

Enzyme catalysis as a chain reaction

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A new type of enzyme kinetic mechanism is suggested by which catalysis may be viewed as a chain reaction. A simple type of one-substrate/one-product reaction mechanism has been analysed from this point of view, and the kinetics, in both the transient and the steady-state phases, has been reconsidered. This analysis, as well as literature data and theoretical considerations, shows that the proposed model is a generalization of the classical ones. As a consequence of the suggested mechanism, the expressions, and in some cases even the significance of classical constants (K_m and V_{max}), are altered. Moreover, this mechanism suggests that, between two successive enzyme-binding steps, more than one catalytic act could be accomplished. The reaction catalysed by alcohol dehydrogenase was analysed, and it was shown that this chain-reaction mechanism has a real contribution to the catalytic process, which could become exclusive under particular conditions. Similarly, the mechanism of glycogen phosphorylase is considered, and two partly modified versions of the classical mechanism are proposed. They account for both the existing experimental facts and suggest the possibility of chain-reaction pathways for any polymerase.

INTRODUCTION

Enzymes are by far the most efficient catalysts known to date. Even in the case of some enzyme-like compounds which may be structurally and functionally similar to a given enzyme, the catalytic-centre activities are, as a rule, much lower than those of the enzyme itself.

The usual explanation for this very high efficiency is that the enzyme decreases the activation free energy (ΔG^*), by reducing the activation enthalpy (ΔH^*) while increasing the activation entropy (ΔS^*). Many attempts have been made to explain the mechanism responsible for the decrease of ΔG^* (Fisher, 1894; Bruce *et al.*, 1971; Jencks, 1975; Blumenfeld, 1981).

In most papers dealing with this subject, one of the assumptions is the validity of the mechanism developed by Haldane (1930) from that given by Henri (1902) and later confirmed through initial rate determinations by Michaelis & Menten (1913):



This mechanism proposes that completion of every catalytic act results in release of the enzyme in its free form. In fact, it has been shown experimentally that, for some enzymes with polymeric substrates, the enzyme remains bound to the substrate through several catalytic cycles, a phenomenon which is termed 'processivity'. There are also theoretical considerations (described below) which support the same idea.

Boudart (1968) outlined the similarity between catalytic and chain reactions, both having high catalytic-centre activities. They differ in the source of the active centres which, in the first case, are produced by a catalyst, whereas in the chain reaction they are supplied by the system itself.

Considering the above definition, the enzyme reaction is a catalytic reaction. However, we show in the present paper that, by considering a new type of enzyme reaction mechanism and viewing the enzyme as an initiator, the enzyme reaction may be considered as a chain reaction. As a consequence, the classical

view regarding the mechanistic interpretation of the kinetic constants could be changed. Moreover, some unusual types of enzyme kinetic properties (e.g. some kinds of non-hyperbolic behaviour) can be explained in a simple manner. The mechanism proposed here throws new light on the high catalytic efficiency of the enzymes.

THE MECHANISM

We consider that the first stage of the one-substrate/one-product mechanism, formation of the enzyme–substrate complex (ES), can be viewed as an initiation reaction, the ES complex being a very reactive active centre. Similarly, the last stage of the classical mechanism, dissociation of the enzyme–product complex (EP), can be considered as an interruption reaction and the stage where ES is converted into EP as a propagation reaction.

We consider also that there is one additional propagation reaction frequently playing an essential role: the substitution of the reaction product in EP by a new substrate molecule, leading to a new enzyme–substrate complex.

Thus, the suggested mechanism can be represented as shown below:

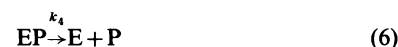
Initiation reaction:



Propagation reactions:

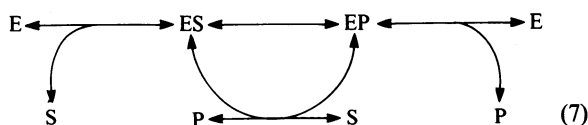


Interruption reactions:



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This sequence of reactions can be briefly summarized as shown below:



In some instances, eqn. (4) can be replaced by a non-concerted version, eqns. (4a) and (4b):



In this formulation, the enzyme acts as an initiator by providing the initial concentration of ES active centres. The propagation reactions yield the product P, while ensuring that enzyme remains in its bound forms, ES and EP.

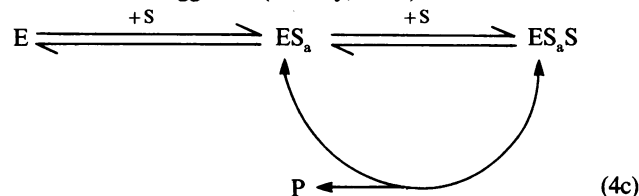
The critical step of the proposed mechanism is that shown as eqn. (4) or eqns. (4a) and (4b). This kind of reaction has been also considered by others in particular enzyme mechanisms. For example, the Theorell–Chance mechanism, initially proposed for alcohol dehydrogenase (Theorell & Chance, 1951), assumes that there is a step where such a substitution occurs when the enzyme–NAD⁺ complex reacts with ethanol to give an enzyme–NADH complex while releasing acetaldehyde. Another example is the Flip Flop mechanism (Lazdunski *et al.*, 1971); here, the binding of a substrate molecule to one enzyme subunit induces the reaction of another substrate, previously bound to a second enzyme subunit.

In fact, eqn. (4) can be considered as a single-stage (concerted) version of eqns. (4a) and (4b). We also point out that in a Flip Flop mechanism, the step where ES reacts with S to give EPS can be considered, at least formally, as arising from a two-stage process where ES is converted into EP, which then reacts with S to give EPS.

The problem of concertedness has been subjected to a more detailed analysis by Jencks (1982). He underlined that, generally, the concerted mechanism does not lead to a lower-energy pathway as compared with a stepwise mechanism. However, in the enzyme reaction, the enzyme has the advantage of concentrating and compressing the reactive groups into its active site. As a result, multifunctional catalysis will be favoured and implicitly the transition state will be stabilized. The intermediate, if it exists, will not be favoured and it may not exist as a significant species of the mechanism.

It must be stressed that the process represented by eqns. (4a) and (4b) requires the existence of different binding sites for S and P. Equally, in eqn. (4), though the ternary complex EPS is not formed, the direct exchange S–P involves the formation of the ternary transition state, EPS; again, separate binding sites for S and P are required. In the case of a polymeric substrate, the enzyme need not have more than one binding site for the substrate. As the substrate is at the same time a product, the S–P exchange is entirely reasonable. In the case of small substrates, separate binding sites for S and P can exist on the enzyme molecule. Indeed, studies on the specificity of enzymes suggest

that many enzymes have several groups, each combining with a particular part of the substrate molecule. Thus, it is possible to imagine complexes containing two molecules of substrate, or two of product, or one substrate and one product. In fact, the assumption of abortive ES₂ complex formation in the case of the inhibition by high substrate concentrations is well known (Dixon & Webb, 1979, p. 127). There would be also situations in which two molecules of substrate may combine with the enzyme at different sites, as in the case of a substrate acting also as an activator (Dixon & Webb, 1979, p. 137). For instance, fumarate hydratase is activated by anions; as the substrates are themselves anions, it is possible that they may function also as activators. Thus the formation of a complex (ES_aS) containing both activator and substrate is suggested (Massey, 1953):

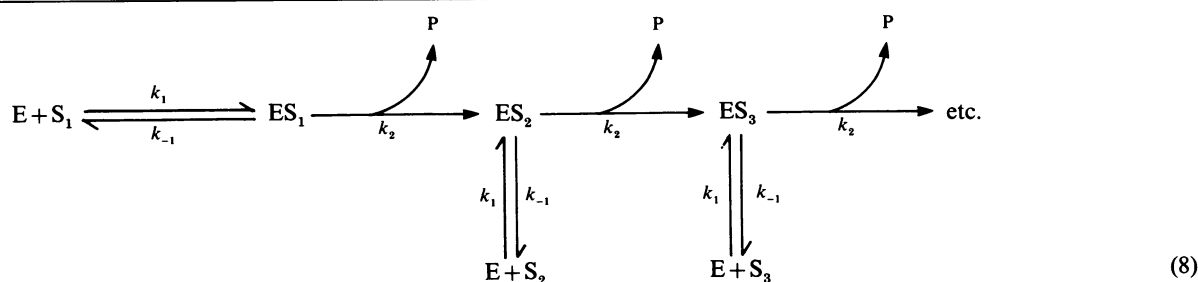


Eqn. (4c) is similar to eqns. (4a) and (4b), but differs in that the complex ES_a is involved instead of EP in the formation of the ternary complex.

The suggested mechanism is also consistent with the ‘induced-fit’ theory elaborated by Koshland (1959). When the enzyme–substrate complex is formed from the free enzyme, conformational changes will be produced in the enzyme molecule which facilitate the reaction between the substrate and the catalytic groups of the active site. This modified enzyme conformation can then be preserved with, at most, minor modifications on passing from ES to EP. According to the classical mechanism, this conformation will be lost as a consequence of the decomposition of EP to E plus P. In a transformation of the type where EP reacts with S to give ES plus P, the enzyme could retain its catalytic conformation between two consecutive reaction cycles. This process would be expected to enhance catalysis by eliminating slow conformational changes and should be energetically more favourable.

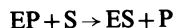
Experimental evidence for similar mechanisms has been given in the case of some enzymes having polymeric substrates. Bayley & French (1957) proposed the term ‘degree of multiple attack, *f*’; it represents the probability that the enzyme will carry out at least one more catalytic act before dissociating from the substrate, after having accomplished one or more catalytic events at the end of a polymer chain. Thus *f* = 0 (multi-chain attack) corresponds to an extreme situation where, between two consecutive catalytic acts, the enzyme undergoes an obligatory dissociation from its polymeric substrate. The other extreme is where *f* = 1 (single-chain attack), which means that the enzyme operates successively on a single substrate molecule without any dissociation of the ES complex.

Bayley & French (1957) pointed out that, for β-amylase acting on a starch molecule, the mechanism can be formulated as follows:



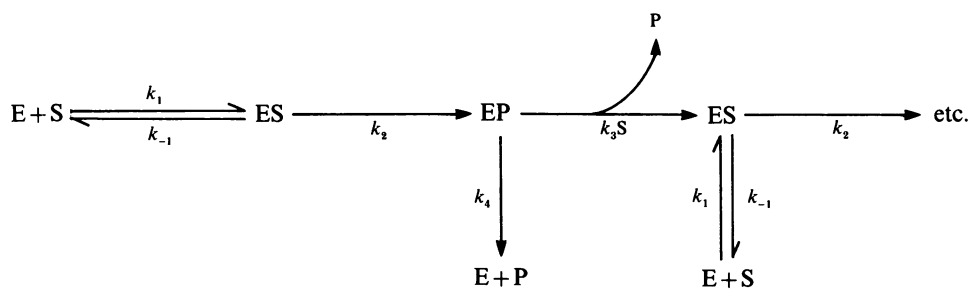
For this mechanism we have $f = k_2/(k_{-1} + k_2)$.

The resemblance of this mechanism to that proposed in the present paper becomes evident if one observes that the complexes $ES_i (i = 1, 2, \dots)$ are at the same time EP complexes with the substrate 'sliding' through the catalytic site. This is formally equivalent to a cyclic process of the type:



Experimentally determined values for f were 0.75–0.80 for β -amylase and 0.88–0.92 for porcine pancreatic α -amylase (Robyt & French, 1970). These values suggest that the chain-reaction mechanism plays a significant role in the mode of action of these amylases. For potato starch phosphorylase, the single-chain attack seems to have a smaller contribution because, in this case, f is equal to 0.3 (Bayley & French, 1957).

Neglecting the elementary steps which correspond to k_{-2} and k_{-3} , a scheme similar to (8) can be imagined for our mechanism:



(9)

In this scheme, the fraction of ES that goes to EP (as opposed to $E + S$) is $k_2/(k_{-1} + k_2)$, and the fraction of EP that goes on to ES (as opposed to $E + P$) is $k_3[S]/(k_3[S] + k_4)$. Since chain reaction requires that these events occur sequentially the propagation probability $f = [k_2/(k_{-1} + k_2)][k_3[S]/(k_3[S] + k_4)]$.

KINETIC ANALYSIS OF THE MODEL

The evolution in time for a reacting system involving a linear chain process is characterized by three main stages: (a) the transient phase, where the initiation reactions are prevalent; (b) the steady-state phase, where the reactions of chain propagation are predominant and the active-centre concentration remains unchanged; and (c) the terminal phase, where the interruption reactions assume major proportions.

The analysis of enzyme reactions proves the existence of all these stages, again in accordance with our model. In the case of enzyme catalysis, the most important are the first and the second stages. They will be analysed in the following sections.

The transient phase

Let us consider the set of reactions given as eqns. (2)–(6) in the initial stage. We assume the approximations $[S] = [S]_{t=0} = S_0$ and $[P]_{t=0} = 0$, which allow us to consider that $k_{-3} = 0$ in the kinetic equations. The rate equations are:

$$dx_1/dt = a_{12} x_2 \quad (10)$$

$$dx_2/dt = a_{22} x_2 + a_{23} x_3 \quad (11)$$

$$dx_3/dt = a_{32} x_2 + a_{33} x_3 + b_3 \quad (12)$$

where $x_1 = [P]$; $x_2 = [EP]$; $x_3 = [ES]$; $a_{12} = k_3[S] + k_4$; $a_{22} = -(k_{-2} + k_4 + k_3[S])$; $a_{32} = k_{-2} + (k_3 - k_1)[S]$; $a_{23} = k_2$; $a_{33} = -(k_{-1} + k_2 + k_1[S])$; and $b_3 = k_1[S]E_0$. This system of differential equations has been obtained by using the enzyme conservation equation $[E] + [ES] + [EP] = [E]_{\text{total}} = E_0$.

Solving this set of equations by applying the Laplace transform

on the eqns. (11) and (12), and knowing that $[EP]_{t=0} = [ES]_{t=0} = 0$, we obtain:

$$\mathcal{L}[x_2(t)] = \int_0^\infty e^{-st} x_2(t) dt = \bar{x}_2(s) = \frac{a_{23} b_3 s^{-1} [s^2 - (a_{22} + a_{23})s + a_{22} a_{23} - a_{33} a_{32}]^{-1}}{a_{23} b_3 s^{-1} [s^2 - (a_{22} + a_{23})s + a_{22} a_{23} - a_{33} a_{32}]^{-1}} \quad (13)$$

By using the Heaviside formula, one obtains $x_2(t)$; substituting in eqn. (10) and integrating, yields the result:

$$\begin{aligned}
 x_1(t) = & a_{12} a_{23} b_3 t / (a_{22} a_{33} - a_{23} a_{32}) \\
 & + [a_{12} a_{23} b_3 / (2\sqrt{\Delta})] \{ -4q\sqrt{\Delta} / (q^2 - \Delta)^2 \\
 & + \exp[-(q - \sqrt{\Delta})t] / (q - \sqrt{\Delta})^2 \\
 & - \exp[-(q + \sqrt{\Delta})t] / (q + \sqrt{\Delta})^2 \} \quad (14)
 \end{aligned}$$

where $q = -(a_{22} + a_{33})/2$ and $\Delta = (a_{22} + a_{33})^2/4 + (a_{23} a_{32} - a_{22} a_{33})$

The above equation can be rearranged to give the following:

$$x_1(t) = A_0 t + A_1 [\exp(-t/\tau_1) - 1] + A_2 [\exp(-t/\tau_2) - 1] \quad (15)$$

where the relaxation times (τ_1 and τ_2) are given by $(q \pm \sqrt{\Delta}) / (q^2 - \Delta)$ and are taken to be different from one another. This equation is in accordance with the general form given by Hijazi & Laidler (1973).

Retaining only the first four terms from the Taylor expansions of the exponentials, it follows that the initial portion of the curve $P(t)$ can be approximated by:

$$P(t) = k_1 k_2 (k_3 S_0 + k_4) S_0 E_0 t^3 / 6 \quad (16)$$

Plotting $P(t)$ as a function of t^3 should yield a straight line with the slope:

$$\varphi(S_0) = k_1 k_2 (k_3 S_0 + k_4) S_0 E_0 / 6 \quad (17)$$

A secondary plot of $\varphi(S_0)/S_0$ as a function of S_0 can then be used to obtain values for $k_1 k_2 k_4$ and $k_1 k_2 k_3$.

As the process goes on (t increases), it reaches the steady-state, characterized by a linear increase in the product concentration with time. The corresponding equation can be derived from eqn. (14) by neglecting the exponentials; the intercept at the origin of this straight line leads to an expression for the lag time.

$$t_{\text{lag}} = \frac{k_{-1} + k_{-2} + k_2 + k_4 + (k_1 + k_3) S_0}{k_1 k_3 S_0^2 + (k_{-1} k_3 + k_1 k_{-2} + k_1 k_4 + k_1 k_2) S_0 + k_{-1} k_{-2} + k_{-1} k_4 + k_2 k_4} \quad (18)$$

A feature of the above formula is the fact that the degree of the denominator is higher than that of the numerator, as opposed to the classical case where both denominator and numerator have the same degree (Darvey, 1968). According to eqn. (18), t_{lag} tends to zero at high S_0 concentration. Eliminating the chain reaction component by putting $k_3 = 0$ in Eqn. (18) we get the expression of t_{lag} for the classical mechanism (Darvey, 1968).

If S_0 can be increased experimentally to a sufficient extent, a

plot of $t_{1/2} S_0$ against S_0 , will tend to a limiting value equal to $(k_1 + k_3)/k_1 k_3$, giving a relationship between k_1 and k_3 . As will be pointed out in the following section, the steady-state rate equation allows determination of k_2 from V_{\max} . Thus, by combining information from the transient and steady-state phases, k_1 , k_2 , k_3 and k_4 could be determined.

The steady-state phase

Let us consider the eqns. (2)–(6) describing the proposed mechanism but include the process $E + P \rightarrow EP$ (with a rate constant of k_{-4}) as another possible initiation reaction. Assuming the steady-state hypothesis for the complexes ES and EP, as well as the enzyme conservation equation $[E] + [ES] + [EP] = E_0$, an equation for the reaction rate will be obtained:

$$v_0 = \frac{(m_0 S_0^2 + m_1 S_0 + m_2 S_0 P_0 - m_3 P_0 - m_4 P_0^2) E_0}{d_0 S_0^2 + d_1 S_0 + d_2 S_0 P_0 + d_3 P_0 + d_4 P_0^2 + d_5} \quad (19)$$

where the various constants are defined as: $m_0 = k_1 k_2 k_3$; $m_1 = k_1 k_2 k_4$; $m_2 = k_2 k_3 k_{-4} - k_1 k_{-2} k_{-3}$; $m_3 = k_{-1} k_{-2} k_{-4}$; $m_4 = k_{-2} k_{-3} k_{-4}$; $d_0 = k_1 k_3$; $d_1 = k_1(k_{-2} + k_2 + k_4) + k_{-1} k_3$; $d_2 = k_1 k_{-3} + k_3 k_{-4}$; $d_3 = k_{-1} k_{-4} + k_2 k_{-4} + k_{-2} k_{-4} + k_{-3} k_4$; $d_4 = k_{-3} k_{-4}$; and $d_5 = k_{-1}(k_{-2} + k_4) + k_2 k_4$.

The squared terms, that is, S_0^2 , $S_0 P_0$ and P_0^2 , are due to eqn. (4). It may be noted that when k_3 and k_{-3} are both equal to zero, eqn. (19) becomes identical with the classical form of the reversible one-substrate/one-product mechanism (Wong, 1975). The inclusion of eqn. (4) results in an altered rate equation, owing to the non-zero coefficients of the squared terms.

Let us analyse the case of a one-substrate irreversible mechanism.

Here we have:

$$v_0 = \frac{(m_0 S_0^2 + m_1 S_0) E_0}{d_0 S_0^2 + d_1 S_0 + d_5} \quad (20)$$

Clearly, setting $k_3 = k_{-3} = 0$ in this case (whereupon $m_0 = d_0 = 0$) leads to the Michaelis–Menten equation. In this situation, $V_{\max} = [k_2 k_4 / (k_{-2} + k_2 + k_4)] E_0$, but inclusion of eqn. (4) gives a different definition: $V_{\max} = (m_0 / d_0) E_0 = k_2 E_0$. The same result can be obtained from the classical case; when the conversions of ES into EP and EP into ES are slow and the complex EP decays rapidly to E and P (i.e. $k_4 \gg k_2$ and $k_4 \gg k_{-2}$), then we get $V_{\max} = k_2 E_0$.

There are two more cases in which the rate equation (eqn. 20) is reduced to the classical Michaelis–Menten-type equation. In the first of these the decomposition of the complexes ES and EP is negligible. Given the high affinity of some enzymes for their specific substrates, such a situation would be plausible. In other words, when $k_4 = k_{-1} = 0$, the contribution of interruption reactions is insignificant; eqn (20) now reduces to:

$$v_0 = \frac{k_2 k_3 S_0 E_0}{k_3 S_0 + k_{-2} + k_2} \quad (21)$$

Although this describes a rectangular hyperbola, the expressions of V_{\max} and K_m do not coincide with their classical ones. Whereas for the classical mechanism we have $V_{\max} = [k_2 k_4 / (k_{-2} + k_2 + k_4)] E_0$ and $K_m = (k_{-1} k_{-2} + k_{-1} k_4 + k_2 k_4) / [k_1(k_{-2} + k_2 + k_4)]$, for the proposed chain-reaction mechanism $V_{\max} = k_2 E_0$ and $K_m = (k_{-2} + k_2) / k_3$. These last two expressions predict that V_{\max} is proportional to the rate constant for conversion of ES into EP and the lower are the two rate constants for this isomerization, the smaller will be the K_m value.

A second case where eqn. (20) reduces to the Michaelis–Menten equation is when the only process in which the complex EP can

participate is of the type given as eqn. (4); i.e. the direct substitution of P by S. With $k_4 = k_{-2} = 0$, eqn. (20) becomes:

$$v_0 = \frac{k_1 k_2 k_3 S_0 E_0}{k_1 k_3 S_0 + k_1 k_2 + k_{-1} k_3} \quad (22)$$

Obviously, here $V_{\max} = k_2 E_0$ and $K_m = (k_2 / k_3) + (k_{-1} / k_1)$, which again differ from the classical expressions. In this particular case V_{\max} does not depend on k_3 , and the Michaelis constant is inversely related to this rate constant. That is, high k_3 values give low K_m values and therefore high enzyme efficiency.

In addition to the situations mentioned above, some other special cases can be derived from eqn. (20). Of particular note are the following (Ferdinand, 1976): (a) hyperbolic curves, if $m_0 d_5 < m_1 d_1$ and $m_0 d_1 > m_1 d_0$; (b) curves passing through a maximum, if $m_0 d_5 < m_1 d_1$ and $m_0 d_1 < m_1 d_0$; and (c) sigmoidal curves with a maximum, if $m_0 d_5 > m_1 d_1$ and $m_0 d_1 < m_1 d_0$.

Thus, the proposed mechanism can explain the occurrence of non-hyperbolic dependence of rate upon the concentration of substrate.

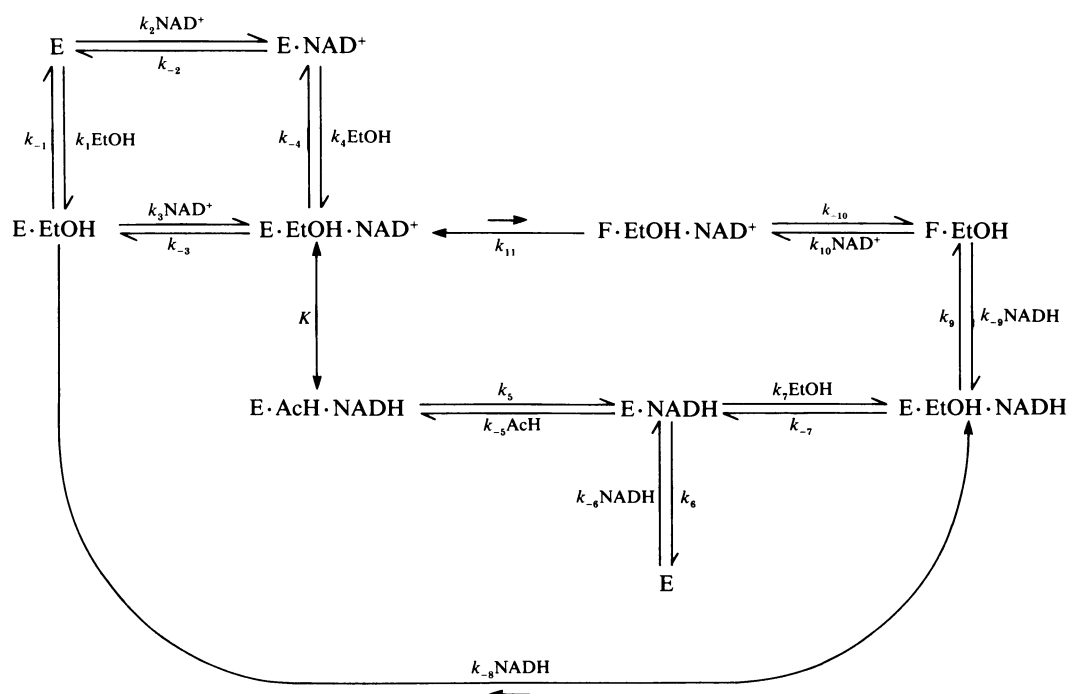
APPLICATIONS

Mechanism of alcohol dehydrogenase

Consider the reaction mechanism (Scheme 1) for horse liver alcohol dehydrogenase (EC 1.1.1.1), as proposed by Hanes *et al.* (1972). In this scheme, EtOH and AcH stand for ethanol and acetaldehyde respectively, whereas F denotes an altered conformation of the enzyme E in the corresponding complexes. This mechanism contains, as well as the classical mechanism, a chain-reaction component in which the reaction can proceed without enzyme being released in its free form after every round of catalysis. Thus, in the forward reaction, a possible pathway could be initiation via the steps E to E·EtOH (hereafter denoted '1' from the corresponding rate constant) and 3, followed by endless repetition of the chain reaction sequence {5, 7, 9, 10, 11}. An alternative process using a different initiation sequence is 2, 4, {5, 7, 9, 10, 11}, whereas the reverse reaction could occur via the steps -6, {-5, -3, -8, -7}. Let us analyse whether the reaction proceeds preferably through these unconventional pathways or through the conventional ones.

For the reverse reaction, it has been shown that the pathway passing through E·EtOH·NADH becomes significant (Hanes *et al.*, 1972) at high level of NADH (i.e. with NADH at a high enough concentration that the E·EtOH complex is diverted to E·EtOH·NADH via step -8). Nevertheless, some catalysis can occur by the classical pathway (-6, -5, -4, -2). The propagation probability (i.e. the probability of chain propagation) is equal to $k_{-3} / (k_{-3} + k_{-4} + k_5)$, whereas the probability of reaction with chain interruption will be $k_{-4} / (k_{-3} + k_{-4} + k_5)$. Substituting the appropriate values given by Hanes *et al.* (1972) results in an interruption probability of 0.337 and a propagation probability of 0.101. Thus it seems likely that, for the reverse reaction, the chain-reaction pathway plays only a minor role in the catalytic mechanism as compared with the conventional pathway. The reason that these two probabilities do not add up to 1.0 is that there is a third fate for the branchpoint complex E·EtOH·NAD⁺. In addition to chain interruption (via step -4) and chain propagation (step -3) there can be backward movement (i.e. the forward reaction) through step 5 after rapid equilibration to E·AcH·NADH.

In the case of the forward reaction, at low ethanol concentration, catalysis takes place via steps 1, 3, 5, 6 or 2, 4, 5, 6; that is the chain reactions are not involved. However, at higher ethanol concentrations the pathways consisting of steps 1, 3, {5, 7, 9, 10, 11} or 2, 4, {5, 7, 9, 10, 11} also become involved. Indeed,



Scheme 1. Mechanism of horse liver alcohol dehydrogenase

This Scheme is redrawn from that described by Hanes *et al.* (1972); EtOH and AcH represent ethanol and acetaldehyde respectively, and F is an altered conformation of the enzyme (E). The central complexes (E·EtOH·NAD⁺ and E·AcH·NADH) are in rapid equilibrium with one another. The steps from F·EtOH·NAD⁺ to E·EtOH·NAD⁺ and from E·EtOH to E·EtOH·NADH are taken to be practically irreversible.

when the steps 1 and 4 are saturated by ethanol, the chain-propagation probability will be $k_7[\text{EtOH}]/(k_7[\text{EtOH}] + k_6 + k_5[\text{AcH}])$ and the probability of chain interruption is $k_6/(k_7[\text{EtOH}] + k_6 + k_5[\text{AcH}])$. Assuming k_7 to be of similar magnitude to the value of k_4 given by Hanes *et al.* (1972), the propagation probability in the absence of acetaldehyde is 0.597 when the ethanol concentration is equal to its K_m (0.5 mM). As the ethanol concentration increases, the propagation probability also increases (e.g. 0.937 at $[\text{EtOH}] = 5$ mM), whereas the interruption probability decreases. Thus the chain-reaction pathway 1, 3, {5, 7, 9, 10, 11} becomes more important than any other pathway and at very high ethanol concentrations, this chain-reaction pathway is used exclusively.

Mechanism of glycogen phosphorylase

Rabbit muscle phosphorylase a (EC 2.4.1.1) catalyses the reversible phosphorolysis of the $\alpha(1 \rightarrow 4)$ -glycosidic linkage from the non-reducing ends of glycogen. Initial-rate (Engers *et al.*, 1970a) and equilibrium-exchange studies (Engers *et al.*, 1970b) demonstrated that the kinetic mechanism of this enzyme is a rapid-equilibrium random Bi Bi; that is, there is random addition of substrates and the interconversion of the ternary complexes is the rate-limiting step in the reaction sequence.

We propose two possible mechanisms derived from that suggested by Engers *et al.* (1970a,b). These mechanisms involve chain-reaction patterns which we believe are credible because, for a related enzyme (potato phosphorylase), it has been shown that single-chain attack occurs ($f = 0.3$; Bayley & French, 1957).

In the first of these mechanisms, there is a ternary complex composed of phosphorylase (E), α -D-glucose 1-phosphate (PG) and a glycogen molecule containing $(n-1)$ glucose units (G_{n-1}). This reacts with a phosphate ion (P), eliminating PG and forming a second ternary complex $E \cdot P \cdot G_n$. This is a homologue of $E \cdot P \cdot G_n$ from which it originates through the two previous steps.

The complete mechanism can be represented as shown in Scheme 2(a).

The crucial step in chain-reaction propagation is the one where $E \cdot P \cdot G_{n-1}$ reacts with P to give $E \cdot P \cdot G_n$ plus PG. The direct substitution of PG by P obviously requires a transition state in which, as well as the glycogen macromolecule, both GP and P are bound to the enzyme. Although this may seem unlikely at first sight, it has been demonstrated that the enzyme binds glucose 1-phosphate through independent binding sites for the glucose and phosphate moieties (Martin *et al.*, 1986). Thus we may imagine a transition state in which the phosphate is bound on the phosphate-binding site while glucose 1-phosphate is fixed only by the glucose-binding site.

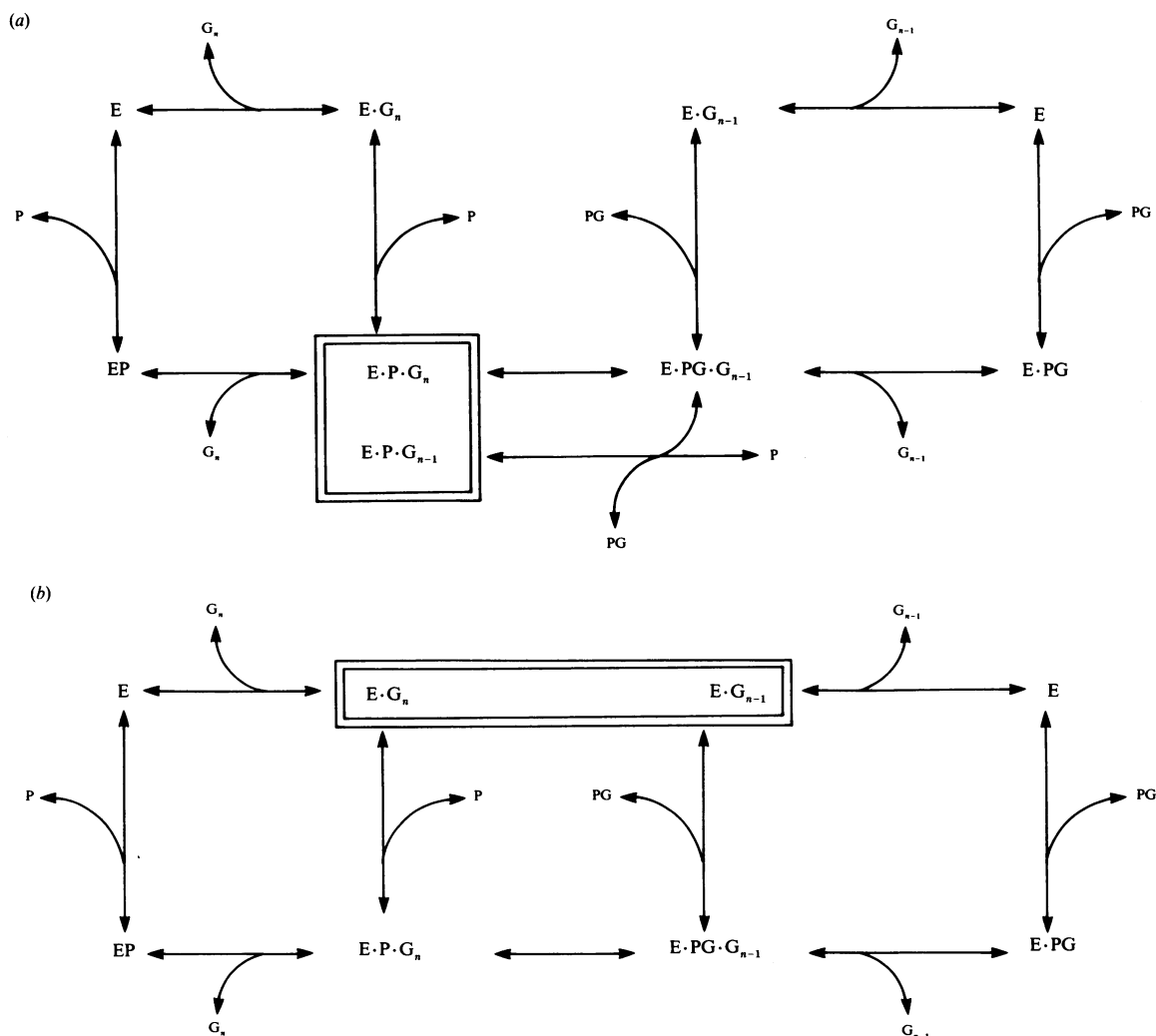
According to this mechanism, the repetitive cycle of catalysis is the pair of reactions starting from $E \cdot P \cdot G_n$ and involving ternary complex conversion followed by direct substitution of PG by P. Since $E \cdot P \cdot G_{n-1}$ so formed is homologous with the initial ternary complex, the cycle is effectively complete and can begin again.

The second phosphorylase mechanism (Scheme 2b) can be directly derived from that proposed by Engers *et al.* (1970a,b). We will consider that there is in fact a single type of enzyme-glycogen binary complex and not two, as one of the substrates (glycogen G_n) is a homologue of one of the products (glycogen G_{n-1}). The above mechanism contains both the classical mechanism and the chain-reaction mechanism. The latter involves the repetitive cycle:



It can be observed that the critical step of this mechanism is, in fact, the non-concerted version of the critical step in the previous mechanism.

Both mechanisms proposed above could also explain the fact that the double-reciprocal plots for phosphorylase have a considerable curvature (Engers *et al.*, 1970a). To test the validity of



Scheme 2. Mechanism of rabbit muscle glycogen phosphorylase

This Scheme is derived from the mechanism proposed by Engers *et al.* (1970*a,b*); P and PG represent phosphate ion and α -D-glucose 1-phosphate respectively, and G is glycogen containing the number of glucose units indicated by its subscript. Double boxes surround homologous complexes. The concerted variant is shown in (a) whereas the non-concerted variant is given in (b).

these mechanisms, or to discriminate between them, new experiments are needed. First of all it would be necessary to determine the degree of multiple attack, f , in order to evaluate the contribution of the suggested type of mechanisms to the catalytic process. To decide which of the first and second mechanisms has a greater contribution to the actual catalytic process, it is necessary to determine experimentally the extent to which the binding of P and expulsion of GP is a concerted step.

CONCLUSIONS

In the present paper we have considered enzyme catalysis as a chain-reaction system and have derived equations describing both the transient and steady-state phases. This model does not conflict with the classical type of enzyme mechanism, which may be regarded as a limiting case of the more general mechanism.

Our approach leads to a partly modified understanding of the enzyme catalysis. Thus, considering the enzyme as an initiator of a chain reaction, a catalytic act is not completed by release of the product from the enzyme molecule. As the chain-reaction propagates, several catalytic acts can be accomplished between an enzyme binding and a subsequent enzyme release. The analysis of the postulated mechanisms of alcohol dehydrogenase and of

glycogen phosphorylase show that the reaction sequence presented here is not simply a formal possibility, but its occurrence is also supported by experimental data from the literature.

This approach gives new expressions for the constants of the enzyme reaction in both the transient state (i.e. the lag time) and the steady state (V_{\max} and K_m). In the particular cases considered above in which the rate equations reduced to the classical Michaelis-Menten-type equation, the interpretations of V_{\max} and K_m are altered. Since V_{\max} depends linearly on the rate constant of $ES \rightarrow EP$ isomerization and is independent of any other rate constants, it simply reflects the efficiency of enzyme-substrate complex conversion into the enzyme-product complex and is unrelated to the rate constant of enzyme-product-complex decomposition to product and free enzyme. Further, K_m does not necessarily reflect the strength of substrate binding to the enzyme, but it can give information about the ratio between the rate constant of $EP + S \rightarrow ES + P$ transformation and the rate constants of $ES \leftrightarrow EP$ isomerization.

The extent to which these types of pathways contribute to the mechanism for a given enzyme can, in some instances, be evaluated directly from the kinetic constants of the classical mechanism if it contains chain-reaction pathways, as is shown here for alcohol dehydrogenase. In other cases where the chain-

reaction mechanism involves new steps as compared with the classical mechanism, new experimental measurements would be needed. Transient-phase measurements could be useful in this regard. For instance, the dependence of t_{lag} on S_0 (eqn. 18) can give evidence for mechanism obeying the eqns. (2)–(6). For polymerases, it is likely that there always exists a chain-reaction type of contribution to their mechanisms and the second mechanism we discussed for phosphorylase should also hold for any polymerase having ternary complex mechanism with random addition of substrates and random release of products. We propose that other aspects of enzyme catalysis should also be reconsidered by taking into account the new type of mechanism suggested in the present paper.

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