EXPRESSION, PURIFICATION AND ACTIVITY ASSESMENT OF RECOMBINANT ESCHERICHIA COLI PURINE DEAMINASES

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Deaminases are enzymes that catalyze deamination reactions in purine metabolism and help to maintain purine homeostasis in cells. Disturbances in these pathways can contribute to harmful accumulation of purine-related metabolites and lead to metabolic imbalance. Purines themselves are essential biomolecules across all living systems. They are building blocks of DNA and RNA, central to cellular energy metabolism (ATP), and participate in intracellular and extracellular signalling through cyclic nucleotides and purinergic signalling networks [1]. Because altered purine metabolism and purinergic signalling have been associated with diverse pathological conditions, systematic biochemical characterization of enzymes involved in purine conversion remains relevant for both basic research and potential application-oriented work [2].

The aim of this study is to establish recombinant production and purification of Add and AdeC proteins in *Escherichia coli* and to confirm their functional activity using the adenine/adenosine and their modified derivatives as substrates, including product-formation analysis by the chromatography methods. *E. coli* Rosetta strains were transformed with expression constructs encoding Add and AdeC. Recombinant protein expression was induced with IPTG, and His-tagged proteins were purified using Ni^{2+} affinity chromatography. Expression and purity of proteins were assessed by SDS-PAGE. Enzymatic activities were tested using adenine/adenosine and their methylated conterparts as substrates, and TLC and HPLC were used to monitor substrate conversion and quantification of reaction products. HPLC analysis demonstrated enzymatic conversion of adenine to xanthine in the presence of the recombinant AdeC enzyme, indicating that the purified protein retained catalytic function after purification. However, the enzymatic activity was not detected when methylated adenine was used. Further experiments are aimed at the testing of the enzymatic activity of Add using the adenosine and it's methylated counterparts to establish the enzymatic activity and specificity of corresponding purine deaminases.

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