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Apparent Enzyme Cooperativity Caused By Inactivation

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비활성화가 효소의 조절에 미치는 영향에 대한 이론적 고찰

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Summary

Ornithine transcarbamylase is the enzyme which catalyzes the condensation reaction of L-ornithine and carbamyl phosphate to produce L-citrulline. The enzyme is now known to be an allosteric enzyme in the presence of a metal ion, zinc. The metal affects the enzyme mechanism as an allosteric efffector or an irreversible inhibitor. The combination of both roles produces the time-dependent apparent enzyme cooperativity. The computer simulation has been applied to understand how metal-induced, time-dependent inactivation of enzyme exerts positive cooperativity. With the equation derived from the MWC model, the results show that over the incubation time of enzyme and metal ion n_H^{app}, the Hill constant, and $S_{0.5}$ increases. These changes are all observed experimentally. Therefore the reaction scheme presented in this paper is a model to explain the time-dependent enzyme cooperativity without the hysteresis.

Introduction

Anabolic ornithine transcarbamylase (EC 2.1.3.3) is the enzyme that catalyzes the first step of the urea cycle, the synthesis of L-citrulline from the condensation of L-citrulline and carbamyl phosphate. Because L-arginine is the penultimate product of the urea cycle, transcarbamoylation of L-ornithine in mammals is also a step in the pathway of arginine biosynthesis. The urea cycle is the major pathway for the removal of excess nitrogen. For eukaryotes, half of the reactions in the cycle is located in the cytoplasm while the other half in the mitochondrial matrix.

In general, ornithine transcarbamylase is not known to be the enzyme regulating the flux of

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metabolites in the urea cycle. Even though *E. coli* ornithine transcarbamylase is consisted of three identical subunits, the enzyme strictly follows the Michaelis-Menten kinetics for both substrates, L-ornithine and carbamyl phosphate. However, it has been reported that the metal ion, zinc, induces nonlinear sigmoidal kinetics in the ornithine saturation of *E. coli* enzyme (Lee *et al.*, 1990). In the presence of zinc ion, binding of L-ornithine is modulated in the cooperative manner. The effects of zinc ion on the enzyme have two different facets. One is the induction of an allosteric binding of L-ornithine to the carbamyl phosphate bound enzyme-zinc ternary complex, and the other is the time-dependent reduction of the enzyme activity in the enzyme-zinc binary complex. In the latter effect of zinc on the enzyme, time-dependent increase of Hill constant has been observed in ornithine saturation curve.

Sigmoidal velocity curves are usually taken as evidence of positive cooperativity. Cooperativity occurs when more than one kinetic pathway exists for an enzyme reaction as a result of subunit interactions or of non-equilibrium processes (Richard and Cornish-Bowden, 1987). Metal ions have known to be the source of artificial sigmoidicity (Morrison, 1979 : London and Steck, 1969). The concern, now, is about how metal-induced enzyme inactivation creates the sigmoidal substrate saturation curve. The intent of the analysis presented here is to determine whether metal ion induced, time-dependent inactivation contributes towards the apperance of the non-hyperbolic saturation profiles observed for *E. coli* ornithine transcarbamylase in the presence of zinc ion.

Computer simulation has been employed to answer this question. A general model was constructed for cooperative ligand interactions with enzymic subunits in the presence of metal-induced inactivation. Saturation curves were calculated for widely varied model parameters. The curves were fit to the Hill equation, and the apparent Hill constant was used as the index of sigmoidicity.

Materials and Methods

1. Reaction Scheme

$$E+Zn \underset{-k_b}{\overset{k_b}{\rightleftharpoons}} E' \cdot Zn \xrightarrow{k_{isc}} K \cdot Zn$$

Scheme I

Scheme I is a mechanism for the modification of an enzyme by a metal ion, zinc (Lee *et al.*, 1990). Zinc binds the enzyme reversibly at the first stage, then the metal bound enzyme isomerizes to be inactivated at the second stage, which is irreversible. Ornithine transcarbamylase is a trimer with one L-ornithine site per subunit. If each subunit can be inactivated only when zinc is bound to it, then inactivation, the second step in the Scheme I, of the trimer proceeds in three sequential steps as depicted in Scheme II.

$$E_3 \xrightarrow{k_1} E_2E' \xrightarrow{k_2} EE'_2 \xrightarrow{k_3} E_3$$

Scheme II

In this Scheme, $E_nE'_{n-j}$ is the enzyme with n subunits in the active conformation, E, and n-j subunits in the inactive conformation, E'. Each k_j is a rate constant and is first-order with time as long as association and dissociation of zinc and the enzyme are fast relative to the conformational change of E to E'. The magnitude of k_j depends on zinc concentration. For example, k_2 is propotional to the fractional saturation with zinc of the two subunits of E_2E' in the E conformation. Fractional saturation with zinc in turn depends on ornithine concentration

because zinc and ornithine compete for the same site. In the event that k_1 is much smaller than k_2 and k_3, transformation of E_3 to E'_3 becomes a concerted process.

Scheme II places no restriction on the expression relating zinc concentration to zinc saturation. Moreover, E_3, E_2E' and EE'_2 can each have different saturation curves. This condition permits in our model differential changes in zinc and ornithine affinity between trimers of different compositions and permits cooperativity of zinc binding. Accordingly, zinc binding, the first step in Scheme I, can be noncooperative, positively cooperative, or negatively cooperative. E'_3 will be omitted from further consideration because it has no bearing on the outcome of the analysis.

2. Cooperative Ligand Binding

To incorporate cooperative binding of zinc into our model, each type of trimer (E_3, E_2E' and EE'_2) is assumed to have two states, R and T, conforming to the MWC model of positive cooperativity. Other restrictions placed on the model are : (a) zinc and ornithine compete on each subunit of a trimer, and (b) binding of carbamyl phosphate is independent of zinc and ornithine binding. The expression for the fractional saturation of an n-subunit oligomer by one ligand, A, in the presence of a competitive ligand, B, is given by Equation 1 (Kuo, 1983) :

$$Y_A = \frac{\alpha(1+\alpha+\beta)^{n-1} + Lc\alpha(1+c\alpha+d\beta)^{n-1}}{(1+\alpha+\beta)^n + L(1+c\alpha+d\beta)^n}$$

where

$$\alpha = \frac{[A]}{K_{RA}}, \quad \beta = \frac{[B]}{K_{RB}}, \quad c = \frac{K_{RA}}{K_{RB}}$$

$$d = \frac{K_{RB}}{K_{TB}}, \quad \text{and } L = \frac{[T_0]}{[R_0]}$$

In this equation, Y_A is fractional saturation with A, $[R_0]$ and $[T_0]$ are the concentration of the free enzyme in the R and T states, L is the intrinsic allosteric equilibrium constant in the absence of ligand, K_{RA} and K_{TA} are the dissociation constants from the R and T states, respectively, and K_{RB} are the analogous dissociation constants for B. For ornithine saturation, A=ornithine and B=zinc : for zinc saturation, A=zinc and B=ornithine.

The numerator and denominator of Equation 1 each has two principal terms. The first term in the denominator represents number of sites for A in the R states, and the first term in the numerator represents the number of these sites occupied by A. The second term in the numerator and denominator are the corresponding values for The T state. If a process is proportional to the number of sites filled by A but does not discriminate between the R and T states, then the rate of the process will be equal to the numerator of equation 1 multiplied by a single rate constant. If the process occurs at different rates for R and T states, however, then the two terms in the numerator will be multiplied by different rate constants as shown in Equation 2 :

$$\text{Rate}_A = \frac{\alpha(1+\alpha+\beta)^{n-1}k_R + Lc\alpha(1+c\alpha+d\beta)^{n-1}k_T}{(1+\alpha+\beta)^n + L(1+c\alpha+d\beta)^n}$$

where Rate_A is the rate of the process as a function of saturation with A, and k_R and k_T are the rate constants for the R and T states, respectively. If the process is catalysis, then A is ornithine, Rate_A is the rate of citruline synthesis, and k_R and k_T are the catalytic rate constants for the two states. If the process is inactivation, then A is zinc, and Rate_A is the rate of activity or fluorescence loss, and k_R and k_T are first-order rate constants for the change of conformation E to conformation E'.

The MWC model is normally applied only to oligomeric proteins composed of identical

subunits with the assumption that all binding sites in each states are equivalent (the symmetry requirment). In Scheme Ⅱ, E_2E' and EE'_2 have two kinds of subunits, E and E', which may or may not have identical ligand binding properties. The realistic assumption is that there are changes in both catalysis and binding. However, since zinc does not dissociate from E' and ornithine cannot bind ternary comlex of E · cp · Zn without the metal first departing, one may legitimately ignore the E' subunit and treat E_3 as a trimer, E_2 E' as a dimer, and EE'_2 as a monomer. One can simply substitute new values of k_R and k_T into Equation 2 for E_2E' and EE'_2. For example, if there are no inactivations between E and E', then the rate constants for E_2E' and EE'_2 are simply two-third and one-third of the value for E_3, respectively. The number n in Equation 2 varies from 1 to 3 depending on the enzyme forms. Interactions between E and E' should lead to lower rate constants for E_2E' and EE'_2. In the treatment presented below, no communications between E and E' are assumed. The likely consequences of interactions between the two types of subunit will be addressed in Fig. 1.

3. Enzyme Activity As Function Of Incubation Time

Each form of the enzyme (E_3, E_2E', and EE'_2) has one equation relating it to its rate of catalysis and another relating to its rate of inactivation. All six equations have the form of Equation 2. The fraction of enzyme in each form after an incubation time, predicted by Scheme Ⅱ is :

Equation 3 $[E_3]=e^{-k_1t}$

Equation 4 $[E_2E']= k_1\left[\frac{e^{-k_1t}}{(k_2-k_1)} + \frac{e^{-k_2t}}{(k_1-k_2)}\right]$

Equation 5 $[EE'_2] = k_1k_2\left[\frac{e^{-k_1t}}{(k_2-k_1)(k_3-k_1)}\right]+$

$$\left[\frac{e^{-k_2t}}{(k_1-k_2)(k_3-k_2)} + \frac{e^{-k_3t}}{(k_1-k_3)(k_2-k_3)}\right]$$

If the specific activities of E_3, E_2E', and EE'_2 are represented by a_{E_3}, $a_{E_2E'}$, and $a_{EE'_2}$, then the sum of activity of all enzyme forms is given by Equation 6 and the fractional activity remaining at thime t is given by Equation 7 :

Equation 6

$$A_{total}(t) = a_{E_3}[E_3]+a_{E_2E'}[E_2E']+a_{EE'_2}[EE'_2]$$

Equation 7

$$A_{total}(t) = a_{E_3}e^{-k_1t}+a_{E_2E'}k_1\left[\frac{e^{-k_1t}}{(k_2-k)_1}+\frac{e^{-k_2t}}{(k_1-k_2)}\right]+$$

$$a_{EE'_2}k_1k_2\left[\frac{e^{-k_2t}}{(k_2-k_1)(k_3-k_1)}+\frac{e^{-k_3t}}{(k_1-k_2)(k_3-k_2)}\right.$$

$$\left.+\frac{e^{-k_1t}}{(k_1-k_3)(k_2-k_3)}\right]$$

The value of k_1, k_2, k_3, a_{E_3}, $a_{E_2E'}$, and $a_{EE'_2}$ in Equation 3-7 are each calculated from an equation having the form of Equation 1.

4. Computer Simulation

The model in its most general form is given by Equation 7. The inputs to the equation are the incubation time, t, and a list of paired concentrations of ornithine and zinc. The addition of carbamyl phosphate marks the end of enzyme incubation with zinc because this substrate stops enzyme inactivation. The substrate form of ornithine is taken to be the zwitterionic species whose δ-amino moiety is deprotonated. The outputs of Equation 7 are velocity saturation data. These data are then fit to the Hill equation to give an apparent Hill constant, n_H^{app}, and a half saturating ligand concentration, $S_{0.5}$ or $I_{0.5}$

Results and Discussions

The effective concentration of inhibitory zinc

decreases as ornithine concentration increases due to metal chelation by the amino acid. At a fixed level of zinc, the rate of the inactivation is greater at low than at high concentration of ornithine. In kinetic assays of ornithine saturation, enzyme is added to a series of test tubes containing buffer, a fixed quantity of zinc, but varied amount of ornithine. Thus enzyme in different test tubes is preincubated with different concentration of inhibitory zinc and inactivated to different degrees. Fig. 1 and Fig. 2 illusterate with use of Equation 7 how this phenomenon produces a time-dependent Hill constant in kinetic experiments. The same analysis also explains the time-dependent phenomenon in the zinc saturation curve.

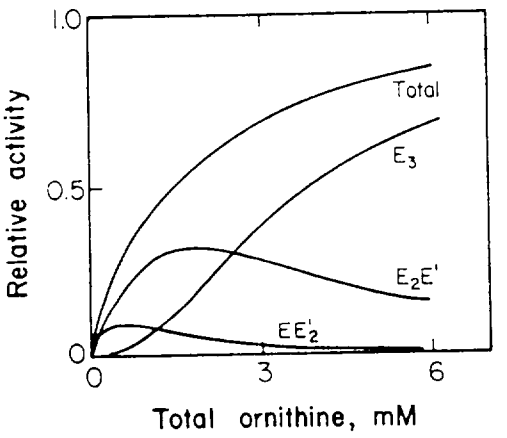

Fig. 1. Changes of n_H^{app} resulting from differential inactivation of enzyme subunits. Results are shown for simulated ornithine saturation curves obtained for enzyme species, composed of the active E and the inactivated E′ subunits, preincubated for an arbitary period of time with ornithine and zinc (0.3mM). The parameters used are : for ornithine, k_d=1 mM during preincubation and 0.3 mM during assay; for zinc, k_d=0.1 mM for the T-state enzyme and 10 mM for the R-state enzyme during preincubation and $k_d=\infty$ for both states during assay. The ratio of $[T_0/R_0]$ is set at 100. If this ratio is lowered from 100 to 10, the n_H^{app} of the E_2E' curve increases to 3.2 while that of the 'total' enzyme curve drops to 0.6. When t is O, the E_3 curve becomes the 'total' enzyme curve and n_H^{app} is unity.

Fig. 1. shows an ornithine saturation curve generated with arbitary parameters to give a n_H= 1 prior to inactivation (t=0). with the same parameters, the saturation curves of each enzyme species (E_3, E_2E', and EE'_2) at the end of an arbitary inactivation period (t=0) are also illusterated. Since the distribution of the different enzyme species is governed by the concentration of inhibitory zinc which in turn is governed by ornithine concentration, the activity of each enzyme species reflects their concentration as a function of ornithine concentrations. In this Figure, It is assumed that there are no interactions between E and E′, so the activities of the E_2E', and EE'_2 species are simply two-thirds and one-third that of E_3. It can be seen that at high concentration of ornithine, most of the enzyme is inactivated. The activity of the fully active species is sigmoidal against ornithine concentration with n_H of 2.2, but the sum of the activities of all enzyme species for t>O is not sigmoidal and has an n_H of 0.8. It follows then without contribution of the partially active intermediate (E_2E', and EE'_2), the n_H^{app} is significantly greater than the true n_H. Since the experimentally observed n_H^{app} always increases with incubation time, either E_3 is the only active species or E_2E', and EE'_2 have much less than two-thirds and one-third of the activity of E_3. Both possibilities require that there are interactions between the E and E′ subunits. The active site in ornithine transcarbamylase is in the interface between adjacent subunits, so it is reasonable to speculate that the concentration of the E′ subunit affects the binding and catalytic

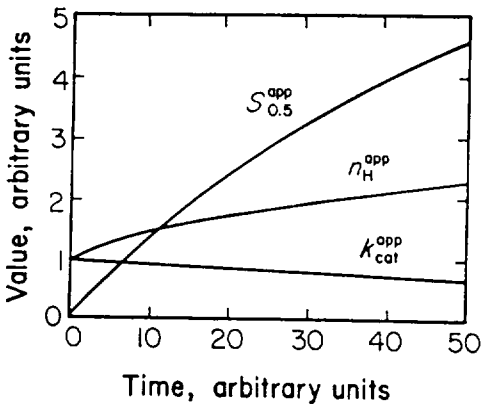

Fig. 2. Effect of inactivation on the steady-state kinetic parameters. Results are shown for simulated ornithine saturation curves obtained for enzyme preincubated with ornithine and a fixed quantity of zinc followed by initiation of reaction with addition of carbamyl phosphate. The K_M used for ornnithine is 0.3 mM, the concentration of zinc is arbitary, and the n_H for the metal is set at unity.

effeciency of the E subunit.

Fig. 2. shows the time-dependence of k_{cat}, $S_{0.5}$, and n_H^{app} resulting from enzyme inactivation as depicted by Scheme I (i.e., only E_s is active). The Hill constant at time zero is chosen to be 1.0. It is seen that over time k_{cat} decreases slightly, n_H^{app} increases moderately, and $S_{0.5}$ increases drastically. These changes are all observed experimentally (Lee et al., 1990).

Conclusion

Metal-induced, time-dependent enzyme inactivation creates not only the sigmoidicity of substrate saturation curve but also the time-dependence of enzyme kinetic parameters. On the other hand, in the absence of enzyme inactivation, the Hill coefficient observed in substrate saturation curve is a valid measure of cooperativity. The result underscores the significance of zinc regulation on ornithine transcarbamylase.

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〈국문초록〉

비활성화가 효소의 조절에 미치는 영향에 대한 이론적 고찰

오니틴 트랜스카바밀라제는 오니틴과 카바밀인산의 축합반응으로 생성되는 시트룰린의 합성을 촉진시키는 효소이다. 이효소는 금속아연이온에 의하여 효소활성이 조절된다는 사실이 알려져 있다. 즉 금속 아연이온은 조절리간드와 비가역성 저해제로서 작용을할 하며 이 두가지 역활이 병합되어 반응시간에 영향을 받는 겉보기 효소 조절가능을 한다. 본 논문에서는 이와같은 현상이 실험적으로 관찰되는 것에 대하여 이론적 모델을 제시하기 위한 것이며, 제시한 MWC 모델에 맞는 속도반응식을 유도한 후 컴퓨터를 이용한 모의 실험을 하였다. 그 결과 실제 실험에서의 결과와 잘 일치되었다. 즉 효소와 금속아연이온의 반응시간에 따라 Hill constant, $S_{0.5}$ 등 반응에 관여되는 상수들이 변화하였다.