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A perspective into ligand–receptor affinities using complex numbers

(stoichiometric binding constant/ghost binding constant/binding equations)

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ABSTRACT **The binding of ligands by a receptor can be expressed in an equation derived by a thermodynamic stoichiometric approach in which the stoichiometric equilibrium constants are all positive and real. An alternative binding equation can be derived algebraically in which the equilibrium constants can have values that are complex numbers. Some consequences of this situation are explored, and numerical values of the ghost equilibrium constants are evaluated for a number of ligand–receptor complexes.**

To obtain numerical values for affinities of receptors for ligands from equilibrium binding experiments, one can follow two different paths of formulation. The classical thermodynamic approach defines appropriate equilibrium constants in terms only of the stoichiometry (and concentrations) of the species participating in the multiple equilibria. In consequence, equations for extent of binding of ligand contain a limited number of equilibrium constants, regardless of the extent of uptake of ligand by receptor. Alternatively, one can introduce explicitly the assumption of specific binding sites on the receptor and define binding constants for each site. In this formulation, however, the binding constant for a specific site may change markedly as the extent of occupancy of other sites on the receptor varies. Hence, it becomes necessary to denominate a large array of such constants or to introduce variable interaction coefficients. So the total number of such parameters increases dramatically for multiple ligand binding, unless one assumes a specific model to constrain the interactions between sites.

Equations for Amounts of Bound Ligand

In the thermodynamic approach, the moles of ligand L bound per mole of receptor R can be related to the concentration of free ligand (1) by the equation

$$B = \frac{K_1(L) + 2K_1K_2(L)^2 + \cdots + j(K_1K_2\cdots K_j)(L)^j + \cdots}{1 + K_1(L) + K_1K_2(L)^2 + \cdots + (K_1K_2\cdots K_j)(L)^j + \cdots}, \quad [1]$$

where K_j (which is always real and positive) represents the stoichiometric equilibrium constant for the successive stoichiometric step j,

$$RL_{j-1} + L = RL_j; \qquad K_j = \frac{(RL_j)}{(RL_{j-1})(L)}. \quad [2]$$

An alternative algebraic expression for B, derived from the fundamental theorem of algebra, is

$$B = \frac{K_\alpha(L)}{1 + K_\alpha(L)} + \frac{K_\beta(L)}{1 + K_\beta(L)} + \cdots + \frac{K_\omega(L)}{1 + K_\omega(L)} + \cdots. \quad [3]$$

The coefficients K_ω have the dimensions of equilibrium constants and can be combined to yield functions of the stoichiometric equilibrium constants $K_1, K_2, \ldots K_j$. However, they do not correspond to any particular step in the successive stoichiometric equilibria of Eq. 2 or to the binding of ligand by any specific actual site on the receptor. They may be thought of as equilibrium constants for "ghost" sites (1), hypothetical sites with equilibrium constants K_ω that in Eq. 3 faithfully reproduce the experimentally observed dependence of B on (L).

Complex Numbers in Ghost Equilibrium Constants

When the uptake of one mole of ligand enhances the affinity of the receptor for the second mole—that is, when $K_2 > K_1/4$, in which case the binding is generally called "cooperative," the ghost site constants are complex numbers, appearing in conjugate pairs. To illustrate, let us consider a bivalent receptor, which becomes saturated when two moles of ligand are bound. Then

$$K_\alpha = a + bi; \qquad K_\beta = a - bi, \quad [4]$$

where $i = \sqrt{-1}$ and the coefficients a and b are real numbers. For example, for the binding of Ca^{2+} by calbindin (2) (Table 1),

$$K_\alpha = 1.10 \times 10^8 + 2.63 \times 10^8 i;$$

$$K_\beta = 1.10 \times 10^8 - 2.63 \times 10^8 i. \quad [5]$$

Alternatively, in exponential form,

$$K_\alpha = Ae^{i\theta}; \qquad K_\beta = Ae^{-i\theta}, \quad [6]$$

where $A = \sqrt{(a^2 + b^2)}$ and $\theta = \arctan(b/a)$. For Ca^{2+}/calbindin,

$$K_\alpha = 2.9 \times 10^8 e^{0.38\pi i}; \qquad K_\beta = 2.9 \times 10^8 e^{-0.38\pi i}.$$

Some Relationships in a Bivalent System

For a bivalent ligand–receptor system, the maximum value of $\tan(b/a)$ is infinity. In that case $\arctan(b/a)$, that is, θ, is $\pi/2$, and Eq. 3 for moles of ligand bound by a divalent receptor becomes

$$B = \frac{Ae^{i\frac{\pi}{2}}(L)}{1 + Ae^{i\frac{\pi}{2}}(L)} + \frac{Ae^{-i\frac{\pi}{2}}(L)}{1 + Ae^{-i\frac{\pi}{2}}(L)}. \quad [7]$$

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Table 1. Characterizing constants for ligand–receptor complexes

Ligand	Receptor (ref.)	Stoichiometric binding constant	Ghost binding constant*	Real/imaginary index, cos θ
Ca^{2+}	Calbindin (2)			
	Wild type	$K_1 = 2.2 \times 10^8$	$K_\alpha = 2.9 \times 10^8\, e^{0.38\pi i}$	0.37
		$K_2 = 3.7 \times 10^8$	$K_\beta = 2.9 \times 10^8\, e^{-0.38\pi i}$	
	Mutant M2	$K_1 = 1.7 \times 10^8$	$K_\alpha = 1.7 \times 10^8$	
		$K_2 = 5 \times 10^5$	$K_\beta = 1 \times 10^6$	
Leucine	Isopropylmalate synthase (1)†	$K_1 = 0.48 \times 10^5$	$K_\alpha = 1.13 \times 10^5\, e^{0.43\pi i}$	0.22
		$K_2 = 2.5 \times 10^5$	$K_\beta = 1.13 \times 10^5\, e^{-0.43\pi i}$	
Fe^{3+}	Ovotransferrin (1)†	$K_1 = 23$	$K_\alpha = 22$	
		$K_2 = 0.57$	$K_\beta = 0.6$	
Anions	Transferrin (3)			
Bicarbonate		$K_1 = 4.6 \times 10^2$	$K_\alpha = 3.9 \times 10^2$	
		$K_2 = 6 \times 10^1$	$K_\beta = 7 \times 10^1$	
Phosphate		$K_1 = 1.5 \times 10^4$	$K_\alpha = 1.2 \times 10^4$	
		$K_2 = 2 \times 10^3$	$K_\beta = 2.4 \times 10^3$	
Vanadate		$K_1 = 2.8 \times 10^7$	$K_\alpha = 2.3 \times 10^4$	
		$K_2 = 4 \times 10^6$	$K_\beta = 5 \times 10^6$	
Trifluorodihydroxy-propyl phosphonate	Aspartate transcarbamoylase (trimer) (1)†	$K_1 = 2.89 \times 10^4$	$K_\alpha = 2.89 \times 10^4$	
		$K_2 = 0.0295 \times 10^4$	$K_\beta = 5.1 \times 10^2\, e^{0.41\pi i}$	0.28
		$K_3 = 0.0875 \times 10^4$	$K_\gamma = 5.1 \times 10^2\, e^{-0.41\pi i}$	
Acetylcoenzyme A	Pyruvate carboxylase (1)†	$K_1 = 2.08 \times 10^5$	$K_\alpha = 2.17 \times 10^5$	
		$K_2 = 1.64 \times 10^4$	$K_\beta = 7.6 \times 10^4\, e^{0.45\pi i}$	0.16
		$K_3 = 3.67 \times 10^5$	$K_\gamma = 7.6 \times 10^4\, e^{-0.45\pi i}$	
		$K_4 = 1.30 \times 10^4$	$K_\delta = 1.31 \times 10^4$	
O_2	Sheep hemoglobin (4)	$K_1 = 0.1124$	$K_\alpha = 0.30\, e^{0.24\pi i}$	0.73
		$K_2 = 0.1974$	$K_\beta = 0.30\, e^{-0.24\pi i}$	
		$K_3 = 0.1475$	$K_\gamma = 0.17\, e^{0.70\pi i}$	−0.59
		$K_4 = 1.996$	$K_\delta = 0.17\, e^{-0.70\pi i}$	
	Human hemoglobin, cross-linked (5)	$K_1 = 0.3497$	$K_\alpha = 0.49\, e^{0.20\pi i}$	0.81
		$K_2 = 0.1409$	$K_\beta = 0.49\, e^{-0.20\pi i}$	
		$K_3 = 0.2686$	$K_\gamma = 0.39\, e^{0.69\pi i}$	−0.57
		$K_4 = 2.802$	$K_\delta = 0.39\, e^{-0.69\pi i}$	

*For the divalent receptors these constants were evaluated analytically from the stoichiometric binding constants. For the multivalent receptors the ghost binding constants were evaluated by J. Nocedal (Northwestern University) by numerical analysis. The computations were done with the MATLAB software package (MATLAB Reference Guide, The Math Works, 1992).
†Original sources of data are cited in ref. 1.

Recalling the relation

$$e^{ix} + e^{-ix} = 2 \cos x, \qquad [8]$$

we can reduce Eq. 7 to

$$B = \frac{2A^2(L)^2}{1 + A^2(L)^2}. \qquad [9]$$

From this it can be shown that

$$\log \frac{B}{2 - B} = \log A^2 + 2 \log(L). \qquad [10]$$

This is the classical Hill equation for maximally cooperative binding of a ligand L by a divalent receptor.

For any value of θ, substitution of exponential expressions for K_α and K_β in Eq. 3 for a divalent receptor gives

$$B = \frac{Ae^{i\theta}(L)}{1 + Ae^{i\theta}(L)} + \frac{Ae^{-i\theta}(L)}{1 + Ae^{-i\theta}(L)}. \qquad [11]$$

Again, recalling the relation of Eq. 8, we can convert Eq. 11 into

$$B = \frac{A(2 \cos \theta)(L) + 2A^2(L)^2}{1 + A(2 \cos \theta)(L) + A^2(L)^2}. \qquad [12]$$

Comparison of the respective polynomials in the denominators of Eqs. 1 and 12 leads to the relations

$$K_1 = 2A \cos \theta \qquad [13]$$

$$K_1K_2 = A^2. \qquad [14]$$

As has been shown previously (3),

$$K_1 = K_\alpha + K_\beta \qquad [15]$$

$$K_1K_2 = K_\alpha K_\beta. \qquad [16]$$

Inserting the exponential notation for the complex numbers K_α and K_β, one obtains

$$K_1 = 2A \cos \theta, \qquad [17]$$

$$K_2 = \frac{A}{2 \cos \theta}, \qquad [18]$$

and

$$\frac{K_1}{K_2} = 4 \cos^2 \theta. \qquad [19]$$

In the limit of $\theta = 0$—that is, when the imaginary part of the complex number vanishes,

$$\frac{K_1}{K_2} = 4, \qquad [20]$$

as has been demonstrated repeatedly by statistical arguments (6). At the other limit where $\tan(b/a)$ reaches a maximum, and $\theta = \pi/2$, one finds that

$$\frac{K_1}{K_2} = 0. \quad [21]$$

From Eq. 6 it also follows that

$$\frac{K_\alpha}{K_\beta} = e^{2i\theta}. \quad [22]$$

At the limiting value of $\theta = 0$

$$K_\alpha = K_\beta, \quad [23]$$

and as $\theta = \pi/2$, $e^{2i(\pi/2)}$ becomes -1, so

$$K_\alpha = -K_\beta. \quad [24]$$

Under the latter circumstances, because $b \gg a$ (Eq. 4), K_α and K_β became purely imaginary numbers,

$$K_\alpha = be^{i\frac{\pi}{2}}; \qquad K_\beta = be^{-i\frac{\pi}{2}} \quad [25]$$

and

$$K_1 = K_\alpha + K_\beta \rightarrow 0, \quad [26]$$

even though neither term, K_α or K_β, is zero. Because

$$K_1K_2 = K_\alpha K_\beta = b^2, \quad [27]$$

it also follows that

$$\lim_{K_1=0} K_2 = \infty, \quad [28]$$

in agreement with Eq. 21.

Multivalent Ligand–Receptor Complexes

Turning to a trivalent system, in which saturation of receptor is attained with three bound ligands, we recognize that Eq. 3 will have three terms. If the binding is cooperative—that is, if the ratio of the successive stoichiometric equilibrium constants K_2/K_1 or K_3/K_2 is greater than the statistical values (6), then one pair of the constants K_α, K_β, and K_γ will be a complex conjugate. Thus, we can convert Eq. 3 to the form

$$B = \frac{A(2\cos\theta)L + 2A^2(L)^2}{1 + A(2\cos\theta)L + A^2(L)^2} + \frac{K_\gamma(L)}{1 + K_\gamma(L)}. \quad [29]$$

For a tetravalent ligand–receptor complex with cooperative binding, there can be one or two pairs of complex conjugate values of the ghost constants K_ω. For the latter case, the equation for B becomes

$$B = \frac{A_1e^{i\theta_1}(L)}{1 + A_1e^{i\theta_1}(L)} + \frac{A_1e^{-i\theta_1}(L)}{1 + A_1e^{-i\theta_1}(L)} + \frac{A_2e^{i\theta_2}(L)}{1 + A_2e^{i\theta_2}(L)} + \frac{A_2e^{-i\theta_2}(L)}{1 + A_2e^{-i\theta_2}(L)}. \quad [30]$$

For each conjugate pair we can substitute an expression of the form of Eq. 12. Because there are two pairs of conjugate terms and the moles of bound ligand at saturation n is 4, Eq. 30 becomes

$$B = \sum_{\ell=1}^{2\left(=\frac{n}{2}\right)} \frac{A_\ell(2\cos\theta_\ell)(L) + 2A_\ell^2(L)^2}{1 + A_\ell(2\cos\theta_\ell)(L) + A_\ell^2(L)^2}. \quad [31]$$

If in any specific system only one pair of complex roots appears, then

$$B = \frac{A(2\cos\theta)(L) + 2A^2(L)^2}{1 + A(2\cos\theta)L + A^2(L)^2} + \sum_{\omega_1}^{\omega_2} \frac{K_\omega(L)}{1 + K_\omega(L)}. \quad [32]$$

It is evident that for the general case where saturation of receptor is reached with n moles of bound ligand

$$B = \sum_{\ell=1}^{s\le n/2} \frac{A_\ell(2\cos\theta_\ell)(L) + 2A_\ell^2(L)^2}{1 + A_\ell(2\cos\theta_\ell)(L) + A_\ell^2(L)^2} + \sum_{\omega_1}^{\omega_{n-2s}} \frac{K_\omega(L)}{1 + K_\omega(L)}. \quad [33]$$

Numerical Values for Ligand–Receptor Complexes

A few specific numerical illustrations, for trivalent and tetravalent ligand–receptor systems (Table 1) manifesting enhancing interactions, may be enlightening.

The trimer form of aspartate transcarbamoylase binds trifluorodihydroxypropyl phosphate with stoichiometric binding constants K_1, K_2, K_3 of 2.89×10^4, 0.0295×10^4, and 0.0875×10^4, respectively (Table 1). Clearly the affinity for ligand is enhanced after the second stoichiometric step, but it is not enhanced after the first. Thus, K_α is a real number (Table 1), but K_β and K_γ form a conjugate pair.

For the tetravalent system acetylcoenzyme A-pyruvate carboxylase (Table 1), enhancement in binding is evident from the large increase in K_3 compared with K_2 (Table 1), but it is not evident in the other stoichiometric steps. Thus, for this binding, K_α and K_δ are real numbers, but K_β and K_γ constitute a conjugate pair.

In contrast, tetravalent oxygen–hemoglobin binding (4, 5) manifests enhancing interactions at each of the successive steps (Table 1). All the ghost constants are complex numbers, forming two conjugate pairs.

The coefficient A in the ghost binding constants (Table 1) reflects the modulus, the geometric sum, of the coefficients of the real and imaginary parts of the complex values of K_ω. It is also of interest to have a measure of the *relative* contribution of the real and imaginary components of the ghost constants. One such gauge would be $\cos\theta$ because it expresses the argument of the complex value K_ω on a scale that varies from 1 to zero as the enhancement of K_j over K_{j-1} increases. This real/imaginary index, $\cos\theta$, is also listed in Table 1 for each ligand–receptor system manifesting enhancing interactions in binding.

Free Energy Changes

Corresponding to any equilibrium constant, there must be a (standard) free energy change. Thus, we may write

$$\Delta G^\circ_\omega = -RT\ln K_\omega. \quad [34]$$

For a bivalent ligand–receptor system, Eq. 6 applies, and we obtain

$$\Delta G^\circ_\alpha = -RT\ln A - RTi\theta; \qquad \Delta G^\circ_\beta = -RT\ln A + RTi\theta. \quad [35]$$

For example, for the leucine/isopropylmalate combination (Table 1), if R is in calories and T is 298 K,

$$\Delta G^\circ_\alpha = -6900 - 807i; \qquad \Delta G^\circ_\beta = -6900 + 807i. \quad [36]$$

In Eq. 4, the real number coefficients a and b can have any numerical value. Hence, in Eq. 6, the modulus A is not constrained in value. However, the maximum value of b/a is infinity, and the minimum value is zero. Thus θ spans the range between $\pi/2$ and zero. Consequently, in Eq. 35, the value of $RTi\theta$ (at 298 K) ranges from $930i$ (in calories) to zero.

Conclusion

In view of Eq. 3, it is clear that the binding of ligand by receptor can be expressed as a linear combination of terms with parameters that may be complex numbers. Although each of the terms contributing to B has a format that is similar to that for a specific binding site, it is clear from the presence of complex numbers, associated with enhancing interactions between successive K_j values, that the individual terms cannot be assigned to real sites. For other reasons, the same conclusion can be reached when there are damping interactions between K_j values (1). If one wishes to use the term binding site for any single term in Eq. 3, it is perhaps most descriptive to call it a ghost site, one that is a representation of all the complex interactions between ligand and receptor. It is not possible to visualize such a ghost site in any structural sense. It is the linear sum of their interactions in a multivalent ligand–receptor complex that is manifested in the observed value of B, a real number.

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