Theoretical studies on ectoine synthase

J. Andrys-Olek[1], J. Heider[2], T. Borowski[1]

[1] *Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Niezapominajek 8, 30-239, Cracow, Poland*
[2] *Philipps-University Marburg, Karl-von-Frisch-Str. 8, 35041 Marburg, Germany*
justyna.andrys@ikifp.edu.pl

Ectoine, a chemical chaperone produced by bacteria to counter osmotic stress, has gained significant interest from pharmaceutical and cosmetic industries due to its hydrating and cell-protective properties [1]. However, the reaction mechanism of its final synthesis step remains elusive. This step is catalyzed by ectoine synthase (EctC), a Fe^{2+}-dependent homodimeric cytoplasmic protein. To address this knowledge gap, we combined Mössbauer spectroscopy, molecular dynamics simulations and QM/MM calculations to investigate (1) the coordination geometry of the Fe^{2+} ion, (2) the geometry of an active site of enzyme-substrate complex (3) and finally propose a mechanism for the EctC-catalyzed reaction [2,3].

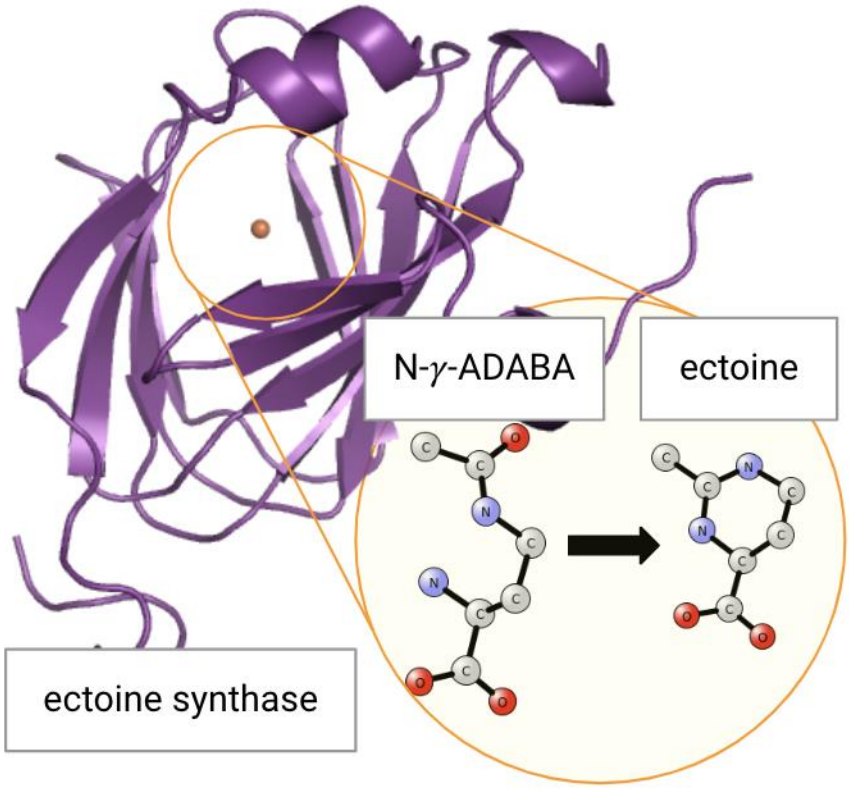

Figure 1. Model of EctC monomer with a scheme of catalyzed reaction of N**-γ-**ADABA cyclization [3].

Our findings indicate that the ligands needed to fill the first coordination sphere of the Fe^{2+} cofactor are the three amino acids (Glu57, Tyr84, and His92), along with one water molecule and one hydroxide ion. The latter two act as critical proton donors and acceptors during the cyclization reaction. Molecular dynamics simulations of the *Paenibacillus lautus* EctC (PlEctC)

protein in dimeric form show that the presence of the substrate stabilizes the protein structure, notably affecting a short helix near the entrance to the active site [4]. Furthermore, amino acids crucial for substrate binding were identified as Trp21, Arg25, Asn38, Thr40, and Tyr52, which is consistent with previous experimental data [4,5]. The studies shine new light on the active site geometry and ligand interactions, providing insights into the dynamic nature of EctC-N-γ-ADABA complex and energetics of the ectoine biosynthesis reaction. This knowledge can help to design an efficient biocatalyst, which can be used in industrial production of ectoine.

References

[1] Hermann, L.; Mais, C.N.; Czech, L.; Smits, S.H.J.; Bange, G.; Bremer, E. Biol. Chem. 2020, 401, 1443–1468. DOI:10.1515/hsz-2020-0223
[2] Andrys-Olek, J.; Borowski, T.; Heider, J. Catalysts 2023, 13(1), 124. DOI:10.3390/catal13010124
[3] Andrys-Olek, J.; Kluza, A.; Tataruch, M.; Heider, J.; Korecki, J.; Borowski, T.; Chem. Eur. J. 2024, e202304163. DOI:10.1002/chem.202304163
[4]. Czech:, L.; Höppner, A.; Kobus, S.; Seubert, A.; Riclea, R.; Dickschat, J.S.; Heider, J.; Smits, S.H.J.; Bremer, E. Sci. Rep. 2019, 9, 364. DOI:10.1038/s41598-018-36247-w
[5]. Widderich, N.; Kobus, S.; Höppner, A.; Riclea, R.; Seubert, A.; Dickschat, J.S.; Heider, J.; Smits, S.H.J.; Bremer, E. PLoS ONE 2016, 11, e0151285. DOI:10.1371/journal.pone.0151285

Acknowledgements

This research was funded in part by the National Science Centre, Poland, grant number: UMO-2022/45/B/ST4/01411. We gratefully acknowledge Polish high-performance computing infrastructure PLGrid (HPC Centers: ACK Cyfronet AGH) for providing computer facilities and support within computational grant no. PLG/2022/015898.