

X-ray crystallographic studies of a novel histone demethylase PHF8

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Introduction

The reversible process of histone methylation and demethylation has been implicated multiple biological processes including heterochromatin formation, X-inactivation, genomic imprinting, and silencing of homeotic genes. Aberrant histone methylation has been linked to a number of human diseases such as leukemia and prostate cancers. Defects in PHF8 were associated with diseases such as cleft lip, cleft palate, X-linked mental retardation (XLMR) and pathogenesis of autism. These findings link PHF8 to cognitive function and midline formation¹³. Here, we show that human PHF8 is a novel H3K9me2/1 demethylase and solve the crystal structures of the catalytic-core domain of PHF8 with α -KG in the presence of Fe²⁺.

Results and Discussion

X-ray diffraction studies were performed at liquid nitrogen gas at the Photon Factory (NE3A and NW12A). The crystals are diffracted to 2.1 Å resolutions. The data were processed using program HKL2000. The crystals belong to the space group P2₁2₁2₁. The unit cell dimensions were determined as $a = 52.09$ Å, $b = 52.53$ Å and $c = 134.82$ Å. Using the structure of JHDM1a as the search model (PDB 2YU2), molecular replacement solutions were found using PHASER. The crystal contains one protein per asymmetric unit, giving a crystal solvent content of 54%. The model was built manually in the program COOT, and refinement was carried out with

CNS and REFMAC5.

The overall structure of c-PHF8 consists of 16 α -helices and 12 β -strands, arranged in a manner similar to the structure of known JMJC domain-containing histone demethylases. Interestingly, a strong hydrophobic core, composed of F246, I248, F279, W282, F292, F293, I318, I349 and L353, is adjacent to the active center. An examination of the structure shows that the disease-causing mutation of PHF8 causes an alteration the F279 in hydrophobic core adjacent to the active center of the enzyme, and thus resulting in the loss of enzymatic activity.

From biochemical data, we found that PHF8 is a novel H3K9me2/1 demethylase. The structure of the catalytic core domain of PHF8 reveals a conserved overall fold of the JMJC domain-containing demethylase, a distinctive substrate specificity of H3K9. Thus, our structural and biochemical results presented here have provided mechanistic insights into the molecular function of PHF8 and the pathogenesis of PHF8 aberration in humans.

References

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