Inhibition mechanism of SAHA in HDAC: a revisit†

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Histone deacetylase (HDAC) has been identified as a remarkable drug target against cancer and other human disorders,1,2 and four classes (class I, IIa, IIb, IV) exist among the 11 zinc-dependent HDAC isozymes. So far, there are four FDA-approved anticancer drugs targeting HDAC, i.e., SAHA (vorinostat, Merck),3 FK-228 (romidepsin, Gloucestor),4 PXD-101 (belinostat, Spectrum)5 and LBH-589 (panobinostat, Novartis).6 SAHA is a well-studied and most famous HDAC pan-inhibitor.7,8 However, the essential protonation state of SAHA (see Fig. 1) has been debated theoretically and experimentally for more than ten years.9–12 In 2002, Duca et al. computationally predicted that the pKₐ value would increase from 9.4 (for isolated SAHA)13 to ~12.7 upon binding to the zinc ion in the active site of TACE (a TNF-α converting enzyme), whose active site is similar to HDAC.9 Later, Gallinari and Bradner9,14,15 experimentally found that H843Y in HDAC7 and HDAC4 (both belonging to class Ia) would significantly increase the binding affinity to LAQ-824, whose structure is very similar to SAHA, while it is a conserved tyrosine residue in class I HDAC (Y308/306 in HDAC2/8), as shown in Fig. 1. Meanwhile, Vanommeslaeghe and Wiest11,16 computationally suggested that SAHA ought to be deprotonated due to the presence of the conserved Y308 in class I HDAC. From then on, the “Class-dependent” (or “Tyr-dependent”) hypothesis was extensively accepted; in this hypothesis, SAHA is deprotonated (namely negative SAHA) in class I HDAC but protonated (namely neutral SAHA) in class IIa HDAC due to the distinct residue conservatism (Y308 vs. H843). In contrast, our previous quantum mechanics/molecular mechanics (QM/MM) molecular dynamics (MD) simulations12,13,17 supported the view that the negative SAHA is thermodynamically unfavorable even with the existence of Tyr 306 in HDAC8. Very recently, Wiest et al.18 further put forward the viewpoint that the deprotonation of SAHA in HDAC8 is “Model-dependent” (namely “QM-size” dependent) in their static QM/MM (ONIOM) calculation, in which negative SAHA is more stable if the two Asp residues (see the conserved D–H dyads shown in Fig. 1) are considered in the QM region while neutral SAHA is more stable if using a smaller QM region as we used in a previous study.12

Meanwhile, on the basis of isothermal titration calorimetry (ITC) experiments in 2014, Srivastava et al.19 suggested that SAHA may release a proton to the external buffer upon binding to HDAC8, but as the determined enthalpy and entropy difference is tiny it is thus still difficult to definitely identify the feasibility of the proton transfer reaction between SAHA and His142 (as shown in Fig. 1). Nevertheless, that study was the first to clarify the SAHA protonation state in the enzyme–inhibitor complex experimentally. In summary, the protonation state of SAHA, as well as its regulatory mechanism in HDAC, is still not clearly known even though so many theoretical and experimental studies have been performed.

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11 Electronic supplementary information (ESI) available: Detailed structural basis comparisons of the HDAC2/7/8–SAHA complexes are summarized in Fig. S1 and Table S1. More detailed discussion of the choice of reaction coordinates (RC) and reaction free energy profiles is given in Fig. S2–S6 and Tables S2–S6. More detailed results regarding the “Metal-dependent” inhibition mechanism are provided in Fig. S7–S14 and Table S7. For additional computational details and discussion of the two water molecules (WAT1/2) see Fig. S15–S18 and Tables S8–S10. See DOI: 10.1039/c5cp05633k

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In fact, now the deprotonation on the basis of the two structural classes is still not very clear. Herein all available crystals for the HDAC2/8/7-hydroxamate complexes are reviewed and summarized in Table S1 (ESI†). Fig. 1 and Fig. S1 (ESI†). Intriguingly, the second metal site in HDAC2 is distinct from HDAC8 and HDAC7 (Ca2+ in HDAC2 but K+ in HDAC8/7, see Fig. 1), and this conserved metal binding site in HDAC is related to its catalytic activity experimentally.18,20−22 It should be clearly emphasized that the K+ ion in the second metal site (7 Å from the catalytic center Zn2+, see Fig. 1) was kept in our previous modelling as most experiments had proved it to be conserved, whereas it is absent in Wiest’s model. In this sense, it is unwarranted to compare our previous simulations with Wiest’s modelling of HDAC8, since the employed computational models are different not only in terms of their number of atoms in the QM region, but also in their choice of theoretical method (QM/MM MD vs. static ONIOM). Another essential but ignored structural component is the water binding site (WAT1/2 are determined for HDAC2 crystals,23 as shown in Fig. 1, see Table S1 and Fig. S1, ESI† for details), which construct a stable hydrogen bond network with Y308/G154 and SAHA. For HDAC8, it is also found that one water (WAT1) is present in several recent human HDAC8 crystal structures (1VKG,24 1W22,25 and 2VSX,26 see details in Fig. S1, ESI†) or two waters (WAT1/2) are observed in Schistosoma mansoni HDAC8 crystal structures (4BZ6, 4BZ7 and 4BZ9,27 see details in Fig. S1, ESI†), while the WAT1/2 were missed in the early “1T69” and “2VSX” crystals.24,26 HDAC7 (3C10 and 3C0Z28) also has one crystal water (WAT2) there. To our best knowledge, the functional role of the water binding site had never been carefully investigated until 2014, when Srivastava pointed out the importance of the resident water molecules in the active site of HDAC8 experimentally.19

For our computation, all the computational models are shown in Tables S2−S6 (ESI†). The HDAC2-SAHA (4LXZ),23 HDAC8-SAHA (1T69)24 and HDAC7-SAHA (3C0Z)28 complexes were used as the initial structures. A state-of-the-art Born−Oppenheimer ab initio quantum mechanics/molecular mechanics molecular dynamics (QM/MM MD)22,29−34 method combined with unbiased sampling and umbrella sampling35,36 technologies was used to capture all data presented in this work. The free energy profiles were mapped out by determining the probability distributions of the reaction coordinate and pieced together with the WHAM37,38 program on the basis of the last 20 ps (25 ps for each window) QM/MM trajectories along the proton transfer reactions in all HDAC models. All other computational details are presented in the ESI†.

To detect the two structural regions and “Model-dependence” in the theoretical modelling as Wiest suggested, a benchmark test on the complete HDAC−SAHA complexes with the existence of the second metal site and water binding site was performed at the B3LYP/(6-31G*, SDD) level (the same as Wiest’s and our previous calculations). The corresponding free energy profiles for the wild type HDAC2 (see Fig. 2(a), for conciseness further details are shown in Fig. S3, ESI†) and HDAC8 (see Fig. S4, ESI†) have been mapped out, by considering six kinds of QM regions, including the classical smallest QM subsystem (Cs-QMS, as defined in our previous work17,31) and the classical bigger QM region (Cb-QMS, as defined in Wiest’s work for HDAC818), were calculated. Our simulations reflect the model-dependence either in HDAC2 or HDAC8. Apparently, the two water molecules should be considered in the QM subsystem (see Fig. 2(a)). Since the reaction free energy profiles almost show no change (blue vs. pink curves in Fig. 2(a)) if Y308 is further considered in the QM region, the choice of “BQMS” is big enough for the following QM/MM MD simulations to probe the proton transfer reaction of SAHA in HDAC.

As shown in Fig. 2(b), considering the negative SAHA is about 4.4 and 2.5 kcal mol−1 more stable than the neutral one in HDAC7 (class IIa) and HDAC8 (class I) respectively, SAHA should be deprotonated in HDAC7/8: thus this contradicts the
previous “Class-dependent” hypothesis. Regarding HDAC2, the proton prefers to be shared nearly half-and-half between SAHA and H145 as the neutral/negative SAHA shares an almost equivalent stability and because the interconversion barrier is so small (ca. 2.3 kcal mol\(^{-1}\)). That is, although HDAC2/8 belong to class I HDAC with highly similar conserved tyrosine and the SAHA binding sites, SAHA could present different protonation states. Since the existence of WAT1/WAT2 is not consistent in various crystal structures, several other possible HDAC8/7/2 computational models with various water binding sites are also discussed in the ESI† (Fig. S15–S18 and Tables S8–S10), and all these simulations proved that negative SAHA would be stabilized either by only one or by both water molecules in the water binding site.

To further identify the role of Y308/Y306 in regulating the deprotonation of SAHA in class I HDAC, mutant modelling was performed on HDAC2, and their free energy profiles are summarized in Fig. 2(c) and (d) respectively. For the Y308H single mutant, the negative SAHA is unstable while the protonated state is more stable in HDAC2. In striking contrast, negative SAHA is much more stable (\(-5.1\) kcal mol\(^{-1}\)) than neutral SAHA in HDAC8 via Y306H. Thus the regulatory role of the conserved tyrosine on the protonation state of SAHA is of opposite polarity, and further contradicts the “Class-dependent” hypothesis that the conserved tyrosine could increase the deprotonation ability of SAHA in class I HDAC. Therefore, we conclude that Tyr308/306 is not the determinative structural basis for the protonation of SAHA.

To figure out the real protonation mechanism of SAHA in HDAC, a Ca\(^{2+}\) → K\(^+\) single mutation was performed on HDAC2, thus stabilizing the negative SAHA. Meanwhile, negative SAHA was destabilized in HDAC8 via a K\(^+\) → Ca\(^{2+}\) single mutation. This indicated that the existence of K\(^+\) is favourable for stabilizing negative SAHA. Even more, the negative SAHA would be further stabilized if the second metal is deleted in HDAC2/8 (see Fig. S5 and S6, ESI†). Therefore, the lower positive charge on the second-metal binding site would promote the deprotonation of SAHA. More intriguingly, if Y308H is performed after Ca\(^{2+}\) → K\(^+\) mutation in HDAC2, the negative SAHA would be further stabilized, as shown in Fig. 2(c). This indicates that the contribution of Y308 to the deprotonation of SAHA is reversed between wild type and Ca\(^{2+}\) → K\(^+\) mutant HDAC2. Moreover, a function reversal similar to Y306 is also observed in HDAC8 (Fig. 2(d)). Thus we conclude that functional role of Y308 is dependent on the second metal site, so the next query is how it remotely regulates the protonation state of SAHA.

From Fig. 3, for the Ca\(^{2+}\) → K\(^+\) mutant in HDAC2, the hydrogen bond (HB) interaction in the D179–H145 dyad is
strengthened, and the HB distance decreases from 1.96 to 1.70 Å. As charge transfer is facile through the “metal–dyad–SAHA–Zn” linker network, the negative charge on the H145:N d would be reduced from −0.36 to −0.51, which would facilitate proton transfer from SAHA:O1 to H145:N d. Meanwhile, the positive charge on the zinc ion is decreased from 0.93 to 0.82, thus the coordinative ability of Zn²⁺ is reduced, and as a result the Zn²⁺–O2 (SAHA) coordination bond becomes longer (from 2.16 to 2.43 Å). But the Zn²⁺–O1 (SAHA) coordination bond is still well maintained (2.31 Å) and thus the acidity is not decreased to polarize the O1–H bond of SAHA, which would promote the proton transfer reaction. As a result, the neutral SAHA would survive for less than 1 ps (Table S7, ESI†) in the metastable state (namely the structure in the dashed-line frame in Fig. 3), and then immediately and spontaneously achieve the final stable negative SAHA state, which is consistent with the very low reaction barrier (0.9 kcal mol⁻¹) shown in Fig. 2(c).

At the negative SAHA state in HDAC2, Zn²⁺ is nearly 4-fold coordinated, and the Zn²⁺–O1 (SAHA) coordination bond becomes stronger (2.22 Å) as the negative charge on SAHA:O1 would be increased followed by the proton transfer, while SAHA:O2 (SAHA) would not chelate Zn²⁺ and instead forms a strong hydrogen bond with WAT2 (more discussion on WAT2 is presented in the ESI†). Moreover, a similar structural rearrangement and electron-transfer effect also exists in HDAC8 (see details in Fig. S10–S12, ESI†). Thus the reverse function of Y308/Y306, stabilizing or destabilizing the negative SAHA in HDAC2/8, is dependent on the second metal site (Ca²⁺ vs. K⁺). This further confirms that the second metal site is the most critical controller of the protonation of SAHA, not the conserved Y308 as emphasized in the previous “Class-dependent” hypothesis.¹⁰,¹¹

In conclusion, our ab initio QM/MM MD simulations powerfully contradict the previous “Class-dependent” hypothesis that the protonation state of SAHA is determined by distinct residue conservatism (Y308 in class I HDAC but H843 in class IIa HDAC). Instead, we found that the functional role of Y308 is dependent on the variety of the second metal ion. A novel proof-of-principle...
“Metal-dependent” mechanism is elucidated herein: the deprotonation of SAHA is mostly modulated by the metal ion (Ca$^{2+}$/K$^+$) in the second metal site. Meanwhile, the water binding site around the second zinc coordination shell is also a necessary consideration in QM/MM simulations. Therefore, it is critical to pay attention to and utilize the second metal site and water binding site in future selective inhibitor design towards HDAC, which existed extensively but were ignored in most previous theoretical and experimental studies on HDAC. On the basis of the detailed free energy profiles [27 PMF profiles and more than 150 simulation windows] in our modelling with the presence of WAT1/2 (water binding site) in the QM region and the remote second metal site in MM region, we conclude that negative SAHA is much more stable and will be prevalent in HDAC7 and HDAC8 while it is indistinguishable for HDAC2 theoretically. As Srivastava found, the magnitude of the deprotonation for SAHA-like inhibitors is dependent on the type of the “linker/cap” regions and this feature is intrinsic to its binding modes in HDAC8 experimentally, thus we advocate strongly that in future it is essential to bridging theoretical and experimental investigations to finally define the protonation state of SAHA as well as its analogues upon binding to each HDAC isoform. Meanwhile, we believe that the new “Metal-dependent” mechanism would open a new avenue to utilize the second metal site and the water binding site for isoform-selective inhibitor design. Indeed, we have obtained isoform-selective HDAC inhibitors that could distinguish HDAC1 and HDAC2, whose sequence similarity is over 98%, by taking advantage of the different reactivity due to the different metal ions in HDAC1 and HDAC2.

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Notes and references


