Reversible Inhibitions

22

Reversible inhibitors, especially the product and dead-end inhibitors, provide valuable insights to establish enzyme kinetic mechanisms. We have acknowledged earlier (Chap. 18) that reversible nature of inhibition has to be established before embarking on its use to study enzyme mechanisms. How these reversible inhibitors are employed in enzyme kinetic analyses is discussed in this chapter.

The nature of kinetic experiments conducted and the information sought from reversible enzyme inhibition data are as follows:

1. Monitor initial velocity "v" by varying the concentration of one substrate at different fixed concentrations of the inhibitor. If the enzyme reaction in question involves more than one substrate, then the concentration of all other substrates (other than the one whose concentration is varied) is fixed.
2. The $v \rightarrow [S]$ data are plotted in the double reciprocal format (double reciprocal plots) to generate a series of curves – one for each fixed concentration of the inhibitor. These patterns are analyzed qualitatively.
3. Gradual changes in the slope and/or intercepts, as a function of the fixed inhibitor concentration, are noted. An inhibitor may affect the first-order rate constant (V_{max}/K_M which is reflected in slope changes) or the zero-order rate constant (V_{max} as reflected in intercept changes) or both.
4. On quantitative analysis of slope and intercept changes, various kinetic constants including K_I values are evaluated. Depending upon whether the slope/intercept increases as a linear function of $[I]$ or not, the inhibition may also be classified as *linear*, *hyperbolic*, or *parabolic*.

Inhibition analyses through double reciprocal plots are most useful as their slope and/or intercept effects tell directly about the effect of that inhibitor on the rate constants. At times the *Dixon plot* is a useful alternative (Dixon 1953). Like before, we monitor initial velocity "v" but by varying the concentration of the inhibitor at different fixed concentrations of the substrate in question. If the enzyme reaction

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involves more than one substrate, then the concentration of all substrates (other than the one whose concentration is varied) is fixed. The $1/v \rightarrow [I]$ data are plotted for every fixed concentration of the substrate. There are a few advantages of Dixon analysis. Since we are primarily varying $[I]$, relatively high, fixed $[S]$ values could be used. For the double reciprocal analysis however, data at lower $[S]$ values are very essential for accuracy of analysis (see Chap. 17 Enzyme Kinetic Data: Collection and Analysis, in Part II). Dixon plots are diagnostic for nonlinear inhibitions (see below). A drawback of the Dixon plot patterns is, unlike the double reciprocal analysis, slope and intercept effects cannot be directly interpreted. It is also difficult to infer which of them is/are nonlinear. Finally, these plots are the best way to study the interaction of different inhibitors of the same enzyme through multiple inhibition analysis. This is particularly valuable in the analysis of enzymes regulated by multiple inhibitors such as glutamine synthetase (see Chap. 37 Regulation of Enzyme Activity, in Part V).

Three common inhibition patterns (as double reciprocal plots) observed in reversible inhibition studies are described below. An understanding of these concepts through cartoons is also informative (Tayyab 1990).

22.1 Competitive Inhibition

The scheme representing interaction equilibria for a competitive inhibitor with the enzyme is given below (Fig. 22.1).

A competitive inhibitor affects only the slope of a double reciprocal plot. Therefore, a series of lines that intersect on the Y-axis are obtained (see Fig. 22.2 below). Using the equilibrium assumption (Chap. 16, More Complex Rate Expressions, in Part II), a rate equation may be derived to represent this situation:

$$v = \frac{V_{\max}[S]}{K_M\left(1 + \frac{[I]}{K_I}\right) + [S]}$$

This equation is identical to classical Michaelis–Menten equation except that $(1 + [I]/K_I)$ term multiplies the K_M in the denominator. The equation and the equilibria do not make explicit as to whether the inhibitor binds to the active site or a site elsewhere on the enzyme. Both isosteric and allosteric inhibitors behave identical kinetically. It is natural to expect that a competitive inhibitor structurally related to either a substrate or a product occupy the active site. Malonate and

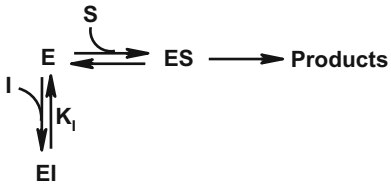

Fig. 22.1 Equilibria representing interaction of a competitive inhibitor with the enzyme

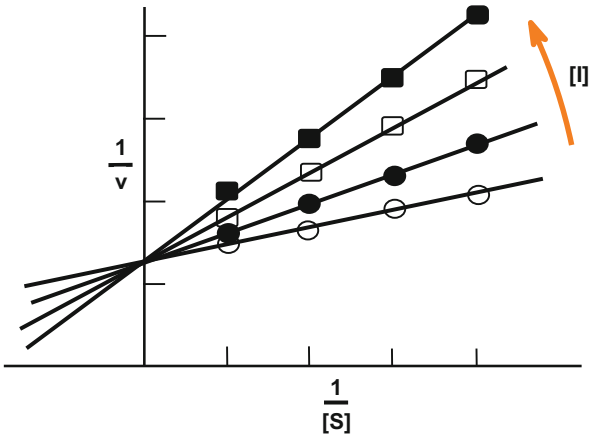

Fig. 22.2 Double reciprocal plots for the competitive inhibition of the enzyme with *S* as the varied substrate

fumarate are well-characterized succinate dehydrogenase competitive inhibitors of this kind. In all other cases, therefore, additional data are required to establish where a competitive inhibitor binds.

22.1.1 Determination/Evaluation of Kinetic Constants and Replots

The double reciprocal form of the rate equation

$$\frac{1}{v} = \frac{K_M}{V_{max}}\left(1 + \frac{[I]}{K_I}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

may be used to determine the K_I for the competitive inhibitor. Only the slope of the double reciprocal plot changes as a function of inhibitor concentration:

$$\text{slope} = \frac{K_M}{V_{max}}\left(1 + \frac{[I]}{K_I}\right)$$

In a simple competitive inhibition as represented in the scheme above, the replot of slope→[*I*] yields a straight line; analysis of its slope and intercept (actually intercept/slope of this line) gives the K_I. The slope replot may not be linear however. While such nonlinear slope replots are diagnostic of inhibitor interaction, extracting a K_I from such analysis is not straightforward. The nonlinearity of inhibition is better visualized by Dixon analysis. These Dixon plots of $1/v \rightarrow [I]$ are based on the rearranged format of the following double reciprocal form for competitive inhibition:

$$\frac{1}{v} = \frac{1}{V_{\max}} \frac{K_M}{[S]K_I}[I] + \frac{1}{V_{\max}}\left(1 + \frac{K_M}{[S]}\right)$$

22.1.2 Interpretation

The presence of a competitive inhibitor affects only the first-order rate constant ($V_{\max}/K_M$; 1/slope of Lineweaver-Burk plot). The $V_{\max}$ is not affected and the lines intercept on the Y-axis (where $[S] \rightarrow \infty$).

This is expected according to the equilibria described in the competition scheme. While *S* and *I* compete for the free enzyme (form *E*), at infinite [*S*], all the enzyme will be in the *ES* form – hence no inhibition by *I* occurs. At any finite level of [*S*] however, there is a proportion of *E* available for *I* to bind, and inhibition results. These arguments do not assume anything about the equilibrium dynamics – leading to mutually exclusive binding of *I* and *S* to the enzyme form *E*. Clearly the kinetic consequence is the same whether (a) *I* displaces *S* at the active site (isosteric competitive) or (b) I binds elsewhere on *E* but changes its conformation such that *S* cannot access the active site (allosteric competitive). In any case, inhibition completely overcome by large excess of substrate is a hallmark of competitive inhibition. Finally, with competitive inhibitors that are nonlinear, slope replots may either be hyperbolic or parabolic. Such nonlinear competitive inhibition is indicative of more complex scheme of interaction between the inhibitor and the enzyme. For instance, a hyperbolic inhibition may result when a ternary complex of *IES* also forms where this complex is active but is less productive than the *ES* complex. A parabolic competitive inhibition may occur due to multiple inhibitor molecules binding to the substrate binding site.

22.2 Uncompetitive Inhibition

The scheme representing interaction of an uncompetitive inhibitor with the enzyme is shown below (Fig. 22.3). An inhibitor affecting only the intercept of a double reciprocal plot yields a series of lines that are parallel to each other (see Fig. 22.4 below). Using the equilibrium assumption (Chap. 16, More Complex Rate Expressions, in Part II), a rate equation may be derived to represent this situation:

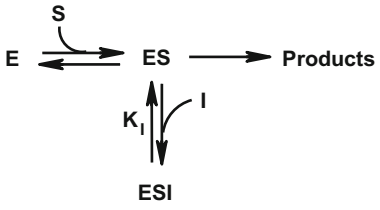

Fig. 22.3 Equilibria representing interaction of an uncompetitive inhibitor with the enzyme

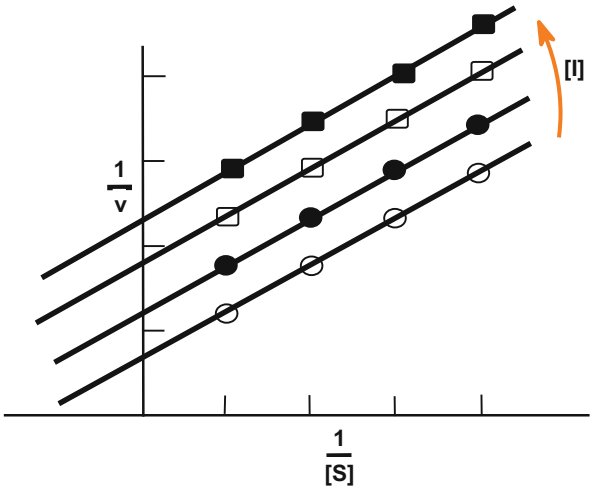

Fig. 22.4 **Double reciprocal plots for uncompetitive inhibition of the enzyme with *S* as the varied substrate**

$$v = \frac{V_{\max}[S]}{K_M + [S]\left(1 + \frac{[I]}{K_I}\right)}$$

This equation is identical to the Michaelis–Menten equation except that the [*S*] term in the denominator is multiplied by a factor (1 + [*I*]/K_I). From the way the equilibria are represented, it is obvious that an uncompetitive inhibitor binds to *ES* complex but not to *E* (free enzyme). It is however conceptually challenging to imagine how an inhibitor can bind only the enzyme–substrate complex.

22.2.1 Determination/Evaluation of Kinetic Constants and Replots

The double reciprocal form of the rate equation

$$\frac{1}{v} = \frac{K_M}{V_{\max}}\frac{1}{[S]} + \frac{1}{V_{\max}}\left(1 + \frac{[I]}{K_I}\right)$$

could be used to determine the K_I for the uncompetitive inhibitor. The intercept of the double reciprocal plot changes with the inhibitor concentration according to the relation:

$$\text{intercept} = \frac{1}{V_{\max}}\left(1 + \frac{[I]}{K_I}\right)$$

The replot of intercept→[*I*] data for an uncompetitive inhibitor gives a straight line; analysis of its slope and intercept (actually intercept/slope of this line) gives the K_I. If the intercept replot is nonlinear, then extracting a K_I from such analysis is not

straightforward; nonlinear curve fitting for the data could be resorted to. The nonlinearity of inhibition is better visualized by Dixon analysis based on the following double reciprocal form of equation for uncompetitive inhibition:

$$\frac{1}{v} = \frac{1}{V_{\max}} \frac{1}{K_{\mathrm{I}}} [I] + \frac{1}{V_{\max}} \left(1 + \frac{K_{\mathrm{M}}}{[S]}\right)$$

22.2.2 Interpretation

An uncompetitive inhibitor affects only the zero-order rate constant ($V_{\max}$; 1/intercept of LB plot). Since $V_{\max}/K_{\mathrm{M}}$ is not affected, a pattern with parallel set of lines (with no slope change) is obtained (Fig. 22.4).

According to the scheme for uncompetitive inhibition, at low [*S*] most of the enzyme will be in *E*; very little *ES* exists for *I* to bind. Hence this inhibitor will not affect the slope (first-order rate constant). However the Y-axis intercepts (where $[S] \rightarrow \infty$) do change because all enzymes will be in *ES* form and *I* binds to it. This also means uncompetitive inhibition (unlike competitive inhibition) cannot be overcome by increasing [*S*]. Intercept replots, whenever nonlinear, may either be hyperbolic or parabolic. Such nonlinear uncompetitive inhibition is indicative of more complex scheme of interaction between the inhibitor and the enzyme.

Inhibition of (a) arylsulfatase by hydrazine and (b) 5-enolpyruvylshikimate-3-phosphate synthase (EPSP synthase) by glyphosate are two interesting examples of uncompetitive inhibition. Uncompetitive inhibitors do not bind free enzyme but only the *ES* complex (Cornish-Bowden 1986). To visualize the physical picture of how this happens is quite a challenge – particularly with single-substrate enzyme reactions. The problem is how to imagine that the inhibitor has no affinity to (or binding site on) the free enzyme but one gets created in the *ES* form. A conformational change in the enzyme to reveal this site may be invoked. Alternately, the inhibitor may bind the enzyme-bound substrate itself. Uncompetitive inhibitions, more common in multi-substrate enzyme mechanisms, are of diagnostic value in elucidation of the kinetic mechanism. An uncompetitive dead-end inhibition by an analog of *B*, with *A* as the varied substrate, is diagnostic of an ordered mechanism.

22.3 Noncompetitive Inhibition

Interaction of a noncompetitive inhibitor with the enzyme may be represented by the following equilibria (Fig. 22.5). A noncompetitive inhibitor affects both slope and intercept of a double reciprocal plot. Normally such a pattern shows a common point of intersection by a series of lines (see Fig. 22.6 below). Using the equilibrium

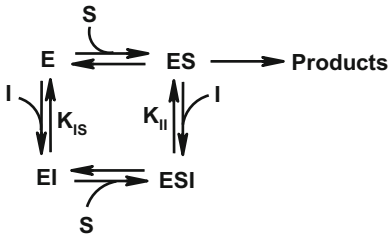

Fig. 22.5 Equilibria representing interaction of a noncompetitive inhibitor with the enzyme

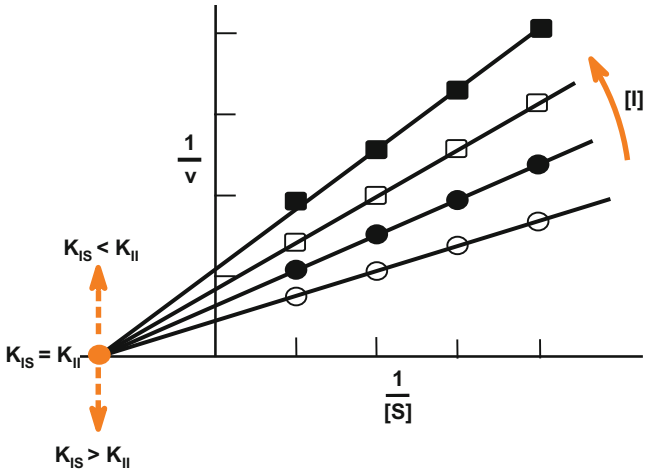

Fig. 22.6 Double reciprocal plots for noncompetitive inhibition of the enzyme with S as the varied substrate

assumption (Chap. 16, More Complex Rate Expressions, in Part II), a rate equation may be derived (appendix to this chapter) to represent this situation:

$$v = \frac{V_{\max}[S]}{(K_M + [S])\left(1 + \frac{[I]}{K_I}\right)}$$

Again, this equation is similar to the Michaelis–Menten form except that the entire denominator (K_M + [S]) is multiplied by a factor of (1 + [I]/K_I). It is obvious that a noncompetitive inhibitor combines the virtues of both a competitive (binds E) and an uncompetitive (binds ES) inhibition. Hence both slope and intercept effects are, respectively, observed.

22.3.1 Determination/Evaluation of Kinetic Constants and Replots

The double reciprocal form of the rate equation

$$\frac{1}{v} = \frac{K_M}{V_{max}}\left(1 + \frac{[I]}{K_I}\right)\frac{1}{[S]} + \frac{1}{V_{max}}\left(1 + \frac{[I]}{K_I}\right)$$

could be used to determine the K_I for the noncompetitive inhibitor. From the way the noncompetitive inhibition equilibria are represented, it is not necessary that the inhibitor affinity to E and ES be identical. Therefore the K_I obtained from the slope (termed more specifically as K_{IS}) and the intercept (termed K_{II}) may have different numerical values. In this sense, the slope and intercept of the double reciprocal plots change with the inhibitor concentration as shown below:

$$\text{slope} = \frac{K_M}{V_{max}}\left(1 + \frac{[I]}{K_{IS}}\right)$$
$$\text{intercept} = \frac{1}{V_{max}}\left(1 + \frac{[I]}{K_{II}}\right)$$

The two secondary plots (i.e., replots of slope→[I] data and intercept→[I] data) for a simple noncompetitive inhibitor give straight lines. The two sets of data may be now analyzed for K_I values, just the way we did before for competitive and uncompetitive inhibitors, respectively. It is possible that the slope replot, the intercept replot, or both of them may be nonlinear; such nonlinearity of inhibition is better visualized by Dixon analysis. A nonlinear curve fitting strategy will also be needed to obtain relevant K_I values.

22.3.2 Interpretation

A noncompetitive inhibitor affects both the zero-order rate constant (V_{max}; 1/intercept of the double reciprocal plot) and the first-order rate constant (V_{max}/K_M; 1/slope of the double reciprocal plot). According to the scheme for noncompetitive inhibition, the inhibitor binds an enzyme form both at low [S] (where form E predominates) and high [S] (where form ES predominates) conditions. Since Y-axis intercepts (where [S]→∞) do change, noncompetitive inhibition (unlike competitive inhibition) cannot be overcome by increasing [S].

Noncompetitive inhibition (with few exceptions, see below) is manifested as an intersecting set of lines in the double reciprocal analysis. The coordinates of the point of intersection when evaluated (also refer to intersecting patterns in Chap. 19, Analysis of Initial Velocity Patterns) are

Vertical (1/v) coordinate of the cross-over point $= \frac{1}{V_{max}}\left(1 - \frac{K_{IS}}{K_{II}}\right)$ and
horizontal (1/[S]) coordinate of the cross-over point $= -\frac{K_{IS}}{K_{II}}$.

The point of intersection is determined by the relative magnitudes of K_{IS} and K_{II}. If $K_{IS}/K_{II} = 1$, then the intersection is at the left of origin and on the X-axis at $1/v = 0$. This is a situation where $K_{IS} = K_{II}$ and is often known as pure noncompetitive inhibition. It is perfectly possible that $K_{IS} \neq K_{II}$, and such cases are at times referred to as *mixed inhibition*! Thus depending on the relative values of K_{IS} and K_{II}, the point of intersection is located above, on or below the X-axis (arrows in Fig. 22.6 indicate how the point of intersection moves).

Noncompetitive inhibition may also be viewed as a combination of two extremes, i.e., competitive (slope effect alone) and uncompetitive (intercept effect alone). When $K_{IS}/K_{II} \rightarrow 0$, it ends up being competitive (K_{II} is infinite; I has no affinity for ES), but with $K_{IS}/K_{II} \rightarrow \infty$, it simply collapses to being uncompetitive (K_{IS} is infinite; I has no affinity for E). However, if K_{IS} is very large (but not infinity!), then the lines in a noncompetitive pattern may appear parallel but actually intersect far away to the left of the origin. While it is easy to conclude that a set of lines intersect, we need to be cautious in concluding that a pattern is really parallel.

Whenever intercept/slope replots are nonlinear, they may either be hyperbolic or parabolic. So long as either slope or intercept (or both) replots are linear, a crossover point is observed in the noncompetitive pattern. If no crossover point is observed, but a series of "magic lines" are observed, then the inhibition is nonlinear in both slope and intercept effects.

22.4 Reversible Inhibition Equilibria: Another Viewpoint

The equilibria between the enzyme, substrate, and the inhibitor may be treated by one other approach. It is useful to appreciate this kinetic representation as one also finds some enzyme kinetic literature presented in this way. While different sets of nomenclature and/or representation are no doubt confusing, they need to be understood in order to fully savor the richness of enzyme kinetic literature. In this equilibrium treatment, dissociation constants for ES complex (K_S), EI complex (K_I), and k_{cat} are as shown in Fig. 22.7.

Two additional factors included in this scheme are (i) α is the factor by which I changes the affinity of S to E and (ii) β is the factor by which I alters the rate of product formation from ES complex. In the equilibrium scheme, both K_S and K_I are affected by the same α value by the presence of the other ligand. On thermodynamic

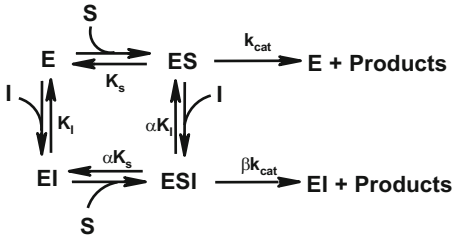

Fig. 22.7 A general equilibrium scheme for the interaction of an inhibitor with different enzyme forms

Table 22.1 Kinetic significance of α and β values

Effect	Nature	Lines intersect	Parameter value	
Complete inhibition	Competitive	On Y-axis	$\alpha = 0$	$\beta = 0$
	Noncompetitive	On X-axis	$\alpha = 1$	$\beta = 0$
	Mixed	Above X-axis	$\alpha < 1$	$\beta = 0$
	Mixed	Below X-axis	$\alpha > 1$	$\beta = 0$
	Uncompetitive		$\alpha >> 1$	$\beta = 0$
Partial inhibition			$\alpha =$ any	$0 < \beta < 1$
Activation			$\alpha =$ any	$\beta > 1$

grounds, ΔG° is a path-independent function, and equilibrium constants (K_S, K_I, αK_S, and αK_I) are directly related to corresponding ΔG° values. As long as E goes to form the same *ESI* complex, by either route, the constant α multiplying K_S and K_I will be identical.

22.4.1 Significance of α and β Values

The values of α and β provide useful information on the binding interactions and catalysis. Their relationship with the earlier classification of inhibitor types is summarized in the table above (Table 22.1).

This representation is particularly suited to describe enzyme activation and partial inhibition. A complete inhibitor will prevent the breakdown of *ESI* to products, and therefore $\beta = 0$. A partial block of this step is described by a value of β between 0 and 1. On the other hand, an activator will have a value of β greater than 1. We note that depending on the value of α, partial inhibitors and activators can also be classified. The inhibition constants obtained from the slope (K_{IS}) and the intercept (K_{II}) effects for a noncompetitive inhibitor (see the earlier discussion at noncompetitive inhibition and Fig. 22.6) are related to the α value as $K_{IS}/K_{II} = \alpha$. The corresponding rate equation will be

$$v = \frac{V_{max}[S]}{K_M\left(1 + \frac{[I]}{K_I}\right) + [S]\left(1 + \frac{[I]}{\alpha K_I}\right)}$$

For nonzero values of α other than unity, the lines do not intersect on the X-axis; these cases are denoted as mixed inhibitors in this treatment (see Table 22.1 above).

22.5 IC_{50} and Its Relation to K_I of an Inhibitor

The magnitude of K_I value for an inhibitor reflects its strength of interaction with the enzyme. The K_I being dissociation constant – the smaller its value, the more potent is the inhibitor. At times it is not feasible to rigorously determine the K_I value for an

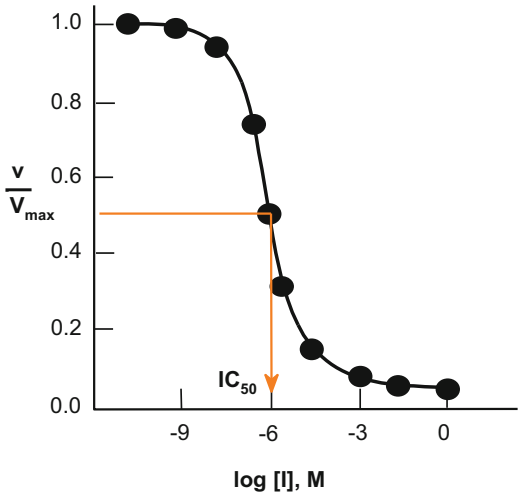

Fig. 22.8 Dose-response curve of enzyme activity as a function of inhibitor concentration. In this semi-log plot, fractional enzyme activity is plotted versus logarithm of inhibitor concentration

inhibitor. One other measure of relative inhibitory potency is the IC_{50} value. This is the concentration of inhibitor required to achieve 50% inhibition of enzyme activity under a defined set of assay conditions. The enzyme reaction velocity, IC_{50}, and the inhibitor concentration are related as shown:

$$\frac{v_i}{v} = \frac{1}{1 + \frac{[I]}{IC_{50}}}$$

where v_i is the rate in the presence of inhibitor I and v is the control rate in the absence of I. One plots fractional activity (v_i/v) as a function of log[I]. The IC_{50} can be graphically gleaned from such a dose-response curve (Fig. 22.8).

In practice, the inhibitor concentration is varied over several log values (at a single fixed [S]) to obtain estimates of IC_{50}. Presenting inhibitory potency of molecules as IC_{50} values is very popular in pharmaceutical research. For instance, a series of potential inhibitors against a particular enzyme target may be screened and ranked quickly and conveniently according to their IC_{50} values.

It is possible to relate IC_{50} values of an inhibitor to its corresponding K_I provided the type of inhibition is known (Brandt et al. 1987). For example, the relationship between the K_I, K_M, [S], and IC_{50} value can be derived for a competitive inhibitor as shown.

By definition, when $[I] = IC_{50}$, we have $v_i = v/2$. Substituting respective velocity equations

$$\frac{V_{max}[S]}{K_M\left(1 + \frac{IC_{50}}{K_I}\right) + [S]} = \frac{V_{max}[S]}{K_M + [S]}\frac{1}{2}$$

On simplifying we get

$$\frac{K_M}{K_I}IC_{50} = K_M + [S] \text{ and therefore } IC_{50} = K_I\left(1 + \frac{[S]}{K_M}\right)$$

This equation represents the Cheng-Prusoff relationship for a competitive inhibitor (Cheng and Prusoff 1973). It can be used to calculate K_I for an enzyme (at any given $[S]$) from the corresponding IC_{50} value. Although the IC_{50} determinations are popular for their experimental simplicity, two precautions are mandated.

1. It is important to maintain and report the assay conditions along with the IC_{50} value. Otherwise, different IC_{50} values are not comparable. Among other things, the substrate concentration used in the assay influences the measured IC_{50} value (see from equation above).
2. Cheng-Prusoff relationships for different inhibitor types (e.g., uncompetitive and noncompetitive) are not the same. Therefore IC_{50} values may be compared strictly between compounds exhibiting same mode of inhibition. When in doubt the IC_{50} values must be cross-checked by performing a rigorous double reciprocal analysis.

IC_{50} values and Cheng-Prusoff relationships are commonly used for high-throughput inhibitor screening of a series of structurally related molecules. Such analysis is of immense practical value in quantitative structure activity relationship (QSAR) studies (see Chaps. 23 and 28). In the final analysis, however, there is no substitute for a rigorously determined K_I value of an inhibitor.

Appendix

The rate equation for noncompetitive inhibition may be derived starting from an equilibrium assumption (hence K_S replaces K_M) and the scheme shown in Fig. 22.5. In this equilibrium *E*, *ES*, *EI*, and *ESI* are the four different enzyme forms, and *ESI* form is nonproductive. Defining any three equilibrium constants will automatically define the fourth (note: state function). In order to evaluate the concentration of the *ES* complex, we use the following relations:

$$[ES] = \frac{[E][S]}{K_S}; \quad [EI] = \frac{[E][I]}{K_{IS}}; \quad \text{and} \quad [ESI] = \frac{[ES][I]}{K_{II}} = \frac{[E][S][I]}{K_S K_{II}}$$

Evaluating the fraction of total enzyme ($[E_t]$) present in the *ES* form, we get

$$f = \frac{[ES]}{[E_t]} = \frac{[ES]}{[E] + [ES] + [EI] + [ESI]}$$

Substituting for the concentrations of all enzyme forms from the three equilibrium relationships in terms of $[E]$,

$$f = \frac{\frac{[E][S]}{K_S}}{[E] + \frac{[E][S]}{K_S} + \frac{[E][I]}{K_{IS}} + \frac{[E][S][I]}{K_S K_{II}}} = \frac{\frac{[S]}{K_S}}{1 + \frac{[S]}{K_S}} + \frac{[I]}{K_{IS}} + \frac{[S][I]}{K_S K_{II}}$$

Simplifying (multiplying both the numerator and the denominator by K_S) further, we obtain

$$f = \frac{[S]}{K_S + [S] + \frac{K_S}{K_{IS}}[I]} + \frac{1}{K_{II}}[S][I].$$

This fraction ($f = [ES]/[E_t]$) may be now plugged in to obtain the rate equation:

$$v = f.V_{max} = \frac{V_{max}[S]}{K_S + [S] + \frac{K_S}{K_{IS}}[I]} + \frac{1}{K_{II}}[S][I]$$

This equation can be easily rearranged to the more familiar form:

$$v = \frac{V_{max}[S]}{K_S\left(1 + \frac{[I]}{K_{IS}}\right) + [S]\left(1 + \frac{[I]}{K_{II}}\right)}$$

Of course, if $K_{IS} = K_{II}$, then this simply becomes

$$v = \frac{V_{max}[S]}{(K_S + [S])\left(1 + \frac{[I]}{K_I}\right)}.$$

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