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14. ABSTRACT <p>Polyvalent protein shells (capsids) are useful platforms for the display of molecules of interest (MOI) on their surface. The resulting polyvalent reagents act as efficacious prophylactic vaccines and therapeutics. The coat protein subunits of Tomato Bushy Stunt Virus (TBSV) and structurally similar Norwalk viruses, when expressed in insect cells, spontaneously self assemble to form protein shells. The self-assembly of the coat protein mutants of TBSV resulted in two types of nanoparticles: small (60 subunit) and the regular size (180 subunit) capsids. These protein shells (capsids) can be used for the display of 60-180 copies of peptides/proteins of the pathogens of concern.</p> <p>Previously, it has been shown that antibodies raised against various cytotoxins (e.g., ricin and Shiga toxin) render protection against the potential toxin attack. The proposed polyvalent reagents, which display various peptide/protein fragments of ricin would act as prophylactic vaccines of the ricin toxin. As a proof of concept we have successfully generated a reagent displaying one of the ricin peptides (RTA, 95-110) on the surface of TBSV. Generation of other reagents and estimation of their efficacy are underway. Continued support is requested for the creation of new and novel reagents that render protection against the different types of cytotoxins.</p>					
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INTRODUCTION

The proposal titled "Creation of Polyvalent Decoys of Protein Cytotoxins as Therapeutics and Vaccines" entails displaying multiple (60-180) copies of antigenic regions of ricin toxin and other similar cytotoxins. These polyvalent reagents have been suggested to work as prophylactic vaccines against the respective cytotoxins (Mourez et al., 2001). Towards this end we have proposed to create display platforms of protein shells by over-expressing the coat proteins of Tomato Bushy Stunt Virus (TBSV) and a structurally similar Norwalk virus in the first year of the contract. The unique 2-domain subunit structure of the above coat protein subunits facilitate the display of any proteins/peptides of interest either by replacing the C-terminal P-domains of the coat protein subunits or appending at the end of the P-domain.

BODY OF REPORT

STATEMENT OF WORK:

Expression and structural characterization of the polyvalent display platform of altered (mutant) capsids of Tomato Bushy Stunt Virus (TBSV) and Norwalk Viruses (month 1-12).

Generation of protein shells of TBSV and Sinsiro virus capsid proteins in insect cells:

The capsid protein subunits of TBSV and Sinsiro (Norwalk) viruses have been expressed in insect cells successfully using baculovirus system. Full length (CP-FL) and a N-terminal deletion mutant (CP-N Δ 52) of the TBSV capsid protein form native like (T=3) protein shells of 32nm in diameter made up of 180 protein subunits, while the longer N-terminal deletion mutants, CP-N Δ 72 and CP-N Δ 82 form smaller 21nm diameter (T=1) particles with 60 subunits (Figure 1). Similarly, the full-length coat protein Sinsiro virus expressed in insect cells spontaneously forms 35-40nm protein shells made of 180 subunits.

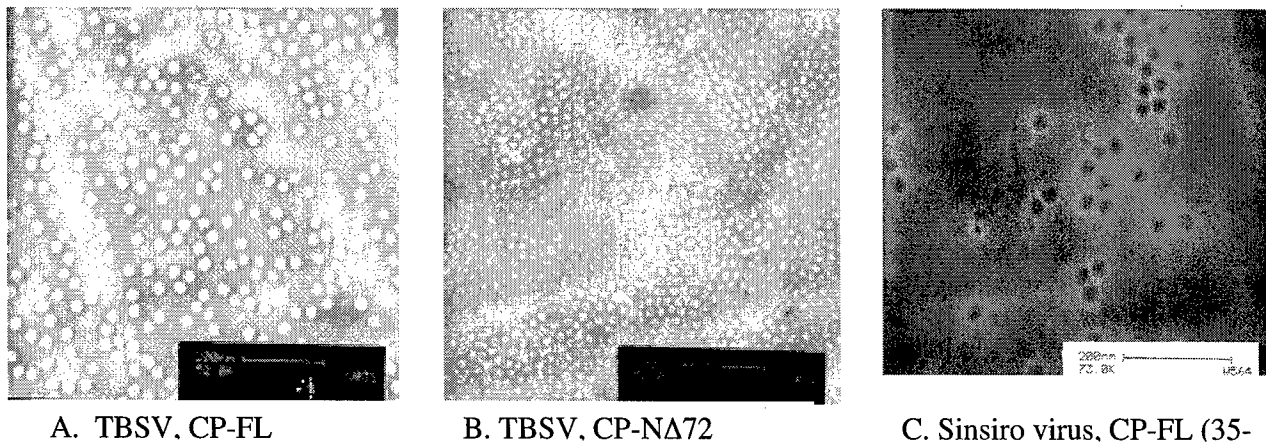


Figure 1. A) Electron micrographs of the 32nm nanoparticles (protein shells) assembled from the full-length TBSV coat protein. B) Smaller 21nm nanoparticles assembled from the coat protein (CP-N Δ 72) with the first 72 amino acids deleted. C) 35-40nm size protein shells made of full-length coat protein of Sinsiro virus. All the samples were visualized by coating with 1% uranyl acetate stain.

Cryo-electron microscopy and image reconstruction methods were employed to determine the structures of respective particles at about 20Å resolution (Figure 2). The above results suggest that each kind of protein shells is made uniformly with high fidelity.

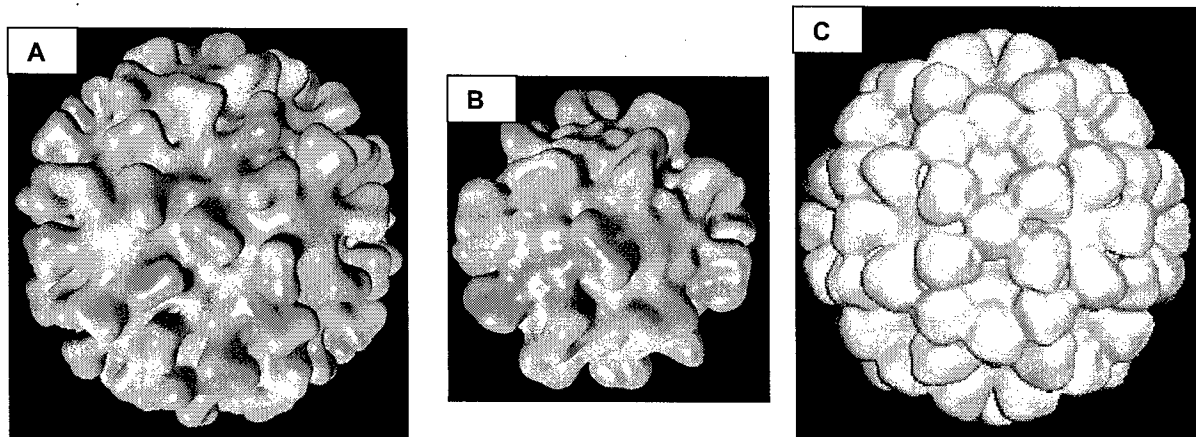


Figure 2. Cryo electron microscopy reconstructions of A) Protein shell (32nm) made of CP-NΔ52 of TBSV B) Smaller (21nm) particles made of TBSV CP-NΔ72 and C) Protein shell (~38nm) made from the full length coat protein of Sinsiro virus.

Creation of polyvalent reagents displaying an RTA peptide on TBSV capsids:

Having successfully generated two types of proteins shells as display platforms, a polyvalent reagent displaying multiple copies of RTA peptide of residues 95-110 was created by genetically appending the peptide at the C-terminal end of the TBSV coat protein. It has been shown that residues 95-110 or ricin-A chain creates

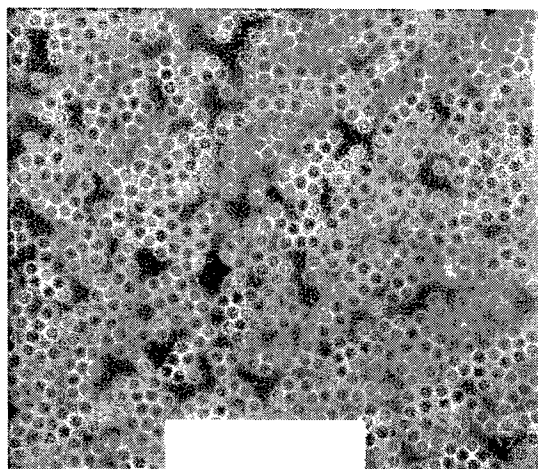


Figure 3. Electron micrograph showing the protein shells of TBSV with 180 copies of the RTA peptide of residues 95-110 displayed on the surface.

neutralizing antibodies that render protection against the ricin toxin (Olson et al., 2004). This novel reagent contains 180 copies of the RTA peptide displayed on the surface of TBSV capsid. We propose that the new reagent will be highly efficacious compared to single molecules/peptides on molar basis in generating antibodies against the ricin, hence provide protection against the toxin attack.

Figure 3 shows an electron micrograph of the TBSV capsids displaying the RTA peptide. The figure 2 clearly shows slightly larger and rather corrugated surface of the TBSV-RTA proteins shells compared to the native protein shells. Efforts are well underway to display larger domains of RTA chain on TBSV capsids.

KEY RESEARCH ACCOMPLISHMENTS:

- 1) Expression/Creation of two types of nanoparticles of TBSV capsid proteins as potential display platforms of molecules of interest.
- 2) Creation of another nanoparticle platform from Sinsiro virus capsid protein using the baculovirus system.
- 3) Structural characterization of the above nanoparticles using cryo-electron microscopy and image analysis.
- 4) Creation of polyvalent reagent displaying the 180 copies of the RTA peptide of residues 95-110 as a prophylactic ricin vaccine.

CONCLUSIONS:

We have made a number of important advances in the past year by generating a 3 different proteins shells of varying sizes for the purposes of displaying molecules of interest and their potential use as vaccines and therapeutics. These nanoparticles are currently being used to display different segments of RTA chains there by creating potential vaccines for ricin toxin.

References:

- Mourez, M., Kane, R. S., Mogridge, J., Metallo, S., Deschatelets, P., Sellman, B. R., Whitesides, G. M., and Collier, R. J. (2001). Designing a polyvalent inhibitor of anthrax toxin. *Nat Biotechnol* **19**(10), 958-61.
- Olson, M. A., Carra, J. H., Roxas-Duncan, V., Wannemacher, R. W., Smith, L. A., and Millard, C. B. (2004). Finding a new vaccine in the ricin protein fold. *Protein Eng Des Sel* **17**(4), 391-7. Epub 2004 Jun 08.

APPENDIX: Publications from W81XWH-04-2-0027

Catherine Hsu, Pratik Singh, Wendy Ochoa, Darley J. Manayani, Anette Schneemann, Vijay S. Reddy :
Characterization of polymorphism displayed by the coat protein mutants of Tomato Bushy Stunt Virus (TBSV) (manuscript in preparation).