The International Journal of Biochemistry & Cell Biology 41 (2009) 736–739

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Molecules in focus

Zn(II)-dependent histone deacetylase inhibitors: Suberoylanilide hydroxamic acid and trichostatin A

Rachel Codd[a,*], Najwa Braich[a], Joe Liu[a], Cho Zin Soe[a], Amalie A.H. Pakchung[b]

[a] School of Medical Sciences (Pharmacology) and Bosch Institute, University of Sydney, Camperdown, NSW 2006, Australia
[b] School of Chemistry, University of Sydney, Camperdown, NSW 2006, Australia

ARTICLE INFO

Article history:
Received 16 April 2008
Received in revised form 2 May 2008
Accepted 2 May 2008
Available online 3 August 2008

Keywords:
Zn(II)-dependent histone deacetylases
Suberoylanilide hydroxamic acid
Trichostatin A
Hydroxamic acids
Chemical biology

ABSTRACT

Suberoylanilide hydroxamic acid (SAHA, vorinostat, Zolinza®) and trichostatin A (TSA) are inhibitors of the Zn(II)-dependent class I and class II histone deacetylases (HDACs), which are enzymes that operate in concert with histone acetyltransferases (HATs) to regulate the acetylation status of the ε-amino group of lysine residues of nucleosomal histones in chromatin. An increased level of histone acetylation resulting from the SAHA or TSA inhibition of Zn(II)-dependent HDACs relaxes the chromatin structure and upregulates transcription. The links made in the 1990s between the inhibition of HDAC activity and the suppression of tumor growth have brought the design of HDAC inhibitors (HDACi) to the forefront of oncology research. SAHA has anticancer activity against hematologic and solid tumors and has been approved by the FDA for the treatment of cutaneous T-cell lymphoma. The increased molecular-level understanding of class I and class IIa HDACs from X-ray crystallography highlights differences in the residues distal to the active site and in the cavity size, which has implications for HDACi substrate specificity and enzyme mechanism. Results from HDAC-focussed activity-based protein profiling experiments may lead to the design of molecules that are class-specific HDACi.

1. Introduction

The regulation of gene transcription in eukaryotes is directed in part by the dynamic changes in the structure of chromatin between 'open' and 'closed' forms which itself reflects the structure of the constituent histone-DNA nucleosomal packages. Post-translational modifications to histones, which include acetylation, methylation, phosphorylation, ubiquitinylation, ADP-ribosylation and deimination, contribute to the structural remodelling of chromatin (Minucci and Pelicci, 2006). These epigenetic changes are thought to play a role in the onset and progression of cancer, which is fueling drug development efforts that target chromatin remodelling enzymes (Bolden et al., 2006).

Of these post-translational modifications, the reversible acetylation of histones is the most abundant (Fischle et al., 2002). The acetylation status of the ε-amino group of lysine residues of nucleosomal histones is regulated by the activity of histone acetyltransferases (HATs) and histone deacetylases (HDACs). Generally, there is a positive correlation between the level of histone acetylation and transcriptional activity: hyperacetylation (upregulated HAT and/or downregulated/inhibited HDAC activity) relaxes the chromatin structure ('open' form) and increases transcriptional activity while hypoacetylation (downregulated HAT and/or upregulated HDAC activity) condenses the chromatin structure ('closed' form) and decreases transcriptional activity. HDACs have been grouped into four classes. Class I, class II (which have been further divided into class IIa and class IIb) and class IV HDACs are Zn(II)-dependent metallohydrolases. Class III HDACs are NAD^+-dependent enzymes called sirtuins, which are structurally unrelated to class I, class II or class IV HDACs (de Ruijter et al., 2003; Minucci and Pelicci, 2006).

Suberoylanilide hydroxamic acid (SAHA, *N*-hydroxy-*N*′-phenyloctanediamide, vorinostat, Zolinza®) is a synthetic hydroxamic acid, which is structurally related to the natural product, trichostatin A (TSA, 7-[4-(dimethylamino)phenyl]-*N*-hydroxy-4,6-dimethyl-7-oxo-(2*E*,4*E*,6*R*)-2,4-heptadienamide), produced by selected strains of *Streptomyces platensis*, *Streptomyces hygroscopicus* Y-50 or *Streptomyces sioyaensis*. Hydroxamic acids have a high affinity to biometals, including Fe(III), Ni(II) and Zn(II), which confers significant value upon these agents in biomedicine (Codd, 2008). The *R*-isomer of TSA was one of the first noted inhibitors of HDAC (HDACi) which increased in an enantioselective fashion, the levels of histone acetylation in various mammalian cell lines (Yoshida et al., 1990). The synthesis of SAHA and its potency to induce differentiation of murine erythroleukemia (MEL) cells was first reported in 1996; SAHA or the first generation HDACi, hexam-

* Corresponding author. Tel.: +61 2 9351 6738; fax: +61 2 9351 4717.
E-mail address: rcodd@med.usyd.edu.au (R. Codd).

1357-2725/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biocel.2008.05.026

ethylenebisacetamide, induced differentiation of MEL cells at 2 μM or 5 mM, respectively; SAHA shows inhibition at nanomolar concentrations for both class I and class II HDACs via coordination to the catalytic Zn(II) (Richon et al., 1998).

2. Structure

SAHA and TSA (Fig. 1) comprise a hydroxamic acid-based metal-binding domain that coordinates the catalytic Zn(II) in the HDAC active site, a 5 (TSA) or 6 (SAHA)-membered carbon-based linker that mimics the C_α functional group of lysine, and a hydrophobic motif that interacts with the periphery of the HDAC binding pocket (Fig. 2A). These features have been identified from structure activity relationships as requirements for an effective HDACi (Mai, 2007).

Due to space limitations, this article develops further SAHA-bound and not TSA-bound HDAC structures (one TSA-bound HDAC structure (Finnin et al., 1999) is given in Fig. 2A and B (panel i)). X-ray crystal structures of SAHA bound to: human HDAC8 (Somoza et al., 2004); a class I HDAC homologue from *Aquifex aeolicus* (Finnin et al., 1999); a class IIb HDAC homologue from *Bordetella/Alcaligenes* strain FB188 (Nielsen et al., 2005); and human HDAC7 (Schuetz et al., 2008) have been solved. In these structures, the catalytic

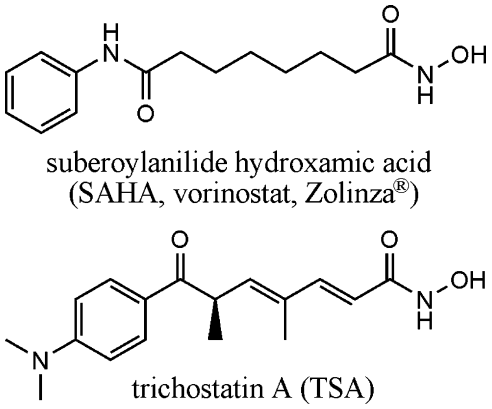

Fig. 1. Structure of suberoylanilide hydroxamic acid (SAHA) and trichostatin A (TSA).

Zn(II) is coordinated by two monodentate Asp residues and one His residue (Fig. 2B). In the absence of SAHA, the Zn(II) is bound to a water molecule which is the predicted source of the nucleophile that attacks the carboxyl group of the acetylated Lys substrate during catalysis (Finnin et al., 1999). The contiguous His residues that

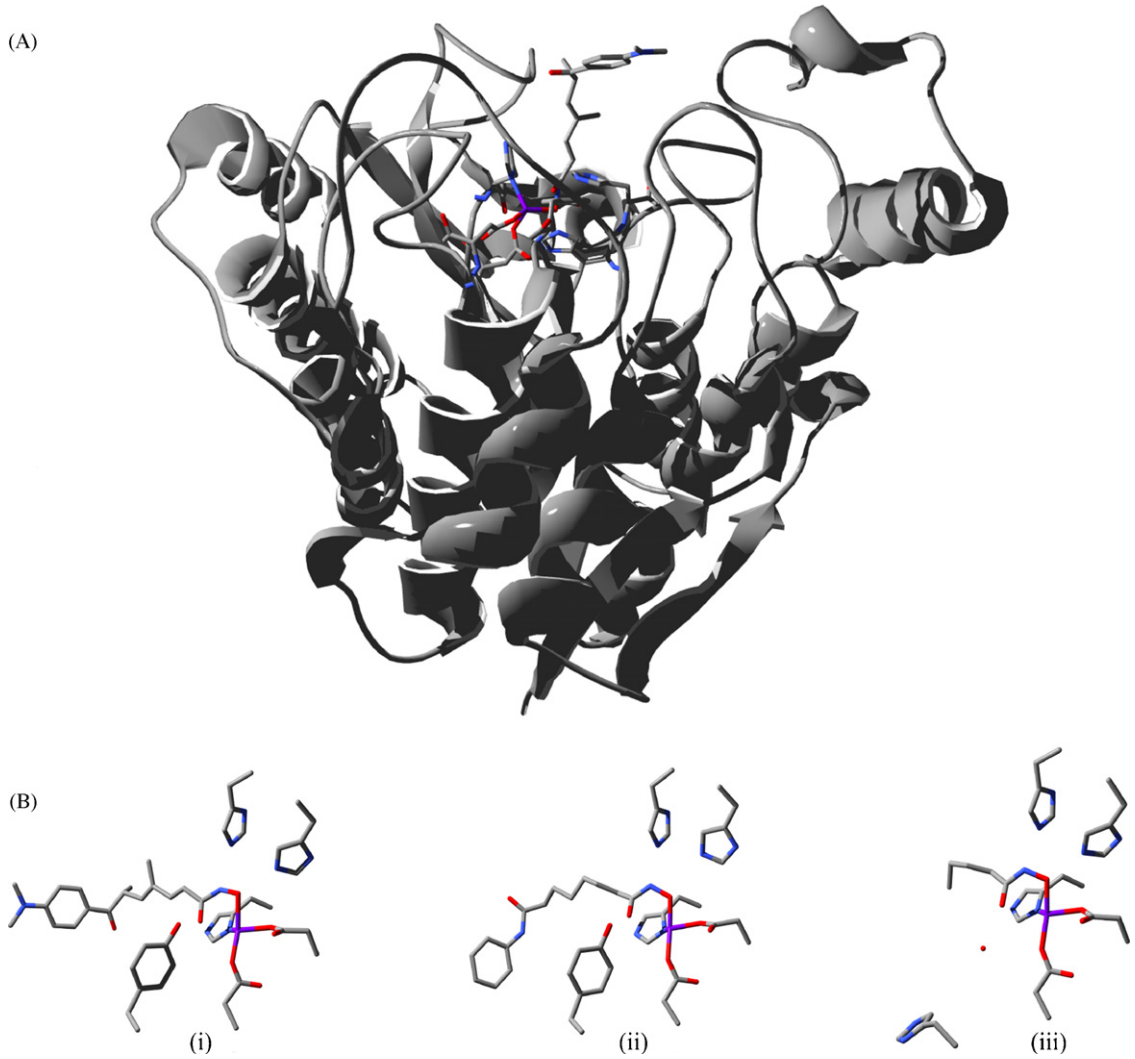

Fig. 2. (A) TSA-bound to the class I HDAC homologue from *A. aeolicus* (PDB: 1C3R) and (B) TSA (i) or SAHA (ii) bound to the catalytic Zn(II) site of the class I HDAC homologue from *A. aeolicus* (PDB: 1C3R or 1C3S, respectively; Finnin et al., 1999) or SAHA (iii) with poorly defined electron density of the *N*-phenylpropanamide region, bound to the class IIa human HDAC7 (PDB: 3C0Z; Schuetz et al., 2008).