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Theoretical Study of Tautomerization and Conformations of His64 in Human Carbonic Anhydrase II

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[Introduction]

The specific manner of Histidine 64 (His64), in which the transfer of productive proton is mediated during catalysis of CO_2 hydration reaction in human carbonic anhydrase II (HCAII), has been explored on the basis of structural information. X-ray analysis of HCAII suggests that His64 undergoes a pH-dependent conformational change in which either an "in" or "out" conformation can be adopted in the active site cleft at a neutral pH [1], whereas ^{15}N-NMR analysis of HCAII suggests that the interconversion of two $N_{\delta 1}$-H and $N_{\varepsilon 2}$-H tautomers of His64 can associate with the proton-transfer in which imidazole nitrogen atoms serve as both a donor and acceptor [2].

Our previous work reported that the existence of the π-stacking interaction between Trp5 and the "out" conformation of His64 in HCAII [3]. We also determine the relation of the π-stacking interaction in the His64 rotation of motion on a simple model, consist of Trp5, Gly63, His64 and Ala65. The result indicates that the π-stacking interaction cause an increase in energy barrier of rotational motion [4,5].

In this study, we performed *ab initio* calculation with electronic correlation (MP2) for the imidazole in the Trp5-containing cluster models constructed to estimate the energetic effect of the tautomerization of His64 coupled with the ionization of zinc-bound water *via* water-bridge on the rotation of χ_1 angle of His64 in the active site cleft of HCAII. The goal of our study is to detail the mechanism responsible for the catalytic manner of human carbonic anhydrase II.

[Calculation Methods]

The three-dimensional structure of HCAII was obtained from the X-ray coordinate file (Protein Data Bank (PDB) code: 2CBA). The active site residues (ASR; Trp5, Tyr7, Gly63, His64, Ala65, Asn67, His94, His96, His119, Thr199, and Thr200), zinc ion, and four oxygen atoms of water molecules (named ZnO, w1a, w2, and w3a) were extracted to obtain the cluster model, as shown in Fig. 1, then two types of cluster models were constructed: models with/without three oxygen atoms, w1a, w2, and w3a, as shown in Fig. 1.

The hybrid meta-GGA density functional theory (DFT) calculations, M06-2X, has produced reliable models including transition metals and hydrogen bonding interactions. By using this method, the optimized structure was obtained. In addition, the second-order Møller-Plesset perturbation theory (MP2) is a reliable method for models having electron correlation,

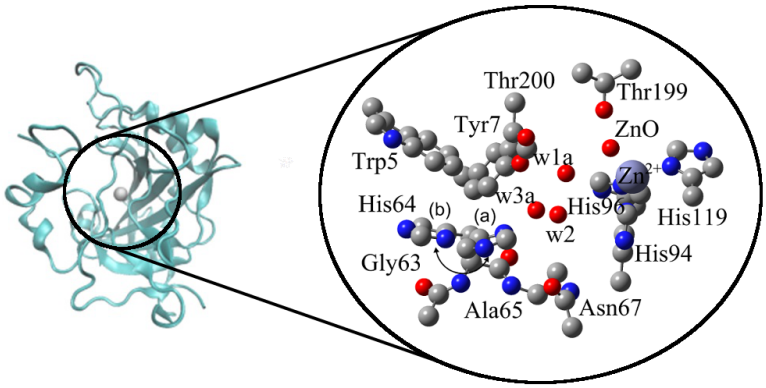

Figure 1. The representation of the active site of the HCA II

allowing the estimation of the structure having π-stacking interaction between two aromatic rings (His64 and Trp5). All calculation were performed on the Gaussian 09 program package, using 6-31G(d,p) basis set and solvent ether (ε=4.24).

[Results and Discussion]

Comparative analysis of rotational energy surfaces indicates that a formation of water-cluster having water-bridge between His64 and zinc-bound solvent in cleft stabilizes the "in" conformation, compared to "out", especially when the protonated form in interconversion is adopted in the way of the tautomerization (protonated form), the energetic difference for the water-cluster formation in the cleft is large (-13.6 kcal/mol) to cause the change of stability of conformation. As a result, the "in" conformation becomes 9.34 kcal mol^{-1} more stable than the "out" conformation.

The interaction energy analysis of the water-cluster to the active site residues shows that energetic contributions of protonated form and $N_{\delta 1}$-H tautomer of His64 to the water-bridge formation are -16 and -8.5 kcal/mol, respectively, while that of $N_{\varepsilon 2}$-H tautomer is -4.3 kcal/mol. This can be compatible with the experimental result in which the imidazole group intrinsically tends to the $N_{\varepsilon 2}$-H tautomer in histidine residues, unless a hydrogen bond interacts with the δ1-nitrogen of the imidazole ring.

In addition to the favorable interactions of the water-cluster with the protonated form and $N_{\delta 1}$-H tautomer, these forms of His64 have a productive proton transferred from zinc-bound water in the proton-relay process. Considering them, the ring of His64 having the productive proton cannot rotate/swing from "in" to "out". Our data indicates that the $N_{\varepsilon 2}$-H tautomer has the lowest rotation energy barrier (5.9 kcal/mol) compared to another forms, which have a limited possibility or rotate. These results are significantly inconsistent with that of simulations in which TIP3P bulk water model was used for HCAII [6]. These suggest that there is no occasion for His64 having the productive proton to rotate from "in" to "out" in proton-transfer process during catalysis of HCAII.

[References]

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