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UCRL-18971
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NOV 5 1969

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October 1969

AEC Contract No. W-7405-eng-48

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Master Reaction Concept: Its Validity in Integrated Biological Systems

Abstract. The maximum rate of a biological process consisting of a series of linked reversible enzymatic reactions is determined by the slowest reaction in the series. This master (or bottleneck) reaction is a consequence of the fact that enzymatic reactions approach zero-order kinetic behavior as they near their maximum rate.

Over sixty years ago Blackman, without proof, proposed the concept of a master (or bottleneck) reaction, stating that the rate of a biological process is limited by the pace of the slowest factor (1). Several workers have criticized Blackman's concept, both conceptually and mathematically (2). However, Perret has defended the master reaction concept, pointing out that the criticism was based on first-order chemical kinetics, whereas biological reactions are of all feasible orders from virtually zero upwards (3). We present a proof for the master reaction concept based on reversible enzyme kinetics.

We base our argument on a simplified model of a biological system consisting of N consecutive reversible Michaelis-Menten type enzymatic reactions. (The case of a <u>single</u> enzyme-substrate complex undergoing successible transformations (4) should not be confused with this case.) The product of each enzymatic reaction is the substrate for the next. There is one external limiting substrate, S.

$$S + E_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} SE_1 \underset{k'_{-1}}{\overset{k'_1}{\rightleftharpoons}} P_1 + E_1$$

$$P_1 + E_2 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} P_1E_2 \underset{k'_{-2}}{\overset{k'_2}{\rightleftharpoons}} P_2 + E_2$$

$$\vdots$$

$$\vdots$$

$$P_{n-1} + E_n \underset{k_{-n}}{\overset{k_n}{\rightleftharpoons}} P_{n-1}E_n \underset{k'_{-n}}{\overset{k'_n}{\rightleftharpoons}} P_n + E_n$$

$$\vdots$$

$$P_{N-1} + E_N \underset{k_{-N}}{\overset{k_N}{\rightleftharpoons}} P_{N-1}E_N \underset{k'_{-N}}{\overset{k'_N}{\rightleftharpoons}} P_N + E_N$$

E denotes the enzyme, P the products of the enzymatic reactions, and k the rate constants. Catalysis occurs in the substrate-enzyme-complexes (such as $P_{n-1}E_n$). In this general case no reaction steps are considered to be irreversible; irreversibility is approached only by virtue of a large negative free energy change.

The velocity of the $n^{\underline{th}}$ reaction, v_n, is found by assuming that the concentration of enzyme is much smaller than the concentration of its substrate, and by assuming steady state conditions. Steady state may be assumed even if we consider an autocatalytic process such as balanced cellular growth since we express enzyme concentrations per unit of cellular mass (which is independent of <u>total</u> cellular mass). The solution (5) is given here with v_n implicitly expressed:

$$[P_{n-1}] = \frac{[P_n](V_{Bn} + v_n) + K'_n v_n}{K'_n/K_n(V_{Fn} - v_n)} , \qquad (1)$$

where $[P]$ and $[P_{n-1}]$ are concentrations of species P_n and P_{n-1}; v_n is the net rate of enzymatic reaction, $V_{Fn} = k'_n[E^o_n]$, the maximum forward rate; $V_{Bn} = k_{-n}[E^o_n]$, the maximum backward rate; $[E^o_n]$ is the total concentration of the $n^{\underline{th}}$ enzyme; $K_n = (k_{-n} + k'_n)/k_n$; $K'_n = (k_{-n} + k'_n)/k'_{-n}$.

All of the individual reactions give equations of the form of equation (1). We obtained the solution for the whole system of N equations by realizing that at steady state $v = v_1 = v_2 = \ldots\ldots = v_n = \ldots\ldots = v_N$ and by making successive substitutions until the concentration of S, [S], is related only to v and $[P_N]$, the concentration of final product. If P_N is a cellular constituent and cellular growth is balanced, then $[P_N]$ is constant with time (when expressed per unit of cellular mass). The solution is:

$$[S] = \sum_{n=1}^{N} \frac{K_n \prod_{i=1}^{n} (V_{B_{i-1}} + v)}{\prod_{i=1}^{n} \frac{K'_{i-1}}{K_{i-1}} (V_{Fi} - v)} + \frac{[P_N] \prod_{n=1}^{N} (V_{Bn} + v)}{\prod_{n=1}^{N} \frac{K'_n}{K_n} (V_{Fn} - v)}, \qquad (2)$$

where V_{Bo} is defined equal to zero, and K'_o/K_o is defined equal to one. If, for example, we have two enzymatic reactions, N = 2, and :

$$[S] = \frac{[P_2](V_{B2} + v)(V_{B1} + v)}{\frac{K'_2}{K_2} \frac{K'_1}{K_1} (V_{F2} - v)(V_{F1} - v)} + \frac{K_2 v (V_{B1} + v)}{\frac{K'_1}{K_1} (V_{F2} - v)(V_{F1} - v)} + \frac{K_1 v}{(V_{F1} - v)}. \qquad (3)$$

In equation (2) the maximum rate for the system is set by, <u>and cannot exceed</u>, the maximum forward rate of the smallest V_{Fn}, since [S] approaches infinity as v approaches the smallest V_{Fn}. This can be easily seen by substituting any

two numerical values for V_{F2} and V_{F1} into equation (3). As v approaches the smaller of the two numerical values corresponding to V_{F1} or V_{F2}, [S] must approach infinity. The slowest V_{Fn} is the master reaction for the system: a barrier which cannot be exceeded. An interesting corollary is that when [S] is large (i.e. not limiting), the system rate is operating near the maximum rate set by the master reaction.

Such a limiting step or master reaction cannot occur in chemical reactions of higher than zero order (6), but can occur in enzymatic reactions since zero-order is approached as the enzyme sites become saturated with their substrate.

Care must be used not to carry the master reaction concept too far. Even though the slowest reaction sets the maximum rate for the system, it does not entirely determine the relationship of overall rate as a function of limiting substrate concentration. At low substrate concentrations, [S], more than one of the reactions in series may influence the overall rate. We have in fact used equation (2) as a basis to explain deviations of experimental data from some of the simpler models used to describe the substrate-limited growth of micro-organisms (7).

Insight into the chemical kinetics of such integrated biological processes as cellular growth, respiration and photosynthesis appears possible using the approach presented here.

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References and Notes

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