

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

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Sterolibacterium denitrificans, a denitrifying bacterium that under anaerobic conditions mineralize cholesterol [1] is a source of new region- and chemoselective enzymes that can be considered as interesting for the industry. During the cholesterol degradation pathway, the initial degradation steps are taking place via a ring A oxidation and isomerisation to cholest-4-en-3-one by cholesterol dehydrogenase/isomerase (AcmA, Anaerobic cholesterol metabolism) and further oxidation 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 side chain, using water as an oxygen donor in a reaction catalyzed by steroid C25 dehydrogenase (S25DH) [3] Fig. 1.

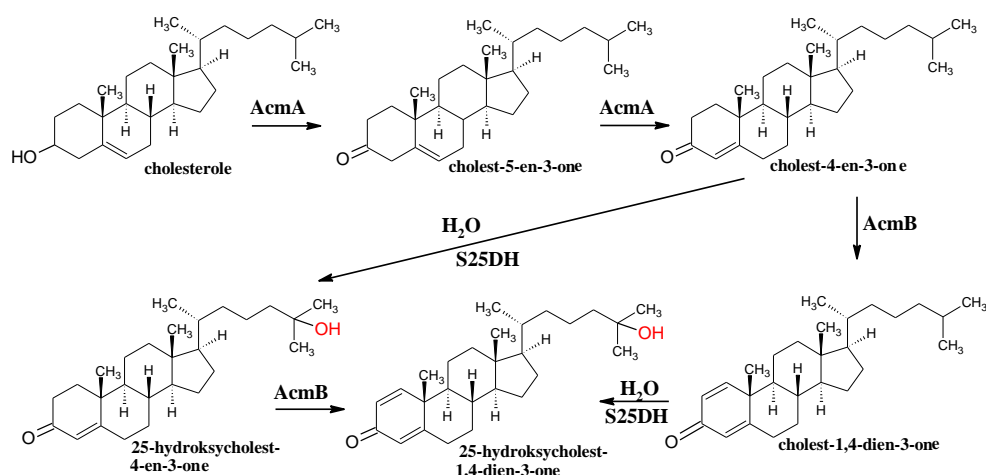


Fig. 1. Initial steps of cholesterol degradation pathway with formation of cholest-1,4-dien-3-one and 25-hydroxylated steroids.

In our work a purified S25DH and crude protein fractions of AcmB were tested as catalysts in water 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 product of oxidation was extracted using SPE (40-100 ml reactors containing app. 20 mg of a substrate) and products were identified with HPLC-MS and NMR.

Literature

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[3] J. Dermer and G. Fuchs, J. Biol. Chem, 2012, 287, 3690

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