A Modified Graphical Method for Determination of Equilibrium Constants

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A modification is described of the method of Dixon (1965) for determining equilibrium constants (K) of combinations of the type $A + B \rightleftharpoons AB$. The method obviates drawing of a tangent to the curve at the origin.

The method described by Dixon (1965) involves the drawing of a tangent. It may not always be possible to arrange conditions such that the total concentration of B (b_t) is of the same order as K , particularly if B is an enzyme. Under conditions where b_t is small compared with K the accuracy in determining b_t may be poor as a result of errors in drawing of the tangent. Even under conditions where b_t is relatively large it may be advisable to avoid drawing a tangent to the curve. A slight modification of the method allows one to determine the same quantities without drawing any tangent.

METHOD

Let us assume that we have a reversible equilibrium of the type $A + B \rightleftharpoons AB$ such that a_t is the total added concentration of the activator A, and b_t is the total amount of the

apoenzyme B. A graph relating the velocity (v) of the reaction catalysed by the enzyme to the activator concentration (a_t) will be of the form shown in Fig. 1. Horizontal lines are drawn to represent V , the maximum velocity with excess of A, and $V/2$, half maximum velocity. A chord is drawn joining the origin and the point on the curve where $v = 3V/4$. The distance on the $V/2$ line between the points where it intercepts the curve and the chord gives K .

THEORY

Dixon (1965) obtained two expressions for the equilibrium constant K of the reaction:

$$K = \frac{ab}{p} = \frac{(a_t - p)(b_t - p)}{p} = \left(\frac{a_t}{p} - 1\right)(b_t - p) \quad (1)$$

$$K = a_t' - \frac{V}{2k} \quad (2)$$

Fig. 1. Graphical method for determining the dissociation constant K , as applied to a flavoprotein oxidase (cf. Dixon, 1965). The ordinate shows the velocity (v) of the oxidase reaction in arbitrary units, and the abscissa the total concentration (a_t) of activator FAD. For other quantities see the text.

where a , b and p are the concentrations of A, B and AB respectively; the subscript t denotes total concentration (i.e. the sum of concentrations of free and bound forms of the substance) and absence of subscript denotes concentrations of the free form; a'_t is the concentration of a_t when $v = V/2$; and k is the velocity constant of the reaction catalysed by AB.

For the point on the curve where $v = 3V/4$, let the value of a_t be a'_t .

Then:

$$p = \frac{v}{k} = \frac{3V}{4k}, b_t = \frac{V}{k} \text{ and } b_t - p = \frac{V}{k} - \frac{3V}{4k} = \frac{V}{4k}$$

Hence from eqn. (1):

$$K = \left(a'_t \cdot \frac{4k}{3V} - 1 \right) \frac{V}{4k} \quad (3)$$

$$\therefore K = \frac{a'_t}{3} - \frac{V}{4k}$$

Solving for K by eliminating V/k from eqns. (2) and (3):

$$K = \frac{2a'_t}{3} - a'_t$$

EXTENSIONS

As with Dixon's (1965) method, other quantities can be determined from the same diagram.

Since:

$$b_t = \frac{V}{k}$$

From eqn. (2):

$$\frac{b_t}{2} = a'_t - K$$

Hence if a distance equal to K is set off on the $V/2$ line to the left of a'_t , and a line from the origin through this point extended to cut the V -line, b_t is obtained (see Fig. 1).

The other quantities (a , b and p for any point on the curve, and K) can be determined as described by Dixon (1965).

DISCUSSION

The method, being based on the same assumptions as Dixon's (1965) method, is subject to the same limitations. The only advantage claimed is that one avoids the difficulty of drawing a tangent at the origin, which might present greater difficulties than drawing a chord through two widely separated points on a curve.

REFERENCE

Dixon, M. (1965). *Biochem. J.* **94**, 760.