THE KINETICS OF ENZYME ACTION

II. THE TERMINAL PHASE OF THE REACTION

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Several approximate rate equations describing the reaction scheme of MICHAELIS AND MENTEN[1],

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + Q, \tag{1}$$

have been discussed in a previous paper[2]. These were based on certain assumptions as to the relative magnitudes of the concentrations of reactants. The present communication considers another approximation, where only a small fraction of the total enzyme concentration is combined in the enzyme–substrate complex (*i.e.* $[E] \gg [ES]$). This assumption is not necessarily valid over the entire reaction for most enzymes but is certainly true in the initial and in the last stages of the reaction.

The above restriction simplifies the kinetics of reaction scheme (1) to those of two consecutive first order reactions with the first reaction step being reversible. A general treatment of the kinetics of reversible consecutive first order reactions was published by RAKOWSKI in 1906[3]. A soluble differential equation relating product concentration and time is obtained by the application of the law of mass action to reaction scheme (1) on the assumption that the concentration of uncombined enzyme remains constant.

$$\frac{d^2x}{dt^2} + k_1(K_m + e)\frac{dx}{dt} - k_1k_3\,e(a-x) = 0 \tag{2}$$

The symbols for concentration at time t which are used in deriving equation (2) are $[E] = e$, $[S] = a-x-p$, $[ES] = p$, and $[Q] = x$. In the previous paper[2], new units of time and concentration were introduced so that the three rate constants (k_1, k_2 and k_3) might be replaced in the rate equations by a single parameter r. The following nomenclature was used: $a_s = a/K_m$, $x_s = x/K_m$, $e_s = e/K_m$; $p_s = p/K_m$ and $rk_3 = k_2 + k_3 = k_1 K_m$. Thus equation (2) becomes

$$\frac{d^2x_s}{d(k_3t)^2} + r(1 + e_s)\frac{dx_s}{dk_3t} - re_s(a_s - x_s) = 0 \tag{3}$$

Upon integration, equation (3) gives

$$(a_s - x_s) = c_+ \cdot \exp\left\{\frac{-r(1+e_s) + [r^2(1+e_s)^2 - 4re_s]^{1/2}}{2}\,k_3t\right\} - c_- \cdot \exp\left\{\frac{-r(1+e_s) - [r^2(1+e_s)^2 - 4re_s]^{1/2}}{2}\,k_3t\right\} \tag{4}$$

The two constants of integration c_+ and c_- can only be defined algebraically by the

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boundary conditions of the initiation of the reaction, $x_s = 0$ and $p_s = 0$ when $t = 0$. Then c_+ and c_- are given by

$$\frac{r(1 + e_s) \pm [r^2(1 + e_s)^2 - 4re_s]^{1/2}}{2[r^2(1 + e_s)^2 - 4re_s]^{1/2}} a_s \qquad (5)$$

When equation (4) is used to describe only the last stages of the reaction and the assumption $[E] \gg [ES]$ is not valid throughout the reaction then c_+ and c_- remain undefined. Whether the assumption is valid throughout the reaction may be ascertained from a prior knowledge of e, a and K_m, since the maximum concentration of the enzyme-substrate complex is necessarily smaller than $2ae/(K_m + e + a)$.

Equation (4) formulates $(a_s - x_s)$ as the difference between a larger exponential term with a small rate of decay and a smaller exponential term which decays more rapidly. Both terms are significant during the initial phase of the reaction but towards the end of the reaction the first term becomes dominant, particularly so if the decay constants are of different orders of magnitude. The coefficient of k_3t in this dominant exponential term has already been discussed in the previous paper[2], as it is the "limiting slope" at the end of the reaction of the function relating p_s with x_s.

Fig. 1 illustrates the divergence of the present treatment from the numerical solution of the complete rate equation for the case $e_s = 1$, $a_s = 1$ and $r = 2$. The derivation of the numerical solution has been discussed in the previous paper[2].

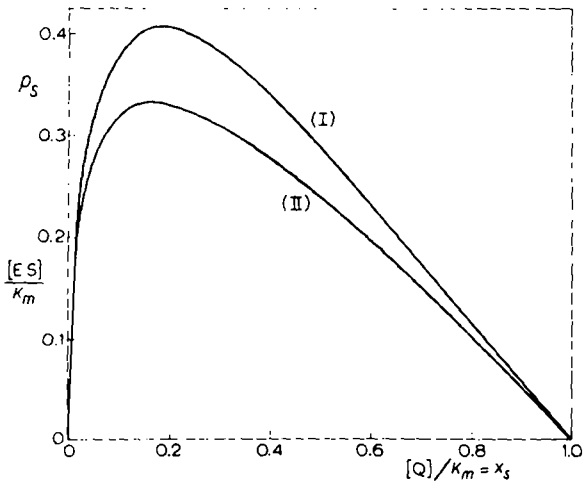

Fig. 1. Comparison for the case $e_s = 1$, $a_s = 1$ and $r = 2$, of (I), the solution of the differential equation obtained on the assumption $[E] \gg [ES]$, with (II), the numerical solution of the complete rate equation for the Michaelis-Menten reaction scheme.

The curve for the present treatment was calculated from

$$(1 - x_s) = 1.207 \exp(-0.5858\, k_3t) - 0.207 \exp(-3.414\, k_3t) \qquad (6)$$

and

$$p_s = \frac{dx_s}{dk_3t} = 0.707[\exp(-0.5858\, k_3t) - \exp(-3.414\, k_3t)] \qquad (7)$$

by assigning arbitary values to k_3t. As during the reaction p_s becomes of comparable magnitude to e_s, the values predicted for p_s by equation (7) are higher than those of the numerical solution. This divergence disappears for those cases where the assumption $[E] \gg [ES]$ is strictly valid.

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The first exponential term in equations (6) and (7) becomes increasingly dominant as the reaction proceeds. Above $x_s = 0.5$, the magnitude of the second exponential term is less than 1% of the first, and therefore p_s becomes a linear function of x_s.

$$p_s = 0.5858(1 - x_s) \tag{8}$$

This "limiting slope" of the complete solution requires for its derivation no assignment of numerical values to the constants c_+ and c_-.

MORALES AND GOLDMAN[4] derived the above two-term exponential equation (4) to describe the product concentration during the terminal phase of the reaction, using however, different algebraic symbols and postulating the assumptions made in a different manner. They suggested that through the use of an electronic computer a unique set of values for the reaction parameters might be established by trial. However, the two constants c_+ and c_- must also be considered as unknown for most reactions. The fact that numerical values have to be assigned to several unknowns in order to fit the theoretical function to experimental data, may preclude attainment of reasonable accuracy.

MORALES AND GOLDMAN[4] considered two favourable cases in which the decay constants reduce to simple functions of the individual rate constants. In the first case, where the assumption made was $k_2 \gg (k_3 + k_1 e)$, they suggested that one decay constant tends to zero and the other to $-k_2$. However, it is the numerically smaller decay constant which produces the dominant exponential term. This smaller decay constant, instead of tending to zero, in fact becomes $-k_3 k_1 e/k_2$. In the second case, where $k_3 \gg (k_2 + k_1 e)$, the decay constant $-k_1 e$ is again numerically much smaller than $-k_3$ and is therefore dominant over the latter.

The similarity between the two cases is more evident if they are formulated in terms of specific units. In both instances $(k_2 + k_3) \gg k_1 e$ (*i.e.* $1 \gg e_s$) and therefore equation (4) simplifies to

$$(a_s - x_s) = c_+ \cdot \exp(-e_s k_3 t) - c_- \cdot \exp(-r k_3 t) \tag{9}$$

Under the first assumption r is very large $(k_2 \gg k_3)$, whilst in the second r tends to unity $(k_3 \gg k_2)$. In each case $e_s k_3 t \ll r k_3 t$.

As the stated conditions for both cases require that $K_m \gg e$, they may both be described by the BRIGGS-HALDANE "steady state" approximation which at the end of the reaction becomes

$$\frac{d(a-x)}{dt} = -\frac{k_3 e(a-x)}{K_m} = -\frac{k_3 k_1 e}{k_2 + k_3}(a-x) \tag{10}$$

On integration this gives

$$(a-x) = c \cdot \exp\left(-\frac{k_3 k_1 e}{k_2 + k_3} t\right) \tag{11}$$

The dominant exponential term for both cases is thus given by equation (11). Experimentally the two cases are not distinguishable from a study of the terminal phase of the reaction; some other criterion is required to determine the relative magnitude of k_2 and k_3.

ACKNOWLEDGEMENTS

I wish to thank Dr. E. M. CROOK for helpful advice, and the Medical Research Council for a personal grant.

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SUMMARY

A differential equation is presented describing the Michaelis-Menten scheme under the assumption that [E] $\gg$ [ES], an approximation which is valid in the initial and in the last stages of an enzymic reaction. The solution of this equation relates the product concentration to a two-term exponential function in time. Under most conditions only one term is significant during the terminal phase of the reaction. When $K_m \gg$ [E], this dominant term is given by the Briggs-Haldane "steady state" approximation.

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Received January 28th, 1957

THE REACTION OF β-MERCAPTOPYRUVATE WITH LACTIC DEHYDROGENASE OF HEART MUSCLE*

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INTRODUCTION

Recent studies dealing with the mechanism of desulfuration of cysteine in certain microorganisms (KUN *et al.*[1,2]) suggested that β-mercaptopyruvic acid is a precursor of H_2S. An enzyme system catalyzes the release of some form of sulfur from β-mercaptopyruvate which then oxidizes 2 moles of cysteine to cystine and H_2S. A similar series of reactions has been suggested previously by MEISTER *et al.*[3] and shown to occur in mammalian liver. These workers demonstrated that β-mercaptopyruvate may arise by transamination from cysteine. CHATAGNER AND SAURET-IGNAZI[4], postulating an analogous mechanism, also attribute a central rôle to β-mercaptopyruvate in the H_2S producing multienzyme system of mammalian liver. Furthermore the participation of β-mercaptopyruvate as a sulfur donor in enzymic transsulfuration reactions was pointed out by WOOD *et al.*[5,6] who used cyanide as a sulfur acceptor. Since the crystalline rhodanase of SÖRBO[7] does not react with β-mercaptopyruvate it is likely that liver cells contain several transsulfurases.

This apparently manifold rôle of β-mercaptopyruvate in intermediary metabolism led to a search for further enzymic reactions which this substance may undergo. As a part of this study this paper describes the reaction of reduced diphosphopyridine nucleotide (DPNH) and crystalline lactic dehydrogenase (LDH) with β-mercaptopyruvate.

* This investigation was supported by research grants of the U.S. Public Health Service (Heart, H-2897 and Cancer, C3211).
** Established investigator of the American Heart Association, Inc.

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