Construction of recombinant chimeric proteins on the basis of SV40 virus major coat protein VP1

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In order to change the tropism and immunogenicity of SV40 major surface protein VP1, chimeric virus-like particles forming proteins were constructed. According to the known structure of SV40 VP1, two surface-exposed sites were selected and hybrid VP1 genes containing inserted HBV surface antigen HBsAg encoding sequence were constructed. Hybrid genes were expressed in the yeast *Saccharomyces cerevisiae*. As was expected, chimeric SV40 VP1 VLPs which carried foreign polypeptides exposed on the surface of particles were efficiently expressed in the yeast *S. cerevisiae* and formed VLPs. It was demonstrated that SV40 VP1 derived VLPs tolerated the insertion of foreign sequences of different origin and length, suggesting them as a promising carrier moiety for vaccine development.

Key words: polyomaviruses, tropism, immunogenicity

INTRODUCTION

Polyomaviruses are non-enveloped double-stranded DNA viruses which are species-specific and exhibit a high level of tissue tropism. The simian monkey virus SV40 is one of the thirteen members of the family *Polyomaviridae* [1]. The SV40 virus major capsid protein VP1 generated by expression in yeast self-assembles spontaneously turns into virus-like particles (VLPs) whose structure and immunogenicity resemble that of virions. VLPs tolerate the insertion and surface exposure of foreign epitopes and potentiate their immunogenicity [2–4]. In this study, chimeric VLPs were generated using the SV40 major capsid protein from SV40. The major objective of our present study was to establish the SV40-VP1 as a new carrier moiety for foreign polypeptide sequences that allow the generation of chimeric VLPs.

MATERIALS AND METHODS

Generation of expression plasmids. All DNA manipulations were performed according to standard procedures [5]. Enzymes and kits for DNA manipulations were purchased from Fermentas AB. Recombinants were screened in *E. coli* DH5α. PCR amplification, cloning, transformation and expression in yeast of the polyomavirus VP1 and derivatives in yeast have been recently described recently [4, 6]. Purification of polyomavirus VP1 and derivatives was carried out as described in [6].

Electron microscopy. CsCl gradient fractions containing recombinant VP1 were placed on 400-mesh carbon coated palladium grids. Samples were stained with 2% aqueous uranyl acetate solution and examined using a JEM-100S electron microscope.

RESULTS AND DISSCUSSION

Selection of potential insertion sites in SV40 VP1. First of all, SV40 VP1 protein was expressed in the yeast *S. cerevisiae* in order to test the efficiency of VLPs formation in the yeast expression system. The results of expression confirmed that SV40 VP1 efficiently formed VLPs in yeast (Figure, a). Purified VLPs yielded 35–40 mg/l of yeast culture.

To define the potential insertion sites in SV40 VP1 for foreign polypeptide sequences in SV40 VP1, we took advantage of the known three-dimensional structure of SV40 VP1 and of our recent experience with the related polyomaviruses [4, 7]. The selected sites were localized in the four loops exposed to the surface of the virus particle: Site 1 – BC-loop, 73–

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74 aa; Site 2 – EF-loop, 198–199 aa; Site 3 – F-G1-loop, 228–229 aa; Site 4 – HI-loop, 273–274 aa. In this paper we present the data on insertion of foreign sequences in two external loops, BC and HI of SV40 VP1.

Introduction of *Bgl*II recognition site into BC-loop at the position 220–225 nt. To enable insertion of foreign epitopes into the BC loop of SV40 VP1 protein, the recognition sequence of restriction endonuclease *Bgl*II was created introduced in the SV40 VP1 gene at 220–225 nt, corresponding to the amino acids 74–75 of the encoded protein. Plasmid pBS-SV-VP1 was used as a template for PCR with primers SVBC5 and SVBC3.

SVBC5: 5'GAA **AGA TCT** TTT ACA G**G**T GAC TCT CCA GAC 3'

SVBC3: 5'AAA **AGA TCT** TTC AGC TGC TAA GCT TTT ACT 3'

*Bgl*II recognition sequences are underlined, non-complementary nucleotides are printed in bold. The product of the reaction was cleaved with *Bgl*II and ligated on to itself. The modified SV40 VP1 gene in the resulting plasmid pBS-SV-VP1-BC was sequenced. The original sequence AAA CAG (220–225 nt) changed into AGA TCT. The encoded amino acids Lys-Gln (74–75 aa) were substituted for Arg-Ser.

Introduction of *Bgl*II recognition site into HI-loop encoding sequence at the 820–825 nt. To enable insertion of foreign epitopes into the HI loop of SV40 VP1 protein, the recognition sequence of restriction endonuclease *Bgl*II was introduced in the SV40 VP1 gene at 820–825 nt, corresponding to amino acids 274–275 of the protein. Plasmid pBS-SV-VP1 was used as a template for PCR with primers:

SVHI5: 5'AAC **AGA TCT** GGA ACA CAG CAG TGG AAG GGA 3'

SVHI3: 5'TCC **AGA TCT** GTT GGT AAA CAG CCC ACA AAT 3'

*Bgl*II recognition sequences are underlined, non-complementary nucleotides are printed in bold. The product of the reaction was cleaved with *Bgl*II and ligated on to itself. The modified SV40 VP1 gene in the resulting plasmid pBS-SV-VP1-HI was sequenced. The original sequence ACT TCT (820–825 nt) changed into AGA TCT. The encoded amino acid Thr (274) was substituted for Arg.

Insertion of HBV HBsAg 101–169 aa encoding sequence in the structure of SV40 VP1. Human hepatitis B virus major surface antigen HBsAg major antigenic determinants encoding sequence, corresponding to the full length hydrophilic external loop from 101 aa to 169 aa were amplified with specific primers:

S-S101D: 5'CTG **TGA TCA GGT GGA TCT GGT** GGT ATG TTG CCC GTT TGT CCT 3'

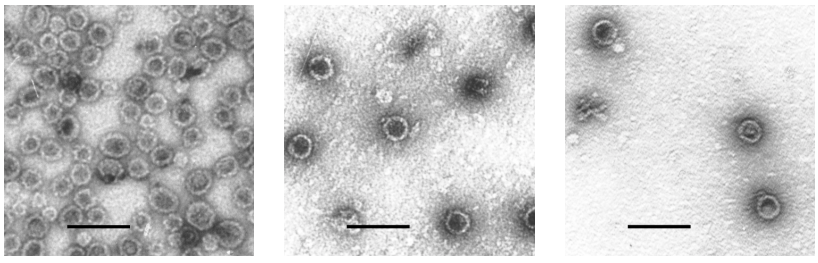
S-S169R: 5'CTG **AGA TCT TCC ACC AGA TCC ACC** GAA ACG GGC TGA GGC CCA CTC 3'

During amplification, a linker sequence containing Gly-Gly-Ser-Gly-Gly and cloning sites for restriction endonucleases *Bcl*I and *Bgl*II were inserted. The recognition sequences for restriction endonucleases are underlined, linker-encoding nucleotides are printed in bold. Plasmids pBS-SV-VP1-BC and pBS-SV-VP1-HI were cleaved with *Bgl*II and ligated with the amplified fragment encoding HBV S 101–169 aa flanked with Gly-Gly-Ser-Gly-Gly. Plasmids with the correct orientation of the insert were selected. The SV40 VP1 gene with the insert in the resulting plasmids pBS-SV-VP1-BC-S[101–169] and pBS-SV-VP1-HI-S[101–169] were sequenced.

Expression of chimeric proteins in yeast. The resulting two hybrid genes were inserted into the yeast expression vector and expressed in the yeast *S. cerevisiae*. The expression level and VLPs formation were investigated. Both chimeric proteins formeds VLPs (Figure, b and c).

CONCLUSIONS

To the best of our knowledge, this is the first report on the expression of SV40 VP1 as VLPs in yeast. Moreover, we have shown that SV40 VP1 VLPs tolerate insertions of large foreign sequences in two different positions, thus suggesting them as a promising carrier moiety for developing a new experimental vaccine. The SV40 VP1 system is suitable for the presentation of different epitopes on the surface of VLP.



A **B** **C**

Figure. Negative staining electron microscopy of SV40 VP1 (A), SV40 VP1 containing
HBV HBsAg 101–169 aa inserted at BC loop (B) and HI loop (C). Magnification x100000. Bar – 100 nm

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References

1. Cole CN, Conzen SD. In: Knipe DM and Howley PM (eds): Fields Virology, edition 4. Philadelphia, Lippincott Williams & Wilkins, 2001: 2141–96.
2. Ulrich R, Nassal M, Meisel H and Kruger DH. Adv Virus Res 1998; 50: 141–82.
3. Pumpens P and Grens E. FEBS Lett 1999; 442: 1–6.
4. Gedvilaite A, Frömmel C, Sasnauskas K et al. Virology 2000; 273: 21–35.
5. Sambrook J and Russell DW. Molecular cloning. A Laboratory Manual. 3rd edition. CSHL Press: Cold Spring Harbor, New York; 2001.
6. Sasnauskas K, Buzaite O, Vogel F et al. Biol Chem 1999; 380: 381–6.
7. Liddington RC, Yan Y, Moulai et al. Nature 1991; 354: 278–84.

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CHIMERINIŲ BALTYMŲ KONSTRAVIMAS PANAUDOJANT SV40 VIRUSO PAGRINDINĮ PAVIRŠIAUS BALTYMĄ VP1

S a n t r a u k a

Norint pakeisti SV40 viruso paviršiaus baltymo VP1 formuojamos dalelės tropizmą, buvo pabandyta modifikuoti VP1 koduojantį geną, į jo struktūrą įterpiant sekas, koduojančias žmogaus hepatito B viruso paviršiaus baltymo pagrindinius epitopus. Remiantis rentgeno struktūrinės analizės duomenimis, modifikacijai parinktos virusinės dalelės paviršiuje eksponuojamos kilpos. Modifikavus geną ir į modifikacijos vietas įvedus hepatito viruso sekas, genas buvo patalpintas į mielių ekspresijos sistemą. Paaiškėjo, kad sukonstruotas baltymas mielėse formuoja elektroniniu mikroskopu matomas į virusą panašias daleles, kurios savo paviršiuje eksponuoja hepatito viruso paviršiaus epitopus.