Conformational Effect (Induced-Fit) on Catalytic Activity of α-Chymotrypsin

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The kinetics for the hydrolyses of *p*-nitrophenyl esters of acetic acid and certain amino acid derivatives mediated by α-chymotrypsin have been studied. The kinetics are a function of the medium viscosity, which indicate that the enzyme must change its conformation during the reaction. Detailed analysis of the dependence of kinetic rate constants on the medium viscosity has revealed that the induced-fit conformational adjustment of enzyme plays a crucial role in its catalytic activity.

Keywords : Induced-Fit Theory / α-Chymotrypsin / Medium Viscosity / Catalytic Activity

According to the induced-fit theory [1], conformation of an enzyme changes depending on the structure of a substrate when it forms an enzyme-substrate (ES) complex with the substrate so that the catalytic functional groups in the active site of the enzyme are arranged according to their most appropriate positions for the chemical reaction. Although the above idea clearly explains the extraordinary rate enhancements by enzyme catalysis, there has been no report on the participation of the conformational change in the enzymatic reactions. If the conformational change is associated with the reaction of a chymotrypsin, a series of reaction kinetics as a function of medium viscosity will surely contribute to endorse the proposed process. Thus, we studied the kinetics of a series of reactions of α-chymotrypsin with *p*-nitrophenyl acetate and certain amino acid derivatives as substrate [2]. The simplified reaction scheme is illustrated in Scheme 1, which will be revised as the discussion proceeds.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\longrightarrow} \text{Acyl-E} \overset{k_3}{\longrightarrow} E + P_2$$

S = **1**: Ac-*p*NP, **2**: Z-Ala-*p*NP, **3**: Ac-Gly-*p*NP

Scheme 1

The viscosity of the medium was changed by adding appropriate amount of glycerol to buffer solutions of α-chymotrypsin as a viscogen. Figure 1 illustrates the dependence of k_{cat}, k_2 and k_3, respectively, on medium viscosity. The rate constant k_2 decreases as the medium viscosity increases in all the substrates studied, which reveals that a chemical reaction that takes place between a substrate and the enzyme must be associated with the change in the conformation of the enzyme in order to accommodate an acyl group in its pocket appropriately to form the acyl enzyme.

An acyl enzyme with an unfavorable conformation,

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Figure 1. Dependence of kinetic parameters on medium viscosity: **1**, **2**, **3**: (a) k_{cat}, (b) k_2, (c) k_3.

i.e., a conformation that was not adopted to accommodate the acyl group, tends to eject the acyl group from its pocket so as to stabilize itself as the free enzyme. Thus, the decrease in k_2 is coupled by an increase in k_3. However, the increase in k_3 with the increase in medium viscosity is not monotonic. Instead, it begins to decrease at about $\eta \approx 1.6$ mPa s. This is accounted for by the presence of an additional process in this step, which becomes unfavorable as the medium becomes more viscous. We assign this additional process to the product releasing step with a rate constant of k_4. This assignment proposes that the conformation suitable for the acyl enzyme is also suitable for releasing P_2, the acid part of the substrate. In other words, the release of free acid from the enzyme of undistorted conformation is not a simultaneous process. The release of P_2 requires energy corresponding to the release of distortion energy that exists in the conformationally distorted enzyme, and Scheme 1 is now revised to Scheme 2 by taking into account the induced-fit conformational adjustment for the release of P_2.
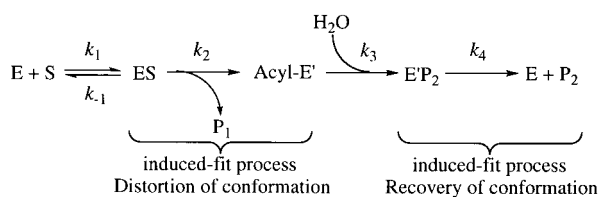
Scheme 2

The shapes of the curves for k_{cat} shown in Fig. 1(a) are similar to those for k_3/k_4 shown in Fig. 1(c), which stems from the fact that this step is more sensitive process to the effect of induced-fit conformational adjustment than the k_2 step and the chemical (catalytic) reactivity of the enzyme is regulated by this process. It has been known that the rate-determining step in the enzymatic hydrolysis of an ester is the step in which the acyl enzyme is hydrolyzed into a free enzyme and an acid. Absolute values for k_2 and k_3, as well as substrate

independence of the k_2 step shown in Fig. 1(b), also support the idea that the hydrolysis of acyl enzyme is (at least in part) the rate-determining step of the present reaction under normal conditions. However, interestingly, the rate-determining step is shifted to the k_2 step in the reactions occurring in highly viscous media for certain substrates. An exception is the reaction with **1**, where the rate-determining step is always in the k_3/k_4 step even in the most viscous medium studied.

The observation that the maximum position in the plot of k_3/k_4 step for **1**, if any, shifts to higher viscosity than the other amino acid substrates indicates that the reaction of this substrate is less sensitive to induced-fit conformational adjustment than the reactions of other substrates. The result obtained herein has good agreement with that obtained by proton inventory kinetics [3], where **1** was found to be associated by the movement of one proton whereas other amino acid substrates were associated with the movement of two protons at their respective transition states. In other words, **1** is not an appropriate substrate for studying the catalytic activity of α-chymotrypsin. Although, conventionally, **1** has been employed as a typical substrate in the study of catalytic activity of α-chymotrypsin, one should be very careful in extending the results obtained with **1** as a substrate to the discussion of the general mechanism of α-chymotrypsin hydrolysis.

Consequently, we now believe that both acylation/deacylation and release of products P_1/P_2 are dependent on the induced-fit conformational adjustment in the hydrolysis of an ester by α-chymotrypsin. More results should be accumulated using substrates of various types before we reach a conclusion.

References
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