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SOME BIOCHEMICAL THRESHOLD MECHANISMS

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Certain arrangements of enzymatic (bimolecular) subsystems lead to characteristic threshold-type response. Two simple cases of such systems are studied here in terms of steady state behavior and explicit relationships between system and curve parameters. It is found that the curvature of the threshold curve is directly related to the equivalent Michaelis constant and, in the case of saturated threshold curve, the slope of the curve at the idealized threshold is limited by the ratio of saturation to threshold. This slope may be appreciably increased up to a stepwise response at the threshold if a multisubstrate complex of the enzyme is the only species which affects the enzyme mediated transport.

Saturation phenomenon occurring in enzymatic reactions can result in certain threshold traits in systems containing bimolecular subsystems. Quite simple systems which are examples of such threshold mechanisms may be found. More generally, this type of threshold behavior can arise as a result of the nonlinearities inherent in bimolecular reactions.

If two observable components of a system, X and Y, are connected either unidirectionally as in Figure 1a, or reversibly, as it is shown in Figure 1b, the relationship between the steady state value of Y, Y_∞, and the steady state value of X, X_∞, is linear unless some of the arrows stand for nonlinear transport. Considering Y as a response to X, we may write in the general case

$$\dot{Y} = p(X)X - q(Y)Y \tag{1}$$

where $p(X)$ and $q(Y)$ are nonlinear rate coefficients, whose form is limited to

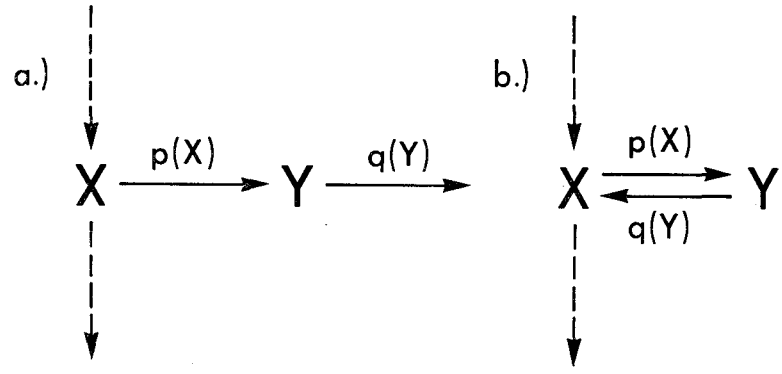

Figure 1.

rational functions, if bimolecular subsystems are the carriers of the corresponding transport mechanism.

The simplest threshold mechanism of this kind arises from an enzyme mediated loss of Y: the enzyme, E, combines with the substrate, Y, to form a complex, C, according to the typical bimolecular reaction

$$\dot{C} = k_1 EY - (k_2 + k_3)C \tag{2}$$

so that the equation for Y (if $p(X) = p = \text{const}$) reads as follows

$$\dot{Y} = pX - k_1 EY + k_2 C. \tag{3}$$

We have an additional equation for E, namely

$$\dot{E} = \mathscr{E} - aE - \dot{C} \tag{4}$$

for an open system, i.e. the enzyme is produced with the rate $\mathscr{E}$ and inactivated with the rate aE; for a closed system, $\dot{E} = -\dot{C}$, and, therefore,

$$E + C = E_0, \tag{5}$$

where E_0 is the total amount of enzyme.

For the closed system, then, the steady state of (2) and (3) with the use of (5) is given by the following formulas:

$$C_\infty = \frac{E_0 Y_\infty}{K + Y_\infty} \tag{6}$$

and

$$pX_\infty = \frac{k_3 E_0 Y_\infty}{K + Y_\infty} + qY_\infty, \tag{7}$$

with the equivalent Michaelis constant

$$K = \frac{k_2 + k_3}{k_1}. \tag{8}$$

Here a linear loss of Y was added, since otherwise after saturation of the enzyme no steady state would be possible. Solving (7) for Y_∞, we obtain a threshold curve (see heavy line in Figure 2):

$$Y_\infty = \frac{1}{2q}\left[\sqrt{(k_3E_0 + qK - pX_\infty)^2 + 4qKpX_\infty} - (k_3E_0 + qK - pX_\infty)\right]. \tag{9}$$

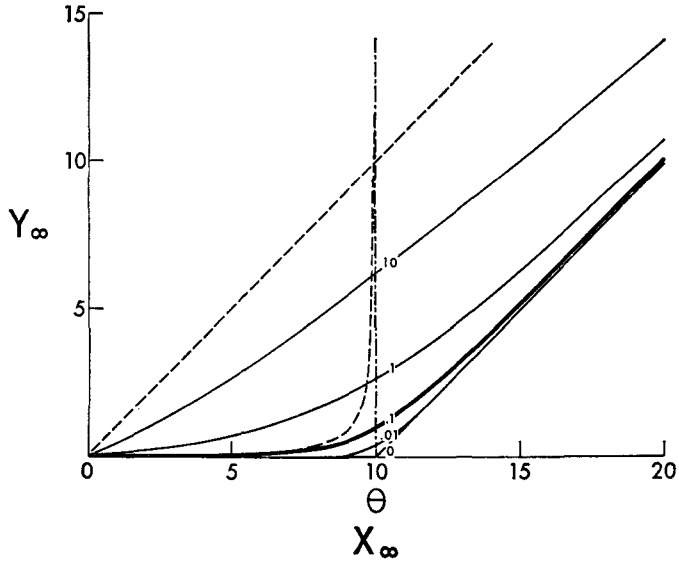

Figure 2. Steady-state plot of (2) and (3) with (5) as shown by (9) or (13)—heavy line. The dashed lines show the two components of the heavy line. The thin lines identify the threshold curves for several values of K, the equivalent Michaelis constant. The idealized curve, i.e. $K = 0$, is also shown [cf. (14)]

Parameters of the threshold curve are now easily identified if X_∞ is plotted as a function of Y_∞ through the following expression, the inverse of (9),

$$X_\infty = \frac{\frac{k_3E_0}{p}Y_\infty}{K + Y_\infty} + \frac{q}{p}Y_\infty. \tag{10}$$

The result is the sum of two terms, the two dashed lines in Figure 2. We note that for Y_∞ large enough $Y_\infty \gg K$, the straight line

$$X_\infty = \frac{k_3 E_0}{p} + \frac{q}{p} Y_\infty \quad \text{or} \quad Y_\infty = \sigma(X_\infty - \Theta) \tag{11}$$

ensues. The threshold Θ and the slope σ may then be defined in terms of parameters of the model, viz.,

$$\Theta = \frac{k_3 E_0}{p} \quad \text{and} \quad \sigma = \frac{p}{q}, \tag{12}$$

so that (9) takes the form

$$Y_\infty = \tfrac{1}{2}\{\sqrt{[K - \sigma(X_\infty - \Theta)]^2 + 4\sigma K X_\infty} - K + \sigma(X_\infty - \Theta)\}. \tag{13}$$

Real threshold curves have always finite curvature in the neighborhood of the threshold. The higher the curvature, the sharper is the threshold and the closer is the real curve to an idealized one. For, given threshold Θ and slope σ, the real curve approaches an idealized one as $K \to 0$, as one can expect from (7). In fact, the idealized threshold curve is

$$\lim_{K=0} Y_\infty = \tfrac{1}{2}\sigma[|X_\infty - \Theta| + (X_\infty - \Theta)], \tag{14}$$

that is, $Y_\infty = 0$ up to $X_\infty = \Theta$; beyond that point it increases linearly with X_∞ (cf. Figure 2). (Note that $K \to 0$ for given k_3 requires according to (8) that $k_1 \gg k_2 + k_3$, or, the rate constant for the forward enzymatic reaction, k_1, must be increasing indefinitely as compared with the backward rate constant k_2 and the constant for catalytic return of the enzyme, k_3.)

In an open steady state system we cannot have such a nonlinear response of the system as it is represented by a threshold curve. Since in the steady state [cf. (4)]

$$E_\infty = \frac{\mathscr{E}}{a}, \tag{15}$$

the (5) is not true anymore. Then, instead of (6) we have only

$$C_\infty = \frac{k_1}{k_2 + k_3} E_\infty Y_\infty \tag{16}$$

which, when substituted into (3) yields

$$pX_\infty = \left(\frac{k_1 k_3}{k_2 + k_3} \frac{\mathscr{E}}{a} + q\right) Y_\infty, \tag{17}$$

a linear relationship between the steady state values of the two variables. We thus conclude that a threshold mechanism is feasible in the type of system we consider only if the system is effectively closed, or, when the relaxation time of the enzyme is far larger than that of the (Y, C) subsystem. We note here that we tacitly assumed larger relaxation time for the X component as compared with that of (Y, C) subsystem.

No specifications of the relative magnitudes of relaxation times of the components Y and C of the subsystem (Y, C) need be given. However, it is commonly true that the elementary bimolecular process have far smaller relaxation time than other processes encountered in the system, thus leading to a stratified temporal hierarchy in the system. Then, we may write (3) with the use of (6) as

$$\dot{Y} = pX - \frac{mY}{K + Y} - qY \tag{18}$$

where the linear loss qY was included as in (7) and k_3E_0 was replaced by the maximal loss of Y, m, due to enzymatic (bimolecular) process. We introduced here the stratified temporal hierarchy concept to justify belatedly (1) and to drive at possible generalizations.

Comparing (1) with (18) we have

$$q(Y) = \frac{m}{K + Y} + q \tag{19}$$

while $p(X) = p$. We know that the threshold property of the system response is due to the form of $q(Y)$ and, hence, we may resort to a simplified form of the steady state of (18), namely, for $K = 0$ (idealized threshold curve), while keeping the form of $p(X)$ general. Such a consideration leads to

$$Y_\infty = \frac{1}{2q}[\,|m - p(X_\infty)X_\infty| - (m - p(X_\infty)X_\infty)] \tag{20}$$

analogous to (9) when $k_3E_0 = m$, $K = 0$ and pX_∞ was replaced by $p(X_\infty)X_\infty$. Accordingly, $Y_\infty = 0$ for $0 < p(X_\infty)X_\infty \leqslant m$ and

$$Y_\infty = \frac{p(X_\infty)X_\infty - m}{q} \quad \text{for } p(X_\infty)X_\infty > m. \tag{21}$$

The threshold is defined by

$$p(\Theta)\Theta = m. \tag{22}$$

Now we choose a specific functional form for $p(X)$. The simplest nonconstant form in view of the note below (1) is

$$p(X) = \frac{n}{K' + X}, \tag{23}$$

e.g. the transport process from X to Y is enzymatic (or the like) in nature. However, we will proceed with somewhat generalized form, viz.,

$$p(X) = \frac{nX^{c-1}}{K' + X^c} \tag{24}$$

since it does not present any greater difficulty in arriving at the relationship between the parameters of the curve and those of the model. This form results from an assumption that an enzyme needs to combine with c molecules of the substrate X in a sequence of bimolecular reactions before it becomes active in the transport of X into Y. We recall that this form is an approximation to the actual one containing all the powers of X in the denominator, from 0 to c, but it is a sufficient one to point out the trend in a more realistic situation.

Using (22), we find explicitly the threshold in terms of the system parameters

$$\Theta^c = \frac{mK'}{n - m} \tag{25}$$

and define the initial slope as the slope of the curve at the idealized threshold

$$\sigma = \left(\frac{dY_\infty}{dX_\infty}\right)_{X_\infty = \Theta} = \left(\frac{n}{q}\frac{cK'X^{c-1}}{(K' + X^c)^2}\right)_{X_\infty = \Theta} = \frac{c}{nqK'}\frac{(mK')^{c-1}}{(n - m)^{c-3}} \tag{26}$$

In the steady state, (1) reads in this case

$$\frac{nX_\infty^c}{K' + X_\infty^c} = \frac{mY_\infty}{K + Y_\infty} + qY_\infty \tag{27}$$

yielding for $X_\infty \to \infty$

$$n = \frac{mY_{\infty\,\max}}{K + Y_{\infty\,\max}} + qY_{\infty\,\max} \tag{28}$$

thus arriving at a new curve parameter, $Y_{\infty\,\max}$, the maximal value of Y_∞ which cannot be exceeded however high value X_∞ will reach (Figure 3). Since we consider the idealized case (sharp threshold), $K = 0$, (28) simplifies to

$$n = m + qY_{\infty\,\max}. \tag{29}$$

Equations (25), (26) and (29) comprise a full definition of the parameters of an idealized threshold curve with saturation. When we inquire to which extent these three parameters of the curve, the threshold Θ, the initial slope σ and the saturation $Y_{\infty\,\max}$ are independent, we rewrite (25) after eliminating n by use of (29) as

$$mK' = qY_{\infty\,\max}\Theta^c \tag{30}$$

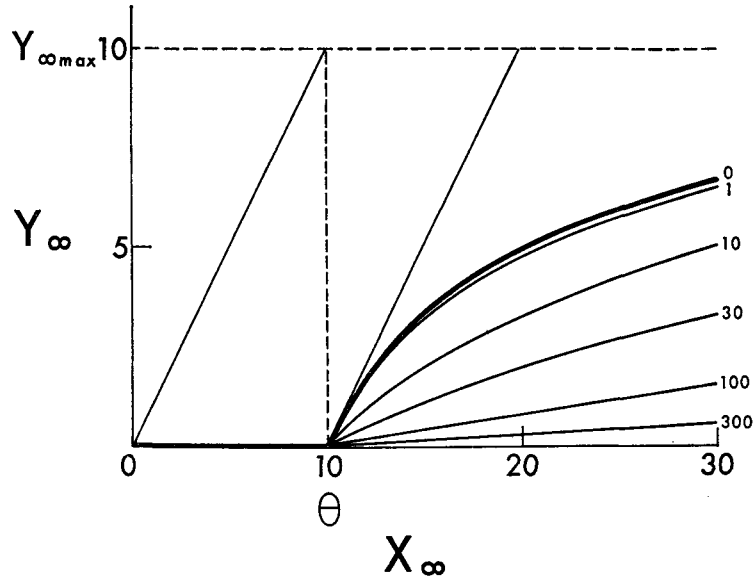

Figure 3. The threshold curves with saturation for various values of the equivalent Michaelis constant K' ($K = 0$). Threshold Θ and saturation $Y_{\infty\,\max}$ are for all curves the same so that for given c ($c = 1$), m and n were computed from (30) and (29), respectively

and the product $\sigma\Theta^c$ [or that of (25) and (26), using (29) again]

$$\sigma\Theta^c = \frac{c(mK')^c}{qK'(qY_{\infty\,\max})^{c-2}(m + qY_{\infty\,\max})} \tag{31}$$

to obtain

$$\sigma = \frac{c(\Theta^c)^{c-1}Y_{\infty\,\max}}{K' + \Theta^c}. \tag{32}$$

Hence, for given threshold Θ and saturation $Y_{\infty\,\max}$, the initial slope σ depends upon the equivalent Michaelis constant for X to Y, K', and the number of molecules c of the substrate X required for the transport to be carried out. While K' only decreases the initial slope σ, c increases the initial slope very effectively.

For $c = 1$, the initial slope σ_1 is

$$\sigma_1 = \frac{Y_{\infty\,\max}}{K' + \Theta} \tag{33}$$

so that the highest attainable slope is the ratio of saturation $Y_{\infty\,\max}$ to the threshold Θ (Figure 3), when $K' = 0$.

In Figure 4 a family of threshold curves with saturation is given for several values of c. As (32) suggests, the initial slope increases rapidly with c: for $c = 2$, $\sigma_2 = 2Y_{\infty\,\max}$ and for $c = 5$, $\sigma_5 = 5Y_{\infty\,\max}\Theta^{15}$ (both given for $K' \ll \Theta$).

To summarize, we see that the properties of a threshold curve with saturation differ from that without saturation mainly in the above-threshold properties so that if the restrictive condition $K = 0$ is lifted, a somewhat sigmoidal curve follows (Figures 5 and 6). However, it was assumed throughout that $n > m$ [cf. (29)] to use the term "threshold curve" meaningfully.

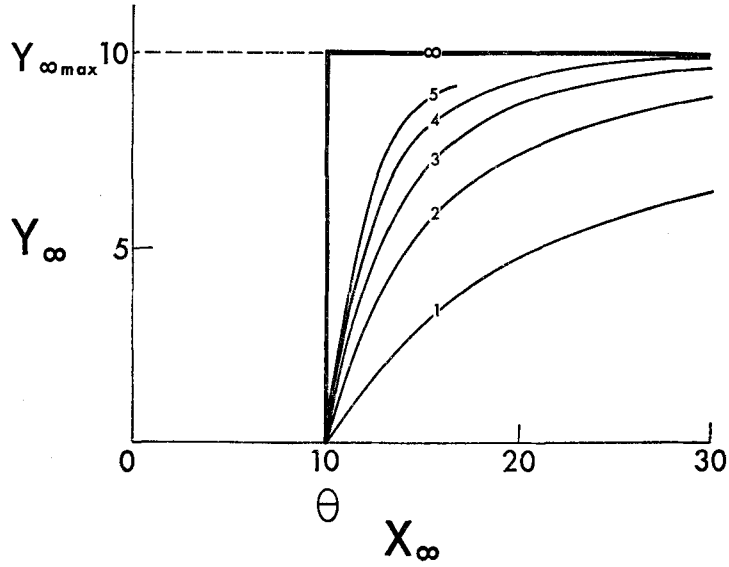

Figure 4. The threshold curves with saturation for different c. Values of the equivalent Michaelis constants are $K = 0$ and $K' = 1$ (close to the maximal initial slope curve, cf. Figure 3) and for the same Θ and $Y_{\infty\,\max}$ of the family, m and n were computed

More complex bimolecular transport mechanisms would result in higher degree of the polynomials of the rational functions $p(X)$ and $q(Y)$ such that the rates $p(X)X$ and/or $q(Y)Y$ are nonmonotonous and thus could give rise to peculiar "threshold curves." The threshold, if at all, is less clearly defined and the relationship between the parameters of the curve and the parameters of the model might not be obtainable in an explicit form.

The systems with threshold characteristics discussed here are by no means the only (bimolecular) threshold mechanisms. Here we were concerned with the elaboration of the suggestion how behavior of enzymatic reactions may facilitate the understanding of threshold urinary excretion (Ličko, 1963).

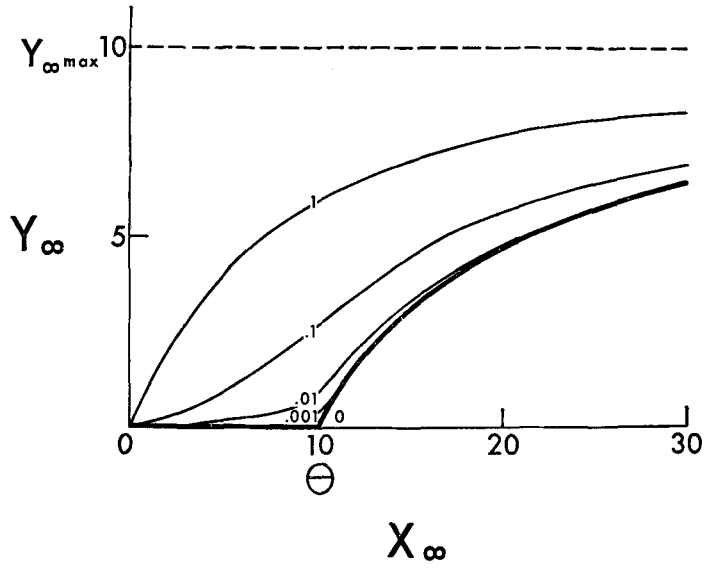

Figure 5. The threshold curves with saturation for different values of K (non-idealized curves). Value of the equivalent Michaelis constant is $K' = 1$; number of substrate molecules required for the activity of enzyme is $c = 1$. For the same Θ and $Y_{\infty\ max}$, m and n were computed from (30) and (29), respectively

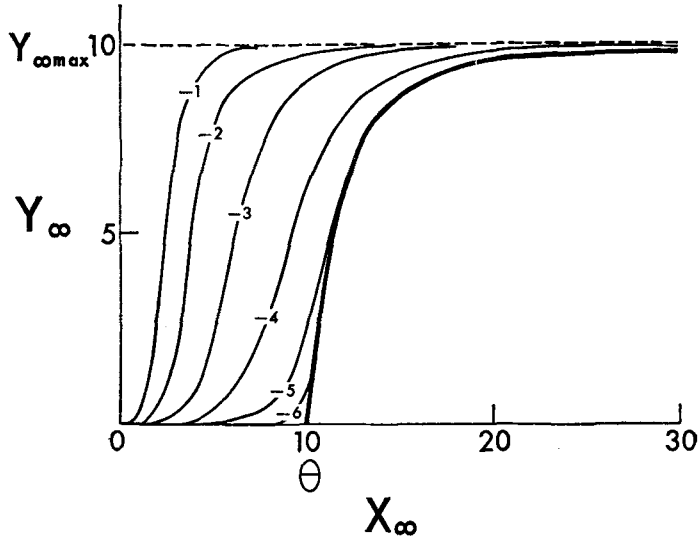

Figure 6. The threshold curves with saturation for different values of $\log_{10} K$ (non-idealized curves). Value of the equivalent Michaelis constant is $K' = 1$; number of substrate molecules required for the activity of enzyme is $c = 5$. For the same Θ and $Y_{\infty\ max}$ m and n were computed from (30) and (29), respectively

Walter *et al* (1967) studied biochemical binary logic and, using the assumption that in a chain of enzymatic reactions a sigmoid relationship between the activity of an enzyme and the concentration of a substrate exists, they showed stepwise response of the system to increasing substrate concentration. This threshold-like character of the response implies another way of looking at bimolecular threshold mechanisms. Although the stepwise response is not an essential property of the systems we were interested in (it can occur only for $c \to \infty$), the advantage may be found in the comprehension of the role of the system parameters in shaping the response curve and, vice versa, what values of the system parameters must be implemented in order to obtain a threshold curve with certain characteristics. The latter is appreciated when modeling of complex biochemical systems is attempted and certain properties of the threshold curves impose restrictive conditions on biochemical interpretation.

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LITERATURE

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