Mechanism of Histone Deacetylase 8

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ABSTRACT: Histone deacetylases (HDACs) are the key enzymes in histone deacetylation and play critical role in gene expression regulation. Though its structure has been revealed for a long time, its reaction mechanism remained ambiguous for years. Here we give a review of the development of the interpretation of HDACs working mechanism and introduce the several reasonable models.

■ INTRODUCTION

Post-translational modification is an important process in the later stage of protein biosynthesis, regulating the functional activities of the protein and its interaction with other molecules in cell. It contains various chemical changes on proteins, such as alkylation, acetylation, phosphorylation, and so on. Histone as the protein which organizes DNA into chromatin, its modification plays a vital role in gene expression. Histone deacetylases (HDACs), which remove the acetyl functional group on the lysine residue in the N-terminal tails of core histones after translation, have been found related to both cancer and non-cancer disorders, becoming the target of novel drugs and therapeutic approaches[1,2].

Based on the working mechanism, eukaryotic HDACs can be divided in to two families, zinc-dependent family and NAD^+-dependent family[3]. One member of the zinc-dependent family, HDAC8 has been studied in most detail, however, its working mechanism and the roles of metal cations in it have been debatable for years. As shown in Figure 1[4], the crystal structure of HDAC8 gives that there is one zinc ion, as the catalytic center, and two potassium ions in this enzyme. Scientists have worked hard to elucidate the roles they play in deacetylation and develop HDAC inhibitors with higher selectivity and less toxicity.

■ REACTION MECHANISM OF HDAC8

Based on the crystal structure of HDAC8, there are two histidine residues, H142 and H143, being close to the zinc ion spatially and in turn coordinated to two aspartates D176 and D183. These four residues are conserved in several human HDAC isoforms, while in other human HDACs D183 is replaced by a Q or N. That's why H142 and H143 are the focus of HDAC working mechanism study. In addition, as

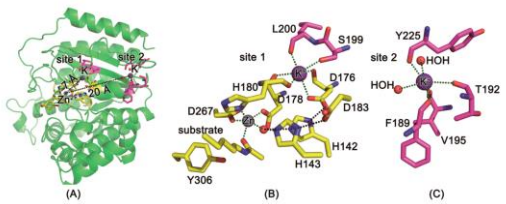


Figure 1. (A) crystal structure of HDAC8 (pdb code: 2V5W) with active-site Zn^{2+} and two K^+ ions and K^+ binding (B) site 1 and (C) site 2.

the potassium ion binding in site 2 is about 20 Å away from the zinc center, it is regarded to regulate the activity by an allosteric effect instead of directly participate in the hydrolysis reaction. Therefore, we mainly focus on the zinc ion and the potassium in site 1 for mechanism study.

There are mainly two mechanisms proposed independently by different research approaches (shown in Scheme 1)[4]. There is no doubt that the zinc center here works as a Lewis acid to activate the water molecule, making it more polar thus easier to be deprotonated to form a nucleophile, which would attack the carbonyl carbon of acetyl lysine. The problem is, which histidine residue here act as the general base to abstract the proton. The first mechanism proposed by Finin et al. in 1999 suggested that H143 is protonated and H142-D176 acts as a general base. Consistent with that, Gantt et al. carried out a further research and draw a conclusion that the K^+ binding in site 1 inhibits the activity of HDAC8 since it would like to reduce the Lewis basicity of D176, as shown in Figure 2, thus the protonated H142 (B1 in Scheme 1) would not be stabilized so well and the reaction become less thermodynamically favorable.

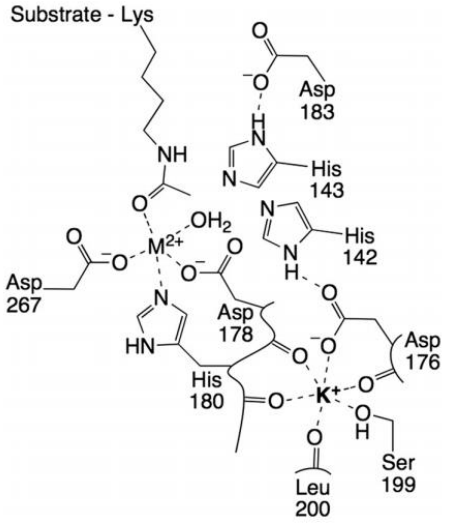
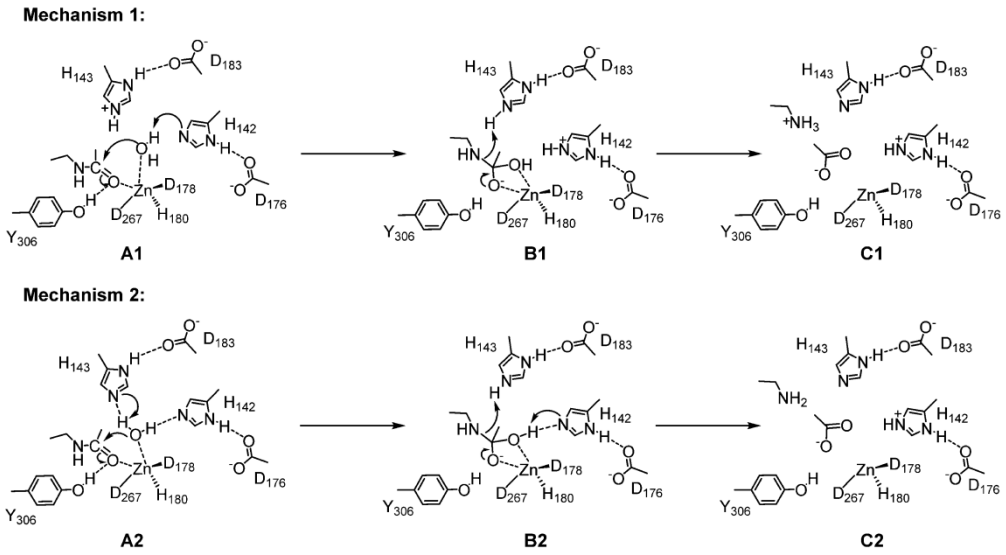


Figure 2. HDAC8 active site and K^+ in site 1.
The site 1 K^+ is coordinated by the backbone carbonyl oxygens of Asp-176, Asp-178, His-180, and Leu-200 and by the side chains of Asp-176 and Ser-199. The carboxylate of Asp-176 also forms a hydrogen bond with His-142.[6]

The second mechanism proposed by Zhang et al., unlike the works mentioned previously, is not a conclusion from mutation experiments, but from a theoretical model which was built basing on data of the crystal structure of HDAC8. Surprisingly, they reported that the K^+ in site 1 was supposed to



Scheme 1.the two reaction mechanism of HDAC8

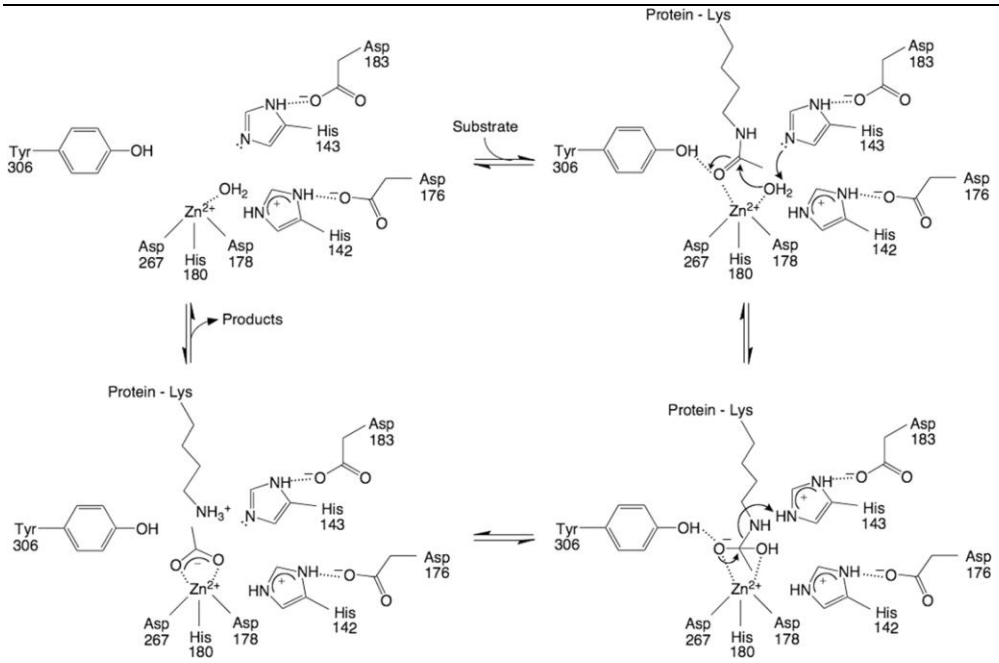


Figure 3. HDAC8 reaction mechanism proposed by Gantt et al.

activate HDAC8 because in their model the presence of K^+ would significantly increase the positive charge on Zn^{2+}, which makes it a stronger Lewis acid, and the negative charge on nitrogen atom (N^{δ}) of His143, strengthening its basicity. Both of these two effects promote the reaction.

It is obvious that one of them must be wrong. Later, Chen et al. has published their work in 2014, which supports the first mechanism and the inhibitory role of K^+ in site 1 through a computational method.

In that work they not only investigated the mechanism through substrate-HDAC8 complex information, but also studied the protonation state of an inhibitor, suberoylanilide hydroxamic acid (SAHA), through the structure of SAHA-HDAC8. That enables them to interpretate the question in a novel perspective that it is the shift of pKa of substrates and inhibitors when they bind to the zinc that push the reaction to a point that where the two His-Asp dyads play an active role. It is inspiring that they figured out that the mechanism of HDAC8 is a combination of the ones of both serine and metalloprotease. Neither of them is good model alone.

However, it is not the end of HDAC8 working mechanism study. As the second mechanism seems to be less reasonable in the lack of supporting experiment evidence, there is another possible working mechanism of HDAC8 consistent with their QM/MM results developed from experiments proposed by Gantt et al. later. As shown in Figure 3[8], though in the previous research they found K^+ in site 1 inhibits HDAC8 activity, which contrasts with Zhang's mechanism, their recent work agrees with Zhang's hypothesis that H143 is a single general-base-general-acid catalyst, while H142 is an

electrostatic catalyst. The most appropriate interpretation of HDACs reaction mechanism is still waiting to be found.

■ CONCLUSION

Due to the unique structure of HDAC, which has characteristics of both serine protease and metalloprotease, the reaction mechanism of it used to be ambiguous. Here we conclude the development of the study of it. So far, there are several computational models and hypothesis matching some of the experimental phenomenon, respectively. And the one proposed by Chen et al. also provides us a new way to design novel HDAC inhibitor. It is feasible to improve the selectivity of HDAC inhibitor and reduce its toxicity by controlling the pKa of it to maintain its activity while optimizing the other part of the molecule.

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