Shapes of Velocity Curves in Multiunit Enzyme Kinetic Systems*

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ABSTRACT

Multiunit enzyme kinetic mechanisms incorporating multiple reactants and different forms and degrees of inhibition, activation, cooperativity, and allosteric effects may be reflected by the shape of the corresponding velocity of reaction curves. In this work we set up a general model for such velocity curves and examine the qualitative behavior of velocity shapes for different classes of biochemical parameters. Special attention is given to various extensions of the Monod-Wyman-Changeux model with the purpose of establishing analytic results characterizing its velocity curve. Such results can provide a help in discriminating between various competing models.

1. INTRODUCTION

This and the paper, Karlin [12], describe a mathematical approach to the study of enzyme kinetics and allostery using known models of enzyme action and control. The cases dealt with are single substrate reactions, in enzymes with multiple subunits. The approach used should allow one to understand better their physical (experimental) implications. Two particularly possible relevant aspects of this work pertain to the analysis of the shapes of velocity curves for the "Monod model" and the comparison of three measures of cooperativity treated extensively in Karlin [12] and their application to some velocity functions.

The basic model of enzyme-substrate interaction has the following structure. Let E, S, ES denote the enzyme, substrate, and complex (bound enzyme-substrate), respectively. When substrate is available, E and S can

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combine at the active sites, and ES accumulate rapidly over a transient phase, attaining a pseudo-steady-state reaction-velocity level. After forming a complex, the enzyme frees itself, releasing a "transformed" substrate—the product. With increasing initial concentration of S, the velocity of product formation will asymptote toward a well-defined limit, the saturation velocity. Thus, the reaction velocity (also called initial velocity and pseudo-steady-state velocity) of an enzyme catalyzed reaction is a function of the concentrations of substrates and of possible effectors involved in the reaction.

Multiunit enzyme-kinetic mechanisms incorporating multiple reactants and different forms and degrees of inhibition, activation, cooperativity, and allosteric effects may be reflected by the behavior of the corresponding reaction-velocity curves. Through the study of these differences, with reference to the number of active sites participating in the reaction, some insights into the nature of the interactions between enzyme subunits and the type of binding and catalytic mechanism involved may be uncovered.

The reaction-velocity curve of an enzyme composed of identical and independent active sites manifests the classical Michaelis-Menten hyperbolic curve, $V(x)=xV_\infty/(x+K_M)$ (K_M is the Michaelis-Menten constant), asymptoting to V_∞ as x gets large. Cooperativity and allosterism can yield velocity curves deviating from this simple hyperbolic shape. We define hyperbolicity as described by a concave velocity curve, while sigmoidicity denotes a shape that is initially convex and subsequently concave with only one inflection point. Multiple inflection points for $V(x)$ indicate "undulations" or "bumps."

Bardsley and Childs [1, 6] considered general velocity curves of the form

$$V(x)=\frac{\sum_{i=1}^{n}\alpha_i x^i}{1+\sum_{j=1}^{m}\beta_j x^j} \qquad \text{for all} \quad \alpha_i,\beta_j>0, \quad m\geqslant n.$$

They attempted to bound the number of positive zeros of $V'(x)$ and $V''(x)$. Trivial bounds are the degrees of the numerators in V' and V'', which are $n+m-2$ and $2m+n-3$, respectively. These authors reduced the bounds to $2n-3$ and $3n-3$, respectively; the latter is no improvement for $n=m$. Another aspect of their work [2, 3] provided a classification of the possible shapes of $V(x)$ for $m,n\leqslant 4$.

In this work we propose to study shapes of velocity curves for specific enzyme-kinetic mechanisms, correlating classes of biochemical parameters of enzymatic reactions with the qualitative properties of the velocity curves. A general multiunit class of models is set forth in Sec. 2, and then a number of results characterizing the associated reaction-velocity curves are elaborated. In particular, sufficient conditions for hyperbolicity and sigmoidicity are established. In this perspective a detailed discussion of generalized forms of the Monod-Wyman-Changeux model is presented. The shapes of related Scatchard plots are also ascertained. Some mathematical details and proofs are relegated to the appendices.

2. AN N-UNIT REACTION-VELOCITY FUNCTION

For completeness and clarity we briefly review the standard formulation of a general kinetic model for an enzyme E involving N active sites. When i sites have a substrate S attached, the complex is labeled C_i, $i=0,\ldots,N$. C_0 represents the state of free enzyme where all sites are unoccupied. The reaction activated by E can be schematized as follows:

$$\cdots C_{i-1} \underset{\tilde{\mu}_i}{\overset{\tilde{\lambda}_{i-1}}{\rightleftharpoons}} \; C_i \; \underset{\tilde{\mu}_{i+1}}{\overset{\tilde{\lambda}_i}{\rightleftharpoons}} C_{i+1} \cdots, \qquad i=1,\ldots,N-1, \quad \gamma_0=\tilde{\mu}_0=\tilde{\lambda}_N=0,$$
$$C_i \xrightarrow{\gamma_i} C_{i-1}+P \tag{2.1}$$

P being the product released at rate γ_i when i sites are occupied, $\tilde{\lambda}_i$ the rate of binding of substrate to an additional site, and $\tilde{\mu}_i$ the dissociation rate resulting in a decrease by one in the number of active sites. Let $\mu_i=\tilde{\mu}_i+\gamma_i$, $i=1,\ldots,N$.

The initial abundance of substrate S as compared to the total initial enzyme concentration, denoted by $(E)_0$, yields at steady state the relative complex amounts $C_i=\tilde{\lambda}_0\tilde{\lambda}_1\cdots\tilde{\lambda}_{i-1}C_0/\mu_1\cdots\mu_i$, $i=1,\ldots,N$, where we use C_i not only to represent the state C_i but also its concentration.

Set

$$\tilde{\pi}_i=\frac{\tilde{\lambda}_0\tilde{\lambda}_1\cdots\tilde{\lambda}_{i-1}}{\mu_1\mu_2\cdots\mu_i} \quad \text{and} \quad \tilde{\pi}_0=1. \tag{2.2}$$

Then $C_i=\tilde{\pi}_iC_0$, $i=0,\ldots,N$. The *velocity of reaction* is the rate of total product formation,

$$V=\sum_{i=1}^{N}\gamma_iC_i=C_0\sum_{i=1}^{N}\gamma_i\tilde{\pi}_i. \tag{2.3}$$

The conservation law of total enzyme concentration entails $(E)_0=\sum_{i=0}^{N}C_i=C_0\sum_{i=0}^{N}\tilde{\pi}_i$ and so $C_0=(E)_0/\sum_{i=0}^{N}\tilde{\pi}_i$. Substituting into (2.3) leads to the classical formula

$$V=\frac{(E)_0\sum_{i=1}^{N}\gamma_i\tilde{\pi}_i}{\sum_{i=0}^{N}\tilde{\pi}_i}. \tag{2.4}$$

The following stipulations are usual.

Assumption 1. $\tilde{\lambda}_i = \lambda_i S$, $i = 0, \ldots, N-1$. That is, the rate of binding of substrate to an additional active site is proportional to the substrate concentration S.

Let $\pi_i = \lambda_0 \lambda_1 \cdots \lambda_{i-1}/\mu_1 \mu_2 \cdots \mu_i$, $i = 1, 2, \ldots, N$, $\pi_0 = 1$. Then (2.4) becomes

$$V(S) = \frac{(E)_0 \sum_{i=1}^{N} \gamma_i \pi_i S^i}{\sum_{i=0}^{N} \pi_i S^i} \tag{2.5}$$

Assumption 2. $\gamma_i = ik_{+2}$, $i = 1, \ldots, N$. This assumption means that the catalytic activities of the separate units are independent with an intrinsic constant rate of product formation, k_{+2}.

With this assumption

$$V(S) = \frac{(E)_0 k_{+2} \sum_{i=1}^{N} i \pi_i S^i}{\sum_{i=0}^{N} \pi_i S^i}. \tag{2.6}$$

Let $V_\infty = N(E)_0 k_{+2}$, and set $P(S) = \sum_{i=0}^{N} \pi_i S^i$; then

$$V(S) = \frac{SP'(S) V_\infty}{NP(S)}. \tag{2.7}$$

The binding curve or saturation function which measures the fraction of sites bound by a ligand to a protein has the same form as in (2.7) with $V_\infty = 1$. Any results relevant to $V(S)$ will apply to binding curves, *mutatis mutandis*.

Noncooperative model. $\lambda_i = (N-i)k_{+1}$, $\tilde{\mu}_i = ik_{-1}$, $\mu_i = i(k_{-1} + k_{+2})$, $i = 1, \ldots, N$, reflecting an enzyme with independent and identical active sites. In this case

$$\pi_i = S^i K^i \binom{N}{i}, \qquad i = 0, \ldots, N,$$

where $K = k_{+1}/(k_{-1} + k_{+2})$, implying

$$P(S) = \sum_{i=0}^{N} S^i K^i \binom{N}{i} = (1 + KS)^N$$

and

$$V(S)=\frac{SP'(S)V_\infty}{NP(S)}=\frac{SV_\infty}{S+K_M},$$

where $K_M=1/K$ is the associated Michaelis-Menten constant.

3. GENERAL RESULTS FOR THE VELOCITY OF REACTION

Under Assumption 2 we obtain a velocity curve of the form (2.7). We next summarize for easy reference some elementary facts in two propositions.

PROPOSITION 3.1

Let $V(x)$ be a velocity curve of the form (2.7). *Then*:

(i) *$V(x)$ is a monotone increasing function of x, for any rate constants $k_{+2}, \lambda_i, \tilde{\mu}_i$.*

(ii) $\lim_{S\to\infty} V(x)=V_\infty$, *which entails that $V''(x)<0$ for large x.*

(iii) $V'(0)=(\pi_1/\pi_0)V_\infty/N=(\pi_1/\pi_0)(E)_0 k_{+2}$.

(iv) *Apart from the multiplicative constant V_∞,*

$$V''(x)=2\frac{P''(x)}{P(x)}-2\left[\frac{P'(x)}{P(x)}\right]^2-3\frac{xP'(x)P''(x)}{P(x)^2}$$
$$+x\frac{P'''(x)}{P(x)}+2x\left[\frac{P'(x)}{P(x)}\right]^3;$$

in particular $V''(0)=[2/(\pi_0)^2][2\pi_0\pi_2-\pi_1^2](E)_0k_{+2}$.

PROPOSITION 3.2

If the γ_i are increasing, then $V(x)$ is a monotone increasing function of x for $S>0$.

Two basic general results on shapes of velocity curves are highlighted next. For simplicity we normalize $V(x)$ so the $V_\infty=1$. The proofs appear in Appendix A.

Shape of $V(x)$ when $\{\pi_i/\pi_{i-1}\}_{i=1}^N$ is increasing (not necessarily strictly). Let $K_i=\pi_i/\pi_{i-1}=\lambda_{i-1}/\mu_i$, $i=1,\ldots,N$. The quantity K_i is the equilibrium constant between states C_{i-1} and C_i.

RESULT I

Let $V(x)=xP'(x)/NP(x)$. If $\{K_i\}_{i=1}^N$ is an increasing sequence, then $V(x)$ is sigmoidal.

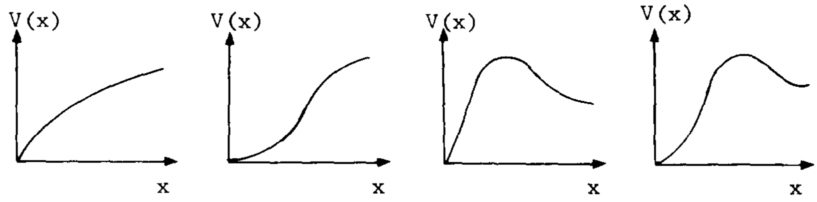


FIG. 1.

Shape of $V(x)$ when $\{\pi_i/\pi_{i-1}\}_{i=1}^N$ is concave. ($\{K_i\}_{i=1}^N$ is concave if and only if $(K_{i-1}+K_{i+1})/2 \leqslant K_i$, $i=1,\ldots,N-1$.)

RESULT II

Let $V(x)=xP'(x)/NP(x)$. *If* $\{K_i\}_{i=1}^N$ *is a concave sequence, then* $V(x)$ *is either sigmoidal or hyperbolic, and* $2K_2>K_1$ *implies the former,* $2K_2<K_1$ *the latter.*

RESULT III

Let $V(x)$ *be a velocity curve with the property that for any real constants a and b,* $a>0$, $V(x)-ax-b$ *has at most three positive roots. Then only four shapes for* $V(x)$ *are possible, as in* Fig. 1.

The above figure describes all possible velocity curves for a *dimer*, as then $V(x)$ is a ratio of quadratics and $V(x)-(ax+b)$ can have at most three positive roots; cf. [4]. In particular, for a dimer $V(x)$ cannot have undulations in its increasing portion.

The velocity curve of a *trimer* can produce two inflection points (or "bumps"). Consider $P(x)=(x^2+\alpha)(x+\beta)$, $\alpha,\beta>0$, and $V(x)=xP'(x)/P(x)$. Then the possibilities are as in Fig. 2.

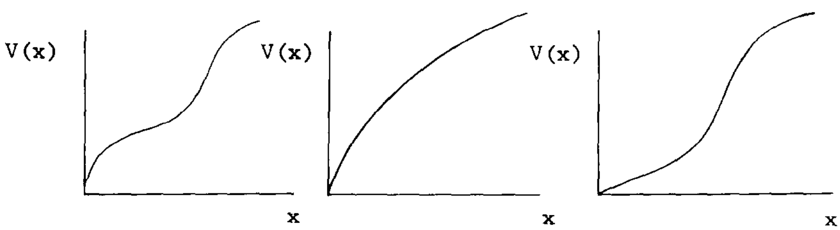


FIG. 2.

SOME RELEVANT EXAMPLES OF VELOCITY CURVES

(a) Let $P(x)=\sum_{i=0}^{N}x^i$, $\pi_i=1$, $i=0,\ldots,N$, i.e., K_i are coincident, so that enzyme state C_i occurs with concentration C_0x^i, x the substrate concentration. The corresponding velocity curve is

$$V(x;N)=\frac{\sum_{i=1}^{N} ix^i}{N\sum_{i=0}^{N} x^i}=\frac{1}{N}\left[\frac{Nx^{N+2}-(N+1)x^{N+1}+x}{x^{N+2}-x^{N+1}-x+1}\right],$$

$$V'(0)=\frac{1}{N},\qquad V''(0)=\frac{2}{N}>0.$$

Results I and II apply here. It follows that $V(x;N)$ is sigmoidal. The Michaelis-Menton constant of $V(x;N)$ for each N is $K_M=1$, and

$$\lim_{N\to\infty} V(x,N)=\begin{cases}0 & \text{for } x<1,\\ 1 & \text{for } x>1,\end{cases}$$

showing that $x=1$ is an increasingly sharpened threshold point as $N\to\infty$.

(b) Consider $P(x)=\prod_{i=1}^{N}(x+\alpha_i)$; $\alpha_i>0$, $i=0,\ldots,N$, where $P(x)$ has N negative roots $-\alpha_i$, $i=1,2,\ldots,N$. Then correspondingly

$$V(x)=\frac{xP'(x)}{NP(x)}=\frac{1}{N}\sum_{i=1}^{N}\frac{x}{x+\alpha_i},$$

and since

$$V''(x)=-\frac{2}{N}\sum_{i=1}^{N}\frac{\alpha_i}{(x+\alpha_i)^3},$$

$V(x)$ is strongly hyperbolic.

(c) If $P(x)=\prod_{i=1}^{N}(x^2+a_i)$, where $P(x)$ has $2N$ pure imaginary roots $x=\pm i\sqrt{a_i}$, $a_i>0$, then $V''(x)$ can exhibit at most $2N-1$ sign changes, and this upper bound can be attained.

4. THE MONOD-WYMAN-CHANGEUX (MWC) MODEL

Monod et al. [15] modeled cases of the existence of modifier molecules that underlie the enzyme-substrate activity when the enzyme admits two conformational states, the R (relaxed) state and the T (tense) state. The different enzyme-substrate complexations from 0 to N sites occupied are denoted by $R_0,\ldots,R_N$ in the R state, and $T_0,\ldots,T_N$ in the T state. The N

active sites are identical and independent in both the R and the T states. Monod et al. postulated an allosteric mediated transition between states R_0 and T_0. These authors restricted attention only to the case of equal catalytic performance per conformational state. The extended model, allowing for different catalytic rates, yields the standardized reaction-velocity relationship

$$V(x)=\frac{x(1+x)^{N-1}+L\theta cx(1+cx)^{N-1}}{(1+x)^{N}+L(1+cx)^{N}} \tag{4.1}$$

(see [8]), where x denotes the initial substrate concentration scaled by the Michaelis-Menton constant of the R state. Here, L is the ratio of the transition rates between R_0 and T_0, c the ratio of the Michaelis-Menten constants for the separate R and T states, and θ the ratio of the saturation velocities in the T and R configurations. For simplicity, set $V_R=1$ where V_R is the saturation velocity in the R state.

Of particular interest is the case $\theta=1$, corresponding to $V_R=V_T$, i.e., $\gamma_i^R=\gamma_i^T$. Biologically, this describes either a model where the R and T states have the same catalytic activity (the case treated by Monod et al.) or a model for the binding-velocity curve of a protein possessing two conformational forms.

In the model (4.1) we set, with $\theta=1$,

$$P(x)=(1+x)^{N}+L(1+cx)^{N}.$$

Then $V(x)$ has the form described in (2.7), i.e., $V(x)=xP'(x)/NP(x)$, V_∞ normalized to 1. We can deduce

PROPOSITION 4.1

With $\theta=1$ the velocity curve of the kinetic MWC model (4.1) *is a monotone increasing function of x. For $\theta\neq 1$ this is not necessarily the case, and $V(x)$ can attain a maximum before extending to its asymptote. More precisely, when $(1-\theta)(1-c)\geq 0$, then $V(x)$ in* (4.1) *is increasing to its asymptote. For $(1-\theta)(1-c)<0$, $V(x)$ is not necessarily monotone.*

Monod et al. [15] gave numerical curves with $\theta=1$, exhibiting hyperbolic and sigmoidal shapes. Goldbeter [11] verified numerically for $\theta=0.1$, $N=6$, $L=10^5$, $c=0.1$ that $V(x)$ can describe a "small undulation," i.e., a pair of inflection points.

We showed numerically that even for $\theta=1$, $N=4$, $L=43$, $c=0.2$, $V(x)$ can have undulations such that

$$V''(x)\leq 0 \quad \text{for} \quad x\leq 0.14 \text{ and } x\geq 0.52,$$

$$V''(x)\geq 0 \quad \text{for} \quad 0.14\leq x\leq 0.52.$$

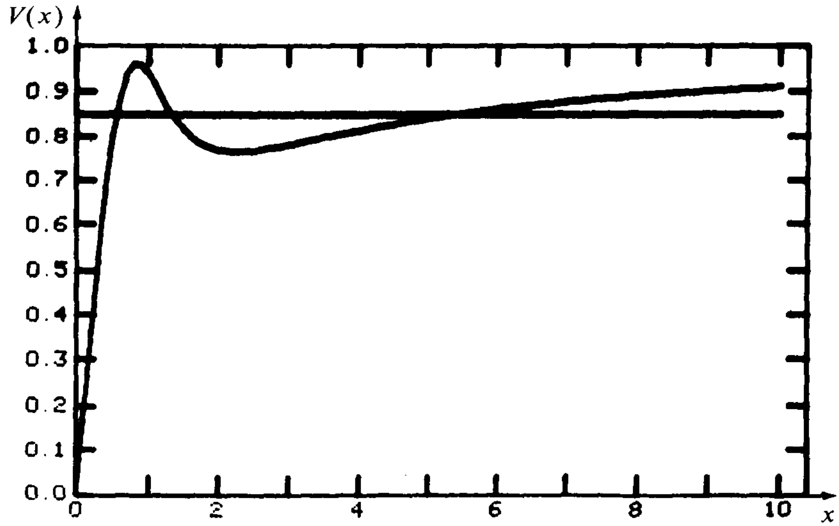


FIG. 3. $N=10$, $L=100$, $c=0.2$, $\theta=10$.

Further information on the nature of the generalized MWC model velocity curve is now presented.

RESULT IV

(i) *The kinetic MWC-model velocity curve* $V(x)$ *of* (4.1) *is monotone increasing if* $c<1$, $\theta\leqslant 1$ *or if* $c>1$, $\theta\geqslant 1$. *Furthermore,* $V(x)-a$, $a>0$ *admits at most three roots.*

(ii) *With* $c=0$, $V(x)$ *has hyperbolic or sigmoidal shape, according as* $\operatorname{sign} V''(0)=\operatorname{sign}[L(N-1)-1]$ *is negative or positive, respectively.*

(iii) $V(x)$ *admits at most three intersections with an increasing line passing through the origin, and for* $c<1$, $\theta c>1$ *or* $c>1$, $\theta c<1$, *this upper bound is reduced to* 1.

Remark. The bounds of Result IV are attainable, and also the kinetic MWC-model velocity curve (4.1) can reach a maximum prior to its asymptote. Figure 3 shows results for $N=10$, $L=100$, $c=0.2$, $\theta=10$.

5. MODELS WITH TRANSITIONS BETWEEN SEVERAL CONFORMATIONAL FORMS

Consider an enzyme E with N active sites and K possible conformational states. Let $f_1,\ldots,f_K$ denote the free-enzyme steady-state concentrations in the K different conformational forms $F_1, F_2,\ldots, F_K$. We denote by $d_\nu^{(j)}$ the concentration of E in the jth conformational form with ν sites occupied, $\nu=1,\ldots,N$, $j=1,\ldots,K$.

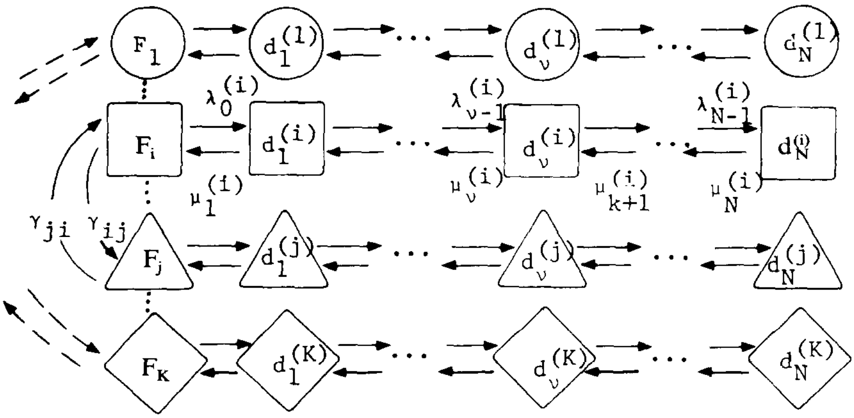


FIG. 4.

We allow transitions between F_i and F_j at rate $\gamma_{i,j}$, $i, j = 1, \ldots, K$ and general binding and dissociation rates $\lambda_\nu^{(i)}$, $\mu_\nu^{(i)}$ within the ith conformational array, as described schematically in Fig. 4.

Let $\gamma_\nu^{(i)}$ denote the rate of product formation when the enzyme system is in conformational state i with ν occupied sites. In this extended model the equilibrium concentrations are calculated as follows. Define $P_{ij} = \gamma_{ij}/\sum_{j=1}^{K}\gamma_{i,j}$, and determine f_i as the unique (up to a constant factor) positive solution of $\sum_{i=1}^{K} f_i P_{ij} = f_j$, $j = 1, 2, \ldots, K$. Define $\pi_\nu^{(i)} = \lambda_0^{(i)}\lambda_1^{(i)} \cdots \lambda_{\nu-1}^{(i)}/\mu_1^{(i)}\mu_2^{(i)} \cdots \mu_\nu^{(i)}$, $1 \leqslant \nu \leqslant N$, $\pi_0^{(i)} = 1$. Then $d_\nu^{(i)} = \pi_\nu^{(i)} f_i$, $\nu = 0, 1, \ldots, N$, $i = 1, 2, \ldots, K$, is (up to a constant factor) the steady-state concentration of the enzyme complex in conformational state i having ν active sites.

The corresponding reaction-velocity curve is

$$V(x) = e\frac{\sum_{i=1}^{K}\sum_{\nu=1}^{N}\gamma_\nu^{(i)}\pi_\nu^{(i)}f_i}{\sum_{i=1}^{K}\sum_{\nu=1}^{N}\pi_\nu^{(i)}f_i}$$

where e is a normalizing constant. Under the MWC-like specialization

$$\lambda_\nu^{(i)} = (N-\nu)Sk_{+1}^{(i)}, \quad \mu_\nu^{(i)} = \nu(k_{-1}^{(i)} + k_{+2}^{(i)})$$
$$\gamma_\nu^{(i)} = \nu k_{+2}^{(i)}, \qquad K^{(i)} = k_{+1}^{(i)}/(k_{-1}^{(i)} + k_{+2}^{(i)}). \tag{5.1}$$

(see Sec. 3), the velocity curve of this *generalized MWC model* with K conformational states takes the form

$$V^*(x)=\frac{x\sum_{i=1}^{K} Q_i L_i(1+Q_i x)^{N-1}\theta_i}{N\sum_{i=1}^{K} L_i(1+Q_i x)^{N}}, \tag{5.2}$$

with

$$L_i=\prod_{j=1}^{i-1}\frac{F_{j+1}}{F_j}, \qquad Q_i=\prod_{j+1}^{i-1}\frac{K^{(i+1)}}{K^{(i)}}$$

and where θ_i measure the relative saturation velocities for the different conformation forms. The velocity curve $V^*(x)$ in (5.2) reduces to (4.1) for $K=2$.

The associated binding curve is obtained by setting $\theta_i \equiv 1$:

$$V(x)=\frac{x\sum_{i=1}^{K} Q_i L_i(1+Q_i x)^{N-1}}{\sum_{i=1}^{K} L_i(1+Q_i x)^{N}}. \tag{5.3}$$

The generalization of Result I is

RESULT V

If Q_i and θ_i are monotone together (more generally, share the same ordering), then $V^*(x)$ of (5.2) is monotone increasing.

6. DIAGNOSTIC PLOTS

A widely used graph is the double reciprocal plot, i.e., $1/V(x)$ versus $1/x$. This data transformation can be useful to determine the behavior of V near zero concentration. As $x\to\infty$ the graph of $1/V$ versus $1/x$ approaches an asymptote of slope $1/\pi_1$ and intercept $-[2\pi_0\pi_2-\pi_1^2]$, so that $V''(0)>0$ means a negative intercept and $V''(0)<0$ a positive intercept (cf. Proposition 3.1). It is conceivable that in some cases it will be easier to obtain the intercept of the asymptote in the double reciprocal plot, than the initial behavior of V.

Levitzki and Koshland [14] used the double reciprocal plot in order to assess the extent of positive or negative cooperativity in terms of the Hill equation (cf. Karlin [12])

$$V = \frac{x^N}{x^N + K_M} V_\infty .$$

They infer that convex or concave behavior of $1/V$ with respect to $1/x$ is of diagnostic value for ascertaining cooperativity (positive and negative, respectively) relative to small or large substrate concentration levels. An analysis of the classical Monod model was done following these lines by Bardsley and Childs [1, 6]. See also Ricard et al. [17] for a simulation study on double reciprocal plots for tetramers.

Application of the double-reciprocal-plot criterion for positive or negative cooperativity in the case $N=2$, i.e., for dimeric enzymes, is of particular interest. Let

$$V(x) = \frac{\alpha_1 x + \alpha_2 x^2}{1 + \beta_1 x + \beta_2 x^2}, \qquad V''(0) = \alpha_2 - \alpha_1 \beta_1 .$$

In the double reciprocal plot we have for $y = 1/V$ and $z = 1/x$ that

$$y(z) = \frac{z^2 + \beta_1 z + \beta_2}{\alpha_1 z + \alpha_2} \quad \text{and} \quad y''(0) = \frac{2}{(\alpha_2)^2}\left[\alpha_2(\alpha_2 - \alpha_1\beta_1) + \alpha_1^2 \beta_2\right],$$

so that $V''(0) > 0$ entails $y''(0) > 0$. But the converse does not necessarily hold, i.e., an initial convex double reciprocal plot does not compel sigmoidicity of the velocity curve.

For N-unit enzymes,

$$V(x) = \frac{\alpha_1 x + \cdots + \alpha_N x^N}{1 + \beta_1 x + \cdots + \beta_N x^N}, \qquad V''(0) = \alpha_2 - \alpha_1 \beta_1$$

and

$$y''(0) = 2\left[\beta_{N-2} - \frac{\alpha_{N-2}\beta_N}{\alpha_N} - \frac{\beta_{N-1}\alpha_{N-1}}{\alpha_N} + \left(\frac{\alpha_{N-1}}{\alpha_N}\right)^2 \beta_N\right],$$

so that there is no clear-cut relation between $V''(0)$ and $y''(0)$. In particular, we can have $V''(0)>0$ with $y''(0)<0$.

The nature of cooperativity or the real shape of a velocity curve cannot be determined by the behavior of V for x near zero or near infinity, as this is determined by the coefficients of the first and last powers of x only. For dimers these coefficients are the same.

Another common way to present data in enzyme kinetics is to plot $S(x)=V(x)/x$ against $V(x)$. This is called the *Scatchard plot*, and in the following we propose to investigate the relationships between the shapes of velocities of reaction and their Scatchard plots. We concentrate on velocity curves of the form $V=[xP'(x)/NP(x)]V_\infty$, where $P(x)=\sum_{i=0}^{N}\pi_i x^i$.

(i) Straightforward computations yield that $S(0)=V'(0)$ and $2S''(0)=V''(0)(\pi_0)^2$, so that an initial increase in $S(x)$ implies an initially sigmoidal velocity curve and conversely.

(ii) $\{\pi_{i+1}/\pi_i\}_{i=0}^{N-1}$ *increasing yields a monotone decreasing Scatchard plot.*

Proof. For $a>0$, consider

$$S(x)-a=\frac{\frac{1}{N}\sum_{i=0}^{N}(i+1)\pi_i\left[\frac{\pi_{i+1}}{\pi_i}-\frac{Na}{i+1}\right]x^i}{\sum_{i=0}^{N}\pi_i x^i}.$$

But $Na/(i+1)$ decreases convexly, so that the sequence

$$\left\{\frac{\pi_{i+1}}{\pi_i}-\frac{Na}{i+1}\right\}_{i=0}^{N-1}$$

has at most one sign change for π_{i+1}/π_i increasing, and by the Descartes rule of signs, we infer that $S(x)-a$ has at most one positive real root. This property holds for all $a>0$, by which we can conclude that $S(x)$ is monotone decreasing.

(iii) $\{\pi_{i+1}/\pi_i\}_{i=0}^{N-1}$ *concave yields a monotone decreasing Scatchard plot if* $2\pi_0\pi_2>\pi_1^2$; *otherwise the Scatchard plot initially increases, but then decreases monotonely after reaching a unique maximum.*

The proof paraphrases that of (ii) above.

(iv) For the classical MWC model

$$S(x)=\frac{(1+x)^{N-1}+L\theta c(1+cx)^{N-1}}{(1+x)^N+L(1+cx)^N},$$

referring to the results of Sec. 4 and noting that $x[S(x)-a]=V(x)-ax$, we deduce that *for* $(1-c)(\theta c-1)\geq 0$ the Scatchard plot is monotone increasing, and otherwise it can produce at most three intersections with a horizontal line.

(iv) If $P(x)=\prod_{i=1}^{N}(x+\alpha_i)$, $\alpha_i>0$, then $S(x)=\sum_{i=1}^{N}1/(x+\alpha_i)$ is monotonely decreasing for $x>0$.

(v) If $P(x)=\prod_{i=1}^{N}(x^2+\alpha_i)$, $\alpha_1>0$, then $S(x)=\sum_{i=1}^{N}x/(x^2+\alpha_i)$, so that $S(0)=S''(0)=S(\infty)=0$, $S'(0), S'(\infty)>0$, and $S''(0), S''(\infty)<0$, determining the initial and the asymptotic shape of $S(x)$.

These results provide another graphical representation by which to discriminate between different models. See Gibson and Levin [10] for an application of similar ideas.

7. DISCUSSION

In this study we concentrated mostly on mechanisms satisfying Assumptions 1 and 2 of Sec. 2. Within this general framework the initial velocity of reaction has the form

$$V(x)=\frac{xP'(x)}{NP(x)}V_\infty,$$

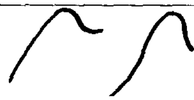
$$P(x)=\sum_{i=0}^{N}\pi_i x^i, \qquad \pi_i\geq 0, \quad i=0,\ldots,N,$$

where x is the initial substrate concentration and V_∞ the limiting saturation velocity. This velocity curve is always monotone increasing, and we characterized its shape for various choices of $P(x)$. The qualitative shape of velocity curves studied in Secs. 2–5 and of some additional cases are described in Table 1.

Our results on the MWC model can serve as diagnostic aids for deciding about its relevance. This problem is of special importance when the velocity curve plotted from the data shows positive cooperativity, while the Koshland et al. models [13] cannot be excluded. It is interesting to note (Proposition 4.1) that the velocity curve of the kinetic MWC model, in contrast to the binding MWC model (where $\theta=1$) is not necessarily monotone. The use of Table 1 and Result IV can help reject the MWC model hypothesis in some situations.

Further information that can be drawn from velocity curves concerns the form and level of interaction and cooperativity between active sites. More details on measures of cooperativity can be found in [12].

TABLE 1

$P(x)=\sum_{i=0}^{N}\pi_i x^i,\ V(x)=\dfrac{xP'(x)}{NP(x)},\ \pi_i\geqslant 0,\ i=0,\ldots,N$	Shape
$K_i=\dfrac{\pi_i}{\pi_{i-1}}$ increasing	 Sigmoidal
$K_i=\dfrac{\pi_i}{\pi_{i-1}}$ concave	 Sigmoidal or hyperbolic
$\pi_i=1,\ 1=0,\ldots,N$	 Sigmoidal
$\prod_{i=1}^{N}(a_i x+\alpha_i),\ a_i,\alpha_i\geqslant 0,\ i=0,\ldots,N$	 Strongly hyperbolic
$\prod_{i=1}^{N}(x^2+a_i),\ a_i\geqslant 0,\ i=1,\ldots,N$	 Up to $2N-1$ inflection points
$(x^2+\alpha)(x+\beta),\ \alpha,\beta>0$	
$(x+a)^2+\alpha,\ a,\alpha>0$	
$(1+x)^N+L(1+cx)^N,\ L,c>0$	
Kinetic MWC model with two conformational forms: $V(x)=\dfrac{x(1+x)^{N-1}+xLc\theta(1+cx)^{N-1}}{(1+x)^N+L(1+cx)^N}$	$c<1$ $\theta<1$ $c>1$ $\theta\geqslant 1$  $c<1$ $\theta>1$ $c>1$ $\theta<1$ 
Generalized MWC model with K conformational forms: $V(x)=\dfrac{x\sum_{i=1}^{K}Q_iL_i(1+Q_ix)^{N-1}\theta_i}{\sum_{i=1}^{K}L_i(1+Q_ix)^N}$	If Q_i and θ_i share the same ordering, then $V(x)$ is monotone increasing. When $\theta_i\equiv 1$, $V(x)$ is positively cooperative. (see [12])

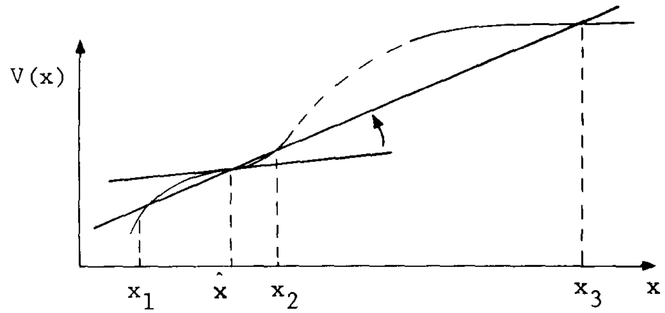


FIG. 5.

In this modeling analysis our aim is to construct a frame of reference relevant to the analysis of actual data. A complementary role is played by statistical methodology, where the problem is that of estimation of parameters (e.g., see Cleland [7], Box and Hill [5], or Pritchard et al. [16]). A comprehensive analysis of data should incorporate information from both directions.

APPENDIX A

To prove Results I–III we first establish a lemma.

LEMMA 1A

Let $V(x)$ be a velocity curve satisfying the following properties: (i) $V(0)=0$; (ii) $V(x)>0$ *for all* $x>0$, *entailing* $V'(0)>0$; (iii) $\lim_{x\to\infty}V(x)=V_\infty<\infty$. *If there exists a concentration level $\hat{x}$ such that $V'(\hat{x})>0$ and*

$$\begin{aligned} V''(x)&<0 \quad \text{for} \quad x<\hat{x}, \\ V''(x)&=0 \quad \text{for} \quad x=\hat{x}, \\ V''(x)&>0 \quad \text{for} \quad x>\hat{x}, \end{aligned} \tag{A.1}$$

then there will be an increasing line crossing $V(x)$ at four points on the positive axis.

Proof. Take the tangent to $V(x)$ at $\hat{x}$ and tilt slightly in a positive direction. The new line will cross $V(x)$ at two neighboring points of $\hat{x}$, at $x=\hat{x}$, and at a fourth point whose existence is assured by the asymptotic limit of $V(x)$ (see Figure 5).

We can now proceed to prove a general proposition (cf. Result II).

LEMMA 2A

Let a, b be arbitrary constants, $a>0$, and $V(x)$ a monotone increasing velocity curve. If $V(x)-(ax+b)$ has at most three positive roots, then $V(x)$ has only hyperbolic or sigmoidal shapes, i.e., at most one inflection point.

Proof. The existence of at most three roots contradicts the conclusion of Lemma 1A, therefore excluding the verification of its assumptions. In other words, the relations described by (A.1) are precluded, and a concave portion of $V(x)$ cannot be followed by a convex portion, so that we must have a hyperbolic or sigmoidal shape.

Let $V(x)=xP'(x)/NP(x)$, $P(x)=\Sigma_{i=0}^{N}\pi_i x^i$, V being the general velocity curve obtained under Assumption 2 with V_∞ normalized to one. We form

$$V(x)-(a'x+b')=\frac{Q(x)}{NP(x)}, \tag{A.2}$$

where $Q(x)=-b\pi_0+\Sigma_{i=1}^{N}[(i-b)\pi_i-a\pi_{i-1}]x^i-a\pi_N x^{N+1}$, $a=Na'$, $b=Nb'$. Then if $Q(x)$ has at most three positive roots, V is either hyperbolic or sigmoidal, by Lemma 2A.

We proceed to the proof of Result I.

To this end, consider in (A.2) the polynomial $Q(x)=-b\pi_0+\Sigma_{i=1}^{N}[(i-b)\pi_i-a\pi_{i-1}]x^i+a\pi_N x^{N+1}$. We have to distinguish two cases:

(i) $b<0$, so that $i-b>0$; then examination of the sequence

$$\left\{\frac{\pi_i}{\pi_{i-1}}-\frac{a}{i-b}\right\}_{i=1}^{N}$$

allows us to conclude, since $K_i=\pi_i/\pi_{i-1}$ is increasing, that the coefficients in $Q(x)$ exhibit at most three sign changes. It follows by invoking the Descartes rule of signs that $Q(x)$ vanishes on the positive axis at most three times.

(ii) $b>0$; for $i<b$ we have $(i-b)\pi_i-a\pi_{i-1}<0$. If $i>b$, we study again $\{\pi_i/\pi_{i-1}-a/(i-b)\}$ and uncover an upper bound of 2 for the sign changes among the remaining coefficients in $Q(x)$. Again, the Descartes rule of signs implies that $Q(x)$ possesses at most three positive real roots. On account of Lemma 2A we may conclude that $V(x)$ is either sigmoidal or hyperbolic. The initial shape of $V(x)$ is ascertained from $\text{sign}[V''(0)]=\text{sign}[2\pi_0\pi_2-\pi_1^2]=\text{sign}[2K_2-K_1]$. But $K_i\uparrow$ implies $K_2>K_1$ and therefore $V''(0)>0$, so that $V(x)$ is always sigmoidal.

The proof of Result II paraphrases the analyses of Result I with the adoption of the concavity assumption on $\{K_i\}$ in place of the monotonicity.

APPENDIX B. SKETCH OF PROOF OF RESULT IV

The assumption $c=0$ reduces (4.1) to

$$V(x)=\frac{x(1+x)^{N-1}}{(1+x)^N+L}.$$

Let $P(x)=\sum_{i=0}^{N}\pi_i x^i=(1+x)^N+L$. Thus, $V(x)=xP'(x)/NP(x)$ is monotone increasing. We note that $\lim_{x\to\infty}V(x)=V_\infty=1$. Then

$$\pi_0=1+L, \qquad \pi_i=\binom{N}{i}, \quad i=1,\ldots,N$$

$$K_1=\frac{\pi_1}{\pi_0}=\frac{N}{1+L}, \quad K_i=\frac{\pi_i}{\pi_{i-1}}=\frac{N-i+1}{i} \quad \text{is decreasing in } i=2,\ldots,N.$$

In order to describe $V(x)$ we have to compare $K_i=(N-i+1)/i$ with $a/(i-b)$, $a>0$, $i=2,\ldots,N$, and we will rely on the representation of $Q(x)$ in (A.2). We distinguish two cases.

(i) $b<0$: then the coefficients of $Q(x)$ have at most three sign changes in the order $+-+-$.

(ii) $b>0$: then the signs in $Q(x)$ are in the arrangement $--+--$.

In both cases there are at most three sign changes and therefore at most three positive roots to $Q(x)$, so that, in accordance with Lemma 2A, $V(x)$ is either hyperbolic or sigmoidal.

The velocity curve (4.1) of the kinetic MWC model displays the same qualitative properties with $c<1$, $L>1$, $\theta<1$ as with $c>1$, $L<1$, $\theta>1$. This comes about by the simultaneous transformation of variables $c\to 1/c$, $L\to 1/L$, $\theta\to 1/\theta$. In order to prove that $V(x)$ of (4.1) is monotone under the conditions of Result IV, we examine $V(x)-a$ for a constant a and ascertain the number of sign changes. This reduces to the study of the function $x(1+x)^{N-1}+L\theta cx(1+cx)^{N-1}-a[(1+x)^N+L(1+cx)^N]$.

The coefficient of x^k is

$$\binom{N-1}{k-1}[1+L\theta c^k]-a\binom{N}{k}[1+Lc^k]. \tag{B.1}$$

Note that

$$\frac{N}{k}=\binom{N}{k}\Big/\binom{N-1}{k-1}$$

is monotone decreasing in k. Now for all $c>0$ and $\theta\geqslant 1$,

$$\frac{1+Lc^k}{1+L\theta c^k}<1.$$

Now consider

$$\frac{1+Lc^k}{1+L\theta c^k}-\gamma \qquad (\gamma<1). \tag{B.2}$$

We have for the numerator of (B.2) $c^kL(1-\gamma\theta)+1-\gamma$, and if $c>1$, then the signs can only be in the order $+(\mp)$. It follows that $(1+L\theta c^k)/(1+Lc^k)$ is decreasing or is increasing for k increasing. This implies that (B.1) exhibits at most one sign change for each $a>0$, and the proof when $c>1$, $\theta \geqslant 1$ is established. The validation for the case $c<1$, $\theta \leqslant 1$ ensues by similar means.

The other statements of Secs. 3, 4, and 5 can be proved by further refinements of the methods outlined above.

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