BIOCHEMICAL JOURNAL LETTERS

What does I_{50} mean?

Studies of enzyme inhibition are an important part of the overall enzyme research. Inhibitors are very effective tools for studying the active site and reaction mechanism of enzymes. Usually K_i (inhibition constant, i.e. dissociation constant of the enzyme–inhibitor complex) is used to measure the effectiveness of an inhibitor. Unfortunately, a parameter called I_{50} (concentration of inhibitor at which the enzyme is inhibited by 50%) is still used (see, e.g., De Vrij *et al.*, 1983). Here we would like to show the interrelation between K_i and I_{50} for different types of inhibition and how I_{50} can misrepresent the inhibitory capability of an inhibitor. We will consider reversible inhibition and the simple two-step Michaelis-type reaction only:

$$E + S \underset{}{\overset{K_s}{\rightleftharpoons}} ES \xrightarrow{k_{+2}} E + P \qquad (1)$$

where E, S, ES and P are enzyme, substrate, enzyme–substrate complex and product, respectively. The expression for the reaction rate for eqn. (1) is given in eqn. (2):

$$v_0 = \frac{V[S]}{K_s + [S]} \qquad (2)$$

where V is the limiting (maximum) reaction rate, equal to $k_{+2} \times [E]_0$.

Let us examine the relationship between K_i and I_{50} for different types of inhibition.

(*1*) *Competitive type.* In this case of competitive inhibition the reaction rate in the presence of inhibitor (v_i) is:

$$v_i = \frac{V[S]}{K_s(1 + [I]/K_{ic}) + [S]} \qquad (3)$$

where I is inhibitor and K_{ic} is the inhibition constant for a competitive inhibitor. From eqns. (2) and (3):

$$v_o/v_i = \frac{K_s\,(1 + [I]/K_{ic}) + [S]}{K_s + [S]} \qquad (4)$$

when $[I] = I_{50}$, $v_i = v_o/2$, and from eqn. (4) we can derive:

$$I_{50}/K_{ic} = 1 + [S]/K_s \qquad (5)$$

(all other expressions for I_{50}/K_i are derived in analogous ways).

So K_{ic} is always less than I_{50} and, depending on the values of K_s and [S], the ratio can be of any value greater than 1, i.e. $1 < I_{50}/K_{ic} < \infty$. In this case I_{50} will always underestimate the inhibiting capacity of the inhibitor ($I_{50} < K_{ic}$). However, if the initial substrate concentration is much lower than K_s, K_{ic} will be almost equal to I_{50}.

(*2*) *Uncompetitive type.* In this case

$$I_{50}/K_{iu} = 1 + K_s/[S] \qquad (6)$$

where K_{iu} is the inhibition constant for an uncompetitive inhibitor. Here also $1 < I_{50}/K_{iu} < \infty$ and I_{50} underestimates the inhibiting capacity of the inhibitor. However, for the uncompetitive type of inhibition, initial substrate concentration must be much larger than K_s for the ratio I_{50}/K_{iu} to approach 1.

(*3*) *Pure non-competitive type.* In this case only, $I_{50} = K_i$ and I_{50} can be a true measure of the inhibitory capacity of the inhibitor.

(*4*) *Mixed type.* Let us use the general scheme for enzyme inhibition (Berezin & Martinek, 1971):

$$\begin{array}{ccccc} E + S & \overset{K_s}{\rightleftharpoons} & ES & \xrightarrow{k_{+2}} & E + P \\ {\scriptstyle K_i}\uparrow\downarrow & & \uparrow\downarrow{\scriptstyle \alpha K_i} & & \\ EI + S & \overset{\alpha K_s}{\rightleftharpoons} & EIS & \xrightarrow{\beta k_{+2}} & EI + P \end{array} \qquad (7)$$

For eqn. (7):

$$I_{50} = K_i\left[\frac{\alpha(K_s + [S])}{K_s(\alpha - 2\beta) + [S](1 - 2\beta)}\right] \qquad (8)$$

As can be easily seen from eqn. (8) $I_{50} \neq K_i$ and I_{50} depends on [S], K_s, α and β. I_{50} can be equal to K_i only if $\alpha = 1$ and $\beta = 0$, i.e. when the inhibitor is of pure non-competitive type. This has been stated earlier.

A few special cases of the mixed type of inhibition need comment. (i) When $\alpha = 1$, $\beta = 0.5$, the ratio I_{50}/K_i is equal to infinity; (ii) when $\alpha = \beta = 0.5$, the ratio I_{50}/K_i has a negative value, which by definition has no physical meaning; (iii) eqn. (8) can be rearranged as:

$$K_i/I_{50} = 1 - \frac{2\beta}{\alpha} + \frac{(1-\alpha)[S]}{\alpha(K_s + [S])} \qquad (9)$$

and in a given case, K_I/I_{50}, depending on the values of α, β, K_s and [S], can be less, equal to or greater than 1. Hence I_{50} can either underestimate or overestimate the inhibiting capacity for mixed inhibition.

In conclusion, it should be noted that I_{50}, the concentration of inhibitor at which the enzyme is inhibited by 50%, is not a constant like K_I and hence is not recommended to be used as a measure of inhibition.

The author is indebted to Prof. B. Chance for his encouragement and support.

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(*Received 4 July 1983*)

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