Virus Research 153 (2010) 244–249

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Analysis of expression and glycosylation of avian metapneumovirus attachment glycoprotein from recombinant baculoviruses

Lizhong Luo[a,*], Krista Nishi[a], Erin MacLeod[a], Marta I. Sabara[a], Yan Li[b]

[a] National Centre for Foreign Animal Disease, Canadian Food Inspection Agency, 1015 Arlington Street, Winnipeg, Manitoba, Canada R3E 3M4
[b] National Microbiology Laboratory, Public Health Agency of Canada, 1015 Arlington Street, Winnipeg, Manitoba, Canada R3E 3R2

ARTICLE INFO

Article history:
Received 30 June 2010
Received in revised form 5 August 2010
Accepted 7 August 2010
Available online 14 August 2010

Keywords:
Avain metapneumovirus
Glycosylation of attachment glycoprotein
Baculovirus expression

ABSTRACT

Recently, we reported the expression and glycosylation of avian metapneumovirus attachment glycoprotein (AMPV/C G protein) in eukaryotic cell lines by a transient-expression method. In the present study, we investigated the biosynthesis and O-linked glycosylation of the AMPV/C G protein in a baculovirus expression system. The results showed that the insect cell-produced G protein migrated more rapidly in SDS-PAGE as compared to LLC-MK2 cell-derived G proteins owing to glycosylation differences. The fully processed, mature form of G protein migrated between 78 and 86 kDa, which is smaller than the 110 kDa mature form of G expressed in LLC-MK2 cells. In addition, several immature G gene products migrating at 40–48 and 60–70 kDa were also detected by SDS-PAGE and represented glycosylated intermediates. The addition of the antibiotic tunicamycin, which blocks early steps of glycosylation, to insect cell culture resulted in the disappearance of two glycosylated forms of the G protein and identified a 38 kDa unglycosylated precursor. The maturation of the G protein was completely blocked by monensin, suggesting that the O-linked glycosylation of G initiated in the trans-Golgi compartment. The presence of O-linked sugars on the mature protein was further confirmed by lectin *Arachis hypogaea* binding assay. Furthermore, antigenic features of the G protein expressed in insect cells were evaluated by ELISA.

1. Introduction

Avian metapneumovirus (AMPV) is an emerging respiratory viral pathogen in turkeys, causing severe economic loss to the turkey industry (Alexander, 1997). Although AMPV has been reported principally in turkeys (Panigrahy et al., 2000) cases have also been identified in ducks, pheasants, guinea fowl and ostriches (Cook, 2000) and has been associated with swollen-head syndrome of chickens (Maharaj, 1994; Pattison and Chette, 1989). AMPV belongs to the family *Paramyxoviridae*, subfamily *Pneumovirinae*, and genus *Metapneumovirus* (Collins and Crowe, 2007; Pringle, 1999). Like all members of the paramyxovirus family, the AMPV is a negative-sense, nonsegmented single-stranded RNA virus that contains eight genes, namely; nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), second matrix (M2), small hydrophobic (SH), attachment protein (G), and RNA-dependent RNA polymerase (L) in the order 3′-N-P-M-F-M2-SH-G-L-5′ (Fig. 1). Based on nucleotide sequence divergence in the attachment glycoprotein genes and their antigenic differences, AMPV isolates have been classified into four subgroups AMPV/A-D (Bayon-Auboyer et al., 2000; Lwamba et al., 2005). The antigenic diversity of AMPV likely corresponds to nucleotide variability in the three envelope glycoproteins: SH, G and F (Bayon-Auboyer et al., 2000; Luo et al., 2005, 2009).

Similar to HRSV and HMPV, the G gene of APMV/C is the most divergent structural protein among isolates (Govindarajan et al., 2004; Toquin et al., 2003). It is considered to play a key role in viral attachment to cell membranes (Levine et al., 1987) and the induction of a protective immune response (Bennett et al., 2005). Analysis of the nucleotide sequence of the AMPV/C G protein gene was shown to consist of 783 nucleotides coding for 252 amino acids. The deduced amino acid sequence of the APMV/C G protein contains a single hydrophobic transmembrane domain that is located near the N-terminus (amino acids 32–54) as an uncleaved signal peptide and a membrane anchor (Toquin et al., 2003; Govindarajan et al., 2004). Furthermore, the G protein is heavily glycosylated and 50% of the molecular weight of the mature protein (*M*r 110,000) may be provided by carbohydrates, predominantly O-linked with some N-linked sugars. Consistent with this degree of glycosylation, the AMPV/C G protein has a 30% content of serine and threonine, which are potential acceptor sites for O-linked sugars (Govindarajan et al., 2004; Toquin et al., 2003). In addition, the G protein also contains high level of proline residues (6.3%) and five potential N-linked glycosylation sites (Toquin et al., 2003) (Fig. 1). The excessive potential O-glycosylation sites and high level of proline residues suggest a heavily glycosylated mucin-like structure for the G protein. Thus,

* Corresponding author. Tel.: +1 204 789 2149; fax: +1 204 789 2038.
E-mail address: lizhong.luo@inspection.gc.ca (L. Luo).

0168-1702/$ – see front matter.
doi:10.1016/j.virusres.2010.08.008

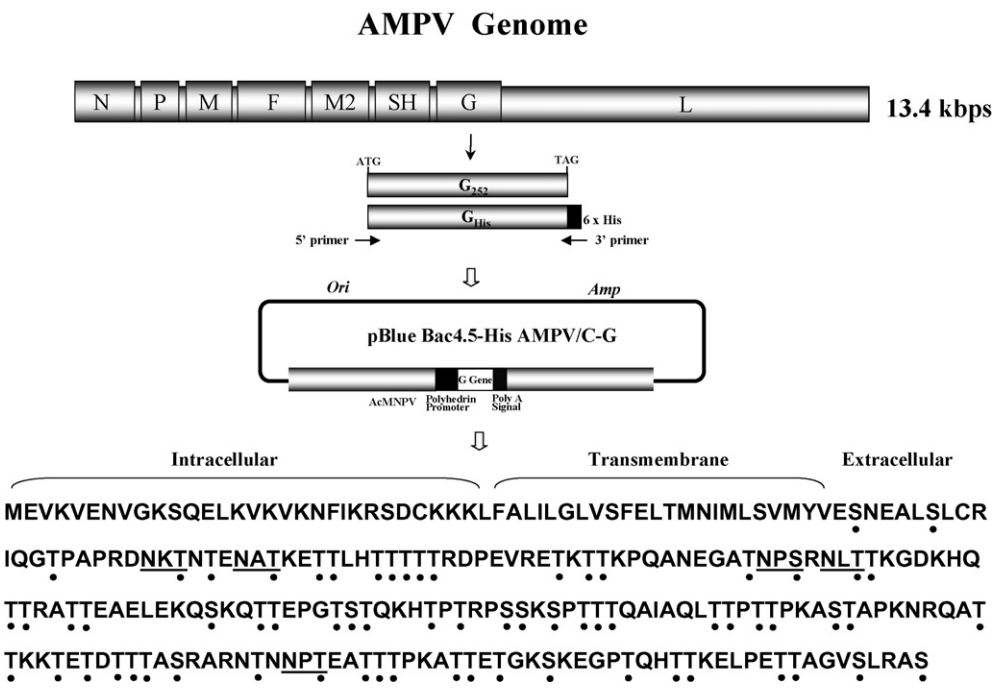

Fig. 1. Construction and expression of AMPV/C G glycoprotein gene. A 756 bps segment containing the G genes of AMPV/C was amplified and cloned into the baculovirus transfer vector, pBlueBac4.5, under the control of the polyhedrin promoter. The ATG start and TAG stop codons for the G gene are indicated by a bar. The primer set used for amplication is indicated by arrows. A six-histidine tag at carboxyl terminus of the construct G_{His} is indicated. The black dots represent potential acceptor sites for O-linked sugars.

it is important to determine the extent of glycosylation of the glycoprotein.

The baculovirus expression system is widely considered to be an excellent tool for recombinant glycoprotein production. Insect cells are capable of performing many of the same processing events required for the production of biologically active proteins as in mammalian cells (Indyk et al., 2007). However, the difference between the two cell lines is that mammalian cells produce more complex N-glycans containing terminal sialic acids and insect cells produce simpler N-glycans with terminal mannose residues. In this report, we analyzed the biosynthesis and O-linked glycosylation of the AMPV/C G protein in the baculovirus expression system.

2. Materials and methods

2.1. Construction of recombinant plasmid

The AMPV Colorado strain Lot Number: 193ADV9902 (Animal and Plant Health Inspection Service, National Verterinary Service Laboratories, Ames, Iowa) was propagated in QT-35 cells (Sabara et al., 2003). Virion-associated RNA was extracted from infected cells using the RNeasy Mini Kit (Qiagen, Toronto, Ontario, Canada) according to the manufacturer's instruction. The G protein gene coding for 252 amino acids was amplified by RT-PCR with primers AMPV/C G 5F (5′-AACATGGAGGTCAAGGTAGAGAATG-3′) and AMPV/C G 3R (5′-GCTAACTTGCTCTTAAACTAACTCCTG-3′), which were based on the published sequence of the G gene (GenBank accession number AY579780). The amplified G gene product was cloned into the baculovirus transfer vector, pBlueBac4.5/V5-His (Invitrogen Burlington, Ontario, Canada). In addition, the G gene containing a six-histidine tag at the carboxyl terminus was also constructed (Fig. 1). The resulting recombinant plasmids were designated, respectively, as pBlueBac4.5-AMPV/C G_{252} or pBlueBac4.5-AMPV/C G_{His}. The constructs were also confirmed by DNA sequencing using the ABI 377 sequencer with a fluorescent dye terminator kit (Applied Biosystems, Streetsville, Ontario, Canada).

2.2. Generation of recombinant baculovirus

The recombinant baculovirus was generated by cotransfecting SF21 cells with 0.5 μg linearized wild-type AcNPV DNA and 1 μg of the transfer vector pBlueBac4.5-AMPV/C G_{252} or pBlueBac4.5-AMPV/C G_{His} according to the manufacturer's instructions (Invitrogen, Burlington, Ontario, Canada). After cotransfection, the culture supernatant was harvested and titrated on SF21 cells. Polyhedrin-negative plaques were selected and purified by consecutive plaque picking and used to produce a virus stock of 1×10^8 plaque forming units (PFU)/ml (Luo et al., 2010). The G gene insertion was confirmed using a polymerase chain reaction (PCR) screening procedure followed by DNA sequencing of insertional junctions. Recombinant viruses, designated Ac-Bac-AMPV/C-G_{252} and Ac-Bac-AMPV/C-G_{His}, were verified by Western blot analysis.

2.3. Expression analysis and immunoblotting

SF21 cells were infected with recombinant virus Ac-Bac-AMPV/C-G_{His} at a multiplicity of infection (MOI) of 5 PFU/cell and incubated at 27 °C. After an appropriate incubation time, whole cell lysates were prepared in sample buffer containing sodium dodecyl sulphate (SDS) and β-mercaptoethanol and electrophretically separated on a 10% SDS-polyacrylamide gel (PAGE) using a procedure described by Luo et al. (2010). Fractionated proteins were then transferred onto an immunoblot polyvinylidene difluoride (PVDF) membrane sheet (BioRad, Mississauga, Ontario, Canada). Hyperimmune sera, specific for AMPV subgroup C and anti-His antibody diluted 1:1000 were used to probe the G_{252} and G_{His} proteins. After incubation with horseradish peroxidase-conjugated anti-mouse antibody, the immunoblots were visualized using enhanced chemiluminescent substrate (Pierce, Ottawa, Ontario, Canada).