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Theoretical Study of a π-stacking Interaction Effect to the Orientation of His64 of Carbonic Anhydrase

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[Introduction]

Carbonic Anhydrase (CA) is zinc-containing enyme that catalyses the reversible hydration of carbon dioxide to form bicarbonate and excess proton.

$$CO_2 + H_2O \leftrightharpoons HCO_3^- + H^+$$

Human Carbonic Anhydrase II (HCA II) has the fastest value among those of CA isozymes. Histidine at position 64 is accepted to facilitate the transfer of the productive proton from the zinc-bound water to a buffer molecule in bulk-water through intervening hydrogen bonded water molecules.

The final step of proton transfer has been assumed to be connected with a rotational or swinging motion of the side chain of His64 because this residue has two conformations, "in" and "out". The properties of the imidazole side chain of His64 should be tuned by Trp5 because the indole ring is located near to imidazole ring of His64 (~4 Å), in which the π-stacking interaction can occur in such aromatic rings. According to the crystal structure, the indole ring of Trp5 planar parallel to the imidazole ring of the "out" conformation of His64 is an off-centered structure, in which a face-to-face of π-stacking interaction should be formed to stabilize the two aromatic rings.[1] A researcher reported that His64 has a potential to be rotated in the catalysis by using molecular dynamic simulations.[2] However, the indole ring of Trp5 has not been considered in the model system.

In order to investigate the possibility that the indole ring of Trp5 interrupts the rotational motion of His64, we constructed the His64 and Trp5-containing model, and estimate the effect of the π-stacking interaction energy.

[Experiment]

We constructed a model system that consists of His64 and Trp5 (Figure 1). The coordinates were obtained from the protein database (PDB) file of the crystal structure of HCA II (2CBA). The χ1 angle of His64 was adjusted manually to clearly see the interaction energy between His64 and Trp5. The pKa value for the imidazole ring is approximately 7.0, both the acid and base forms are present. The acid form is imidazolium

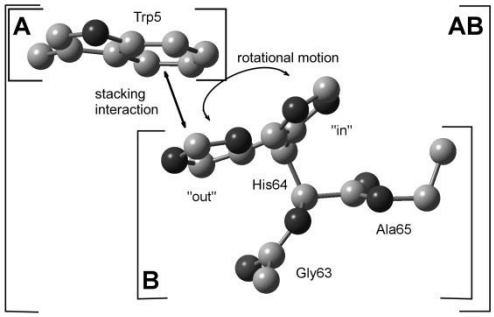

Figure 1. The model system included His64 and Trp5.

ion, and the base form is the Nδ1-H tautomer and the Nε2-H tautomer. Considering that, we simulated all three forms of imidazole.

The density-functional theory (DFT) method was employed to optimize the position of hydrogen atoms in model system. The position of hetero atoms (C, N, and O) was fixed. Considering electron-electron interaction, DFT method was not enough to estimate the π-stacking interaction. The second-order Moller-Plesset perturbation theory applied to the optimized structure from MP2 calculation.

[Results and Discussion]

The calculated energy values were plotted as a function of the χ1 angle of the His64 to investigate the profile of the π-stacking interaction. We superimposed two curves of the energy data at the biggest χ1 angle value. There was expected to be the lowest π-stacking interaction at this angle (Figure 2). The π-stacking interaction occurs at the χ1 angle around -50°. At this χ1 angle, His64 form a face-to-face parallel interaction with Trp5. The curve of three types of imidazole shows that addition Trp5 to the model system could stabilize the structure. The imidazolium form preferred the "out" conformation, similar with the model system reported by Maupin.[1] However Maupin only reported one curve in tautomeric form, probably the Nδ1-H tautomer, because it preferred "in" conformation. In our result, the Nε2-H tautomer preferred the "out" conformation. In our result, the Nδ1-H tautomer preferred the "in" conformation, while the Nε2-H tautomer preferred the "out" conformation. However Maupin only reported one curve in tautomeric form, probably the Nδ1-H tautomer, because it preferred "in" conformation. The different of preferred orientation of two tautomers could support the tautomerism of His64 to the proton transfer mechanism.[3]

The addition of Trp5 also increases the energy barrier that could restrict the rotational motion of His64. We need more investigation to verify the effect of π-stacking interaction in the catalytic mechanism of HCA II.

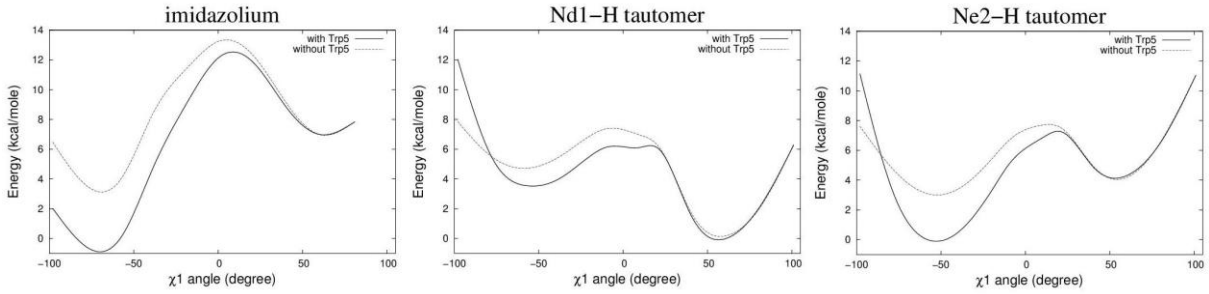

Figure 2. The energy profile for each types of imidazole of His64.

1. D.N. Silverman and R. McKenna: *Acc. Chem. Res.* **40** (2007) 669.
2. C. Maupin and G.A. Voth: *Biochemistry*. **46** (2007) 2938.
3. H. Shimahara, *et al.*: J. Biol. Chem. **282** (2007) 9646.