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„Bakteryjne Δ^1-dehydrogenazy 3-ketosteroidowe – struktura, mechanizm reakcji i zastosowanie w biokatalitycznym odwodornieniu leków steroidowych"

Streszczenie w języku angielskim

3-Ketosteroid Δ^1-dehydrogenases (Δ^1-KSTD) are enzymes belonging to the class of FAD-dependent oxidoreductases (EC 1.3.99.4) that catalyze a key step in the microbial breakdown of ketosteroids, phytosterols and cholesterol. These proteins are responsible for the regio- and stereoselective dehydrogenation of 3-ketosteroids, i.e. the introduction of a double bond between the C1 and C2 atoms of the sterane A-ring. The main object of this PhD thesis is Δ^1-KSTD isolated from the facultative anaerobic bacteria *Sterolibacterium denitrificans* (AcmB). AcmB is a protein associated with the *S. denitrificans* inner membrane, which *in vitro* forms massive aggregates with a mass above 600 kDa. It also has an unusually low pH optimum that equals 6.0 (the pH optimum of most Δ^1-KSTD is in the range 7–10) and a broad, compared to other enzymes of this class, substrate spectrum.

As part of this PhD thesis, the process of recombinant AcmB expression in *Escherichia coli* was optimized and the process of protein purification and disaggregation using non-ionic detergents was developed. The quaternary structure of AcmB was characterized using gel filtration and dynamic light scattering, while the crystal structure of AcmB was solved using X-ray crystallography.

The analysis of the obtained results allowed formulation the hypotheses describing the aggregation enzyme mechanism and structural features increasing its affinity to C17-substituted 3-ketosteroids, as well as to indicate the key amino acids involved in the reaction catalyzed by AcmB. Moreover, to characterize AcmB biochemistry and to determine the optimal reaction conditions for the synthesis of 1-dehydro-3-ketosteroids, the temperature optimum of the dehydrogenation reaction, enzyme thermostability, its pH optimum dependent and independent on the artificial electron acceptors presence and the effect of pH on the enzyme stability were determined.

As part of the catalytic characterization of AcmB, the steady-state kinetic parameters for AcmB and selected artificial electron acceptors were determined. Moreover, to verify the hypothesis suggesting that only a few Δ^1-KSTD have the ability to dehydrogenate

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C17-substituted 3-ketosteroids, and to determine the AcmB's native substrate, kinetic studies for AcmB and Δ^1-KSTD from *Rhodococcus erythropolis* (KSTD1) were conducted with androst-4-en-3,17-dione and cholest-4-en-3-one, in the presence of 2-hydroxypropyl-β-cyclodextrin as a solubilizer.

In order to verify the hypothetical, postulated in the literature mechanism of the Δ^1-KSTD reaction, the role of key amino acids in the active site of AcmB was determined using site-directed mutagenesis. Moreover, the steady-state analysis of the kinetic isotope effect (KIE) was performed for AcmB and KSTD1 using the direct and competitive method and the pre-steady-state analysis of the KIE was performed using the stopped-flow technique. The KIE studies and the kinetic effect of the solvent viscosity measured for KSTD1 allowed to indicate the rate-limiting step of the reaction catalyzed by Δ^1-KSTD.

Finally, the identification of the optimal reaction conditions, including the most effective enzyme fraction and the optimal artificial electron acceptor, allowed development of an efficient, AcmB-catalyzed process for the synthesis of Δ^1-3-ketosteroids. The aim of this part of the work was to perform the large-scale synthesis of the pharmacologically active Δ^1-3-ketosteroids, including Δ^1-diosgenone – a 3-ketosteroid saponin derivate, which exhibits biocidal, anti-inflammatory and anticancer properties and which enzymatic Δ^1-dehydrogenation has not been previously described in the literature.

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