FSA-Catalyzed Carboligation: New Synthetic Opportunities from an Ancient Enzyme Scaffold

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1. Introduction

Aldol reactions constitute a powerful methodology for carbon-carbon bond formation in synthetic organic chemistry. Biocatalytic carboligation offers a green, uniquely regio- and stereoselective tool to perform this transformation. New enzymes from the transaldolase scaffold, such as fructose-6-phosphate aldolase (FSA) from E. coli, were recently shown to be unusually flexible in their substrate scope,[1] which renders them particularly valuable for the synthesis of complex polyfunctional targets.[2] So far, wild-type FSA has been demonstrated to utilize dihydroxypropanone, hydroxypropanone, 1-hydroxy-butane, and hydroxyethanal as aldol donor components for highly stereoselective carboligation reactions. Here we present our study to broaden the substrate scope even further for larger donor nucleophiles.

Figure 1. Aldol formation catalyzed by variant fructose-6-phosphate aldolases (FSA*)

2. Substrate Synthesis

A range of structurally varied compounds was prepared, taking into account the absolute requirement of the enzyme for the (C=O)-CH2OH moiety. A selection is listed in Figure 2.

3. Protein Engineering

In order to prepare the wt-FSA enzyme to accommodate larger nucleophiles, the active site-pocket was enlarged by rationally designed mutations (Figure 3). In particular, replacement of L163-building up a hydrophobic wall with interactions to the non-reactive part of the donor – by smaller residues was anticipated to make room for larger aliphatic substrates.

Figure 3. Differences in active-pocket-space between the wt and the L163A Mutant

4. Mutant Screening

Figure 4. Mutant-screening with different artificial substrates after 2 h

5. Product Characterization

Figure 5. 13C- and HSQC-NMR of new product (beta-anomer and open-chain form).

Conclusion

This study demonstrates that simple rationally reengineered protein variants, which have an expanded active site volume, allow to convert an unusually broad substrate range. We believe that the high stability of the decameric protein fold facilitates such variations. Further work in this direction is currently in progress.

References

Biotrans 2011, Italy, Giardini Naxos