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ALLOSTERIC PROPERTIES OF ENZYMES WITH "PING-PONG" MECHANISM

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SUMMARY

Allosteric properties of enzymes exhibiting the ping-pong mechanism, which is characterized by the oscillation of enzyme protein between multiple stable enzyme forms during reaction, have been analyzed in terms of the partial equilibrium theory recently introduced by Cha (*J. Biol. Chem.*, 243 (1968) 820). It is disclosed that the enzyme exhibiting the ping-pong mechanism is endowed with the unique property that the homotropic and heterotropic effects of ligands are determined by the kind of stable enzyme form which undergoes allosteric transition.

INTRODUCTION

Regulatory enzymes are often characterized by complicated kinetic patterns including sigmoidal relationships between initial velocities and substrate concentrations which can be accounted for by postulating the cooperative interactions between ligands simultaneously bound to the enzyme molecule. As a theoretical interpretation for these interactions, several types of models have been proposed based on the concept of allosteric transition, *i.e.* reversible conformational changes of the enzyme molecule induced by the binding of ligands[1-3].

Apart from these allosteric properties, various types of kinetic mechanisms are known dependent on the order of the binding of substrates and release of products within the reaction sequence involving two or more substrates and products[4]. These mechanisms, though by themselves responsible for the complicated kinetic patterns often encountered in the enzyme kinetic studies, are supposed not to involve a molecular transition of enzyme protein upon the binding of substrates. This actually holds true for the sequential* mechanism in which all substrates bind to the enzyme before any products are released. Cooperative kinetics, if observed for this type of enzyme reaction, must be readily analyzed in terms of the above-mentioned allosteric models by assuming that molecular transition is induced by the binding of substrates to the enzyme protein.

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* The terms sequential and stable enzyme form are used here exactly according to be nomenclature or description proposed by Cleland[4].

So far as the enzyme with ping-pong mechanism is concerned, however, a more complicated situation is anticipated in kinetic analysis of its allosteric properties, because such an enzyme exists in two or more stable enzyme forms* between which it oscillates during the reaction[4]. These stable enzyme forms are interconvertible in the reaction sequence through the transitory complex as a result of the actual group transfer reaction involving covalent linkages between substrate molecules and enzyme protein. In this case, the formation of new stable enzyme forms involves the "chemical modification" of the original enzyme protein. It is well known that the enzyme-catalyzed chemical modification of allosteric enzymes such as phosphorylase[5], glycogen synthetase[6] and glutamine synthetase from *Escherichia coli*[7] caused a striking alteration of the physical properties, *e.g.* association–dissociation of subunits, affinities to effectors, *etc.* It may be possible, therefore, to assume that an interconversion between stable enzyme forms during the ping-pong type reaction sequence could exert some influences on the allosteric properties of the enzyme, though the stable enzyme form in the ping-pong mechanism is quite different from the enzyme form generated by the enzyme-catalyzed chemical modification in a sense that the former is in fact a transitory intermediate of a reaction sequence while the latter is actually the stable end-product of a reaction catalyzed by the modifying enzyme.

The purpose of this paper is to analyze the cooperative kinetics shown in the ping-pong type reaction sequence in terms of the theory of concerted allosteric transition[1]. The paper deals with the derivation of the simple rate equations which can offer valuable insight into the reaction mechanism. Some characteristics of the proposed model are also discussed.

THEORETICAL

Kinetic models of the reaction in which the enzyme with ping-pong mechanism undergoes the allosteric transition

The ping-pong bi-bi mechanism is chosen in the present study as a simple representative of the enzyme reaction in which the enzyme exists in two or more stable forms during reaction. This mechanism is summarized in the kinetic scheme in Diagram I where E is a free enzyme while E' stands for another stable enzyme form. Substrates which bind to E and E' are shown as A and B, respectively, while products which bind to E and E' are designated D and C, respectively.

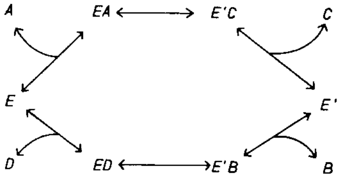

Diagram I

If the enzyme responsible for the ping-pong mechanism reaction is supposed to consist of multiple subunits and to be subjected to concerted allosteric transition[1], either E or E' can exist in equilibrium between states R and T. When the affinities of the substrates for the T state are assumed to be negligible, the reaction scheme in Diagram I represents the R state with only one subunit bound with the ligands, which could be further stabilized by binding more ligands successively on other subunits. To save the space of presentation, such a situation is simply illustrated as the kinetic scheme in Diagrams IIa and IIb by denoting the number of subunits by n. In Diagram IIa, E is assumed to be in equilibrium with the T state (E_T) while E' is assumed to be in equilibrium with the corresponding T state (E'_T) in Diagram IIb.

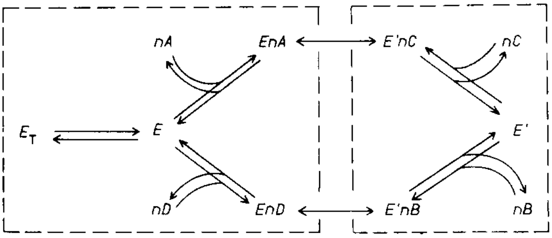

Diagram IIa

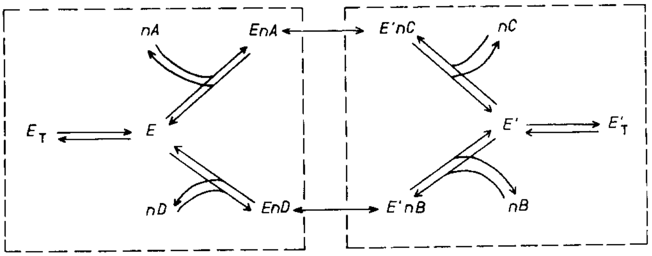

Diagram IIb

Since two stable enzyme forms, E and E', are equally responsible for the enzyme activity in the ping-pong mechanism, Diagrams IIa and IIb represent equally probable kinetic models for the reaction in which the enzyme functioning by the ping-pong mechanism as its "basal" catalytic property undergoes the concerted allosteric transition. Another possibility to be taken into consideration is that the conversion of one stable enzyme form to another fails to exert any influence on the allosteric property of the enzyme protein. Under this condition, both E and E' will undergo allosteric transition with equal allosteric constants as shown in Diagram IIc.

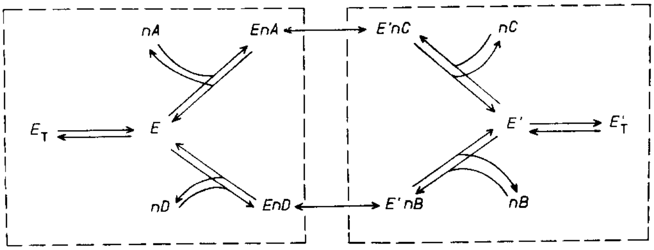

Diagram IIc

Since it may be appropriate to refer to these reaction mechanisms as "allosteric ping-pong", these three types of mechanism shown in Diagrams IIa, IIb and IIc will be called "allosteric ping-pong I", "II" and "III", respectively.

Derivation of rate equations

Generally speaking, the rate equations for the ping-pong mechanisms are derived under the steady-state assumption while most of the complicated cooperative kinetics have been so far analyzed on the basis of the rapid equilibrium assumption which is sometimes more useful than the steady-state treatment in leading to simpler rate equations and constants. In deriving the rate equations for the allosteric ping-pong mechanism, we introduced an assumption that the actual group transfer reactions between two stable enzyme forms, E and E', are much slower than the binding and dissociation of the reaction components occurring on either enzyme form, as first proposed by Cha[8] as a reasonable restriction on the full steady-state kinetics. The kinetic schemes in Diagram II then consist of two "rapid equilibrium segments", the one concerning the binding and dissociation of ligands on E and the other on E', as indicated by dotted-line squares. These two segments are connected to each other through the slow steps limiting the overall reaction rate as indicated by the single-lined double-headed arrows in Diagram II. Under this "partial equilibrium" assumption, following rate equations, Eqns 1, 2 and 3, are derived for the allosteric ping-pong mechanisms of Type I (Diagram IIa), Type II (Diagram IIb) and Type III (Diagram IIc), respectively.

$$\frac{v}{n} = \frac{k_1 k_2 A B e_0}{k_1 K_B A\left(1 + \dfrac{B}{K_B}\right) + k_2 K_A B\left\{1 + \dfrac{A}{K_A} + \dfrac{L}{\left(1 + \dfrac{A}{K_A}\right)^{n-1}}\right\}} \tag{1}$$

$$\frac{v}{n} = \frac{k_1 k_2 A B e_0}{k_1 K_B A\left\{1 + \dfrac{B}{K_B} + \dfrac{L}{\left(1 + \dfrac{B}{K_B}\right)^{n-1}}\right\} + k_2 K_A B\left(1 + \dfrac{A}{K_A}\right)} \tag{2}$$

$$\frac{v}{n} = \frac{k_1 k_2 A B e_0}{k_1 K_B A\left\{1 + \dfrac{B}{K_B} + \dfrac{L}{\left(1 + \dfrac{B}{K_B}\right)^{n-1}}\right\} + k_2 K_A B\left\{1 + \dfrac{A}{K_A} + \dfrac{L}{\left(1 + \dfrac{A}{K_A}\right)^{n-1}}\right\}} \tag{3}$$

In these equations, K_A and K_B represent the intrinsic dissociation constants of EA and $E'B$, respectively, k_1 and k_2 the rate constants of the rate-limiting steps involved in the decomposition of EA and $E'B$, respectively, L the allosteric constant and e_0 the total concentration of the enzyme. For the sake of simplicity, the reaction in the reverse direction was neglected in deriving these equations; *i.e.* v is the initial velocity in a strict sense.

It should be noted that the concept adopted here for deriving rate equations can be expanded to analyze more complicated situations. Theoretically, the allosteric properties of the enzyme which oscillates between three or more stable enzyme forms can be analyzed in a similar way by assuming that each of the stable enzyme forms constructs its own "rapid equilibrium segment" in which binding and dissociation of ligands occurs rapidly, and between which the reaction involving actual group transfer intervenes as a slow rate-limiting step. Moreover, the "heterotropic effect" induced by the binding of modifiers is also analyzed in the same way, because the effect is likewise confined to the "rapid equilibrium segment" to which the stable enzyme form affected by the modifier belongs; an example being shown in Eqn 4 for the heterotropic effect of modifier (M) stabilizing the R state in the mechanism of Type I, with the intrinsic dissociation constant of the enzyme–modifier complex being K_{M}.

$$\frac{v}{n} = \frac{k_1k_2ABe_0}{k_1K_{\mathrm{B}}A\left(1 + \dfrac{B}{K_{\mathrm{B}}}\right) + k_2K_{\mathrm{A}}B\left\{1 + \dfrac{A}{K_{\mathrm{A}}} + \dfrac{L}{\left(1 + \dfrac{A}{K_{\mathrm{A}}}\right)^{n-1}\left(1 + \dfrac{M}{K_{\mathrm{M}}}\right)^{n}}\right\}} \tag{4}$$

This equation is based on the assumption that the modifier is directly bound to E; such a binding is essentially required for its role as a positive allosteric effector, but the binding to E', if it occurs, has no influence on the rate equation.

Characteristics of the reaction models

The rate equations derived above clearly reveal the striking feature of the reaction models as follows. If the allosteric transition occurs on one stable enzyme form, cooperative kinetics are observed only for the ligands which bind directly to this enzyme form. In contrast, the enzyme exhibits normal Michaelis–Menten kinetics with respect to the ligands bound to another enzyme form. This holds true for either the homotropic or the heterotropic effect. Since the interconversion between stable enzyme forms in ping-pong mechanisms involves a "chemical modification" of enzyme protein probably at an area close to the substrates binding sites, it is possible to assume that each stable enzyme form has its own characteristic physical properties and hence that only one of the enzyme forms undergoes allosteric transition. Such a situation, if it occurred, could be checked experimentally by studying which substrate exhibits cooperative kinetics. In other words, if the ping-pong mechanism enzyme exhibits cooperative interaction only with respect to some of the substrates but obeys Michaelis–Menten kinetics in response to a change of concentration of other substrates, it is highly probable that not all of the stable enzyme forms undergo allosteric transition as shown by the present rate equations, Eqns 1 and 2. Phosphofructokinase from a bacterial source[9] probably provides a good example for such a situation; it showed a strong cooperation with respect to Fru-6-P but showed a hyperbolic pattern with respect to ATP. The Lineweaver–Burk plots with respect to ATP gave a series of parallel lines, in conformity with the ping-pong mechanism. These kinetic properties seem to be interpretable in terms of the present model of Type II; *i.e.* the phosphorylated enzyme which binds Fru-6-P does (but free enzyme which binds ATP does not) undergo an allosteric transition. Another example for such a specific type of allosteric ping-pong mechanism will be presented in the subsequent paper.

Of course, cooperation can be observed with respect to all the substrates in the reaction mechanisms belonging to Type III as revealed by Eqn 3.

DISCUSSION

The most extensive theoretical considerations of the allosteric properties of enzymes have so far been given to single-substrate reactions. With two-substrate and three-substrate enzymes, which in fact are more commonly encountered, it has usually been implicitly assumed that the cooperative interaction with any kind of substrate occurs independent of the interactions with others and that all substrates are equally effective in displacing the equilibrium between the different conformational states of the enzyme protein. The failure of any substrate to exhibit cooperative interactions, if observed, has been explained by further assuming that it binds to different conformational states with the same affinity[1]. The kinetic models given in the present paper, however, reveal that an alternative possibility should be also taken into consideration in the case of ping-pong mechanisms in which not all of the substrates exist simultaneously bound to the enzyme protein. The enzyme with ping-pong mechanism oscillates between mutiple stable forms each of which selectively binds a given substrate (or substrates). Allosteric properties are then explained by assuming that one (or more) of these stable enzyme forms exist in different conformational states. The present study has clarified the relation between the allosteric mechanism and the ping-pong one; the equilibrium between the different conformational states of a given stable enzyme form is displaced only by the ligands which directly bind to it. In the case of the present models of Type I and Type II, the interconversion between the stable enzyme forms during the ping-pong type reaction could possibly play a significant role in determining the allosteric property of the enzyme; it looks then as if the conversion from one to another stable form would involve the "desensitization" or "resensitization" of allosteric protein. In this respect, the specific type of allosteric transition such as Type I and Type II could be termed a "half reaction induced-allosteric transition" or "enzyme form-specific allosteric transition".

This striking feature of the allosteric enzymes with ping-pong mechanism appears not to be an artifact arising from the "partial equilibrium" assumption that each stable enzyme form belongs to its own "rapid equilibrium segment" separated from others through the slow reactions. This view is based on the tentative calculation conducted without "partial equilibrium" assumption as follows. Since the straightforward combination of allosteric transition with ping-pong mechanism by means of the full "steady-state" assumption instead of the "partial equilibrium" assumption leads to equations so complex that the kinetic properties may be obscured, the allosteric transition in ping-pong mechanism was simulated by assuming that, in Diagram I, one of the stable enzyme forms, E, isomerizes to E_a. Then, the concentration ratio of E to E_a was calculated under the assumption of full "steady-state" and found to be entirely independent from the concentration of B which does not directly bind to E. Since it is conceivable that the isomerization of E to E_a postulated here mimicks the allosteric transition of E, it may be concluded that the failure of any ligand to stabilize the stable enzyme form to which it does not bind directly is the intrinsic nature of the allosteric transition involved in the ping-pong mechanism regardless of the assumption utilized for deriving the rate equations.

It should be pointed out here that the rate equations given in the present paper have been derived under the assumption of "partial equilibrium" first introduced by Cha[8]. It is evident now that Cha's concept is very valuable in deriving rate equations for such a complicated kinetic situation as encountered in analyzing the allosteric properties of multiple-substrate enzymes. The resultant equations are so simple as to make it promising to determine the rate constants and dissociation constants conforming to the experimentally obtained kinetic patterns[10] by computer simulation studies, which are currently in progress in our laboratory according to the previously proposed procedure by one of the present authors[11].

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