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A NEW MEASURE OF COOPERATIVITY IN PROTEIN-LIGAND BINDING

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An allosteric binding system consisting of a single ligand and a nondissociating macromolecule having multiple binding sites can be represented by a binding polynomial. Various properties of the binding process can be obtained by analyzing the coefficients of the binding polynomial and such functions as the binding curve and the Hill plot. The Hill plot has an asymptote of unit slope at each end and the departure of the slope from unity at any point can be used to measure the effective interaction free energy at that point. Of particular interest in detecting and measuring cooperativity are extrema of the Hill slope and its value at the half-saturation point. If the binding polynomial is symmetric, then there is an extremum of the Hill slope at the half-saturation point. This value, the Hill coefficient, is a convenient measure of cooperativity. The purpose of this paper is to express the Hill coefficient for symmetric binding polynomials in terms of the roots of the polynomial and to give an interpretation of cooperativity in terms of the geometric pattern of the roots in the complex plane. This interpretation is then applied to the binding polynomials for the MWC (Monod-Wyman-Changeux) and KNF (Koshland-Nemethy-Filmer) models.

1. Introduction

If a nondissociating macromolecule has n binding sites and a_x represents ligand activity, then the binding polynomial can be written as

$$N = 1 + \beta_1 a_x + \beta_2 a_x^2 + \ldots + \beta_n a_x^n \quad (1)$$

where the β_i are overall equilibrium constants. The saturation function $y = a_x N'/nN$ describes the fraction of the total number of binding sites which are occupied and this increases from zero to unity as a_x increases from zero to infinity. The Hill plot is the graph of $\log y/(1-y)$ vs. $\log a_x$ and the Hill slope is the slope of the graph at any point. The Hill slope is asymptotic to unity for both large and small values of a_x [2] and positive and negative cooperativity can be defined as Hill slope greater than or less than unity respectively.

For comparative purposes it will be convenient to consider normalized binding polynomials of the form

$$N(x) = 1 + \alpha_1 x + \alpha_2 x^2 + \ldots + \alpha_{n-1} x^{n-1} + x^n \quad (2)$$

which can be obtained from eq. 1 by the substitution $x = \beta_n^{1/n} a_x$.

2. Properties of polynomials

A few useful properties of the roots of polynomials with real coefficients are stated here without proof.

(i) If $a + bi$ is a root, then so is its complex conjugate $a - bi$.

(ii) The total number of roots of a polynomial of degree n is n and the number of nonreal roots is even.

(iii) If the coefficients of a polynomial are all nonnegative as in eq. 2, then any real root is negative.

(iv) If $N(x)$ is a symmetric polynomial so that $\alpha_i = \alpha_{n-i}$ for all i and if the complex number z is a root, then $1/z$ is a root.

(v) If the roots of $N(x)$ are $z_1, \ldots, z_n$, then $N(x) = (x - z_1)(x - z_2)\ldots(x - z_n)$. The product of the roots is $(-1)^n$ and their sum is $-\alpha_{n-1}$

(vi) If the coefficients of a polynomial of degree n are all nonnegative, then no complex number written in the form $r(\cos\theta + i\sin\theta)$ with $-\pi < \theta \le \pi$ can be a root if $|\theta| < \pi/n$ radians.

(vii) If $N(x)$ is a symmetric polynomial, then both half-saturation and an extremum of the Hill slope, called the Hill coefficient, occur at $x = 1$.

3. The Hill coefficient for symmetric polynomials

For binding polynomials of degree n, the maximum possible Hill coefficient is n which occurs for $N(x) = 1 + x^n$. The roots of this polynomial are the n-th roots of -1 which are distributed uniformly around the unit circle in the plane of complex numbers beginning with an angle of π/n radians. The statistically neutral case of n identical but independent binding sites is represented by $N(x) = (1 + x)^n$ which has an n-fold root of -1 and a Hill coefficient of unity. If $N(x)$ has n real roots which are not all equal, then the Hill coefficient is less than unity, indicating negative cooperativity. These cases suggest a description of cooperativity in terms of the geometric pattern in the complex plane of the roots of the binding polynomial. This relationship is now examined in more detail.

For $n = 2$, $N(x) = 1 + \alpha x + x^2$ where $\alpha \ge 0$. The roots are real if $\alpha \ge 2$ and nonreal if $\alpha < 2$. Using property iv above, the roots can be written as $-r$, $-1/r$ with r positive if they are real or as $\cos\theta \pm i\sin\theta$ if they are nonreal. The Hill coefficient is $4/(\alpha + 2)$ which becomes

$$\frac{1}{2}\left[\frac{8r}{(r+1)^2}\right] \text{ if the roots are real and}$$

$$\frac{1}{2}\left[\frac{4}{1-\cos\theta}\right] \text{ if the roots are nonreal.} \tag{3}$$

For $n = 3$, the general symmetric polynomial is $N(x) = 1 + \alpha x + \alpha x^2 + x^3$ which always has a root of -1. Again the other two roots can be written as $-r$, $-1/r$ or as $\cos\theta \pm i\sin\theta$. The Hill coefficient is $(\alpha + 9)/3(\alpha + 1)$ which becomes

$$\frac{1}{3}\left[1 + \frac{8r}{(r+1)^2}\right] \text{ if the roots are real and}$$

$$\frac{1}{3}\left[1 + \frac{4}{1-\cos\theta}\right] \text{ if there are nonreal roots.} \tag{4}$$

For $n = 4$, the general symmetric polynomial is $N(x) = 1 + \alpha x + \beta x^2 + \alpha x^3 + x^4$ which can have 0, 2 or 4 nonreal roots. If there are two real and two nonreal roots, then the real roots are reciprocals of each other and the nonreal roots are both reciprocals and conjugates of each other and so must lie on the unit circle. If there are four nonreal roots, then they may consist of two conjugate pairs on the unit circle or two pairs with angles θ and $-\theta$ on circles of radii r and $1/r$. The roots therefore can be written as one of the following forms:

$$-r_1, -\frac{1}{r_1}, -r_2, -\frac{1}{r_2}$$

$$-r, -\frac{1}{r}, \cos\theta \pm i\sin\theta$$

$$\cos\theta_1 \pm i\sin\theta_1, \cos\theta_2 \pm i\sin\theta_2$$

$$r(\cos\theta \pm i\sin\theta), \frac{1}{r}(\cos\theta \pm i\sin\theta).$$

In any case the Hill coefficient is $(2\alpha + 8)/(2\alpha + \beta + 2)$ which becomes, respectively,

$$\frac{1}{4}\left[\frac{8r_1}{(r_1+1)^2} + \frac{8r_2}{(r_2+1)^2}\right]$$

$$\frac{1}{4}\left[\frac{8r}{(r+1)^2} + \frac{4}{1-\cos\theta}\right]$$

$$\frac{1}{4}\left[\frac{4}{1-\cos\theta_1} + \frac{4}{1-\cos\theta_2}\right]$$

$$\frac{1}{4}\left\{\frac{16r[2r-(r^2+1)\cos\theta]}{(r^2+1-2r\cos\theta)^2}\right\}. \tag{5}$$

Several observations are now in order. The rational functions of r which appear in exs. 3–5 of $|\theta|$ according to property vi of section 2.

For $n = 3$, if $N(x)$ in ex. 2 is symmetric and has a pair of nonreal roots, then they lie on the unit circle. Positive cooperativity occurs, since $H > 1$ from ex. 4 and H increases to a maximum of 3 as $|\theta|$ decreases to its minimum value of $\pi/3$.

For $n = 4$, if $N(x)$ in eq. 2 is symmetric and has a pair of real roots and a pair of nonreal roots, then $N(x)$ can be factored into two linear factors and a quadratic factor. This, however, does not always imply positive cooperativity. In particular, from ex. 5 if $\cos\theta < -2r/(r^2+1)$, then $H < 1$ which indicates negative cooperativity. It is interesting to note that this boundary value of $\cos\theta$ when $H = 1$ is the harmonic mean of the two real roots. Positive cooperativity occurs if $\cos\theta$ is greater than this value and increases as $|\theta|$ decreases. The smallest value of $|\theta|$ which is possible with a pair of real roots is the angle whose cosine is $r/(r^2+1)$ and for this angle the maximum value of H is 5/2 when $r = 1$. If $N(x)$ has two pairs of nonreal roots on the unit circle, then from ex. 5 H is always greater than unity, indicating positive cooperativity. The maximum possible value of H is 4 which occurs when $\theta_1 = \pi/4$ and $\theta_2 = 3\pi/4$ and $N(x) = 1 + x^4$. If $N(x)$ has two pairs of nonreal roots not necessarily on the unit circle lying at angles θ and $-\theta$, then $\pi/2 < \theta < \pi$ and $N(x)$ can be factored into two quadratic factors with positive coefficients. Positive cooperativity does not always occur, since for any θ in this interval, H become less than unity for r sufficiently large. The maximum value of H in this case is 2 for $\theta = \pi/2$ and $r = 1$ when $N(x) = (1 + x^2)^2$.

The general principle which results from this analysis is that cooperativity will be greater the closer the absolute values of the roots are to unity for all roots and the smaller the value of $|\theta|$ for nonreal roots. The pattern of roots is always subject to the restriction that $N(x)$ have nonnegative coefficients and the limiting pattern for maximum cooperativity for a given n is the symmetric distribution of the n-th roots of -1 around the unit circle.

5. Application to MWC and KNF models

The binding polynomial for the MWC model can be written as

$$P(x) = \frac{1}{L+1}\left[(1+x)^n + L(1+cx)^n\right] = \frac{1}{L+1}Q(x).$$

If $L = t^n$, we have

$$Q(x) = (1+x)^n + t^n(1+cx)^n$$

so that if $P(\gamma) = 0$, then

$$\frac{t(1+c\gamma)}{1+\gamma} = \omega \text{ or} \tag{10}$$

$$\gamma = \frac{\omega - t}{-\omega + ct} \text{ where } \omega^n = -1 \tag{11}$$

and conversely. Thus, the roots of $P(x)$ can be found by evaluating eq. 11 for the n n-th roots of -1. If $P(x)$ has a real root, then from eq. 10 ω is real which occurs only for n odd and $\omega = -1$. Therefore, the roots of $P(x)$ are all nonreal with the exception of a single real root when n is odd. If $L = c^{-n/2}$ and $P(x)$ is transformed to the normalized form $N(x)$ in eq. 2, then $N(x)$ will be a symmetric polynomial [2]. The roots of $P(x)$ can be found from eq. 11 using $t = c^{-1/2}$ and a simple calculation shows that they all have the same absolute value. Since the transformation to $N(x)$ is a contraction or dilation of the complex plane, the roots of $N(x)$ must all lie on the unit circle. For the important case $n = 4$, $N(x)$ is symmetric for $L = c^{-2}$ and cooperativity is minimum for $c = L = 1$ when there is a quadruple root of -1. $N(x)$ has the same roots for c^{-1} as for c and cooperativity increases as c approaches zero or infinity and the roots approach the fourth roots of -1.

After a simple transformation, the binding polynomial for the KNF tetrahedral model can be written as

$$T(x) = 1 + 4Ax + 6A^{4/3}x^2 + 4Ax^3 + x^4$$

which is symmetric [1]. The root patterns are quite different from those of the MWC model for $n = 4$. $A = 1$ gives $T(x) = (1+x)^4$. $A > 1$ gives four distinct real roots and the roots spread out and cooperativity decreases as A increases. If $A < 1$, then $T(x)$ has four nonreal roots on the unit circle which all have negative real parts if $A > 3^{-3/4} \cong$

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0.44. If $A < 3^{-3/4}$, two roots have negative real parts and two have positive real parts and as A approaches zero, cooperativity increases and the roots approach the fourth roots of -1. The case of two real and two nonreal roots does not occur.

After transformation the binding polynomial for the KNF square model can be written as

$$S(x) = 1 + 4Cx + 2C(C + D)x^2 + 4Cx^3 + x^4$$

which is symmetric [1]. Having two parameters, this is a versatile model capable of representing any symmetric polynomial of degree four with the exception of $1 + \alpha x^2 + x^4$, $\alpha \neq 0$ and all possible root patterns can occur. For example, for $C = 1$, $D = 2$, $S(x)$ becomes $(1 + x)^4$ having a four-fold root of -1. For $C = 1$, $S(x)$ has two real roots and two nonreal roots on the unit circle if $D < 2$ and has four nonreal roots not on the unit circle if $D > 2$. For $D = 2$, $S(x)$ has four real roots if $C > 1$ and has four nonreal roots on the unit circle if $C < 1$. For a fixed value of D, cooperativity increases and the roots approach the fourth roots of -1 as C approaches zero.

References

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