Structural modelling of a novel molybdoenzyme: steroid C25 dehydrogenase from *Sterolibacterium denitrificans*

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Steroid C-25 dehydrogenase (S25DH) is an oxygen sensitive, αβγ heterotrimeric molybdenum enzyme isolated from cholesterol-degrading, denitrifying bacterium *Sterolibacterium denitrificans* (Chol-1ST) belonging to so-called EBDH-like class of DMSO reductase family [1]. The enzyme catalyzes regioselective hydroxylation at the C-25 tertiary carbon atom of the aliphatic side chain in cholest-4-en-3-one and its derivatives [1].

As the crystal structure of the enzyme is still unknown we developed a homology model of S25DH catalytic α subunit using ethylbenzene dehydrogenase (EBDH) as a template [2] (sequence identity 40%, similarity 96%). The catalytically active structure of the molybdenum cofactor (MoCo) was obtained from QM:MM modeling of the EBDH α subunit. In the catalytically active, oxidized form of the cofactor Mo atom is coordinated by six ligands: four sulphur atoms from two pyranopterin-guanine dinucleotides (MGD-Q, MGD-P), Oδ1 from Asp^{211} residue and catalytically active oxo ligand (Fig. 1).

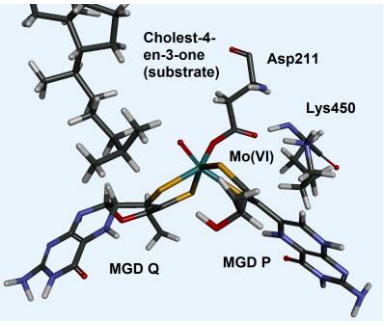

Fig. 1: Active site of S25DH with docked substrate

100 homology models were developed in MODELLER 9v7 and the best models that could accommodate test substrates were used in molecular dynamics (MD) simulations. MD simulations were conducted in Amber12 software in order to obtain reliable model of S25DH in complex with native substrate. 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 was conducted for 30 ns in 303 K under constant pressure.

The obtained results showed that the structure of MoCo is comparable to those obtained in QM:MM studies, confirming quality of the parameterization. Analysis of MD trajectories enabled insight into dynamics of substrate aliphatic chain in the enzyme active site and revealed important interactions between cofactor, amino acid residues, substrate and water molecules present in the active site. The cooled model was used for docking experiments with other substrates as well as QM:MM calculations aimed at the modeling of the reaction mechanism.

Acknowledgements: Financial **s**upport of the National Center of Science grant SONATA UMO-2012/05/D/ST4/00277 and project MOL-MED of Human Capital Operational Programme.

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