Engineered Aldolases with Unusually Broad Nucleophile Specificity

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Enzymatic C-C bond forming reactions are highly attractive because of the unparalleled enantio- and diastereoselectivity of aldolases and related carboxylating enzymes, without a requirement for extensive protective group chemistry. Typically, these enzymes are quite flexible to accept a structurally broad range of aldehydes as electrophilic substrates but have a rather strict specificity for their nucleophilic aldon donor substrate, which limits their range of synthetic applications. [1]

Thus, we are currently attempting to modify the donor substrate scope of transaldolase B (TaB) [2,3] and fructose 6-phosphate aldolase (FSA) from E. coli by protein engineering. [2] Independent of substrate structure, both enzymes catalyse a stereospecific carbonylation resulting in the α-threo configuration. [3]

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**SUBSTRATE SYNTHESIS**

Donor Synthesis
- from: alcohols, aldehydes, aikenes, epoxides
- 1-hydroxyketone synthesis
  - a) RuO₄-catalyzed ketohydroxylation
  - b) KMnO₄ or NaOCl oxidation
- Dihydroxyacetone synthesis
- Cross-acylol condensation
- Epoxy substitution

Acceptor Synthesis
- 3-Hydroxy-propanal, synthesized by hydration of acrolein

**RESULTS**

- Synthesized a panel of various 1-hydroxy-2-alkanones as potential donor substrates and investigated their reactivity for a selected number of structure-based designed FSA variants
- Product stereoselectivity easily determined by NMR analysis of the stable cyclic hemiacetal isomers with 3-hydroxy-propanal as acceptor [3,4]
- Hydroxymethyl unit is an indispensable structural prerequisite for the aldor doner component
- α-branching of the hydroxyl ketones did not result in the formation of the desired products.

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**CONCLUSION**

TaB and variants tolerate only small donor modifications (R = methyl, ethyl, n-butyl, methoxyethyl)
- Structures presenting increasing sterical demand (R = tert-butyl, ethoxy methyl etc.) are for the first time favorably tolerated by engineered FSA variants

- Results indicate that the toolbox for biocatalytic carbonylation can be significantly expanded

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**EXPERIMENTS**

**PRODUCTS**

**REACTION KINETICS**

**ENZYMATIC STUDIES**

<table>
<thead>
<tr>
<th>Nr.</th>
<th>FSA variants</th>
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<tbody>
<tr>
<td>1</td>
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<tr>
<td>2</td>
<td>L107A</td>
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<td>3</td>
<td>A129G</td>
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<td>4</td>
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<tr>
<td>5</td>
<td>L163A</td>
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<td>6</td>
<td>L107A A129G</td>
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</tr>
<tr>
<td>10</td>
<td>L107A A129G L163A</td>
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150 mM donor
100 mM acceptor
50 mM triethanolamin buffer (pH 8.0)
100 μl enzyme solution (2 mg/ml)

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**REFERENCES**