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Why substituting the asparagine at position 35 in *Bacillus circulans* xylanase with an aspartic acid remarkably improves the enzymatic catalytic activity? A quantum chemistry-based calculation study

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ABSTRACT

Xylanases from *Bacillus circulans* (BCX) are known as configuration-retaining glycoside hydrolases, which hydrolyze xylans with two glutamic acid residues (Glu78 and Glu172) serving as catalytic active residues according to a double displacement mechanism. Existing experimental researches show that mutating the asparagines (Asn) to aspartic acid (Asp) at position 35 next to Glu172 can obviously improve the catalytic activity of BCX. To better understand the inherent mechanism for the experimental finding, we performed quantum chemistry calculations on two model systems to mimic the catalyses of wild-type and mutant BCXs. Geometrical structures and relative energies of intermediates and transition states involved in the hydrolysis reactions are given in detail. It is found that in the wild-type model system Asn35 interacts with Glu172 via a loose hydrogen bond, while in the mutant model system Asp35 forms a very tight hydrogen bond with Glu172. The glycosidic bond cleavage is proposed to be the rate-determining step for the hydrolysis reaction, whose barrier varies from 98 to 65 kJ mol^{-1} when Asn35 is replaced by Asp35, showing the presence of Asp35 remarkably reduces the energy demand for the hydrolysis reaction. The present result provides a theoretical elucidation for why a single amino acid substitution can importantly influences catalytic activity of BCX.

1. Introduction

The hydrolysis of glycosidic bonds catalysed by glycosidases can be enhanced by up to 10^{17} fold compared with the uncatalysed reactions [1,2]. In recent years, the hydrolysis mechanism of glycosidic bonds has been studied in detail [3], showing that the glycosidases can be generally classified as inverting and retaining hydrolases based on whether the initial configuration of anomeric carbon is reversed or not after hydrolysis.

Enzymes of endo-β-(1,4)-glycosidase family 11 (or GH 11) called xylanases discovered from both prokaryotic and eukaryotic species are known to be retaining-type hydrolases which share sequence identity ranging from 40 to 90% [4,5]. Therefore, enzymes of this family have similar active site structures. Studies show that two glutamic acid residues which are approximately 5.5 Å apart [6,7] in the catalytic tunnel play essentially important roles in the hydrolysis reaction [5,8,9]. During the reaction proceeding through an imaginary double displacement mechanism [10], one glutamic acid residue acts as a nucleophile and another as a general acid/base catalyst [11–13], and the β-conformation of the anomeric carbon in xylans is maintained after a catalytic cycle.

Despite the similarities among enzymes from GH 11, their pH optima vary greatly from 2 to 11. According to the comparison of the sequences of endo-β-(1,4)-glycosidases conducted by Torronen and Rouvinen in 1997, a residue which is adjacent to the general acid/base catalyst and forms hydrogen bond with it, plays a key role in determining the pH optima: the asparagine (Asn) at this position leads to alkaline pH optimum, while the aspartic acid (Asp) results in the acidic pH optimum [5]. In particular, in 2000, Joshi et al. reported that in *Bacillus circulans* xylanase (BCX) where Glu172 and Glu78 act as the general acid/base catalyst and nucleophile respectively, after the point mutation that changes the residue at position 35 from Asn to Asp, which is hydrogen bonded to Glu172, not only is there a shift of the pH optimum from 5.7 to 4.6, but also the activity of BCX is increased by approximately 20% [11]. This result clearly indicates that the property of the residue at position 35 has an inherent influence on the catalytic activity. However, our knowledge about the detailed mechanism is far from complete.

As is well known, enzyme-catalysed reactions are so fast that the received information from experimental studies regarding

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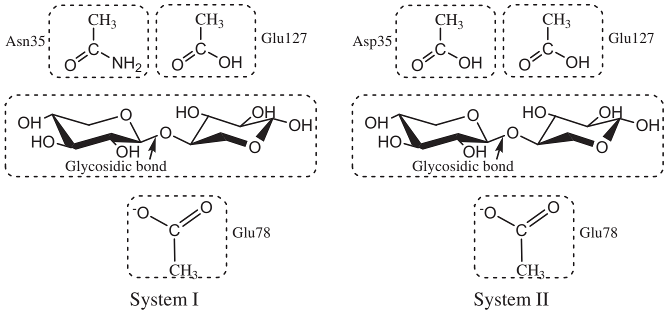


Scheme 1. Schematic diagrams of two model systems used in the presented calculations.

enzyme functioning mechanism is limited and indirect. As an alternative, theoretical studies based on quantum chemistry calculations provide one of the most direct ways to reveal mechanism details at the atomic scale. In order to understand how the point mutation of Asn35 influences the reactivity, here we present a quantum chemistry-based calculation study. Our main intent is to provide theoretical evidence for the experimental finding.

2. Model and calculation details

The crystallographic structure of endo-1,4-β-xylanase was obtained from Protein Data Bank (http://www.pdb.org). The PDB number is 1C5I. Since accurate quantum chemistry calculation is available only for small systems with no more than tens of molecules, we need to use model systems to mimic the catalysed reactions. Here, a xylobiose is considered as the substrate, two acetic acids are used to represent the protonated Glu172 and deprotonated Glu78, which are the two catalytic active residues for BCX [6–9], and another either acetamide or acetic acid functions as the Asn35 or Asp35. The initial relative positions of these species are set according to those in the crystallographic structure. The two model systems are shown in Scheme 1.

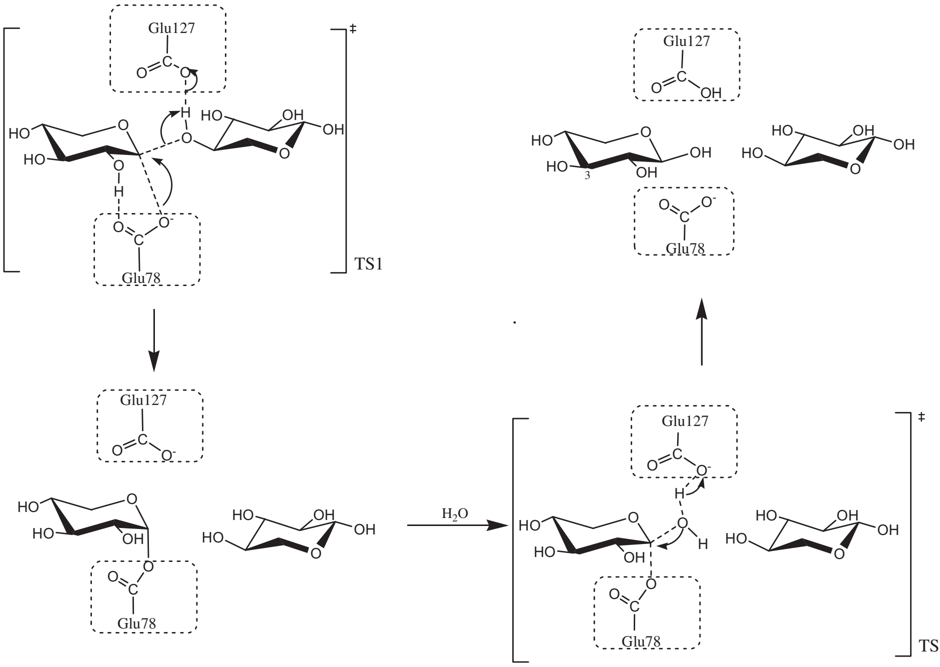
The calculations were performed using the Gaussian 03 program package [15]. In a recent review [16], the hybrid meta BB1K functional [17] was pointed out to be the best functional to accurately calculate activation barriers. In particular, this functional is shown to be appropriate for studying the heavy atom transfer, nucleophilic substitution, association, and unimolecular reactions [18]. So our calculations used the BB1K functional combined with the standard 6-31G(d,p) basis set. Full geometry optimizations of minima and transition states were performed at the selected level of theory. Frequency calculations at the same level of theory have also been carried out to verify all stationary points as minima (zero imaginary frequencies) or first-order saddle points (one imaginary frequency). To identify the minimum-energy paths, the intrinsic reaction coordinate (IRC) [19,20] calculations were conducted in both directions (forward and reverse) from the transition states to the corresponding local minima.

3. Results and discussion

We performed calculations on two model systems (Scheme 1), which adequately capture the key properties concerned here. For simplification, these two systems are denoted as systems I and II, using which we mimic the catalyses of wild-type and mutant BCXs, respectively.

3.1. General description of the catalytic mechanism

Known as retaining enzymes, BCX catalyses the substrate hydrolysis through the double displacement mechanism [10], which leads to the configuration retention of the anomeric carbon in the product [4,5]. According to the general double displacement mechanism [10], the catalysis of xylanases involves two elementary steps [9]: (i) the glycosidic bond is broken under the attack of Glu172 and Glu78, resulting in the inversion of the anomeric carbon configuration and the formation of covalent glycosyl-enzyme intermediate, and (ii) a water molecule attacks the anomeric



Scheme 2. Schematic illustration of the glycosidic-bond hydrolysis mechanism on the active site of *Bacillus circulans* xylanase according to the double displacement mechanism proposed by Koshland [10].