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Minireview

The multiple origins of cooperativity in binding to multi-site lattices

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Abstract Binding events involving the reversible association of ligands with polymeric lattices of binding sites are common in biology and frequently exhibit significant cooperativity in binding. Positive and negative cooperativity in binding may be detected by characteristic changes in binding curves for multiple binding, compared to the binding expected for simple, independent binding events that are based on combinatorial considerations only. Cooperativity arises from ligand-dependent interactions distinct from binding per se. Ligand-dependent nearest neighbor interactions may be of two types referred to as ligand-lattice (which can only occur if a bound ligand is unneighbored) and ligand-ligand (which can occur if two or more bound ligands are adjacent). The molecular mechanisms underlying these two sources of cooperativity are not the same. Identical cooperative binding curves may be produced by changes from unity in parameters representing either one or both of these interaction types. Positive cooperativity may equally result from destabilizing ligand-lattice interactions that disfavor initial, unneighbored binding, stabilizing ligand-ligand interactions that favor subsequent, neighbored binding, or both. The structural origins of these are different, and cooperativity may emerge from multiple structural interactions.

1. Introduction

Much of biology is dependent on and regulated by reversible binding events. The non-covalent reactions of macromolecules with each other and with small molecules are the core of the dynamic chemistry characterizing biology. The data generated to analyze these interactions frequently consist of binding data presented in sometimes ill-defined plots that are interpreted as cooperativity without attention to the limitations of this analysis or the structural implications of models of cooperativity. This article will address several questions about multiple, linked, reversible binding. What is the origin of the cooperativity sometimes observed in these binding reactions? How can it be recognized in binding data? Does cooperative binding, in itself, imply any particular molecular mechanism for its origin? How does the form of the equation used to fit the data limit the structural interpretation of cooperativity? It is often overlooked that a given set of binding data showing cooperativity may be equally well explained by multiple mechanisms in which the structural origins of cooperativity are quite different.

Many macromolecular associations may be described as the multiple binding of ligands to a polymeric lattice of binding sites. We here use the term ligand to mean the smaller, and univalent member of the binding pair; the lattice is larger and contains multiple (at least two) binding sites for the ligand. The binding to be examined then is the association of multiple ligand molecules with a single molecular lattice of binding sites. Examples of such binding that show cooperative interactions include the binding of oxygen to hemoglobin [1], the binding of proteins to single stranded DNA [2], the binding of tropomyosin to F-actin [3], and the binding of MAP2 to tubulin microtubules [4,5].

It is the origin of this cooperative behavior that is the concern of this article. More precisely, it is the origins, for cooperativity can originate through more than one molecular mechanism, although often data are analyzed in a way that only allows one mechanism. Positive cooperativity is frequently taken to imply or require interactions between sites with bound ligands that stabilize the bound state [6,7], but this need not be the case. As we will elaborate, such cooperativity may originate in stabilizing interactions that only occur when two bound ligands are adjacent, or may originate in lattice-destabilizing interactions that only occur when a bound ligand has no neighbors [8].

These two approaches may be described as ligand-ligand and ligand-lattice models. We will refer to these as 'α_2' and 'α_1', respectively, for reasons that will become clear shortly. Before discussing the workings of these models of cooperativity, we will briefly examine the characteristics of cooperative binding that allow it to be recognized.

2. Cooperativity – general features

Cooperativity is detected by examining experimental binding curves (isotherms) [9] (see Fig. 1). Binding may be plotted as fractional saturation of binding sites or as the number bound (y goes from 0 to n, the number of binding sites) vs. the log of the concentration (Fig. 1A), or vs. the concentration (Fig. 1B) of unbound ligand. Alternatively, the binding data may be presented in the oft-used Scatchard plot (Fig. 1C). A single binding site or multiple identical sites reacting independently will yield the plots in Fig. 1 labeled R (for Reference). Positive cooperativity in binding will produce changes in the binding isotherms illustrated by the curves labeled (+): an increase in the slope of the log plot, conversion of the linear plot from hyperbolic to sigmoidal form (not readily apparent in Fig. 1), and appearance of a convex Scatchard plot. Negative cooperativity (plots labeled (−)) results in similarly characteristic alterations in the binding isotherms: a decrease in the slope of the log plot, a change in the

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linear plot, and appearance of a concave Scatchard plot (the presence of more than one class of binding site will yield similar data; here we only consider cases with identical sites). Note that cooperative interactions between binding sites can alter the half-maximally saturating concentration of ligand, as well as the slope of the curve.

We will examine these points sequentially, and will limit our discussion to positive cooperativity. Simple binding to a single site will be examined first. The results with multiple binding sites will then be examined using a model with two linked sites, first without interactions between sites (no cooperativity), then with interactions between sites [10] according to each of the two models described. All of these cases are shown schematically in Fig. 2. For each case, the apparent or experimental binding constants K_1 and K_2 (for the first and second ligand, respectively) will be compared to the intrinsic binding constant k, which describes the binding of a single ligand to an isolated binding site. Only non-overlapping sites are considered here, although the points raised here apply equally to cases with overlapping sites; this has been examined in detail elsewhere [8]. It is important to emphasize that in the discussion to follow, the ligand-dependent interactions between sites do not include the binding per se, which is contained in the binding constant k. In order to emphasize the distinction, the term ligand-dependent interactions will be used. In addition we note that these interactions are all nearest neighbor interactions.

3. Binding to a single site

Binding of a single ligand to a single binding site (Fig. 2, line 1) may involve myriad atomic interactions and rearrangements, whether we consider the binding of two macromolecules, the binding of oxygen to myoglobin, or the binding of a proton to an ionized carboxylic acid. Nonetheless, the summation of the multiple atomic rearrangements required for binding to occur may be rationally combined in a single binding constant that adequately describes the binding event with energetic (thermodynamic) quantities. The binding isotherms for such a reaction are those shown in Fig. 1, curves R. Single site binding is shown schematically in Fig. 2, line 1.

It is convenient to describe these binding events by the use of simple reactions and the parameters describing them. Consider first a single site ∪ binding the ligand X.

$$\cup + X \Leftrightarrow [X] \qquad [1]$$

The association constant k for this reaction is given by the following expression (note that this and all other reaction constants in this discussion are equilibrium constants):

$$k = [[X]] / [\cup][X]$$

where the square brackets represent the concentrations of the enclosed species (activity coefficients are assumed to be unity). The apparent or experimental association constant K (the constant directly available from experimental binding data) for this reaction is equal to the intrinsic or unperturbed constant k. This is not always true with multiple sites as will be seen shortly. The free energy of association is defined as $-RT \ln(k)$. This energy contains the myriad of changes occurring in the formation of the complex

$$[X]$$

These are symbolized by the change of ∪ to □ and X to **X**.

4. Binding to multiple sites

One can imagine combining many identical receptor sites together in a lattice in such a way that they all remain independent. In such a hypothetical case, the fractional binding isotherm for n identical *independent* sites on the lattice would be the same as that for a single site. The curve would look just like curve R in Fig. 1. As long as the sites are independent it does not matter if they are physically linked or not. However, it is likely that in general, combining multiple receptor sites into a structure (or lattice) will result in some change in properties; the sites will no longer be independent. The binding

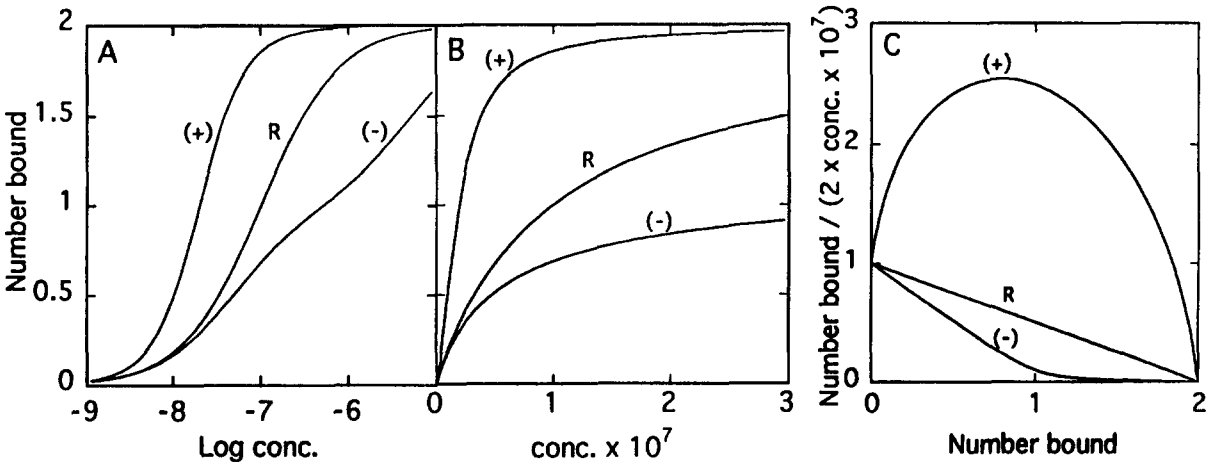

Fig. 1. Binding isotherms showing the saturation of two sites as a function of the concentration of free (unbound) ligand (shown as 'conc'). In (A) the free ligand concentration is presented in logarithmic format while in (B) it is linear. (C) Presents the data in Scatchard format (on the ordinate, the free ligand concentration is multiplied by the number of sites (2), and by the binding constant (10^7) so that the y-intercept for the reference case is equal to 1). The curves labeled R are the reference case, independent sites, unperturbed binding (the same as single site binding). Curves labeled (+) show positive cooperativity; those labeled (−) show negative cooperativity. The intrinsic binding constant k was 10^7 M^{-1} for all curves. For all curves, $\alpha_1 = 1$, but α_2 varied: for (R) $\alpha_2 = 1$; (+) $\alpha_2 = 25$; (−) $\alpha_2 = 0.04$. See text for further details.

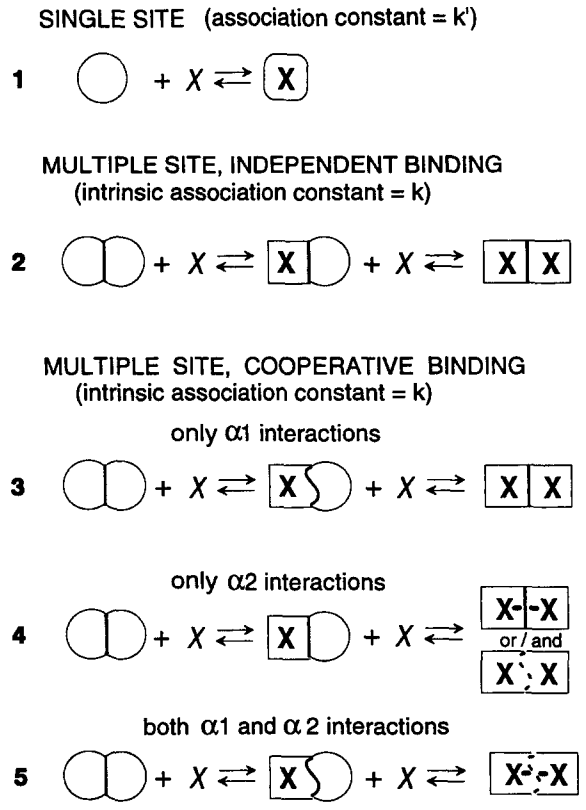

Fig. 2. Models of binding. The binding models discussed in the text are shown in pictorial format. (1) Single site binding demonstrates that the properties of both the ligand (the X) and the site are altered by binding. (2) Multiple independent sites show the same behavior as single sites (though with possibly nonidentical k): the site and the ligand are altered by binding. The interactions between the sites (represented by the line separating them) are unaltered throughout. (3) Multiple site binding with α_1 interactions shows that the site-site interactions are altered by the initial binding. In the doubly liganded species site-site interactions are the same as in the empty state, and there are no interactions between the bound ligands. (4) Multiple site binding with α_2 interactions alters the site-site interactions in the doubly liganded species and/or involves direct interactions between bound ligands. Site-site interactions are unaltered in the singly liganded species. (5) With both α_1 and α_2 interactions, site-site interactions are altered at each binding step.

isotherm for such a matrix of binding sites would differ from curve R in Fig. 1, possibly resembling curves (+) or (−) (positive and negative cooperativity, respectively).

How may these differences be quantitated? What differences in the experimentally determined binding constants K will reveal cooperativity in binding to multiple binding sites? To examine this we use a simplified model with just two identical binding sites on the same molecule (identical intrinsic association constant k) and describe the effect of interactions between such sites. For simplicity, we will focus on interactions that give rise to positive cooperativity.

In the absence of cooperative effects, the nature of the binding curve will be dictated only by the varying number of possible combinations between a ligand and binding site at different levels of saturation. The ratio of the experimental association constants describing binding of a ligand to the two identical independent sites (K_1 and K_2) depends only upon combinatorial terms (i.e. the number of possible ways an event could occur). Thus there is only one state with zero ligands bound or with two ligands bound, but there are two states that have one ligand bounds (since there are two binding sites). The combinatorial factor is just the ratio of the number of states after the binding event to the number before. The value of K is given by the combinatorial factor multiplied by the intrinsic binding constant k: For the first ligand:

$$K_1 = [(\text{no. of one ligand states})/(\text{no. of zero ligand states})]$$
$$k = (2/1)k = 2k,$$

and for the second ligand:

$$K_2 = [(\text{no. of two ligand states})/(\text{no. of one ligand states})]$$
$$k = (1/2)k$$

and therefore the ratio of the experimental binding constants is given by:

$$K_2/K_1 = (1/2)/2 = 1/4$$

The value 1/4 applies only to two independent (non-interacting) sites (for three sites $K_3/K_1 = 1/9$). If the sites interact in some manner, cooperativity may result and the ratio will change. Positive cooperativity produces an increase in the ratio K_2/K_1 to some value greater than 1/4. Negative cooperativity is associated with values less than 1/4 (for two sites).

We will now detail binding to the two-site model, first without and then with cooperative interactions.

4.1. Independent binding to two sites

Consider binding of the ligand X to two identical and independent sites (no ligand-dependent interactions). This is shown schematically in Fig. 2, line 2

∪-∪	⇔ [X]-∪	⇔ [X]-[X]		[2]
{1}	{2kc}	$\{k^2c^2\}$		[2a]
{1}	$\{K_1c\}$	$\{K_1K_2c^2\}$		[2b]

The reaction is shown in line 2. The symbols have the same meaning as for the single site binding discussed above. This reaction assumes that the liganding does not change the interactions between the sites. The terms in lines 2a and 2b give the relative concentrations of each of the three species in line 2. The concentration of the unliganded lattice is used as the standard reference. This is set equal to 1 and all other species concentrations are defined in terms of this reference value, the concentration of the unbound ligand, c, and (in line 2a) the intrinsic binding constant k or (in line 2b) the experimental constants K_1 and K_2. The sum of the concentrations of all the species relative to the reference (unliganded) species is represented by Ξ, and is obtained by summing line 2a or 2b:

$$\Xi = 1 + 2kc + k^2c^2 = 1 + K_1c + K_1K_2c^2$$

Since line 2a and 2b describe the same reaction they must be equivalent. Therefore, $K_1 = 2k$ and $K_2 = k/2$, yielding the ratio of $K_2/K_1 = 1/4$, as required for independent sites In the previous section. Note that although the particular values of the experimental constants K_1 and K_2 depend on the value of

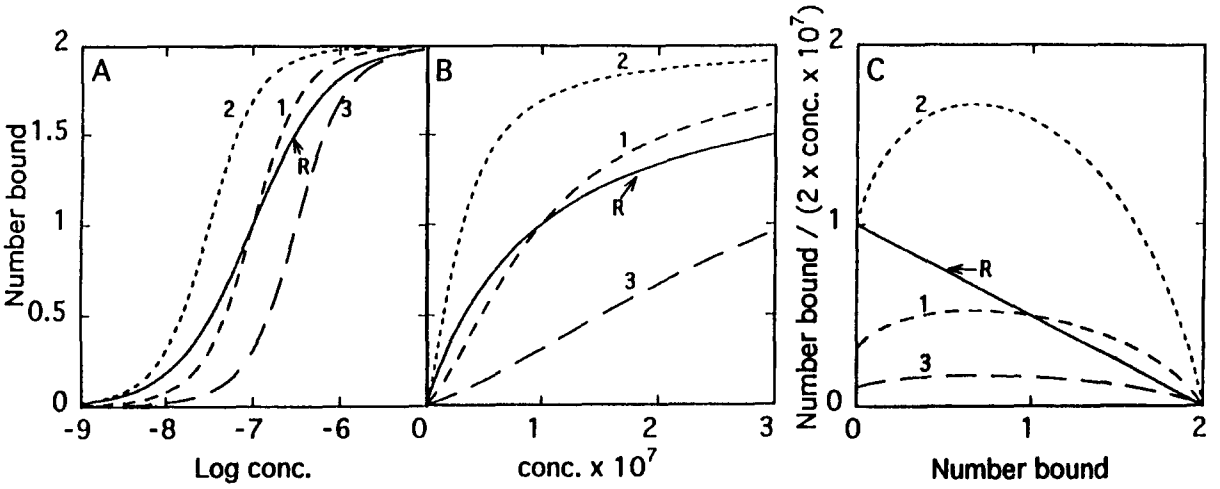

Fig. 3. Positive cooperativity due to α_1, or α_2 interactions or both can produce identical changes in the binding isotherms. The binding data in (A–C) are presented in the same forms as in Fig. 1. Curves labeled R are the reference case: unperturbed binding ($\alpha_1 = 1$, $\alpha_2 = 1$). Cases 1–3 present binding perturbed by (1) α_1 interactions only ($\alpha_1 = 1/(\sqrt{10})$, $\alpha_2 = 1$), (2) α_2 interactions only ($\alpha_1 = 1$, $\alpha_2 = 10$), or (3) both α_1 and α_2 interactions ($\alpha_1 = \alpha_2 = 0.1$). All three curves have the same K_2/K_1 ($= \alpha_2/4\alpha_1^2$) = 2.5. The intrinsic association constant is the same for all curves and equal to 10^7 M^{-1}. If the intrinsic constants were not identical, curves 1, 2, and 3 could be made to exactly superimpose, with any desired value for the half-saturating concentration of free ligand.

the intrinsic constant k, the ratio of K_1 and K_2 does not. It may be demonstrated that binding curves of the fraction bound vs. concentration of free ligand, c, are identical for a single site and for multiple independent sites, so the binding curve for this case is R in Fig. 1.

4.2. Interaction parameters

Interactions between *different regions of* a lattice occur in the absence of ligand as well as with ligand present. The interactions that occur in the completely unliganded state are the reference state for interactions, just as the unliganded state is the reference state for binding per se. When a ligand binds, structural changes induced in the site by the bound ligand will either alter interactions with neighboring sites or not. If no changes in interactions occur, the interaction parameter is defined as equal to 1 (no change from the reference state). If interactions are altered in a way that costs extra free energy then binding is disfavored and the interaction parameter α is <1. On the other hand, if the interactions are energetically favorable compared to the reference state, binding is favored and the interaction parameter α is >1. The interactions between a liganded site and a neighboring unliganded site are monitored by the interaction parameter α_1. Thus, if $\alpha_1 \neq 1$, then the bound ligand altered the interactions between a liganded site and an adjacent unliganded site compared to the interactions between those sites in the absence of ligand. The term α_2 describes interactions between neighboring liganded sites, and therefore if $\alpha_2 \neq 1$, then interactions between a liganded site and an adjacent liganded site are altered by the ligand. This would include, but is not limited to, interactions between the bound ligands.

5. Cooperative binding

We will consider cooperative binding first without specifying the nature of the specific interactions (Hill formulation), and then consider the results of altering one interaction parameter or the other or both.

5.1. The Hill formulation

If the concentration of the singly liganded species, represented by $2kc$ in line 2a above, is taken to be vanishingly small the relative sum of the species becomes:

$$\Xi = 1 + k^n c^n$$

where n is the Hill coefficient [11]. The value becomes 2 for reaction [2] above when the species represented by $2kc$ are, in fact, small enough to be ignored, indicating maximum cooperativity (the maximum value of n is equal to the number of binding sites, here = 2). When the concentration of these species are significant the value of n is smaller than its maximum value. Thus positive cooperativity entails the selective underrepresentation of intermediate states (compared to combinatoric expectation), and the Hill coefficient n reflects this and hence is an index of the cooperative effect.

5.2. Interactive formulations

Cooperative binding may be described in terms of particular interactions between the binding sites. As detailed above, positive cooperativity is characterized by an increase in K_2/K_1 above the value expected from combinatorial factors (here 1/4, for two sites). This may occur as a decrease in K_1, due to what we refer to as α_1 interactions, or as an increase in K_2, due to what we refer to as α_2 interactions. We will consider these in turn. In all of the following, the value of the interaction parameters α_1 and α_2 quantify the ligand-dependent interactions relative to the unliganded reference state. The energy of interaction is given by $-RT \ln(\alpha)$, which, to repeat, is distinct from the energy of binding, given by $-RT \ln(k)$.

5.2.1. Interactions affecting only K_1 (α_1 interactions). The forces that stabilize the (unliganded) polymeric structure of a binding lattice can alter (inhibit) initial ligand binding compared to what would be observed if a single binding site could be evaluated in isolation (the 'intrinsic' binding site character). This is sufficient to cause cooperative binding. Note that this effect is limited to the binding of the initial, unneighbored

ligand; it is not an effect that is propagated to adjacent sites. Such an effect would alter α_2, which here is equal to 1 by definition.

To illustrate this case, consider a binding process in which (a) the first ligand binds under conditions where binding destabilizes the interactions in the unliganded state (shown by the change from ∪ to ∩) and (b) no ligand dependent interactions perturb the complex with two ligands bound. This is shown in Fig. 2, line 3. The reaction may be symbolized as follows:

$$\begin{array}{ccccc} \cup\text{-}\cup & \Leftrightarrow & [X]\text{-}\cap & \Leftrightarrow & [X]\text{-}[X] \\ \{1\} & & \{2kc\alpha_1\} & & \{k^2c^2\alpha_2\} \\ & & \alpha_1 < 1 & & \alpha_2 = 1 \end{array} \quad [3]$$

Here the change from ∪ to ∩ on binding the first ligand represents the destabilizing ligand dependent interactions. The relative concentration of the species with one ligand bound is now $2kc\alpha_1$, with the value of $\alpha_1 < 1$ due to the interactions opposing initial binding. The relative concentration of the species with two ligands bound is $k^2c^2\alpha_2$ with $\alpha_2 = 1$. The experimental constants for this reaction are $K_1 = 2k\alpha_1$ and $K_2 = k\alpha_2/(2\alpha_1)$, so $K_2/K_1 = \alpha_2/(4\alpha_1^2)$. Since $\alpha_2 = 1$, this reduces to $K_2/K_1 = 1/(4\alpha_1^2)$. Since $\alpha_1 < 1$, K_2/K_1 is greater than 1/4, which is diagnostic of positive cooperativity.

We note here that this concept for developing positive cooperativity was first put forth by Coryell [12] in discussing Pauling's model [1] for the cooperative binding of oxygen to hemoglobin. He pointed out that the unliganded-unliganded interaction, rather than the liganded-liganded interaction, could be the predominant stabilizing factor in the development of the cooperative effect.

5.2.2. Interactions affecting only K_2 (α_2 interactions). If binding of the first ligand is not perturbed (inhibited), cooperativity can still occur due to interactions between sites with bound ligand. This includes, but is not limited to, stabilizing interactions between the bound ligands (see Fig. 2, line 4). To generalize this, consider the reaction below, in which *altered* interactions between sites only affect the species binding two ligands. Unlike the previous case, here $\alpha_1 = 1$ and $\alpha_2 > 1$.

$$\begin{array}{ccccc} \cup\text{-}\cup & \Leftrightarrow & [X]\text{-}\cup & \Leftrightarrow & [X]\text{-}[X] \\ \{1\} & & \{2kc\alpha_1\} & & \{k^2c^2\alpha_2\} \\ & & \alpha_1 = 1 & & \alpha_2 > 1 \end{array} \quad [4]$$

Note that here the empty binding site in the single liganded state is unchanged from the unliganded state, since interactions only affect the doubly liganded state. Ratios of the experimental association constants are $K_2/K_1 = \alpha_2/4\alpha_1^2$, here $= \alpha_2/4$. Since $\alpha_2 > 1$, K_2/K_1 is again greater than 1/4, indicating positive cooperativity.

5.2.3. Interactions affecting both K_1 and K_2 (α_1 and α_2 interactions). As we have shown, either a change in K_1 alone, due to α_1 interactions ($\alpha_1 < 1$, $\alpha_2 = 1$), or a change in K_2 alone, due to α_2 interactions ($\alpha_1 = 1$, $\alpha_2 > 1$), is sufficient to result in cooperative binding. Clearly then, a situation in which both α_1 and α_2 interactions occur, affecting both K_1 and K_2, can also result in positive cooperativity in binding (see Fig. 2, line 5). This can occur in many ways. One is a simple combination of the two cases above (i.e. $\alpha_1 < 1$ and $\alpha_2 > 1$). However, the requirement for cooperativity is just that K_2/K_1 ($= \alpha_2/4\alpha_1^2$) $> 1/4$, and there are many additional ways to meet the requirement that $\alpha_2/\alpha_1^2 > 1$. There are literally an infinite number of ways to produce positive cooperativity.

Fig. 3 illustrates three limiting cases for the effects of interactions on the binding curves for two identical sites. All of these cases are derived with the same intrinsic association constant $k = 10^7$ M^{-1}. Case R (the reference case) is that for independent sites, so $\alpha_1 = \alpha_2 = 1$. Cases 1–3 all show positive cooperativity, but due to different mechanisms. Case 1 shows the effect of interactions affecting K_1 only; here $\alpha_1 = 1/(\sqrt{10})$ and $\alpha_2 = 1.0$. Case 2 shows the effect of interactions affecting K_2 only; here $\alpha_1 = 1.0$ and $\alpha_2 = 10$. In Case 3 interactions affect K_1 and K_2; $\alpha_1 = \alpha_2 = 0.1$. The three cases 1–3 all have identical shapes (most evident in panel A) with ratios $K_2/K_1 = 2.5$, which is to say that these three cases all show the same extent of positive cooperativity. This demonstrates that all three models (altered α_1, altered α_2, or altered both) could generate identical binding data if the value of k were allowed to vary. For example, the binding curve for case 2 is exactly produced using α_1 and α_2 from case 3 if k is changed from 10^7 to 10^8 M^{-1}. The shape of the binding curve can be affected equally and indistinguishably by interactions affecting K_1, K_2, or both. Thus, *in the absence of independent data defining the intrinsic association constant k or knowledge of the nature of the ligand-dependent interactions, a set of binding data showing cooperativity can be equally well explained by more than one model.*

6. Molecular mechanisms

In order to gain insight into the molecular mechanisms involved, it is not sufficient only to fit adequately a curve to the data; this can be equally well done by multiple molecular models using one, two, or more parameters. While the three cases 1–3 can generate identical binding data, and can equally well model a given set of binding data (with different intrinsic association constants), the molecular mechanisms underlying these cases are *not* identical. In other words, the three cases do not differ merely by a change in reference state. The molecular interactions that give rise to cooperativity are different in the three cases. Biochemists and molecular biologists frequently use models with only a single cooperativity parameter, usually associated with the doubly liganded species, i.e. an α_2-only model. Since α_1 is equal to 1 by definition in such models, interactions that affect K_2 must be invoked to explain cooperative binding. These α_2 interactions are easily visualized as stabilizing ligand-ligand interactions that occur on the binding lattice, though the stabilizing interactions could be between the two liganded *sites* rather than the ligands bound to them. It is certainly not necessary for bound ligands to interact directly in order to result in cooperative binding. The binding of oxygen to hemoglobin and the binding of tryptophan to *trp* RNA binding attenuation protein [13] are both clearly cooperative and both clearly do not involve direct interactions between bound ligands.

Interactions that affect K_1 are less often invoked and perhaps less easily visualized. However, such interactions can give rise to identical cooperative binding, due only to interactions that stabilize the unliganded state, altering the properties of the multiple binding sites and inhibiting initial (unneigh-

bored) binding, but not affecting subsequent contiguous binding. It is important to emphasize again that this is not a lattice perturbation that is hard to initiate but easy to propagate. These α_1 effects only are relevant to unneighbored, binding and are distinct from site-site (ligand-ligand) effects.

Hemoglobin provides an example. The association of the subunits of the hemoglobin tetramer involves shifts in structure, and alterations in the hydration and electrostatic field around the protein. The resulting constraints on the protein structure result in a significant inhibition of the binding of the first oxygen to deoxyhemoglobin relative to the 'intrinsic' binding that would be seen (as in myoglobin) absent the interactions that stabilize the deoxyhemoglobin lattice (tetramer). Binding of the first oxygen is inhibited but binding of the final oxygen is similar to that for myoglobin (the intrinsic constant). Of course this positive cooperativity does not require any interaction between the ligands (oxygens) bound in adjacent hemes. While the cooperative shape of the binding isotherm for hemoglobin can be produced by pure α_1 effects, the correct shape and correct half-maximal value are obtained only with both α_1 and α_2 effects, where the constant for the binding of oxygen to myoglobin is used for the intrinsic k.

Relevant interactions that stabilize biological polymeric structures are well known. Base-stacking or other neighboring interactions in nucleic acids may favor a conformation that must be disrupted for binding of a protein to occur [14,15]. Bending of the phosphodiester backbone following protein binding to DNA provides a clear example of this [16]. Bending of the DNA can result in positive cooperativity in protein binding to the DNA in the absence of any protein-protein interaction [8,17]. Interactions between exposed C-terminal binding domains of tubulin form a network of charges around the surface of the polymerized microtubule which must be altered to allow docking of microtubule-binding proteins. The protonation of glycine provides a case of pure α_1-driven cooperativity (albeit negative cooperativity) in which the interactions are purely electrostatic [18].

The unifying theme here is that the network of interactions that stabilize a polymeric lattice of binding sites may oppose ligand binding to an isolated unliganded binding site on the lattice. Hence, binding of a second ligand will appear favored energetically, even in the absence of any ligand-ligand interactions on the lattice. Obviously, if altering either K_1 or K_2 can generate identical behavior, altering both also can do so. Consequently, curve 3 in Fig. 3A has the same slope as curves 2 or 1.

7. Conclusions

The three cases can be distinguished if and only if either the intrinsic constant k is known or the nature of the ligand dependent interactions is known. This is rarely the case. The value of the intrinsic constant is inaccessible to direct experiment and must be evaluated based on reasonable assumptions or extrapolation, even in such simple interacting systems as the symmetrical malonic acid [19]. The context of the isolated site is important, and physically isolating a segment of a matrix will not necessarily reveal the intrinsic k for the matrix (due to end group effects, if nothing else). Real biological systems are unlikely to exhibit binding described properly by either pure α_1 or α_2 models. Rather, most systems will likely show interactions affecting both initial and subsequent binding to varying (and yet to be determined) extents. In the absence of explicit evidence, equating positive cooperativity with ligand-ligand interactions appears unwarranted. Multiple molecular mechanisms can give rise to cooperative binding.

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