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COOPERATIVE EFFECTS ON BINDING OF PROTEINS TO DNA

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Equations are derived for description of cooperative binding of large ligands to a homogeneous polynucleotide lattice for a wide variety of binding models. Both short- and long-range interactions between nearest-neighbour bound ligands are taken into account. It is shown that cooperative binding of ligand at high levels of occupancy can be described with good accuracy by the equation derived for the noncooperative binding of the same ligand with an apparent binding constant K_{eff}. A new method is proposed for the analysis of cooperative binding isotherms. It is based on a comparison of the asymptotic behavior of cooperative and noncooperative binding isotherms in the limit when the occupancy of lattice by ligand approaches the saturation level of binding. It is demonstrated that cooperative effects mediated by direct contact between bound ligands can be divided into two classes depending on whether dimeric species or aggregates of unrestricted size are formed by bound ligands on the lattice at high levels of occupancy. These two classes can be easily distinguished on strictly empirical grounds. In particular, if interligand interactions favor the formation of dimeric species on DNA, $K_{eff} \approx a^{1/2}$ where a is the interligand interaction constant. If interligand interactions generate aggregates of unrestricted size, $K_{eff} \approx a^{L+1}$ where L is the size of binding site for the ligand on DNA. We also demonstrate that cooperative systems in which interligand interaction extends over two or more free polymer residues can be distinguished from systems in which only short-range interactions mediated by direct contacts between bound ligands are allowed.

1. Introduction

The cooperative and noncooperative binding of ligands to a homogeneous polynucleotide lattice has attracted much attention from various investigators [1–16]. Crothers [2] and Zasedatelev et al. [3] have noted that binding characteristics of large ligands are markedly distinct from those of small ligands which occupy only one residue upon binding [1]. Exact formulae are derived for description of cooperative and noncooperative binding of large ligands to both finite and infinite polymer chains for a wide variety of models and molecular mechanisms responsible for the origin of binding cooperativity. However, a general procedure for analysis of cooperative binding isotherms and determination of the relevant thermodynamic parameters from experimental data has not yet been reported.

In the present paper we describe a new approach to analysis of experimental binding isotherms which is based on a comparison of the asymptotic behavior of cooperative and noncooperative binding isotherms in the limit when the occupancy of a lattice by a ligand tends to the saturation level.

In order to illustrate the application of the proposed method to various experimental situations we derive binding equations for a general case in which a ligand can bind to a lattice in two different modes. These two modes can be associated with the binding of ligand in two opposite orientations with respect to a polynucleotide lattice. In this particular case we investigate how interaction between nearest-neighbour bound ligands affects the shape of binding isotherms. We demonstrate that cooperative effects mediated by

direct contacts between bound ligands can be divided into two groups which can be distinguished on strictly empirical grounds. These two groups exhibit some resemblance with isologous and heterologous interactions taking place between subunits in oligomeric proteins [17].

Effects of interaction between bound ligands on the shape of the Scatchard isotherm and kinetics of the binding reaction were considered by Schwarz [5,14,15]. Schwarz and Stankowsky [16] have also rigorously treated the case when a ligand can bind in two different modes to a lattice.

In the case when binding occurs in only one orientation (polar binding) the most general binding equations have been obtained by Zasedatelev et al. [3]. Both short- and long-range interactions between nearest-neighbour bound ligands have been taken into account. Some of these equations have also been obtained by McGhee and Von Hippel [4] for the specific case when only short-range interactions between nearest-neighbour bound ligands are allowed. Our purpose here is to demonstrate that cooperative systems in which only short-range interactions between nearest-neighbour bound ligands are allowed can be distinguished from systems in which both short- and long-range interligand interactions are possible.

2. Asymptotic method for analysis of experimental binding isotherms

In order to describe the main idea of our approach we initially formulate a simple binding model which can be generalized in the subsequent sections if required.

Let DNA and ligand molecules exist in solution at constant temperature and pressure. The DNA molecule can be represented as a linear array of N equivalent residues (base-pairs). Each ligand occupies L consecutive residues upon binding and makes them inaccessible for binding to another ligand. The parameter L will be referred to as the size of the binding site for a ligand on a lattice. The binding is also characterized by the intrinsic binding constant K.

Let q be the average number of ligand molecules bound to a lattice. If $N \gg L$ one can neglect the influence of the end effects on the binding equilibrium. In this case it is convenient to consider that all additive thermodynamic characteristics are calculated per polymer residue. For instance, the average number of bound ligands per polymer residue $r = q/N$. The average number of free (unoccupied) polymer residues is equal to $(N - qL)/N = 1 - rL$.

In the noncooperative binding case the binding isotherm of a large ligand to a homogeneous lattice can be calculated from eq. 1 (see refs. 3 and 4).

$$r/m = K(1 - rL)^L/(1 - rL + r)^{L-1} \tag{1}$$

Eq, 1 has a simple probabilistic interpretation. The macromolecular complex with q ligands can be represented as a linear array of $N + qL + q$ elements of two kinds – Lq residues occupied by bound ligands and divided into q separate groups and $N - Lq$ unoccupied residues.

The probability of finding a free (unoccupied) residue on a lattice, P, is equal to

$$P = (N - qL)/(N - qL + q)$$
$$= (1 - rL)/(1 - rL + r) \tag{2}$$

Clearly, the probability of finding a stretch of L consequtive free residues is equal to P^L. Such a stretch may serve as a potential site for binding to another ligand. The average number of potential binding sites calculated per residue at binding density r, $n(r)$, is equal to

$$n(r) = (1 - rL)^L/(1 - rL + r)^{L-1} \tag{3}$$

Combining eqs. 1–3, one can obtain

$$r/m = Kn(r) \tag{4}$$

If the occupancy of polymer with ligand, r, approaches the saturation level of binding r_{max}, the average number of free potential sites for binding of successive ligands approaches zero as $(1 - rL)^L$. At high r values the majority of bound ligands are in contact with nearest-neighbour bound ligands. Only a small number of adjacent ligands are separated by a single free polymer residue intervening between the polymer regions covered by the bound ligands. When $r \to r_{max}$ one can neglect the fraction of nearest-neighbour

bound ligands separated by a gap containing more than one free polymer residue. Clearly, this general type of arrangement of bound ligands on a lattice in the limit when $r \to r_{max}$ is retained in the cooperative systems as well.

Cooperativity can be quantitated via the parameter a, a dimensionless equilibrium constant for transfer of a bound ligand from an isolated binding site on a lattice to a contiguous site. In the case of binding to a contiguous site a bound ligand can interact with one or two adjacent ligands already bound to a lattice; the binding is characterized by cooperative binding constants Ka and Ka^2, respectively.

If r approaches r_{max}, the number of potential binding sites on a lattice tends to zero as $(1 - rL)^L$, in close similarity with the noncooperative binding case. However, in the cooperative binding case, the major part of these sites belongs to a class of contiguous sites in which bound ligands are allowed to interact with nearest-neighbour bound ligands. This means that at binding densities close to the saturation level of binding there is a region on the cooperative binding isotherm which can be described with good accuracy by eq. 5

$$r/m = K_{eff} n(r) \tag{5}$$

Here $n(r)$ is given by eq. 3. K_{eff} is the apparent binding constant which depends on the energy of interaction between nearest-neighbour bound ligands. Eq. 5 states that when $r \to r_{max}$ the cooperative binding isotherm asymptotically approaches a curve calculated for noncooperative binding of the same ligand with an apparent binding constant K_{eff}. The noncooperative curve describing the binding with intrinsic binding constant K will be referred to as the reference isotherm. From eqs. 4 and 5 we deduce

$$\lim_{r \to r_{max}} \frac{(r/m)_E}{(r/m)_R} = \frac{K_{eff}}{K} \tag{6}$$

where $(r/m)_E$ is the experimental value of r/m measured for the cooperative binding case and $(r/m)_R$ the calculated value of r/m for the noncooperative reference isotherm (see eq. 4).

In the subsequent sections we demonstrate the validity of eq. 6 for a wide variety of cooperative systems and derive exact formula for the similarity coefficient K_{eff}/K of cooperative and noncooperative isotherms in the limit when $r \to r_{max}$. Clearly, K_{eff}/K carries information on the nature and magnitude of interactions between bound ligands in the cooperative system. These considerations describe the main idea of the asymptotic method for analysis of experimental binding isotherms. In practical applications it is convenient to use a logarithmic presentation of binding data in the form

$$H(r) = \ln \frac{r}{Km} \cdot \frac{(1 - rL + r)^{L-1}}{(1 - rL)^L} \tag{7}$$

where

$$H(r_{max}) = \ln \frac{K_{eff}}{K} \tag{8}$$

A plot of H vs. r will be referred to as the H plot. Clearly, in the noncooperative binding case, the H plot is a straight line parallel to the horizontal axis. In the cooperative binding case, the H plot is curvilinear but $H(r)$ tends to $\ln(K_{eff}/K)$ when r tends to r_{max}.

3. Cooperative effects mediated by direct contacts between nearest-neighbour bound ligands on a lattice

3.1. Binding equations

Cooperativity of binding can be defined as a tendency of bound ligands to cluster on a lattice. In this section, we demonstrate that cooperative phenomena in one-dimensional systems can be divided into two broad categories depending on the size of clusters formed by bound ligands on a lattice.

Since the DNA double helix possesses a set of 2-fold symmetry axes normal to the DNA helix axis, the majority of large ligands can bind to DNA in the two alternative orientations related by 2-fold rotation. The base-pair sequence establishes the directionality of DNA. Our previous analysis of cooperative effects in these systems [8,9,13] shows that bound ligands can generate isologous and heterologous associations on a lattice.

Monod et al. [17] have pointed out that isologous associations are stabilized by interactions which can generate aggregates of finite size – isologous dimers or tetramers. In an isologous dimer, the two bound ligands are related by 2-fold rotation. By contrast, heterologous associations have no element of symmetry and may contain an unrestricted number of interacting molecules. In a heterologous aggregate, each bound ligand can interact with two nearest-neighbour bound ligands, thus allowing for unrestricted growth of the aggregate.

It should be mentioned that this classification cannot be literally applied to ligand-ligand associations comprising different molecules or molecules which form complexes of different geometry with DNA. However, obvious analogs of isologous and heterologous associations exist. Generally, ligand-ligand associations can be divided into two broad categories depending on whether dimeric ligand species or aggregates of unrestricted size are formed on a lattice by bound ligands at high levels of occupancy. We shall now try to demonstrate that these two classes of cooperative system exhibit markedly different binding characteristics. To accomplish this we derive binding equations for the general case when a ligand can interact in the two modes 1 and 2 with a homogeneous polynucleotide lattice.

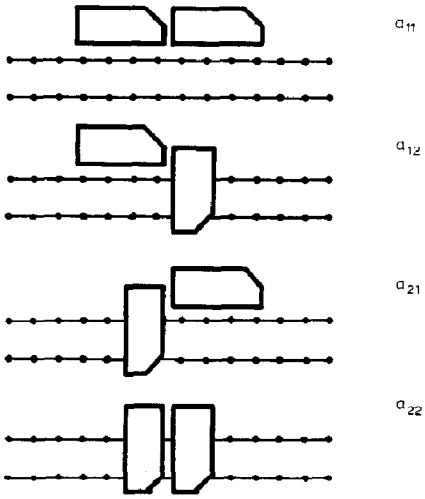

Fig. 1. A scheme illustrating all possible types of interactions between adjacent ligand implicated in the formation of complexes of type 1 and 2 with a polynucleotide lattice. Circles denote DNA bases. A ligand is shown as a bar covering 4 and 3 base-pairs for complexes of type 1 and 2, respectively. a_{11}, a_{12}, a_{21} and a_{22} are ligand-ligand interaction constants defined as described in the text.

Let K_u be the equilibrium association constant for binding of ligand to an isolated site of type u on a lattice and let L_u be the size of the binding site ($u = 1,2$). Bound ligands are allowed to interact, either favorably or unfavorably, with nearest-neighbour bound ligands. To describe these interactions four parameters a_{uv} ($u,v = 1,2$) are required as illustrated in fig. 1. Each parameter a_{uv} is defined as an equilibrium constant for moving a bound ligand from an isolated site of type u on a lattice into a position where it lies in the range of interaction with another ligand implicated in the formation of complex of type v. It is assumed that cooperative interactions are mediated by direct contacts between bound ligands.

Let r_u be the occupancy of u-type binding sites on a polynucleotides lattice ($u = 1,2$). If the left-hand end of a ligand bound at site u can contact the right-hand end of another ligand bound at a site of type v, a ligand-ligand association is formed with interaction constant a_{vu}. Let g_{vu} be the average number of contacts between bound ligands that generate ligand-ligand associations with interaction constant a_{vu}. Of the total number of u-type bound ligands $r_u - g_{1u} - g_{2u}$ have no contact with another bound ligand at the site immediately adjacent to the left-hand end of the occupied u site. Both r_u and g_{uv} are calculated per polymer residue.

The probability that a ligand bound at a site of the u type does not form contact with adjacent ligand bound to the left from a given occupied site is equal to $(r_u - g_{1u} - g_{2u})/r_u$. The probability of finding a bound ligand at an isolated u site on a lattice is equal to the product of probabilities that both the left-hand and the right-hand termini of a given bound ligand are not in contact with adjacent bound ligands $((r_u - g_{1u} - g_{2u})/r_u)((r_u - g_{u1} - g_{u2}/r_u)$. The average number of ligand molecules bound at isolated sites of the u type is $(r_u - g_{1u} - g_{2u})(r_u - g_{u1} - g_{u2})/r_u$.

The condition of thermodynamic equilibrium for binding to an isolated u site can be formulated if one is able to evaluate the number of free

isolated binding sites on a lattice at a given occupancy $r = r_1 + r_2$. The macromolecular complex at occupancy r can be visualized as a linear array of a number of groups of interacting bound ligands (isolated ligand groups) separated by free polymer residues. We note that the free energy of thermodynamic system is not changed if each isolated ligand group moves as a whole along the lattice in both directions until it is separated from adjacent ligand groups by at least one free polymer residue.

The average number of isolated ligand groups can be evaluated if one subtracts from the total number of boundaries between bound ligands on a lattice the number of boundaries separated interacting ligands on the lattice. This quantity divided by N is therefore equal to $r_1 + r_2 - g$ where $g = \sum_{u,v} g_{uv}$ is the average number of boundaries between interacting bound ligands. Summation is carried out over all possible values of u and v ($u = 1,2$; $v = 1,2$).

All possible arrangements of bound ligands on a lattice can be generated by incorporation of free polymer residues between isolated ligand groups arranged in a linear fashion, provided that adjacent ligand groups are separated by at least one free polymer residue. The probability of finding a free (unoccupied) polymer residue, P is equal to

$$P = \frac{1 - r_1 L_1 - r_2 L_2 - (r_1 + r_2 - g)}{1 - r_1 L_1 - r_2 L_2} \tag{9}$$

The probability of finding a stretch of $L_u + 1$ free polymer residues is equal to P^{L_u+1}. Such a stretch may serve as a potential isolated site for binding to another ligand. We can now write the equation for description of isolated site binding in the form of the mass action law:

$$\frac{r_u - g_{1u} - g_{2u}}{r_u} \cdot \frac{r_u - g_{u1} - g_{u2}}{r_u} \cdot \frac{r_u}{m} = K_u P^{L_u+1}(1 - r_1 L_1 - r_2 L_2) \tag{10}$$

The equation for description of cooperative binding events can be obtained in a similar way. Without loss of the generality we consider the situation when a bound ligand at a site of type u interacts only with one out of two of its nearest neighbours bound at an immediately adjacent site of type v lying on the left-hand side of the occupied u site. The probability of occurrence of such a situation on a lattice is equal to $(g_{vu}/r_u)(r_u - g_{u1} - g_{u2})/r_u$, where g_{vu}/r_u is the probability that the left-hand terminus of a bound ligand at site u contacts the right-hand end of another ligand bound at site v.

The number of potential binding sites for which this type of binding can be realized is equal to $P^{L_u}(r_v - g_{v1} - g_{v2})$, where the first multiplier is simply the probability that exactly L_u residues are free and constitute a binding site of type u on the lattice (the binding of ligand to this site is characterized by a cooperative binding constant $K_u a_{vu}$). The second multiplier is equal to the average number of v-type bound ligands having no bound ligand at the immediately adjacent site lying on the right-hand side of the site of type v on the lattice. The equation for the description of cooperative binding events is

$$\frac{g_{vu}}{r_u} \frac{r_u - g_{u1} - g_{u2}}{r_u} \frac{r_u}{m} = K_u a_{vu} P^{L_u}(r_v - g_{v1} - g_{v2}) \tag{11}$$

Eqs. 9–11 represent a system of six simultaneous equations for six unknowns (r_u and g_{uv}; $u = 1,2$; $v = 1,2$). Interestingly, similar equations can be derived for description of binding of two different ligands to a lattice [18].

Similar considerations can be used to demonstrate that the number of boundaries between adjacent ligand molecules bound at sites of types u and v which are separated by i free polymer residues is equal to

$$g_{uv}(i) = P^i g_{uv}/a_{uv}, \ (i > 0). \tag{12}$$

Using eq. 12 one can obtain the following relations:

$$r_1 = g_{11}\left(1 + \frac{P}{(1-P)a_{11}}\right) + g_{12}\left(1 + \frac{P}{(1-P)a_{12}}\right) \tag{13}$$

$$r_2 = g_{22}\left(1 + \frac{P}{(1-P)a_{22}}\right) + g_{21}\left(1 + \frac{P}{(1-P)a_{21}}\right) \tag{14}$$

Substituting numerical values of P $(0 < P < 1)$

into eqs. 9–14 one can evaluate g_{uv}, r_u and r_u/m ($u = 1,2$; $v = 1,2$).

An important general conclusion which can be drawn from eqs. 10 and 11 is that r_u is proportional to P^{L_u}. Since P approaches zero when m tends to infinity one can conclude that at high m values $r_2 \gg r_1$ if $L_2 < L_1$. Increasing free ligand concentration leads to substitution of complexes with greater site sizes by complexes possessing smaller site sizes. We conclude that occupancy of lattice by ligand $r = r_1 + r_2$ cannot diminish when the concentration of free ligand is increased. Using a different mathematical approach Schwarz and Stankowsky [16] have considered competitive-type relations between various modes of ligand binding to a homogeneous lattice and investigated the role of excluded site effects on binding equilibria.

In the most practical applications the two modes of ligand binding can be associated with binding of a ligand in two opposite orientations with respect to a polynucleotide lattice. In this case $L_1 = L_2$ and the two binding modes can coexist on a lattice at high occupancy levels. We now list several implications of eqs. 9–14 for various experimental situations assuming that $L_1 = L_2 = L$.

3.2. Cooperative interligand interactions generating aggregates of unrestricted size on a lattice

(a) If ligand-ligand interaction constants are all identical ($a_{uv} = a$; $u = 1, 2$; $v = 1, 2$) eqs. 10 and 11 reduce to eqs. 15 and 16:

$$\left(\frac{r-g}{r}\right)^2 \frac{r}{m} = K\left(\frac{1-rL-(r-g)}{1-rL}\right)^L \times (1-rL-(r-g)) \qquad (15)$$

$$(r-g)^2 = \frac{g}{a}(1-rL-(r-g)) \qquad (16)$$

where $g = \sum_{u,v=1,2} g_{uv}$, $r = r_1 + r_2$, $K = K_1 + K_2$.

Eqs. 15 and 16 were first derived by Zasedatelev et al. [3] and are equivalent to eqs. 25 and 26 of McGhee and Von Hippel (ref. 4, with corrections given in ref. 19). Fig. 2 shows examples of binding isotherms calculated from eqs. 15 and 16 for $L = 5$ and various values of the parameter a.

In section 3.1, we have found that cooperative binding isotherm in the limit when $r \to r_{max} = 1/L$ can be approximated with a good accuracy by an isotherm calculated for noncooperative binding of the same ligand with an apparent binding constant K_{eff}.

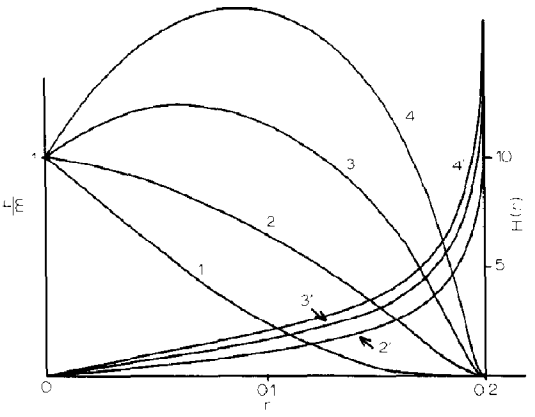

Fig. 2. Theoretical binding isotherms calculated for a system in which interligand interactions can generate aggregates of unrestricted size. Calculations are made according to eqs. 15 and 16 for $L = 5$, $K = 1$ (M^{-1}) and the following values of parameter a: curve 1, $a = 1$; curve 2, $a = 5$; curve 3, $a = 10$; curve 4, $a = 15$. Curves 2′, 3′ and 4′ are H plots of binding data shown in the form of Scatchard isotherms 2, 3 and 4, respectively.

From eq. 16 one can obtain

$$\lim_{r \to r_{max}} \frac{1-rL-(r-g)}{(r-g)^2} = La \qquad (17)$$

Combining eqs. 3, 6, 15, 16 and 17 one can find

$$K_{eff} = Ka^{L+1}. \qquad (18)$$

If $a > 1$, K_{eff} exceeds markedly the magnitude of K.

Combining eqs. 8 and 18 we obtain

$$\lim_{r \to r_{max}} H(r) = (L+1)\ln a \qquad (19)$$

Eqs. 18 and 19 have clear physical meaning. When r approaches r_{max} the majority of bound ligands on a lattice are in contact with adjacent ligands. Only a small population of bound ligands are separated by a single free polymer residue. In order to accommodate a new ligand in this tightly packed array of bound ligands on a lattice a certain rearrangement of bound ligands is required to form a stretch of L empty polymer

residues which may serve as a potential site for binding to another ligand.

The binding to this site is accompanied by the formation of $L+1$ contacts between nearest-neighbour bound ligands. This explains why K_{eff} is proportional to a^{L+1}. Asymptotic behavior described by eqs. 18 and 19 is indicative of a cooperative system in which interligand interactions generate aggregates of unrestricted size on a lattice.

(b) If ligand-ligand interactions are allowed only between adjacent ligands implicated in the formation of complexes of different geometry ($a_{12} = a_{21}$; $a_{11} = a_{22} = 1$), eqs. 9–11 can be simplified to yield eq. 20 ($u = 1,2$):

$$\left(\frac{r_u - g + g/a}{r_u}\right)^2 \frac{r_u}{m} = K_u \left(\frac{1 - rL}{1 - rL + r - 2g + 2g/a}\right)^L \times (1 - rL + r - 2g + 2g/a)$$

$$(r_1 - g + g/a)(r_2 - g + g/a) = \frac{g}{a}(1 - rL + r - 2g + 2g/a) \qquad (20)$$

Combining eqs. 3, 6 and 20 and assuming that $K_1 = K_2 = K$, one can obtain

$$K_{\text{eff}} = 2K\left(\frac{a+1}{2}\right)^{L+1} \qquad (21)$$

This type of asymptotic behavior is a consequence of the fact that ligand-ligand interactions can generate aggregates of unrestricted size in which complexes of type 1 and 2 alternate.

(c) If ligand-ligand interactions are allowed only between adjacent ligands implicated in the formation of complexes of type 2 ($a_{22} = a$, $a_{11} = a_{12} = a_{21} = 1$) eqs. 10 and 11 reduce to

$$\frac{r_1}{m} = K_1\left(\frac{1 - rL}{1 - rL + r - g + g/a}\right)^L \times (1 - rL - g + g/a + r) \qquad (22)$$

$$\frac{r_2}{m} = K_2\left(\frac{1 - rL}{1 - rL + r - g + g/a}\right)^L \frac{ar_2^2}{g}$$

$$(r_2 - g + g/a)^2 = \frac{g}{a}(1 - rL + r - g + g/a) \qquad (23)$$

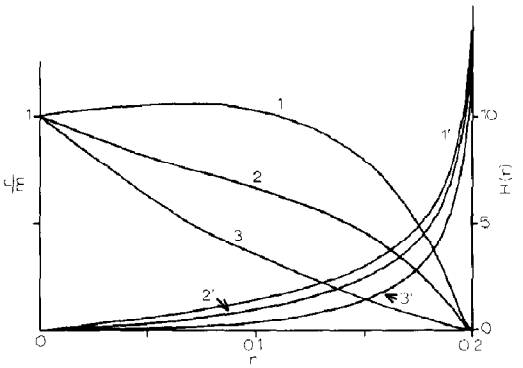

Fig. 3. Binding isotherms calculated for a system in which only interaction between adjacent ligands forming a type 2 DNA complex is allowed. The specific case is considered when $a_{11} = a_{12} = a_{21} = 1$, $a_{22} = a$ (see fig. 1). Calculations are made according to eqs. 22 and 23 for $a = 20$, $L = 5$, $K = K_1 + K_2 = 1$ (M^{-1}) and for various values of K_1/K_2: curve 1, $K_1/K_2 = 1$; curve 2, $K_1/K_2 = 2$; curve 3, $K_1/K_2 = 5$. Curves 1', 2' and 3' represent the binding data summarized in Scatchard isotherms 1, 2 and 3, respectively, in the form appropriate for an H plot.

In this case the shape of calculated binding curves depends on the ratio of binding constants K_1/K_2 (fig. 3). If $K_1 \gg K_2$ the binding isotherms have a shape characteristic of noncooperative ligand binding with binding constant K_1. If there is substantial positive cooperativity ($K_2 a \gg K_1$) a ligand tends to form preferentially complexes of type 2. Interaction between adjacent ligands can generate aggregates of unrestricted size on a lattice.

3.3. Interligand interactions capable of generating only dimeric associations on a lattice

We suggest now that interaction between bound ligands is only allowed when the right-hand end of a ligand bound at a site of type 1 is in contact with the left-hand end of another bound ligand at a site of type 2 (i.e., $a_{11} = a_{22} = a_{21} = 1$; $a_{12} = a$; see fig. 1). In this case eqs. 10 and 11 yield ($u = 1,2$)

$$\frac{r_u - g + g/a}{m} = K_u\left(\frac{1 - rL}{1 - rL + r - g + g/a}\right)^L \times (1 - rL + r - g + g/a) \qquad (24)$$

where $r = r_1 + r_2$. $g = g_{12}$ is the positive root of the quadratic equation

$$g^2(1-a) + g(1 - rL + ar) - ar_1r_2 = 0. \qquad (25)$$

Clearly, g is simply the average number of bound dimers divided by the number of DNA base pairs.

Eqs. 24 and 25 were first derived for description of binding of large ligands in two opposite orientations with respect to a polynucleotide lattice provided that only isologous interactions between nearest-neighbour bound ligands are allowed [9]. Schwarz and Stankowski [16] and Tsushiua and Szabo [12] have derived parameterized equations which are similar to equations reported in refs. 8 and 9. Certain general aspects of statistical mechanics of ligand-DNA interactions in the case when 2-fold rotation of ligand is allowed upon binding have been extensively treated. Exact recursive relations for evaluation of partition function have been derived for description of cooperative binding of large ligands to both homogeneous and heterogeneous polynucleotide lattices [8,13].

From eq. 25 one can find

$$\lim_{r \to r_{max}} g = \frac{1}{2L} \cdot \frac{a^{1/2}}{1 + a^{1/2}} \qquad (26)$$

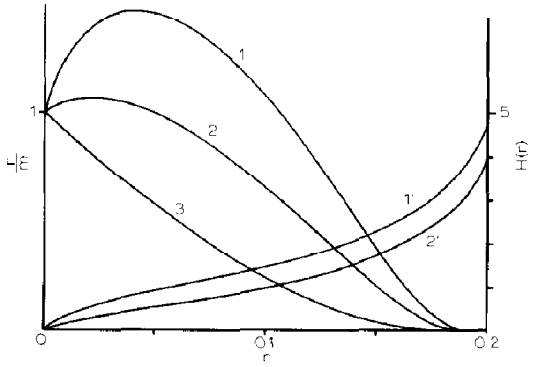

Fig. 4. Binding isotherms calculated for a system in which interligand interactions can generate only dimeric species on a lattice. This corresponds to the specific case when $a_{12} = a$, $a_{11} = a_{21} = a_{22} = 1$ (see fig. 1). Calculation are made according to eqs. 24 and 25 for $a = 100$, $L = 5$, $K = K_1 + K_2 = 1$ (M^{-1}) and various values of K_1/K_2: curve 1, $K_1/K_2 = 1$; curve 2, $K_1/K_2 = 0.1$; curve 3, $K_1/K_2 = 0$. Curves 1′ and 2′ are H plots of binding data corresponding to Scatchard isotherms 1 and 2, respectively.

If $a \to \infty$, $g \to 1/2L$, i.e., the tightest packing of bound ligands is realized in which complexes of the first and second type alternate on a lattice. If a is finite, a partially ordered arrangement of bound ligands arises. Combining eqs. 3, 6 and 24–26 one can obtain

$$K_{eff} = (K_1 + K_2)a^{1/2}\left(\frac{2a^{1/2}}{1 + a^{1/2}}\right)^{L-1} \qquad (27)$$

If $a \gg 1$, K_{eff} is equal to $K_1 + K_2$ multiplied by a factor $a^{1/2}$

This result has a clear physical meaning. If there is substantial positive cooperativity an ordered arrangement of bound ligands arises on a lattice at high levels of occupancy. The bound ligands tend to form dimeric species, each stabilized by interaction energy $-RT$ ln a (R, gas constant; T, absolute temperature). A statistical weighting factor $a^{1/2}$ should be assigned for each bound ligand in a dimer, thus explaining the fact that K_{eff} is proportional to $a^{1/2}$.

Fig. 4 shows examples of binding isotherms calculated from eqs. 24 and 25. One can see that these curves are markedly different from binding isotherms calculated from eqs. 15, 16, 20, 22 and 23 for systems with substantial binding cooperativity. This indicates that cooperative systems in which interligand interactions can generate only dimeric species on a lattice can be easily distinguished from systems in which formation of aggregates of unrestricted size is allowed.

Eqs. 18 and 27 show that asymptotic behavior of binding isotherms in the limit when r tends to r_{max} provides a basis for the systematics of cooperative systems. Generally, cooperative systems can be divided into two classes depending on whether dimeric species or aggregates of unrestricted size are formed by bound ligands on a lattice at high levels of occupancy.

4. Cooperative effects mediated by long-range interactions between bound ligands

4.1. Binding equations

We now turn to a consideration of the binding of polar large ligands for a general case when

long-range interactions between nearest-neighbour bound ligands are allowed. Let $\Delta f(i)$ be the energy of pairwise interaction between nearest-neighbour bound ligands separated by i free polymer residues intervening between the polymer regions covered by the bound ligands. The range of interaction is assumed to be finite spanning l polymer residues ($\Delta f(i) = 0$ if $i > l$). $\Delta f(i)$ will be referred to as the potential of pairwise interaction between bound ligands. Let $g(i)$ be the average number per polymer residue of nearest-neighbour bound ligands separated by a gap containing i free polymer residues. In the noninteracting ligand case,

$$g(i+1)/g(i) = P \tag{28}$$

where P is the probability of finding a free polymer residue on a lattice. In the noninteracting ligand case, all the arrangements of bound ligands on a lattice are equally probable and P can be calculated from eq. 2.

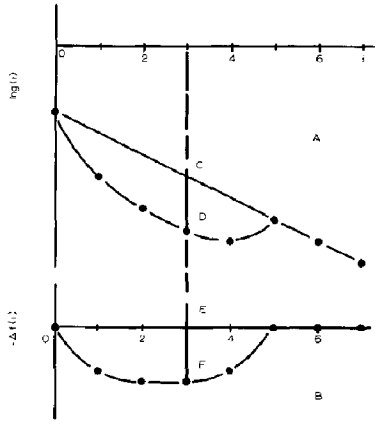

Fig. 5. (A) A plot of ln g vs. i, the distance between nearest-neighbour bound ligands. The deviation of the plot from the straight line characteristic of a noninteracting ligand allows one to determine the dependence of interligand interaction free energy on the separation between nearest-neighbour bound ligands. The specific case is illustrated when interligand interaction extends over 4 base-pairs. (B) The dependence of interaction free energy on the separation bound ligands as revealed from the plot of ln g vs. i shown above. If $i = 3$, the interaction free energy $\Delta f(3) = |FE| = |CD|$ where $|CD|$ is the deviation of plot ln g vs. i from the straight line. The interaction energy is measured in units of RT (R, gas constant; T, absolute temperature).

If bound ligands are allowed to interact with nearest-neighbour bound ligands an analog of eq. 28 is

$$g(i+1)/g(i) = P \exp(-(\Delta f(i+1) - \Delta f(i))/RT) \tag{29}$$

where P is the probability of finding a free polymer residue in the cooperative system under study.

From eq. 29 we obtain

$$\ln g(i) = \ln g(0) + i \ln P + \Delta f(0)/RT - \Delta f(i)/RT \tag{30}$$

In the noncooperative binding case ($\Delta f(i) = 0$ for all i), the plot of ln $g(i)$ vs. i is a straight line with a slope ln P. In the interacting ligand case, the magnitude of deviation of the plot ln $g(i)$ vs. i from the straight line provides information about the precise form of dependence of the interaction energy on the distance between nearest-neighbour bound ligands (fig. 5).

A macromolecular complex with ligand at occupancy r can be visualized as a linear array of groups of interacting bound ligands separated by free polymer regions. In each group bound ligands are allowed to interact only with nearest-neighbour bound ligands belonging to the same ligand group. This means that each ligand group should be separated from adjacent ligand groups by at least $l+1$ free polymer residues. The average number of ligand groups (divided by the total number of polymer residues) is equal to $r - \sum_{i=0}^{l} g(i)$.

The probability of finding a ligand at an isolated site (there is no interaction with any other ligand) is equal to $(r - \sum_{i=0}^{l} g(i))/r)$. The average number of free polymer residues lying outside the regions covered by ligand groups is equal to $1 - rL - \sum_{i=1}^{l} ig(i)$. The probability P of finding a free polymer residue that lies outside the polymer regions covered by groups of interacting ligands is equal to

$$P = \frac{1 - rL - \sum_{i=1}^{l} ig(i) - (l+1)\left[r - \sum_{i=0}^{l} g(i)\right]}{1 - rL \sum_{i=1}^{l} ig(i) - l\left[r - \sum_{i=0}^{l} g(i)\right]} \tag{31}$$

The probability of finding a stretch of $L+l+1$ free polymer residues is equal to P^{L+l+1}. The binding of ligand to such a stretch is described by the association constant K. The equation for description of ligand binding to an isolated site on a lattice can be written in the form of a mass action law:

$$\left(\frac{r-\sum_{i=0}^{l} g(i)}{r}\right)^2 \frac{r}{m} = KP^{L+l+1}\left(1-rL-\sum_{i=1}^{l} ig(i) - l\left(r-\sum_{i=0}^{l} g(i)\right)\right) \quad (32)$$

where

$$r=\sum_{i=0}^{\infty} g(i) \quad (33)$$

$$1-rL=\sum_{i=1}^{\infty} ig(i) \quad (34)$$

Eqs. 30–34 can be rearranged to eqs. 9 and 10 of Zasedatelev et al. [3]. It should be noted that a procedure describing the use of these nonlinear algebraic equations in calculations of binding isotherms has not yet been reported for the general case of arbitrary l. In this section, we demonstrate that the binding relations, eqs. 29 and 32, have obvious and physically interpretable meaning. If we introduce the physically interesting parameter P (see eq. 31) as an intermediate variable these equations can be rearranged into a form which is simpler to use in practical calculations.

$$\frac{g}{a_0}=\left[L\left(\sum_{i=0}^{l} a_iP^i+\frac{P^{l+1}}{1-P}\right)+\sum_{i=1}^{l} ia_iP^i + P^{l+1}\left(\frac{l+1}{1-P}+\frac{P}{(1-P)^2}\right)\right]^{-1} \quad (35)$$

$$r=\frac{g}{a_0}\left[\sum_{i=0}^{l} a_iP^i+\frac{P^{l+1}}{1-P}\right] \quad (36)$$

$$\frac{r}{m}=KP^L\frac{a_0}{g}r^2 \quad (37)$$

where

$$a_i=\exp(-\Delta f(i)/RT),\ g=g(0). \quad (38)$$

Inserting various numerical values of P ($0<P<1$) into eqs. 35–38 one can calculate binding isotherms for any functional form of dependence of interaction free energy on the separation between nearest-neighbour bound ligands.

4.2. Analysis of experimental binding isotherms

From eqs. 7 and 35–38 one can obtain

$$\lim_{r\to 0}\frac{dH}{dr}=2\left[\sum_{i=0}^{l}(a_i-1)\right] \quad (39)$$

$$\lim_{r\to r_{max}} H(r)=(L+1)\ln a_0-L\ln a_1 \quad (40)$$

$$\lim_{r\to r_{max}}\frac{dH}{dr}=-2L^3\left(1-\frac{a_0a_2}{a_1^2}\right) \quad (41)$$

From experimentally measured value of $r_{max}=1/L$ one can estimate the numerical value for binding site size L on the lattice. Eqs. 39–41 represent three simultaneous equations for three unknowns (a_0, a_1 and a_2). This indicates that ligand-ligand interaction constants can be determined if interligand interaction spans over $l\leqslant 2$ free polymer residues. In the general case of arbitrary l, eqs. 39–41 are clearly insufficient for determination of $l+1$ unknowns, and more detailed information about the molecular events responsible for the cooperativity of a binding process is required. In some cases, the exact form of dependence of interaction energy on the separation between nearest-neighbour bound ligands is known from independent experiments. In other cases, interaction energy $\Delta f(i)$ can be approximated by interaction potentials of well-established forms. For example, if dipole-dipole interaction is the leading term in the interaction free energy between bound ligands, $\Delta f(i)$ should be inversely proportional to the square of the distance between bound ligands.

Experimental binding isotherms with obvious upward curvature in the Scatchard representation often exhibit an extended region at high level occupancy where the cooperative binding isotherm coincides with a noncooperative reference

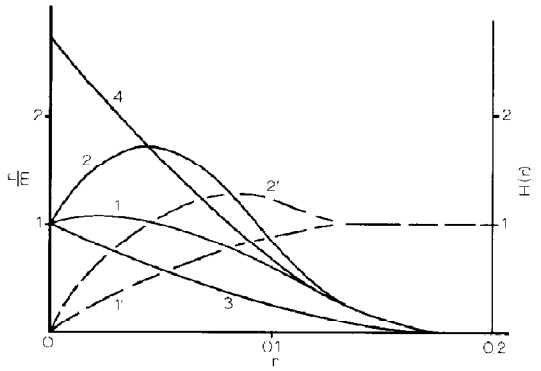

Fig. 6. Binding isotherms calculated for a system in which long-range interaction between nearest-neighbour bound ligands is allowed. Calculations are made according to eqs 35–38 and 42 for the case when interaction between bound ligands is described by a linearly quenching potential (curve 1, $\Delta f = 0$, $d = 0.05RT$) and potential of rectangular form (curve 2, $\Delta f = RT$, $d = 0$) (R, gas constant; T, absolute temperature). Isotherms 3 and 4 are calculated for noncooperative ligand binding with intrinsic binding constant $K = 1$ and $K = 2.72$, respectively (M^{-1}). Curves 1′ and 2′ show the same data converted into terms appropriate for the H plot. Curves 1′ and 2′ correspond to Scatchard isotherms 1 and 2, calculated for $K = 1$ (M^{-1}).

isotherm [20–24]. The existence of such a region on the binding isotherm imposes strong restrictions on the possible form of the interaction potential $\Delta f(i)$. For adequate description of binding isotherms of such a type one should set

$$\lim_{r \to r_{max}} \frac{dH}{dr} = 0; \quad \lim_{r \to r_{max}} \frac{d^2H}{dr^2} = 0.$$

From eqs. 7, 35–38 and 41 one can determine that the interaction potential which satisfies these requirements is

$$\Delta f(i) = \begin{cases} (l+1-i)d + \Delta f, & i \leqslant l \\ 0, & i > l \end{cases} \qquad (42)$$

It is important to investigate how the shape of a binding isotherm depends on the form of interaction potential. For illustrative purposes we shall consider the following specific cases: (1) $\Delta f = 0$, the magnitude of interaction energy $f(i)$ is proportional to i (a linearly quenching potential). (2) $d = 0$, the interaction energy $\Delta f(i)$ assumes the same value within the interval $0 < i < l$ (rectangular potential).

Fig. 6 shows examples of binding isotherms calculated from eqs. 35–38 and 42 for these two limiting cases. If there is substantial positive cooperativity the H plot goes through the maximum for systems in which interaction between bound ligands is described by a rectangular potential whereas no maximum is seen on the curves calculated for the case of a linearly quenching potential.

If interaction between bound ligands is described by a rectangular potential eqs. 39 and 40 yield

$$\lim_{r \to 0} \frac{dH}{dr} = 2(l+1)(a-1) \qquad (43)$$

$$H(r_{max}) = -\frac{\Delta f}{RT} \qquad (44)$$

Interactions between nearest-neighbour bound ligands can be mediated by conformation changes

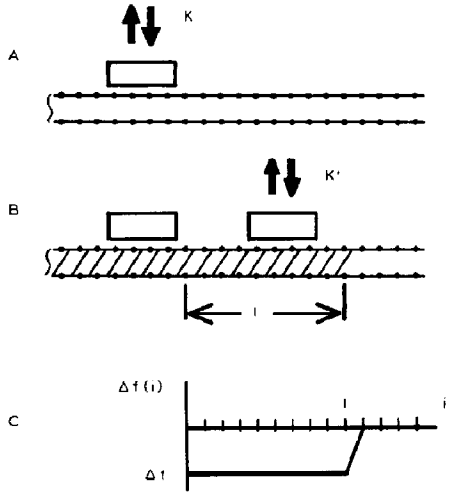

Fig. 7. A model for description of cooperative effects mediated by conformation changes induced in DNA by binding reaction. (A) Binding of ligand to an isolated site on DNA (K, intrinsic binding constant). (B) Cooperative binding mediated by DNA conformation changes induced upon binding of ligand and transmitted beyond the region of immediate ligand-DNA contact. It is assumed that conformationally altered regions extend over l base-pairs in the two directions from a given occupied site. (K', binding constant of ligand to a site lying within the conformationally altered region) (C) Interaction potential of rectangular form which may adequately describe cooperative effects mediated by induced conformational changes.

induced in DNA upon binding of ligand and transmitted beyond the region of immediate ligand-DNA contact. A well-studied example of such a phenomenon is the conformation switch in poly[d(G-C)] · poly[d(G-C)] induced by binding of ethidium as described by Pohl and Jovin [25]. Poly[d(G-C)] · poly[d(G-C)] can exist in the two helical forms, Z and B, which possess markedly different affinities for ethidium. The cooperative nature of the transition from the Z to B form implies that the B-like conformation induced upon binding of ethidium to the Z form of poly[d(G-C)] · poly[d(G-C)] will tend to propagate into adjacent polymer regions affecting the binding properties of other sites. Recently, Breslow and Crothers [10] have described the cooperative binding of ethidium to various synthetic polynucleotides in terms of theory for induced allosteric changes in DNA [10].

Alternatively, cooperative effects can be described in terms of pairwise interaction between nearest-neighbour bound ligands using a potential of rectangular shape. As before, the binding of ligand to an isolated site is characterized by an intrinsic binding constant K. If a ligand induces DNA conformation changes extending over l base-pairs from the two ends of the region covered by bound ligand, the binding of another ligand to a nearby site lying within the interval $0 < i < l$ from a given occupied site is characterized by binding constant $K' = Ka$ (fig. 7). The potential of pairwise interaction between nearest-neighbour ligands can be written as

$$\Delta f(i) = \begin{cases} -RT \ln(K'/K) = -RT \ln a = \Delta f, \\ \quad i \leqslant l \\ 0, \; i > l \end{cases} \tag{47}$$

where a measures cooperative interactions in the system.

This model was applied by Krylov et al. [20] and Nechipurenko et al. [11,21–23] for description of cooperative binding of distamycin analogs to natural and synthetic DNAs. They have found that isotherms calculated for the case when interaction between nearest-neighbour bound ligands is described by a rectangular potential exhibit a good fit with the experimental curves provided that the range of pairwise interaction between ligands extends over several base-pairs. However, isotherms calculated for the case when only short-range interligand interactions are allowed fail to describe the shape of experimental binding isotherms [22].

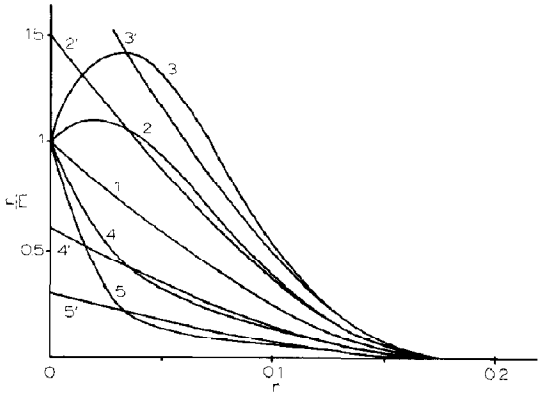

Fig. 8. Binding isotherms calculated for a system in which long-range interactions between nearest-neighbour bound ligands are allowed. The interaction is described with the aid of a potential of rectangular form. Calculations are made according to eqs. 35–38 and 47 for $L = 5$, $K = 1$, $l = 20$ and the following values of parameter a: curve 1, $a = 1$; curve 2, $a = 1.5$; curve 3, $a = 2$; curve 4, $a = 0.6$; curve 5, $a = 0.3$. At high levels of occupancy ($r > 0.1$) each cooperative binding curve can be approximated by the isotherm calculated for noncooperative binding of ligand with an apparent binding constant Ka (see curves 2′–5′).

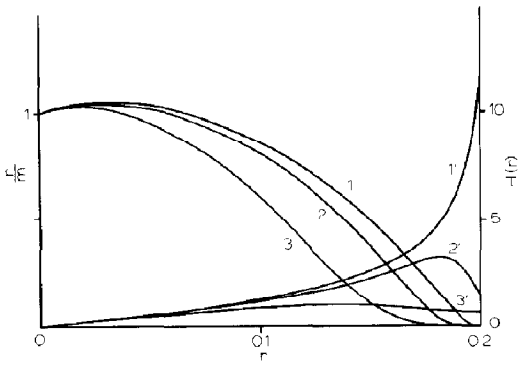

Fig. 9. Binding isotherms calculated from eq. 35–38, 47 for $L = 5$, $K = 1$ and various values of parameters a and l: curve 1, $a = 7$, $l = 0$; curve 2, $a = 4$, $l = 1$; curve 3, $a = 2$, $l = 5$. Curves 1′–3′ show the same binding data in the form of H plots corresponding to Scatchard isotherms 1–3, respectively.

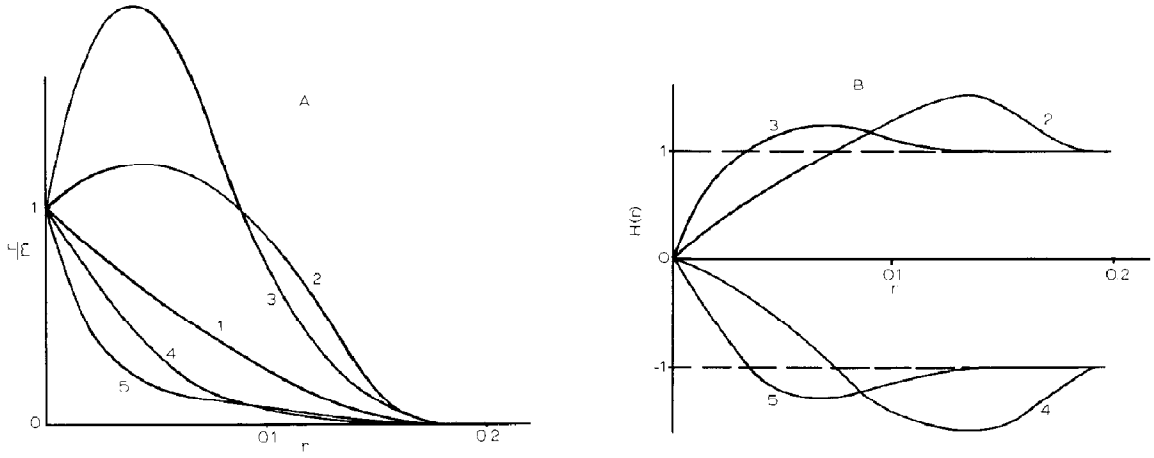

Fig. 10. Binding isotherms calculated for the case when interaction between nearest-neighbour bound ligand is described by potential of rectangular form with $l > L$. (A) Calculations are made according to eqs. 35–38, 47 for $L = 5$, $K = 1(M^{-1})$ and the following values of Δf and l: curve 1, $\Delta f = 0$; curve 2, $\Delta f = -1RT$, $l = 10$; curve 3, $\Delta f = -1RT$, $l = 20$; curve 4, $\Delta f = 1RT$, $l = 10$; curve 5, $\Delta f = 1RT$, $l = 20$ (R, gas constant; T, absolute temperature). (B) The same binding data converted in terms appropriate for H plots. Curves 2–5 correspond to Scatchard isotherms 2–5 in panel A.

Our preference for using this formalism is due to the fact that it allows separation of long- and short-range effects. Clearly, short-range interactions correspond to a limiting case when $l = 0$. In this case eqs. 35–38 reduce to eqs. 15 and 16. Figs. 8–10 show the binding isotherms calculated from eqs. 35–38 and 42 for various values of parameters a and l. As expected, at high levels of occupancy the cooperative binding isotherms coincide with noncooperative isotherms calculated for binding of ligand with intrinsic binding constant Ka.

The first general observation which can be made from a comparison of the binding isotherms presented in fig. 9 is that the size of the region where the cooperative binding isotherm coincide with the noncooperative isotherm depends on the magnitude of l. Decreasing l leads to a decrease in the size of the region where the two isotherms are coincident. The second observation which can be made from figs. 9 and 10 is that the shapes of binding isotherms calculated for $l = 0$ and $l > 1$ are markedly different, especially at high levels of occupancy. This also follows from the limiting expressions describing the asymptotic behavior of binding isotherms when r tends to r_{max}. We note that $\lim_{r \to r_{max}} H(r) = (L + 1)\ln a$ if $l = 0$ and $\lim_{r \to r_{max}} H(r) = \ln a$ if $l > 0$ (see eq. 44).

The limiting slope of the H plot when $r \to r_{max}$ also depends on the magnitude of l. From eq. 41 it follows that $\lim_{r \to r_{max}} (dH/dr) = 0$, if interaction between bound ligands is described by a potential of rectangular form with $l \geqslant 2$. In contrast, $\lim_{r \to r_{max}} (dH/dr) \neq 0$ when $l < 2$. Figs. 9 and 10 show examples of binding isotherms calculated for various values of l. One can see that H plots calculated for $l = 5$ and $l = 20$ lie almost parallel to the horizontal axis at high r values. However, this is not the case for H plots calculated for $l < 2$. This indicates that cooperative systems in which interligand interactions span over small distances can be easily distinguished from systems in which long-range interligand interactions are allowed.

5. Concluding remarks

In the present paper a new approach to analysis of binding isotherms of large ligands to a polynucleotide lattice is reported which is based on a comparison of the asymptotic behavior of cooperative and noncooperative binding isotherms in the limit when the degree of occupancy of lattice by ligand approaches the saturation level of binding.

We find that at high levels of occupancy the magnitude of deviations of a cooperative binding isotherm from the noncooperative reference isotherm is an important diagnostic tool for visual differentiation between various types of binding cooperativity. Based on the asymptotic behavior of Scatchard isotherms in the limit when r tends to r_{max} it is possible on strictly empirical grounds to divide cooperative systems into two broad categories.

We find that the shape of the Scatchard isotherm as well as its asymptotic behavior in the limit when $r \to r_{max}$ can be correlated with the size of the clusters formed by bound ligand on a lattice. Generally, cooperative systems can be divided into two groups depending on whether aggregates of unrestricted size or only dimeric species can be formed by bound ligands upon increased occupancy of a lattice. This can be done regardless of the nature and complexity of interactions in the thermodynamic systems and irrespective of whether binding to a homogeneous or heterogeneous polynucleotide lattice is considered.

To simplify analysis of experimental binding isotherms we propose a new way of plotting the binding data (H plot) and derive exact expressions for limiting characteristics of H and Scatchard plots at low and high extents of binding. These limiting characteristics are useful for visual differentiation between various types of cooperativity.

If interactions between nearest-neighbour bound ligands favor the formation of aggregates of unrestricted size, the cooperative binding isotherm at high levels of occupancy approaches the curve calculated for noncooperative binding with an apparent binding constant $K_{eff} = Ka^{L+1}$. If interaction between bound ligands can generate only dimeric species, $K_{eff} \sim a^{1/2}$ for systems with substantial positive cooperativity.

In the case of binding of polar large ligands which form one type of complex with DNA our analysis shows that cooperative systems in which interaction between nearest-neighbour bound ligands extends over more than two free polymer residues can be distinguished from systems in which ligand-ligand interactions span over shorter distances.

In the general case of the arbitrary interaction range l interpretation of binding data requires more information than binding isotherm themselves can provide. As a result, quantitative conclusions require at least knowledge of how the interaction free energy depends on the separation between nearest-neighbour bound ligands. If such a dependence is unknown, its approximation by a rectangular potential provides a basis for a rough estimation of the range over which interligand interaction spans in the cooperative system. This simplifies selection of the appropriate model for description of binding equilibrium from a number of a priori possible binding models. This also enables one to obtain initial reliable estimates of binding parameters.

Very recently footprinting and chemical protection methods have been developed to obtain more detailed information about protein-DNA interactions. In particular, nuclease digestion experiments have been used to determine the distribution pattern of distances between nearest-neighbour nucleosomes in rat liver chromatin [26]. From an analysis of these data using eq. 30 the conclusion is drawn that positioning of nucleosomes on DNA is governed both by exclusion site effects and cooperative interactions between nucleosomes [27]. Equations derived in this and subsequent papers [27–29] are useful for analysis of results of biochemical experiments designated to locate the sites of ligand-DNA contact and to estimate cooperative interactions between individual binding sites.

References

1 G. Scatchard, Ann. N.Y. Acad. Sci. 15 (1949) 660.
2 D.M. Crothers, Biopolymers 6 (1968) 575.
3 A.S. Zasedatelev, G.V. Gursky and M.V. Volkenstein, Mol. Biol. 5 (1971) 245.
4 J.D. McGhee and P.H. von Hippel, J. Mol. Biol. 86 (1974) 469.
5 G. Schwarz, Biophys. Chem. 6 (1977) 65.
6 I.R. Epstein, Biophys. Chem. 8 (1978) 327.
7 B. Ramanatham and K.S. Shwitz, Biopolymers 17 (1978) 217.
8 G.V. Gursky and A.S. Zasedatelev, Biofizika 23 (1978) 932.
9 Yu. D. Nechipurenko and A.S. Zasedatelev G.V. Gursky, Biofizika 24 (1979) 351.

10 J.L. Breslow and D.M. Crothers, Biochemistry 20 (1981) 3547.
11 Yu. D. Nechipurenko, Biofizika 27 (1982) 391.
12 T. Tsushiua and A. Szabo, Biopolymers 21 (1982) 979.
13 G.V. Gursky and A.S. Zasedatelev, Sov. Sci. Rev. D. Physicochem. Biol. 5 (1984) 53.
14 G. Schwarz and F, Watanabe, J. Mol. Biol. 163 (1983) 467.
15 G. Schwarz, Biophys. Struct. Mech. 2 (1976) 1.
16 G. Schwarz and S. Stankowsky Biophys. Chem. 10 (1979) 173.
17 J. Monod, J. Wyman and J.-P. Changeux, J. Mol. Biol. 12 (1965) 88.
18 Yu. D. Nechipurenko, Mol. Biol. 18 (1984) 1066.
19 J.D. McGhee and P.H. von Hippel, J. Mol. Biol. 103 (1976) 679.
20 A.S. Krylov, S.L. Grokhovsky, A.S. Zasedatelev, A.L. Zhuze, G.V. Gursky and B.P. Gottikh, Nucleic Acids Res. 6 (1979) 289.
21 Yu. D. Nechipurenko, Stud. Biophys. 87 (1982) 217.
22 Yu. D. Nechipurenko, A.S. Krylov, A.S. Zasedatelev and G.V. Gursky Mol. Biol. 18 (1984) 332.
23 Yu, D. Nechipurenko, A.S. Zasedatelev and G.V. Gursky, Mol. Biol. 18 (1984) 798.
24 S.A. Winkle and T.R. Krugh, Nucleic Acids Res. 9 (1981) 3175.
25 F.M. Pohl, T.M. Jovin, W. Baehr J. Helfbrook, Proc. Natl. Acad. Sci. U.S.A. 74 (1977) 188.
26 F. Strauss and A. Prunell, Nucleic Acids Res. 10 (1982) 2275.
27 Yu. D. Nechipurenko, Mol. Biol. (1986) in the press.
28 Yu. D. Nechipurenko and G.V. Gursky, Dokl. Akad. Sci. SSSR 281 (1985) 213.
29 Yu. D. Nechipurenko and M.V. Volkenstein, Dokl. Akad. Sci. SSSR 286 (1986) 216.