Expression and Molecular Dynamics Studies on Effect of Amino Acid Substitutions at Arg344 in Human Cathepsin A

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1. Introduction
Human Cathepsin A (hCath A) is a lysosomal enzyme that exhibits a catalyst function as a serine carboxypeptidase, and also has a protective function for β-galactosidase and neuraminidase-1 through the multimeric complex formation with them [1, 2]. The genetic defect of hCath A causes the lysosomal disease called galactosialidosis. hCath A is synthesized as an enzymatically inactive precursor form so it needs to remove excision peptide, M285−R298 (see Figure 1), to be active. We examined the effect of a single amino acid mutation at R344 on the catalytic activity using molecular dynamics (MD) simulations [3]. In this study, we performed MD and ab initio fragment molecular orbital (FMO) calculations on the wild-type hCath A and R344 mutants in order to understand the effect of mutation on the activation of hCath A.

2. Methods
The X-ray crystallographic structure of the wild-type (WT) hCath A (PDB code; 1IVY [1]) was used as an initial structure for WT and twelve mutants (13 mutants in total, R344X = R (WT), A, D, E, G, I, K, M, N, P, Q, S, and V) at the 344 position. We immersed each mutant structure in a water box (consisting of c.a. 17,500 TIP3P water molecules) and carried out MD simulations (AMBER parm99 force field); the production dynamics run was performed for 1.0 ns in the NPT ensemble (1.0 atom and 300 K) using the periodic boundary condition. The last 0.5 ns trajectory (5,000 snapshots in total) for each production dynamics of 13 mutants was used for the subsequent analyses. To quantify the structural differences among 13 mutants, we characterized snapshot-structures of each mutant in terms of inter-residue (Cα−Cα) distance and ASA (accessible surface area).

To extract structural characteristics of each mutant, we classified 5,000 snapshots in each MD trajectory into 10 clusters of structure by means of RMSD conformational clustering with the K-means method. The RMSD values were calculated between all pairs of snapshots for each mutant by superpositioning of all Cα atoms of the protein. We generated an average structure among structures in the cluster with the largest population for each mutant. Each average structure of 13 mutants was energetically optimized, and then resulting structures were used for the FMO calculation (HF/6-31G: ABINIT-MP).

3. Results and Discussion
The results of MD simulations on the total 13 R344X mutants revealed that only R344D takes on a significantly different conformation of S293, G294, and D295 in the excision peptide from the other 12 mutants; the side-chains of S293 and D295 in R344D are exposed on the protein surface, although those in the other 12 mutants are buried inside the protein (only ASA for D295 are shown in Figure 2). The distributions of ASA other than these three residues were similar in all the mutants.

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The characteristic local conformational difference of S293–D295 in R344D was also found in inter-residue \((C_\alpha-C_\alpha)\) distances. The inter-residue distance of R344D between S293 and D295 takes the largest distance among those of 13 mutants (7.02 Å) (average value for the other 12 mutants, 5.77 Å). These results indicate that the electrostatic interaction between side-chains of S293 (-OH) and D295 (-COO'), which is observed in the X-ray structure of WT, is considerably weakened only in R344D.

Using FMO calculations, we estimated the inter-fragment interaction energy (IFIE) between the \(i\) and \(j\) th residues in 13 mutants, IFIE \((i, j)\), in order to understand the origin of conformational change observed only in R344D. We focused K296 located in the excision peptide. Figure 3 shows IFIE (K296, D266) and IFIE (K296, D332) in WT, R344K, R344A, R344G, R344P, R344E, and R344D mutants. IFIE (K296, D266) is significantly less than IFIE (K296, D332) in all the mutants other than R344D. This dramatic "switching of stability of interaction" could explain the conformational difference of R344D from the other mutants. It is still not clear how the effect of mutation at the 344 residue is transmitted to the excision peptide. However, ionizable residues such as D266 and D332 intervened between the region around the 344 residue and the excision peptide probably play important roles in dictating the subtle structural change of protein through their mutual interactions.

The above comparison analyses for 13 mutants reveal the remarkable conformational difference of the R344D mutants from the other ones. The R344D mutant doesn't mature and exhibit the catalytic activity unlike the other mutants including WT. The results obtained from MD and FMO calculations suggest that the ionizable residues such as K296, D266, and D332 probably govern the conformation of the excision peptide, which could be involved in the recognition of hCath A with the other putative protein [4]. The current results give useful and instructive guides for further experiments and analyses for understanding of maturation process in hCath A.

References

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