GENERALIZATION OF THE MODEL BY MONOD, WYMAN AND CHANGEUX FOR THE CASE OF A REVERSIBLE MONOSUBSTRATE REACTION $S \overset{R,T}{\rightleftharpoons} P$

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1. Introduction

So far it has been assumed in the mathematical analysis of oligomeric enzyme kinetics [1] that the reactions catalysed by such enzymes are irreversible (e.g. see [2–7]). However, many of the known oligomeric and polymeric enzymes catalyse essentially reversible reactions, which should be taken into account in the analysis of open multienzyme systems. In this paper we describe a model of a reversible monosubstrate reaction (a generalization of the model by Monod, Wyman and Changeux [2]) and discuss a possible way of the construction of mathematical models of complex reactions catalysed by regulatory enzymes.

2. Kinetic model

Consider a reversible reaction,

$$S \underset{E(R,T)}{\rightleftharpoons} P, \qquad (1)$$

in which the interconversions of the substrate S and the product P are catalysed by the enzyme E which is present in two conformational forms R and T. Assume that the enzyme E has n identical active sites for S and P and the interactions of S and P with different enzyme forms are described by the following kinetic model:

$$R_0 \underset{l_-}{\overset{l_+}{\rightleftharpoons}} T_0 \qquad (2)$$

$$R_{i-1} + S \underset{ik_{-1}}{\overset{(n-i+1)k_{+1}}{\rightleftharpoons}} R_i \underset{(n-i+1)k_{-2}}{\overset{ik_{+2}}{\rightleftharpoons}} R_{i-1} + P$$

$$T_{j-1} + S \underset{jk'_{-1}}{\overset{(n-j+1)k'_{+1}}{\rightleftharpoons}} T_j \underset{(n-j+1)k'_{-2}}{\overset{jk'_{+2}}{\rightleftharpoons}} T_{j-1} + P$$

Here R_0 and T_0 are the free forms of conformations R and T; R_i $(1 \leqslant i \leqslant n)$ and T_j $(1 \leqslant j \leqslant n)$ are active enzyme–substrate complexes (indices i and j indicate the number of molecules of S bound to the corresponding form, R or T); l_+ and l_- the rate constants for isomerization of the free forms of the enzyme E; $(n-i+1)k_{+1}$, $(n-j+1)k'_{+1}$, ik_{-1}, jk'_{-1}, ik_{+2}, jk'_{+2}, $(n-i+1)k_{-2}$, and $(n-j+1)k'_{-2}$ the rate constants of the elementary steps. The relationships between the rate constants and the indices i and j taken account of the variations in the probability of joining and liberating the molecules of S and P by the enzyme molecules caused by variations in the number of free and occupied active sites [8,9].

Let us introduce the following notations:

$K_S = (k_{-1} + k_{+2})/k_{+1}$ is the Michaelis constant of the form R for the substrate;
$K'_S = (k'_{-1} + k'_{+2})/k'_{+1}$ the same for the form T;
$K_P = (k_{-1} + k_{+2})/k_{-2}$ the Michaelis constant of the form R for the product
$K'_P = (k'_{-1} + k'_{+2})/k'_{-2}$ the same for the form T;
$L = l_+/l_-$ the equilibrium constant of the isomerization

$R_0 \rightleftharpoons T_0$ or an allosteric function in the case where allowance is made for the dependence of L on the concentrations of allosteric effectors [2,3].
$V = nk_{+2}e_0$ is the maximum rate of the conversion $S \longrightarrow P$ catalysed by R (e_0 is the full concentration of the enzyme E), $V = nk'_{+2}e_0$ is the same for T;

$x = k_{-1}/k_{+2}$ is the *asymmetry coefficient* for the breakdown of the enzyme–substrate complex in the case of form R (at $x > 1$ complexes R_i are broken down predominantly in the direction of the substrate formation, at $x < 1$ in the direction of the product formation);
$x' = k'_{-1}/k'_{+2}$ is the asymmetry coefficient in case of form T;
$c_S = K_S/K'_S$ is *non-exclusive binding coefficient* [2] for the substrate S (form R is chosen so that the condition $c_S \leqslant 1$ be fulfilled);
$c_P = K_P/K'_P$ the same for the product P;

$a = \frac{V'}{K'_S} / \frac{V}{K_S}$ the *relative activity* of the form T;

$v = v/V$ the *dimensionless rate* of the substrate-to-product conversion (v is the dimensional rate of this conversion);
$\sigma = [S]/K_S$ the *dimensionless substrate concentration;*
$\pi = [P]/K_P$ the *dimensionless product concentration;*

$\overline{R} = \sum_{i=0}^{n} [R_i]/e_0$ the *relative total concentration of the form* R, or a *state function* [2];

$\overline{T} = \sum_{j=0}^{n} [T_j]/e_0$ the *relative total concentration of the form* T;

$Q = \sum_{i=0}^{n} [R_i] / \sum_{j=0}^{n} [T_j] = \overline{R}/\overline{T}$ a *quotinent function* [2].

3. Mathematical model

Applying the conservation and mass action laws to model (2), we get the following system of stationary relations: $[T_0] = L\,[R_0]$

$$\begin{aligned}
&(n-i+1)\,[R_{i-1}]\,(k_{+1}\,[S] + k_{-2}\,[P]) \\
&\quad - i\,(k_{-1} + k_{+2})\,[R_i] = 0, \\
&(n-j+1)\,[T_{j-1}]\,(k'_{+1}\,[S] + k'_{-2}\,[P]) \\
&\quad - j\,(k'_{-1} + k'_{+2})\,[T_j] = 0,
\end{aligned} \tag{3}$$

$$v = -\frac{d[S]}{dt} = \sum_{i=1}^{n} (ik_{+2}\,[R_i] - (n-i+1)\,k_{-2}\,[P]$$

$$[R_{i-1}]) + \sum_{j=1}^{n} (jk'_{+2}\,[T_j] - (n-j+1)\,k'_{-2}\,[P]$$

$$[T_{j-1}]),$$

and after simple transformations we obtain the equation for the dimensionless rate

$$v = \frac{\sigma - x\pi}{1 + \sigma + \pi}\,\overline{R} + a\,\frac{\sigma - x'\frac{c_P}{c_S}\pi}{1 + c_S\sigma + c_P\pi}\,\overline{T} \tag{4}$$

where

$$\overline{R} = \frac{Q}{1+Q};\quad \overline{T} = \frac{1}{1+Q},\quad Q = \frac{q_n}{L},\quad q = \frac{1+\sigma+\pi}{1 + c_S\sigma + c_P\pi}.$$

In case of a thermodynamical equilibrium of reaction (1), two conditions must simultaneously be fulfilled according to the detailed balance principle:

$$\sigma - x\pi = 0,\quad \sigma - x'\frac{c_P}{c_S}\pi = 0 \tag{5}$$

This yields the relation

$$x = x'\frac{c_P}{c_S}, \tag{6}$$

with consideration for which equation (4) takes the form

$$v = \frac{\sigma - x\pi}{1 + \sigma + \pi}\,\overline{R} + a\,\frac{\sigma - x\pi}{1 + c_S\sigma + c_P\pi}\,\overline{T} \tag{7}$$

or

$$v = \frac{\sigma - x\pi}{1 + \sigma + \pi}\cdot\frac{Q + aq}{1+Q} \tag{8}$$

One remarkable feature of eq. (8) is worth noticing. Its right hand side is a product of two functions which describe two principally different processes associated with the oligomeric enzyme activity. One of the functions

$$\Phi = \frac{\sigma - x\pi}{1 + \sigma + \pi} \tag{9}$$

is a dimensionless *rate law function* for a single active site of the form R. The shape of this function is determined only by the mechanism of elementary interactions of the substrate S and product P with

one active site of the enzyme. In contrast to the rate function Φ, the function

$$\Psi = \frac{Q + aq}{1 + Q} \tag{10}$$

makes allowance for the effect on the enzyme E catalytic activity of indirect cooperative interactions between its active sites and of the conformational transitions R$\rightleftharpoons$T. The function Ψ we shall subsequently term the *regulatory function.*

If enzyme E belongs to class K (i.e. if $V'/V = 1$), then

$$\Psi = \frac{Q + c_S q}{1 + Q} \tag{11}$$

In this particular case at $\pi = 0$ or $\sigma = 0$ equation (8) is identical to the equation derived by Monod, Wyman and Changeux [2], except for the difference in notations.

If enzyme E belongs to class V (i.e. $c_S = c_P = 1$), then

$$\Psi = \frac{1 + aL}{1 + L}. \tag{12}$$

In figs.1 and 2 presented are the families of the curves $v(\sigma)$ and $v(\pi)$ constructed for enzymes of class K (fig.1) and class V (fig.2). The points of intersection of the curves $v(\sigma)$ and $v(\pi)$ with the abscissa axis represent the states of thermodynamical equilibrium of the reaction in which $\sigma = x\pi$. The negative values of the rate imply that the substrate is formed from the product. As seen from equation (8), $v < 0$ if $\sigma < x\pi$. By applying the functions Φ and Ψ, the velocity of a reaction catalysed by an olygomeric enzyme may be represented in a fairly general form

$$v = \Phi \cdot \Psi \tag{13}$$

4. Discussion

The presentation of eq. (8) in the form of (13) may seem far too abstract. However, it is this presentation, free of the specific effects of the catalytic and regulatory enzyme sites, that dictates a very simple way of construction of the theory of complex multisubstrate reactions catalysed by regulatory enzymes. Briefly, this way is the following.

The general form of the regulatory function Ψ, as determined by equation (10), is *invariant* with respect to the action mechanism of the catalytic sites, it depends on the *assumed* mechanism of conformational

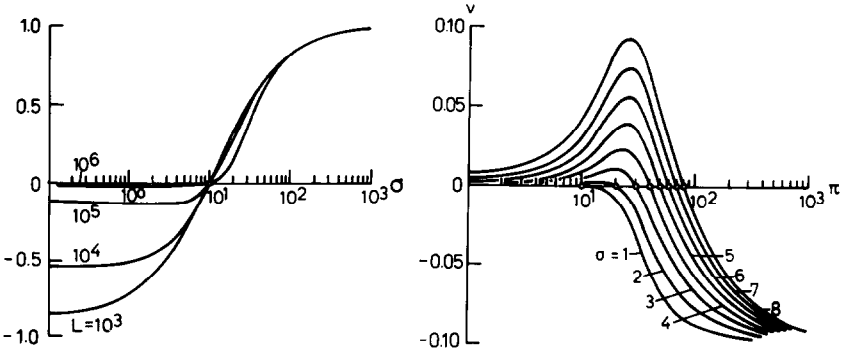

Fig.1. Dimensionless rate $v = v/V$ of reversible reaction (1) as a function of the dimensionless concentrations of the substrate, $\sigma = [S]/K_S$, and of the product, $\pi = [P]/K_P$, for enzyme E of class K as calculated from eq. (8). (Left) Family of the curves $v(\sigma)$ constructed for various values of the isomerization constant L, as shown in the figure, and for $\pi = 10, x = 1, c_S = c_P = 10^{-4}, n = 4$. *Note that the rate of the back reaction ($v<0$) depends drastically on L whereas that of the forward reaction ($v>0$) is only little dependent on L.* This feature of reaction (1) seems to explain the apparent unidirectedness of the action of allosteric effectors on reversible reactions catalysed by olygomeric enzymes. (Right) Family of the curves $v(\pi)$ constructed for various values of the dimensionless substrate concentration, σ, as shown in the figure, and for $x = 0.1, a = 0, c_S = c_P = 0, L = 10^6, n = 4$. *Note that product P acts as an activator of the enzyme when in small and as an inhibitor when in large concentrations.* The activating isosteric effect of product P at $n>1$ may be of an apparent cooperative character (i.e. Hill's coefficient $n_H > 1$ for that portion of the curve $v(\pi)$ where $dv/d\pi > 0$) and is the stronger the smaller x and a and the larger L.

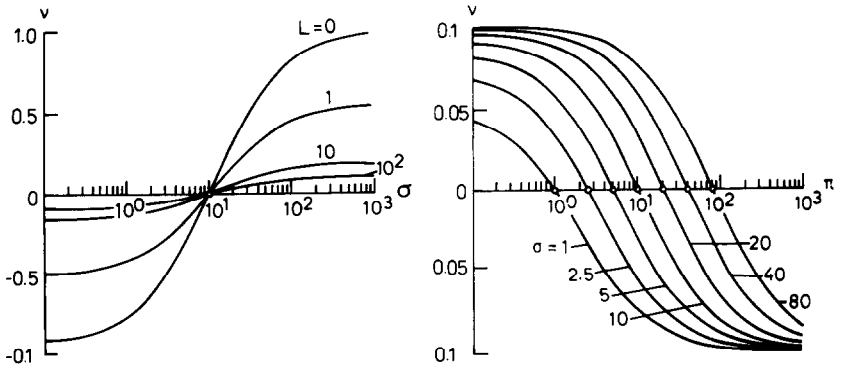

Fig.2. Dimensionless rate $v = v/V$ of reversible reaction (1) as a function of the dimensionless concentrations of the substrate, $\sigma = [S]/K_S$, and product, $\pi = [P]/K_P$, for enzyme E of class V as calculated by eq. (8). (Left) Family of the curves $v(\sigma)$ constructed for various values of the isomerization constant L, as shown in the figure, and for $\pi = 10$, $x = 1$, $a = 0.1$, $c_S = c_P = 1$, $n = 4$. As the regulatory function Ψ of the enzyme of class V (12) is independent of the substrate and product concentrations, *the variation of L similarly affects the rates of the forward ($v > 0$) and back ($v < 0$) reactions.* (Right) Family of the curves $v(\pi)$ constructed for various values of the dimensionless substrate concentration, σ, as shown in the figure, and for $x = 1$, $a = 0.1$, $c_S = c_P = 1$, $L = 10^3$, $n = 4$. *Note that in this case product P acts only as an inhibitor of enzyme E.*

transitions. As will be shown elsewhere, with due regard to the detailed balance principle the generalized versions of the model by Monod, Wyman and Changeux, which make allowance for the existence of isomerizations $R_i \rightleftharpoons T_i$ $(0 \leqslant i \leqslant n)$ [10,11], and under some limitations 'the square model' [5,12] too, may be described by a function of type (10) whatever the mechanism of the catalytic site action (the number of substrates, the order of their binding and the reaction reversibility).

Since the rate law function Φ describes the reaction kinetics of the catalytic site alone with no allosteric or cooperative interactions involved, in most applications there is no need to derive it for oligomeric enzymes which catalyse complex reactions. For many complex (multisubstrate, reversible) reactions the single-site rate law functions Φ have already been derived [7,13–20].

Thus, using the function Φ which may be found in the appropriate publications or derived with the aid of the graph theory [21] or a computer, one can construct the small quotinent function q and the quotinent function Q, and then, using eq. (10), the regulatory function Ψ. The product of $\Phi \cdot \Psi$ yields the dimensionless velocity v for a complex reaction.

In our opinion, two important conclusions result from analysis of model (8). First, *a product(s) of reversible reactions catalysed by olygomeric enzymes can produce a profound isosteric activating or inhibiting effect on the reaction rate* (fig.1, right). The isosteric product activation of an olygomeric enzyme may reveal an apparent cooperative character and is the stronger the greater L and the smaller a and x. Second, *on product accumulation the forward and back reactions catalysed by an olygomeric enzyme of type K may strongly differ in their sensitivity to displacement in the equilibrium $R \rightleftharpoons T$ caused by allosteric effectors or by any physico-chemical factors* (fig.1, left).

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