Virus-like particles of *potato leafroll polerovirus* as potential carrier/delivery system in gene therapy

Elżbieta Sułuja, Ludmiła Strokowskaja, Wlodzimierz Zagórski-Ostoja and Andrzej Pałucha.
Institute of Biochemistry and Biophysics PAS; Ul. Pawińskiego 5a, 02-106 Warszawa, Poland;
alfap@ibb.waw.pl

Potato leafroll virus (PLRV) is the member of the polerovirus genus. The virus posses a small, 23 nm in diameter, isometric virion encapsidating a single-stranded, positive-sense, mono-partite genomic RNA with covalently attached Vpg at the 5' end. The coat protein (CP) of the virus is represented by two forms: i) 23 kDa protein, the product of ORF3 and ii) 65 kDa protein, the product of ORF3 and ORF5 expressed by read-trough of the CP gene stop codon. Virus-like particles (VLPs), when genetically engineered, are unique under macromolecules and are able to present foreign epitopes on their surface and may be competent to encapsidate various types of particles, i.e. nucleic acids.
The main aim of our work, is expression of PLRV-CP based proteins capable to assembly into VLPs in various systems. This modified particles will be tested for their ability to encapsidate RNA or DNA and to assembly into VLPs after engineering of foreign epitopes at the C-terminus of the CP.
In our preliminary studies two types of his-tagged at the N-terminus CP constructs were used for expression in insect cells. One, encoding a protein with the terminal amino acid sequence corresponding to the wild type CP (hCP) and the second with a clathrin binding domain at the tail (hCPc). Expression of hCP and hCPc proteins by a recombinant baculovirus was characterised by Western immuno-blotting with antibodies directed against the PLRV CP. The putative encapsidation of nucleic acids by CP derivatives was shown in DNase I and RNase A protection assays. Transfected with appropriate constructs insect cells were collected 72 and 96 hours post infection and homogenised by vortexing with glass beads in 0.1 M Na-phosphate pH 6.0 and 0.4 M NaCl buffer. After subsequent centrifugation steps, the content of the clarified supernatants was characterised by SDS-PAGE and Western-immunoblot experiments, in which the amount and quality of hCP and hCPc proteins was accomplished. The s-natants were also exposed to DNase I and RNase A digestion for 30 min at 4°C and 37°C in order to eliminate all unprotected nucleic acids from the samples. The obtained results shown that: i) modified CP-based proteins are efficiently expressed in insect cells; ii) low molecular weight nucleic acids from insect cells are protected from degradation by the presence of newly synthesised hCP and hCPc proteins.