Overexpression and Purification of hirame Rhabdovirus Glycoprotein from recombinant *Escherichia coli*

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Summary

Hirame rhabdovirus (HIRRV) is one of the virus infecting flounder. In this study, glycoprotein (G protein) of HIRRV was expressed as about 55kDa glutathione-S-transferase fusion protein (GST-G protein) and it was isolated from *E. coli* inclusion bodies. In order to determine the optimum expression conditions and purification methods for maximum production of GST-G protein, several experiments were performed. The GST-G protein producing *E. coli* was induced by 1mM IPTG at OD_{600} of culture broth were 0.5 and cultivated for another 2 to 12hrs, and then harvested. The harvested cells were sonicated and washed with lysis buffer, and used for testing the optimum induction time by comparing the amount of protein in inclusion body. For denaturation of inclusion body, 8M urea was used and protein refolding by dialysis were carried out using 10mM Tris-HCl (pH 8.0) buffer. The crude protein sample was purified by ion exchange chromatography (DEAE Sephadex A-25) under various pH of 10mM Tris-HCl buffer. And the optimum condition for elution of the GST-G protein was determined by step elution under various NaCl concentration. After ion exchange chromatography, GST-G protein was purified by affinity chromatography using glutathione agarose beads and the bound were eluted by elution buffer containing various concentration of reduced glutathione. Finally, for concentrating the eluted GST-G protein solution, ultrafiltration was used and 4 fold concentrated GST-G protein was obtained.

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