Enzymatic preparation of new dehydrogenated pentacyclic triterpenes of pharmacological importance

The project aims to develop and improve the bacterial enzyme 3-ketosteroid dehydrogenase (KstD), which enables the selective dehydrogenation of steroids and pentacyclic triterpenes (TP) – natural plant-derived compounds with potential therapeutic properties. This enzyme has the ability to introduce a double bond between the carbon atoms C1 and C2 in the A-ring of the molecule, leading to the formation of Δ^1-derivatives – compounds with significantly enhanced biological activity.

Pentacyclic triterpenes are compounds with strong anti-inflammatory, antioxidant, and regenerative properties, widely used in natural medicine, particularly in East Asia and North America. Chemical modifications, such as introducing the Δ^1 double bond, can significantly increase their biological activity, opening the door to the development of new, more effective phytotherapeutics, which may be useful in treating diseases such as cancer, inflammatory disorders, and neurodegenerative conditions.

The project focuses on synthesizing new Δ^1-TP derivatives that demonstrate promising therapeutic potential. However, naturally occurring Δ^1-TPs are rare, which justifies the search for new biotechnological methods that allow for their efficient and environmentally friendly production.

To achieve this, the substrate specificity of 3-ketosteroid dehydrogenase (KstD) – an enzyme capable of transforming steroid compounds and TP into their Δ^1-derivatives – is being studied. The KstD enzyme, isolated during previous research, possesses a unique ability to catalyze the dehydrogenation of TP, making it the first enzyme of its kind. The project will involve a thorough investigation of the enzyme's catalytic properties and structural analysis to understand the mechanisms underlying its specificity toward various substrates.

One of the key aspects of the project is the analysis of the enzyme's region located near the substrate-binding pocket. Variability in this region may be crucial for the enzyme's preference for specific compounds, which will be examined through bioinformatics methods and molecular modeling.

Additionally, the project will include directed evolution of the enzyme to obtain KstD variants with higher activity toward the conversion of TP and steroids. These variants will be tested for their catalytic activity and the corresponding structural changes in the enzyme.

The outcomes of the project will include: i) the development of new Δ^1-TP and Δ^1-steroids with potential therapeutic properties, ii) the creation of new KstD enzyme variants that may serve as the foundation for future application-oriented projects, and iii) the provision of a crystal structure of the enzyme, contributing to a better understanding of its mechanism of action.

The project will not only foster the development of new biotechnological tools but also provide training for a PhD student and enhance the research skills of all project members. Additionally, its implementation will create opportunities for international collaboration and increase the application potential in the field of new phytotherapeutic production.