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An Alternative to Allosterism and Cooperativity in the Interpretation of Enzyme Kinetic Data*

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ABSTRACT: Several kinds of cooperative models have been used to explain sigmoid relationships between velocities and substrate concentrations observed with regulatory enzymes, *e.g.*, allosteric interactions between subunits and multiple binding site models. Even though cooperative models can explain sigmoid data, cooperativitiy is not essential. Models requiring single, independent active sites with multiple reaction pathways by which the binding sites are occupied can also explain sigmoid data. It appears that sigmoid data can only eliminate ordered sequence models (*i.e.*, those with a single reaction pathway) which involve the binding of only one molecule of each substrate to one molecule of enzyme. Any model that conforms to a rate equation of the form, $1/V = a + b[S]^x$, where x is equal to or less than -2, will give a sigmoid relationship between velocity and substrate concentration.

A group of 15 steady-state models which can reduce to equations of the above form are presented. These models do not require cooperativity or the binding of more than one molecule of substrate to each catalytic site at a given time in order to exhibit sigmoidness.

Studies of mechanisms wherein enzyme activities are controlled in living organisms have occupied the attention of a large number of research groups for the past several years. The principal idea which has dominated thinking in this area is that of allosteric interaction suggested by Monod *et al.* (1963, 1965) and extended by Koshland *et al.* (1966). These investigations have emphasized the importance of subunit interactions in the control of enzymatic activity. Other investigators have used multisite models but without the requirement of subunit interactions (*e.g.*, Worcel *et al.*, 1966; Sanwal *et al.*, 1965; Sanwal and Cook, 1966). The essence of all of these ideas is that the binding of a substrate to an enzyme, like the binding of oxygen to hemoglobin, is modified by events at other sites on the protein. The principal evidence used to indicate that such effects are involved is the presence of a sigmoid relationship between velocity and substrate concentration and modification of this relationship by allosteric effectors. Additional evidence has been obtained with some enzyme systems where changes in conformation induced by heat or other conditions have changed a sigmoid relationship to the more usual hyperbolic function.

We have found that any model requiring a single, independent active site can explain sigmoid data if there is more than one reaction pathway leading to the binding of one substrate molecule to the single active site. Any model system, which can reduce to a rate equation which in reciprocal form has dominating terms containing substrate concentrations to powers less than -1, can be used to explain sigmoid results (*e.g.*, 1–3 below). Curves predicted by these equations

$$\frac{1}{V} = a + b[S]^{-2} \quad (1)$$

$$\frac{1}{V} = a + b[S]^{-3} \quad (2)$$

$$\frac{1}{V} = a + b[S]^{-2} + c[S]^{-3} \quad (3)$$

are shown in Figure 1. These curves have been made

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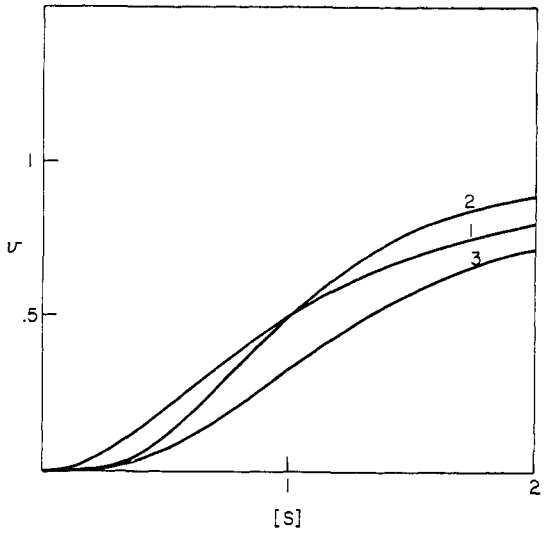

FIGURE 1: Sigmoid curves calculated from eq 1–3. Constants *a*, *b*, and *c* were assigned the value of 1.

consistent with experimental data for allosteric enzymes by adjusting constants *a*, *b*, and *c*. In one enzyme system an equation like 1 above has been shown to hold (Keech and Barritt, 1967).

The purpose of this paper is to describe a group of models which will give sigmoid data when reasonable rate constants are assigned. In this way it will be shown that subunit interactions or multiple binding sites are not always necessary in order to explain the properties assigned to allosteric enzymes.

Detailed Treatment of Illustrative Models

The model enzyme mechanism diagrammed in Figure 2 will be used to demonstrate how a steady-

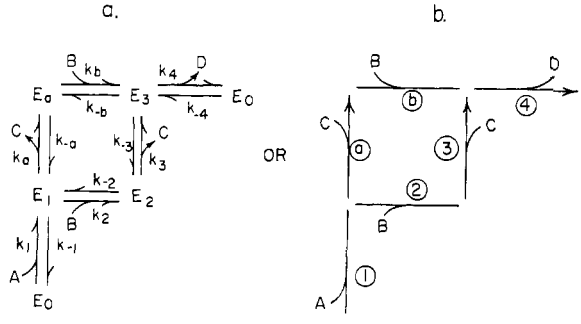

FIGURE 2: Schematic representations. (a) Example 1 (model 13 from Figure 5) written in a completely reversible form, where E_0 is the free form of enzyme and E_1, E_2, E_3, and E_a are enzyme–substrate complexes or other forms of the enzyme. (b) A less complex representation of model 13 with steps a, 3, and 4 considered to be irreversible because products (C and D) are omitted and initial velocities are measured. This form of representation will be used in the remainder of the paper.

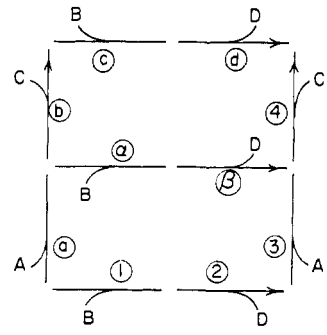

FIGURE 3: Example 2 (model 6 from figure 5). Steps 2, β, b, d, and 4 are considered to be irreversible because products (C and D) are omitted and initial velocities are measured.

state system without cooperative effects can give sigmoid data when reasonable values are assigned to rate constants. In deriving the rate equation for this model we are assuming that the following hold true. (1) That only one molecule of each substrate can associate with one catalytic site at a time. (2) There is no interaction between catalytic sites. (3) There are no allosteric sites. (4) That steady-state conditions obtain during initial velocity measurements. (5) The total enzyme concentration is very much smaller than the concentrations of substrates. Using these assumptions and the mathematical treatment given by Fisher and Hoagland (1968), the following analytically exact rate equation can be written (eq 4). The *S* and *P* terms are assemblies of rate constants and substrate concentra-

$$\frac{1}{\bar{V}} = S_4\left(1 + \frac{1}{P_b}\right) + f_b S_{bb} + f_3\left(S_{33} + S_{23} + \frac{S_{23}}{P_1}\right) + S_{11}$$

$$f_b = \frac{S'_{23}}{S'_{23} + S_{aa}[B]} \qquad f_3 = 1 - f_b \qquad (4)$$

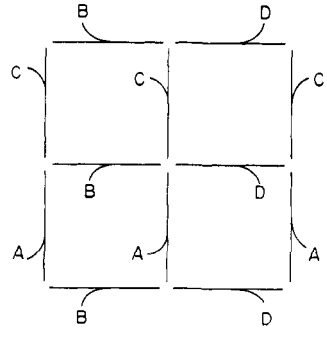

FIGURE 4: A reasonably general model for a two-substrate, two-product enzyme-catalyzed reaction; from Fisher and Hoagland (1968).

tions terms from (Hearon *et al.*, 1959), the "*f*" terms represent the fractional velocities through pathways a,b and 2,3, respectively, and $\hat{V}$ is the velocity per mole enzyme. Equation 4 is written in expanded form in eq 5. Since f_3 and f_b are independent of [A], the reciprocal of [A] is a linear function of the reciprocal of velocity. Whereas non-linearity may be observed with [B], depending upon the values of rate constants.

$$\frac{1}{\hat{V}} = \frac{1}{k_4}\left(1 + \frac{k_{-b}}{k_b[B]}\right) + f_b\frac{1}{k_b[B]} + f_3\left(\frac{1}{k_3} + \frac{1}{k_2[B]} + \frac{k_{-2}}{k_2[B]k_3} + \frac{k_{-1}}{k_1[A]k_2[B]} + \frac{k_{-1}k_{-2}}{k_1[A]k_2[B]k_3}\right) + \frac{1}{k_1[A]} \quad (5)$$

where

$$f_b = \frac{\frac{1}{k_2} + \frac{k_{-2}}{k_2k_3}}{\frac{1}{k_2} + \frac{k_{-2}}{k_2k_3} + \frac{[B]}{k_a}} \qquad f_3 = 1 - f_b$$

In order to show that eq 4 can reduce to the form of eq 1, it is helpful to write it as shown in eq 6. The f_3 term has been removed by substituting $1 - f_b$ and terms containing $1/[B]$ have been collected. A $[B]^{-2}$-containing term can be obtained when $[B]^{-1}$ is multiplied by f_b. Two conditions are necessary in order for the entire equation to reduce to the form of eq 1. The f_b-containing term must be large and positive, and the [B]-containing term in the denominator of f_b must be dominant. These conditions are met if S'_{bb} is very large with respect to $S'_{23}(1 + (1/P_1))$ and $S_{aa}[B]$ is very large with respect to S'_{23}.

$$\frac{1}{\hat{V}} = S_4 + S_{33} + S_{11} - f_bS_{33} + \frac{1}{[B]}\left[\frac{S_4}{P'_b} + S'_{23} \times \left(1 + \frac{1}{P_1}\right) + f_b\left(S'_{bb} - S'_{23} - \frac{S'_{23}}{P_1}\right)\right] \quad (6)$$

TABLE I: Rate Constant Assignments for Example 1.[a]

First-Order Rate Constants	sec^{-1}	Second-Order Rate Constants	M^{-1} sec^{-1}
k_{-1}	10^4	k_1	2×10^6
k_{-2}	10^3	k_2	10^8
k_3	10^3	k_b	10^4
k_4	5×10^2		
k_a	10^2		
k_{-b}	1		

[a] [A] = 10^{-4}; B is the variable substrate.

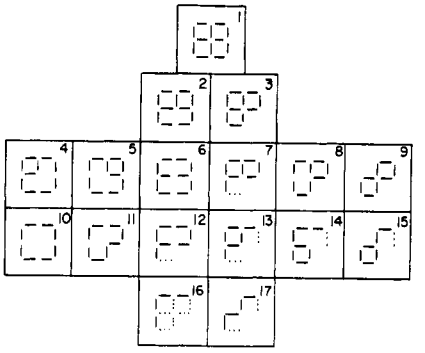

FIGURE 5: Allowed mechanisms from general model in Figure 4; from Fisher and Hoagland (1968).

That is

$$\frac{1}{k_b} \gg \left(\frac{1}{k_2} + \frac{k_{-2}}{k_2k_3}\right)\left(1 + \frac{k_{-1}}{k_1[A]}\right)$$

and

$$\frac{[B]}{k_a} \gg \frac{1}{k_2} + \frac{k_{-2}}{k_2k_3}$$

It is easy to assign reasonable values to the rate constants in this system which meet the above requirements and conform with the law of microreversibility (Table I). Assigned values are consistent with those of reported rate constants as summarized by Eigen and Hammes (1963). A sigmoid curve identical in shape with curve 1 in Figure 1 is obtained when these

TABLE II: Rate Constant Assignments for Example 2.[a]

First-Order Rate Constants (sec^{-1})	1	2
k_{-1}	10^3	10^3
k_2	10^3	10^3
k_{-3}	10^3	10^3
k_4	10^3	10^3
k_{-a}	10^3	10^3
k_b	10^3	10^3
k_{-c}	10^3	10^3
k_d	10^3	2
$k_{-\alpha}$	5×10^2	5×10^2
k_β	5×10^2	5×10^2
Second-Order Rate Constants (M^{-1} sec^{-1})		
k_1	5×10^7	5×10^7
k_3	10^6	10^6
k_a	10^6	10^6
k_c	10^4	10^4
k_α	5×10^7	5×10^7

[a] [A] = 10^{-4}; B is the variable substrate.

TABLE III: A Summary of Allowed Sigmoid Equations for 17 Models.[a]

Model	Direction	Variable Substrate	Allowed Sigmoid Equations		
			$a + \frac{b}{[S]^2}$	$a + \frac{b}{[S]^2} + \frac{c}{[S]^3}$	$a + \frac{b}{[S]^3}$
1	Either	Either	+	+	+
2	Either	Either	+	+	+
3	Either	Either	+	+	+
4	Either	B or C	+	+	+
	Either	A or D	+	−	−
5	Forward	Either	+	−	−
	Reverse	Either	+	+	+
6	Either	B or D	+	+	+
	Either	A or C	+	−	−
7	Forward	A	−	−	−
		B	+	−	−
	Reverse	C	+	+	+
		D	+	−	−
8	Forward	Either	+	−	−
	Reverse	C	+	+	+
		D	+	−	−
9	Either	Either	+	−	−
10	Either	Either	+	−	−
11	Either	Either	+	−	−
12	Forward	A	−	−	−
		B	+	−	−
	Reverse	Either	+	−	−
13	Either	A or D	−	−	−
		B or C	+	−	−
14	Forward	A	+	−	−
		B	−	−	−
	Reverse	Either	−	−	−
15	Forward	Either	+	−	−
	Reverse	Either	−	−	−
16	Either	Either	−	−	−
17	Either	Either	−	−	−

[a] Allowed equations are noted with a plus sign.

values of rate constants are substituted in to eq 6 and velocities are calculated at various concentrations of B.

Example 1 above reduces to a reciprocal equation with a dominating term containing $[B]^{-2}$. Other models can reduce to equations having terms with $[B]^{-2}$ and $[B]^{-3}$ or $[B]^{-3}$ alone. Example 2, shown in Figure 3, will be used to illustrate such a mechanism. Values for rate constants are given in Table II. Those in column 1 give an equation with a dominating term having $[B]^{-3}$. When k_d is changed from 10^3 to 2 (column 2), an equation is obtained with both $[B]^{-2}$ and $[B]^{-3}$ terms. Curves obtained with these two sets of rate constants are identical in shape with curves 2 and 3 in Figure 1.

A Systematic Consideration of Models

Fisher and Hoagland (1968) set up a general model for a two-substrate, two-product enzyme-catalyzed reaction (Figure 4) which was reversible. The 17 models, which they showed to be implicit in this general model, were chosen for systematic consideration in this paper. These models are summarized in Figure 5. The kinds of sigmoid equations which are possible for each substrate in each model are summarized in Table III. It should be emphasized that any of these substrates may exhibit patterns other than those shown in this table depending upon the assigned values of the rate constants. Linear reciprocal plots, substrate inhibition plots, as well as other kinds of nonlinearity may be observed with some of the substrates in some of the models.

All but 2 of these 17 models will allow some degree of sigmoidness. By choosing the proper models and values for rate constants, it is possible to fit a wide spectrum of experimental sigmoid data.

Discussion

The classical oxygen binding curves of hemoglobin have served most usefully in bringing out the importance of cooperative effects in the binding of ligands to proteins. These results have led to the association of sigmoidness with cooperativity which has been applied to enzymes as well as proteins which only bind ligands. From this point of view, a number of model systems have been generated involving subunit interactions, allosteric effects at sites different from the active site of the enzyme and so forth. All of these models involve some aspect of cooperativity. The results presented in this paper show that sigmoidness can be obtained with models wherein there are no requirements for subunit interaction or multiple binding sites.

It is important to note that the models we are using to explain sigmoidness take into account all of the low molecular weight components in the system, do not require the presence of subunits (although their presence is not excluded), and are readily amenable to further kinetic analysis using product inhibition and other approaches which have been described previously (*e.g.*, Fisher and Hoagland, 1968). Furthermore, it should be possible to test these models using isotope-exchange techniques and other related experimental approaches.

Changes in the allosteric properties of an enzyme by heating (Gerhart and Pardee, 1962; Kirschner *et al.*, 1966) or other techniques involving changes in the conformation of the enzyme have been used as evidence for cooperativity in the enzyme system. If it can be assumed that changes in the conformation of the enzyme could result in changes in the magnitude of the rate constants involved in the mechanism, then these non-cooperative models can explain the data equally well. In many of these models a change in one rate constant can induce or eliminate sigmoidness. It seems reasonable to expect that changing the conformation of an enzyme could indeed change the relative magnitudes of rate constants and thereby change its kinetic characteristics. In a similar way an added inhibitor or other effector could modify the relative magnitude of terms in the rate expression in such a way as to change the observed relationships between velocity and substrate concentration. The changes induced by an effector could involve a large conformational change. Alternatively, if the effector binds at or very near the active site, the conformation of the enzyme may remain unchanged.

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