ENGINEERING AMMONIA-LYASES FOR LYSINE TRANSFORMATION: FIRST STEPS TO GREEN PRODUCTION OF ADIPIC ACID

Verónica Sáez-Jiménez[a], Matteo Lambrugui[b], Elena Papaleo[b], Lisbeth Olsson[a] and Valeria Mapelli[a]

[a]Division of Industrial Biotechnology, Department of Biology and Biological Engineering - Chalmers University of Technology, Göteborg, Sweden, [b]Computational Biology Laboratory, Danish Cancer Society Research Center, Copenhagen, Denmark. saez@chalmers.se

INDUSTRIAL BIOTECHNOLOGY

Adipic acid is one of the most important dicarboxylic acid for commercial purposes, mainly used as building block for nylon polymers. Around three million tons per year of adipic acid are produced by chemical synthesis using petrochemical resources. The current chemical production process has serious consequences for the environment. Therefore, the implementation of a by a bio-based process using renewable feedstocks would be highly beneficial for the society and the environment.

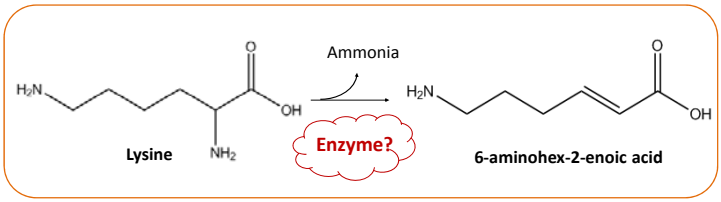


Figure 1: Conversion of L-lysine into 6-aminohex-2-enoic acid as part as the metabolic pathway (not shown) for adipic acid production.

The construction of a microbial metabolic pathway to produce adipic acid using L-lysine as precursor is a potential alternative. The first step of the pathway converts L-lysine into 6-aminohex-2-enoic acid (6-AHEA) (Fig.1), however, no enzymes able to carry out this reaction have been disclosed yet. The main goal of the research is the generation a novel enzyme activity for this conversion.

STUDY OF HISTIDINE and 3-METHYLASPARTATE AMMONIA-LYASES

The enzymatic activity necessary to catalyze the required deamination is defined as ammonia lyase and results in the removal of the α-amino group. Histidine ammonia lyase (HAL) and 3-methylaspartate-ammonia lyase (MAL) are known and characterized enzymes acting on histidine and 3-methylaspartate, respectively. They were selected among the top candidates to be engineered to catalyze the deamination of L-lysine.

HAL from *Pseudomonas putida* and MAL from *Clostridium tetanomorphum* and *Carboxydothermus hydrogenoformans* were recombinantly produced in *E.coli* and purified. The capability of these enzymes to catalyze L-lysine deamination was tested (Figure 2).

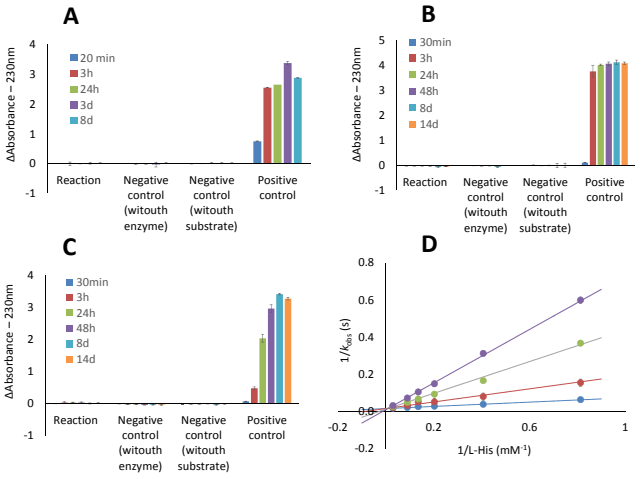


Figure 2: Activity of MAL from Clostridium tetanomorphum (A), MAL from Carboxydothermus hydrogenoformans (B) and HAL from Pseudomonas putida (C) with L-lysine. HAL reaction was performed in Tris 100mM pH 9,2 $ZnCl_2$ 10 µM and MAL reaction mixture consisted of Tris 100mM pH 9, $MgCl_2$ 20mM and KCl 1mM. 10 µg/ml of enzyme and 30 mM of L-lysine were used in all the reactions. Negative controls were made in the absence of enzyme and substrate. D, Inhibition test of HAL with L-lysine. Inverse plots of histidine deamination by HAL in the presence of 0 mM (blue), 20 mM (red), 40 mM (green) and 70 mM (purple) of L-lysine.

None of the native ammonia-lyases tested showed activity towards L-lysine in the tested conditions (Fig. 2 A-C). On the other hand, L-lysine was proven to be a competitive inhibitor of HAL (Fig. 2D). The constant of inhibition (K_i) obtained was 5,6 ± 1,1 mM and the relation K_i / K_m was 1.45, which showed that L-lysine is a strong inhibitor of HAL.

COMPUTATIONAL STUDY AND SITE-DIRECTED MUTAGENESIS ON MAL

Computational structural biology methodologies were combined with protein engineering techniques in order to design MAL mutant enzyme variants potentially active on L-lysine.

In-silico saturation mutagenesis tools were used to model all the possible mutations in the active site or its surroundings that are expected to increase affinity for the L-lysine substrate. Following the results of the structural study and using a rational design, the residues Cys361 and Leu384, located in the binding pocket, were mutated.

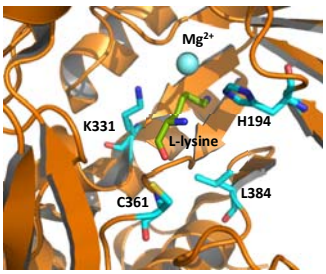
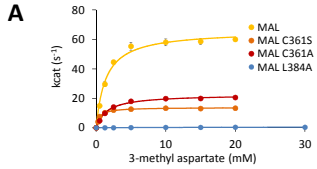


Figure 3: Detail of the crystal structure of MAL from Citrobacter amalonaticus showing the binding pocket with the catalytic residues K331 and H194 and the mutated residues C361 and L384.

The MAL mutant variants were produced and purified, and the activity on the natural substrate (3-methyl aspartate) and L-lysine was tested by monitoring the production of 6-AHEA and the release of ammonia (Fig. 4).The kinetic constants towards 3-methyl aspartate were impaired to different extents and no L-lysine deamination was observed.



	kcat (s-1)	Km (mM)
MAL	66 ± 2	1,4 ± 0,2
MAL C361A	23 ± 1	1,6 ± 0,1
MAL C361S	14 ± 1	0,5 ± 0,04
MAL L384A	1 ± 0,02	23,0 ± 1,4

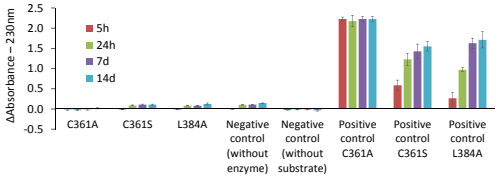


Figure 4: Kinetics and kinetic constants (A) and activity on L-lysine (B) of Clostridium tetanomorphum MAL and mutant variants. Reaction mixtures made in Tris 100mM pH 9, $MgCl_2$ 20mM and KCl 1mM. Activity on L-lysine was measured as the increment of absorbance at 230nm.

CONCLUSIONS

The studied HAL and MAL did not showed the capability to deaminate lysine. On the contrary, L-lysine was showed to be an inhibitor on HAL. Preliminary results obtained in the protein engineering of MAL did not showed positive results. On going studies are being performed.