Introduction

Hydratases provide access to secondary and tertiary alcohols by stereospecifically adding hydroxyl groups to carbon-carbon double bonds without the need for costly cofactor recycling. Also taking into account that many hydration reactions are impossible with current chemistry, enzymatic hydration is highly interesting on an industrial scale\(^1\). Here, oleate hydratase originating from *Elizabethkingia meningoseptica* (OhyA) was structurally and functionally characterized in the presence of the flavin cofactor. A structure-based mutagenesis study in concert with biochemical analyses allowed us to derive the first reaction mechanism for this class of hydratases.

![Figure 1: Stereospecific hydration of oleic acid, yielding (R)-10-hydroxystearic acid, catalyzed by oleate hydratase.](image)

Role of flavin

The 3D structure implied a structural role of FAD, since E122 is located on a loop which becomes more ordered upon cofactor binding. Biochemical analyses proved that the loss of FAD correlated with a loss of functionality (Figure 3A). ThermoFluor measurements indicated that deflavination does not lead to an overall destabilization of the enzyme (Figure 3B). However, OhyA harboring two-electron reduced FAD was 6- to 7-times more active than the oxidized control. Interestingly, some activity of the Y241F variant could be restored upon cofactor reduction (Figure 3C and 3D).

![Figure 4: Proposed reaction mechanism](image)

Reaction mechanism

We propose that the essential E122 activates water and Y241 confers protonation of the double bond in a supposedly concerted reaction (Figure 4).

The role of FAD might be dual:
- Proper organization of the active site
- Stabilization of partial positive charge

Moreover, reduced FAD might take over some protonation activity from Y241, as observed for the Y241F variant\(^2\).

![Figure 3: Investigating the cofactor dependence of OhyA. Cofactor removal via chemical depletion (Apo-OhyA) or by exchanging conserved nucleotide binding residues (G69A, G71A) in the N-terminal Rossmann-type domain (A). ThermoFluor measurements of OhyA and variants (B). UV-Vis absorption spectra (C) and activities (D) of purified OhyA and variants harvesting oxidized and reduced FAD.](image)

Summary and conclusion

\- First 3D structure of a fatty acid hydratase in complex with the flavin cofactor
\- A structure-based reaction mechanism that rationalizes the requirement of FAD
\- These findings will help to develop hydratases for industrial applications in the near future


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