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A comparative analysis of the binding site of *Plasmodium falciparum* histone deacetylase-1 and human histone deacetylase-8

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Abstract

Histone deacetylases (HDACs) are a family of enzymes involved in the modulation of mammalian cell chromatin structure, regulation of gene expression, DNA repair, and stress response. The histone deacetylase enzymes *Plasmodium falciparum* histone deacetylase-1 (*Pf*HDAC-1) and human histone deacetylase-8 (hHDAC 8) have been identified as novel targets for development of antimalarial and antitumor drugs respectively. Homology models of *Pf*HDAC-1 and hHDAC 8 were generated from the crystal structures of HDAC8 and HDLP and IT64 respectively using a restraint guided optimization procedure involving a combination of the Optimized Potentials for Liquid Simulations and the Generalized Born Surface Area (OPLS/GBSA) potential setup. The models were validated using protein structure validation tools. Comparative analysis of their binding sites was also carried out to identify their topology and residue interaction that could be utilized in developing *Pf*HDAC-1 specific inhibitors.

Keywords: Histone deacetylase; *Plasmodium falciparum*; human histone deacetylase; homology model; binding site.

Introduction

Histone deacetylase (HDAC) is a zinc-dependent enzyme involved in the deacetylation of terminal acetylated lysine residues of histone proteins [1-4]. Deacetylation is one of the post-translational chromatin modifications that regulates the epigenetic control of gene expression. This chromatin modification is absolutely necessary since it prepares the chromatin to be accessible to a large number of chromatin interacting proteins [5]. The acetylation and deacetylation processes are controlled by two families of enzymes; histone acetyl transferases (HATs) and HDACs, respectively [6-8]. Gene transcription is controlled by the extent to which the DNA is bound to the histone and the extent of exposure of the positive charge of lysine residues on deacetylation.

In contrast, HATs loosen the histone-DNA binding by acetylating the positively charged lysine residues back to their acetylated form and this leads to the transcriptional activation [9]. The transcriptional repression of various preprogrammed sets of genes including the tumor suppressor gene leads to cancer. These repression and activation of transcription have fundamental regulatory roles in developmental processes and their deregulation has been linked to the progression of cancers and different human disorders [10].

HDAC inhibitors can induce cancer cell death whereas normal cells are relatively resistant to the inhibitor induced cell death [11,12]. HDACs are present in almost all organisms and at least, eighteen types of HDACs have been identified and classified into four broad classes.

First eleven of these eighteen types of HDAC contain divalent Zn^{2+} cation as catalytic machinery, and the other seven are known as sirtuins (1-7). They are NAD-dependent enzymes.

Class I HDACs include HDAC1-3 and 8 whereas Class II is made of HDACs 4-7, 9 and 10. All of the sirtuins are grouped into Class III HDACs, as their mechanism of deacetylation process is different from that of other HDACs. HDAC11, which shares

substantial homology with Class I and Class II HDACs, is the only member of Class IV HDACs. Because of the widespread biological effects of HDACs the inhibition of these enzymes have emerged as a new therapeutic approach to treat many diseases including cancer, neurodegenerative, hereditary, and inflammatory diseases[13-20].

HDAC inhibitors are a structurally distinct class of chemical compounds with unique structural elements to compliment the active site components. The generally known elements present in HDAC inhibitors are: (i) a zinc-binding moiety (ZBM) to co-ordinate with the catalytic metal ion; (ii) a hydrophobic cap group (HCG) to bind at the surface of the tunnel-like active site; and (iii) a hydrophobic linker (HYL) that joins both ZBM and HCG.

In this paper, we report the homology models of the active sites of *Pf*HDAC-1 and hHDAC 8 and the comparative analysis of these models to identify differences in the topology and amino acids that line their active sites.

Materials and methods

Computational resources

The protein sequence of PfHDAC1 was downloaded from the Uniprot server10. The crystal structures 1C3R (PDB code) from HDLP; a HDAC homologue from *A. aeolicus* bacterium and human HDAC8 (1T64 PDB code) were templates used for the building of the PfHDAC 1 model [21].

The X-ray crystal structure of hHdac 8 complexed with Trichostatin A (TSA) (PDB IT64) was recovered from protein data bank (PDB: www.rcsb.org). Protein preparatory wizard and prime was used to remove water molecules, unwanted monomeric units and ligand/ metal ion associated with the protein before it was imported into prime.

The computational studies were done on Ubuntu 10.10 (Maverick Meerkat) Linux desktop. Sequence alignment, homology modeling, loop and side chain refinement were carried out using Prime version 2.1 (Schrodinger LLC, New York, NY, 2009). Maestro version 9.0, which is a graphic unified interphase, was also used to generate the figures. [21]

Results and discussion

Zn^{2+}-binding site

The binding sites of the *Pf*HDAC-1 and hHDAC 8 are made up of a narrow channel that leads to a deep cavity (Figure 1) lined by amino acid residues essential for the enzyme's catalytic machinery. This was in agreement with previous findings. [4, 22] These narrow channels accommodate acetylated lysine, the aliphatic chain and the dimethyl aniline group of the inhibitor during substrate processing and they form several important interactions with the catalytic residue that line up the channel. The upper region of the binding site is primarily hydrophobic while at the bottom of this tunnel is found a Zn^{2+} ion that is useful in substrate processing.

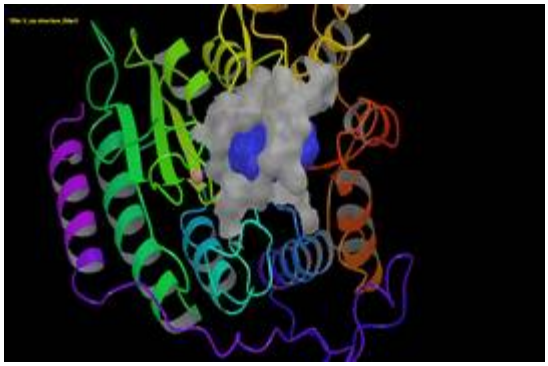

Figure 1. Surface representation of the active site entrance tunnel of hHDAC8 (Blue and White). The ribbons represent the α-helix region (red, blue, green and violet cylinder like coils) and β-strands (green arrows).

The Zn^{2+} ion in both models are pentacoordinated; three with the side chains Asp 174, 262 (Od1), His 176 (Nd1) and Asp 178, 267(O1), His 180 (Nd1) of *Pf*Hdac-1 and hHdac 8 respectively. The other two coordinates are with the hydroxyl group of a water molecule and the oxygen of the carbonyl of the hydroxamate group. In contrast to the *Pf*Hdac model, the hydroxyl group of the hHdac 8 is turned towards the Zn^{2+} ion, where it interacts with the warhead of the inhibitors. Another major role played by this Zn^{2+} ion in the catalytic process is the polarization of the carbonyl group of the acetylated lysine, reducing the overall entropy through coordination to both the substrate and the water molecule therefore increasing the nucleophilicity of the water by lowering the proton's pka value [4].

Inhibitor binding site

The inhibitor is made up of three parts namely; the metal binding domain known as the zinc-binding group (ZGB). This group coordinates to the catalytic metal atom within the binding site. The second part is a linker domain that could be an aliphatic or partly aromatic group, which binds in the hydrophobic channel and positions the ZBG and capping group for interactions in the active sites. Finally, there is a hydrophobic capping group that acts as a surface recognizing entity that can be elaborated upon in order to achieve isozyme selectivity and interacts with residues at the active sight entrance [23-26].

The inhibitor coordinates the Zn^{2+} ion in a bidentate fashion and simultaneously contacts residues likely to be involved in catalysis (His 138,139,Tyr 301 and His 142,143, Tyr 306 for *Pf*Hdac-1 and hHdac8

respectively) by the hydroxamate moiety (Table 1). This moiety is highly involved in a complicated network of hydrogen bonds with His 142,143,180, Asp 178 and Tyr 306 to the extent that His 142 and 143 hydrogen bond to a water molecule; while a third hydrogen bond from Tyr 306 polarizes the carbonyl group of the acetyl lysine in hHdac8 [27]. In *Pf*Hdac-1, the moiety forms hydrogen bond interaction with His 138,139 residues which in turn hydrogen bond with Asp 172 and 262 hence creating a charge relay system whose interactions enhance the basicity of the N(e) thereby allowing it to abstract proton.

Table 1. Key binding site residues of *Pf*HDAC-1 and hHDAC 8 and the nature of their interaction with ligand and/or metal ion.

*Pf*HDAC-1		hHDAC 8	Nature of interaction
1.	Pro 25	–	Hydrophobic interaction
2.	Thr 96	Tyr 100*	Polar interaction
3.	Asp 97	Asp 101	Polar interaction
4.	His 138	His 142	Catalytic residue
5.	His 139	His 143	Catalytic residue
6.	Gly 147	Gly 151	Hydrophobic interaction
7.	Phe 148	Phe 152	Hydrophobic interaction
8.	Asp 172	Asp 176	Catalytic residue
9.	Asp 174	Asp 178	Coordinates Zn^{2+}
10.	His 176	His 180	Coordinates Zn^{2+}
11.	Phe 203	Phe 208	Hydrophobic interaction
12.	Asp 262	Asp 267	Coordinates Zn^{2+}
13.	Leu 269	Met 274*	Hydrophobic interaction
14.	Tyr 301	Tyr 306	Polar/Hydrophobic interaction

* Residues that are not conserved.

The linker region of the inhibitor fits into the narrow channel with hydrophobic walls lined by the residues Gly 151, Phe 152, His 180, Phe 208, Met 274 and Tyr 306 and gives rise to lipophilic interaction with these residues. All the residues that the linker is in contact with are conserved except for Met 274 in hHdac 8, which is Leu 269 in *Pf*Hdac-1 (Table 1 and Figure 4) [22, 23, 26]. A previous report [26] suggests that Met 274 diminishes the hydrophobic character of the pocket that usually accommodates the aliphatic part of the incoming acetylated lysine.

Potassium binding site

Figures 2 and 3 show K^+ ion close to the Zn binding site. According to Vannini *et al* [22], the K^+ ion-binding site close to the Zn binding site is directly connected to this site and is coordinated by the main-chain carbonyl oxygen of Asp 178, His 180 of hHDAC 8 and Asp 174, His 176 of *Pf*HDAC 1 whose side chains are involved in Zn chelation. The presence of this K^+ ion in the active site is assumed to influence the catalytic mechanism of the deacetylation in many ways.

Figure 2. hHDAC 8 receptor-ligand surface with the active site Zn^{2+} (green sphere), K^+ ion (Peach sphere) and the TSA ligand (Ball and Stick representation).

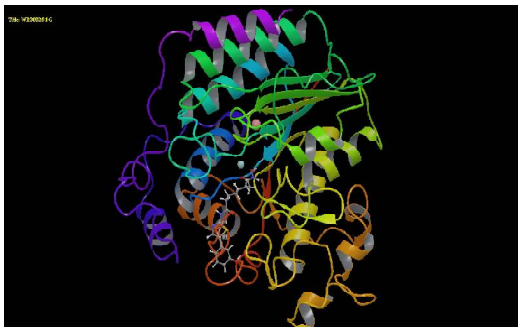

Figure 3. *Pf*HDAC 1 receptor-ligand surface with the active site Zn^{2+} (green sphere), K^+ ion (Peach sphere) and the TSA ligand (Ball and Stick representation).

Some of these include an increase in the positive electrostatic potential in the active site that could stabilize the oxyanion formed in the transition state of the deacetylation reaction, and stabilize the negatively charged acetate product. K^+ ion also influences the correct orientation and properties of Asp 176 and 172 hHDAC 8 and *Pf*HDAC 1 respectively. These residues are believed to form a buried charge-relay system with His 142 and 138 [1]. It has been reported that substrate binding becomes weak without the presence of K^+ ion near the active site in hHDAC 8 [28-30].

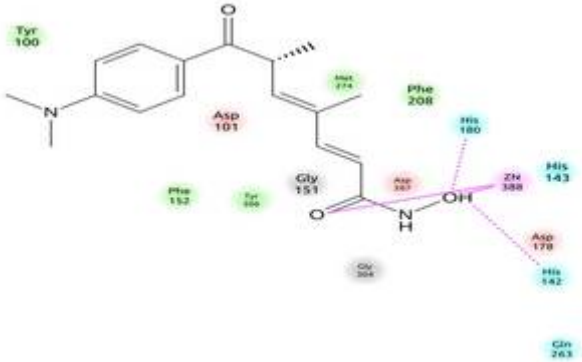

Figure 4. Ligand interaction diagram of hHDAC 8 model showing the critical interactions undergone by TSA (black) and the zinc metal ion (pink). The residues undergoing hydrophobic interactions are shown in green sphere, residues that coordinate to Zn^{2+} are shown in peach sphere, and those residues with polar interaction are shown in blue sphere.

Conclusion

Homology models of the active sites of *Pf*HDAC 1 have been compared to that of hHDAC 8. The delicate differences in the topology and amino acids that line the binding pocket, especially the exterior surface at the entrance of the tubular Zn-bound active site might be exploited to design selective *Pf*HDAC 1 inhibitors as potential antimalarials. With the increasing resistance of malaria parasite to available drugs, there is an urgent demand to develop new antimalarial drugs that will not interfere with the human cell.

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