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# LIMITATIONS IN THE USE OF DIXON PLOTS TO EVALUATE ENZYME INHIBITION

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SUMMARY

Dixon-type plots afford a useful means of distinguishing between competitive enzyme inhibition and non-competitive enzyme inhibition. If in the latter case, however, binding of the inhibitor to the enzyme does affect substrate binding or *vice versa*, then Dixon plots may not be used to differentiate between competitive and non-competitive inhibitors.

In most studies of enzyme inhibition the data are presented graphically either as Dixon plots[1-3] or in Lineweaver–Burk form[4]. In the former procedure, the reciprocal of initial reaction velocity is plotted on the ordinate as a function of inhibitor concentration at various fixed levels of substrate concentration. In the double reciprocal method of Lineweaver and Burk, one records 1/(initial velocity) on the ordinate as a function of 1/[substrate] in the absence and presence of the inhibitor. The advantage of the Dixon plot is that one can readily evaluate the inhibition constant ($K_i$) from the kinetic data. In order to obtain this parameter by the double-reciprocal method, a certain amount of calculation is required.

Eqns. 1 and 2 depict the case of competitive inhibition for a one substrate enzyme system, and in Eqn. 3 is presented the well-known rate equation for this effect.

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E + P \tag{1}$$

$$E + I = EI,\ K_i \tag{2}$$

$$\frac{1}{v} = \frac{1}{V} + \frac{K_m}{V[S]}\left[1 + \frac{[I]}{K_i}\right] \tag{3}$$

In Eqn. 3, $v$, $V$, $K_m$, $S$, $I$ and $K_i$, are taken to be initial reaction velocity, maximal velocity, Michaelis constant, substrate concentration, inhibitor concentration, and inhibition constant, respectively.

In the Dixon plot when $1/v$ is plotted *versus* $[I]$ at different constant levels of $[S]$, the family of curves intersect at a point above the abscissa. The coordinates of this point of intersection are $(-K_i, 1/V)$.

In the case of non-competitive inhibitors, it has been suggested that the lines of the Dixon plot will intersect on the abscissa with coordinates at this point equal to $(-K_i, 0)$[2,3]. Mahler and Cordes[3] also show that mixed inhibitors act indentically to non-competitive inhibitors when graphed as Dixon plots. It is the purpose of this report to show that not only do mixed and non-competitive inhibitors yield different Dixon plots, but that in some cases, mixed inhibitors will in fact resemble competitive inhibitors.

Eqns. 4–7 show the interactions expected for an inhibitor which produces mixed inhibition:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E + P \tag{4}$$

$$E + I = EI,\ K_i \tag{5}$$

$$ES + I = ESI,\ K_{ii} \tag{6}$$

$$EI + S = ESI,\ K_{iii} \tag{7}$$

The rate expression for this mechanism is:

$$\frac{1}{v} = \frac{1}{V}\left[1 + \frac{[I]}{K_{ii}}\right] + \frac{K_m}{V[S]}\left[1 + \frac{[I]}{K_i}\right] \tag{8}$$

where $K_i$ and $K_{ii}$ represent the dissociation constants depicted in Eqns. 5 and 6 and the other kinetic parameters are as described for Eqn. 3. It is important to note that there is no reason to believe that in the mechanism outlined in Eqn. 4, the presence of substrate on the enzyme will or will not influence the binding of the inhibitor to the enzyme. Similar remarks relative to substrate binding can be made when inhibitor is bound to the enzyme. If one compound does not affect the binding of the other, $K_i = K_{ii}$; however, $K_i \neq K_{ii}$ when this is not true.

If one evaluates the coordinates of the point of intersection of the lines in Dixon plots of data conforming to Eqn. 8, the result is $(-K_i, 1/V\,[1 - K_i/K_{ii}])$. In the case of a true non-competitive inhibitor (where $K_i = K_{ii}$) the lines of the Dixon plot will intersect on the abscissa. On the other hand, for mixed inhibitors (where $K_i \neq K_{ii}$) the lines may converge above or below the abscissa depending upon the relationship between $K_i$ and $K_{ii}$. If $K_i$ is appreciably less than $K_{ii}$, the $1/v$ coordinate will be $1/V$ and the inhibition pattern will be indistinguishable from the competitive inhibition case. Of course, if $K_{ii}$ is substantially less than $K_i$, the intersecting lines will converge below the $[I]$ axis.

On the basis of the above considerations, it seems clear that one cannot differentiate between competitive and mixed inhibition in some cases using a Dixon plot. When such distinctions are necessary in arguments supporting a kinetic mechanism of an enzyme's action, it would appear advantageous to use the double-reciprocal method of Lineweaver and Burk[4].

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REFERENCES

1 M. Dixon, *Biochem. J.*, 55 (1953) 170.
2 M. Dixon and E. C. Webb, *Enzymes*, Academic Press, New York, 2nd ed., 1964.
3 H. R. Mahler and E. H. Cordes, *Biological Chemistry*, Harper and Row, New York, 2nd ed., 1971.
4 H. Lineweaver and D. Burk, *J. Am. Chem. Soc.*, 56 (1934) 658.