SUBSTRATE-BASED PROTEIN ENGINEERING OF A FLAVOPROTEIN OXIDASE FOR IMPROVED ALCOHOL OVER-OXIDATION

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The oxidation of alcohols to the corresponding carbonyl compounds represents a convenient strategy for the selective introduction of carbon-acceptor units into carbohydrate-based starting materials from renewable resources. A simple system to accomplish this transformation is by using flavin-containing alcohol oxidases. However, with prim-alcohols, the oxidation does not necessarily stop at the aldehyde stage, but may furnish the carboxylic acid via 'over'-oxidation of the aldehyde hydrate.[1] In order to develop an alcohol oxidase for the efficient transformation of alcohols into carboxylic acids, we chose the recently discovered (5-hydroxymethyl)furfural oxidase (HMFO), which converts not only the eponymous (5-hydroxymethyl)furfural, but also a range of aromatic and allylic alcohols (Figure 1).[2]In order to improve the performance of HMFO for over-oxidation, we anticipated an improved stabilisation of the aldehyde hydrate in the active site to be a crucial factor. After inspection of the HMFO crystal structure, two positions were identified, where hydrogen bond donating and accepting amino acids were introduced, in order to stabilize the gem-diol moiety.[3] Indeed, one of the new HMFO variants exhibited a significantly increased activity for the formation of carboxylic acids from benzylic alcohols.

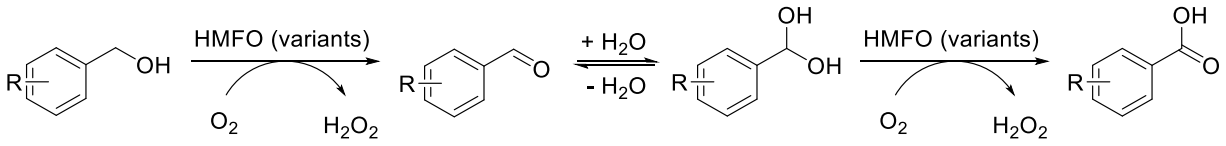

Figure 1 Oxidation of benzylic alcohols to carboxylic acids catalyzed by HMFO variants

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References

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