

## Protein Expression and Immobilization into Viral Polyhedra and Its Application

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Insect viruses produce large, proteinaceous particles called polyhedra, which function to protect progeny virions from hostile environmental conditions. It has been postulated that the polyhedra produced by certain insect viruses could be a useful platform for protein immobilization without destruction of biological activity. The major viral protein in polyhedra, which is called polyhedrin, is produced in massive quantities during viral infection. The cytoviruses (CPV), a member of the family *Reoviridae* is one group of insect viruses that produce polyhedra in cytoplasm. A previous study revealed that fusing enhanced green fluorescent protein (EGFP) to the C-terminus of a *Bombyx mori* CPV (BmCPV) outer capsid protein, VP3, results in its incorporation into polyhedra when the chimeric protein is co-expressed with polyhedrin during BmCPV infection. Therefore, it was considered that the VP3 functioned as an “occlusion signal” which can direct the stable incorporation of foreign proteins into polyhedra. In this study, we demonstrated that the N-terminus of VP3 is necessary for this occlusion of foreign proteins into polyhedra. A large-scale survey revealed that the VP3 occlusion signal could direct the incorporation of the variety of human proteins into polyhedra. Immune reactivity and protein-protein interactions were detected on the surface of polyhedra containing occluded foreign proteins and these particles were shown to be highly stabilized against dehydration. Protein microarrays have important applications for the analysis of protein-protein, substrate-enzyme, DNA-protein, RNA-protein, and ligand-protein interactions. The diversity of individual protein structures, together with the need to maintain all these proteins in a functional state, pose significant problems that must be overcome to develop a broadly useful protein microarray technology. We showed that these particles were arrayed on a glass slide by usual spotting methods. Thus, this approach is well suited for protein expression, protein purification, and the development of protein microarrays.