Rational design of mutations in PyNPs

Rational design targeted mutations of pyrimidine nucleoside phosphorylases to improve kinetics for sugar modified nucleosides

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In Short

- Our group perform molecular dynamics (MD) simulations and hybrid quantum mechanics/molecular mechanics (QM/MM) approaches to study small molecules and biological macromolecules
- In the open conformation of pyrimidine nucleoside phosphorylase (PyNP) both substrates are about 7 Å apart from each other, whereas the distance gets reduced to 3 Å in the closed conformation
- *Thermotoga maretima* (Tma) PyNP shows a higher specific activity with arabinofuranosyluracil (araU) compared to *Geobacillus thermoglucosidasius* (Gt) PyNP, despite sharing a high sequence similarity and the same amino acids important for binding of the nucleoside
- Mutations were made on GtPyNP based on TmaPyNP in the back of the active side
- Each single point mutant of GtPyNP showed a decreased activity for the natural substrates, and almost a complete loss in activity for the mutant containing all substitutions
- All atom MD simulations will be used to find the reason for the decreased activity of the GtPyNP mutants with uridine
- To get a better understanding of the differences between TmaPyNP and GtPyNP all atom MD simulation will be performed with araU as a substrate and without substrate

Pyrimidine nucleoside phosphorylases (PyNPs) catalyze the reversible phosphorolysis of pyrimidine in the presence of phosphat.[1] It is known that PyNPs undergo conformational changes during catalysis in form of rigid body movements within one subunit and therefor opens and closes during catalysis.[2] (Figure 1) Those enzymes have been mainly isolated from thermophilic organisms such as *Thermotoga maretima* (Tma) and *Geobacillus thermoglucosidasius* (Gt).[3] Thermostable PyNPs are interesting since they withstand harsh condition such as acidic pH and organic solvents. Even so, they show to have a wider substrate spectrum compared to their mesophilic counterparts. Especially modifications at the C2' position of the nucleoside are accepted as substrates. Those nucleoside-analogous play an important role in the treatment of cancer and viral infections and are only being synthesized by laborious and inefficient chemical synthesis. Therefore, PyNPs could be valuable catalysts in the synthesis of nucleosides and their analogous, such as arabinosides- (ara) compounds.[4]

Even though TmaPyNP and GtPyNP are both from thermophilic organism, they show differences in optimal temperature for the catalytic reaction as well as different substrate preferences. Nonetheless those enzymes have a 58 % sequence identity and the amino acids important for binding the nucleoside in the active side are similar. It is not clear what exactly causes the different substrate preferences between PyNPs from different origins, despite sharing a high sequence similarity and secondary/quartenary structure. To get a better knowledge of the differences in the active side of the proteins mutations in GtPyNP were made based on the amino acid sequence of TmaPyNP, since TmaPyNP has a higher optimal temperature and a higher specific activity with araU. Four single point mutations in the back of the active side in GtPyNP, as well as a four-point mutant, showed in experiments a decrease in specific activity. (Figure 2)

First attempts to address this question were classical methods such as sequence alignments, crystal structures and homology models of the different PyNPs. Since those methods are not suitable to give an answer to the question of the difference of both PyNPs, all atom molecular dynamics (MD) simulations will be used.

This project aims to get a better understanding of the mutations made in GtPyNP based on TmaPyNP and to obtain a better understanding of the structur function relationship of PyNPs. Since we are investigating the differences of two PyNPs for the binding of substrates, all atom MD simulations are necessary.

Within this project we will investigate the dynamics behaviour of the five mutants with uridine as a substrate and further gain knowledge about the differences between the catalysis of GtPyNP and TmaPyNP with araU.

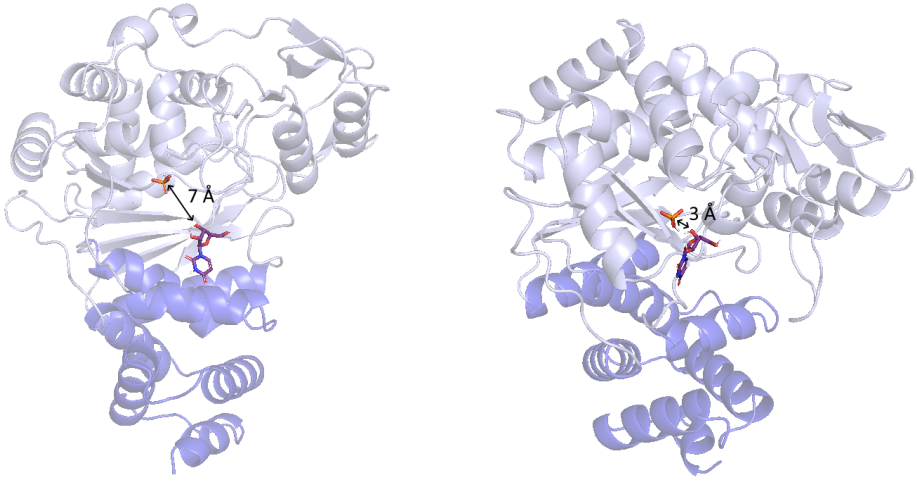

Figure 1: *Ribbon diagram of a GtPyNP monomer. On the left side GtPyNP is in the 'open' conformation and closed on the right side. Each subunit is colored in a darker or lighter shade of the corresponding color. The lighter part represents the α/β -domain and the darker part the α -domain. In the open conformation both substrates are about 7 Å apart from each other, whereas the distance gets reduced to 3 Å in the closed conformation.*

WWW

https://www.tu.berlin/en/biomodeling/about-us

More Information

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[2] M. J. Pugmire and S. E. Ealick, *Struct.* **6**, 1467–1479 (1998). doi:10.1016/S0969-2126(98)00145-2

[3] S. Kamel, I. Thiele, P. Neubauer, and A. Wagner, *BBA Proteins and Proteomics* **1868**, 140304 (2020). doi: 10.1016/j.bbapap.2019.140304

[4] H. Yehia, S. Kamel, K. Paulick, P. Neubauer, and A. Wagner, *Curr. Pharm. Des.* **23**, 6913–6935 (2018). doi: 10.2174/1381612823666171024155811

Project Partners

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EC2 / BIG NSE unisyscat

DFG Subject Area

none

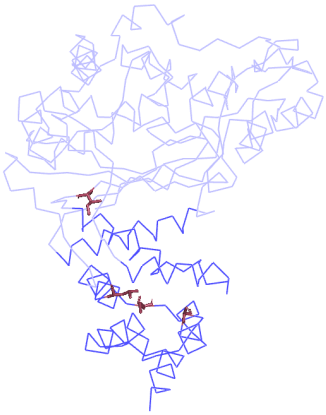

Figure 2: *Ribbon diagram of GtPyNP monomer. Its structure is in the 'open' conformation. The subunit is colored in a darker or lighter shade of the color. The lighter part represents the α/β-domain and the darker part the α-domain. Depicted in red sticks are the mutations made in GtPyNP.*