Selective modifications of sterols performed by enzymes from *Sterolibacterium denitrificans*

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Sterolibacterium denitrificans is a denitrifying bacterium that under anaerobic conditions mineralizes cholesterol [1]. It is a source of new regio- and chemoselective enzymes that can be consider as an interesting biocatalysts for the industry. The initial degradation step of choresterol, ring A oxidation and isomerisation to cholest-4-en-3-one, is catalyzed by cholesterol dehydrogenase/isomerase (AcmA, Anaerobic cholesterol metabolism). This product is further oxidized to cholesta-1,4-dien-3-one by cholest-4-en-3-one-Δ^1-dehydrogenase (AcmB) [2]. Subsequently, both products are hydroxylated at tertiary C25 of the alkyl side chain by steroid C25 dehydrogenase (S25DH) using water as an oxygen donor (Fig. 1) [3].

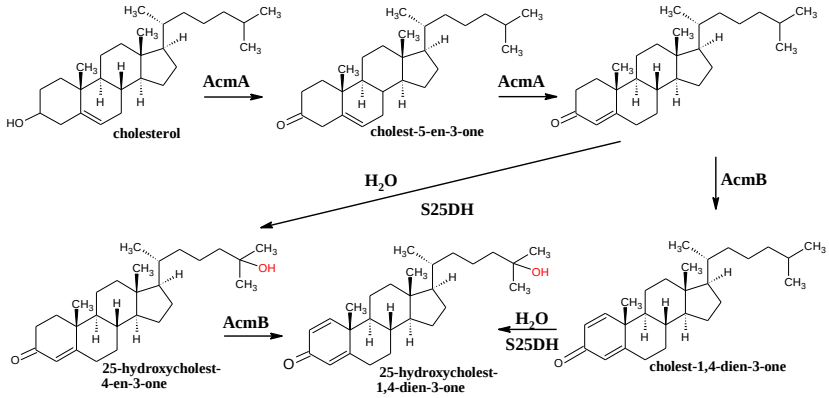

Fig. 1 Initial steps of cholesterol degradation pathway with formation of cholest-1,4-dien-3-one and 25-hydroxylated steroids.

In our work a purified S25DH and crude protein fractions of AcmB were tested as catalyst in batch or fed-batch reactors using various sterols and steroids. For S25DH substrates the reaction rate was monitored by HPLC-MS. For crude AcmB a products of oxidation were extracted using SPE (40-100 ml reactors containing app. 20 mg of a substrate) and analyzed by HPLC-MS and NMR. The S25DH catalyzed hydroxylation in the range of cholesterol derivatives while AcmB was active in introduction of a double bond in some compounds of pharmaceutical interest.

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