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„Improving Catalytic Properties of Biotechnological Enzyme Nitrile Hydratase. Molecular Modeling Approach"

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Abstract

Enzymatic catalysis plays a crucial role in producing compounds used in the synthesis of medicinal drugs, plastics, and food products. Nitrile hydratase (NHase) is a key industrial enzyme, widely employed in the large-scale production of acrylamide from acrylonitrile, with annual outputs reaching thousands of tons.

The primary aim of this doctoral thesis was to use computational modeling to investigate structural changes in NHase, examine its dynamic properties, and propose strategies to improve its biotechnological performance. One of the major challenges associated with NHase is its loss of activity under high temperatures and elevated concentrations (>50%) of its product, acrylamide. Following an introductory chapter outlining the industrial significance of NHase, the thesis includes a comprehensive review of molecular dynamics (MD) studies on enzymes, particularly those with biotechnological applications in non-aqueous solvents. Original simulations were conducted to explore modifications aimed at enhancing NHase thermostability, including connecting its two subunits with peptide linkers. Additionally, a different NHase variant, derived from thermophilic bacteria with naturally high thermostability but low activity, was studied. A specific point mutation was introduced to increase its activity. MD simulations were also employed to investigate potential causes of activity loss in solutions with high acrylamide concentrations. Over the course of 500 ns, the protein retained its overall structure; however, early signs of partial denaturation were observed. The most plausible explanation for the loss of activity is a structural alteration in the of the close vicinity of active site caused by amides, along with impaired transport of amides through the tunnel leading to the active center. Based on the computational findings presented in this thesis, NHase mutants with improved stability were designed and experimentally tested, demonstrating enhanced performance. This study provides valuable insights into the catalytic activity of NHase, contributing to the optimization of its industrial applications.