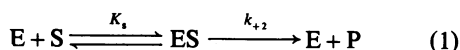


# BIOCHEMICAL JOURNAL LETTERS

## 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:



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):

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

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 ( $v_1$ ) is:

$$v_1 = \frac{V[S]}{K_s(1 + [I]/K_{ic}) + [S]} \quad (3)$$

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

$$v_0/v_1 = \frac{K_s(1 + [I]/K_{ic}) + [S]}{K_s + [S]} \quad (4)$$

when  $[I] = I_{50}$ ,  $v_1 = v_0/2$ , and from eqn. (4) we can derive:

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

(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  $I_{50}$ .

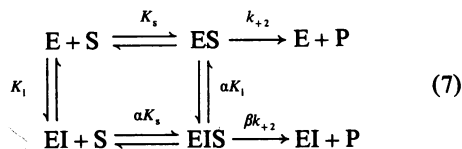
(2) *Uncompetitive type*. In this case

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

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):



For eqn. (7):

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

As can be easily seen from eqn. (8)  $I_{50} \neq K_i$  and  $I_{50}$  depends on  $[S]$ ,  $K_s$ ,  $\alpha$  and  $\beta$ .  $I_{50}$  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:

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

and in a given case,  $K_1/I_{50}$ , depending on the values of  $\alpha$ ,  $\beta$ ,  $K_s$  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  $K_1$  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.

Ali NAQUI

*Johnson Research Foundation, The School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, U.S.A.*

*(Received 4 July 1983)*

Berezin, I. V. & Martinek, K. (1971) *Mol. Biol. (Moscow)* **5**, 347-350

De Vrij, W., Azzi, A. & Konings, W. N. (1983) *Eur. J. Biochem.* **131**, 97-103