The *v versus v*[*I*] Plot*

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Data of the velocity (v) of an enzyme-catalyzed reaction at a fixed concentration of substrate (S) and various concentrations of an inhibitor (I) may be presented in a *v versus* $v[I]$ plot. This plot is the counterpart of the Eadie-Hofstee plot (*v versus* $v/[S]$) which may be used to present data of the velocity at various substrate concentrations.

The Lineweaver-Burk plot [1] and the Eadie-Hofstee plot [2, 3], which are based on linear transformations of the Michaelis-Menten equation, have been used during several decades for graphical estimation of kinetic parameters. With the use of computers parameter estimation can now be done by direct fitting of the Michaelis-Menten equation, but these and other plots are still useful for the graphical presentation of kinetic results.

To depict the relationship between the velocity of an enzyme-catalyzed reaction and the concentration of an inhibitor, one may use the plot described by Dixon [4], in which $1/v$ is plotted against $[I]$, or that proposed by Cornish-Bowden [5] in which $[S]/v$ is plotted against $[I]$. Here I want to call attention to a third graphical presentation in which v is plotted against $v[I]$. To the best of my knowledge, this plot has not been mentioned in the literature.

The rate equation for mixed inhibition

$$v = \frac{V\,[S]}{K_m\left(1 + \frac{[I]}{K_{ic}}\right) + \left(1 + \frac{[I]}{K_{iu}}\right)[S]} \qquad (1)$$

can be arranged so as to show that v is a linear function of $v[I]$

$$v = \frac{-\,(K_m\,K_{iu} + K_{ic}\,[S])}{K_{ic}\,K_{iu}\,(K_m + [S])} \cdot v[I] + \frac{V\,[S]}{K_m + [S]} \qquad (2)$$

where K_m is the Michaelis constant and K_{ic} and K_{iu} represent the competitive and uncompetitive inhibition constants for I, respectively.

As shown in Fig. 1b, plots of *v versus* $v[I]$ at various substrate concentrations form a family of lines that intersect at a point with coordinates $-\,VK_{ic}\,K_{iu}/(K_{iu}-K_{ic})$ and $VK_{iu}/(K_{iu}-K_{ic})$. This point is located in

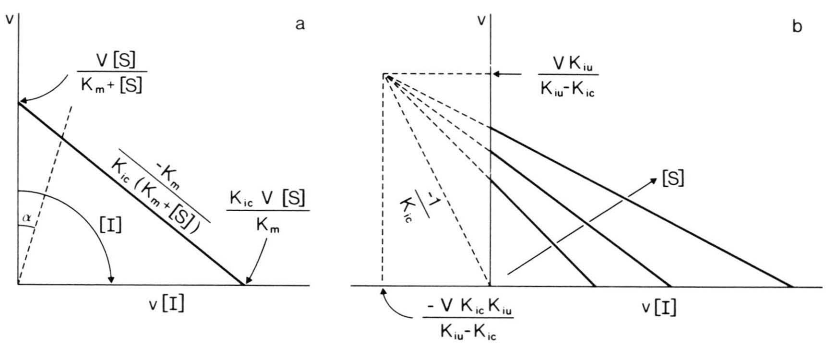

Fig. 1. The *v versus* $v[I]$ plot. (a) Slope and intercepts in case of competitive inhibition. Note that tg $\alpha = [I]$. (b) Plots at various substrate concentrations in case of mixed inhibition.

the 2nd quadrant when $K_{ic} < K_{iu}$, or in the 4th quadrant when $K_{ic} > K_{iu}$; its y-coordinate is either $> V$ or < 0. In case of competitive inhibition ($K_{iu} \rightarrow \infty$) the intersection point is located in the 2nd quadrant, and its y-coordinate equals V (Fig. 2a). For the special

* Symbols and terminology in this paper are according to the NC-IUB Recommendations 1981 (*Eur. J. Biochem.* **126,** 281–291 (1982)); the letter S is used for substrate.

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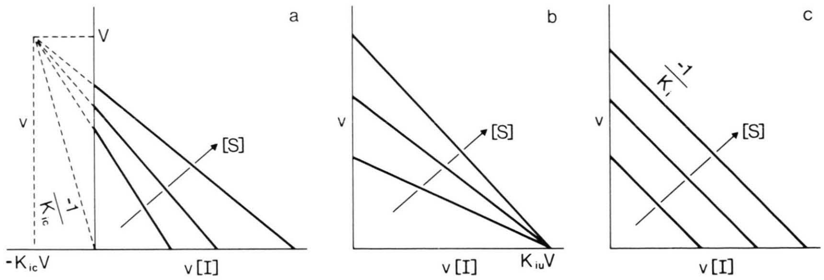

Fig. 2. Plots at various substrate concentrations in case of (a) competitive inhibition; (b) uncompetitive inhibition, and (c) pure non-competitive inhibition, where $K_{ic} = K_{iu} = K_i$.

case of pure non-competitive inhibition where $K_{ic} = K_{iu} = K_i$, a number of parallel lines is obtained, each with a slope equal to $-1/K_i$ (Fig. 2c), and for uncompetitive inhibition ($K_{ic} \rightarrow \infty$) the family of lines intersect at a point with coordinates $K_{iu}V$ and 0 (Fig. 2b).

The *v versus v*[*I*] plot gives direct information about K_{ic}, since the line through the intersection point and the origin (which represents the practically impossible plot for $[S] = 0$) has a slope equal to $-1/K_{ic}$. Competitive and mixed inhibition can be discriminated only when $K_{ic} \geqq K_{iu}$, otherwise V in the absence of inhibitor should be known. The value of the latter parameter is also necessary to find K_{iu}, since the line through the intersection point and $(0, V)$ has a slope equal to $-1/K_{iu}$. This line represents the plot for $[S] \rightarrow \infty$.

For the Dixon plot, contemplations on the discrimination between inhibition types [6] and the information about the inhibition constants are the very same. Here, the x-coordinate of the intersection point equals $-K_{ic}$, whereas the line through intersection point and $(0, 1/V)$ intercepts the x-axis at $-K_{iu}$. In the $[S]/v$ *versus* $[I]$ plot, however, the x-coordinate of the intersection point is equal to K_{iu}, the line through intersection point and $(0, K_m/V)$ intercepts the x-axis at $-K_{iu}$, and unless $K_{iu} \geqq K_{ic}$, the value of K_m/V should be known to discriminate between uncompetitive and mixed inhibition.

The velocity of an enzyme-catalyzed reaction in the presence of an inhibitor may be considered as a function of $[S]$, or as a function of $[I]$. Just as the Dixon plot is the counterpart of the Lineweaver-Burk plot, the plot suggested here is the counterpart of the Eadie-Hofstee plot (Table I). In the last two plots, the independent variable, $[I]$ and $[S]$, respectively, is given by the tangent of the angle between a line through the origin and one of the axes (Fig. 1a). The virtues ascribed to the Eadie-Hofstee plot [7, 8] therefore also apply to the *v versus* $[I]$ plot. For example, deviation from linearity will be much more pronounced in the v versus $v[I]$ plot than in the Dixon-plot. For this reason the plot is especially useful to picture the effect of an inhibitor on a heterogeneous catalytic process, as encountered frequently in transport studies [9, 10].

Table I. Plots based on linear transformations of rate equations as a function of either substrate concentration or inhibitor concentration.

v as function of $[S]$	v as function of $[I]$
Lineweaver-Burk	Dixon
$\frac{1}{v}$ *versus* $\frac{1}{[S]}$	$\frac{1}{v}$ *versus* $[I]$
Eadie-Hofstee	
v *versus* $v \cdot \frac{1}{[S]}$	v *versus* $v \cdot [I]$

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