A Simple Graphical Method for Determining the Inhibition Constants of Mixed, Uncompetitive and Non-Competitive Inhibitors

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A new method is described for plotting kinetic results for inhibited enzyme-catalysed reactions. It provides a simple way of determining the inhibition constant, K'_i, of an uncompetitive, mixed or non-competitive inhibitor.

It has long been common practice to determine the inhibition constants of competitive inhibitors by the method of Dixon (1953), in which the reciprocal velocity, $1/v$, is plotted against the inhibitor concentration, i, at two or more values of s, the substrate concentration. For each s value, the points lie on a straight line, and the extrapolated lines at different s values intersect at a single point, for which $i = -K_i$ and $1/v = 1/V$, where K_i, the inhibition constant, is the dissociation constant of the EI complex, and V is the maximum velocity. The usefulness of this method is limited by the fact that it does not distinguish unambiguously between competitive and mixed inhibitors (Schlamowitz *et al.*, 1969), and, for mixed or uncompetitive inhibitors, it provides no measure of K'_i, the dissociation constant of the EIS complex. In this communication, a new plot is described, which is similar to the Dixon plot but complementary to it, since it provides a simple way of measuring K'_i rather than K_i.

Linear mixed inhibition includes all of the common types of inhibition as asymptotic or special cases, and it will therefore be taken as a general case. It is described by the rate equation:

$$v = \frac{Vs}{K_m[1 + (i/K_i)] + s[1 + (i/K'_i)]}$$

where K_m is the Michaelis constant. It is formally convenient to regard K_i as the dissociation constant of the EI complex, and K'_i as that of the EIS complex, though there are many common cases of product inhibition in multiple-substrate reactions where no such simple interpretation is possible.

From the rate equation, it follows that:

$$\frac{s}{v} = \frac{K_m}{V}\left(1 + \frac{i}{K_i}\right) + \frac{s}{V}\left(1 + \frac{i}{K'_i}\right)$$

and so a plot of s/v against i is a straight line. If two such lines are drawn, at different values of s, they intersect at a point where $i = -K'_i$ and $s/v = K_m[1 - (K'_i/K_i)]/V$. For comparison, the intersection of the lines of a Dixon plot for mixed inhibition occurs at a point where $i = -K_i$, $1/v = [1 - (K_i/K'_i)]/V$. Thus the two plots complement one another and

permit both K_i and K'_i to be measured very simply. If $K_i > K'_i$, the intersection is above the i axis in the plot of s/v against i, and below it in the Dixon plot; the opposite is true if $K_i < K'_i$; and in the special case where $K_i = K'_i$ (simple non-competitive inhibition) the intersections occur on the i axis in both plots.

The asymptotic cases of competitive and uncompetitive inhibition can be generated very easily by inserting the conditions $K'_i \to \infty$ for competitive inhibition, or $K_i \to \infty$ for uncompetitive inhibition. Thus, for competitive inhibition, there is no intersection (i.e. the lines are parallel) in the plot of s/v against i, whereas the intersection in the Dixon plot is given by $i = -K_i$, $1/v = 1/V$. For uncompetitive inhibition, the intersection in the plot of s/v against i is given by $i = K'_i$, $s/v = K_m/V$, whereas there is no intersection in the Dixon plot. These conclusions are illustrated in Fig. 1, which shows that if both plots are used the pattern of results provides an unambiguous indication of the type of inhibition. This is not true of either of the plots alone, since the Dixon plot does not always distinguish competitive from mixed inhibition, whereas the plot of s/v against i does not always distinguish uncompetitive from mixed inhibition.

It is now well established (Johansen & Lumry, 1961; Wilkinson, 1961; Dowd & Riggs, 1965) that the double-reciprocal plot is by far the worst of the commonly used methods of determining K_m and V. Unfortunately, since $1/v$ is also plotted on the ordinate in the Dixon plot, this plot must be expected to provide very poor estimates of K_i, unless the experimental error is very small. By contrast, the plot of s/v against i should provide good estimates of K'_i, since the variance of s/v does not vary greatly with v if v is distributed with uniform variance (Wilkinson, 1961).

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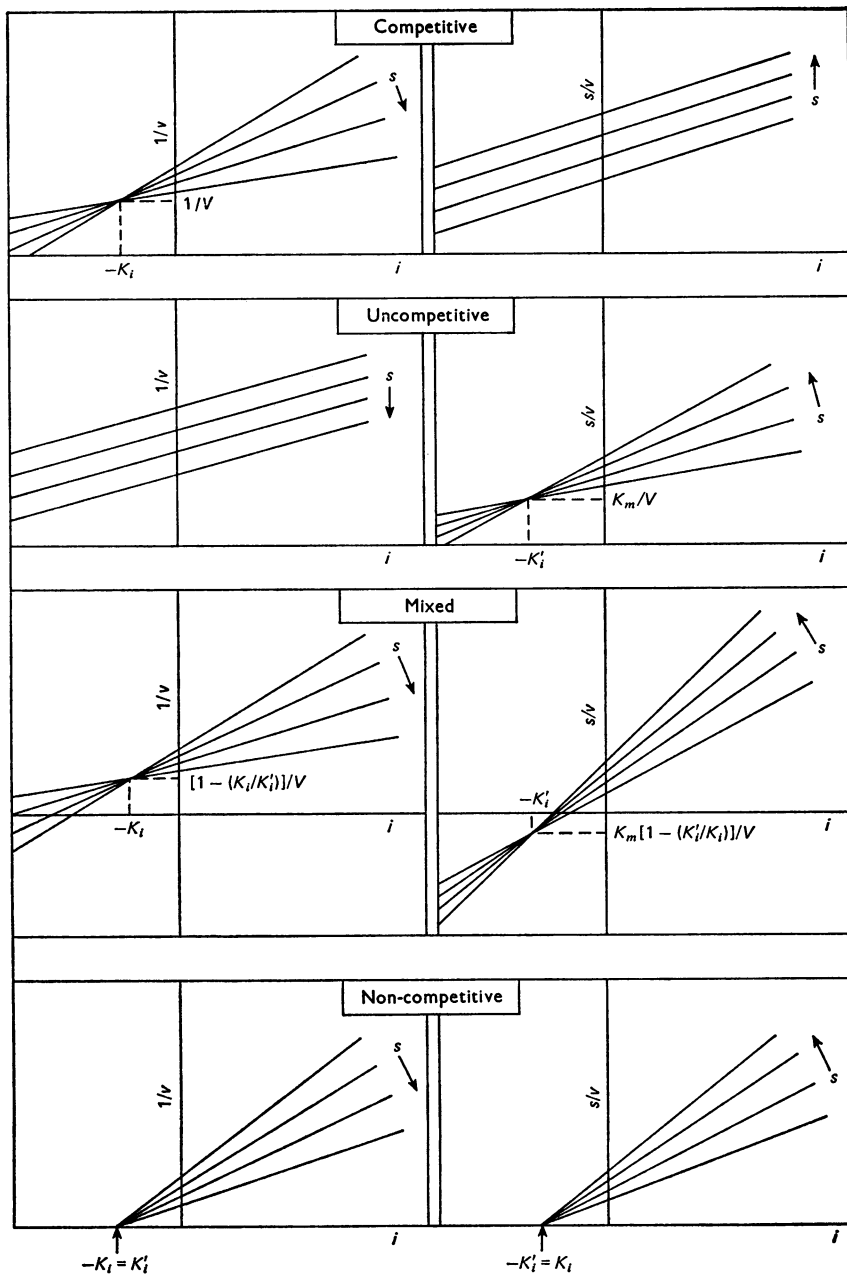
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Fig. 1. *Characteristic plots for the common inhibition types*

Plots of $1/v$ against i (Dixon plots) and of s/v against i for all of the linear types of inhibition. In every case the intersection point in the Dixon plot provides a measure of K_i, whereas the intersection point in the plot of s/v against i provides a measure of K_i'.