Insight into structure and reactivity of steroid C25 dehydrogenase, a molybdenum enzyme hydroxylating sterols.

Rugor A M[a*], **Wójcik A**[b], **Mordalski S**[c], **Staroń J**[c], **Bojarski A**[c], **Szaleniec M**[a]

a Jerzy Haber Institute of Catalysis and Surface Chemistry, PAS, Cracow, Poland
b Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Cracow, Poland
c Institute of Pharmacology, PAS, Cracow, Poland
* ncrugor@cyfronet.pl

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.

Experimental studies with purified S25DH reviled 5 new substrates of S25DH, that together with 4 previously characterized[2] can be divided to two groups: i) 3-keto-sterols that represent 90 – 132 % of S25DH activity for native substrate; ii) 3-hydroxy-sterols and other C3 derivatives that represent not more than 30 % of S25DH activity for native substrate.

Theoretical studies of S25DH consisted of homology modelling of the catalytic α subunit, docking studies for various substrates and enzyme-substrate models relaxation by molecular dynamic simulations. As a result the substrate binding pocked was characterized which in turn allowed elucidation of factors (such as substrate binding mode and frequency of C25-H exposition toward Mo=O) responsible for the experimentally observed difference in reactivity between C3-ketons and C3-alkohol substrate sub-classes. The relaxed in MD S25DH α subunit active site, containing Moco cofactor and [4Fe-4S] cluster, was compared to MD relaxed ethylbenzene dehydrogenase (EBDH)[3] α subunit in order to determine the structural differences. Based on the relaxed structure of the enzyme-substrate complex the cluster models were prepared that allowed initial DFT modeling of the reaction pathway.

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[2] Dermer J., Fuchs G., J. Biol. Chem., 2012, 287, 3690-36916

[3] Szaleniec M., et al., Biochemistry, 2007, 46, 7637-7646

Authors acknowledge the financial support from the project Interdisciplinary PhD Studies "Molecular sciences for medicine" (co-financed by the European Social Fund) and the National Center of Science grant SONATA UMO-2012/05/D/ST4/00277.