J. Iran. Chem. Soc., Vol. 6, No. 2, June 2009, pp. 219-229.

JOURNAL OF THE
Iranian
Chemical Society

Enzyme Inhibition and Activation: A General Theory

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(Received 6 April 2009, Accepted 11 May 2009)

The rate of an enzymatic reaction may be changed by a moderator. Usually, the effect is to reduce the rate, and this is called inhibition. Sometimes the rate of enzyme reaction is raised, and this is called activation. Not only enzyme activation is subject of a less detailed presentation, but also enzyme inhibition and activation are very often discussed independently in enzymology. I attempt to introduce a general model of enzyme inhibition and activation to allow one to interpret inhibition and activation from a mechanistic or physical perspective using the significance of cooperativity as a new approach. The magnitude of interaction between substrate and inhibitor binding sites is given by the α parameter and the magnitude of increasing catalytic reaction constant is given by the β parameter, which both parameter values characterize the type of inhibition and activation. The moderation of mushroom tyrosinse is described by application of the model as a typical.

Keyword: Inhibition, Activation, Cooperativity, Enzyme kinetics

INTRODUCTION

Compounds that influence the rates of enzyme-catalyzed reactions are called modulators, moderators, or modifiers [1]. Usually, the effect is to reduce the rate, and this is called inhibition. Sometimes the rate of enzyme reaction is raised, and this is called activation. Accordingly, the compounds are termed inhibitors or activators. Inhibitors of enzymes are used as pharmaceutical agents in human and veterinary medicine as well as herbicides and pesticides. Enzyme inhibition is usually extensively analyzed due to its great interest both in the study of enzyme mechanisms [2] and in pharmacological studies [3], while enzyme activation is subject of a less detailed presentation, if any at all. While a quantitative understanding of enzyme inhibition is certainly important the actual mechanistic utility of enzyme inhibition is qualitative. When studying these phenomena, one has to understand the molecular events leading to the experimentally observed effects. A number of techniques are available for reaching a basic explanation for the so-called mechanism or mode of action of a substance on the enzyme reaction under investigation. Despite the similitude between the two processes, enzyme inhibition and activation are very often discussed independently in enzymology text-books [4-7].

Inhibitors are usually divided into two groups [1]. The first consists of reversible inhibitors that form noncovalent interactions with various parts of the enzyme surface, which can be easily reversed by dilution or dialysis. The second group comprises irreversible inhibitors that interact with different functional groups on the enzyme surface by forming strong covalent bonds that often persist even during complete protein breakdown. A reversible inhibitor binding to an enzyme may be reduced the enzyme activity as completely or partially inhibition. Considering only the framework of an enzyme reaction exposed to the action of a reversible inhibitor, the degree of inhibition (i) is defined as the reduction of the

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rate divided by the rate of uninhibited reaction:

$$i = (V_o - V)/V_o \quad (1)$$

where V and V_o are the rates of inhibited and uninhibited reactions, respectively.

Many general biochemistry textbooks commonly used in undergraduate and medical school courses revealed that the description of the common types of enzyme reversible inhibition varied from simply stating whether the inhibitor increased or decreased the Michaelis constant (K_m) and maximal velocity (V_{max}) all the way to showing mathematically how the K_m and V_{max} were affected by an inhibitor [8]. Most of the understanding derived from kinetic studies comes from the patterns rather than the actual numbers [8-9].

We can deduce equations to describe the dependence of reaction rate and parameters of the enzyme-catalyzed reaction on the concentrations of substrate and/or moderator. A kinetic analysis of the modification of the reaction rate can be undertaken without any need of prejudging the effect (activation or inhibition) of the moderator on the enzyme. A combined application of our knowledge from thermodynamics and kinetics binding parameters may be used to better understanding and characterization of enzyme inhibition and activation. The purpose of this article is to describe a general model of enzyme inhibition and activation in the point of both kinetic and thermodynamic ligand binding that allows one to interpret inhibition and activation from a mechanistic or physical perspective. This approach has been used for the last 15 years for teaching enzyme inhibition and has been received favorably by the students.

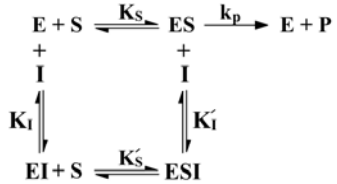
DIFFERENT TYPES OF REVERSIBLE INHIBITION

Four types of reversible inhibition have been introduced by the relation between the velocities of the inhibited and uninhibited reactions:

1) In the *competitive* inhibition type, similarities between the substrate (**S**) and the inhibitor (**I**) exclude simultaneous binding of **I** and **S**. It is obvious that the degree of inhibition is reduced by increasing substrate concentration if the inhibitor competes for the active site with substrate.
2) In the *noncompetitive* inhibition type, **S** and **I** can attach to the enzyme simultaneously since their structures are unlikely to be similar so that they do not compete for the active site. Both **I** and **S** bind to the enzyme in different sites independently. The degree of inhibition is unaffected by change in the substrate concentration.
3) In the *uncompetitive* inhibition type, **I** can attach to the enzyme after binding of **S**. It means that a change in the structure of enzyme should be occurred by binding of **S**, which the inhibitor binding site becomes exposed, so that **I** can bind to its binding site. The degree of inhibition is increased by change in the substrate concentration.
4) In the *mixed* inhibition type, **S** and **I** can attach to the enzyme simultaneously in two different sites dependently. The affinity of binding **S** or **I** is affected by the binding of the other. The degree of inhibition is affected by change in the substrate concentration depends on the interaction between two sites of **S** and **I.**

Mixed type inhibition is often referred to as noncompetitive inhibition, but we (as many other authors [4-5]) employed a more restricted definition for noncompetitive inhibition. It is important to recognize that the term mixed type inhibition does not imply that this type of inhibition is a mixture of different types of inhibition. Mixed type inhibition is a distinct type of inhibition which is characterized by the affect of the inhibitor on the slope and intercept of the Lineweaver-Burk plot due to the interaction between two sites of **S** and **I**.

It is possible to compare four types of reversible inhibitors in a thermodynamics view as shown in Scheme 1. For a mixed type inhibition, the equilibrium dissociation constants of **S** and E (K_S) and **S** and E**I** (K'_S) as well as the equilibrium dissociation constants of **I** and E (K_I) and **I** and E**S** (K'_I) are different due to the interaction between sites of **I** and **S**.



Scheme 1. A scheme for showing the mixed type inhibition to compare four types of reversible inhibitors

Hence, there is an interaction between both sites of **I** and **S**, if the inhibitor is a mixed type. If there is no interaction between both **S** and **I** sites, this results $K_S = K'_S$ and $K_I = K'_I$, the inhibitor is a noncompetitive type due to the presence of independent binding sites for **S** and **I**. If the binding of each **S** and **I** prevents the binding of the other, then $K'_S = K'_I = \infty$, the inhibitor is a competitive type. If the binding of **I** is done only after the binding of **S** to the enzyme, $K_I = \infty$ and $K'_S = 0$, the inhibitor is an uncompetitive type.

The Lineweaver-Burk equation, which may be used to determine the type of inhibition, is [4-5]:

$$\frac{1}{V} = \frac{K_m'}{V_{max}'} \times \frac{1}{[\mathbf{S}]} + \frac{1}{V_{max}'} \qquad (2)$$

V and V_{max}' are the initial and maximum velocities, respectively, and K_m' is the Michaelis-Menten constant in the presence of an inhibitor. The Lineweaver-Burk plot is a plot of 1/V *vs.* 1/[**S**]. The slope and the vertical intercept (Y-int) in the presence of an inhibitor are as below:

$$\text{Slope} = K_m'/V_{max}' = A_0 + A_1\,[\mathbf{I}] \qquad (3a)$$

$$\text{Y-int} = 1/V_{max}' = B_0 + B_1\,[\mathbf{I}] \qquad (3b)$$

A_0, A_1, B_0 and B_1 are constants depend on the type of inhibition. See Table 1.

Following the position of the intersect of the Lineweaver-Burk plots in the presence and absence of inhibitor may be used to determine the type of inhibition as shown in Fig. 1. The Lineweaver-Burk plots in the absence and presence of a

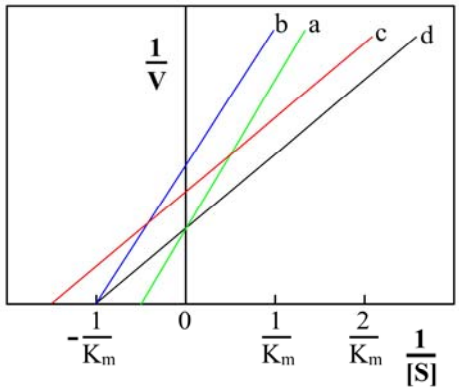


Fig. 1. The position of the intersect of the Lineweaver-Burk plots in the presence (a: competitive, b: noncompetitive, c: uncompetitive) and absence (d) of inhibitor may be used to determine the type of inhibition.

competitive inhibitor in different fixed concentrations are intersected each other on the Y-axis means that V_{max} does not change by the competitive inhibitor. See Fig. 2a. The Lineweaver-Burk plots in the absence and presence of a non-competitive inhibitor in different fixed concentrations are intersected each other on the X-axis means that K_m does not change by the non-competitive inhibitor. See Fig. 2b. The Lineweaver-Burk plots in the absence and presence of an uncompetitive inhibitor in different fixed concentrations are parallel lines means that both K_m and V_{max} change by the uncompetitive inhibitor. See Fig. 2c. The Lineweaver-Burk plots in the absence and presence of a mixed type inhibitor in different fixed concentrations may intersected each other over (if $\alpha > 1$) or below (if $\alpha < 1$) the negative X-axis means that

Table 1. Constant Values of A_0, A_1, B_0 and B_1 for Different Types of Inhibition

	A_0	A_1	B_0	B_1
Competitve	K_m/V_{max}	K_m/K_IV_{max}	$1/V_{max}$	0
Noncompetitive	K_m/V_{max}	K_m/K_IV_{max}	$1/V_{max}$	$1/K_IV_{max}$
Uncompetitive	K_m/V_{max}	0	$1/V_{max}$	$1/K_IV_{max}$
Mixed	K_m/V_{max}	K_m/K_IV_{max}	$1/V_{max}$	$1/\alpha K_IV_{max}$

K_m and V_{max} are the Michaelis-Menten and the maximum velocity in the absence of inhibitor,α is the interaction parameter for binding sites of **S** and **I** and K_I is dissociation equilibrium constant of binding **I** to the enzyme (E).

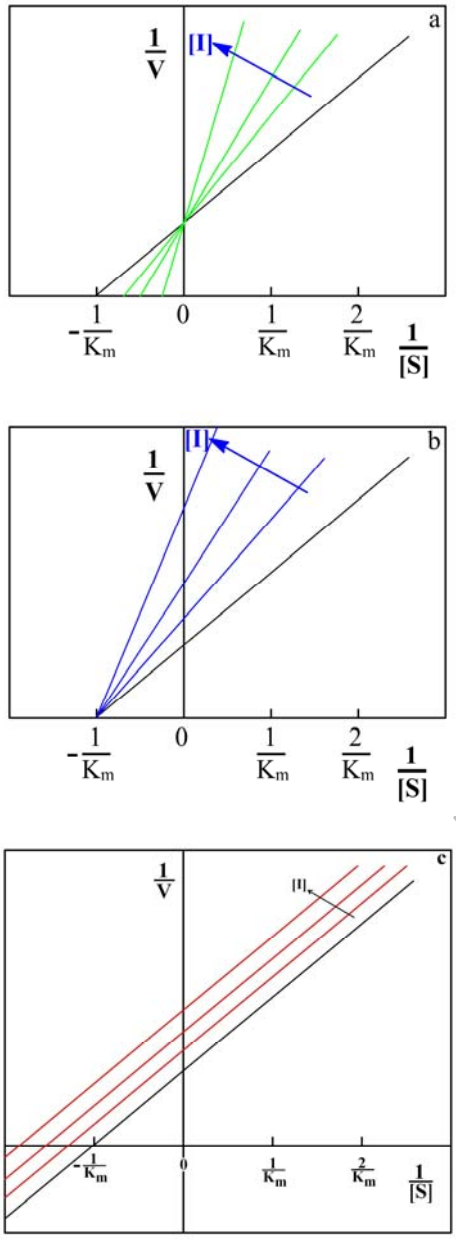


Fig. 2. (a) The Lineweaver-Burk plots in the absence and presence of a competitive inhibitor in different fixed concentrations are intersected each other on the Y-axis. (b) The Lineweaver-Burk plots in the absence and presence of a non-competitive inhibitor in different fixed concentrations are intersected each other on the X-axis. (c) The Lineweaver-Burk plots in the absence and presence of an uncompetitive inhibitor in different fixed concentrations are parallel lines.

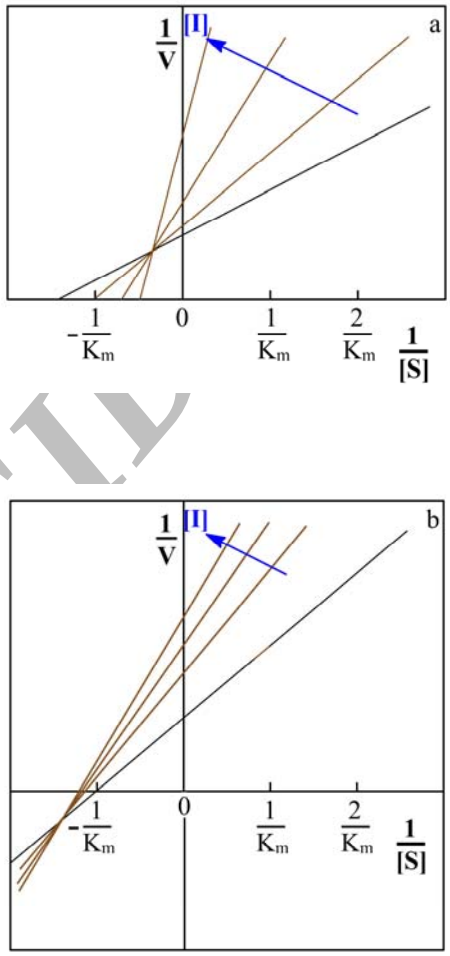


Fig. 3. The Lineweaver-Burk plots in the absence and presence of a mixed type inhibitor in different fixed concentrations may intersected each other over (if $\alpha > 1$: a) or below (if $\alpha < 1$:b) the negative X-axis means that both K_m and V_{max} change by the mixed type inhibitor.

both K_m and V_{max} change by the mixed type inhibitor. See Figs. 3a and b.

The type of depending of slope and Y-int on the inhibitor concentration can also be used to evaluate the type of inhibition according to a plot named the secondary plot. Moreover, the secondary plot can be useful to determine the inhibition constant (K_I) and the interaction value (α) between substrate and inhibitor depending binding sites. See Fig. 4.

DIFFERENT TYPES OF REVERSIBLE ACTIVATION

The activation of an enzyme can be done by increasing the affinity of binding of the substrate to the enzyme ($\alpha < 1$) and/ or increasing the catalytic constant (k_p) value by β ($k'_p = \beta\ k_p$). See Scheme 2. Different types of reversible activation may be observed by the role of two α and β parameters:

1) The moderator may decrease the affinity of binding of the substrate to the enzyme ($\alpha > 1$); however, increase the catalytic constant ($\beta > 1$). If decreasing of the affinity of binding of the substrate to the enzyme overcomes to the increasing of the catalytic constant ($\alpha > \beta$) the moderator will inhibit the enzyme in the low concentration of the substrate and will activate the enzyme in the high concentration of the substrate. See Fig. 5a, which shows the position of the intersect of the Lineweaver-Burk plots. More increasing of the β value respect to the α value can change the inhibition characterization of the moderator to be as an activator even in the low concentration of the substrate. See Fig. 5b, which shows the position of the intersect of the Lineweaver-Burk.
2) In the case of $\alpha = \beta > 1$, which the moderator decreases the affinity of binding of the substrate to the enzyme ($\alpha > 1$) and increases the catalytic constant ($\beta > 1$), the moderator activates the enzyme. The Lineweaver-Burk plots in the absence and presence of the activator in different fixed concentrations are parallel lines; however, in contrast to the uncompetitive inhibition, the K_m value decreases and the V_{max} value increases. See Fig. 5c.
3) In the case of $\alpha = 1$ and $\beta > 1$, the moderator activates the enzyme by increasing the catalytic constant ($\beta > 1$) without any effect on the affinity of binding of the substrate to the enzyme ($\alpha = 1$). This type of activator is not an essential activator because the enzymatic reaction is done without the activator. The Lineweaver-Burk plots in the absence and presence of a non-essential activator in different fixed concentrations are intersected each other on the X-axis means that K_m does not change by the non-essential activator. See Fig. 5d.
4) In the case of $\beta = 1$ and $\alpha < 1$, the moderator activates the enzyme by decreasing the the affinity of binding of the substrate to the enzyme ($\beta = 1$) without any effect on the catalytic constant ($\beta = 1$). This type of activator is not an essential activator because the enzymatic reaction is done without the activator. The Lineweaver-Burk plots in the absence and presence of this type of non-essential activator in different fixed concentrations are intersected each other on the Y-axis means that V_{max} does not change but K_m decreases. See Fig. 5e.

In all types of inhibitors described above $\beta = 0$. Such inhibitors are called *totally inhibitors*. *Partial inhibitors* are said for $\beta < 1$.

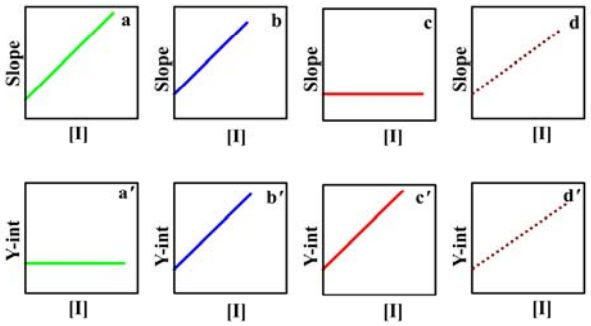
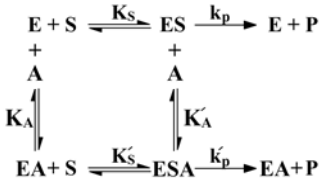


Fig. 4. The type of depending of slope (a, b, c and d for competitive, noncompetitive, uncompetitive and mixed inhibitors, respectively) and Y-int (a′, b′, c′ and d′ for competitive, noncompetitive, uncompetitive and mixed inhibitors, respectively) on the inhibitor concentration can also be used to evaluate the type of inhibition according to a plot named the secondary plot. Moreover, the secondary plot can be useful to determine K_I and α values.



Scheme 2. The activation of an enzyme can be done by increasing the affinity of binding of the substrate to the enzyme and/or increasing the catalytic constant

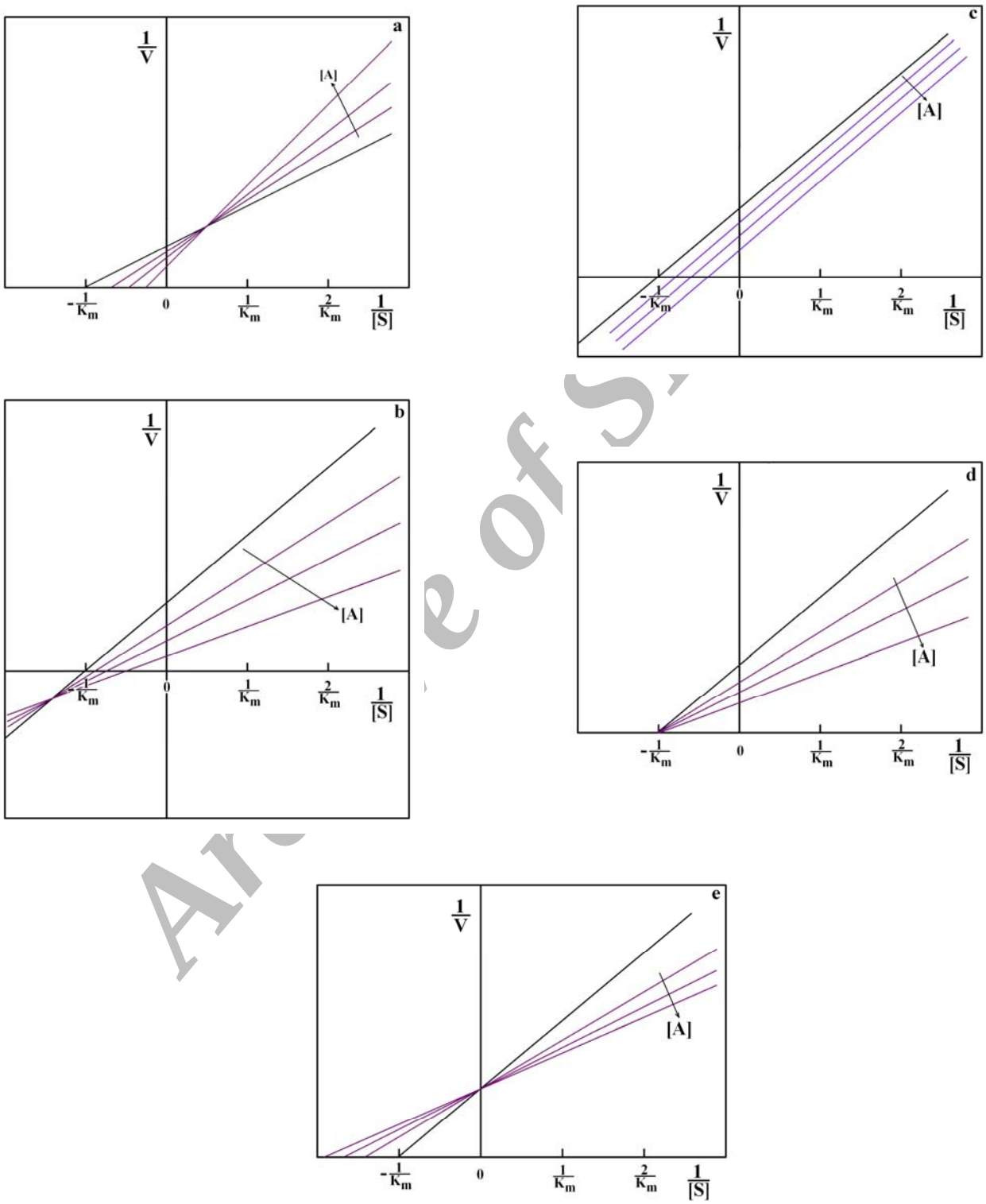


Fig. 5. The position of the intersect of the Lineweaver-Burk plots in the presence (a: $\alpha > \beta > 1$, b: $\beta > \alpha > 1$, c: $\alpha = \beta > 1$, d: $\alpha = 1$ and $\beta > 1$, e: $\beta = 1$ and $\alpha < 1$) and absence of activator may be used to characterize the type of activation.

The Concept of Cooperativity

If binding of a ligand to one site on a macromolecule influences the affinity of other sites, the binding is said to be *cooperative*. Such cooperativity can be positive (binding at one site increases the affinity of others) or *anticooperative* (if the affinity of other sites is decreased). Such effects may be evaluated by the Hill coefficient (n), which is an index of the cooperativity. The value of the Hill coefficient can be useful as a diagnostic test for binding type. If n = 1, the binding is *noncooperative* and all the sites are identical and independent. If n > 1, the binding must be positively cooperative (there is interaction between sites). If n < 1, the macromolecule either has more than one class of sites, or the binding is anticooperative (negatively cooperative) [10-11].

The value of interaction parameter (α) shows extend of the cooperativity between two substrate and moderator binding sites. Different types of interaction between two substrate and moderator binding sites may be occurred by considering the concept of cooperativity:

1) The binding of substrate does not change the affinity of moderator binding sites and *vice versa*, which shows the noncooperativity. It means that $\alpha = 1$ and observes in the noncompetitive inhibition.
2) The binding of substrate decreases the affinity of moderator binding sites and *vice versa*, which shows the anticooperativity. It means that $\alpha > 1$ and observes in the mixed type inhibition.
3) The binding of substrate decreases infinitely the affinity of moderator binding sites and *vice versa*, which shows the anticooperativity infinitely. It means that $\alpha = \infty$ and observes in the competitive type inhibition.
4) The binding of substrate increases the affinity of moderator binding sites and *vice versa*, which shows the cooperativity. It means that $\alpha < 1$ and observes in the mixed type inhibition.
5) Only the binding of substrate leads to the binding of moderator, which shows the cooperativity infinitely. It means that $\alpha = 0$ and observes in the uncompetitive type inhibition.

A GENERAL THEORY FOR INHIBITION AND ACTIVATION

What determine the inhibition or activation characterization of a moderator are values of α and β parameters. Hence, Scheme 2 is a general scheme to evaluate the inhibition and activation together. Both α and β parameters can be deduced from Eq. (4) [4]:

$$\frac{1}{V} = \frac{K_m}{V_{max}} \frac{(1+\frac{A}{K})}{(1+\frac{\beta[M]}{\alpha K})} \times \frac{1}{[S]} + \frac{1}{V_{max}} \frac{\beta-1}{\alpha-\beta} \qquad (4)$$

[M] is the concentration of moderator (**I** or **A**) and K is the inhibition or activation constant. The type of inhibition or activation can be summarized in Table 2 according to different values of α and β. The new insight to the values of α and β is expressed in this review as follows:

For $\beta = 0$, decreasing of the α value from ∞ in the competitive inhibition to one in noncompetitive inhibition and then to zero in uncompetitive inhibition leads to move the intersection of Lineweaver-Burk plots from Y-axis to X-axis and then no intersection (parallel lines), respectively. These boundary values of α parameter (∞, 1 and 0) make a special inhibition. The intersection for Lineweaver-Burk plots may be observed above the X-axis if $\alpha > 1$ (anticooperativity between substrate and inhibitor binding sites) or below the X-axis if $\alpha < 1$ (cooperativity between substrate and inhibitor binding sites); both $\alpha > 1$ and $\alpha < 1$ ranges make mixed type inhibition. Hence, the competitive inhibition ($\alpha = \infty$) is the extreme of anticooperativity and uncompetitive inhibition ($\alpha =$

Table 2. Classification of Moderators by Different Values of α and β

α	β	Type of Moderator
1	1	Not effector
1	0	Noncompetitive inhibition
>1	0	Mixed inhibition
<1	0	Mixed inhibition
∞	0	Competitive inhibition
0	0	Uncompetitive inhibition
1	<1	Partial inhibition
1	>1	Activator
<1	>1	Highly activator
>1	>α	Activator in high substrate concentration

0) is the extreme of cooperativity between substrate and inhibitor binding sites.

The activation of an enzyme can be observed by increasing the cooperativity between substrate and inhibitor binding sites ($\alpha < 1$) and/or increasing the catalytic ($\beta > 1$). The position of the intersection of Lineweaver-Burk plots depends on two α and β parameter values simultanously. Hence, different types of reversible activation may be introduced according to the role of two α and β parameters.

MUSHROOM TYROSINASE INHIBITION AND ACTIVATION

Tyrosinase (EC 1.14.18.1) is a bifunctional enzyme, which catalyzes *o*-hydroxylation of monophenols (cresolase activity) and oxidation of catechols to the corresponding *o*-quinones (catecholase activity) [12–13]. *o*-Quinones follow some reactions, which result in formation of biopolymers like melanin [14]. Tyrosinases are responsible for many biologically essential functions, such as pigmentation, sclerotization, primary immune response and host defense [15]. In mushroom (*Agaricus bisporus*), as well as in fruits and vegetables, the enzyme is responsible for browning, a commercially undesirable phenomenon [16]. MT has a molecular mass of 120 kD, is composed of two H subunits (43 kD) and two L subunits (13 kD) and contains two active sites [17]. Its active site has a di-copper center, resembling that of hemocyanins [18]. Tyrosinase is responsible for the enzymatic browning of fruits and vegetables. In addition to the undesirable color and flavor, the quinone compounds produced in the browning reaction may irreversibly react with the amino and sulfhydryl groups of proteins. The quinone-protein reaction decreases the digestibility of the protein and the bioavailability of essential amino acids, including lysine and cysteine. Therefore, development of high-performance tyrosinase inhibitors is much needed in the agricultural and food fields [19]. Tyrosinase inhibitors have attracted interest recently due to undesired browning in vegetables and fruits in post-harvest handling [20]. Additionally, tyrosinase inhibitors may be clinically used for treatment of some skin disorders associated with melanin hyper-pigmentation and are also important in cosmetics for skin whitening effects [21-22].

To understand the mechanism of the enzyme's action and inhibition, we have attempted to obtain additional information about the structure, function and relationship of MT [23-28]. After introducing two new bi-pyridine synthetic compounds as potent uncompetitive MT inhibitors [29], the inhibitory effects of three synthetic n-alkyl dithiocarbamates, with different tails, were elucidated [30]. The binding process for catecholase inhibition by benzenethiol showed the predominance of hydrophobic interaction in the active site of the enzyme, whereas electrostatic interaction can be important for cresolase inhibition [31-32]. Recently, investigation the inhibitory effects of three new synthesized alkyl xanthates, sodium salts, with different aliphatic tails, of C3, C4 and C5, were described and the kinetics of their inhibition were elucidated for both cresolase and catecholase activities [33-36]. Understanding the role of hydrophobic and electrostatic interactions of inhibitor binding to the active site of the enzyme can lead to the design of new potent MT inhibitors. All moderator constants for recently studied on MT have been summarized Table 3.

The uncompetitive type of inhibition of by [1,4′] bipiperidinyl-1′-yl-4-methylphenyl-methane and [1,4′] bipiperidinyl-1′-yl-4-methylphenyl-methane indicates that these inhibitors bind at a site distinct from the active site and bind only with the enzyme-substrate (ES) complex and not with the free enzyme (E) [29]. Presumably due to binding of the substrate to the enzyme, a conformational change is induced in the enzyme and this establishes a proper site for binding of these two bi-pyridine inhibitors. This site must be formed in a hydrophobic region, which is sufficiently spacious to accommodate these bulky hydrophobic compounds. This proper environment at the inhibitor binding site with high affinity exists to justify the low value of the inhibition binding constants for these two inhibitors. Due of the inhibitory potency of these two compounds and, as mentioned earlier, the great importance of tyrosinase inhibition in medicine and agriculture, it is proposed to study the effect of other bi-pyridine compounds on tyrosinase inhibition.

n-Butyl dithiocarbamate, n-hexyl dithiocarbamate and n-octyl dithiocarbamate, sodium salts, show a greater potency in the inhibitory effect of MT towards the cresolase activity of MT [30]. The MT active site has a di-copper center CuA and CuB [18]. It is probable that these n-alkyl dithiocarbamates coordinate to CuA in the binuclear active site, thus, show a

Table 3. Moderators of Mushroom Tyrosinase at pH 6.8 Phosphate Buffer at 20 °C

Moderator	Effect	K_I (μM)	α	Ref.
[1,4′] Bipiperidinyl-1′-yl-naphthan-2-yl-methanone	Catecholase uncompetitive inhibitor	5.87	0	[29]
[1,4′] Bipiperidinyl-1′-yl-4-methylphenyl-methane	Catecholase uncompetitive inhibitor	1.31	0	
n-Butyl dithiocarbamate, sodium salt	Cresolase competitive inhibitor	0.8	∞	
	Catecholase mixed inhibitor	9.4	2.8	
n-Hexyl dithiocarbamate, sodium salt	Cresolase competitive inhibitor	1.0	∞	[30]
	Catecholase mixed inhibitor	15.1	4.5	
n-Octyl dithiocarbamate, sodium salt	Cresolase competitive inhibitor	1.8	∞	
	Catecholase mixed inhibitor	28.5	4.6	
Benzenethiol	Cresolase competitive inhibitor	0.13	∞	[31]
	Catecholase competitive inhibitor	17.37	∞	
Iso-propyl xanthate, sodium salt	Cresolase mixed inhibitor	9.8	1.2	
	Catecholase mixed inhibitor	12.9	2.0	
Iso-butyl xanthate, sodium salt	Cresolase mixed inhibitor	7.2	4.1	[33]
	Catecholase competitive inhibitor	21.8	∞	
Iso-pentyl xanthate, sodium salt	Cresolase competitive inhibitor	6.1	∞	
	Catecholase competitive inhibitor	42.2	∞	
Ethyl xanthate, sodium salt[a]	Cresolase uncompetitive inhibitor	13.8	0	
	Catecholase mixed inhibitor	1.4	5.7	
Propyl xanthate, sodium salt[b]	Cresolase uncompetitive inhibitor	11.0	0	
	Catecholase mixed inhibitor	5.0	8.0	
Butyl xanthate, sodium salt	Cresolase competitive inhibitor	8.0	∞	[34]
	Catecholase competitive inhibitor	13.0	∞	
Hexyl xanthate, sodium salt	Cresolase competitive inhibitor	5.0	∞	
	Catecholase competitive inhibitor	25.0	∞	

[a]There are two binding sites for ethyl xanthate to MT. At low concentration of ethyl xanthate, which the first binding site is occupied mostly, the enzyme be activated by the ethyl xanthate ($\alpha = 0.62$, $\beta = 1.28$ and $K_A = 1.9$ μM). α and β are increased and K_A is decreased by decreasing the temperature from 20 °C to 10 °C [35]. [b]There are two binding sites for propyl xanthate to MT. At low concentration of propyl xanthate, which the first binding site is occupied mostly, the enzyme be activated by the propyl xanthate ($\alpha = 0.36$, $\beta = 1.21$ and $K_A = 2.7$ μM) [36].

competitive manner of inhibition of cresolase activity. Moreover, K_I values increase in magnitude as the length of the aliphatic tail increases for these compounds, which means that a shorter tail gives a more potent inhibitor. In the case of catecholase activity, mixed inhibition shows that inhibitors can bind both with the free enzyme (E) and enzyme-substrate (ES) complex. Both substrate and inhibitor can be bound to the enzyme with negative cooperativity between the binding sites ($\alpha > 1$) and this negative cooperativity increases with increasing length of the aliphatic tail in these compounds. Diphenol substrates may bind to CUB, while the inhibitor coordinates to the CuA site. Besides, monophenols combine only with oxytyrosinase and diphenols with oxy and met forms [37]. Moreover, if inhibitors show a preferred binding

pattern to the oxy form, the mixed inhibition mode in cathecholase activity may result from the reaction stoichiometry. From another point of view, the nalkyl tails of n-alkyl dithiocarbamates may be responsible for different inhibition constants. K_I is a dissociation constant of the enzyme-inhibitor complex and increasing its value by increasing the length of the aliphatic tail of the inhibitor needs more structural investigation by considering the hydrophobic pocket.

The inhibitory effect of benzenethiol on the cresolase and catecholase activities of MT shows that moderator can inhibit both activities of the enzyme competitively. The inhibitory effect of benzenethiol on the cresolase activity is more than the catecholase activity of MT. The type of binding process is different in the two types of MT activities. The binding process for catecholase inhibition is only entropy driven, which means that the predominant interaction in the active site of the enzyme is hydrophobic, meanwhile the electrostatic interaction can be important for cresolase inhibition due to the enthalpy driven binding process [31].

Three iso-alkyldithiocarbonates (xanthates), iso-propyl xanthate, iso-butyl xanthate and iso-pentyl xanthate, as sodium salts, examined for inhibition of both cresolase and catecholase activities of MT. Lineweaver-Burk plots showed different patterns of mixed and competitive inhibition for the three xanthates and also for cresolase and catecholase activities of MT. In mixed type inhibition, the negative cooperativity ($\alpha > 1$) increases with increasing length of the aliphatic tail in both cresolase and catecholase activities. The cresolase inhibition is related to the chelating of the copper ions at the active site by a negative head group (S^-) of the anion xanthate, which leads to similar values of K_I for all three xanthates. Different K_I values for catecholase inhibition are related to different interactions of the aliphatic chains of xanthates with hydrophobic pockets in the active site of the enzyme [33].

The role of alkyl chain length in the inhibitory effect n-alkyl xanthates on MT activities was also investigated [34]. Lineweaver-Burk plots showed different patterns of mixed, competitive or uncompetitive inhibition for the four xanthates, ethyl xanthate, propyl xanthate, butyl xanthate and hexyl xanthate, sodium salts. The negative cooperativity ($\alpha > 1$) increases with increasing length of the aliphatic tail of these compounds in mixed type inhibition. The length of the hydrophobic tail of the xanthates has a stronger effect on the K_I values for catecholase inhibition than for cresolase inhibition. Increasing the length of the hydrophobic tail leads to a decrease of the K_I values for cresolase inhibition and an increase of the K_I values for catecholase inhibition. There are two binding sites for xanthates to MT. At low concentration of xanthate, which the first binding site is occupied mostly, the enzyme be activated by the xanthate. For ethyl xanthate the activation parameters are: $\alpha = 0.62$, $\beta = 1.28$ and $K_A = 1.9$ μM. α and β are increased and K_A is decreased by decreasing the temperature from 20 °C to 10 °C [35]. For propyl xanthate the activation parameters are: $\alpha = 0.36$, $\beta = 1.21$ and $K_A = 2.7$ μM [36]. Effects of these xanthates are examples show two different behaviors of moderators at different ranges of the moderator concentrations.

ACKNOWLEDGEMENTS

The financial support given by the University of Tehran is gratefully acknowledged.

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