APPLICATIONAL POTENTIAL OF STEROID C25 DEHYDROGENASE FROM STEROLIBACTERIUM DENITRIFICANS

A. Rugor[1], A. **Dudzik[1], M. Tataruch[1], J. Staroń[2], A. Bojarski[2], M. Szaleniec[1]**

1. Jerzy Haber Institute of Catalysis and Surface Chemistry, PAS, Niezapominajek 8, 30-239 Kraków, Poland
2. Institute of Pharmacology, PAS, Smętna 12, 31-343 Kraków, Poland

Steroid C-25 dehydrogenase (S25DH) is an oxygen sensitive, αβγ EBDH-like molybdenum enzyme isolated from cholesterol-degrading, denitrifying bacterium *Sterolibacterium denitrificans* Chol-1ST [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 Fig. 1 [2].

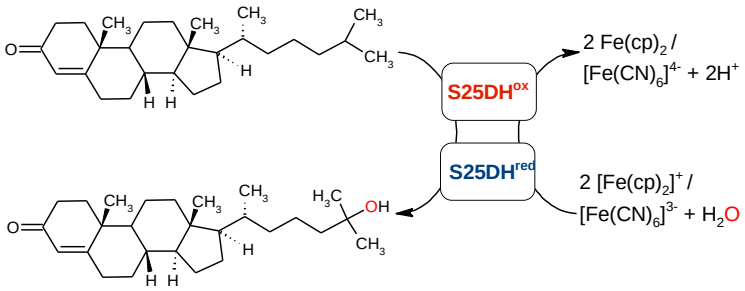

Fig. 1. Scheme of cholest-4-en-3-on oxidation to 25-hydroxycholet-4-en-3-on catalyzed by S25DH. The enzyme in its oxidized form ($S25DH^{ox}$) activates C-25 carbon atom by introduction of OH group and gets reduced ($S25DH^{red}$). The oxidized enzyme is re-constituted by external electron acceptor ($[Fe(cp)_2]^+$ - ferrocenium (III) or $Fe(CN)_6]^{3-}$ - ferricyanide) and a water molecule.

The enzymatic reaction catalyzed by S25DH is an alternative to a complex organic synthesis of 25-hydroxylated steroids (such as 25-hydroxylated cholesterol or 25-hydroxycholecalciferol) that shows regulatory and drug potential.

S25DH purified under aerobic conditions was used to convert substrates under optimized conditions using homogenous and immobilized system. The reaction was conducted with cholest-4-en-3-one, cholest-1,4-dien-3-one, cholest-4,6-dien-3-one, cholesterol and cholesterol succinate showing that enzyme is able to regioselectively hydroxylate a range of structural analogues of its native substrate.

As the crystal structure of the enzyme is still unknown we have developed the homology model of α catalytic subunit S25DH. MD simulations and docking experiments followed by QM studies enabled probing of enzyme catalytic characteristics which is a first step in

understand the catalytic mechanism of this novel enzyme.

References:

[1] Dermer, J., and Fuchs, G. J. Biol. Chem.. 2012, 287, 36905–36916

[2] Chiang, et al. J. Bacteriology 2008, 190, 905-914.

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

Authors acknowledge the financial support from grant projects: LIDER/33/147/L-3/11/NCBR, SONATA 012/05/D/ST4/00277 and project Human Capital Operational Programme MOL-MED.