

Tytuł projektu
Poprawianie właściwości katalitycznych enzymu biotechnologicznego hydratasa nitrylowa za pomocą modelowania molekularnego.
Project title
Improving Catalytic Properties of Biotechnological Enzyme Nitrile Hydratase. Molecular Modelling Approach.
Dyscyplina /Area of science
Nauki fizyczne
PROJECT DESCRIPTION
<p>Project goals</p> <ul style="list-style-type: none"> • To use molecular modelling techniques (molecular dynamics, protein ligand docking, bioinformatics) to improve catalytic properties of nitrile hydratase (NHase) • To improve thermal stability and activity in high concentration of amide of NHase • To find variants of NHase with improved catalytic activity towards selected nitriles, including enantiomeric nitriles and dinitriles • To find mechanism of nitrile hydratase activation • To suggest pipeline for semi-rational design of industrial enzymes mutational variants <p>Outline</p> <p>Enzymes are proteins of enormous importance. Enzymes facilitate specific chemical reactions and this feature is widely used in modern biotechnology. The main enzyme of this project is nitrile hydratase (NHase). NHase is present in bacteria colonizing polluted soils. It may purify the natural environment of bacteria from toxic compounds such as nitriles through hydration of “bad” nitriles to corresponding mild amides. The catalysis is possible due to the presence of a non-standard catalytic center containing either iron or cobalt ion in the specially folded protein. This enzyme was successfully used in the biotechnological utilization of nitriles and synthesis of valuable amides (like vitamin PP or acrylamide). Annually, over 600,000 tons of nitriles are converted into useful chemicals.</p> <p>Natural NHases have drawbacks. These enzymes are thermally unstable. After a few hours of exoenergetic reaction the protein permanently loses its catalytic activity. Therefore, bioreactors need to be cooled down and this is expensive. Hydratases show limited selectivity towards nitriles and sometimes even a stereoselectivity. Unfortunately, native enzymes (WT) have low catalytic activity.</p>

In this PhD project we will try to remove drawbacks of WT enzyme, like low thermal stability, thorough computational scanning of selected physical parameters and linking them to amino acid substitutions in NHase. Another task will be improving selectivity towards selected nitriles and amides. There is also a great need for raising tolerance of this enzyme towards substrates and products of the enzymatic reaction. Poorly understood issue is activation of NHase by metallo-chaperones. In this project investigations will be made mainly by using computer physics, molecular modelling techniques and a bioinformatics analysis.

This PhD project will be conducted in close cooperation with the School of Biotechnology, Jiangnan University, Wuxi, China. NHase properties, predicted through modelling, will be checked experimentally in the lab.

Work plan

1. Mastering molecular dynamics and docking techniques for nonstandard proteins
2. Design mutational variants of nitrile hydratase with desirable properties
3. Finding general schemes for semi-automatic analysis of molecular dynamics simulations for proteins with improved thermal stability
4. Elucidating the mechanism of nitrile hydratase activation by its activators.

Literature

- A. Miyanaga, et al.; *Biochem Biophys Res Commun* 288(5) (2001) 1169
Y. Xia, Ł. Peplowski, et al.; *Biotechnol. Bioeng.* 116(3) (2019), 481-489
Z. Cheng, Ł. Peplowski, et al.; *Biotechnol. Bioeng.* 115(3) (2018), 524-535
Y. Xia, W. Cui, Z. Cheng, Ł. Peplowski, et al.; *ChemCatChem* 10(6) (2018), 1370-1375
Z. Cheng, W. Cui, Y. Xia, Ł. Peplowski, et al.; *ChemCatChem* 10(2) (2018), 449-458
S.M. Thomas, et al.; *Trends in Biotechnol.* 20(6) (2002) 238.
Ł. Peplowski, et al.; *Chem. Phys. Lett.* 467 (2008), 144-149
Ł. Peplowski, et al.; W. Nowak; *J. Mol. Model.* 13 (2007), 725-730

Required initial knowledge and skills of the PhD candidate

- ➔ Analytical thinking
- ➔ Eager to learn
- ➔ Understanding of molecular biology and physics
- ➔ Knowledge about biotechnology and microbiology (critical for cooperation with School of Biotechnology, Jiangnan University, China)
- ➔ Understanding of basic chemistry, physics and biology/biotechnology
- ➔ Eager to work hard

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