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Recombinant expression, purification and PEGylation of DNA Ligases

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Abstract:
Background: Reagent proteins such as DNA ligases play a central role in the global reagents market. DNA ligases are routinely used and are vital in academic and science research environments. Their major functions include sealing nicks by linking the 5'-phosphorylated end to a 3'-hydroxyl end on the phosphodiester backbone of DNA, utilizing ATP or NADP molecules as an energy source. Objective: The current study sought to investigate the role of PEGylation on the biological activity of purified recombinant DNA ligases. Method: We produced two recombinant DNA ligases (Ligsv081 and LigpET30) using E. coli expression system and subsequently purified using affinity chromatography. The produced proteins were conjugated to site specific PEGylation or non-specific PEGylation. FTIR and UV-VIS spectroscopy were used to analyze secondary structures of the PEG conjugated DNA ligases. Differential scanning fluorimetry was employed to assess the protein stability when subjected various PEGylation conditions. Results: In this study, both recombinant DNA ligases were successfully expressed and purified as homogenous proteins. Protein PEGylation enhanced ligation activity, increased transformation efficiency by 2-fold for plasmid ligations and reduced the formation of protein aggregates. Conclusion: Taken together, site-specific PEGylation can potentially be explored to enhance the biological activity and stability of reagent proteins such as ligases.