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THE USE OF DIXON PLOTS TO STUDY ENZYME INHIBITION

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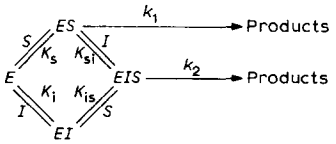
SUMMARY

1. The use of the Dixon method of plotting enzyme inhibition data is considered for cases where the interaction between the inhibitor and enzyme is more complex than for classical competitive and non-competitive inhibition. It is concluded that the use of Dixon plots in conjunction with Lineweaver–Burk plots can be a valuable aid in the identification of inhibition behaviour.

2. The inhibition patterns considered include mixed, hyperbolic-competitive and two forms of uncompetitive inhibition.

In studies of the effects of reversible enzyme inhibitors, many authors present data in the form of Dixon plots[1] in which the reciprocal of the initial reaction velocity v is plotted against the inhibitor concentration (i) at various fixed concentrations of substrate (s). Recently, Purich and Fromm[2] have criticised the use of this method for cases in which there is mixed inhibition and suggest that $1/v$ *versus* $1/s$ plots of Lineweaver and Burk[3] are preferable. While not disagreeing with Purich and Fromm[2], I consider that a case can be made for retaining Dixon plots provided they are used in conjunction with double reciprocal plots. The use of both methods of plotting allows not only ambiguities[2] to be recognised but can also provide much extra data.

Eqn 1 shows a general scheme for the interaction of an enzyme with substrate and inhibitor.



If rapid equilibration of all enzyme–ligand complexes is assumed, the rate equation describing the mechanism (Frieden[4]) is of the Michaelis–Menten form depicted in Eqn 2.

$$\frac{1}{v} = \frac{1 + i/K_{si}}{V(1 + k_2 i/k_1 K_{si})} + \frac{K_s}{Vs} \cdot \frac{1 + i/K_i}{1 + k_2 i/k_1 K_{si}} \qquad (2)$$

For a mechanism as general as that shown in Eqn 1 there is no linear relationship between $1/v$ and i although double reciprocal plots would be linear. Under certain conditions Eqn 2 simplifies to a linear Dixon form and hence a test of whether experimental data can be fitted to straight lines can help in the choice of a suitable mechanism for a particular enzyme under study. Some useful cases are considered below.

Case I. $k_2 = 0$: *EIS* does not break down to form products. This mechanism is considered by Purich and Fromm[2] and double reciprocal plots are characteristic of mixed inhibition. Provided no interpretation of the kind of inhibition is made on the basis of whether Dixon plots of data recorded at different substrate levels intersect above, below or on the i axis, such plots furnish values for $-K_i$ (from the i value at the point of intersection) and $(1 - K_i/K_{si})/V$ (from the value for $1/v$ at the intersection). Values for K_i are usually obtainable with good accuracy by the Dixon method.

Case II. $k_1 = k_2$: I affects the binding of S but the rate of formation of products from enzyme–substrate complexes is unchanged. This kind of inhibition has been called hyperbolic-competitive (Mahler and Cordes[5]) because the rate equation for such a scheme (Eqn 3) indicates that double reciprocal plots have a common point of intersection on the $1/v$ axis.

$$\frac{1}{v} = \frac{1}{V} + \frac{K_s}{Vs} \cdot \frac{1 + i/K_i}{1 + i/K_{si}} \tag{3}$$

The mechanism is indistinguishable from normal competitive inhibition by double reciprocal plots, but Dixon plots are hyperbolic. The use of both methods of plotting allows a distinction to be made between true or hyperbolic-competitive inhibition.

Case III. $k_2 = 0$, $K_i = \infty$: this is classical uncompetitive inhibition[6] in which it is assumed that the inhibitor binds only to the *ES* complex. The well known rate equation is:

$$\frac{1}{v} = \frac{1 + i/K_{si}}{V} + \frac{K_s}{Vs} \tag{4}$$

Dixon plots (like double-reciprocal plots) at various substrate concentrations are parallel straight lines with a slope[5] of $1/VK_{si}$ and intercept[5] on $1/v$ axis equal to $(1 + K_s/s)/V$.

Case IV. $K_{si}/K_i = k_2/k_1$: the ratio of the binding constants for I to E and ES, respectively, is equal to the reciprocal of the ratio of the rate constants describing the breakdown of the *ES* and *EIS* complexes. The rate equation (Frieden[4]) becomes:

$$\frac{1}{v} = \frac{1 + i/K_{si}}{V(1 + i/K_i)} + \frac{K_s}{Vs} \tag{5}$$

Eqn 5 predicts parallel Lineweaver–Burk plots characteristic of uncompetitive inhibition but Dixon plots are hyperbolic and become independent of i as i/K_{si} and i/K_i become much greater than unity. Limiting values of $1/v$ correspond to $(K_i/K_{si} + K_s/s)/V$ and enable the ratio of the binding constants for I to be determined approximately. Thus when uncompetitive inhibition has been detected by parallel Lineweaver–Burk plots, the use of Dixon's method should distinguish between the mechanisms presented in Cases III and IV.

Although uncompetitive behaviour is rare with enzymes acting on single sub-

strates it has been shown to occur with certainty with at least one enzyme[7]. Also for real systems Case IV may represent a more likely situation than assuming that inhibitor can only bind to an enzyme–substrate complex.

CONCLUSIONS

It is true that by suitable replotting of the slopes and intercepts of double-reciprocal plots as a function of i (Cleland[8,9]) similar information to that obtainable from Dixon plots may be gained. The accumulation and processing of sufficient data to allow unambiguous conclusions to be drawn from the replotting of intercepts and slopes are likely to be lengthy exercises, however. The ease with which data may be collected and plotted by Dixon's method makes its use worth considering particularly in the first stages of an investigation.

REFERENCES

1 M. Dixon, *Biochem. J.*, 55 (1953) 170.
2 D. L. Purich and H. J. Fromm, *Biochim. Biophys. Acta*, 268 (1972) 1.
3 H. Lineweaver and D. Burk, *J. Am. Chem. Soc.*, 56 (1934) 658.
4 C. Frieden, *J. Biol. Chem.*, 239 (1964) 3522.
5 H. R. Mahler and E. H. Cordes, *Biological Chemistry*, Harper and Row, New York, 2nd edn, 1971.
6 M. Dixon and E. C. Webb, *Enzymes*, Longmans, London, 2nd edn, 1964.
7 W. H. Fishman and N. K. Ghosh, *Adv. Clin. Chem.* 10 (1967) 255.
8 W. W. Cleland, *Biochim. Biophys. Acta*, 67 (1963) 104.
9 W. W. Cleland, *Biochim. Biophys. Acta*, 67 (1963) 173.