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RELATIONSHIP BETWEEN THE INHIBITION CONSTANT (K_I) AND THE CONCENTRATION OF INHIBITOR WHICH CAUSES 50 PER CENT INHIBITION (I_{50}) OF AN ENZYMATIC REACTION*

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Abstract—A theoretical analysis has been made of the relationship between the inhibition constant (K_I) of a substance and the (I_{50}) value which expresses the concentration of inhibitor required to produce 50 per cent inhibition of an enzymic reaction at a specific substrate concentration. A comparison has been made of the relationships between K_I and I_{50} for monosubstrate reactions when noncompetitive or uncompetitive inhibition kinetics apply, as well as for bisubstrate reactions under conditions of competitive, noncompetitive and uncompetitive inhibition kinetics. Precautions have been indicated against the indiscriminate use of I_{50} values in agreement with the admonitions previously described in the literature. The analysis described shows K_I does not equal I_{50} when competitive inhibition kinetics apply; however, K_I is equal to I_{50} under conditions of either noncompetitive or uncompetitive kinetics.

MANY DRUGS are believed to exert their biological effect as a consequence of enzyme inhibition. One approach to the understanding of the mechanism of action of such drugs has been to study the effect of drug concentration on the rate of reaction of an isolated enzyme. Several approaches have been used to describe the extent of inhibition such as I_{50} (concentration of inhibitor producing 50 per cent inhibition), $(I/S)_{50}$ (concentration of inhibitor relative to substrate concentration producing 50 per cent inhibition), and K_I (the dissociation constant of the enzyme–inhibitor complex, or the reciprocal of the binding affinity of the inhibitor to the enzyme).

Although the relationship between the inhibition constant (K_I) and I_{50} of a competitive inhibitor of a monosubstrate reaction has been discussed,[1,2] a detailed comparison of such a relationship for either bisubstrate reactions when competitive, noncompetitive or uncompetitive inhibition kinetics exist, or for monosubstrate reactions when the latter two types of inhibition kinetics apply has not been presented. An understanding of the relationship between I_{50} and K_I under these conditions and the theoretical basis for their determination is critical to appropriate interpretation of the experimental data, as well as for comparison of the literature values of I_{50} or $(I/S)_{50}$. Blakley[3] has indicated the limitations in the use of $(I/S)_{50}$ relative to the K_I.

Although what is presented is no doubt readily apparent to the enzyme kineticist, those who are less familiar with enzyme kinetics and yet concerned with studying the effect of drugs on enzymes may find this communication useful.

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THEORETICAL ANALYSIS

Several kinetic situations are described below that have the following limitations: (1) the reaction in the absence of the inhibitor follows a simple Michaelis–Menten equation; (2) the rate of the reaction depends on the amount of the enzyme–substrate complex; (3) a rapid equilibrium steady state method is used;[4] and (4) only reversible inhibitors are discussed.

Reactions involving one substrate

$$V_0 = \frac{V_{\max} S}{K_m + S} \tag{1}$$

$V_{\max}$ = maximum velocity; V_0 = velocity in the absence of the inhibitor; K_m = Michaelis constant of the substrate (S); S = substrate concentration.

Case I. When a competitive inhibitor (I) is present.

$$\begin{array}{l} E \overset{S}{\leftrightharpoons} ES \rightarrow E + P \\ \quad \updownarrow I \\ \quad EI \end{array}$$

$$V_I = \frac{V_{\max} S}{K_m\left(1 + \dfrac{I}{K_I}\right) + S} \tag{2}$$

V_I = velocity in the presence of inhibitor; I = inhibitor concentration; K_I = dissociation constant of EI.

When $I = I_{50}$, $V_0 = 2\,V_I$, then

$$\frac{2V_{\max} S}{K_m\left(1 + \dfrac{I_{50}}{K_I}\right) + S} = \frac{V_{\max} S}{K_m + S}.$$

By rearrangement:

$$I_{50} = K_I\left(1 + \frac{S}{K_m}\right). \tag{3}$$

Equation (3) is identical to that described by Webb:[2]

$$\left(\frac{I}{S}\right)_{50} = \frac{K_I}{K_m} + \frac{K_I}{S}. \tag{4}$$

The equation derived by Baker[1] makes the assumption that $V_0 = V_{\max}$ in the beginning of the derivation:

$$\left(\frac{I}{S}\right)_{50} = \frac{K_I}{K_m} - \frac{K_I}{S} \tag{5}$$

which is different from equation (4). However, since most investigators have $S \gg K_m$ in their assay condition, there would be no significant difference between equations (4) and (5), and hence these equations may be transformed into equation (6).

$$\left(\frac{I}{S}\right)_{50} = \frac{K_I}{K_m}. \tag{6}$$

Since I_{50} will depend on the substrate concentration used in the assay (equation 3), it is impossible to compare I_{50} values from one laboratory with those from another unless identical assay conditions are used. Baker[1] has indeed considered the substrate concentration by his use of $(I/S)_{50}$. Appropriate comparison of the effect of one compound relative to another may be made, provided that $S \gg K_m$, and both compounds are competive inhibitors. Without prior determination of the type of inhibition such compounds exert, the relative values of $(I/S)_{50}$ have questionable meaning. Thus, for example, one might have assumed that 3-*N*-methyl-5-iodo-2′-deoxyuridine would be like 5-iodo-2′-deoxyuridine, a competitive inhibitor of thymidine kinase when thymidine is the variable substrate. However, uncompetitive inhibition is observed with *N*-methyl-5-iodo-2′-deoxyuridine when thymidine is the variable substrate, and competitive inhibition kinetics when ATP-Mg^{2+} is the variable substrate.[5]

Having determined that the two compounds being compared are indeed competitive inhibitors, one can effectively use $(I/S)_{50}$ values for the purpose of comparison. If the concentrations of inhibitors *A* and *B* required to produce 50 per cent inhibition at a particular substrate concentration are significantly different yet close, one may amplify the difference by augmenting the substrate concentration.

Case II. When a noncompetitive inhibitor is present.

$$\begin{array}{ccc} E & \overset{S}{\rightleftharpoons} & ES \rightarrow E + P \\ {\scriptstyle I}\Big\Updownarrow{\scriptstyle K_{IS}} & & {\scriptstyle I}\Big\Updownarrow{\scriptstyle K_{Ii}} \\ EI & \rightleftharpoons & ESI \end{array}$$

$$V_I = \frac{V_{\max} S}{K_m\left(1 + \frac{I}{K_{IS}}\right) + S\left(1 + \frac{I}{K_{Ii}}\right)}. \tag{7}$$

When $I = I_{50}$, $V_0 = 2\ V_I$ and:

$$\frac{V_{\max} S}{K_m + S} = \frac{2V_{\max} S}{K_m\left(1 + \frac{I_{50}}{K_{IS}}\right) + S\left(1 + \frac{I_{50}}{K_{Ii}}\right)}.$$

By rearrangement of the above equation:

$$I_{50} = (K_m + S)\Big/\left(\frac{K_m}{K_{IS}} + \frac{S}{K_{Ii}}\right). \tag{8}$$

When $K_{IS} = K_{Ii}$, that is when the affinity of inhibitor to the free enzyme (*E*) and the enzyme–substrate complex (*ES*) is the same, then equation (8) may be transformed into:

$$I_{50} = K_{Ii} \text{ or } K_{IS}, \text{ or more simply, } K_I. \tag{9}$$

Since $S \gg K_m$ in most assays performed, then equation (8) may be transformed into:

$$I_{50} = 1 \Big/ \left(\frac{K_m}{S} \frac{1}{K_{IS}} + \frac{1}{K_{Ii}} \right)$$

and hence,

$$I_{50} = K_{IS} \Big/ \left(\frac{K_m}{S} + \frac{K_{IS}}{K_{Ii}} \right). \tag{10}$$

Provided that $K_m/S \ll K_{IS}/K_{Ii}$ (since K_m/S may be adjusted) apply, equation 10 may then be transformed into either:

$$I_{50} = K_{Ii} \tag{11}$$

or

$$\left(\frac{I}{S} \right)_{50} = 1 \Big/ \left(\frac{K_m}{K_{IS}} + \frac{S}{K_{Ii}} \right). \tag{12}$$

Thus it is quite apparent that there is no value in comparing the effect of inhibitors on the basis of $(I/S)_{50}$, because I_{50} equals K_I (equation 11).

It should be emphasized that the dependence of I_{50} on S is different from that observed above with the competitive inhibitor.

Case III. When an uncompetitive inhibitor is present.

$$\begin{array}{ccccc} E & \rightleftharpoons & ES & \rightarrow & E + P \\ & & {\scriptstyle K_I} \uparrow\downarrow {\scriptstyle I} & & \\ & & ESI & & \end{array}$$

$$V_I = \frac{V_{\max} S}{K_m + \left(1 + \dfrac{I}{K_I} \right) S}. \tag{13}$$

When $I = I_{50}$, $V_0 = 2\ V_I$, then

$$\frac{V_{\max} S}{K_m + S} = \frac{2 V_{\max} S}{K_m + \left(1 + \dfrac{I_{50}}{K_I} \right) S}.$$

Rearrangement of the above equation results in:

$$K_m + S = S \frac{I_{50}}{K_I},$$

and

$$I_{50} = K_I \left(1 + \frac{K_m}{S}\right). \tag{14}$$

When $S \gg K_m$, equation (14) may be simplified into

$$I_{50} = K_I. \tag{15}$$

In this situation, I_{50} is independent of S provided that $S \gg K_m$. Thus there is no value to express the data in terms of $(I/S)_{50}$.

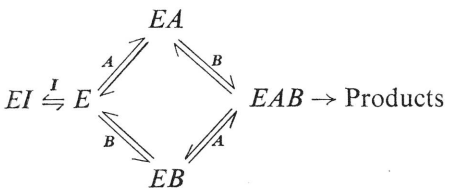
Reactions involving two substrates

When both substrates are added sequentially to the enzyme, the reaction follows either a rapid equilibrium random or ordered mechanism. The rate equation[6–8] is:

$$V_0 = \frac{V_{\max} AB}{K_{ia}K_b + K_aB + K_bA + AB} \tag{16}$$

K_{ia} = dissociation constant of substrate A.

Case IV. If the inhibitor competes for the free enzyme (E) with either substrate A or B in a random mechanism reaction, or with the first substrate in an ordered sequential mechanism, then



or

$EI \overset{I}{\leftrightharpoons} E \overset{A}{\rightleftharpoons} EA \overset{B}{\rightleftharpoons} EAB \rightarrow$ Products, and the rate equation will be:

$$V_I = \frac{V_{\max} AB}{K_{ia}K_b \left(1 + \frac{I}{K_I}\right) + K_aB + K_bA + AB}. \tag{17}$$

By mathematical treatment similar to that performed in the previous cases, and when $V_0 = 2\,V_I$, $I = I_{50}$, then

$$\frac{2V_{\max} AB}{K_{ia}K_b \left(1 + \frac{I_{50}}{K_I}\right) + K_aB + K_bA + AB} = \frac{V_{\max} AB}{K_{ia}K_b + K_aB + K_bA + AB}.$$

This equation may be rearranged into:

$$I_{50} = K_I \left(1 + \frac{K_a}{K_{ia} K_b} B + \frac{A}{K_{ia}} + \frac{AB}{K_{ia} K_b}\right). \tag{18}$$

When $K_a = K_{ia}$, equation (18) may be simplified to:

$$I_{50} = K_I \left(1 + \frac{A}{K_a}\right)\left(1 + \frac{B}{K_b}\right), \tag{19}$$

which basically is similar to equation (3); however, equation (19) takes into account an additional substrate.

Equations (18) and (16) may also be transformed into:

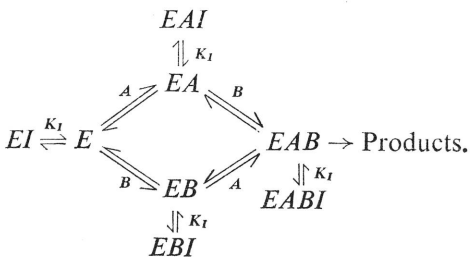
$$I_{50} = \frac{V_{\max}}{V_0} \frac{A}{K_{ia}} \frac{B}{K_b} K_I. \tag{20}$$

Since most assays are performed under optimal conditions in which either $V_0 = V_{\max}$, or $A \gg K_a$ and $B \gg K_b$, then both equations (19 and 20) may be transformed into:

$$I_{50} = \frac{A}{K_{ia}} \frac{B}{K_b} K_I. \tag{21}$$

Thus the I_{50} value will depend on the concentration of both substrates A and B.

Case V. When the reaction follows either an ordered sequential or a rapid equilibrium random mechanism, the inhibitor acts as a noncompetitive inhibitor and can bind to all of the enzyme species in the reaction with the same affinity:



Then, the velocity is described by:

$$V_I = \frac{V_{\max} AB}{(K_{ia}K_b + K_aB + K_bA + AB)\left(1 + \frac{I}{K_I}\right)}. \tag{22}$$

When $V_0 = 2\ V_I$, $I = I_{50}$, and upon substitution of equation 16 for V_0 and equation (22) for V_I, one obtains:

$$K_{ia}K_b + K_aB + K_bA + AB = \frac{I_{50}}{K_I}(K_{ia}K_b + K_aB + K_bA + AB)$$

and hence,

$$I_{50} = K_I. \tag{23}$$

Thus the value of I_{50} does not depend on the substrate concentration and will be equal to K_I. The I_{50} value obtained under these conditions can be compared between laboratories without concern with the substrate concentration.

Case VI. When the reaction follows an ordered sequential mechanism, the inhibitor can compete with either the first substrate A for the free enzyme, or with the second substrate for the EA complex, or both:

$$\begin{array}{ccccc} E & \overset{A}{\rightleftharpoons} & EA & \overset{B}{\rightleftharpoons} & EAB \rightarrow \text{Products.} \\ {\scriptstyle K_{IS}}\Vert & & \Vert{\scriptstyle K_{Ii}} & & \\ EI & \overset{A}{\rightleftharpoons} & EAI & & \end{array}$$

Then

$$V_I = \frac{V_{\max} AB}{K_{ia}K_b\left(1 + \dfrac{I}{K_{IS}}\right) + K_aB + K_bA\left(1 + \dfrac{I}{K_{Ii}}\right) + AB}. \tag{24}$$

When $V_0 = 2\,V_I$, $I = I_{50}$, and by combining equations (23) and (16), one obtains:

$$K_{ia}K_b + K_aB + K_bA + AB = I_{50}\left(\frac{K_{ia}K_b}{K_{IS}} + \frac{K_bA}{K_{Ii}}\right).$$

This equation may be transformed into:

$$I_{50} = \left(\frac{V_{\max}}{V_0} AB\right) \Big/ \left(\frac{K_{ia}K_b}{K_{IS}} + \frac{K_bA}{K_{Ii}}\right)$$

$$= \left(\frac{V_{\max}}{V_0}\right) \Big/ \frac{K_b}{B}\left(\frac{1}{K_{IS}} \cdot \frac{K_a}{A} + \frac{1}{K_{Ii}}\right). \tag{25}$$

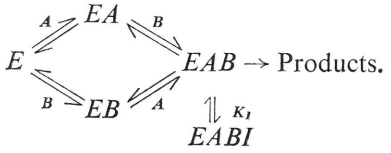
Under the rare condition when $K_{Ii} = K_{IS}$ and $V_0 = V_{\max}$, equation (25) can be simplified to:

$$I_{50} = K_I \cdot \frac{B}{K_b} \tag{26}$$

and

$$\left(\frac{I}{B}\right)_{50} = \frac{K_I}{K_b}. \tag{27}$$

Case VII. When the reaction follows an ordered sequential or rapid random mechanism, and the inhibitor can only bind to the *EAB* complex:



Then,

$$V_I = \frac{V_{\max} AB}{K_{ia}K_b + K_aB + K_bA + \left(1 + \frac{I}{K_I}\right) AB}. \tag{28}$$

When $V_0 = 2\,V_I$, $I = I_{50}$, and by combining equations (28) and (16), one obtains:

$$K_{ia}K_b + K_aB + K_bA + AB = \frac{I_{50}}{K_I} AB,$$

which may be transformed into:

$$I_{50} = \frac{V_{\max}}{V_0} K_I.$$

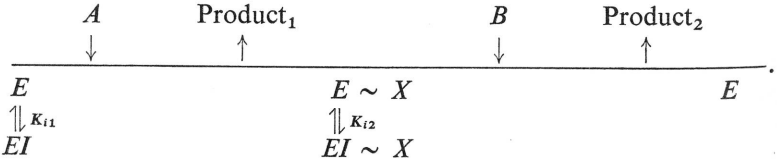
Thus, when

$$V_0 = V_{\max},\ I_{50} = K_I. \tag{29}$$

When the reaction follows a "ping-pong" mechanism[6–8] and involves two substrates, the rate equation will be:

$$V_0 = \frac{V_{\max} AB}{K_bA + K_aB + AB}. \tag{30}$$

Case VIII. An inhibitor, *I*, affects both forms of the enzyme, *E* and $E \sim X$:



The rate equation[9] is:

$$V_I = \frac{V_{\max} AB}{K_b\left(1 + \frac{I}{K_{i2}}\right) A + K_a\left(1 + \frac{I}{K_{i1}}\right) B + AB}. \tag{31}$$

When $I = I_{50}$, $V_0 = 2\ V_I$, and by combining equations (30) and (31), one obtains:

$$K_bA + K_aB + AB = \left(\frac{K_b}{B}\frac{1}{K_{i2}} + \frac{K_a}{A}\frac{1}{K_{i1}}\right) I_{50} \tag{32}$$

and

$$I_{50} = \frac{V_{max}}{V_0} \Big/ \left(\frac{K_b}{B}\frac{1}{K_{i2}} + \frac{K_a}{A}\frac{1}{K_{i1}}\right). \tag{33}$$

Although K_{i1} is generally not equal to K_{i2}, in the specific situation when K_{i1} does equal K_{i2}, equation (31) can be transformed into:

$$I_{50} = \left(1 + \frac{AB}{K_bA + K_aB}\right) K_{i1}. \tag{34}$$

Case IX. When the reaction follows a ping-pong mechanism, an inhibitor affects only one form of the enzyme—E or $E \sim X$. The rate equation will be:

$$V_I = \frac{V_{max}\, AB}{K_bA + K_a\left(1 + \dfrac{I}{K_{i1}}\right) B + AB} \tag{35}$$

or

$$V_I = \frac{V_{max}\, AB}{K_b\left(1 + \dfrac{I}{K_{i2}}\right) A + K_aB + AB}. \tag{36}$$

When $I = I_{50}$, $V_0 = 2\ V_I$, and by combining equations (35) and (30), one obtains:

$$K_bA + K_aB + AB = K_bA\frac{I_{50}}{K_{i1}}$$

or

$$I_{50} = K_{i1}\left(1 + \frac{K_aB}{K_bA} + \frac{B}{K_b}\right). \tag{37}$$

Similarly, equation (36) can be transformed into:

$$I_{50} = K_{i2}\left(1 + \frac{K_aB}{K_bA} + \frac{A}{K_a}\right). \tag{38}$$

DISCUSSION

The effect of an enzyme inhibitor in a variety of situations has been analyzed. Before the equations described above may be used, one must determine the type of

inhibition involved. This is readily established by applying the rules discussed by Cleland.[6–8] The relationship between K_I and I_{50} varies. For instance, when a noncompetitive or an uncompetitive inhibitor is studied in a monosubstrate enzymatic reaction, I_{50} will be equal to K_I, provided certain conditions are met (equations 11 and 15). However, if the inhibitor is a competitive inhibitor, I_{50} will be equal to K_I $(1 + S/K_m)$ (equation 3). The equations (3, 8, 14, 18, 23, 25, 29, 33, 37) have described the relationship of I_{50} to K_I when both the type of inhibitor and the reaction mechanism vary. It is readily apparent that the relationship of I_{50} to K_I is dependent upon the type of inhibition and the mechanism of the reaction. It has been established that $(I/S)_{50}$ may not be used in the absence of such knowledge without producing great uncertainties as to its meaning. K_I does not equal I_{50} when competitive inhibition kinetics apply; however, K_I is equal to I_{50} under the conditions of either noncompetitive or uncompetitive kinetics.

When a group of inhibitory compounds have an identical mechanism of action, a direct comparison of the I_{50} values among them will suffice to determine the relative efficacy, provided the assays are performed under the same conditions. However, in certain cases, when the K_I value of each compound is required, it may be impractical to perform the kinetic studies required to determine the K_I for each. In this situation, it is still possible to calculate the K_I values, provided one knows the K_I of one compound, by using the relationship:

$$\frac{(I_{50})_1}{(I_{50})_2} = \frac{(K_I)_1}{(K_I)_2}.$$

This may be done without knowing the reaction mechanism or type of inhibitor in detail, except for Cases II, VI and IX, in which a certain assumption must be made before this general rule applies. In Cases II and VI, the assumption is that $K_{IS} = K_{ii}$, and in Case IX, that $K_{i2} = K_{i1}$.

When comparing the I_{50} values of compounds that inhibit a specific enzyme derived from the same source, but reported from different laboratories, a few important factors must be considered: (1) Are the assay conditions the same? (2) Do the compounds have the same reaction mechanism for their inhibitory effect?

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