Abstract

The latest model of tertiary structure of capsid protein of potato virus X (PVX CP) was used as a template to design new insertion sites suitable for the preparation of PVX-based antigen presentation system. Based on this model, seven insertion sites (A-G) located in putative surface loops were tested. As an antigen inserted into these sites was used 17 amino acids long epitope derived from human papillomavirus type 16 E7 oncoprotein (E7 epitope) fused with either 6xHis tag or StrepII tag in both possible orientations (6xHis-E7 and E7-6xHis, StrepII-E7 and E7-StrepII).

Prior to plant expression, modified PVX CPs were expressed in *Escherichia coli* MC1061. The results showed that only PVX CP carrying StrepII-E7 or E7-StrepII in the insertion site A formed virus particles.

The results from transient expression experiments with modified PVX CPs in *Nicotiana benthamiana* showed that only the insertion site A (located between 24th and 25th amino acid in the PVX CP) could tolerate all tested inserts. Importantly, viral particles were detected only in the presence of StrepII tag and their stability was affected by the insert orientation (StrepII-E7 vs. E7-StrepII) as only the viral particles presenting E7-StrepII could be purified.

Besides the preparation of PVX-based antigen presentation system, an evaluation of DNA damaging potential of PVX on *Nicotiana tabacum* var. Xanthi by the comet assay was performed. Based on this assay, it was shown that PVX caused DNA damage and the extent of DNA damage correlated with amount of PVX.