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Antigenic structure analysis of glycosylated protein 3 of porcine reproductive and respiratory syndrome virus

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Abstract

The function of the glycosylated protein 3 (GP3), a porcine reproductive and respiratory syndrome virus (PRRSV) associated protein is poorly known. In the present study, the gene encoding GP3 (ORF3), lacking the highly hydrophobic domain in the N- and C-termini was expressed as GST-fusion proteins in *E. coli*. Monoclonal antibodies (MAbs) against GP3 were developed and used to probe a series of GP3 peptides using ELISA. After precise analysis by sequential deletion of the terminal amino acid residues from each peptide, the minimal epitopes recognized by the MAbs were localized to W^{74}CRIGHDRCGED^{85} and Y^{67}EPGRSLW^{74}. The epitope sequences were well conserved among most of the North American-type isolates, with the exception of two amino acid mutations in both epitopes in a few of these isolates. Mutational analysis revealed that these mutants were not recognized by any of the five MAbs, indicating that genetic variation could lead to altered antigenicity. Eight out of nine peptide fragments, 58–72aa, 73–87aa, 88–101aa, 102–115aa, 50–65aa, 66–81aa, 80–95aa and 94–109aa were recognized by PRRSV-positive pig serum as determined by Western blot analysis. The results herein may elucidate partially the antigenic structure of GP3 and variations of PRRSV.

Keywords: PRRSV; GP3; Epitope

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS), characterized by reproductive failure in late term gestation sows and respiratory disease in pigs of all ages, is a major problem in the swine industry worldwide. Porcine reproductive and respiratory syndrome virus (PRRSV), a member of the family *Arteriviridae*, is the causative agent. Glycosylated protein 3 (GP3), encoded by ORF3, is comprised of 254 amino acids (aa), with a very low identity between European and North American isolates (Mardassi et al., 1995; Meng et al., 1995; Meulenberg et al., 1995; Murtaugh et al., 1995). Mardassi (1995), Murtaugh (1995), Katz (1995) and coworkers reported that the most important cause of this difference is the first 35aa, which are 29% indentical among European and North American isolates. However, the first 30 aa of GP3 constitute a signalling peptide, which are removed during protein maturation. Thus, this region cannot affect the biological characteristics of GP3 notably. After analyzing the degree of antigenic variability in a series of English isolates using monoclonal antibodies, Drew et al. (1995) determined that the C-terminus of GP3 varied highly among the different isolates. In addition, GP3 was found to be associated with the mature virion as a structural protein in the European-type isolates (Meulenberg et al., 1995; van Nieuwstadt et al., 1996). However, the GP3 of the Quebec IFA-Klop (a typical North American isolate) is a soluble non-virion associated secreted protein (Gonin et al., 1998, 1999; Mardassi et al., 1998). In the life cycle of the equine arteritis virus (EAV), also a member of *Arteriviridae*, GP3 is crucial to the process of forming infectious virions (Molenkamp et al., 2000). The antibody against GP3 plays a role in viral clearing (Plana et al., 1997). Antigenicity and epitope information of glycosylated protein 3 is scarce. In the present study, we identified two epitopes using MAbs and seven epitopes using PRRSV-positive pig sera with a series of overlapping peptides.

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2. Materials and methods

2.1. Virus, cell and serum

The PRRSV CH-1a strain (GenBank accession no. AY0362626) was maintained in our laboratory. The SP2/0 cells were preserved in our laboratory. The PRRSV CH-1a positive pig sera were kindly provided by the PRRSV research team of the Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, China.

2.2. Construction of expression vectors

Based on the analysis by bio-software DNAStar (DNASTAR, Inc., Madison, WI), we amplified the highly antigenic domain (50–240aa) by reverse transcriptase polymerase chain reaction (RT-PCR), with lacking the signal peptide sequence and the hydrophobic domains of the N- (1–49aa) and C-termini (241–254aa). For cloning directionally into the expression vector pGEX-6p-1 (Amersham Life Sciences, Piscataway, NJ), *Bam*H I and *Xho* I recognition sites were introduced in the 5′-end of forward and reverse primers, respectively (Table 1). We verified directionality of the recombinant plasmid with restriction analysis and nucleotide sequencing. Plasmids were transformed into *E. coli* BL21 (DE3) (Novagen, Darmstadt, Germany) cells for expression. Recombinant protein expression was induced with 1 mM isopropyl-D-thiogalactopyranoside (TPTG, Amersham Life Sciences). Cells were harvested by centrifugation and the pellets were suspended in phosphate buffered saline (PBS; pH 7.4). After being analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting with pig serum, the fusion-expressed truncated GP3 (rtGP3) protein was purified by Glutathione Sepharose 4B Redi-Pack Column affinity chromatography according to the manufacturer's instructions (Amersham Life Sciences). The purity of the rtGP3 was analyzed on SDS-PAGE.

2.3. Generation of MAbs

We immunized the BALB/c mice with purified rtGP3 and generated GP3 monoclonal antibodies according to the methodology of Zhang and Rong (1987). Hybridoma culture supernatants were screened by indirect enzyme-linked immunosorbent assay (ELISA). Briefly, ELISA plates were coated overnight with prokaryotic-expressed rtGP3 (5 μg/well) and blocked for 2 h with 4% non-fat milk at 37 °C, followed by three washes with phosphate buffered saline/Tween-20 (Tianxiang, Jiangsu, China). The plates were then incubated with hybridoma supernatant at 37 °C for 1 h. The bound MAbs were detected with HRP-conjugated goat anti-mouse IgG (Sigma–Aldrich, St. Louis, MO), and developed with *o*-phenylenediamine dihydrochloride (OPD, Sigma–Aldrich). Each sample was analyzed in triplicate. Furthermore, the supernatant of the positive cell clones were tested by indirect immunofluorescence assays (IFA) using Marc-145 cells infected by PRRSV, as described by Mardassi et al. (1994).

2.4. Broad epitope mapping with overlapping GP3 protein fragments

Based on the analysis by the OLIGO6.24 (Wojciech, USA) bio-software program, the truncated ORF3 gene was divided into two overlapping fragments for PCR amplification. To facilitate directional cloning into the pGEX-6p-1 expression vector, we incorporated the *Bam*H I and *Xho* I sites into the primers (Table 1). The recombinant plasmids were transformed into *E. coli* BL21 (DE3) (Novagen) cells for expression. After SDS-PAGE analysis, the expressed proteins were used to coat the wells of the ELISA plates at a concentration of 5 μg/well, and were then probed with five anti-GP3 MAbs, as described above.

2.5. Screening by peptides

Based on the epitope analysis using larger fragments of the GP3 described above, the N-terminal of GP3-P1 (49 through 116 aa), which did not overlap with GP3-P2, was divided into 9 additional overlapping fragments (GP3EP1–GP3EP9), described in Table 2. To express these polypeptides, we synthesized complementary oligonucleotide pairs encoding each peptide, and annealed and cloned them into the *Bam*H I and *Xho* I sites of pGEX-6p-1, resulting in nine recombinant plasmids. The GST-fusion proteins were expressed and used to screen the MAbs by ELISA, as described above.

Table 1
RT-PCR primers used for amplifying the ORF3 fragments

Name	Sequence of PCR primers[a]	Position	
		in genomic	in GP3 protein
tORF3	5′GTT*GGATCC*ATGTTTTCTTTTGAACTCACGGT3′ 5′TCG*CTCGAG*AGGACGAGTCGCCATGCCTAAGG3′	12811–13397	49–240
ORF3-P1	5′ GTT*GGATCC*ATGTTTTCTTTTGAACTCACGGT3′ 5′ CCA*CTCGAG*TTAAAACCAATTGCCGCCGTC3′	12811–13216	49–179
ORF3-P2	5′ TTC*GGATCC*TTCAGCTACACGGCCCAGTTCCA3′ 5′ TCG*CTCGAG*TTAACGAGTCGCCATGCCTAAGG3′	13033–13397	116–240

[a] For directional cloning, *Bam*H I and *Xho* I recognition sites (written in italics) were introduced into the forward and reverse primers, respectively.