Short Communication

Graphical Determination of Relative Concentrations of Enzyme and Endogenous Inhibitor by Dilution

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Schwimmer et al. (8) developed a simple graphical method for the determination of relative enzyme concentration in a crude extract containing endogenous inhibitor. The method requires only the measurement of the reaction rates of several dilutions of the extract. It is the purpose of this communication to show that the same method can be used for finding the relative concentration of inhibitor in the extract and to point out limitations of the method.

From equation 1 (2) for noncompetitive, low affinity inhibition,

\[ v_e/v_i = 1 + i/K_i \]  

Schwimmer et al. (8) derived

\[ E/v_i = 1/k_i + \frac{k_2}{k_1 K_i} E \]  

where \( v_e \) = the velocity of enzyme action in the absence of inhibitor, \( v_i \) = the velocity in the presence of inhibitor at concentration \( i \), \( K_i \) = the dissociation constant of the enzyme-inhibitor complex, \( E \) = the amount of tissue present in the enzyme reaction mixture, \( k_i \) is a proportionality constant for the relation of \( v_i \) to \( E \), and \( k_2 \) is a proportionality constant for the relation of \( i \) to \( E \). It was pointed out that, when \( E/v_i \) is plotted against \( E \), equation 2 produces a straight line which intercepts the \( E/v_i \) axis at \( 1/k_i \) (Fig. 1). Since \( v_e \) is the product of \( k_i \) and \( E \), \( E \) divided by \( 1/k_i \) gives \( v_e \) for that value of \( E \), i.e., the enzyme activity expected if no inhibitor were present.

The possibility of using this method to determine the relative amount of endogenous inhibitor in the extract was not mentioned by Schwimmer et al. (8). Substituting \( k_i E \) for \( v_e \) in equation 1 and rearranging gives

\[ \frac{i}{K_i} = \frac{E/v_i}{1/k_i} - 1 \]  

It follows that for any value of \( E \), \( i/K_i \) can be obtained by subtracting 1 from the quotient of \( E/v_i \) divided by the \( E/v_i \) intercept (Fig. 1). Where \( K_i \) is not known, it is convenient to define inhibitor concentration in arbitrary units such that 1 unit is the amount of inhibitor which reduces the reaction rate of an appropriate quantity of enzyme by 50% under standard assay conditions. Units of inhibitor will then be equal to \( i/K_i \) and can be determined directly from equation 3 after graphical determination of \( 1/k_i \).

Schwimmer et al. (8) pointed out that an equation of the same form as equation 2 can be obtained from the equation for competitive inhibition. For the competitive case, the equation parallel to equation 3 is

\[ \frac{i}{K_i + (K_i/K_m) \cdot s} = \frac{E/v_i}{1/k_i} - 1 \]  

where \( s \) is the concentration of substrate and \( K_m \) is the Michaelis constant. One unit of a competitive inhibitor could be defined as the amount which in the presence of a given concentration, \( s \), of substrate slows the enzyme reaction rate by 50%. Units of competitive inhibitor would then be equal to \( i/(K_i + [K_i/K_m] \cdot s) \), and these would be given directly by equation 4.

It should be stressed that equation 1 and hence also the equations derived from it are applicable only to low affinity inhibition, i.e., to enzyme inhibition for which the concentration of inhibitor bound to enzyme is negligible compared to the total inhibitor concentration (1). The appropriate equation for noncompetitive, high affinity inhibition would be equation 5, which is derived from the general equation (1) for this type of inhibition.

![Graphical method for the determination of relative concentrations of enzyme and endogenous inhibitor present in a tissue extract. Tissue concentration, \( E \), in the enzyme reaction mixture is the abscissa; the ratio of \( E \) to the reaction rate, \( v_e \), is the ordinate. A low affinity inhibitor gives a straight line which intercepts the ordinate at \( 1/k_i \), thus giving the specific activity of enzyme in the absence of inhibitor. Relative concentration of inhibitor present when \( E = x_i \) can be obtained from \( (y_i + 1/k_i) - 1 \). A high affinity inhibitor produces a curve rather than a straight line. For calculation of these hypothetical graphs, the same value of \( k_i \) was chosen for low and high affinity inhibition. However, \( k_i \) was much smaller and \( K_i \) was very much smaller for the high affinity inhibition.

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Here $k$ is a proportionality constant for the relation of reaction rate in the absence of inhibitor to total enzyme concentration, and other terms are defined as above. As shown in Figure 1, equation 5 leads not to a straight line, but rather to a curve which has the limit of $1/k_i$ as $E$ approaches zero and a limit of $1/(k_i - kK)$ as $E$ becomes large. The higher the affinity of inhibitor for enzyme and hence the smaller the $K_i$, the more rapidly the curve will approach the horizontal. An inhibitor forming an undissociable complex with the enzyme would give a line which was parallel to the horizontal line found in the absence of inhibitor and which would intercept the ordinate at $1/(k_i - kK)$.

It should also be noted that any increase in enzyme specific activity following dilution, whether resulting from the presence of low affinity inhibitor or from completely unrelated factors, will produce deviation from the horizontal line expected in the absence of inhibitor when $E/v_i$ is plotted against $v_i$. Schwimmer et al. (8) and Frost et al. (4) utilized equation 2 to determine the amount of invertase present in potato tuber extracts containing an endogenous inhibitor. Dialyzed crude extracts from potato tubers show increased specific activity of invertase following dilution (3–5, 8); therefore a diagonal line is produced when $E/v_i$ is plotted against $E$. If enough points are included, we consistently find that the diagonal line is slightly convex rather than straight, with the most pronounced curvature at low values of $E$ (Fig. 2).

The question is not only whether the line is straight. It is whether the increased specific activity that accompanies dilution and the consequent deviation from a horizontal line obtained by plotting $E/v_i$ against $E$ are due to the presence of a low affinity inhibitor. Apparently such is not the case. The only invertase inhibitor which has been found in potato tubers (5, 6) forms an essentially undissociable complex with the enzyme (3, 6). Dilution of partially purified invertase gives no more increase in specific activity when this inhibitor is present than when it is absent (3). Likewise, the inhibitor causes no deviations from the horizontal if $E/v_i$ is plotted against $E$ (as can be seen by plotting data from Fig. 5 of Ref. 3 in this manner).

One must conclude that the diagonal line shown in Figure 2, like those previously reported (4, 8), results from the presence of factors other than the inhibitor which has been identified (5, 6) to date, and that the method is inappropriate for the system for which it was devised (8). This emphasizes the importance of ascertaining, before employing the graphical method: (a) that over a wide range of concentrations a plot of $E/v_i$ against $E$ actually gives a straight line; and (b) that the increases in enzyme specific activity arising from dilution result from the presence of an inhibitor possessing a low affinity for the enzyme.

**LITERATURE CITED**