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

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**Introduction**

Histone deacetylases (HDACs), Acetylpolymamine-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**

- 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**

**Inhibitor Complex**

- **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 %**

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Boc-Lys(TFA)-AMC</th>
<th>Boc-Lys(Ac)-AMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>100.0 ± 2.3</td>
<td>100.0 ± 3.6</td>
</tr>
<tr>
<td>H143A</td>
<td>1.0 ± 0.1</td>
<td>no activity</td>
</tr>
<tr>
<td>H144A</td>
<td>1.4 ± 0.1</td>
<td>no activity</td>
</tr>
<tr>
<td>Y313F</td>
<td>97.5 ± 3.3</td>
<td>no activity</td>
</tr>
<tr>
<td>Y313H</td>
<td>84.7 ± 2.8</td>
<td>no activity</td>
</tr>
</tbody>
</table>

**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 acetylpolymamine-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**