DEAMINATION REACTIONS AS PART OF THE METABOLIC PATHWAY FOR THE PRODUCTION OF ADIPIC ACID

Verónica Sáez-Jiménez[a], Željka Sanader Maršić[b], Matteo Lambrugui[b], Robin van Havere[a], Elena Papaleo[b], Lisbeth Olsson[a], Valeria Mapelli[a]

[a]Division of Industrial Biotechnology, Chalmers University of Technology, Göteborg, Sweden
[b]Computational Biology Laboratory, Danish Cancer Society Research Center, Copenhagen

Deamination of lysine or β-lysine (**Fig. 1**) are enzymatic reactions that have a high biotechnological interest as part of the metabolic pathways for the production of adipic acid, that is the industrially most important di-carboxylic acid. However, enzymes able to deaminate these substrates have not been identified so far.

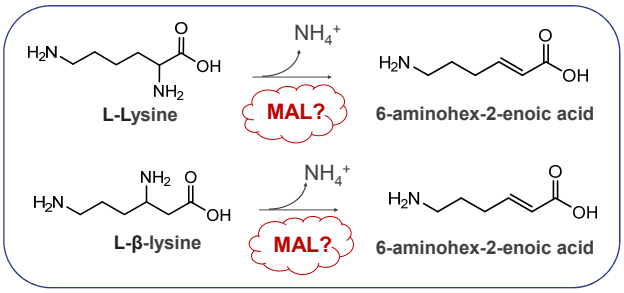

Figure 1: *Conversion of L-lysine or L-β-lysine into 6-aminohex-2-enoic acid as part of the metabolic pathway for adipic acid production from L-lysine.*

We selected 3-methylaspartate-ammonia lyase (MAL, EC 4.3.1.2) as an enzyme that potentially could act on or could be engineered to act on the target substrates

Here, we aimed at studying MAL, characterizing the binding and inhibition properties of the target substrates and other five additional chemicals with a similar structure to both the natural and the target substrates were also tested (**Fig. 2**, left panel).

1. The substrates spatially fit in the pocket

Docking results showed that all the tested substrates spatially fit in the catalytic pocket (**Fig. 2**). However, the binding affinities obtained for the tested ligands are lower than for the natural substrate, suggesting that the ligands are weak or very transient binders.

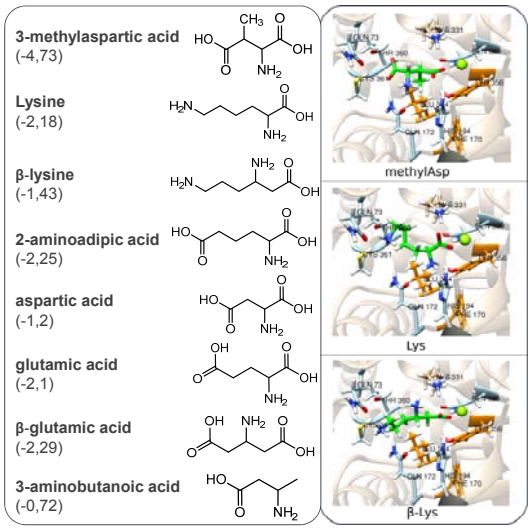

Figure 2. *Left panel, comparison of the natural substrate and the docked ligands with the binding affinities values (kcal/mol) in brackets. Right panel, MAL catalytic pocket with the natural substrate position (upper part) and the example of the complexes of MAL with β-Lysine and lysine obtained from docking results.*

2. β-lysine is a competitive inhibitor

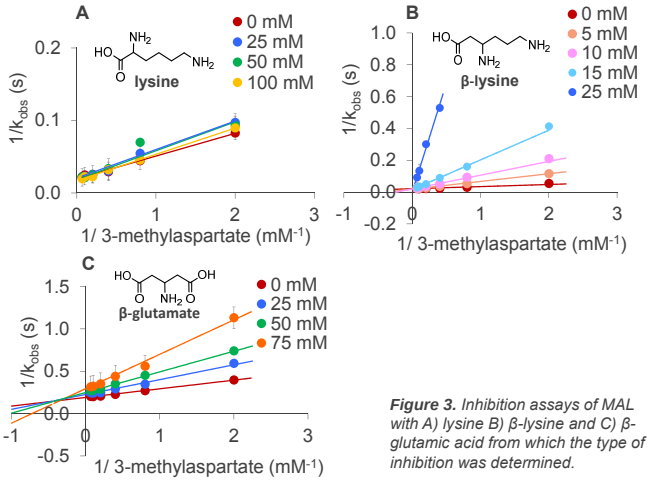

Figure 3. *Inhibition assays of MAL with A) lysine B) β-lysine and C) β-glutamic acid from which the type of inhibition was determined.*

β-lysine and β-glutamic acid are competitive and mixed inhibitors of MAL, respectively, indicating that they bind close to or in the catalytic pocket (**Fig. 3**). Interestingly, lysine does not inhibit MAL. These results suggest that the amino group positioned in the β-carbon (as in β-lysine and β-glutamic acid) is needed for the binding of substrate in MAL.

3. Aspartate shows a poor binding in MAL compared to 3-methylaspartate

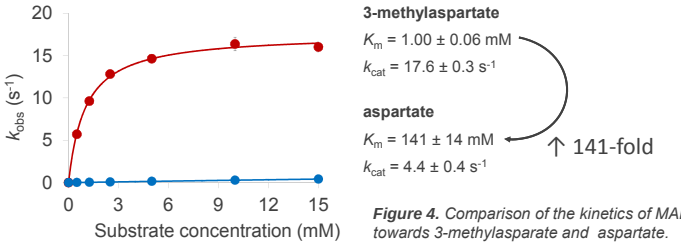

Figure 4. *Comparison of the kinetics of MAL towards 3-methylasparate and aspartate.*

Of the 7 tested compounds, MAL was active only towards aspartate, showing a decrease in the apparent affinity constant of 141-fold compared to 3-methyl-aspartate (**Fig. 4**).

These results suggest that the presence of the methyl group (in 3-methylasparate) and the interactions that it forms in the catalytic pocket are essential for the proper binding of the substrate.

CONCLUSIONS

The binding of the substrate in MAL is highly structured. Of the seven tested substrates, only β-lysine and β-glutamic acid provide a chemical structure suitable to form the hydrogen bonds needed for the proper positioning of the substrate in MAL binding site (showing a non-polar group in the α-carbon correctly oriented in the hydrophobic pocket and an amino group in the β-carbon).

This study provides the groundwork necessary for future studies on engineering MAL substrate specificity.

Kræftens Bekæmpelse
FORMAS
novo nordisk fonden