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Protein display by bovine herpesvirus type 1 glycoprotein B

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ABSTRACT

Glycoprotein B (gB) of bovine herpesvirus 1 (BHV-1), a major component of the viral envelope, is essential for membrane fusion during entry and cell-to-cell spread. It is cleaved in the *trans*-Golgi network by the proprotein convertase furin. Integration of the open reading frame (ORF) encoding a mutated gB with a second furin cleavage site and mature boIFN-α as intervening peptide between the amino-terminal (NH_2) and carboxy-terminal (COOH) gB subunits yielded recombinant BHV-1/gB2FuIFN-α which, unexpectedly, express gB with an enlarged NH_2-subunit of 90 kDa. Here we show that boIFN-α-specific antibodies bind to the 90 kDa gB subunit and efficiently neutralize BHV-1/gB2FuIN-α infectivity. We also show that inactivated BHV-1/gB2FuIN-α virions induce an antiviral state in cells incubated with UV-inactivated particles. These results demonstrate that the 90 kDa protein is a NH_2-subunit/boIFN-α fusion protein whose boIFN-α domain is biologically active. To verify that BHV-1 gB is suitable for the display of (glyco)proteins on the surface of virions we constructed BHV-1 recombinants expressing within gB the first 273 amino acids of the NH_2-subunit (HA1) of avian influenza haemagglutinin, either flanked by two furin cleavage sites or with only one cleavage site between a gB/NH_2_HA1 fusion protein and the COOH subunit. The resulting recombinant BHV-1/gB2FuHA1 expressed gB from which 55 kDa HA1 was excised and secreted. In contrast, gB from BHV-1/gB_NH_2HA1 infected cells retained HA1 as fusion protein with the NH_2-subunit. Immunoblotting and neutralization analyses revealed that HA1 is incorporated into the envelope BHV-1/gB/NH_2_HA1 particles and exposed to the exterior of virions. Thus, this novel approach enables display of polypeptides and (glyco)proteins of at least 273 amino acids on viral particles which is of particular interest for development of novel diagnostics and vaccines as well as for, e.g. gene therapy applications especially when biologically active ligands need to be presented.

1. Introduction

The subtilisin-like endoprotease furin is a ubiquitous protease that cleaves protein precursors in the secretory pathway after the consensus sequence -arginine-Xaa-lysine or arginine-arginine (R-X-K/R-R; X = any amino acid). Furin is mainly localized in the *trans*-Golgi network and plays important roles in, e.g. embryogenesis, homeostasis and in diseases like Alzheimer's disease, cancer, anthrax and Ebola fever (Nakayama, 1997; Thomas, 2002). Envelope glycoproteins of pathogenic viruses such as hemagglutinin of influenza virus, gp160 of human immunodeficiency virus and F_0 proteins of Sendai virus, measles virus, and respiratory syncytial virus (RSV) require cleavage at consensus furin sites to become fusogenic which is required for infectivity (Klenk and Garten, 1994; Nakayama, 1997). After cleavage, disulfide bonds covalently link the amino-terminal (NH_2-) and carboxy-terminal (COOH-) subunits of the respective glycoprotein. The F proteins of human and bovine RSV (BRSV) share a so far unique feature among the furin-cleaved fusion proteins. The respective F_0 proteins are cleaved twice

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which results in release of a short oligopeptide of 27 amino acids, pep27 (Gonzalez-Reyes et al., 2001; Zimmer et al., 2001). For BRSV it has been shown that pep27 is secreted as a biologically active member of the tachykinin family (Zimmer et al., 2003). We demonstrated recently that pep27 of BRSV can be replaced by bovine interleukins which are secreted into the culture medium of cells infected with the respective recombinants (König et al., 2004). Most of the conserved glycoproteins B (gB) of herpesviruses which are major components of the respective virions and essential for virus entry and direct spread from cell-to-cell (Spear and Longnecker, 2003), are also cleaved by furin (Kopp et al., 1994), although gB of several herpesviruses such as herpes simplex virus type 1 and type 2 is not cleaved (Claesson-Welsh and Spear, 1986; Eberle and Courtney, 1982). Mutagenesis of the furin recognition sequence in gB of the betaherpesvirus human cytomegalovirus and the alphaherpesviruses pseudorabiesvirus and bovine herpesvirus 1 (BHV-1) – members of the subfamily *Alphaherpesvirinae*, genus *Varicellovirus* – demonstrated that cleavage of gB is not required for replication in cell culture (Kopp et al., 1994; Strive et al., 2002) which led us to test whether this domain of BHV-1 gB might tolerate more extensive sequence modifications (Keil et al., 2005). We showed that the introduction of a second furin cleavage site and intervening cargo proteins into gB of BHV-1 was compatible with the function of gB for BHV-1 induced membrane fusion processes. This was demonstrated by isolation of viable BHV-1 recombinants that expressed the green fluorescent protein or bovine alpha interferon (boIFN-α) as furin-excisable proteins, which were secreted biologically active from cells infected with the respective recombinants (Keil et al., 2005). We observed, however, that cleavage of gB with embedded boIFN-α by furin yielded only minor amounts of the 72 kDa NH_2-subunit of BHV-1 gB but produced an additional abundant cleavage product of 90 kDa which was also found in BHV-1/gB2FuIFN-α virions. We assumed that furin failed to cleave gB2FuIFN-α at the authentic furin cleavage site (FCS-1) and that the 90 kDa protein represents a fusion protein between the NH_2-subunit and boIFN-α (Keil et al., 2005). In the present study we verify this assumption, demonstrate that boIFN-α is incorporated into recombinant virus particles and show that the gB-NH_2IFN-α fusion protein is biologically active. We further demonstrate that amino acids 1–273 of the HA1 subunit of influenza virus haemagglutinin can be expressed and secreted from within a BHV-1 gB chimeric protein, or displayed on virions.

2. Materials and methods

2.1. Cells and viruses

Madin–Darby bovine kidney (MDBK), and bovine pharyngeal (KOP/R) cells were kindly provided by Roland Riebe (Collection of Cell Lines in Veterinary Medicine, Friedrich-Loeffler-Institut, Insel Riems, Germany) and grown in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum, 2.4 mM L-glutamine, 100 U of penicillin per ml, and 100 μg of streptomycin per ml. Cell cultures were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. Wild-type BHV-1 strain Schönböken (wtBHV-1) was obtained from O.C. Straub (Tübingen, Germany). BHV-1 recombinants BHV-1/boIFN-α, BHV-1/gB2FuIFN-α and BHV-1/gB^{rev} have been described previously (Höhle et al., 2005; Keil et al., 2005).

2.2. Construction of plasmids

All cloning procedures were carried out by standard methods (Sambrook et al., 1989). The sequences of all cloned PCR fragments were determined using the Prism Big Dye Terminator v1.1 cycle sequencing kit (Applied Biosystems) and analyzed on an automatic sequencer (ABI-377, Applied Biosystems). To obtain the ORF encoding amino acids 1–273 (nucleotides 51–869, numbering according to GenBank accession number CY033185.1) of the mature HA1 subunit of AIV/duck/Vietnam/NCVD01/2005(H5N1) which encompass the receptor binding subdomain (Ha et al., 2002), in the appropriate form for integration into pgB-Asc as described by Keil (2009), the respective fragments were amplified from cloned cDNA (kindly provided by Walter Fuchs, FLI, Insel Riems, Germany) with primers gB2FuH5Viet+ (TACTCGAGCCATGGCGCGCCGCGGAGCAGACCAGATTTGCATTGGTTACC) or gB-NH_2H5Viet+ (TACTCGAGCCATGGCGCGCCAGGGAGCAGACCAGATTTGCATTGGTTACC), and FuH5Viet− (TAGCTCGAGCGGCGCGCCCGCTTGGTGTTGCAGTTACCATATTCCAATTC). The PCR fragments were cleaved with AscI and ligated into the AscI-cleaved gB transfer vector pgB-AscI (Keil, 2009). After sequence verification, the resulting plasmids pgB2FuH5 and pgB-NH_2H5 were used for integration into the genome of BHV-1.

2.3. Construction of BHV-1 recombinants

MDBK cells were cotransfected with 5 μg of recombination plasmid and 1 μg of purified genomic gB^{-}BHV-1 DNA as described previously (Keil et al., 2005). Virus progeny from the culture supernatants was titrated on MDBK cells and recombinant virus was plaque purified to homogeneity (Keil et al., 2005).

2.4. Antibodies

Mouse monoclonal antibody (MAb) 42/18/7 is specific for the NH_2-subunit of BHV-1 gB (Keil et al., 2005). Rabbit serum directed against the COOH gB subunit and rabbit anti-HA(H5Vietnam) serum have been described previously (Keil et al., 2005; Pavlova et al., 2009). The antiserum against boIFN-α was raised in rabbits (Kühnle et al., 1996) using purified 6xHis tagged boIFN-α expressed by recombinant baculovirus in SF9 insect cells following procedures recommended by the suppliers of the reagents ("Bac-to-Bac Baculovirus Expression Systems" manual, Invitrogen; The QIAexpressionist handbook 5th edition, Qiagen).

2.5. Western blotting

Proteins were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE), transferred to nitrocellulose filters, and probed with monoclonal