EXPERIMENTAL AND THEORETICAL STUDIES ON STEROID C25 DEHYDROGENASE FROM *STEROLIBACTERIUM DENITRIFICANS*

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Steroid C-25 dehydrogenase (S25DH), a new molybdenum enzyme isolated from denitrifying bacterium *Sterolibacterium denitrificans* Chol-1ST [1], catalyzes regioselective hydroxylation at the C-25 tertiary carbon atom of the aliphatic side chain in cholesterol and its derivatives. The enzyme is express only under anaerobic and catalyzes transfer of the oxygen atom from a water molecule to the substrate [2].

Reaction catalyzed by S25DH is proposed as an alternative method for synthesis of 25-hydroxycholesterol (25-HC) in place of the currently used multistep chemical synthetic procedure. 25-HC is an important regulatory compound that is involved in a complex regulation of cells of the human immunological system [3]. However, up to date lack of a cheap commercial source of 25-HC limits studies of its physiological and immunological role and its potential medical application.

S25DH was purified under anaerobic conditions and aerobic with ferrocenium (III) tetrafluoroborate as an oxygen protectant [1, 4]. Then the enzyme was tested as a catalyst for production of 25-hydroxylated cholesterol.

In this presentation we show an optimized reaction conditions for synthesis of 25-HC in aqueous medium. The main obstacle to high conversion, i.e. poor solubility of cholesterol, was circumvented by substitution of cholesterol with its more soluble derivatives such as cholesteryl hemisuccinate or addition of solubilizers such as a mixture of β-cyclodextrin and short chain alcohols or glycols.

Moreover, we present the homology model of S25DH based on the template of ethylbenzene dehydrogenase (EBDH) from *Aromaticum aromatoleum* EbN1 [4] (identity 40 %, similarity 96 %). Molecular dynamic simulations and docking experiments for known S25DH substrates we used to study the substrates binding mode and crucial interactions between amino acid residues and the docked ligands.

References:

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