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Local fluctuation control of papain by changing a highly fluctuating residue

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ABSTRACT

To control the local fluctuation of the amino acid residues of papain, ARG59, a highly fluctuating residue in papain, has been changed to GLY. We investigated the binding properties of 2-10GLY (peptides with between 2 and 10 glycine residues) to the modified papain structure via molecular dynamics and docking simulations. The change of the ARG59 residue to GLY alters the binding sites for some peptides, and changed its substrate specificity. Furthermore, the modification alters the binding stability of some peptides. Thus, control of the local fluctuations of residues in proteins has the potential to alter the protein's function.

1. Introduction

Fluctuation of the whole protein structure is the combination of the fluctuations of all of the residues in the protein. Fluctuation of the local protein structure can be controlled by modification of some residues in the protein. Local fluctuation control of protein structure would allow us to customize proteins to suit a wide variety of needs, because the fluctuations of proteins influence their functions [1–4]. Highly fluctuating residues in proteins are interesting for target modification. There are many highly fluctuating residues in proteins, and their modification has great potential.

The relationship between the residues in a protein and its function has been studied for a number of years, and the function of proteins can be improved by modifying the residues. However, the contributions of the local fluctuation control to the function of proteins is not well understood.

The purpose of this present study was to clarify the contributions of the local fluctuation control to the function of the protein to create new functional proteins that are suitable for a wide range of applications. The papain cysteine protease was chosen as a model protein. Papain is important in drug design [5–7], and is also used in a wide variety of fields, such as food processing and medical practice [8]. Furthermore, new functional devices could be fabricated by immobilizing papain on the surfaces of solids and controlling the activity of papain.

The functions of proteins have been analyzed by molecular dynamics (MD) simulations [9–12]. In this study, we analyzed the binding properties of some peptides to the time-resolved structures of modified papain using both MD and docking simulations. 2-10GLY (peptides consisting of 2-10 GLY residues) were chosen as the peptides, to estimate the contributions to the bindings of peptides of different chain length. ARG59 was chosen as the highly fluctuating residue on the basis of a significant correlation between the binding of 6-10GLY to sites near the active center of papain and the fluctuation of ARG59 [13]. ARG59 was changed to GLY to attempt to decrease the local fluctuations of papain.

We report the effect of local fluctuation control by changing ARG59 to GLY in papain on the activities of papain on the basis of the binding properties of 2-10GLY to fluctuating PAPAIN_ARG59GLY (papain modified ARG59 to GLY).

2. Methods

The MD simulations of PAPAIN_ARG59GLY were performed using the software package AMBER 9.0 [14]. The ff03ua force field [15] was used for the MD simulations. The structure of PAPAIN_ARG59GLY was constructed from the Protein Data Bank (PDB) data of papain (PDB code: 1bp4) using AMBER 9.0. The PAPAIN_ARG59GLY system was solvated with 10191 TIP3P water molecules in a cubic box [16]. Three-dimensional periodic boundary condition were adopted, and the pressure and temperature were kept constant using the Berendsen algorithm [17]. The long-range electrostatic interactions were calculated by the particle mesh Ewald (PME) method [18]. To reduce the computational effort, only bond lengths involving hydrogen atoms were constrained by the SHAKE method [19]. The integration time step of the MD simulations was set to 2 fs. The procedure for our simulations is as follows. First, the initial structure was optimized by potential energy minimization (3500 steps of the steepest descent method and 1500 steps of the conjugate gradient method). Next, MD simulations were performed for the minimized structure. The temperature of the system was gradually increased from 5 to 300 K in 52 ps and then maintained at 300 K for 4000 ps.

The structural dynamics of PAPAIN_ARG59GLY was analyzed using the ATOMICFLUCT (atomic positional fluctuations) in AMBER

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9.0. The ATOMICFLUCT is defined for each residue by the following equation:

$$\sum_{a=N_{res}}^{N_{res+1}-1} m_a\langle\Delta r_a^2\rangle \Big/ \sum_{a=N_{res}}^{N_{res+1}-1} m_a, \Delta r_a = r_a - \langle r_a\rangle, \quad (1)$$

where a is the atom number, res is the residue number, N_{res} is the number of the first atom in the residue res, m_a is the mass of each atom, and r_a is the coordinate of each atom.

The docking simulations between PAPAIN_ARG59GLY and the peptides were performed using the software package AutoDock 4.0 [20,21]. AutoDock 4.0 is a suite of programs that make it possible to predict how ligands bind to large macromolecules. The Lamarckian genetic algorithm (LGA) was used as the search engine. Step sizes of 2.0 Å for translation and 50 degree for rotation were chosen. For each of the 100 independent runs, 10000 LGA operations were generated on a single population of 150 individuals. The operator weights for crossover, mutation, and elitism were set to 0.80, 0.02, and 1, respectively. The Solis and Wets algorithm was the local search method used by the LGA, and the contraction and expansion factors were 0.5 and 2.0. The time-resolved structural data of PAPAIN_ARG59GLY were derived from our MD simulation results. The structural data of the peptides was determined using AMBER 9.0. We explored the binding of the peptides to the all of the surfaces of the time-resolved PAPAIN_ARG59GLY structures, as well as to regions surrounding the binding sites of the peptides on the surfaces.

3. Results

The MD simulations of the PAPAIN_ARG59GLY system were performed for 4 ns. The structural changes in PAPAIN_ARG59GLY were estimated by the root mean square deviation (RMSD) of the structure at each time step compared to the initial structure. The RMSD increased for the first 3000 ps and then converged.

The ATOMICFLUCTs (from 3001 to 4000 ps) of papain and PAPAIN_ARG59GLY are shown for each residue in Table S1 in Supplementary Material. The differences of the ATOMICFLUCTs between papain and PAPAIN_ARG59GLY are also shown for each residue in Table S1 in Supplementary Material. The differences of the ATOMICFLUCTs were large for RESIDUES_ATOMICFLUCT (ARG41, THR42, GLY43, ASN44, ARG58, ARG-GLY59, ARG93, GLU99, LYS100, VAL113, GLN118, TYR123, LYS139, TYR197, THR204, LYS211, and ASN212).

The average structure for each 100 ps between 3001 and 4000 ps, e.g., AVE_3001_3100_PS is the average structure from 3001 to 3100 ps, were calculated for PAPAIN_ARG59GLY. Docking simulations were then performed for 2-10GLY to all of the average structures (AVE100).

The binding free energies for each GLY peptide to each average structure are shown in Table S2, Table S3, and Table S4 in Supplementary Material. Figures 1 and 2 show top and side views of 2-8GLY on AVE_3001_3100_PS and AVE_3401_3500_PS. All of the 2-8GLY peptides bind to sites away from the active center for AVE_3001_3100_PS. 2-7GLY bind to sites away from active center and 8GLY binds to sites near the active center for AVE_3401_3500_PS. Figure 3 shows the hydrophilic and hydrophobic sites of PAPAIN_ARG59GLY. There are hydrophilic sites near the active center. The thiol (SH) group of the CYS25 residue of papain attacks the peptide bonds. The averages of the distances between heavy atoms in 2-10GLY and S-CYS25 (S atom of CYS25) of PAPAIN_ARG59GLY were calculated for AVE100, and are shown in Table S5, Table S6, and Table S7 in Supplementary Material.

For 2-7GLY, the averages of the distances between heavy atoms in the peptide and S-CYS25 of PAPAIN_ARG59GLY were large for all the average structures. (Figure 4). Conversely, for 8-10GLY the averages of the distances varied with time (Figure 5).

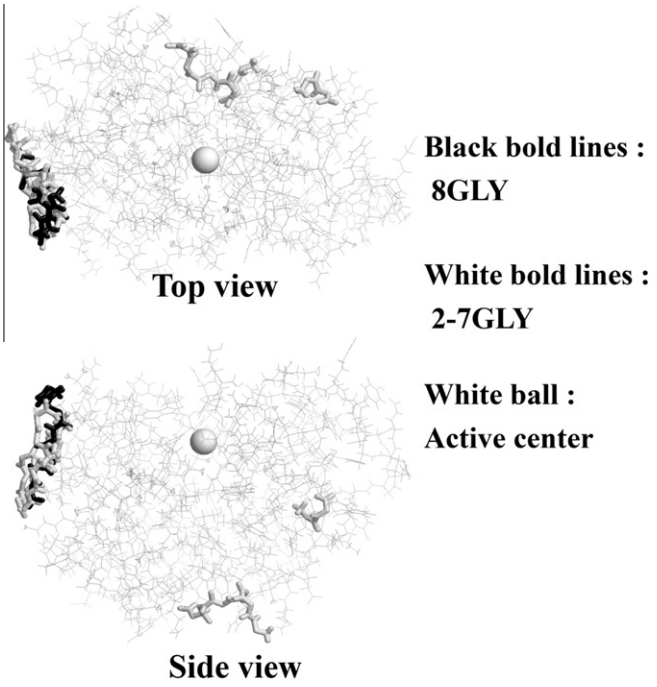

Figure 1. Top and side views of 2-8GLY on AVE_3001_3100_PS.

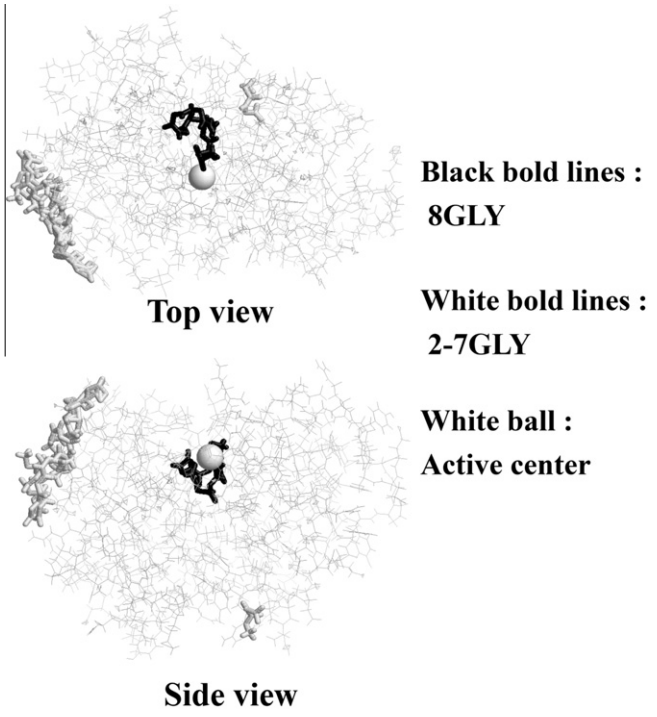

Figure 2. Top and side views of 2-8GLY on AVE_3401_3500_PS.

The averages of the distances between the heavy atoms of 8GLY and S-CYS25 of PAPAIN_ARG59GLY were significantly smaller for 8GLY_SMALL (AVE_3401_3500_PS and AVE_3501_3600_PS). The average of the binding free energies of 8GLY for 8GLY_SMALL was −5.17 kcal/mol, and the standard deviation of these energies was 0.86 kcal/mol. AVE_8GLY_SMALL (the average structure of 8GLY_SMALL) and AVE_WITHOUT_8GLY_SMALL (the average structure of AVE100 without 8GLY_SMALL) were calculated. The RMSDs of AVE_8GLY_SMALL from AVE_WITHOUT_8GLY_SMALL were esti-