Facilitation of Expression and Purification of Antimicrobial Peptide by Fusion with Baculoviral Polyhedrin in *Escherichia coli*

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Abstract

Several fusion strategies have been developed for the expression and purification of small antimicrobial peptides (AMPs) in recombinant bacterial expression systems. However, some of these efforts have been limited by product toxicity on host cells, product proteolysis, low expression, poor recovery yield, and sometimes absence of post-modifications required for biological activity. In the present work, we investigated the use of the baculoviral polyhedrin (Polh) protein as a novel fusion partner for production of a model AMP (halocidin 18 amino acid subunit Hal18) in *Escherichia coli*. The useful solubility properties of Polh as a fusion partner facilitated expression of the Polh-Hal18 fusion protein (~33.6 kDa) by forming insoluble inclusion bodies in *E. coli*, which could be easily purified by inclusion body isolation and affinity purification using the fused hexahistidine tag. The recombinant Hal18 AMP (~2 kDa) could then be hydroxylamine cleaved from the fusion protein and easily recovered by simple dialysis and centrifugation. This was facilitated by the fact that Polh was soluble in the alkaline cleavage reaction but became insoluble during dialysis at a neutral pH. Reverse phase HPLC was used to further purify the separated recombinant Hal18, giving a final yield of 30% recovery with >90% purity. Importantly, recombinant and synthetic Hal18 peptides showed nearly identical antimicrobial activities against *E. coli* and *Staphylococcus aureus*, which were used as representative Gram-negative and Gram-positive bacteria, respectively. These results demonstrated that baculoviral Polh can provide an efficient and facile platform for production or functional study of target AMPs.

References

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