Virus-like particles of potato leafroll virus as potential carrier system for nucleic acids

Elżbieta Suluja, Ludmila Strokowskaja, Włodzimierz Zagórski-Ostoja and Andrzej Pałucha

Institute of Biochemistry and Biophysics PAS; Warszawa, Poland; e-mail: alfap@ibb.waw.pl

Received: 15 March, 2005; revised: 14 July, 2005; accepted: 28 July, 2005
available on-line: 15 September, 2005

Potato leafroll virus is a member of the polerovirus genus. The isometric virion is formed by a coat protein encapsidating single-stranded, positive-sense, mono-partite genomic RNA with covalently attached viral protein at the 5’ end. The coat protein of the virus exists in two forms: i) a 23 kDa protein, the product of the coat protein gene, and ii) a 78 kDa protein, the product of the coat protein gene and an additional open reading frame expressed by read-through of the coat protein gene stop codon. The aim of this work was the expression of potato leafroll virus coat protein-based proteins that would be able to assemble into virus-like particles in insect cells. These modified particles were tested for their ability to encapsidate nucleic acids. Two types of N-terminally His-tagged coat protein constructs were used for the expression in insect cells: one, encoding a 23 kDa protein with the C-terminal amino-acid sequence corresponding to the wild type coat protein and the second with additional clathrin binding domain at the C-terminus. The expression of these two proteins by a recombinant baculovirus was characterized by Western immunoblotting with antibodies directed against potato leafroll virus. The protection or putative encapsidation of nucleic acids by these two coat protein derivatives was shown by DNase I and RNase A protection assays.

Keywords: Virus-like particles, encapsidation

Virus-like particles formed by the structural elements of viruses have received considerable attention over the past two decades. When expressed in a suitable heterologous system, viral structural proteins involved in capsid or envelope formation often self-assemble into VLPs in the absence of other viral components usually required for virus assembly, such as multiple structural or non-structural proteins and viral genomes. The protein–protein interactions in VLPs are relatively strong and can result in the formation of stable structures. Depending on the nature of the viral protein, such structures can be modified on their surface in order to introduce foreign epitopes, and they may be used to encapsidate non-viral nucleic acids or small proteins.

Potato leafroll virus particles consist of a single-stranded positive sense RNA about 5.8 kb long encapsidated in the major coat protein of 23 kDa (Mayo et al., 1989; van der Wilk et al., 1989). Sequence analysis of a Polish isolate of PLRV (Palucha et al., 1994) confirmed the presence of several open reading frames. The viral CP gene is expressed in two forms: as a 23 kDa protein and a 78 kDa fusion protein after read-through of the stop codon during translation of the CP gene (Bahner et al., 1990).

The assembly of VLPs of PLRV in insect cells infected by a recombinant baculovirus containing the CP gene, modified at the N-terminus by a His-tag (MHHHHHHGDGGGGKDGK), was previously reported (Lamb et al., 1996). The sedimentation coefficient of such VLPs in sucrose gradients was similar to that of viral particles, suggesting the presence of encapsidated nucleic acids. In our study we expressed two types of VLPs, one with the C-terminus of the CP corresponding to the wild type protein and the second with a clathrin binding domain attached to the end of the CP. Protection of small nucleic acids by the PLRV CP was shown in a DNase I and RNase A protection assay.

*Paper was presented at the International Review Conference on Biotechnology, Vienna, Austria, November 2004.

Abbreviations: BCIP, 5-bromo-4-chloro-3-indolyl phosphate; CP, coat protein; NBT, 4-nitro blue tetrazolium chloride; PBS, phosphate-buffered saline; PLRV, potato leafroll virus; VLPs, virus-like particles.
RESULTS AND DISCUSSION

To express in insect cells the coat protein of potato leafroll virus modified at the N-terminus, two recombinant baculoviruses were constructed. BhCP, which contain cDNA encoding the CP unmodified at the C-terminus and BhCPc carrying cDNA with the gene modified by addition of a clathrin binding domain sequence. The modified constructs were obtained using PCR amplification and subcloning of PCR products (Fig. 1). The cloned DNA from pHfCP and pHfCPc was amplified by PCR using universal primers and the products were sequenced. These plasmids were used for all protein expression experiments.

Proteins present in extracts from Sf9 cells infected with non-recombinant baculoviruses or from Sf9 cells infected with a recombinant one containing either the hCP or hCPc constructs were fractionated by SDS/PAGE. Staining with Coomassie brilliant blue showed a polypeptide of about 27 kDa in cells infected with BhCP and of about 28 kDa in cells infected with BhCPc, that were not present in cells infected by non-recombinant baculovirus (Fig. 2).

In immunoblotting experiments two major polypeptides of 27 kDa for the BhCP recombinant and 28 kDa for the BhCPc recombinant, corresponding to the Coomassie-stained bands, were detected. Additional polypeptides of about 24 kDa and 25 kDa appeared for hCP and hCPc, respectively, probably as a result of protease activity in insect cells (Fig. 3).

A fraction of soluble proteins carrying VLPs from recombinant baculoviruses BhCP and BhCPc and similarly prepared proteins from non-recombinant baculovirus were treated with DNase I and RNase A. After extraction the remaining nucleic acids were separated on 1.5% agarose gel and the DNA was purified. It was shown that small heterologous nucleic acids were protected in the presence of the modified coat proteins of PLRV. The size of the putatively encapsidated nucleic acids is rather small and lies between the size of tRNA and the size of a single-stranded RNA of 200 nucleotides.

Our preliminary results show that the coat protein of potato leafroll virus is a good candidate for further experiments leading to the engineering of native or modified virus-like particles which could protect or encapsidate small nucleic acids. Additional in vitro assembly studies on PLRV VLPs will be
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Figure 1. Scheme of cloning of the PLRV CP gene and construction of donor FastBac plasmids.

Acknowledgements

This work was supported by a mini grant SBM 07 funded by the Warsaw School of Molecular Biology, PhD studies at the Institute of Biochemistry and Biophysics, to E.S.

Figure 2. SDS/PAGE of proteins from Sf9 cells.
Lane 1, cells infected with BhCP after 72 h; lane 2, cells infected with BhCP after 96 h; lane 3, cells infected with BhCPC after 72 h; lane 4, cells infected with BhCPC after 96 h; lane 5, prestained molecular mass marker (Fermentas), lane 6, cells infected with non-recombinant baculovirus; lane 7, CP of PLRV. Arrows indicate the infection-specific polypeptides. The gel was stained with Coomassie brilliant blue.

Figure 3. Immunoblotting of proteins from Sf9 cells.
Lane 1, cells infected with BhCP after 72 h; lane 2, cells infected with BhCP after 96 h; lane 3, cells infected with BhCPC after 72 h; lane 4, cells infected with BhCPC after 96 h; lane 5, prestained molecular mass marker (Fermentas), lane 6, cells infected with non-recombinant baculovirus; lane 7, CP of PLRV. Anti-PLRV antibodies linked to alkaline phosphatase were visualized by the NBT/BCIP assay.

Figure 4. Non-denaturing 1.5% agarose gel with nucleic acids derived from PLRV VLPs treated with DNase I and RNase A for 1 h at 37°C.
Lanes 1 and 2, cells collected after 72 and 96 h after infection with non-recombinant baculovirus; lanes 3 and 4, cells collected 72 and 96 h after infection with BhCP; lanes 5 and 6, cells collected 72 and 96 h after infection with BhCPC; lane 7, RNA Ladder High Range (Fermentas) 0.2, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 6.0 kb; lane 8, yeast tRNA.

focused on encapsidation of nucleic acids like oligonucleotides, PNA or siRNA.

Acknowledgements

This work was supported by a mini grant SBM 07 funded by the Warsaw School of Molecular Biology, PhD studies at the Institute of Biochemistry and Biophysics, to E.S.
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