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Some General Aspects Regarding the Interpretation of Binding Data by Means of a Scatchard Plot

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Abstract. The question is quantitatively examined which general information can be obtained from experimental data of ligand binding to macromolecules if represented by a Scatchard plot. In particular, the curvature as well as the intercepts and slopes at both coordinate axes are analyzed assuming rather general conditions including cooperative behavior (with applications to some simplified cases of interest in practice). The results should provide a basis for attempts to elucidate the binding mechanism of a system encountered in experimental work.

Key words: General Binding — Scatchard plots — Cooperativity.

Introduction

In studies of ligand binding to macromolecules one usually determines the binding ratio $\nu = c_a/c_p$, *i.e.* the concentration of bound ligand, a, per concentration of the polymer, as a function of the concentration, c_A, of the free ligand, A. Graphical representation of such binding data in terms of a Scatchard plot (Scatchard, 1949), ν/c_A versus ν, has become quite popular because it can be applied in a straight-forward way and also in simple cases yields a straight line which immediately leads to the basic parameters of the underlying binding process.

Deviations from a straight line are quite sensitive indicators of various complicating effects such as the occurrence of different types of binding sites (*e.g.*, see Weder *et al.*, 1974), cooperative interaction (*e.g.*, see Dahlquist, 1974) or multiple-contact binding sites, *i.e.* sites composed of more than one equivalent binding element so that a partial overlapping of potential binding sites becomes possible (McGhee and v. Hippel, 1974). The shape of the experimentally obtained curve can be interpreted yielding qualitative predictions about the binding mechanism. Assuming a simple model even the respective binding parameters may be evaluated. However, there seems to be no general analysis how quantitative conclusions can be drawn from the course of a Scatchard plot if no special mechanism is assumed beforehand. Pertinent information of this kind appears to be rather useful for any approach to elucidate a given binding process. A number of relevant aspects will be discussed in this article.

Basic Parameters

We note that for any given type r of equivalent binding sites a parameter g_r exists which is defined as the respective maximum number of such sites available on one macromolecule. The total binding ratio can then be written as

$$\nu = \Sigma_r g_r \theta_r, \tag{1}$$

where θ_r denotes the fraction of binding sites of type r actually occupied by the ligand. This is called its degree of binding or saturation. Naturally θ_r must be zero at $c_A = 0$ and increase monotonously upon increasing c_A. For $c_A \to \infty$ we have $\theta_r \to 1$ provided that binding to one type does not affect the number of sites of other types. Then ν asymptotically approaches a maximum according to $\nu \to \nu_\infty = \Sigma_r g_r$.

This will not apply for instance, if potential sites are blocked upon binding. Such blocking reduces ν_∞ due to smaller limits of θ_r (Schwarz, 1975).

Binding of the free ligand molecule A on some site of type r is to be generally formulated as

$$A + u_r \overset{(K_r)}{\rightleftharpoons} a_r \tag{2a}$$

with u_r, a_r standing for a site which is unoccupied or occupied, respectively, by the ligand under consideration. The binding constant K_r is defined as the equilibrium constant of that step according to

$$K_r = \frac{[a_r]}{c_A\,[u_r]}. \tag{2b}$$

We note that K_r could depend on the state of other sites of the same or different type, in other words this means that we allow for cooperative effects in the binding of A.

There may be ligands B_1, B_2, ... which also bind to the same sites. Such competitive binding will naturally affect the binding of A. Let us consider here only the non-cooperative case as far as the B_i are concerned. We denote the concentration of binding sites which are not occupied by neither A nor any B_i as $[o_r]$ whereas $[(b_i)_r]$ stands for the concentration of sites occupied by B_i. With a binding constant K_{ir} for B_i it follows that

$$[(b_i)_r]/[o_r] = K_{ir} c_i \tag{3}$$

(c_i = concentration of free B_i) leading to an apparent binding constant

$$K_r = \frac{K_{or}}{1 + \Sigma_i K_{ir} c_i}. \tag{4a}$$

It approaches the intrinsic binding constant

$$K_{or} = \frac{[a_r]}{c_A\,[o_r]} \tag{4b}$$

when all $c_i \to 0$. Clearly, K_r is always decreased upon adding more of the competitive ligands.

Cooperativity and the Shape of Scatchard Plots

A basic mechanism of cooperative interaction assumes that certain clusters of binding sites exist, each comprising n_H sites, which can only be occupied

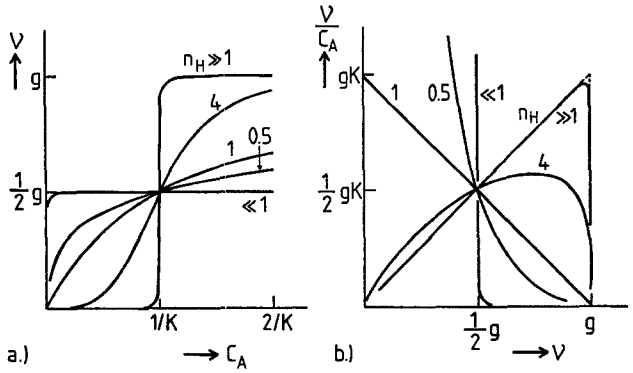

Fig. 1. Graphs of ligand binding data for various degrees of cooperativity as described by the Hill coefficient n_H. *(a)* Plots of the binding ratio ν versus the free ligand concentration c_A for given parameters g and K (see text), *(b)* Respective Scatchard plots

simultaneously ("all-or-none" model). For one type of equivalent sites this leads to a binding ratio described as

$$\nu = g \frac{(Kc_A)^{n_H}}{1 + (Kc_A)^{n_H}}, \tag{5}$$

where K can be interpreted as a mean binding constant.

In the simple case of no cooperativity we have $n_H = 1$ so that

$$\frac{\nu}{c_A} = K(g - \nu). \tag{6}$$

A Scatchard plot thus results in a straight line yielding gK and g by extrapolation to the coordinate axes. If on the other hand, the Scatchard plot displays curvature, some cooperativity must be involved, provided there is really only one type of binding sites and no multiple-contact.

In practice it usually turns out that such cooperative binding can be formally described by Eq. (5) known as Hill's relation (*e.g.*, see van Holde, 1971). It applies over a wide range of concentrations except at the limits of small ν and $g - \nu$. The so determined (positive) exponent n_H, however, will generally not be an integer. It is called the Hill coefficient of the system and serves as an empirical measure of the strength of cooperative interaction. Positive and negative cooperativity are characterized by $n_H > 1$ and $n_H < 1$, respectively.

By means of (5), with no restriction on the value of n_H, we may therefore examine the effect of cooperativity on the shape of binding curves and Scatchard plots. We note that in addition to $\nu/c_A \rightarrow 0$ for $\nu \rightarrow g$ (though practical extrapolation may be rather uncertain for extreme positive or negative cooperativity) one must always have $\nu/c_A = gK/2$ at $\nu = g/2$. Thus K can always be evaluated from the Scatchard plot as the ratio of the ordinate and the abscissa for the point taken at half the maximum value of ν.

Positive cooperativity leads to sigmoidal ν versus c_A curves as illustrated by the examples in Fig. 1. This well-known fact is apparently paralleled by a hump shaped Scatchard plot. Such curves can be qualitatively understood if one takes

into consideration that in the middle region of the transition a sharper change of ν on c_A occurs than in the non-cooperative case. Accordingly ν/c_A must also exhibit a greater slope in its dependence on ν up to a certain point where the plot eventually turns around towards the g-value on the abscissa axis. For extremely strong cooperativity we have nearly constant $c_A = 1/K$ at practically any value of ν so that the Scatchard plot again becomes a straight line (this time passing through the origin) with a positive slope of K in contrast to zero cooperativity where the slope equals $-K$. These qualitative considerations can be extended by a quantitative treatment based on a calculation of the derivatives. We obtain

$$\frac{d(\nu/c_A)}{d\nu} = \left\{ g \frac{n_H - 1}{n_H} - \nu \right\} \cdot \frac{K}{g - \nu} \left(\frac{\nu}{g - \nu} \right)^{1/n_H} \tag{7}$$

$$\frac{d^2(\nu/c_A)}{d\nu^2} = -\frac{(n_H - 1)}{{n_H}^2} \cdot \frac{g^2 K}{\nu (g-\nu)^2} \left(\frac{g - \nu}{\nu} \right)^{1/n_H}. \tag{8}$$

Thus any positive cooperativity is equivalent to a negative curvature of the Scatchard plot (as determined by a negative second derivative) or, in other words, to a convex shape (for experimental examples see for instance Carayon and Carella, 1974; Sukow and Sandberg, 1974). Furthermoore, it can be readily recognized that there is always a maximum which is located at an abscissa position of

$$\nu_{\max} = g(n_H - 1)/n_H. \tag{9}$$

In principle, the parameter n_H may therefore be evaluated from $\nu_{\max}$ although this method must not be expected to yield fairly accurate values (Dahlquist, 1974) since the maximum is either too flat or too close to ν_∞. More accuracy can be achieved (except for very strong cooperativity) if one utilizes the ordinate value which is

$$\left(\frac{\nu}{c_A} \right)_{\max} = (gK/n_H)\,(n_H - 1)^{1 - \frac{1}{n_H}}. \tag{10}$$

At any rate we conclude that the maximum is monotonously shifted towards the upper right part of the diagram when the cooperativity is increased (at constant K). The upper bounds for $\nu_{\max}$ and $(\nu/c_A)_{\max}$ are obviously equal to g and gK, respectively. These coordinates thus characterize a rather high degree of (positively) cooperative binding (see Fig. 1 b).

On the other hand, we see that negative cooperativity is always reflected by Scatchard plots exhibiting positive curvature and concave shape. Neither a maximum nor a minimum can occur. Going to greater negativity leads to a sharper and sharper decay of the curve around $\nu = g/2$ (see Fig. 1 b). Note that in such cases experimental data at a sufficiently high degree of binding are particularly important for a correct interpretation. Lack of these data may result in confusing the curve with a straight line having an intercept on the abscissa which is in-between $g/2$ and g.

We may now turn to a system having different but independent types of binding sites which do not involve multiple contacts. The respective binding curves according to (5) and Fig. 1 a simply superimpose each other, *i.e.*

$$\nu = \Sigma_r g_r \frac{(K_r c_A)^{n_{H,r}}}{1 + (K_r c_A)^{n_{H,r}}}. \tag{11}$$

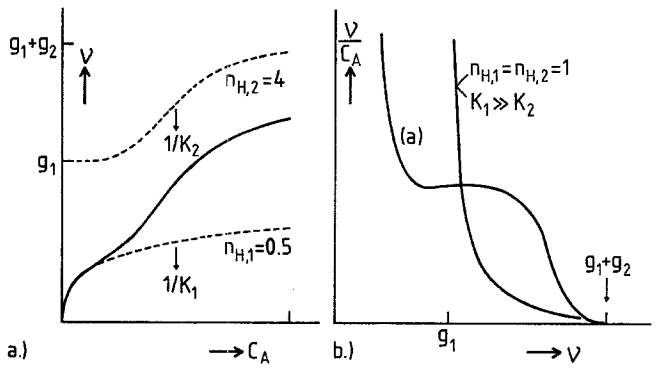

Fig. 2. Examples of binding curves in case of two different types of binding sites. *(a)* Overall binding ratio *vs.* free ligand concentration (solid curve) composed of individual ν-values (indicated as dashed curves) which are determined by the same K but clearly different cooperativity, *(b)* Scatchard plots of the cases given in part *a* and one with sites of zero cooperativity but largely different K, respectively

Under favorable circumstances the individual binding curves can be recognized and analyzed from the overall curve. This is especially true if a distinct difference in cooperativity exists as can be seen for the example given in Fig. 2: The positively cooperative binding sites cause a humped part in the Scatchard plot while outside its range the negatively cooperative character of the other type of binding sites is reflected. Clearly, points of inflection implying a switch in the sign of curvature can only be due to the occurrence of different types of binding sites, at least one of them exhibiting positive cooperativity. Plots of such appearence have been actually encountered experimentally (*e.g.*, see Wagner, 1969; Danchin, 1972).

In cases where the degree of cooperativity of the different types is about the same a separation of the individual binding curves becomes rather difficult. This is because on superimposing them a more or less broadened curve arises which can hardly be distinguished from a curve referring to uniform binding sites with an average K and a slightly decreased cooperativity. This is demonstrated in Fig. 2b for two different and non-cooperative types of binding sites. With increasing cooperativity, however, a certain difference in the K-values will nevertheless suffice to produce characteristic humps in the Scatchard plot. As illustrated in Fig. 3 these may be utilized to recognize or even evaluate the individual sites.

From the above considerations we conclude that negative curvature or even a maximum in a Scatchard plot indicates at least one type of binding site with positive cooperativity. The magnitude of the latter, however, can generally not be determined quantitatively from the shape and position of the hump since these will also be dependent on the properties of other types of binding sites if such exist.

Positive curvature in a Scatchard plot apparently has no unequivocal causes. It could be due to negative cooperativity, superposition of different types of non-cooperative binding sites or—as to be pointed out below—multiple-contact effects.

As a matter of fact a satisfactory analysis of experimental binding data will only be possible on the basis of a definite model of the underlying mechanism. Nevertheless, we want to point out that even without a special model mechanism

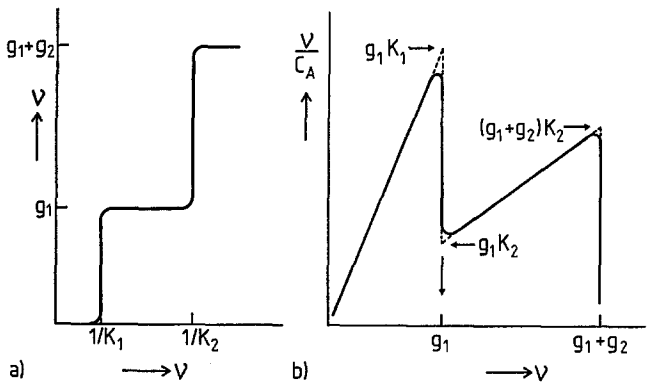

Fig. 3. Behavior of two highly cooperative types of binding sites. *(a)* Overall binding ratio *vs.* free ligand concentration, *(b)* the resulting Scatchard plot with two characteristic humps, each corresponding to one type of sites. For medium cooperativity they will of course tend to be less sharp but more broadened

in mind certain parameters, namely the intercepts and slopes at the coordinate axes, may be evaluated from a Scatchard plot and can be interpreted in quantitative terms under very general circumstances. Together with other pertinent information the results are particularly useful to elucidate the kind of mechanism responsible for the binding process.

Quantitative Significance of Low Saturation Data

At sufficiently low c_A we have also a small value of ν, *i.e.* low saturation of the macromolecule with bound ligand. We may there expand ν in a Taylor series according to

$$\nu = \alpha_1 c_A + \alpha_2 c_A^2 + \ldots \tag{12}$$

While α_1 represents the Intercept I_0 of the Scatchard plot on the ordinate axis, its initial slope S_0 turns out as α_2/α_1. Both these quantities are ordinarily accessible experimentally by extrapolation. Thus it will be useful to relate them to basic parameters of the binding reactions. For the present multiple-contact effects and blocking of sites are to be excluded.

Since we must take into account that the binding constant K_r generally depend on the state of other sites owing to cooperative interaction, any binding site will be classified according to the number of such interactions which it is subjected to under the given circumstances. First we consider those occupied binding sites with no cooperative interaction because all their potential partners are unoccupied. As far as type r is concerned their concentration follows from the mass action law as

$$c_{a,r}^{(0)} = c_{u,r}^{(0)} \cdot K_r^{(0)} c_A, \tag{13}$$

where $c_{u,r}^{(0)}$ stands for the concentration of corresponding unoccupied binding sites and $K_r^{(0)}$ denotes the appropriate binding constant. It refers to the nucleation step which must occur before any cooperative interaction can take place. Thus $\Sigma_r c_{a,r}^{(0)}$

represents the predominant part of c_a in the limit of $\nu \to 0$. Now we turn to those occupied binding sites of type r which interact with just one other occupied site. If the latter is one of type s the respective concentrations may be written

$$c^{(1)}_{a,rs} = c^{(1)}_{u,rs} \cdot K^{(1)}_{rs} c_A \tag{14}$$

with $K^{(1)}_{rs}$ being the inherent cooperative binding constant. Introducing z_{rs}, the number of sites r which may cooperatively interact with one site s, we obtain

$$c^{(1)}_{u,rs} = z_{rs} c^{(0)}_{a,s} + \mathrm{O}(c_A^2), \tag{15}$$

where the first term on the right yields the concentration of all sites r which interact with isolated occupied sites in s (and O means "of the order of"). Among them may be some which are either occupied or also counted with a different s but these turn out to have probabilities being of at least second order in c_A. The same is true regarding the effect of non-isolated occupied sites in s. Inserting (15) in (14) and neglecting terms of the order of c_A^3 and higher yields

$$c^{(1)}_{a,r} = \Sigma_s z_{rs} K^{(1)}_{rs} c_A c^{(0)}_{a,s}. \tag{16}$$

This is of the order of c_A^2. Occupied binding sites with more than one cooperative interaction must have probabilities of at least the order of c_A^3. Thus we need not consider them as long as we are only interested in the first two terms of the Taylor expansion (12). Summing up (13) and (16) results under these circumstances in

$$c_a = \Sigma_s K^{(0)}_s c_A \{1 + \Sigma_r z_{rs} K^{(1)}_{rs} c_A\} c^{(0)}_{u,s}. \tag{17}$$

Neglecting again O (c_A^2) we have

$$c^{(0)}_{u,s} = g_s c_p - \Sigma_\sigma (z_{s\sigma} + \delta_{s\sigma}) c^{(0)}_{a,\sigma}$$

($\delta_{s\sigma} = 1$ for $\sigma = s$, otherwise $\delta_{s\sigma} = 0$) where we may then set $c^{(0)}_{a,\sigma} = K^{(0)}_\sigma \; c_A \cdot g_\sigma c_p$. Inserting this in (17) yields

$$\nu = \Sigma_s K^{(0)}_s c_A \{1 + \Sigma_r z_{rs} K^{(1)}_{rs} c_A\} \{g_s - \Sigma_\sigma (z_{s\sigma} + \delta_{s\sigma}) g_\sigma K^{(0)}_\sigma c_A\}$$

resulting in

$$\alpha_1 = I_0 = \Sigma_s g_s K^{(0)}_s$$

and

$$\alpha_2 = \Sigma_s g_s K^{(0)}_s \Sigma_r \{z_{rs} q^{(1)}_{rs} - (z_{rs} + \delta_{rs})\} K^{(0)}_r.$$

The parameter $q^{(1)}_{rs}$ is defined according to

$$K^{(1)}_{rs} = q^{(1)}_{rs} K^{(0)}_r \tag{18}$$

and represents a measure of the cooperative interaction between two sites of type r and s; it will be determined in the usual way by the respective standard free enthalpy of reaction contributed by that interaction. Positive and negative cooperativity are characterized by $q^{(1)}_{rs} > 1$ and < 1, respectively. The initial slope of the Scatchard plot can now be written

$$S_0 = \frac{\alpha_2}{\alpha_1} = \Sigma_s \varkappa_s \Sigma_r \{z_{rs} q^{(1)}_{rs} - (z_{rs} + \delta_{rs})\} K^{(0)}_r \tag{19}$$

with

$$\varkappa_s = \frac{g_s K_s{}^{(0)}}{\Sigma_\sigma g_\sigma K_\sigma{}^{(0)}}.$$

While the intercept on the ordinate axis is essentially determined by those sites having the highest value of $g_s K_s^{(0)}$ the situation is generally more complicated regarding the initial slope. Simplifications would result from the lack of interaction between different types of sites so that we may set $z_{rs} = z_r \delta_{rs}$ and $q_{rr}^{(1)} = g_r^{(1)}$. This case of purely superimposed cooperative binding to independent sets of binding sites leads to an initial slope of

$$S_0 = \Sigma_r \varkappa_r \{z_r q_r^{(1)} - (z_r + 1)\} K_r^{(0)} \tag{20}$$

Quantitative Significance of High Saturation Data

At rather high c_A we may expand the binding ratio according to

$$\nu = \beta_0 + \beta_1 c_A^{-1} + \ldots \tag{21}$$

and eventually neglect terms of the second or higher order in $1/c_A$. With β_0 being the intercept of the Scatchard plot on the abscissa axis, I_∞, its final slope S_∞ follows as

$$S_\infty = \beta_0/\beta_1 .$$

In case the macromolecule is nearly saturated with ligands, almost all occupied and non-occupied binding sites will have their maximum number of cooperative interactions with bound ligand. The respective concentrations are to be denoted $c_{a,r}^{(\infty)}$ and $c_{u,r}^{(\infty)}$. The corresponding binding constant $K_r^{(\infty)}$ relates them to each other according to

$$c_{u,r}^{(\infty)} = \{c_{a,r}^{(\infty)}/K_r^{(\infty)}\} c_A^{-1} .$$

The total number of occupied binding sites follows then as

$$\begin{aligned} c_{a,r} &= g_r c_p - c_{u,r}^{(\infty)} + O(c_A^{-2}) \\ &= g_r c_p \{1 - (1/K_r^{(\infty)}) c_A^{-1}\} + O(c_A^{-2}) . \end{aligned}$$

This leads immediately to

$$\beta_0 = \nu_\infty = \Sigma_r g_r \tag{22}$$

and

$$\beta_1 = -\Sigma_r g_r / K_r^{(\infty)} .$$

The final slope thus turns out as

$$S_\infty = -\frac{\Sigma_r g_r}{\Sigma_r g_r / K_r^{(\infty)}} . \tag{23}$$

Its reciprocal value can therefore be interpreted as a mean of the reciprocal final binding constants, $K_r^{(\infty)}$, with the g_r as weight factors.

Special Cases

(a) Let us consider a system which is assumed to involve only one type of binding sites. Then we have

$$I_0 = g K^{(0)}, \qquad I_\infty = g$$

$$S_0 = \{z q^{(1)} - (z+1)\} K^{(0)}, \qquad S_\infty = -K^{(\infty)} .$$

From the intercepts and the final slope we may thus determine the number of binding sites as well as the two nucleation binding constants (note that $K^{(\infty)}$ refers to the formation of a nucleus of unoccupied sites). Since the initial slope can also be written

$$S_0 = z(q^{(1)} - 1)K^{(0)} - K^{(0)}$$

we conclude that a greater (smaller) slope than in the non-cooperative case (which would be $-K^{(0)}$) indicates positive (negative) cooperativity in the interaction between only two occupied sites. We can, however, determine only the product $z(q^{(1)} - 1)$. In order to evaluate the parameter $q^{(1)}$ we must know the number z. Fortunately this may be anticipated in most cases on the basis of a definite model mechanism. For instance, if a linear lattice of binding sites with nearest neighbor cooperativity is given, we have $z = 2$. In this case the system can be completely described by means of the parameters g, $q(= q^{(1)})$ and $K = qK^{(0)}$ provided end effects can be neglected (Schwarz, 1970). The final binding constant follows here as

$$K^{(\infty)} = q^2 K^{(0)}.$$

Accordingly we have

$$S_0/S_\infty = -(2q - 3)/q^2$$

i.e. the final slope is in a certain way related to the initial one. Clearly the final decay of the plot is much more pronounced than the initial rise if some positive cooperativity is present. On the other hand, the plot must exhibit less descent near its end than at its start if negative cooperativity is involved. More complex behavior is naturally to be expected in cases of cooperative interaction beyond nearest neighbor or at least two different types of binding sites. A more detailed discussion of these experimentally interesting problems will be given elsewhere (Schwarz, 1975).

Cooperative binding to allosteric enzymes may be briefly mentioned since it has aroused great interest in biochemistry as a potential mechanism of regulation (Monod *et al.*, 1965). In the case of homotropic allosterism the ligand binds to $n(> 1)$ cooperatively interacting equivalent sites (each located on a different subunit of the protein). Here we thus have $g = n$ and $z = n - 1$.

Turning now to systems with different types of binding sites we confine ourselves to non-cooperative ones. Since the two intercepts are $\Sigma_r g_r K_r$ and $\Sigma_r g_r$, respectively, a straight line between these points would correspond to the Scatchard plot of only one set of equivalent sites with a $g = \Sigma_r g_r$ and a K being equal to an average binding constant

$$K_{av} = \Sigma_r g_r K_r / \Sigma_r g_r.$$

The corresponding average slope is $S_{av} = -K_{av}$. The actual value of the initial slope according to (19) turns out as

$$S_0 = -\frac{\Sigma_r g_r \Sigma_r^2}{\Sigma_r g_r K_r} = S_{av} - \frac{\Sigma_{r>s}\Sigma_s g_r g_s (K_r - K_s)^2}{I_0 \cdot I_\infty} < S_{av}.$$

On the other hand, we find

$$S_\infty = -\frac{\Sigma_r g_r}{\Sigma_r g_r / K_r} = S_{av} + \frac{\Sigma_{r>s}\Sigma_s \frac{g_r g_s}{K_r K_s}(K_r - K_s)^2}{I_\infty \Sigma_r g_r / K_r} > S_{av}.$$

This means that the slopes generally have the properties which are characteristic for negative cooperativity in a system of only one set of binding sites. The occurrence of non-cooperative but different types of binding sites could therefore be confused with the phenomenon of negative cooperative binding if no other pertinent information about the sites is available.

Multiple-Contact Binding

We have already drawn attention to the special case of binding sites made up by more than one equivalent binding contact. Let us assume we have N such contacts on a macromolecule. A certain combination of them forms a binding site for a ligand, each comprising $n(>1)$ contacts. Under these circumstances potential binding sites may overlap each other so that the binding of one ligand will generally eliminate more than one site from further binding.

The total number of available sites in the limit of $\nu \to 0$, g_0, will depend not only on N and n but also on the way in which the contacts are arranged (*e.g.*, $g_0 = N - n + 1$ for a linear lattice). At any rate, $g_0 \leq N$. Confining ourselves to zero cooperativity the concentration of bound ligand can be calculated as

$$c_a = c_u \cdot K c_A \tag{24}$$

with the binding constant K and the instantaneous concentration of unoccupied potential binding sites, c_u. The latter is represented according to

$$c_u = g_o c_p - \gamma(\nu) c_a, \tag{25}$$

where $\gamma(\nu)$ denotes the average number of potential binding sites eliminated by one of the already bound ligands. This quantity must monotonously decrease with increasing ν because the probability that a newly occupied site overlaps with a site which already has been eliminated by a previously bound ligand becomes the higher the more ligands are bound.

Thus the general condition

$$\gamma'(\nu) < 0$$

applies to the first derivative with regard to ν. The maximum value of γ is naturally reached in the limit of $\nu \to 0$ [this $\gamma(0)$ is, for instance, found to be $2n-1$ for a linear lattice of contacts].

Owing to (24) and (25) we obtain

$$\nu/c_A = K\{g_o - \nu\gamma(\nu)\}.$$

The first and second derivatives result as

$$(\nu/c_A)' = -K\{\gamma(\nu) + \nu\gamma'(\nu)\}$$

$$(\nu/c_A)'' = -K\{2\gamma'(\nu) + \nu\gamma''(\nu)\}.$$

This yields

$$I_o = g_o K, \quad S_o = -\gamma(0) K. \tag{26}$$

Moreover, we note that at least for small ν the curvature must be positive.

Introducing now g_∞ ($\leq N/n$), *i.e.* the maximum of ligands which can be bound to a macromolecule, the condition $\nu/c_A \to 0$ at $\nu \to g_\infty$ is seen to imply

$$g_o = g_\infty \gamma(g_\infty).$$

Because of

$$I_\infty = g_\infty$$

the average slope of the Scatchard plot must be

$$S_{av} = -K\gamma(g_\infty)$$

so that

$$S_o < S_{av}. \tag{27}$$

This, as well as the positive curvature, reflects the same behavior also observed for plots obtained in the case of superimposed non-cooperative binding sites or negative cooperativity.

For an approach to S_∞ we start from the point that the number of contacts available for potential unoccupied binding sites must be a quantity proportional to $(g_\infty - \nu)$ if the system is sufficiently close to complete saturation with bound ligands. The probability to find there appropriate combinations of n contacts yielding a free binding site will then be proportional to the n-th power of that quantity. Accordingly, we likewise have under these circumstances

$$\frac{\nu}{c_A} \sim (g_\infty - \nu)^n. \tag{28a}$$

Because of $n > 1$ this results in positive curvature together with

$$S_\infty = 0. \tag{28b}$$

Obviously the plot must be expected to turn flatter and flatter near the abscissa axis the greater the number of contacts needed for a binding site. This behavior, too, makes it generally difficult to distinguish multiple-contact binding purely on the basis of the shape of a Scatchard plot.

A more general and detailed treatment of linear systems involving multiple-contact and blocking effects will be given in another article (Schwarz, 1975).

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