c)^m} \quad [13]$$

The ratio $K_{eff}(c)/K$ displays the interactions between bound ligands; $K_{eff}(c)/K \equiv 1$ if these interactions are absent ($W=0$ and $a=1$). To reveal the mechanism of origin of the adsorptive phase transition, let us compare the influence on $K_{eff}(c)/K$ of equivalent contact and long-range interactions. Equivalent interactions give the same value of $K_{eff}(c)/K$ at saturation level when almost all base pairs are covered by ligands, i.e. for $c \rightarrow c_{max} = 1/(rm)$.

As follows from comparison of Eqs. [10], [13] for the case of pure long-range interactions ($a=1$),

$$K_{eff}(c_{max}) = K \cdot A(c_{max}) = K \cdot \exp(2Wc_{max}). \quad [14]$$

For the case of contact interactions,

$$K_{eff}(c \rightarrow c_{max}) = K \cdot a^2$$

because binding of every new ligand gives rise to two additional contacts with the two neighboring bound ligands if c is high enough and there are few free base pairs. It means that for equivalent long-range and contact interactions

$$A(c_{max}) = a^2 \quad \text{or} \quad a = \exp(W \cdot c_{max}). \quad [15]$$

Figure 6 represents dependencies of $\ln[K_{eff}(c)/K]$ on c and the binding curves, $c(c_o)$, for equivalent long-range ($W=4$, $a=1$) and contact ($W=0$, $a=54.6$) interactions

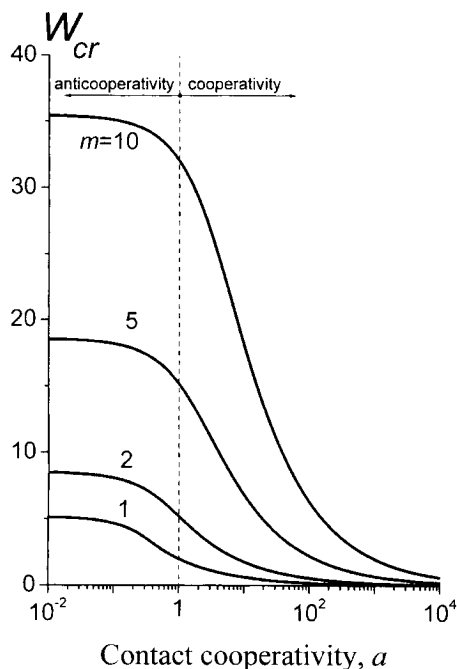


Figure 5: Dependence of W_{cr} on the factor of contact cooperativity (a) for various ligand length (m) and $r=1$. m values are shown in the figure.

calculated using Eq. [13] where c and c_0 are obtained from Eqs. [5]-[9] for $r=m=1$. The curve $\ln[K_{eff}(c)/K]$ corresponding to pure long-range interaction is represented by a straight line with the slope $2 \cdot W=2 \cdot 4=8$ (see Eq. [14]).

The dependence $\ln[K_{eff}(c)/K]$ for contact cooperativity is more complex. At low and high degrees of binding, the dependence rapidly elevates while, at the central part, the elevation is slowed down. Such a dependence is explained by few number of contacts between bound ligands at very low c for which $K_{eff} \approx K$. With an increase in c , the possibility of ligand-ligand contact increases and new ligands are included into already formed blocks of interacting ligands. Every new bound ligand forms a contact. For such a mode of binding, $K_{eff} \approx a \cdot K$. The change in effective binding constant from K to $a \cdot K$ explains the first sharp increase in the curve $\ln[K_{eff}(c)/K]$ at low concentrations. At the saturation level ($c \rightarrow c_{max}$), most of base pairs are already covered by ligands and the possibility that a new bound ligand forms the two contacts with its two neighbors is high. This part of the curve corresponds to $K_{eff} \approx a^2 \cdot K$. The change in K_{eff} from $a \cdot K$ to $a^2 \cdot K$ at high c accounts for the second sharp increase of the binding curve at high c .

As follows from Figure 6a, the contact interaction that does not give rise to adsorptive phase transition ($a=54.6$) has much lower slope of the central part of the curve $\ln[K_{eff}(c)/K]$ in comparison with the equivalent long-range interaction ($W=4$) that causes phase transition. For long-range interaction, the slope is equal to $2 \cdot W=2 \cdot 4=8$ that is higher than the threshold value for the rise of the adsorptive phase transition, $2 \cdot W_{cr}=2 \cdot 2=4$. For contact interaction with $a=54.6$ the slope is equal to 3.5 that is lower the threshold value 4. Thus, a sufficiently high value of the slope in the central part of the curve $\ln[K_{eff}(c)/K]$ corresponds to the adsorptive phase transition.

In Figure 7, dependencies $\ln[K_{eff}(c)/K]$ are plotted for several pairs of equivalent long-range interaction and contact cooperativity. For long-range interaction, the central part of the curves characterized by much steeper slope in comparison with equivalent contact interaction. Pure contact interaction does not provide sufficient slope (that is equal to 4) in the central part of the curve $\ln[K_{eff}(c)/K]$ even for $a=3000$ (the slope is equal to 3.93).

Let us consider the slope in the central part of the curve $\ln[K_{eff}(c=0.5c_{max})/K]$ as a function of a (Figure 8). As was mentioned above, the phase transition arises only if this slope is greater than 4 for $c=0.5c_{max}$ and $m=r=1$. Pure contact cooperativity is characterized by a lower slope at the central part of the curve $\ln[K_{eff}(c)/K]$ for any a value (Figure 8, $W=0$). This limit is gained only in the case of infinite positive contact cooperativity ($a \rightarrow \infty$).

Thus pure contact cooperativity can not give rise to adsorption with the character of phase transition because it does not provide a sufficient slope in the central part of the curve. However its combination even with a weak long-range interaction may give a sufficient slope that is higher than 4 for $r=m=1$. For a general case ($m \geq 1$), the critical slope value ($2 \cdot W_{cr}$) in the central part of the considered function is dependent on m and $W_{cr}(m)$ can be found from Figure 3.

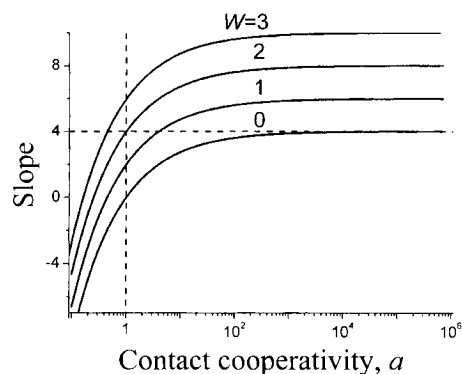


Figure 8: Dependencies of the slope of the function $\ln[K_{eff}(c)/K]$ on a for $c=0.5c_{max}$, $m=r=1$ and various W : 0; 1; 2; 3. The horizontal dash line corresponds to the threshold slope sufficient for occurrence of the adsorptive phase transition.

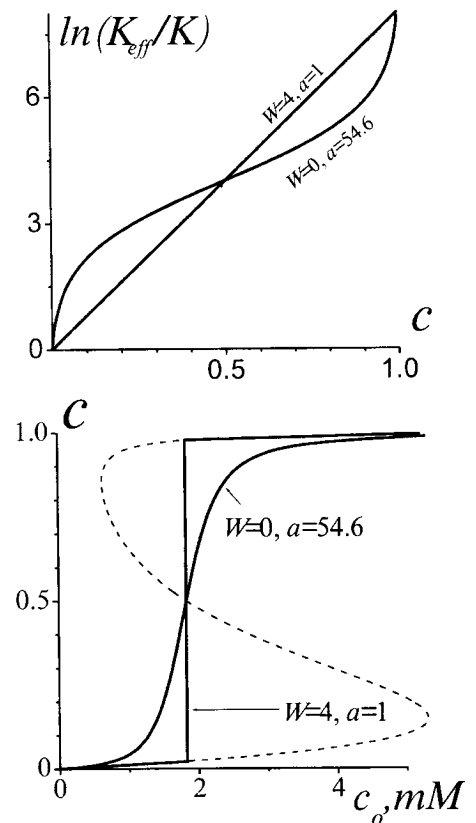


Figure 6: Dependencies of $\ln(K_{eff}/K)$ on the degree of ligand binding (c), and the binding curves, $c(c_0)$, for equivalent contact and long-range interactions between bound ligands. 1 - contact interaction ($a=54.6$, $W=0$, $m=r=1$), 2 - long-range interaction ($a=1$, $W=4$, $m=r=1$).

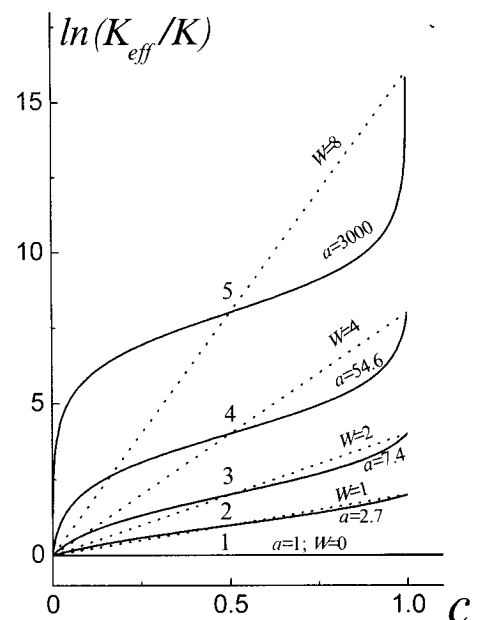


Figure 7: Dependencies of $\ln[K_{eff}(c)/K]$ for pure contact cooperativity ($a > 1$, $W=0$, solid line) and equivalent pure long-range cooperativity ($W > 0$, $a=1$, dash line) for $m=r=1$. 1. $a=1$ - $W=0$, for both curves $K_{eff}(c)/K=1$; 2. $a=2.7$ - $W=1$; 3. $a=7.4$ - $W=W_{cr}=2$; 4. $a=54.6$ - $W=4$; 5. $a=3000$ - $W=8$.

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