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LINEAR COOPERATIVE BINDING OF LARGE LIGANDS INVOLVING MUTUAL EXCLUSION OF DIFFERENT BINDING MODES

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A rigorous treatment is given for mutually exclusive multiple mode cooperative binding on a linear structure of equivalent binding "contacts". This will be of special interest with regard to larger ligands implicating the possibility that there are different kinds of binding interactions with more than one monomeric subunit of a linear biopolymer. Quantitative evaluation of binding properties is shown to be essentially based on calculating the largest root of an algebraic equation. The whole procedure can be practically executed by means of a fairly simple computer program. Various typical examples comprising only two modes are discussed in more detail. For some nucleotide–polylysine systems definite binding parameters have been determined from pertinent experimental data.

1. Introduction

Unspecific binding of ligands to a linear array of binding sites on a macromolecular structure is frequently encountered in systems of biological relevance. The various non-trivial manifestations of such linear binding thus deserve to be thoroughly studied from a physico-chemical point of view.

Some general aspects concerning the theoretical treatment of the equilibrium binding properties have recently been reviewed and especially applied to an analysis of the behavior exhibited by systems comprising a single class of binding sites so that only one mode of binding occurs [1].

A distinct binding mode is characterized by a definite structure of the ligand-polymer complex. In general, this may involve

(i) some degree of cooperative interaction, i.e. a change of binding strength induced by ligands bound somewhere else on the array, as well as

(ii) a "multiple contact" effect for larger ligands, i.e. the fact that a binding site consists of a certain number of equivalent basic binding "contacts".

The latter effect implies that potential sites can overlap or, when occupied, may leave behind gaps of an insufficient number of contacts which cannot be occupied in the mode under consideration (see fig. 1a). This phenomenon has been discussed with special reference to the repressor–DNA system [2]. Naturally it must be taken into account for any linear binding where the ligands are much larger than the polymer subunits if these exert equivalent binding forces. Such is indicated, for instance, in the case of "melting"proteins which bind unspecifically to single-stranded nucleic acids [3].

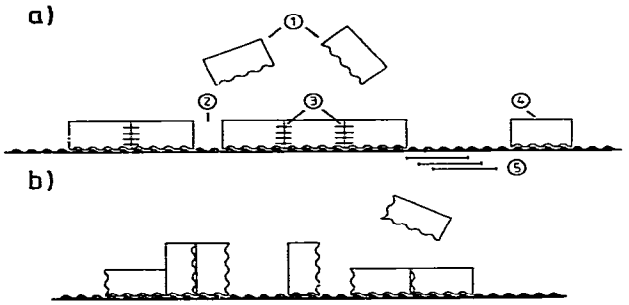

Fig. 1. Schematic illustration of linear binding with multiple contacts (contacts being indicated by ripple pattern). a) Single-mode binding case comprising free ligand molecules (1), gaps insufficient for a binding site (2), cooperative interaction between nearest neighbors (3), isolated ("nucleated") bound ligands (4), and overlapping potential binding sites (5). b) Dual-mode binding of one free ligand with mutual exclusion.

The multiple contact effect apparently applies to all polyions binding an extensive ligand primarily by means of electrostatic attraction. In particular we refer to (poly)peptide–(poly)nucleotide interaction due to protonated aminoacid side groups and the negatively charged phosphate. A pertinent system has recently been investigated in this laboratory by means of equilibrium dialysis, namely poly-L-lysine and ATP. The data (represented in terms of Scatchard plots) clearly indicate the existence of multiple contacts and at least two different cooperative binding modes [4].

So far an appropriate and rigorous extension of the theory to more than one binding mode has not been given. It is complicated by possible interferences between the different modes. We shall especially consider mutual exclusion which means that all contacts are open to any binding mode but only one at a time (see fig. 1b). This seems to be a very likely condition in many cases encountered in practice, at any rate it should be true for the electrostatic binding mentioned above.

Previously an approximate approach was proposed assuming for each mode the same equilibrium distribution between occupied and vacant binding contacts which would be applicable if the respective mode occurs alone [1]. The data obtained for the binding of nucleotides to polylysine have been quantitatively interpreted on this basis [4]. In this article a rigorous theory will be presented. As noted already the binding modes involved are of fundamental biophysical significance, especially with regard to protein-nucleic acid interactions. Our treatment provides a general basis for adequate evaluation and interpretation of pertinent experimental data. (In this context one may envisage, for instance, the possibility of determining the binding of a ligand, not otherwise obtainable, from the measured behavior of an added appropriate indicator ligand). Application to a system of practical interest may permit considerable simplifications owing to special conditions to be considered in relation to the actual data. We feel, however, that the more universal aspects fully warrant the following largely theoretical analysis.

The macromolecular structure under consideration (usually a linear polymer) can be conceived as a linear lattice of equivalent binding contacts (which may be for instance monomer subunits or individual electric charges). A given binding mode i then corresponds to a binding site comprising n_i contacts in a row and is described by a nucleation binding constant K_i^0 (applicable to binding unaffected by cooperative interaction) as well as one or more appropriate cooperativity parameters. Accordingly any contact may exist in one of a number of different states, generally to be expressed by the symbol r. These are specified as f, standing for a vacant contact, and $a_i^{(l)}$, denoting the lth contact occupied by a bound ligand molecule a_i subject to mode i (note that $1 \leqslant l \leqslant n_i$).

The theoretical treatment can be based on the methods developed for the linear Ising model [1]. Most general is the matrix method. It even permits taking into account finite lattice lengths which introduce so-called end effects [5]. However, since these may usually be neglected in practice, two more readily applicable computational procedures will be utilized here, namely the doublet closure and the sequence generating functions method, respectively. In section 2, the formalism is outlined in a general way. An application to the more practical case of two competing modes will be given in sections 3–5.

2. Standard cooperative binding in general

2.1. Introducing basic binding probabilities

Cooperative interaction is first assumed to be restricted to immediately neighboring bound ligands. We shall call this the standard cooperative binding case.

Under such circumstances a comparatively simple relation applies with regard to the probability that n successive contacts on an infinite lattice are in the sequential state $r_1 r_2 \ldots r_n$, namely the so-called doublet closure [1]

$$\{r_1 r_2 \ldots r_n\} = \{r_1\} \cdot (r_1 r_2)(r_2 r_3) \ldots (r_{n-1} r_n) . \qquad (1)$$

By $\{\ldots\}$ we express the absolute probability of the included sequential binding state while the parentheses denote the conditional probability of the respective doublet states, for example

$$(r_1 r_2) = \{r_1 r_2\}/\{r_1\} ,$$

i.e., the probability that the contact immediately to the right of a contact known to be in state r_1 is in the state r_2.

The doublet closure provides a fairly intuitive and straight-forward basis for an analysis of the equilibrium

behavior encountered for an arbitrary number of m mutually excluding standard cooperative modes of binding.

As will be seen, any equilibrium binding property can be quantitatively expressed in terms of variables describing the frequency of the various junctures between different modes, formally including vacant ranges as mode $i = 0$. These variables are the significant conditional probabilities of doublets, namely

$$x_{ij} = (a_i^{(n_i)} a_j^{(1)}) \quad (i, j = 0, 1, 2, ..., m) , \tag{2}$$

where in particular $n_0 = 1$, $a_0^{(1)} = f$. The other conditional probabilities evidently have trivial values, 0 or 1. Since any $a_i^{(n_i)}$ must be followed by one of the $a_j^{(1)}$ we have the $m+1$ relations

$$\sum_j x_{ij} = 1 . \tag{2a}$$

Therefore only $m(m+1)$ of these variables are independent.

2.2. Relating probabilities to binding constants

The x_{ij} may be calculated from appropriate thermodynamic parameters of the system. These are conveniently defined in connection with the equilibrium constants, K_{ij}, of a number of elementary binding reactions which are generally written as

$$.. \underbrace{ff ... f}_{n_i \text{ vacant contacts}} a_j^{(1)} .. + A_i \overset{(K_{ij})}{\rightleftharpoons} .. \underbrace{f a_i^{(1)} .. a_i^{(n_i)}}_{\text{bound ligand } a_i} a_j^{(1)} .. \tag{3}$$

($i = 1, 2, .. m$; $j = 0, 1, 2, .. m$). It concerns the mode i binding of a free ligand, A_i, to the left side of a contact subject to mode j while the contact on the other side of the newly occupied site is still vacant. We note that possibly the A_i is identical for different binding modes (which applies to the case that the same ligand binds in different modes).

Nucleation reactions of the individual modes correspond to $j = 0$. They are described by the basic binding constants K_i^0 ($\equiv K_{i0}$). In addition we introduce quantities q_{ij} so that in general

$$K_{ij} = q_{ij} K_i^0 , \tag{4}$$

(implying $q_{i0} = 1$). Apparently these q_{ij} measure the extent of cooperative interaction between modes i and j ($i, j \neq 0$).

The law of mass action applied to (3) now provides conditions relating the x_{ij} to the K_{ij}. Taking advantage of the doublet closure we find

$$\frac{\{f a_i^{(1)} .. a_i^{(n_i)} a_j^{(1)}\}}{\{ff ... f a_j^{(1)}\}} = \frac{x_{0i} x_{ij}}{(x_{00})^{n_i} x_{0j}} = K_{ij} c_i \quad (i \neq 0) , \tag{5}$$

where c_i stands for the concentration of A_i. Defining basic dimensionless concentration parameters as

$$s_i = K_i c_i \quad (K_i \equiv K_{ii}) , \tag{6}$$

we first obtain by putting $i = j \neq 0$

$$x_{ii} = (x_{00})^{n_i} s_i . \tag{7a}$$

In addition, dividing any of the equations (5) for a $j \neq 0$ by the one for nucleation ($j = 0$) leads to

$$x_{00} x_{ij} = q_{ij} x_{0j} x_{i0} . \tag{7b}$$

Together with (2a) the $m + m^2$ relations (7a, b) may be used to express any of the probabilities x_{ij} in terms of the thermodynamic binding parameters s_i and q_{ij}.

We suggest a practical procedure to be developed in the following way. Summing up (7b) for a running index $k > 0$ instead of j, considering $\Sigma_k x_{ik} = 1 - x_{i0}$, and defining $\xi_i = x_{0i}$ eventually yields

$$\xi_0 = \left(\xi_0 + \sum_k q_{ik} \xi_k\right) x_{i0} = Q_i x_{i0} , \tag{8}$$

where

$$Q_i = 1 + \sum_{k>0} (q_{ik} - 1) \xi_k . \tag{8a}$$

Introducing (8) into (7b) immediately leads to

$$x_{ij} = q_{ij} \xi_j / Q_i \quad (\text{for any } i, j) , \tag{9}$$

which actually proves to be true even for zero subscripts (when the respective q-values are formally taken as unity). In order to determine the ξ-quantities we put

$$x_{00} \equiv \xi_0 = 1/\lambda = 1 - \sum_{k>0} \xi_k , \tag{10a}$$

(note that λ corresponds to the λ_0 used previously [1]) combine (7a) with (9) for $i = j \neq 0$ and finally arrive at

$$q_{ii} (\lambda^{n_i} / s_i) \xi_i = 1 + \sum_{k>0} (q_{ik} - 1) \xi_k . \tag{10b}$$

Taking λ as an independent parameter to be determined later, this is a set of m linear equations for the ξ_i so that

by means of Cramer's rule

$$\xi_i = D_i(\lambda)/D(\lambda) \quad (i > 0)\,, \tag{10c}$$

where the D-terms stand for the appropriate determinants. These depend on the s- as well as q-parameters and are polynominals in λ of the order $n = \Sigma_{i>0}\, n_i$ or less.

According to (10a) it follows

$$(1-\lambda)D(\lambda) - \lambda \sum_i D_i(\lambda) = 0\,, \tag{11}$$

which is an algebraic equation of the order $n+1$ for computing λ. Because of its physical meaning, the λ is also subject to some extra constraints, namely $\lambda \geqslant 1$ and $0 \leqslant \xi_i \leqslant 1$. It turns out that only the largest root of (11) satisfies these conditions. The determination of this λ is actually the decisive step in the calculation of equilibrium binding properties at given thermodynamic parameters. Subsequently the ξ_i can be immediately obtained by means of (10c) and then the x_{ij} according to (9). The whole procedure may readily be executed on a programmable desk calculator.

It should be emphasized that the above definition of basic thermodynamic parameters indeed determines any binding step, not only those according to (3). The binding constant for a_i to the immediate *right* of a_j is found to be

$$K'_{ij} = q_{ji} K_i^0 = K_{ji}\,, \tag{12a}$$

(implying $q_{0i} = 1$). Therefore growth of a sequence of bound ligands in mode i proceeds with the same binding constant K_{ii} no matter to which side a newly bound ligand is added. Binding of A_i to n_i vacant contacts in-between modes j' on the left and j on the right is in general subject to the binding constant

$$K_{j'ij} = q_{j'i} q_{ij} K_i^0\,. \tag{12b}$$

This includes (4) and (12a) as special cases (with $j' = 0$ or $j = 0$, respectively).

2.3. Calculating binding curves

The fraction of contacts occupied by ligands in mode i, θ_i, describes the respective degree of binding as a function of free ligand concentration(s) at given thermodynamic parameters K_i^0 and q_{ij}. We note

$$\{a_i^{(l)}\} = \theta_i/n_i\,,$$

so that the obvious relations

$$\sum_j \{a_j^{(n_j)} a_i^{(1)}\} = \{a_i^{(1)}\}\,,$$

can be transformed to

$$\sum_j x_{ji}(\theta_j/n_j) = \theta_i/n_i\,. \tag{13}$$

This provides a set of linear equations to express any θ_i/n_i in terms of the x-quantities.

We find it more appropriate to first calculate the functions

$$\phi_i = \theta_i/\theta_0\,, \tag{14a}$$

each of which represents an apparent equilibrium "constant" for occupying vacant contacts in mode i (if $i > 0$). Once the ϕ_i are determined we have immediately

$$\theta_i = \phi_i \Big/ \sum_{j=0}^{m} \phi_j \quad (\text{note } \phi_0 = 1)\,. \tag{14b}$$

It follows from (9) and (13) that

$$\phi_i = n_i \xi_i \sum_{j=0}^{m} (q_{ji}/Q_j)(\phi_j/n_j)\,. \tag{15a}$$

This set of linear equations for the ϕ-functions has a rather simple solution in the "symmetric" case of $q_{ij} = q_{ji}$, namely

$$\phi_i = n_i \xi_i Q_i \lambda\,, \tag{15b}$$

as is easily verified by insertion into the equations.

Being now solely concerned with real binding modes we take in the following all subscripts i, j, k etc. > 0. The concentration of macromolecules to which the ligands are bound may be expressed in moles of polymeric subunits per volume, c_p. With g_0 contacts on a subunit, the amount of mode i bound ligand per such subunit then evidently becomes

$$\nu_i = (c_a)_i/c_p = g_0(\theta_i/n_i)\,. \tag{16}$$

This binding ratio is the quantity usually determined in experimental work and plotted versus free ligand concentration as an appropriate binding curve. We shall here briefly discuss the general situation in the limiting cases of low and high ligand concentrations.

If all c_i are sufficiently small we certainly have ξ_i,

$s_i \to 0$ and $\xi_0 \to 1$. Then from (10b)

$$\xi_i = s_i/q_{ii} ,$$

which finally results in

$$\nu_i = g_0 K_i^0 c_i . \tag{17}$$

This must of course reasonably be expected since it corresponds to independent binding on a large excess of contacts where the probability of interaction of ligands can be neglected.

Turning now to large ligand concentrations we may only consider different binding modes of the same free ligand species (note that for different species any one of them will eventually suppress binding of the competing ones if its concentration is sufficiently increased). Accordingly all c_i are taken as being identical. At $c_i \to \infty$ we naturally have $\Sigma_k \xi_k \to 1$. Thus there must be at least one non-zero limiting value of a ξ_i. On the other hand it follows from (10b) that generally

$$\xi_i/\xi_j = [(K_i^0 Q_i)/(K_j^0 Q_j)]\, \xi_0^{n_i - n_j} .$$

Since $\xi_0 \to 0$ we can easily conclude for any two modes where $n_i < n_j$ that $\xi_j \to 0$. Owing to (15a) then also $\theta_j = \theta_0 \phi_j \to 0$. In other words, the mode(s) with the minimum n-value, $n_{\min}$ will finally saturate all binding sites while any other modes are suppressed no matter which q- and K-values they may have. Since the total binding ratio can be written as

$$\nu = g_0 \sum_i \frac{\theta_i}{n_i} = g_0 \left\{ \frac{1-\theta_0}{n_{\min}} - \sum_i \theta_i \left(\frac{1}{n_{\min}} - \frac{1}{n_i} \right) \right\} ,$$

we find at $c_i \to \infty$

$$\nu \to \nu_\infty = g_0/n_{\min} , \tag{18}$$

and at lower c_i always $\nu < \nu_\infty$.

3. Only two not specifically interacting standard cooperative modes

We may develop the quantitative treatment of the above binding model in more detail assuming there are only two modes. This makes the computational procedure less involved and will probably suffice for many relevant systems of practical interest. It seemingly applies to the binding of nucleotides on basic polypeptides [4]. In that latter case the situation is additionally simplified because of an apparent lack of specific interaction between different modes. In the above general model this condition is described by $q_{ij} = 1$ for any $i \neq j$. It will be adopted in the following.

The basic equations (10b) now reduce to

$$q_i \lambda^{n_i} \xi_i = [1 + (q_i - 1)\xi_i] s_i \quad (i = 1, 2) ,$$

(where $q_i \equiv q_{ii}$). Then we have at once

$$\xi_i = s_i/[q_i \lambda^{n_i} - (q_i - 1)s_i] . \tag{19}$$

On the basis of (15b) this directly leads to

$$\phi_i = n_i q_i s_i \lambda^{n_i + 1}/[q_i \lambda^{n_i} - (q_i - 1)s_i]^2 . \tag{20}$$

Once λ is known we can then immediately write down θ_i according to (14b) as a function of thermodynamic parameters and free ligand concentration(s). In order to determine λ we must solve the equation $1 - \xi_1 - \xi_2 = 1/\lambda$ expressed as

$$\xi(\lambda) \equiv \frac{s_1}{[q_1 \lambda^{n_1} - (q_1 - 1)s_1]} + \frac{s_2}{[q_2 \lambda^{n_2} - (q_2 - 1)s_2]} = 1 - \frac{1}{\lambda} . \tag{21}$$

Owing to their physical meaning the individual ξ_i must be positive and not greater than unity. This implies $\lambda^{n_i} > s_i$, or in other words, the appropriate solution of (21) can only be found at

$$\lambda > \lambda'_m = \max [s_1^{1/n_1}, s_2^{1/n_2}] .$$

Now we note that $\xi(\lambda) > 1$ at $\lambda = \lambda'_m$ and then decreases steadily towards 0 when $\lambda \to \infty$. On the other hand, $1 - 1/\lambda$ increases steadily towards unity under the same circumstances. Consequently there must be just one value of λ which satisfies (21) and also is subject to

$$\lambda > \lambda_m = \max [1, s_1^{1/n_1}, s_2^{1/n_2}] .$$

Since (21) is apparently equivalent to an algebraic equation of the $(n_1 + n_2 + 1)^{\text{th}}$ order there may be other roots below λ_m but these are to be disregarded. It will always be the largest root which is the meaningful one.

In practice, this λ-value can in any case easily be evaluated by means of even a small programmable calculator. Starting with $\lambda = \lambda_m$ we simply increase λ until the expression $\xi(\lambda) - 1 + \lambda^{-1}$ becomes sufficiently close to zero. Subsequently the θ_i may immediately be

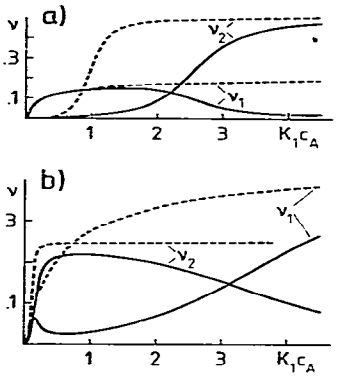

Fig. 2. Typical examples of binding curves for two mutually exclusive standard modes ($q_1 = 1$: non-cooperative, $q_2 = 100$: positively cooperative). The solid curves represent the individual ν_i ($i = 1, 2$) as plotted versus $K_1 c_A$ ($= s_1$), c_A being the free ligand concentration. Superposition of ν_1 and ν_2 yields the overall binding curve. Dashed curves indicate the course of the ν_i if binding were independent. a) Less contacts required by the cooperative mode 2 ($n_1 = 4 > n_2 = 2$, $K_2/K_1 = 1$). Note that in this case the cooperative binding mode is appreciably weakened (in comparison with independent binding) but eventually suppresses the noncooperative mode 1 (which in the independent case saturates very gradually). b) Less contacts required by the non-cooperative mode 1 ($n_1 = 2 < n_2 = 4$, $K_2/K_1 = 10$). Note that there the situation is essentially reversed. Mode 1 binding becomes weaker but completely displaces mode 2 in the limit of high ligand concentrations.

computed on the basis of (20). We have in this way calculated typical examples of binding curves. They are shown in figs. 2a, b and 5a. The procedure can quite analogously be extended to more than two not specifically interacting binding modes of the standard type.

4. Non-standard cooperative modes

In case we deal with cooperative interaction beyond nearest neighbor contacts the doublet closure does not apply. The general matrix method may still be employed but becomes even more involved. Instead we take advantage of the sequence generating functions method [1,6]. It provides a very elegant computational procedure but its results are not so readily interpreted in intuitive physical terms (such as probabilities of binding states).

Fundamentally the occurrence of contacts associated with mode i binding following mode j will be described by a respective sequence generating function of the dummy variable x, namely

$$U_{ji}(x) = u_{ji}^{(1)} x^{-1} + u_{ji}^{(2)} x^{-2} + \ldots \quad (j \neq i) .$$

Here $u_{ji}^{(k)}$ stands for the statistical weight of a sequence of just k contacts in mode i which starts after a mode j contact (mode 0 formally included). With these functions a matrix $\mathbf{U}(x) = (U_{ij})$ is formed ($U_{ii} = 0$) and then the secular equation

$$|\mathbf{U}(x) - \mathbf{I}| = 0 , \tag{22}$$

solved for the variable x ($\mathbf{I}$ being the identity matrix). Only the largest root, λ, is significant. From it, one obtains

$$\theta_i = n_i \, \partial \ln \lambda / \partial \ln s_i \quad (i > 0) , \tag{23}$$

provided any binding constant applicable to mode i is defined as a specific factor times a certain K_i (which may be chosen arbitrarily) and $s_i = K_i c_i$ [1].

We shall apply this method to those modes which are apparently involved in the binding of ATP to poly(L-lysine) [4]. The first one has been called dimeric because it consists of a nucleation step described by a binding constant K_1^0 and only one cooperative growth step with a binding constant $K_1 = q_1 K_1^0$. Contiguous to the so formed bound dimer another nucleation step may occur, then again a cooperative step and so on. This implies interaction up to $n_1 + 1$ nearest neighbor contacts. The second mode is of the standard type introduced before. Specific interaction between the different modes can again be neglected. The statistical weight of any vacant contact may be arbitrarily set equal to unity so that

$$U_{10} = U_{20} = x^{-1} + x^{-2} + x^{-3} + \ldots = 1/(x - 1) ,$$

furthermore

$$U_{01} = U_{21} = \frac{s_1}{q_1} x^{-n_1} + \frac{s_1^2}{q_1} x^{-2n_1} + \frac{s_1^3}{q_1^2} x^{-3n_1} + \ldots$$

$$= s_1 (x^{n_1} + s_1)/(q_1 x^{2n_1} - s_1^2) ,$$

$$U_{02} = U_{12} = \frac{s_2}{q_2} x^{-n_2} + \frac{s_2^2}{q_2} x^{-2n_2} + \frac{s_2^3}{q_2} x^{-3n_2} + \ldots$$

$$= s_2/q_2 (x^{n_2} - s_2) .$$

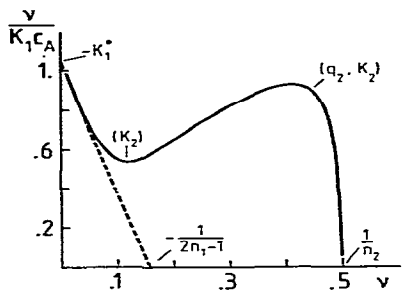

Fig. 3. Dimensionless Scatchard plot ($K_1^{-1}\,\nu/c_A$ versus ν) with parameters as in fig. 2a but with $K_2/K_1 = 4$. It is indicated how the characteristic parameters can be estimated from a practical Scatchard plot (i.e. ν/c_A versus ν): $K_1^0 = K_1$ from the ordinate intercept, n_1 and n_2 from the extrapolated intercepts on the ν-axis, K_2 and q_2 from the positions of the minimum and maximum, respectively (see text).

Insertion in (22) leads to the relation

$$1 - 1/\lambda = \xi_1 + \xi_2 ,$$

where now

$$\xi_1 = s_1(\lambda^{n_1} + s_1)/\lambda^{n_1}(q_1\lambda^{n_1} + s_1) , \tag{24}$$

while ξ_2 remains as in (19). Obviously this relation is perfectly analogous to the equation (21). We conclude here that λ must be the only existing root subject to

$$\lambda > \max\,[1, (s_1/\sqrt{q_1})^{1/n_1}, s_2^{1/n_2}] . \tag{25}$$

Accordingly λ can also be calculated by means of the previously indicated computer program after having it adjusted to the present ξ- and ϕ-functions.

Because of (23) we find that

$$\theta_i = n_i s_i \lambda\,[\partial\xi_1/\partial s_i + \partial\xi_2/\partial s_i] ,$$

which leads to

$$\phi_1 = \frac{n_1 s_1\,[q_1(\lambda^{n_1} + s_1)^2 - (q_1 - 1)s_1^2]}{\lambda^{n_1 - 1}(q_1\lambda^{n_1} + s_1)^2} , \tag{26}$$

and the expression ϕ_2, respectively, which was already given for standard modes in (20).

We note that the general computational procedure to calculate binding curves is actually the same as before. Only the way to derive the specific ξ- and ϕ-functions associated with individual binding modes had to be chosen differently.

5. Scatchard plots and the analysis of experimental data

In practical binding studies with one free ligand species A (its concentration being c_A) the measured results are frequently presented as a Scatchard plot, i.e. ν/c_A versus ν. In the framework of our model (assuming different binding modes of a single ligand so that all $c_i \equiv c_A$) a theoretical fit will require the calculation of

$$\nu/c_A = g_0 \sum_i K_i \frac{\theta_i/n_i}{s_i} , \tag{27}$$

for any ν and appropriate choice of the parameters n_i, q_i, K_i for the individual modes.

The extrapolated intercept on the ordinate ($\nu \to 0$) is generally found as

$$I_0 = g_0 \sum_i K_i^0 , \tag{28}$$

while the initial slope becomes

$$S_0 = \frac{1}{I_0} \sum_i \left[2(q_i - 1)(K_i^0)^2 - (2n_i - 1)K_i^0 \sum_j K_j^0 \right] . \tag{29}$$

Let us consider a simple example comprising only two standard modes, one of them having no cooperative interaction, as presented in fig. 2a. This apparently applies to the case of mononucleotide binding to basic polypeptides [7]. We may vary the individual parameters and examine the appearance of the Scatchard plot. A graphic illustration is given in fig. 3. We find that a clear manifestation of both modes can only be expected for a rather narrow range of K_2/K_1 somewhat above unity. There we have a first part with a negative slope indicating the non-cooperative mode followed by a pronounced hump which reflects the cooperative mode. At lower values of K_2/K_1 the plot is largely determined by the non-cooperative mode, the cooperative one will eventually contribute at very high c_A but the corresponding ν/c_A becomes comparatively small and may be overlooked in practice. On the other hand, the first mode becomes suppressed if $K_2 \gg K_1$ so that solely the hump can be seen.

When both modes can be clearly recognized in the plot all five parameters (i.e. n_1, K_1, n_2, q_2, K_2) may be evaluated from it rather independently. We have $K_1^0 = K_1 \approx I_0/g_0$ and then obtain n_1 from the initial slope $S_0 \approx -(2n_1 - 1)K_1^0$. The final intercept on the ν-axis yields g_0/n_2. The onset of the cooperative mode

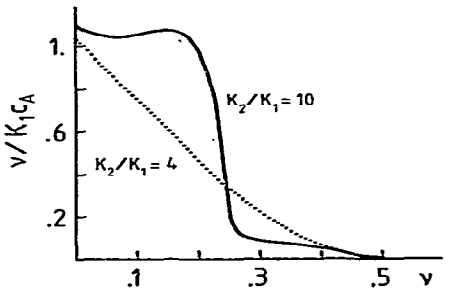

Fig. 4. Typical appearance of Scatchard plots for parameters as in fig. 2b ($n_1 < n_2$). Both modes can only be distinguished from each other around $K_2/K_1 = 10$ (solid curve). Dotted curve applies to $K_2/K_1 = 4$.

occurs at $c_A \approx 1/K_2$ which is slightly to the right of the minimum of the plot (there we have $\nu/c_A/\nu \approx K_2$). The position of the hump primarily depends on q_2 and K_2. Rough values of the parameters estimated in this way have to be refined by means of pertinent curve fitting. We have applied the method to the GMP-polylysine data [7] already discussed in connection with our previous approximation approach [4]. These rigorous results are compiled in the first row of the table together with the earlier ones where they differ. Since the first mode occurs alone at lower ν there will be only some changes of the parameters describing the cooperative mode.

It should be emphasized that a somewhat different picture will be observed if $n_1 < n_2$. Then the non-cooperative mode must at any rate eventually suppress the cooperative one at high c_A. As depicted in fig. 4 this leads to a characteristic "tail" following the hump.

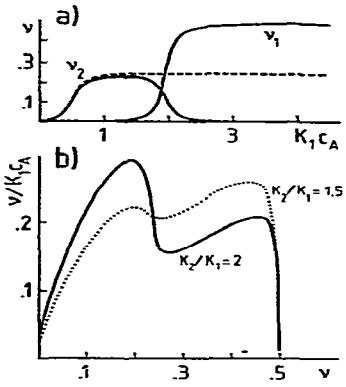

Fig. 5. Typical binding behavior of two mutually exclusive standard modes of pronounced positive cooperativity ($q_1 = q_2 = 100$) with $n_1 = 2 < n_2 = 4$. a) Plots of ν_1, ν_2 versus $K_1 c_A$ for $K_2/K_1 = 2$. Note that the somewhat more strongly binding mode 2 appears at lower c_A (dashed curve indicates its course if it binds independently) but is displaced by the weaker one at higher c_A because the latter needs less contacts for a binding site. b) Scatchard plots for $K_2/K_1 = 2$ (solid curve) and $K_2/K_1 = 1.5$ (dotted curve).

However, at K_2/K_1 appreciably above 10 indications of the non-cooperative mode would be difficult to recognize in practice.

Fig. 5 illustrates the case of two standard modes which are both of considerable positive cooperativity ($q_1 = q_2 = 100$). They are manifested in the Scatchard plot by two respective humps but again a narrow range of K_2/K_1 is required. Outside of it the more weakly binding one practically disappears.

Finally we come to a rigorous reevaluation of our

Table 1
Parameters for the binding of GMP and ATP to polylysine. The data of refs. [4] and [7] were evaluated according to our theory and compared with the approximate analysis given in ref. [4]. The parameter values of ref. [4] are given in parentheses where they differ from the results of the rigorous theory. A full graphical representation of the theoretical curves fitted to the data is dispensed with because this is essentially the same as given before.

Binding experiment	K_1 [mM^{-1}]	q_1	n_1	K_2 [mM^{-1}]	q_2	n_2	fig. in ref. [4]
GMP/polylysine [7] pH 7.0	1.65	1	1.9	4.7(2.7)	500(245)	1.61(1.60)	5
ATP/polylysine [4] pH 7.0	145	38	3.8	51.5 (32)	200(140)	2.9	2a
ATP/polylysine [4] pH 5.3	44	100(88)	7.14	7(5.8)	20(33)	3.2(3.05)	2b

recent data of the ATP—polylysine system [4]. The newly obtained parameters for modes 1 (dimeric) and 2 (standard cooperative), respectively, are also presented in the table. Again essentially some changes with regard to the second mode are observed. This concerns those parameters which can only be evaluated from that part of the plot where both modes contribute to it.

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