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Quantum chemical study on the deacylation step of human chymase

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Abstract

Human chymase (HC) is a family of serine proteases and changes angiotensin I (AngI) for angiotensin II (AngII). This production of AngII by HC is the bypass pathway of renin–angiotensin system and has higher production ability than that. In this study, the deacylation mechanism by human chymase was revealed through quantum chemical calculations. The model reaction system was solved using ab initio MO method with Hartree-Fock level. It was found that HC had two elementary reactions via tetrahedral intermediate and proton relay did not occur through this deacylation step. Further we compared the structural differences between HC and acetylcholinesterase, which suggested that the surroundings of catalytic triad decided whether proton relay occurs or not in an enzyme.

Keywords: Human chymase; Mechanism of human chymase; Deacylation mechanism; Serine protease

1. Introduction

Hypertension is one of the striking factors inducing various lifestyle diseases (arteriosclerosis, brain infarction, cerebral apoplexy, aortic aneurysm, etc.). Hypertension is thought to be a result from the increase of circulating blood volume and/or the constriction of blood vessels. A treatment of hypertension is to decrease the circulating volume and/or to slack the blood vessels. Angiotensin II (AngII) is one of the factors which constrict blood vessels. Control the concentration of AngII is regarded as an effective treatment for hypertension, because AngII is a vasopressor principle. Renin–angiotensin system (RAS) was known for the main production pathway of AngII (Fig. 1). Recently, it is reported that the production of AngII in local organs is not only due to the pathway by RAS but also to the bypass pathway by serine proteases (Fig. 1) [1]. Human chymase (HC) is a family of serine proteases [2,3]. HC has higher ability for production of AngII than RAS [1,4,5]. It is considered that AngII produced by HC contributes to the remodeling of blood vessel rather than the regulation of blood circulation. However, the details of HC is still unclear [6–8]. Clarifying the mechanism of HC is important, because the development of specific inhibitor for chymase becomes popular [9–12] and the development of effective medicine is expected [5]. HC breaks a peptide bond between Phe8 and His9 which locates at the second position from C terminal of AngI and exchanges AngI for AngII [13,14]. This mechanism is a hydrolysis and consists of a sequence of acylation and deacylation steps. Active center of HC is a catalytic triad which composed of His57, Asp102 and Ser195. It is well known that the configuration of this catalytic triad resembles same kinds of serine protease such as trypsin [15], etc. Therefore, the catalytic mechanism of HC is assumed to be similar to serine protease's.

There are many reports about the mechanism of peptide hydrolysis by serine protease using experimental and theoretical method [16–21]. In 1969, Blow and co-workers proposed that 'Charge-relay theory' [16] to explain the activity of Ser residue in catalytic triad during catalytic reaction of chymotrypsin. But Kossiakoff and co-workers indicated that the proton relay did not occur, on a basis of experimental data obtained by neutron diffraction of trypsin with an inhibitor, MonoIsopropylPhosphonyl (MIP) [19,20]. In this structure, His residue in catalytic triad has two protons and becomes a positively charged. Cleland, Frey and others proposed 'Low-Barrier Hydrogen Bond

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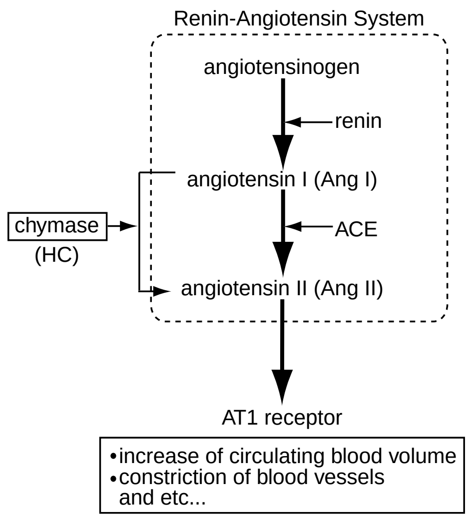

Fig. 1. Flowchart of renin–angiotensin system (RAS). RAS is known as the main production pathway of angiotensin I (AngI) from angiotensinogen. Human chymase (chymase) also changes AngI for angiotensin II (AngII) in local organs, which is a bypass reaction of AngI→AngII conversion. When AngII is bound to AT1 receptor, several very important functions are induced.

(LBHB) theory' which means that hydrogen atom stays between His and Asp (Glu) (His···H···Asp(Glu)) when pK_a value between proton donor and proton acceptor are same [21]. Accordingly, it seems to be plausible that proton relay does not occur in hydrolysis reaction by serine proteases. But the definite conclusion has not been submitted yet. In this study, the reaction process of HC has been examined by performing quantum chemical calculations, and we discuss on the proton relay in the catalysis of serine proteases.

2. Methods

To construct a model system, Ser-acylenzyme intermediate was extracted from the X-ray crystallographic structure that is a complex of HC and substrate inhibitor, succinyl-Ala–Ala–Pro–Phe–chloromethylketone (CMK) (pdbcode: 1PJP) [22]. To construct substrate–enzyme (ES) complex, CMK was replaced with peptide chain of Ile–His–Pro–Phe. This ES complex was placed in rectangular parallelepiped box (63 Å×65 Å×61 Å). About 4105 water molecules were generated around HC and energy minimization was executed using molecular mechanics (MM) method. A cut off distance (13.0 Å) was applied for the computation of the van der Waals forces. Energy minimization was performed for a whole system with a periodic boundary condition. In the computation of energy minimization, the steepest descent method was used for the early cycles and the conjugated gradient method was used for the later. The program package used was Insight II/Discover 2000. Next, a model reaction system for quantum chemical (QC) calculations was constructed using the optimized structure from MM calculation. This model reaction system contains catalytic triad and its surrounding residues (Fig. 2). This structure was fully optimized using QC calculation. Because this structure was based on the X-ray crystallographic structure, we regarded this structure as a standard initial conformation for this study. Asterisked atoms in Fig. 2 were fixed through the calculation. Opened circles indicated that carbon atoms were replaced with hydrogen atoms. The model reaction system was solved using ab initio MO method with Hartree–Fock level. The minima and the transition states on the potential energy hypersurface were obtained by geometry optimization using the energy gradient method. Frequency analysis was executed for the optimized structure of the transition state (TS) to check the presence of only one imaginary frequency in the vibrational modes. The steepest descent paths from the TS were calculated for both forward and reverse directions of the vibrational mode corresponding to the imaginary frequency, and the minimum points on both sides of the TS were determined. The above procedure provided the lowest energy reaction path connecting a reactant and a product via TS. The intrinsic reaction coordinate (IRC) calculations were performed to obtain the steepest descent paths for both forward and reverse directions from TS [23]. To re-evaluate the value for potential energy differences, energy calculations were performed with density functional theory (DFT) method including the effect of electron correlation and the second-order Møller Plesset perturbation (MP2) calculation [24,25] in every stationary point. Becke's three parameter functional [26] incorporating the LYP correlation term [27,28] was used in DFT calculation. The basis set used was 6-31G** [29,30]. To consider the effects of the inside environment in the protein, we used SCRF (Onsager model) method [31–34] with the condition that dielectric constant of 20.0. This method was already shown to represent the protein environment well [35–38]. The program package used was GAUSSIAN 98 [39].

3. Results

3.1. First elementary reaction: formation of TI

First step of the deacylation is the formation of tetrahedral intermediate. The transition state of this step (TS1) was determined by approaching oxygen atom of water molecule to carbon atom of inhibitor, I5H. TS1 of this study is shown in Fig. 3. This TS1 structure has only one imaginary frequency vibrational mode (681.5875i cm^{-1}). The arrows in Fig. 3 (TS1) show the direction for the vibrational mode corresponding to an imaginary frequency, which indicates the pathway of this reaction. Decomposition of O–H_ε bond of water molecule (1.34 Å) and formations of covalent bonds between oxygen atom of water and carbon atom of I5H (2.60 Å) and between N_ε and H_ε (1.14 Å) simultaneously occurred.

Next, the steepest descent paths were calculated for the forward and reverse directions of the vibrational mode, and