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Original article

The structural fluctuation of the histamine oxidase by molecular dynamics simulation

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Introduction

Histamine is the causative substance of poisoning by red-fleshed fish, such as tuna, mackerel and so on [1]. Histidine in the fish is converted to the histamine by microorganism after the death of the fish [2]. Histamine is the intercellular messenger, especially for the immunity [3]. Thus, the symptom of histamine poisoning is allergic-like reaction [1].

In order to control quality of fish meat, we made the histamine sensor, containing histamine oxidase (HOD), from a bacterium *Arthrobacter cystallopoietes* KAIT-B-007 [4]. HOD contains copper ion and converts histamine to imidazole acetaldehyde, releasing H_2O_2 and NH_3. The expiration date of the sensor depends on the stability of HOD. Thus, a mutant enzyme with higher stability is expected. Before the determination of target locations of mutation, the regional flexibility of HOD should be understood. In the present study, we performed the molecular dynamics (MD) simulation of HOD in order to obtain its structural information.

Materials and methods

For the MD simulation, Amber14 was used [5]. Although the 3D structure of protein is essential for the MD simulation as an initial structure, there is no available structure of HOD. Thus, we performed homology modeling by SWISS-MODEL [6]. Amine oxidase from *A. globiformis* (PDB ID: 1w4n [7]) has 62% similarity with *A. cystallopoietes* HOD and thus was used as a template. The resultant structure of HOD started with Pro15. HOD contains topa quinone (TPQ) at the 388th residue. Thus, the parameter of TPQ was constructed with Amber tools by TIP.cif in PDB. HOD contains a disulfide bond between the 323rd and 349th residues and, in the simulation, this disulfide bond was connected. To the HOD, 42,877 of TIP3P water, 109 of Na^+, 77 of Cl^- were added. In the simulation, ff14SB was used. Cut off distance for real space of particle mesh Ewald method was set to 8 Å. The constant volume condition was adopted in the energy minimization. For the solvent and ions energy minimization, 1,000 steps of energy minimization with potential restraint of HOD for 500 kcal·mol^{-1}·Å^{-2} was performed. Then, whole system energy minimization was performed for 2,500 steps. For the MD simulation, the bond lengths involving hydrogen atoms were kept by SHAKE algorism to enable the time step of 2 fs, and the temperature of the system was controlled by Langevin thermostat with the collision frequency of 2 ps^{-1}. The step size was 2 fs. For the solvent relax, 20 ps of MD simulation was performed with potential restraint of HOD for 10 kcal·mol^{-1}·Å^{-2} to the structure after energy minimization under constant volume condition at 300 K. In the constant pressure condition, Berendsen barostat with the relaxation time of 2 ps was adopted. Then, MD simulations (100 ns × 5) were performed at 300 K and 1 bar. Root mean square distance (RMSD) was calculated for the each residue by the main chain C_α, C and N. The last 50 ns for each simulation was used for the calculation of root mean square fluctuation (RMSF) of the main chain C_α, C and N, as the indicators for the regional flexibility of the molecule.

Results and discussion

The RMSD value was fluctuated around 3 Å after 10 ns (Fig. 1A). Thus, we calculated RMSF from 50 to 100 ns. The average value of RMSD from 50 to 100 ns was 3.19±0.18 Å (n = 5). The average of RMSF was 1.14±0.98 Å (n = 616) (Fig. 1B). The residues whose RMSF values were over 3 Å were His57 (outer loop region), Ala214-Thr230 (outer region), Thr362-Asn369 (inner β sheet), Pro465-Asn474 (outer loop region), Thr628 and Leu629 (C-terminus) (Fig. 1C).

For the copper ion in HOD, Amber 14 offers non-bond model, bond model and cationic dummy atom model. In the present study, we chose non-bond model. In Amber 14, there are many non-bond models. In the

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present study, frcmod.ionslrcm_cm_tip3p, which well reproduces the experimental hydration free energy and ion-oxygen distance values, was chosen. Copper ion in HOD binds to His437, His439 and His598, and was detached from these amino acids after a few nano seconds. In the present study, we performed MD simulation with positional restraint of these His residues and copper ion for 5 ns, but the ion was easily detached after the removal of the restraint. Thus, in the present study, 100 ns of MD simulation was performed without restraint. For the more accurate structural evaluation for the region, which interacts with copper ion, MD simulations with other ion parameter or longer restraint should be performed. Although detachment of the copper ion was observed, the fluctuated region as described above was distant from these His residues. Thus, these region(s) could be the candidates for the more stabilized mutant.

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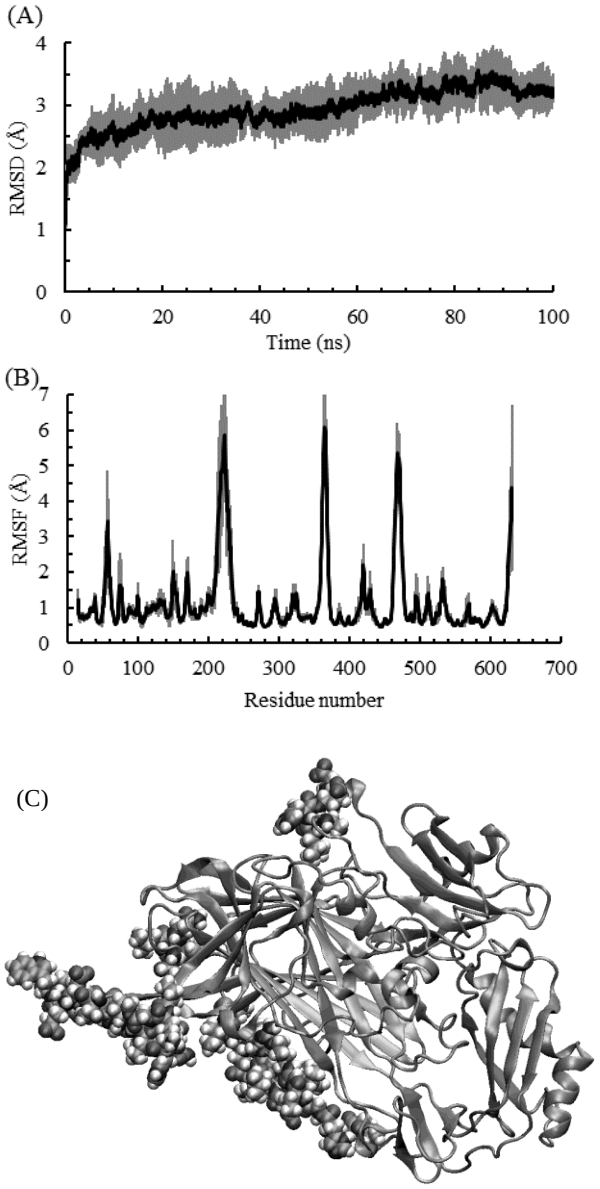

Fig. 1. The fluctuation of histamine oxidase (HOD). (A) The root mean square distance (RMSD, black) and its standard deviation (SD, gray, n = 5) of HOD. Before the calculation of RMSD, the root mean square fitting to the 1st snapshot was performed. (B) The root mean square fluctuation (RMSF) and its SD (n = 5) of HOD. (C) The structure of HOD after the 100 ns of molecular dynamics simulation, shown as new cartoon model. The residues whose RMSF values were over 3 Å are shown as van der Waals model.