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Unexpected Deacetylation Mechanism Suggested by a Density Functional Theory QM/MM Study of Histone-Deacetylase-Like Protein

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Class I and II histone deacetylases (HDACs) constitute a very important family of zinc-dependent enzymes that catalyze the cleavage of acetyl groups from acetyl−lysine residues in histone N-terminal tails.[1] They are critically involved in regulating chromatin structure and gene expression, and the aberrant recruitment of HDACs has been associated with a variety of diseases, notably cancer.[2a] The detailed knowledge of their catalytic mechanism is of high importance, as HDACs have been established as very promising targets for the development of anti-tumor drugs.[2b,c]

The first hypothesized catalytic mechanism originated from the crystallographic study of HDLP, a histone-deacetylase-like protein that is widely used as a model for zinc-dependent HDACs.[3] Both HDLP and HDACs have a highly conserved catalytic domain and are generally assumed to share a common mechanism. In the active site of HDLP, the zinc atom is coordinated by side chains of Asp168, Asp258, His170, as well as by the oxygen of the amido group of the inhibitor. The residues in the second coordination sphere include Tyr297 and the His131−Asp166 and His132−Asp173 dyads, which are also expected to play an important role in the catalytic reaction. The very recent structural studies[4] indicate that human HDAC8 has essentially the same active site as HDLP. On the basis of their crystal structures, Finnin et al.,[3] followed by others,[4] postulated a catalytic mechanism for HDACs in which the first reaction step is analogous to the "hydroxide mechanism" for zinc proteases:[5] a zinc-bound water acts as a nucleophile, and the Zn^{2+} is five-fold coordinated during the reaction process. However, recent experimental studies[6] suggested that the transition state of HDACs may not be analogous to zinc proteases. In fact, the ligand environment is quite different between these two families of enzymes. In zinc proteases, such as carboxypeptidase and thermolysin, Zn^{2+} is bound to one Glu/Asp and two His residues,[7] which have a total of −1 charge, rather than one His and two Asp residues in HDACs with a total charge of −2.

To characterize the catalytic mechanism for HDACs, we have carried out density functional theory QM/MM studies on the deacetylation reaction catalyzed by the HDLP. The calculations are based on the pseudobond ab initio QM/MM approach,[8] which has been successfully employed in the study of several enzymes.[9] The QM subsystem, including the zinc atom, the coordinated ligands (H170, D168, D258), and the chemically active moieties (H131, H132, a water molecule, and the acetylated lysine), is treated by the hybrid B3LYP functional with a Stuttgart ECP/basis set[10] for the zinc atom and a 6-31G* basis set for all other atoms. All other residues and the surrounding water molecules are described by a molecular mechanical force field (8682 atoms). The reaction energy profile was then evaluated by single point energy calculations at the B3LYP/6-311+G** QM/MM level. All calculations were carried out with modified versions of the Gaussian03[11] and TINKER programs.[12]

First we carried out various calculations aiming to characterize the previously hypothesized mechanism,[3] in which Zn^{2+} is always five-fold coordinated, a doubly protonated H132 serves as a general acid, and a singly protonated H131 serves a general base. The QM subsystem consists of 84 atoms. Although the water moiety is bound to the metal in the gas phase or in the absence of substrate,[13] our calculations indicate that the zinc atom prefers a four-fold coordination and the active site water (or hydroxyl) molecule does not directly bind to zinc in the protein environment with the presence of the substrate. Instead, this water molecule is stabilized by forming a hydrogen bond with H131, H132, and D168, respectively (see Figure 5S). Meanwhile, it is found that the reactant state with doubly protonated H131 and singly protonated H131 is about 5 kcal mol^{-1} more stable than the previously suggested one[3] with the reverse protonation states. The first catalytic reaction step, starting from the most stable reactant complex, results in the expected tetrahedral carbon intermediate. However, the 25.9 kcal mol^{-1} calculated barrier at the B3LYP/6-311+G** QM/MM level is not consistent with the activation barrier of 16.9−20.6 kcal mol^{-1} estimated from experimental evaluation of k_{cat} (6×10^{-3} to 2.8 s^{-1})[4a] using transition state theory. Thus, we are motivated to explore whether another catalytic mechanism is more probable.

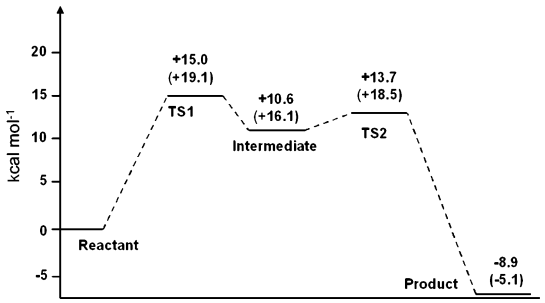

Figure 1. Energy profile of the deacetylation reaction catalyzed by HDLP. Relative energies are given with respect to the reactant at the B3LYP/6-31G* level (using a Stuttgart ECP/basis for Zn). B3LYP/6-311+G** QM/MM single point energies are given in parentheses.

On the basis of the careful analysis of the hydrogen bond network in the active site, both H131 and H132 have been proposed to be singly protonated in the HDLP−inhibitor complexes[3] (see Figure 3c in ref 3). Recent structural results on HDAC8 also support this protonation configuration.[4b] Thus, it would be reasonable to assume that both H131 and H132 are singly protonated in the enzyme−substrate complexes. On the basis of this alternative protonation configuration, we have carried out similar DFT QM/MM calculations to characterize its reaction mechanism, in which the QM subsystem has 83 atoms. The calculated overall reaction energy profile is shown in Figure 1 with the calculated barrier of 19.1 kcal mol^{-1} at the B3LYP/6-311+G** QM/MM level. It is 7.8 kcal/mol lower than the mechanism with one doubly protonated histidine residue and is more consistent with the experimentally determined k_{cat} for histone deacetylases.[4a] Therefore, our calculations suggested a more probable deacetylation mechanism, as shown in Figure 2.

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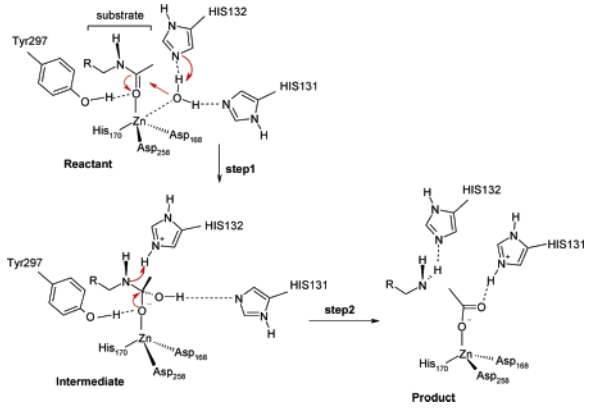

Figure 2. New proposed mechanism of the hydrolysis of acetyl−lysine (substrate) by the HDLP enzyme. Numbering of the residue is based on the HDLP protein sequence.[3]

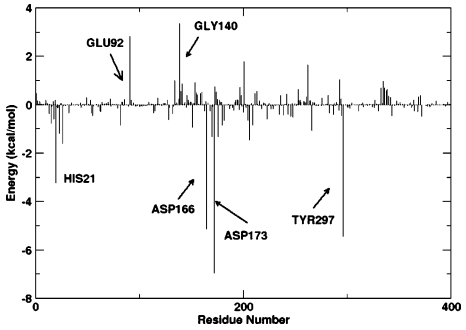

Figure 3. Individual MM residue electrostatic contribution to the transition state stabilization or destabilization: $(E_{MM} \rightarrow QM)_{TS1} - (E_{MM} \rightarrow QM)_{R}$. The negative number indicates that the residue stabilizes the transition state and vice versa. His21 and Glu92 form a salt bridge so that their effects are more or less canceled. The carbonyl oxygen of Gly140 forms a hydrogen bond with the amide hydrogen of the substrate so that its main role is likely to be the substrate binding.

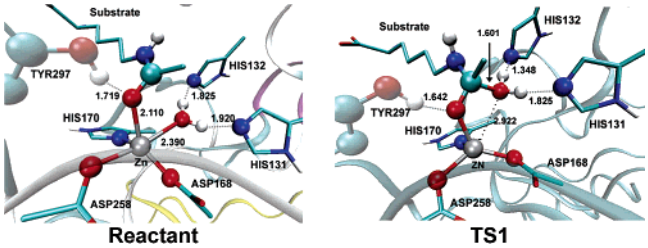

Figure 4. The enzyme active site structures of the determined reactant and first transition state. The distances are in angstroms.

In this mechanism, both histidine residues in the second coordination shell are singly coordinated in the reactant state. The nucleophilic water oxygen attack of the carbonyl carbon to form the tetrahedral intermediate is the rate-determining step and is facilitated by H132, which accepts one proton from the water. The transition state is strongly stabilized by Y297, D166, and D173, as shown in Figure 3. Y297 forms a hydrogen bond with the carbonyl oxygen of the substrate, while D166 and D173 form dyads with H131 and H132, respectively. These results emphasize the key catalytic roles played by both dyads and the residue Y297, which are consistent with experimental mutation results.[14] In the second step of the reaction, H132 serves as a general acid to transfer one proton to the amide nitrogen, which assists the rupture of the amide bond and leads to the lysine and acetic acid product. Meanwhile, the proton spontaneously transfers from acetic acid to H131. Although the water molecule is weakly coordinated to the zinc atom in the reactant in Figure 4 ($Zn-O_{water} = 2.39$ Å, while the average distance for the zinc-coordinated water molecule[15] is 2.12−2.15 Å), the water immediately departs from the metal when the reaction proceeds. During the rest of the reaction process, the zinc atom clearly has a tetrahedral coordination (see Figure 4 and Figure 7S). Therefore, the key catalytic role of the zinc atom is to activate the carbonyl group of the amide toward nucleophilic attack. In comparison with two controversial mechanisms proposed for thermolysin,[16,17] the present scheme in Figure 2 is more similar to the "reverse protonation mechanism".[7,17]

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Supporting Information Available: Additional details on calculation procedures and results, complete refs 4b and 11, and PDB files of the determined minima and TS's for the new proposed mechanism. This material is available free of charge via the Internet at http://pubs.acs.org.

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