Mutations of Cypovirus Polyhedrin and Applications of Polyhedra to Protein Nanocontainers

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ABSTRACT

Cypoviruses are insect viruses that produce micrometer-sized protein crystals called polyhedra. Virus particles are occluded in polyhedra. Recently we have developed a novel method for protein immobilization into polyhedra. It is possible to use these polyhedra to device ultra-stable protein nanocontainers. However, a weak point of them is that polyhedra dissolve only in high pH condition (pH>10.5). It seems important to carry out structure-based engineering of polyhedrin to derive mutants for multiple purposes. At a packing contact, we have identified a cluster of tyrosine (Fig. 1), deprotonation of which is likely to cause disruption of the lattice at alkaline pH. In this study, we substituted these tyrosine residues by other amino acids. We show that the substitutions of some residues in a cluster of tyrosine lead to modify a solubility of polyhedra. The results suggest that the modified polyhedra can serve as the basis for the development of nanoparticles for biotechnological applications.

Keywords : virus, polyhedra, protein, solubility

1 INTRODUCTION

Cypovirus (CPV) belong to the genus *Cypovirus* of the family *Reoviridae* [1, 2]. Viruses belonging to the family *Reoviridae* are characterized by the presence of capsids made up of concentric icosahedrally symmetric layers of proteins organized in one, two or three shells containing 10 to 12 segments of linear, double-stranded RNA (dsRNA). Insect larvae infected by CPV are characterized by the production of massive amounts of polyhedrin protein, which crystallizes in the cell cytoplasm forming occlusion bodies named polyhedra that incorporate numerous virus particles. CPVs are unique amongst the *Reoviridae* because of this unusual encapsulation of virus within protein micro-crystals and also because the virus particles have only a single capsid shell.

The polyhedra are the result of the crystallization of a virus-encoded protein, polyhedrin, late during the viral infection, and many virus particles are occluded into the polyhedra to protect against extracellular environment. Another function of the polyhedra is to ensure the delivery of virus particles to the target intestinal cells. The polyhedra are only dissolved by high alkaline condition of the insect midgut lumen and then the occluded viruses are released [2].

The complete nucleotide sequence of the silkworm *Bombyx mori* CPV (BmCPV) was the first to be determined. Its genome is composed of 10 discrete equimolar double-stranded RNA (dsRNA) segments. Viruse particles of BmCPV are composed of viral capsid protein 1 (VP1) (151kDa), VP2 (142kDa), VP3 (130kDa), VP4 (67kDa), VP5 (33kDa). The atomic structure of BmCPV polyhedra has been determined to a resolution of 2Å [3]. The crystals have body centered cubic symmetry with a 103Å unit cell (Fig. 2)

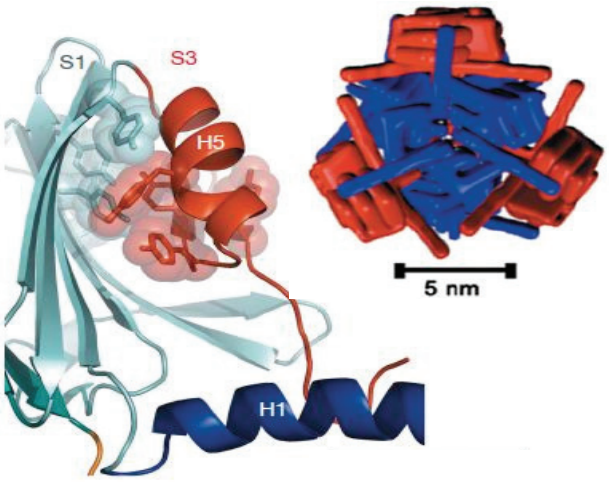

Fig. 1 Tyrosine cluster and N-terminal helix (H1). Unpaired buried negative charges are introduced at pH.10.5 in a tyrosine cluster found between S1 and S3.

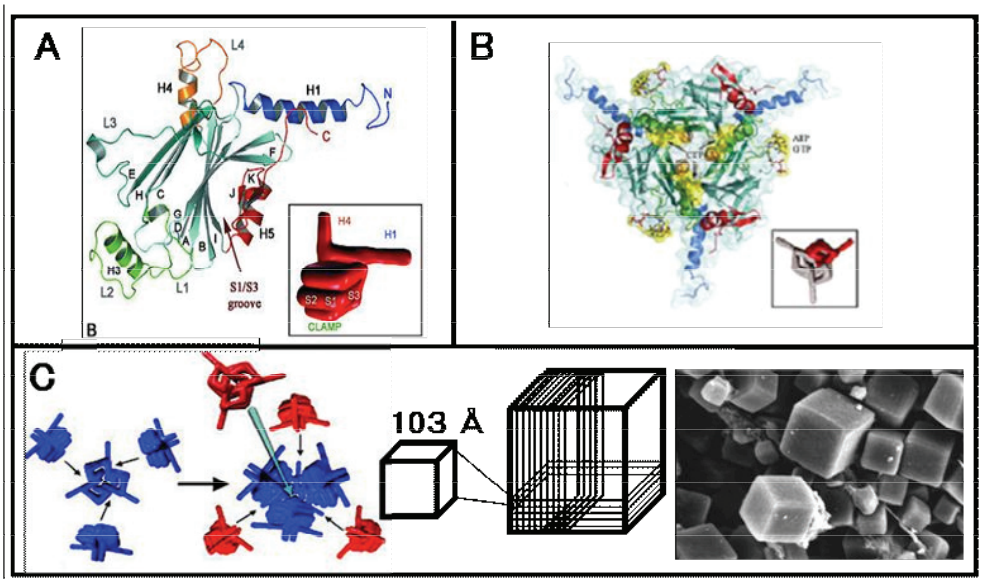

Fig. 2 View of polyhedra (A) Polyhedrin (B) Polyhedrin trimer (C) Unit cell structure with a cell edge 103 Å

We have previously identified the immobilization signals for the incorporation of foreign proteins into BmCPV polyhedra. The GFP fused with the immobilization signal are expressed with BmCPV polyhedrin and then GFP are immobilized into polyhedra. The green fluorescence was observed from polyhedra and GFP was released from polyhedra in alkaline condition. This immobilization signal is derived from an outer capsid protein (VP3) which is a turret protein of BmCPV particles [4]. A large number of different VP3-GFP fusion proteins were subsequently constructed to identify the minimal region of VP3 required to target the GFP fusion construct to polyhedra. Based upon the results of these analyses, it was concluded that the occlusion of GFP into polyhedra required the region of VP3 between amino acids 1 and 79 and this amino acid sequence also was able to direct the immobilization of other foreign proteins into polyhedra [5]. The N-terminal 79 residues of VP3 form a separate domain on the outside of the turret [6]. The N-terminal 79 residues of VP3 were named the immobilization signal [5] or polyhedrin-binding domain [6].

The atomic structure of BmCPV polyhedrin showed that the N-terminal H1 α-helix of polyhedrin plays a significant role in cross-linking and stabilizing polyhedra. We have showed that the polyhedrin H1-helix can also function as a polyhedrin recognition signal and can be used like the VP3 N-terminal sequence to target foreign proteins into polyhedra. The H1 helix plays a central role in the architecture of polyhedra evident from the extent of crystals contacts with four neighboring trimers. Polyhedra are stable in reagents that would normally be expected to denature proteins (concentrated urea, SDS, acid etc.).

Polyhedra do however dissolve readily above pH 10.5 and the deprotonation of a buried cluster of tyrosines (pKa ~10.1) may provide a clue for the disassembly mechanism. Larval alkaline proteinases degrade polyhedrin further contributing to the efficient release of virus from polyhedra in the mid-gut [3]. Consequently H1 helix was identified to contact a cluster of tyrosine at a packing contact and deprotonation of tyrosine residue is likely to cause disruption of the lattice at very alkaline pH. It is perhaps possible to study the effect of substitution of these tyrosine residues by other amino acids. In this study we show that the substitutions with some residues in the tyrosin cluster lead to modify a solubility of polyhedra.

2 RESULTS

2.1 Point mutations of BmCPV polyhedrin

We constructed several mutants of BmCPV polyhedrin with substitutions of tyrosine residue (Y42, Y232, and Y240) in the cluster and in the vicinity of the cluster (Fig.1). The tyrosine residues were replaced with other all amino acid residues. These mutant polyhedrins are introduced in a baculovirus expression vector system and expressed under control of baculovirus polyhedrin promoter. Expression of most of mutant BmCPV polyhedrin decreased and polyhedra-like structure was not observed. But when Y240 was replaced with polyhedra (N), expression of Y240N polyhedrin was similar as wild-type polyhedrin and a small number of hexahedral polyhedra-like structures was observed (Fig.3).

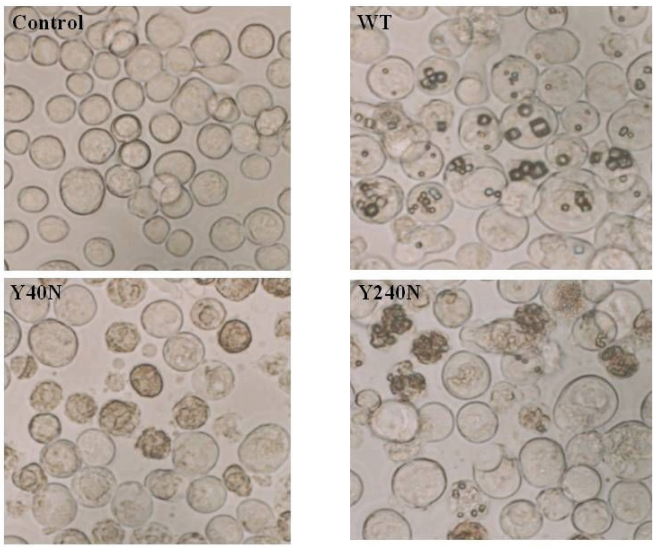

Fig. 3 Cells infected with recombinant baculoviruses expressing wild-type BmCPV polyhedrin (WT), Y40N polyhedin, Y240N polyhedrin and uninfected cells (Control).

2.2 Co-expression of Y240N with wild-type

A mutant BmCPV polyhedrin Y240N was co-expressed with wild-type BmCPV polyhedrin to produce a large amount of polyhedra. Multiplicity of infection of two recombinant baculoviruses expressing wild-type and Y240N polyhedrin was assayed and insect cells were infected with a ratio of 1: 5 of wild-type and Y240N. Cells were incubated for 7 days. Hexahedral polyhedra-like structures were observed, but the size of Y240N polyhedra was smaller than wild-type polyhedra.

3.2 Solubility of chimeric and wild-type polyhedra

The chimeric polyehedra were recoverd and washed for purification with a centrifugation. To study solubility of chimeric polyedra consisted of Y240 and wild-type polyhedrin, the chimeric polyhedra were incubated in several conditions of pH 8.0, 8.5 (Tris-HCl buffer) and 9.0, 10 (Na_2CO_3/$NaHCO_3$ buffer) for 24h at room temperature. Then samples were centrifuged and the supernatants were collected. The supernatants were subjected and SDS-PAGE and western blot analysis using anti-BmCPV polyhedrin antibody. Solubilized polyhedrin of the chimeric polyhedra (Y240N/WT) was detected in the supernatants after incubation in weak alkaline conditions (pH8.0 and pH8.5). This result showed that the solubility of Y240N/WT polyhedra was changed. In Addition, the surface of Y240N/WT polyhedra was observed by scanning electron microscope after the incubation of several pH conditions (Fig.4).

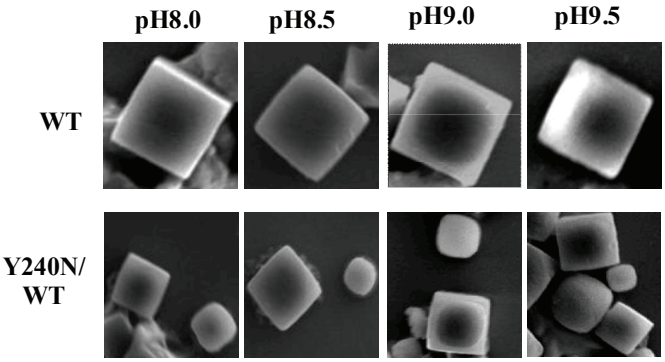

Fig. 4 Observation of surface of polyhedra incubated in alkaline buffer by scanning electron microscope. Buffers were pH8.0 and pH8.5 in Tris-HCl and pH9.0 and pH9.5 in Na_2CO_3-$NaHCO_3$. WT polyhedra and Y240N/WT chimeric polyhedra were incubated with each buffer for 24h at room temperature.

3.3 Nanocontainer of chimeric polyhedra

Fibroblast Growth Factor 2 (FGF2) is a member of FGF family. The biological and physiological properties of FGF-2, FGF-7 and epidermal growth factor (EGF) immobilized into polyhedra with either H1 or VP3 immobilization signal [7]. In this case we also try to immobilize FGF-2 into WT and Y240N/WT chimeric polyhedra by use of these two immobilization signal. Then the biological activity and slow release of FGF-2 immobilized into the chimeric polyhedra will be compared with WT polyhedra.

DISCUSSION

It was demonstrated that solubility of BmCPV polyhedra was changed by substitution of tyrosine residue in the tyrosine cluster or in the vicinity of the cluster. Y42 and Y232 are in tyrosine cluster and they play an important role in polyhedrin crystallization. These mutations drastically affected the expression level of mutant polyhedrin. On the other hand, expression was not affected by a replacement of Y240 with asparagines and a small number of hexahedral polyhedra-like structures were observed. In addition, the solubility of the chimeric polyhedra Y240N/WT was changed and solubilization of Y240N/WT polyhedra was observed in a weak alkaline condition (pH8 and 8.5). Basic function of polyhedra is to protect virions for many years from some environmental conditions, foreign proteins incorporated into polyhedra are also stabilized. In principle, then, to take one example, it is possible to use these polyhedra to make ultra-stable protein chips, in which the protein of interest is protected from dehydration and other damage, as well as presented at the surface. The next steps will be to carry out structure-based engineering of polyhedrin to derive mutants for various purposes, so that, for instance, the crystals can be dissolved and reconstituted at less extreme pH values, with the aim of controlling the release of the incorporated protein. Thus, a further angle of interest to the remarkable polyhedrin structure is that it opens the door to exciting prospects in nanotechnology.

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