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Competition of Two Substrates for a Single Enzyme

A SIMPLE KINETIC THEOREM EXEMPLIFIED BY A HYDROXY STEROID DEHYDROGENASE REACTION

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1. If two compounds are substrates for a single enzyme, and do not form any ternary complex with the enzyme or combine directly with each other, then the total initial rate of reaction for a mixture of the two compounds may be greater than the rate for either compound alone, or may lie between the rates for the compounds alone. It is the concentration of the compound with the higher maximum velocity that determines which applies, and there is one concentration of the compound of higher maximum velocity at which the total rate of reaction is independent of the presence or absence of the substrate of lower maximum velocity. The values concerned are derived. 2. An example is given of 5α-androstan-3-one and 5α-androstane-3,16-dione as substrates competing for a hydroxy steroid–NAD oxidoreductase (EC 1.1.1.53).

When substrates A and B react with a single enzyme E without formation of any ternary complex containing A and B, then in mixtures of A and B each substrate acts as a competitive inhibitor of the other:

$$A + E \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} AE \xrightarrow{k_{+3}} E + \text{products}$$

$$B + E \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} BE \xrightarrow{k_{+4}} E + \text{products}$$

If it is possible to measure separately the reaction rate for each substrate in such mixtures, various well-known graphical methods are available to demonstrate the simple competitive nature of the inhibition (Webb, 1963; Dixon & Webb, 1964, p. 315). In some cases, however, the rate of reaction of the individual substrates in the mixture is not easily measured, although the total rate of reaction, measured, for example, by determining the rate of formation of a common product, may be easily accessible. Steady-state treatment leads to eqn. (1) (Laidler, 1958; Dixon & Webb, 1964, p. 84):

$$v_t = \frac{(V_A/K_A)a + (V_B/K_B)b}{1 + a/K_A + b/K_B} \qquad (1)$$

where v_t is the total initial rate of reaction, a and b are the concentrations of A and B respectively and V_A, V_B, K_A and K_B are the usual kinetic constants given by $V_A = k_{+3} \times$ (total enzyme concentration), $V_B = k_{+4} \times$ (total enzyme concentration), $K_A = (k_{+3} + k_{-1})/k_{+1}$ and $K_B = (k_{+4} + k_{-2})/k_{+2}$.

In the past there have been various statements about the consequences of this relationship (Thorn, 1949; Foster & Niemann, 1951; Hakala, Glaid & Schwert, 1956; Dixon & Webb, 1964, p. 84; Reiner, 1964). A useful theorem is now derived.

Eqn. (1) written in reciprocal form becomes:

$$y = (ex + c)/(fx + V_A c) \qquad (2)$$

where $y = 1/v_t$, $x = 1/a$, $c = 1/K_A$, $e = 1 + b/K_B$ and $f = V_B b/K_B$. For a constant value of b eqn. (2) shows that y is not in general a linear function of x. As $x \to \infty$, $y \to e/f$, so there is a horizontal asymptote at $(K_B + b)/V_B b$.

Eqn. (2) becomes linear in the trivial case of $f = 0$, i.e. $b = 0$, and in the interesting case of $f = eV_A$, i.e. $b = V_A K_B/(V_B - V_A)$. In the latter case $y = 1/V_A$ for all values of x. This has physical significance only when $V_B > V_A$.

The general curve represented by eqn. (2) intersects the line for $b = 0$ when:

$$(ex + c)/(fx + V_A c) = (x + c)/V_A c \qquad (3)$$

Eqn. (3) has roots $x = 0$ and $x = (cV_A/f)(e - 1) - c$, which is $(V_A - V_B)/K_A V_B$. The values of y are $1/V_A$ and $1/V_B$ respectively. Both roots are independent of b. The second root has physical significance only when $V_A > V_B$.

Differentiation of eqn. (2) with respect to x gives $dy/dx = (ceV_A - fc)/(fx + V_A c)^2$. The sign of dy/dx is the sign of the numerator for all real values of x, so $dy/dx <$, $=$ or > 0 according to whether $fc >$, $=$ or $< ceV_A$, i.e. $b >$, $=$ or $< V_A K_B/(V_B - V_A)$.

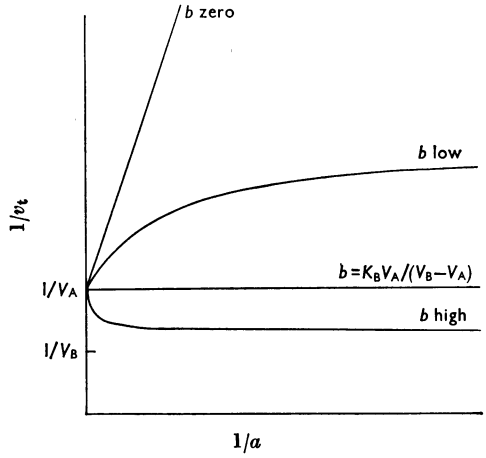

Fig. 1. Effect on total velocity, v_t, of altering the concentration, a, of substrate A at various fixed concentrations, b, of the competing substrate B is shown in this reciprocal plot for $V_A < V_B$. There is one value of b for which the curve becomes a horizontal line. For higher values of b the curves lie between the line for $b=0$ and their horizontal asymptotes (not shown). For lower values of b the curves lie below both the line for $b=0$ and their horizontal asymptotes. The line for $b=0$ represents substrate A alone and the horizontal asymptote represents substrate B alone.

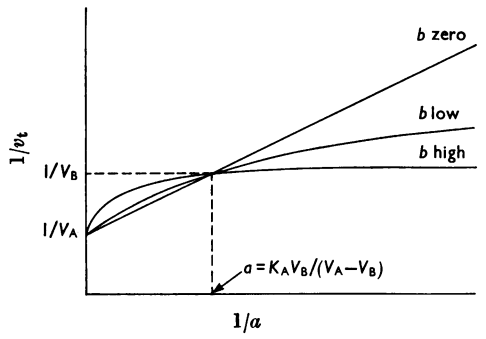

Fig. 2. Effect on total velocity, v_t, of altering the concentration, a, of substrate A at various fixed concentrations, b, of the competing substrate B is shown in this reciprocal plot for $V_A > V_B$. To the left of the common point the curves lie between the line for $b=0$ and their horizontal asymptotes (not shown), whereas to the right of the common point the curves lie below both the line for $b=0$ and their horizontal asymptotes. The line for $b=0$ represents substrate A alone and the horizontal asymptote represents substrate B alone.

The cases of physical significance are therefore as represented by Figs. 1 and 2. Fig. 1 shows the effect of varying the concentration of the substrate with the lower maximum velocity in the presence of various concentrations of the substrate of higher maximum velocity. Fig. 2 shows the effect of varying the concentration of the substrate of higher maximum velocity in the presence of various concentrations of the substrate of lower maximum velocity. The significance of the horizontal line for $b=K_BV_A/(V_B-V_A)$ in Fig. 1 is, of course, the same as the significance of the common point for $a=K_AV_B/(V_A-V_B)$ in Fig. 2. It may be stated as follows. The total initial rate of reaction for the mixed substrates is greater than the rate for either substrate alone for all values of the concentration of the substrate of lower maximum velocity, provided that the concentration of the substrate of higher maximum velocity is less than a certain value. When it exceeds this value, then for all concentrations of the substrate of lower maximum velocity the total rate for the mixture lies between the rates for the substrates separately. The value of this critical concentration of the substrate with the higher maximum velocity is given by the product of its K_m value and the maximum velocity of the other substrate divided by the difference of the maximum velocities. At this concentration the total initial rate is independent of the presence or absence of the substrate of lower maximum velocity, and is equal to this lower maximum velocity.

Evaluation of the critical concentration does not necessarily involve calculation. It can be obtained from Lineweaver–Burk plots for the single substrates by drawing a line parallel to the abscissa axis through the intersection of the upper plot with the ordinate axis, so that it intersects the lower plot, and then reading off the reciprocal of the concentration corresponding to this point of intersection, which is the concentration required. For an equimolar mixture of the substrates, $a=b=s$ (say), the straight line obtained by plotting $1/v_t$ against $1/s$ passes through the same point. Indeed, the family of straight lines for constant-ratio mixtures, i.e. $a=rb$, where r is a constant, all pass through the point provided that one uses the concentration of the substrate of higher maximum velocity as s for plotting. Utilization of the special case of constant-ratio mixtures in this way provides a convenient test for mutual competitive inhibition. In any particular case the common point may or may not lie in the range directly accessible to experiment. It does, however, always occur at a finite concentration to which, when it is not directly accessible, the linear plots may be extrapolated.

An application of this treatment is shown in Figs. 4 and 5. The enzyme was a crystalline preparation of 20β-hydroxy steroid–NAD oxidoreductase (EC 1.1.1.53) from *Streptomyces hydrogenans* (Hübener, Sahrholz, Schmidt-Thome, Nesemann & Junk, 1959), which catalyses reduction of the 3-oxo group in certain 5α-steroids (Pocklington & Jeffery, 1968). 5α-Androstan-3-one and 5α-androstane-3,16-dione were used as substrates. The rate of reaction of the single substrates and the total rate

of reaction of mixed substrates were measured by the rate of oxidation of NADH, which was determined spectrophotometrically at 340 nm. (Pocklington & Jeffery, 1968). The lines shown (Figs. 3–5) were calculated for $V_{max.} = 0{\cdot}96\,\mu$mole/min. and $K_m = 2{\cdot}63 \times 10^{-5}$ M for 5α-androstan-3-one and $V_{max.} = 25{\cdot}3\,\mu$moles/min. and $K_m = 4{\cdot}20 \times 10^{-5}$ M for 5α-androstane-3,16-dione. These values give the critical concentration as 1·66 μM-5α-androstane-3,16-dione. Experimental values, shown as points, scattered close to the theoretical curves and were consistent with mutual competitive inhibition.

The treatment may generally be useful whenever it is convenient to measure the total reaction rate but not the reaction rates of the individual components of the mixture. The total rate of reaction of two penicillins with a penicillinase, for example, can be followed by measuring the rate of formation of the common product H^+, but the rate of formation of the individual penicilloic acids is not easily measured. On the other hand when esters are hydrolysed by esterases either a common product (e.g. *p*-nitrophenol from two *p*-nitrophenyl esters) or the products of the individual substrates (e.g. oestrone and dehydroepiandrosterone from their sulphates) may be convenient to measure. In certain enzyme-catalysed rearrangements (e.g. steroid 5-en-3-ones to the corresponding 4-en-3-ones) the change generates a common chromophore, which serves for measurement of the total rate of

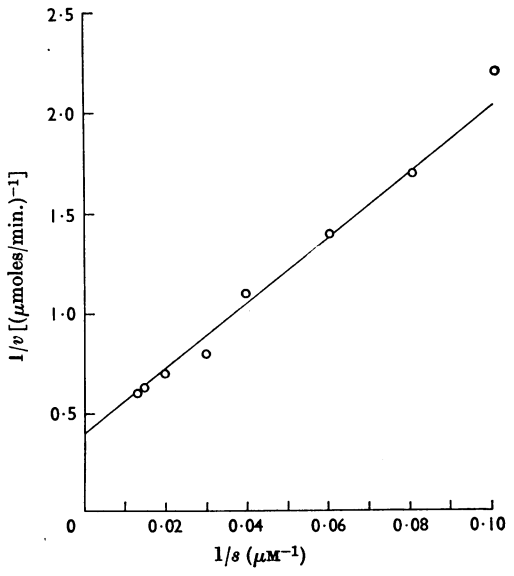

Fig. 3. Effect of substrate concentration on reaction rate for 20β-hydroxy steroid–NAD oxidoreductase at pH 5·2 with 145 μM-NADH at 25°. The substrate was 5α-androstane-3,16-dione (B).

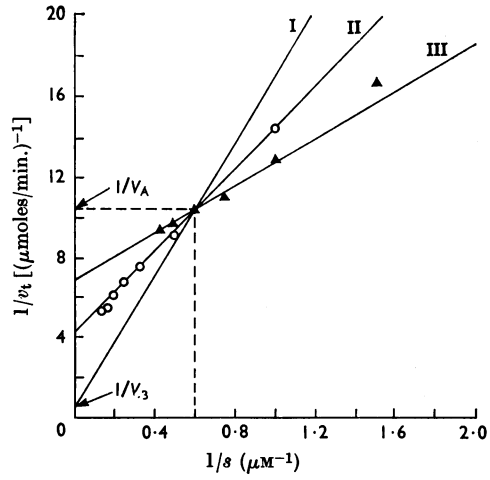

Fig. 4. Effect of substrate concentration on total reaction rate for 20β-hydroxy steroid–NAD oxidoreductase at pH 5·2 with 145 μM-NADH at 25°. Curve I, 5α-androstane-3,16-dione (B); curve II, line calculated for mixture of 5α-androstan-3-one (A) and B in constant ratio $a/b = 10$ (○, experimental points); curve III, line calculated for mixture of A and B in constant ratio $a/b = 30$ (▲, experimental points).

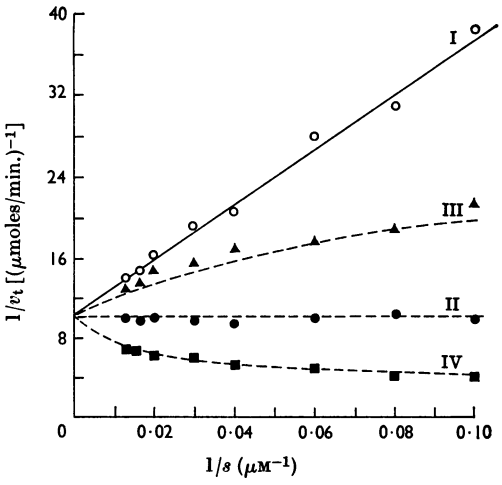

Fig. 5. Effect of substrate concentration on total reaction rate for 20β-hydroxy steroid–NAD oxidoreductase at pH 5·2 with 145 μM-NADH at 25°. Curve I, 5α-androstan-3-one (A) (○, experimental points); curve II, line calculated for mixture of A (concentration varied) with 5α-androstane-3,16-dione (B) at constant concentration of 1·66 μM (●, experimental points); curve III, curve calculated for mixture of A (concentration varied) with B at constant concentration of 0·56 μM (▲, experimental points); curve IV, curve calculated for mixture of A (concentration varied) with B at constant concentration of 5 μM (■, experimental points).

reaction. The mathematical treatment described is therefore potentially applicable to a variety of different types of enzymic reaction. The method most suitable for evaluating the kinetics in a particular case will depend on the particular circumstances.

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