F328 AcNPV Membrane Fusion Mechanism: Mutational Analysis of Hydrophobic Region I of gp64 Glycoprotein

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The baculovirus gp64 glycoprotein is a major envelope fusion protein in budded virus (BV). The gp64 is activated by the acidification of the endosome, resulting in the fusion of the viral envelope with the endosome membrane. To investigate the role of the amino acids within hydrophobic region I and it's adjacent region of gp64, substitution mutants of gp64 glycoproteins was constructed by PCR-derived site-directed mutagenesis. After the mutated gp64 plasmid DNAs were transfected into Sf9 cells, oligomerization, surface localization and fusion activity of transiently expressed gp64 glycoproteins were investigated. All of the mutant gp64 glycoproteins except cysteine-228 were able to form trimers and were transported to the cell surface. Both C228A (Cys-228 to Ala-228) and C228WK232L (Cys-228 to Trp-228 and Lys-232 to Leu-232) mutants which were not able to form trimers and not transported to the cell surface did not show any detectable fusion activity. K225R (Lys-225 to Arg-225), K232R (Lys-232 to Arg-232) and D233E (Asp-233 to Glu-233) mutants showed fusion activity similar level to that of wild type gp64. K225E (Lys-225 to Glu-225), K225L (Lys-225 to Leu-225), K232E (Lys-232 to Glu-232), K232L (Lys-232 to Leu-232), D233K (Asp-233 to Lys-233), D233L (Asp-233 to Leu-233) and I224E (Ile-224 to Glu-224) showed slightly reduced fusion activity compared to wild type gp64. Although L229AL230A (Leu-229 to Ala-229 and Leu-230 to Ala-230), A227K (Ala-227 to Lys-227) and I231K (Ile-231 to Lys-231) were able to form trimers, these mutants were not able to retain their low pH-dependent fusion activity. But L229VL230V (Leu-229 to Val-229 and Leu-229 to Val-230), A227V (Ala-227 to Val-227) and I231V (Ile-231 to Val-231) were able to retain their low pH- dependent fusion activity. These results suggest that the hydrophobicity of Alanine-227, Leucine-229, Leucine-230 and Isoleucine-231 in the hydrophobic region I plays critical role in membrane fusion activity. Also Cysteine-228 may be involved in either oligomerization or/and disulfide bridge formation that is important for fusion activity.

F329 Expression of the Human Rotavirus VP7 Glycoprotein Using a Baculovirus Vector System

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Rotavirus is a major cause of severe gastroenteritis in young children and animals throughout the world. The outer capsid glycoprotein VP7 of rotavirus has been considered to play critical role in early event of the viral infection and to induce the synthesis of neutralizing antibody. In this study, cDNA coding for VP7 of human rotavirus obtained from Korean patient (Y14 strain) was subcloned into baculovirus vector and the VP7 glycoprotein was expressed in insect cells. The cDNA coding for VP7 was inserted next to the strong polyhedrin promoter of pBlueBacIII, a baculovirus transfer vector. The transfer vector and wild type AcNPV DNA were co-transfected into Sf9 cells to generate recombinant virus (rAcVP7-211). A putative recombinant virus was obtained and isolated by three rounds of plaque purification. The results of PCR (Polymerase Chain Reaction), southern blot analysis and occlusion minus phenotype of recombinant virus confirmed the insertion of the cDNA coding VP7 within the recombinant virus. The recombinant virus was infected in Sf9 cells and the cell extract and tissue culture fluid was tested by SDS-PAGE and Western blot analysis with monoclonal antibody against synthetic peptide containing 20 amino acids within VP7 conserved region. The molecular weight of recombinant VP7 was estimated to be 36 kDa which is about the same size with the native VP7. Tunicamycin treatment in the culture media showed that the recombinant VP7 was also glycosylated. Application of the recombinant VP7 glycoproteins for developing subunit vaccine for rotavirus infection is also discussed.