APPLICATION OF T4 COVALENT FUSION FOR STRUCTURAL STUDIES OF BACTERIAL MEMBRANE TRANSPORTERS

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Investigation of the transmembrane proteins found in bacteria is important, among others, to gain knowledge how to effectively control certain pathogens. Molecular chaperones such as T4 lysozyme or antibodies are found to be efficient for studying the structure of membrane proteins. Our aim is to covalently fuse T4 lysozyme to the C terminus of *Hemophilus influenzae* HI0610 membrane protein to study the structure of this fusion protein by X-ray crystallography after cloning, expression, purification and crystallization.

HI0610 transmembrane protein was cloned into pTTQ18 plasmid by using recombinant DNA technology. T4 lysozyme was covalently fused to the target protein during the cloning. The protein expression was followed by SDS gel-electrophoresis to investigate the presence of the required macromolecule. Optimization of protein expression was also performed where IPTG concentration, temperature and time dependence were tested. Subsequently, the protein was purified by affinity chromatography and the efficiency of Ni-NTA and Talon resins was compared. The target protein was further purified by size exclusion and ion exchange chromatography.

T4 lysozyme was successfully fused to HI0610 fucose-proton symporter by genetic engineering. Gel-electrophoresis confirmed that the target macromolecule was expressed in an appropriate amount. Optimal conditions for protein expression were the following in Luria-Broth medium: 37°C, 12 h of induction time, 0.5 mM IPTG. During affinity chromatography, the target protein was recovered in high purity. Investigating the eluted proteins obtained during protein purification using two affinity columns, we concluded that the Ni-NTA resin bound the target protein more efficiently than that of Co^{2+} ion containing Talon resin. However, after affinity chromatography further purification steps were necessary to reach the highest protein purity. Therefore, size exclusion and cation exchange chromatography were also performed to remove the contaminating proteins.

The final analysis of the purified concentrated sample revealed no detectable contaminations even by the sensitive silver staining and become suitable for crystallization and structural studies.