AN ADDITIONAL POSSIBILITY OF USING THE HILL COEFFICENTS

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The possibility of using the Hill coefficients to estimate the first $K^0_{0.5(A1)}$ and the second $K^0_{0.5(B1)}$ constant for the active center of two-subunits of enzymes is considered. The method to calculate $K^0_{0.5(A1)}$ and $K^0_{0.5(B1)}$ constants is discussed.

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Introduction

A considerable number of papers have been published in enzymology dealing with the properties of allosteric (associated as subunits) enzymes. These studies suggested positive or negative cooperativity on the basis of the mechanism of the their action on a substrate. The kinetics of the studies invariably showed the presence of the Michaelis-Menten kinetics.[1-9], in which a saturation curve is obtained when the initial velocity v_0 of the reaction is plotted against the substrate concentration as is shown in curve *a*, Figure 1.

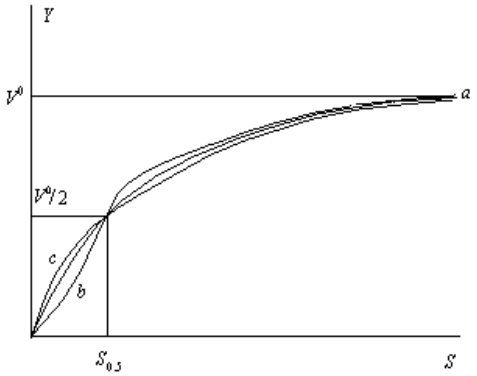

Figure 1. The reaction progress curve showing the dependence on progress curve of velocity changes v_0 on the concentration of substrate *S* when the Hill coefficient $h = 1$ (curve *a*, bold line), curve *b* if $h > 1$, and curve *c* when $h < 1$. *Y* – the parameter of enzyme saturation; $V^0 \rightarrow Y$ (by $S >> K^0_m$).

The aim of this investigation was to obtain the information not only on the catalytic activity (V^0), but also on the strength of binding (K^0_m) between the enzyme and the substrate.[3, 7-9, 11, 14-16] The (Eq. 3) is obtained when the Hill coefficient *h* is equal to 1.

The dependence of the initial velocity of the reactions, catalyzed by cooperative enzymes, is given by the Hill equation (1), whereas the Michaelis-Menten equation is expressed in Equation (2).

$$v_0 = V^0 \frac{S_0^h}{S_0^h + S_{0.5}^h}, \quad (1)$$

$$v_0 = V^0 \frac{S}{S + K_m^0} = V^0 \frac{S}{S + S_{0.5}}, \quad (2)$$

where V^0 is the maximum reaction velocity reached at the maximum substrate concentration *S* as given in equation (2) wherein K^0_m is the Michaelis constant which is equal to the substrate concentration at which the reaction rate is half of V_{max}.

The comparison of the equation (1) with the equation (2) suggests that at $h = 1$ $S^h_0 = S$ and $S^h_{0.5} = K^0_m$.

In enzymology the value of the Michaelis constant K^0_m is characteristic of the enzyme signifying the strength of binding between the enzyme and the substrate; the lower is the value of the K^0_m constant, the greater is the binding strength between the enzyme and the substrate i.e. the substrate conversion is more effective (V^0 increases). The Hill coefficient *h* in equation (1) determines the number of substrate-binding centers on the molecule of the allosteric enzyme and the strength of the binding between the active centers and the substrate. The Hill coefficient *h* is dimensionless.[17] As a general rule, when $h > 1$, indicates the positive cooperativity in a process of substrate conversion; the binding of the second molecule on the second active center of the enzyme increases the initial velocity v_0 of conversion of the first substrate on the first active center of the enzyme (Fig. 1, curve *b*); if the Hill coefficient is $h < 1$, the binding of the second substrate (on the second active center of the enzyme) makes the binding of the first substrate on the first active center weaker, hence the velocity v_0 of the conversion of the first substrate slows down pointing to the negative cooperativity in the mechanism of enzyme action (Fig. 1, curve *c*).

The equation (2) is used to test the data for the strength of the binding of an enzyme to a substrate (an inhibitor or activator), assuming that $h = 1$).[1, 6, 18-20]

$$V^0 \frac{S_0^h}{S_o^h + S_{0.5}^h} \rightarrow V^0 \frac{S}{S + K_m^0} \quad (3)$$

It can be useful if $h = 1$ or it is not larger than the error interval:

$$S_{0.5}^h \rightarrow S_{0.5}^1 = K_m^0. \quad (4)$$

Let us consider one of the most common "parallel-subsequent" schemes of the catalytic conversion of the substrate by the enzymes consisting of two E_A and E_B subunits:

$$NS + \left(\frac{E_A}{E_B}\right) \rightarrow \left(\frac{E_A S_3}{E_B^*} \rightarrow \frac{E_A^*}{E_B S_2}\right) + Pr(s_1) \rightarrow \left(\frac{E_A^*}{E_B S_2} + \frac{E_A S_1}{E_B^*}\right) + Pr(s_2) + K \quad (5)$$

where E_A is a subunit located on the "upper" part of the enzyme and E_B is on the lower part, (Eq. 5) is associated with noncovalent powers of interaction i.e. hydrogen, hydrophobic etc.

There exists an additional possibility of using the Hill coefficients when $h > 1$ or $h < 1$.

Positive cooperativity ($h > 1$). Let us consider that the value for the Hill coefficient $h = 1.75$ (dimensionless units). If this coefficient is written as a sum of two numbers: $h = (1.0 + 0.75)$, Eq. (4) can be rewritten as:

$$S_{0.5}^{1.75} \rightarrow S_{0.5}^1 S_{0.5}^{0.75} = K_m^0 S_{0.5}^{0.75}, \quad (6)$$

where $S^1{}_{0.5}$ is the concentration of first substrate bound to the "upper" subunit E_A of the enzyme.

The relationship $h = 1$ is necessary and sufficient condition (according to Michaelis-Menten theory) for the conversion of the first substrate on subunit E_A into reaction product $Pr(s_1)$, and the remaining value 0.75 characterizes the strength of binding of the second substrate on the second subunit E_B.

It follows that at half value of the substrate concentration, $v_0 = V^0/2$. Taking $S_{0.5} = 4.4 \times 10^{-4}$ M (for ease of analysis), the value of $S_{0.5(A)}$ for the binding of the first substrate on the active center of subunit E_A is given by the equation (7):

$$S_{0.5(A)} = K^0{}_{mA} = \frac{4.4 \times 10^{-4}\ \text{M}}{(1 + 0.75)} = 2.514 \times 10^{-4}\ \text{M}. \quad (7)$$

Similarly the value of $S_{0.5(B)}$ (for the binding of the second substrate on the active center of subunit E_B) is expressed by the equation (8):

$$S_{0.5(B)} = K^0{}_{mB} = \frac{2.514 \times 10^{-4}\ \text{M}}{0.75} = 3.35 \times 10^{-4}\ \text{M}. \quad (8)$$

The values of the parameters h and $S^1{}_{0.5}$ are reported very often.[1-7,12,18-22]

Negative cooperativity ($h < 1$). Assuming $h = 0.85$, an analysis similar to that described in equations (1 – 8) can be made. Thus it implies that the binding strength of the second molecule of the substrate (S) on the second subunit E_B of the enzyme, expressed by the equation (10), is more efficient than the binding strength of the molecule of the first substrate S on subunit E_A, which is expressed by the equation (9)

$$S_{0.5(A)} = K^0{}_{mA} = 4.4 \times 0.85 \times 10^{-4}\ \text{M} = 3.74 \times 10^{-4}\ \text{M}, \quad (9)$$

and molecule of the second substrate S on subunit E_B:

$$S_{0.5(B)} = S_{0.5(A)} \times 0.85 = 3.74 \times 0.85 \times 10^{-4}\ \text{M} = 3.179 \times 10^{-4}\ \text{M}. \quad (10)$$

Symmetrically-opposite position of the Hill coefficient (h) in (Eqs. 9 – 10) with regard to (Eqs. 7 and 8) is in agreement both with the symmetry of the position of curves b and c (Fig. 1) and with the ratio of the values of the relevant coefficients ($h < 1$) and ($h > 1$).

Eqs. (7, 8 and 9, 10) can be useful for the calculation of the values of $K^0{}_{mA}$ and $K^0{}_{mB}$ constants in order to gain the insight into the strength of the binding of the enzyme to a substrate not only on the first $K^0{}_{mA}$ but also on the second $K^0{}_{mB}$ active center of two-subunit enzymes.

The above could probably be applied to the analysis of data of not only bi- (E_A/E_B) and tetra-($E_{A1}E_{A2}/(E_{B1}E_{B2}$)) but two-subunit enzymatic complexes of more higher order ($E_{A1}E_{A2}E_{A3}/(E_{B1}E_{B2}E_{B3}$) as well.

Table 1. An additional possibility of using the Hill coefficients.

Sources of enzyme	pH	$S_{0.5}$, mM	n_H	K_m, mM	K_{mA}, mM	K_{mA}, mM	K_{mB}, mM
L, plant.	5.5	0.65[21]	1.1	0.77		*0.59**	*5.91**
	7.8	0.22[21]	1.0	0.23	0.23 [21]	*0.22*	
	8.5	0.36[21]	1.1	0.39		*0.327*	*3.273*
L. acido.	6.0	0.27[21]	1.1			*0.245*	*2.45*
	7.6	0.37[21]	1.8			*0.206*	*0.257*
	8.3	0.36[21]	2.5			*0.144*	*0.096*
Mtb FolB		0.48 μM[22]	2.0			*0.00024*	*0.00024*
G360P		75 mM[16]	0.68			*51.0*	*34.7*
ATPase		63 μM[23]	0.6			*0.038*	*0.023*

*Iitalicized figures have been obtained using Eqs. 7 – 10. *L. plant* – phosphofructokinase from *L. plantarum*; *L. acido* - phosphofructokinase from *L. acidophilus*; *G360P* – mutant CTP synthase from *Lactococcus lactis*; *Mtb FolB* – 7,8-dehydroneopterin aldolase from *M. tuberculosis*; *ATPase* – Na/K-ATPase from Pig Kidney

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