Crystal structure of a Class IIb Histone Deacetylase Homologue from *Pseudomonas aeruginosa*

A. Krämer[1], J. Carrera-Casanova[2], T. Wagner[1], Ö. Yildiz[2], F.J. Meyer-Almes[1]

[1] University of Applied Science, 64287 Darmstadt, Germany [2]Max Planck Institute of Biophysics, 60438 Frankfurt am Main, Germany

Introduction

Histone deacetylases (HDACs), Acetylpolyamine-Amidohydrolases (APAHs) and Acetoin Utilization Proteins (AcuCs) belong to an ancient protein superfamily known as the histone deacetylase superfamily[1]. Especially Histone deacetylases raised much attention due to their important role in the cell cycle and differentiation and therefore, they became a novel target for chemo therapy. The HDACs are classified in four groups based on their sequence and domain organization: Class 1, 2a, 2b, 3 and 4.[2] Here we present the first structure of a prokaryotic Histone like protein from *P. aeruginosa,* which shows the highest homology with class 2b HDACs.

Overall Structure

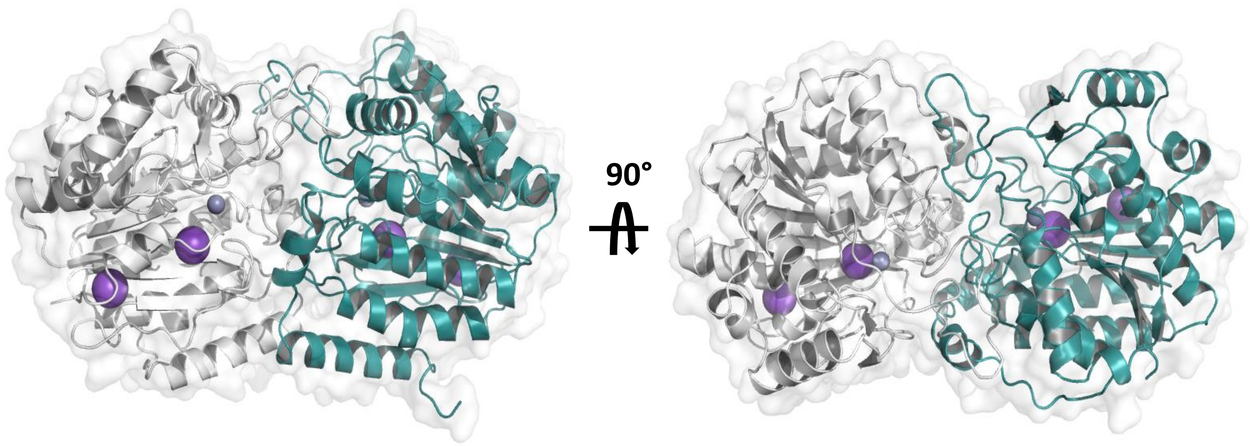

Fig 1: Cartoon representation of PA3774. The Protein is a homo-dimer. One monomer is colored in teal and the other one in grey. The zinc ions are shown as blue and the potassium as purple spheres.

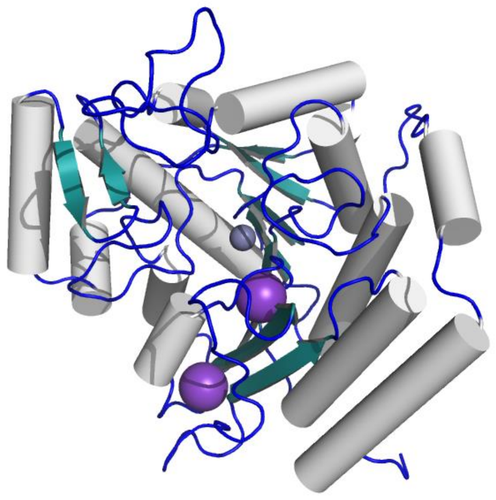

Fig 2: Monomer view: Helices colored in grey, β-sheets colored in teal, loop regions in blue. Potassium ions are shown as purple spheres and the Zn ion as blue sphere.

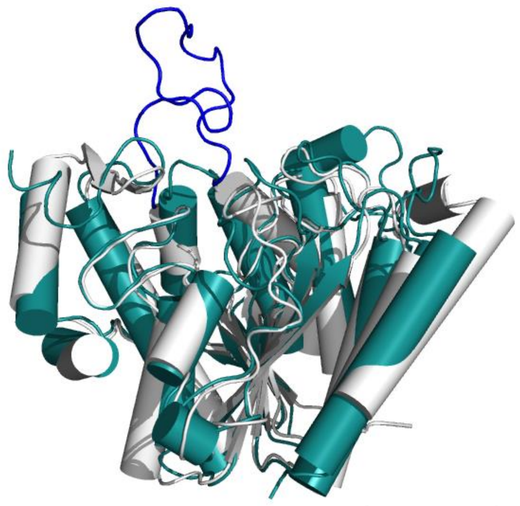

Fig 3: Structure comparison of PA3774 (grey) and HDAC8 (teal) [3]. The backbone structure is highly conserved. The key difference is a flexible loop region insert (colored in blue) is involved in dimer formation.

- Dimer with 41 kDa per monomer
- Open α/β fold: central eight stranded parallel β-sheet surrounded by 14 helices and two smaller antiparallel β-sheets
- Penta-coordinated zinc ion in the active site, two octahedral-coordinated potassium
- 35% sequence identity with the second domain HDAC6 (belongs to class 2b HDAC)
- Flexible loop region is involved in dimer formation

Data Collection

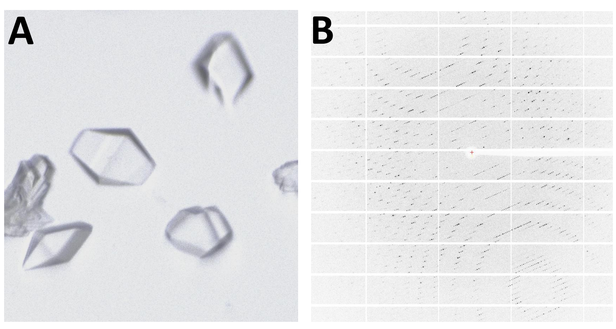

Fig 4: A Crystals of PA3774 grown in 0.5 M K_2HPO_4, 0.5 M Na_2HPO_4, 0.1 M $(NH_4)_2SO_4$ pH 7.5 at 20°C. The size of the crystals were between 0.05 and 0.30 mm **B** Diffraction image collected at the Swiss light source (SLS) in Switzerland.

Data collection and refinement statistics

Dataset	PA3774 SATFMK	PA3774 H143A
Space group	$P4_12_12$	$P4_12_12$
Cell dimensions	a=b= 81.7 c=205.2 α=β=γ=90°	a=b= 81.5 c=205.3 α=β=γ=90°
I/sd(I)	16.0 (2.1)	16.9 (4.8)
Wavelength (Å)	0.97902	0.97903
Resolution range (Å)	75.95 - 1.71	75.80 - 1.99
Overall observations	578699	750443
Unique reflections	75585	48272
Completeness (%)	100 (100)	99.4 (94.1)
Multiplicity	7.3 (8.0)	16.4 (11.7)
Rmerge	0.088 (1.175)	0.135 (0.343)
Rcryst	0.1806	0.1930
Rfree	0.1974	0.2285

Inhibitor Complex

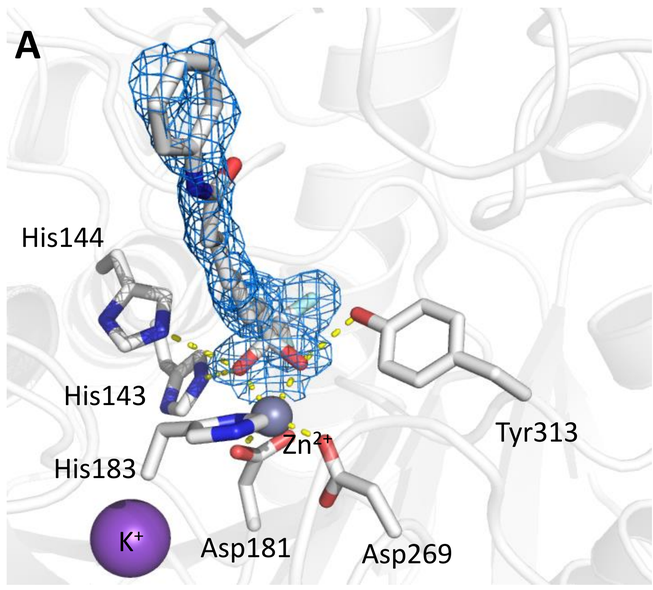

Fig 5: A Binding of the highly affine SATFMK to the active site. Essential H-Bonds are indicated as yellow dash lines. The electron density omit map is contoured at 1σ. **B** Structure of SATFMK **C** IC50 measurement of SATFMK.

- PA3774 gets inhibited by most common HDAC inhibitors
- SATFMK binds with highly affinity (IC50 < 9.7 nM)
- Electron density indicates that the ketone is binding in its hydrated form

Mutational Studies

Motivation:

- Mutation of every amino acid, which seems to make excessive bonds to the inhibitor molecule in Figure 5 to non-reactive one
- Additionally Y313 was mutated to Histidine, which is the typical motif in class 2a HDACs

Relative activities of PA3774 mutants in %

Mutant	Boc-Lys(TFA)-AMC	Boc-Lys(Ac)-AMC
Wild type	100.0 ± 2.3	100.0 ± 3.6
H143A	1.0 ± 0.1	no activity
H144A	1.4 ± 0.1	no activity
Y313F	97.5 ± 3.3	no activity
Y313H	84.7 ± 2.8	no activity

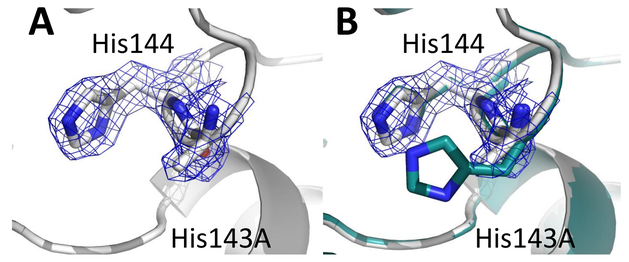

Fig 6: A Close up of H143A mutant with electron density omit map contoured at 1σ. **B** Overlay with the native structure (teal). The backbone structure remains unchanged by the mutation

Results:

- Every mutated amino acid is essential for the mechanism
- The structure of the H143A mutant proofs that no change in the backbone structure is responsible for the activity loss
- Interesting are the Y313 mutants due to the fact of their complete loss against the acetylated substrate and the nearly unaffected turnover against the trifluoroacetic substrate

Summary & Conclusion

- This is the first solved structure of a lysine deacetylase from the human pathogen *P. aeruginosa*.
- Since the high sequence homology to HDAC class 2b, this structure could serve as a model for this class of enzymes
- The protein is annotated as an acetylpolyamine-amidohydrolase (APAH) but it is not able to metabolize any tested acetyl-polyamine. On the contrary, it shows great activity against common HDAC substrates, but the natural substrate is still unknown
- (De-)Acetylation of prokaryotic proteins has become a fascinating new field[4].

References

1. Leipe, D.D. and D. Landsman, Histone deacetylases, acetoin utilization proteins and acetylpolyamine amidohydrolases are members of an ancient protein superfamily. Nucleic Acids Res, 1997. 25(18): p. 3693-7.
2. Haberland, M., R.L. Montgomery, and E.N. Olson, The many roles of histone deacetylases in development and physiology: implications for disease and therapy. Nat Rev Genet, 2009. 10(1): p. 32-42.
3. Vannini, A., et al., Crystal structure of a eukaryotic zinc-dependent histone deacetylase, human HDAC8, complexed with a hydroxamic acid inhibitor. Proc Natl Acad Sci U S A, 2004. 101(42): p. 15064-9.
4. Ouidir, T., et al., Proteomic profiling of lysine acetylation in pseudomonas aeruginosa reveals the diversity of acetylated proteins. Proteomics, 2015.