MD and QM:MM studies of steroid C25 dehydrogenase catalytic subunit

Agnieszka Rugor[1], Anna Wójcik[1], Stefan Mordalski[2],
Andrzej Bojarski[2], Maciej Szaleniec[1]

[1] *Jerzy Haber Institute of Catalysis and Surface Chemistry, PAS, Kraków, Poland*

[2] *Institute of Pharmacology, PAS, Kraków, Poland*

Steroid C-25 dehydrogenase (S25DH) is an oxygen sensitive, heterotrimeric molybdenum enzyme isolated from a cholesterol-degrading, denitrifying bacterium *Sterolibacterium denitrificans* (Chol-1ST) [1]. In nature the enzyme converts cholesterol derivatives such as cholest-4-en-3-one and cholest-1,4-dien-3-one to 25-hydroxylated products. Biochemical studies showed that it belonging to the so-called EBDH-like class of DMSO reductase family.

We decided to investigate the structure and catalytic characteristic of S25DH in more details due to the fact that : i) the mechanism of such a reaction is still unknown, ii) the knowledge of this class of enzymes is based only on relatively well studied ethylbenzene dehydrogenase (EBDH) [2] and iii) the regioselective hydroxylation of sterols is a source of compounds with an application potential.

As the crystal structure of the enzyme is still unknown we developed a homology model of S25DH catalytic α subunit using EBDH as a template (sequence identity 40%, similarity 96%). The obtained model was subjected to molecular dynamics (MD) simulations with substrates (Fig. 1): cholest-4-en-3-one (black), cholest-1,4-dien-3-one (green), cholest-4,6-dien-3-one (magenta) and cholesterol (blue). MD simulations (AMBER force field) were conducted in order to obtain a reliable model of S25DH in complex with selected substrates. Missing parameters for the MoCo and the Fe-S cluster were derived from the quantum mechanical (QM) calculations. The model comprised of 14450 atoms and 30 000 water molecules in a periodic box extending 10 Å from the protein surface. The MD production stage was conducted for 30 ns in 303 K under constant pressure.

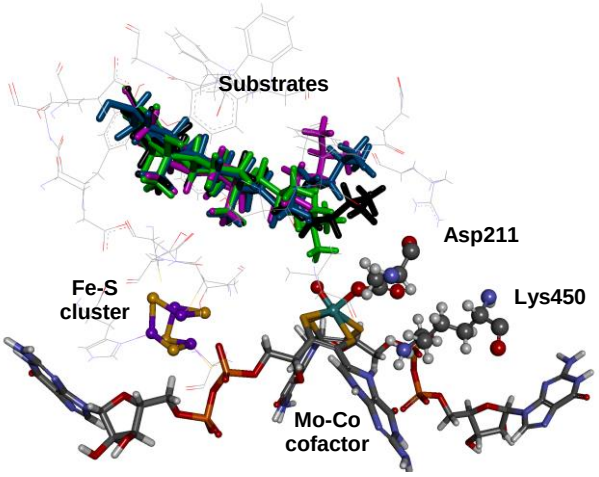

Fig. 1. S25DH active site with docked substrates after MD.

The results were analyzed using MMPBSA.py to calculate the protein : substrate interaction energy. Theoretical results were compared with experimental kinetic parameter for each substrate. The final structure of the enzyme-substrate complex was minimized with QM:MM approach.

Literature

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[2] Szaleniec M., et. al., Biochemistry 2007, 46, 7637

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