Hysteresis in Dynamic Enzyme Models

Proper identification of the actual status of an enzymatical reaction requires the elucidation of the concentration histories of the participants during the process. Analysis of relationships among the concentrations of reaction intermediates and the antecedent and subsequent participants can reveal discriminating characteristics in the dynamic behavior of the investigated models.[1,2] In this paper the results of studies on the relationship between an intermedier and a subsequent participant are described.

The simple model was based on Henri's original stoichiometric equation:[3]

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E + P \tag{1}$$

where E enzyme reacts reversibly with S substrate and/or P product in order to form an ES enzyme substrate complex. Equation (1) covers three types of enzymatic catalysis depending on the ratio velocity constants, namely k_2/k_1 and k_3/k_4, which ratios are abbreviated K_s and K_p, respectively.

Initial E (E_0) was invariably chosen to be 1.0. With the variation of initial S (S_0) different ($E_0/(S_0)$ ratios were obtained in 1.0–0.001 range. Table I presents the values of velocity constants used for the simulation.

TABLE I

Values of Velocity constants for Computer Simulation of Enzyme Kinetics

	k_1	k_2	k_3	k_4
$K_s > K_p$	0.005	0.1	0.1	0.01
$K_s = K_p$	0.1	0.01	0.01	0.1
$K_s < K_p$	0.1	0.01	0.1	0.01

The change of (ES) and (P) with time were investigated in cases $K_s > K_p$, $K_s = K_p$, and $K_s < K_p$, using an IBM 360/75 computer and System/360 Continuous System Modeling Program (CSMP) as well as FORTRAN IV languages for the program construction.[4–6] The step size for the Runge–Kutta integration routine used in the CSMP program was invariable chosen to be 0.05.

In the case of $K_s > K_p$, the (ES) was asymptotically approaching a maximum value. In the $K_s = K_p$ case an oscillation of the (ES) and the (E) values between a maximal value and a value below the maximal one indicated an unstable condition in the system, however, no true maximum of the (ES) value was observed.[6] The (ES) — time curve had a maximum only in the case of

$K_s < K_p$, regardless of the $(E_0)/(S_0)$ ratio. These data verified that the steady-state assumption is correct (only for a theoretical moment) in the $K_s < K_p$ case. These findings were in accordance with those made by Walter and Morales[7] using a TR–48 type analog computer for simulation of kinetics of a reversible enzyme model. True (ES) maximum was experimentally observed by Chance in the case of horseradish peroxidase[8] and indirectly obtained from experimental results by Nanninga in the case of myosine-ATPase catalyzed hydrolysis of ATP.[9]

Both dP/dt and the (ES) values changed during the time course of the process. When plotting dP/dt values versus (ES) two basically different types curves were obtained depending on the K_s/K_p relationship. In cases $K_s > K_p$ and $K_s = K_p$, regardless of the $(E_0)/(S_0)$ ratio, with the increase of (ES) and the progress of time, the dP/dt vs. (ES) curve climbed up to a maximum followed by a significant decrease (Figs. 1 and 2). During the computer simulation the formation of P started when the (ES) reached a value according to a logical statement in the program. This explains the shift of starting point of the dP/dt vs. (ES) curve at the X-axis. The sigmoid character of the curve indicates an explicit dependency of dP/dt on (ES) which changes with the time.

In the case of $K_s < K_p$ the dP/dt values showed also an increase up to a maximum with the increase of (ES) and the progress of time, however, beyond a given (ES) value the curve displayed a counterclockwise turn. The shape of the curve obtained in $K_s < K_p$ case depended on the $(E_0)/(S_0)$ ratio. At $(E_0)/(S_0) = 0.2$ the curve had a sigmoid character and, beyond its clockwise turn, a "crossover" shape (Fig. 3). At $(E_0)/(S_0) = 0.02$ the increase of (ES) and the

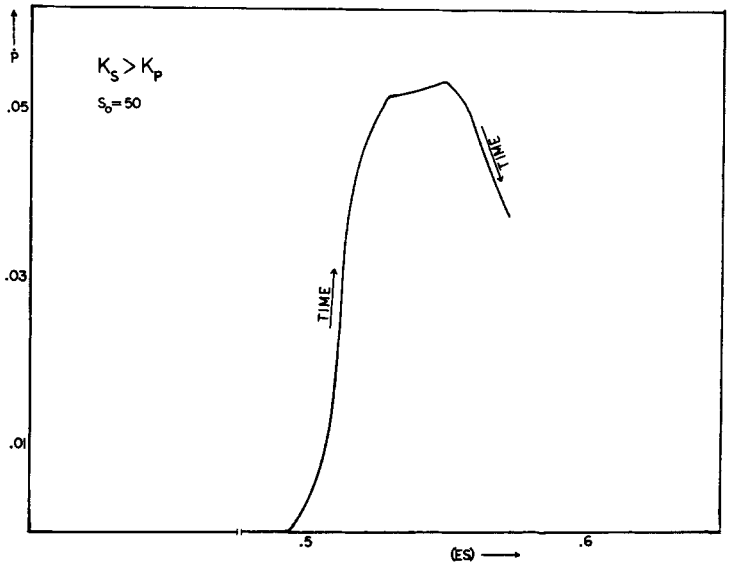

Fig. 1. Change of $\dot{P}$ value with (ES) and time in case $K_s > K_p$ $(S_0 = 50)$.

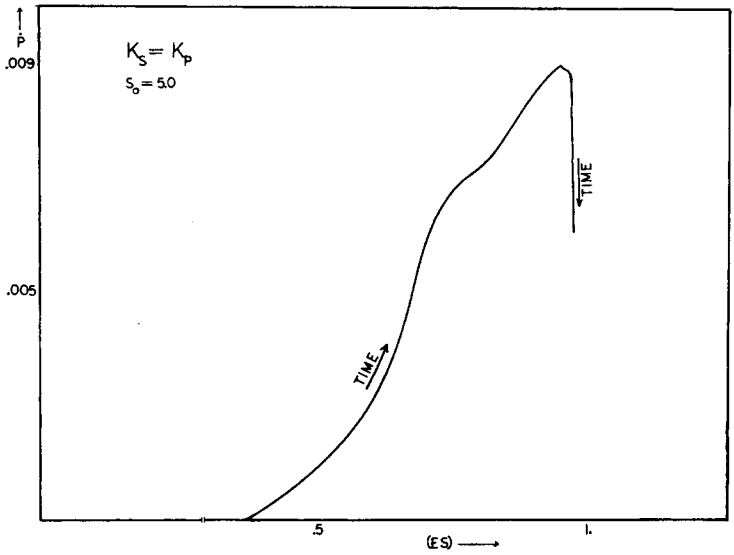

Fig. 2. Change of $\dot{P}$ value with (ES) and time in case $K_s = K_p$ $(S_0 = 5.0)$.

progress of time resulted in, first, a linear increase of dP/dt values, which was altered by a further progress of time from a straight line to a banana-shaped trajectory (Fig. 4).

A banana-shaped curve was also observed by Tanner by plotting the rate of gluconic acid formation as a function of gluconolactone concentration[10] and analyzing Chance's peroxidase case[8] with a computer.[11] The curve obtained when the product rate was graphed as a function of (ES) complex concentration and the reaction time is called a hysteresis curve.

Comparing the relationship between dP/dt and (ES) in all three cases of the single enzyme–single substrate model (eq. (1)) hysteresis curve was observed only in that case where the steady-state assumption was correct. True maximum value of the intermedier was found to be the condition of transformation of straight line relationship of dP/dt and (ES) into the hysteresis curve.

The unexpected finding of "crossover" behavior of the hysteresis loop requires further analysis (Fig. 3). Here there is a change from counterclockwise to clockwise hysteresis. This takes place when the E_0 and S_0 "concentrations" are in the same order of magnitude. The hysteresis curve was obtained in consecutive reactions where the rate of formation of a subsequent participant was plotted against a generated precursor.[10,11] The major advantage of the detailed analysis of this type curve is that it can reveal the actual status of a consecutive process and can give information on the concentration relationship between the participants. This may be helpful in the construction of algorithms for process identification of such complex biochemical processes as feedback inhibition and/or

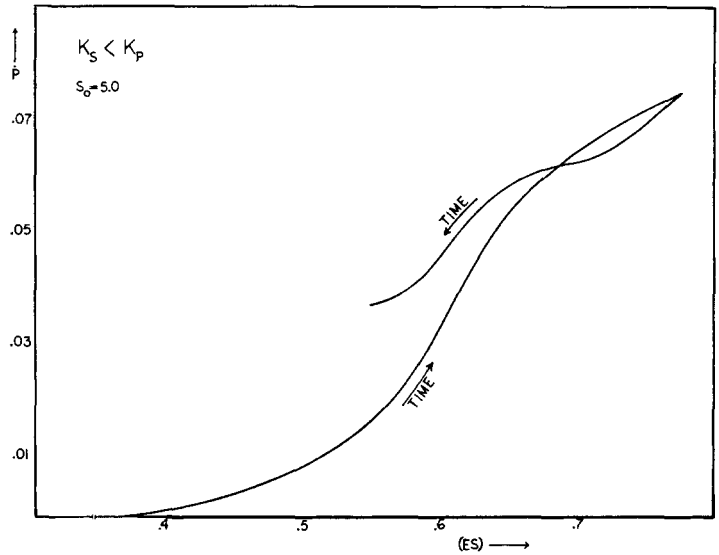

Fig. 3. Change of $\dot{P}$ value with (ES) and time in case $K_s < K_p$ $(S_0 = 5.0)$.

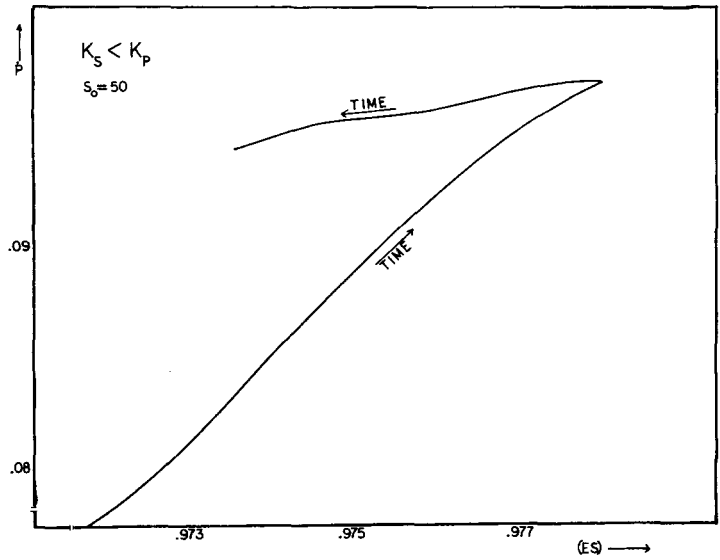

Fig. 4. Change of $\dot{P}$ value with (ES) and time in case $K_s < K_p$ $(S_0 = 50)$.

enzyme induction. The proper process identification is the prerequisite of the adaptive control of fermentation processes.

This work was supported by the New York State Science and Technology Foundation Grant, SSF(7)—4.

References

1. D. Garfinkel, L. Garfinkel, M. Pring, S. B. Green, and B. Chance, *Ann. Rev. Biochem.*, **39,** 473 (1970).
2. B. B. Edelstein, *J. Theoret. Biol.*, **29,** 57 (1970).
3. V. Henri, *Lois Generales de l'Action des Diastases*, Hermann, Paris, 1903.
4. "System/360 Continuous Modeling Program," User's Manual, IBM Application Program (1969).
5. F. Stuart, *FORTRAN Programming*, Wiley, New York, 1970.
6. L. K. Nyiri, G. M. Tóth, and V. Corso, to be published.
7. C. Walter and M. Morales, *J. Biol. Chem.*, **239,** 1277 (1964).
8. B. Chance, *J. Biol. Chem.*, **151,** 553 (1943).
9. L. Nanninga, *Biochem. Biophys Acta*, **60,** 112 (1962).
10. R. D. Tanner, *Biotechnol. Bioeng.*, **12,** 831 (1970).
11. R. D. Tanner, Paper presented at the 69th National Meeting of Amer. Inst. of Chemical Engineers, Cincinnati, Ohio (1971).

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Accepted for Publication June 9, 1971