Virology 513 (2018) 65–74

Contents lists available at ScienceDirect

Virology

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

Identification of N-linked glycosylation sites in the spike protein and their functional impact on the replication and infectivity of coronavirus infectious bronchitis virus in cell culture

Jie Zheng[a,b,1,2], Yoshiyuki Yamada[c,2], To Sing Fung[a,2], Mei Huang[b], Raymond Chia[c], Ding Xiang Liu[a,*]

[a] South China Agricultural University, Guangdong Province Key Laboratory Microbial Signals & Disease Co, and Integrative Microbiology Research Centre, Guangzhou 510642, Guangdong, People's Republic of China
[b] School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive, Singapore 63755
[c] Institute of Molecular and Cell Biology, 61 Biopolis Drive, Proteos, Singapore 138673

ARTICLE INFO

Keywords:
Coronavirus
Infectious bronchitis virus
Virus infectivity
Spike protein
N-linked glycosylation
Cell-cell fusion
Infectious cDNA
Clone

ABSTRACT

Spike (S) glycoprotein on the viral envelope is the main determinant of infectivity. The S protein of coronavirus infectious bronchitis virus (IBV) contains 29 putative asparagine(N)-linked glycosylation sites. These post-translational modifications may assist in protein folding and play important roles in the functionality of S protein. In this study, we used bioinformatics tools to predict N-linked glycosylation sites and to analyze their distribution in IBV strains and variants. Among these sites, 8 sites were confirmed in the S protein extracted from partially purified virus particles by proteomics approaches. N-D and N-Q substitutions at 13 predicted sites were introduced into an infectious clone system. The impact on S protein-mediated cell-cell fusion, viral recovery and infectivity was assessed, leading to the identification of sites essential for the functions of IBV S protein. Further characterization of these and other uncharacterized sites may reveal novel aspects of N-linked glycosylation in coronavirus replication and pathogenesis.

1. Introduction

Coronaviruses are positive stranded RNA viruses. A typical coronavirus consists of few basic structural components. These include the membrane (M), peplomer-like protein spike (S) and envelope (E) protein on the viral envelope, and the nucleocapsid (N) protein which wraps the genomic RNA inside the particles. Some coronaviruses encode an additional protein, the hemagglutinin-esterase (HE), a glycoprotein that forms smaller spikes on the exterior in addition to the S proteins.

Infectious bronchitis virus (IBV) is the coronavirus that plagues the domestic fowl *Gallus gallus*. Similar to other coronavirus S protein, IBV S protein is a type I glycoprotein and forms the peplomers on virion particles giving the crown-like appearance. The protein contains two glycopolypeptides S1 (90 kDa) and S2 (84 kDa) in equimolar proportions (Cavanagh, 1983) (Fig. 1a). The S1 subunit is believed to form the globular head of the protein and contains a receptor binding domain (Kubo et al., 1994). The carboxy terminal S2 subunit, however, is conserved among all coronavirus spikes and forms a stalk-like structure that is embedded in the membrane (Masters, 2006). Overall this gives the spike protein a teardrop shaped structure (Masters, 2006). Mutagenesis of the terminal heptad repeats and the predicted fusion peptides severely compromises SARS-CoV S protein-mediated cell-cell fusion (Petit et al., 2005). S protein-mediated cell-cell fusion is also dependent on a cysteine rich domain in the protein itself (Chang et al., 2000). Yet another point mutation, glutamine to leucine at position 294 of the IBV spike S1 subunit hampers processing of the protein into a matured protein capable of being translocated to the cell surface (Shen et al., 2004).

One aspect of the S protein that remains largely unexplored is the role of its glycans. Glycans are mainly involved in protein post-translational modification and folding. One of its most common forms is the N-linked glycosylation. This involves a high mannose core being attached to the amide nitrogen of asparagine (N), within a conserved motif Asn-X-Ser/Thr (where X is any amino acid except for proline). In the ER, this mannose core is added in the form of a block of fourteen

* Correspondence.
E-mail address: dxliu0001@163.com (D.X. Liu).
[1] Current address: Department of Molecular Medicine, The Scripps Research Institute, Jupiter, FL 33458, USA.
[2] Equal contribution.

http://dx.doi.org/10.1016/j.virol.2017.10.003
Received 25 July 2017; Received in revised form 25 September 2017; Accepted 2 October 2017
0042-6822/ © 2017 Published by Elsevier Inc.

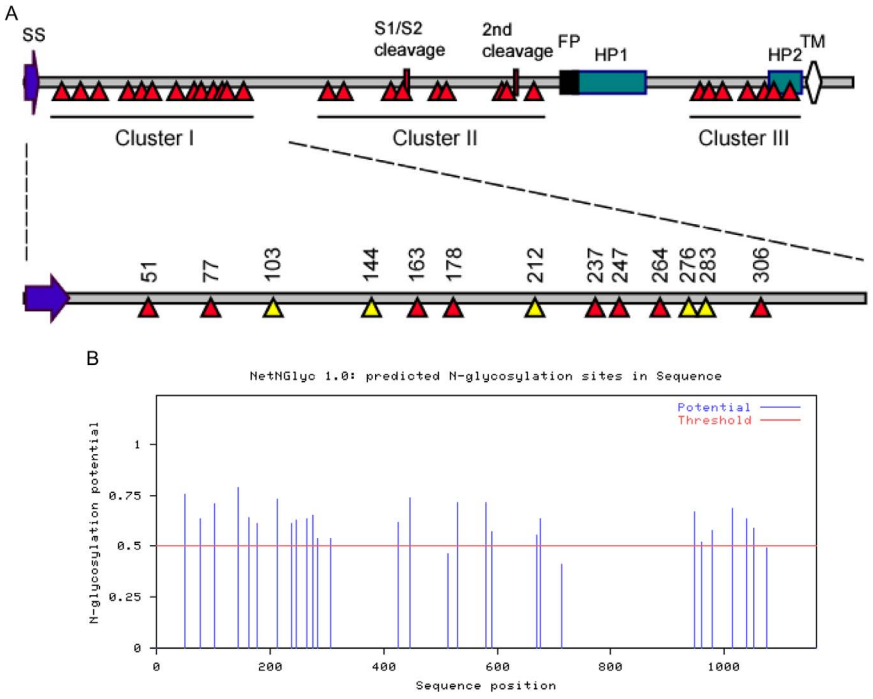

Fig. 1. a Diagram showing the IBV spike protein with different functional domains indicated. Signal sequence(SS), amino acids 1–18; S1, amino acids 19–537; S2, amino acids 538–1162; Heptad Repeat 1 (HP1), amino acids 790–911; Heptad Repeat 2 (HP2), amino acids 1056–1089; Trans-membrane domain (TM), amino acids 1097–1118. Also indicated are the putative N-linked glycosylation sites in three clusters, and amino acid positions of the N-linked glycosylation sites in Cluster I. The relative importance of these N-linked glycosylation sites in Cluster I is indicated with colored triangles, with red indicating less importance and yellow indicating critical importance. b The 29 putative glycosylation sites on the IBV spike protein as predicted by NetNGlyc 1.0 software. The threshold and glycosylation potential are shown.

sugars, $Glc_3Man_9GlcNAc_2$ (Balzarini, 2007). The mannose oligosaccharide then moves through the ER and Golgi apparatus, during which it is altered to form different structures (Vigerust and Shepherd, 2007). Coronavirus S proteins typically contain 23–30 N-linked glycosylation sites, depending on the species in question. The protein is post-translationally glycosylated in the ER (Delmas and Laude, 1990), following which it is transported through the Golgi apparatus where high mannose oligosaccharides are trimmed and the protein further acylated.

The importance of glycans in S protein is likely to extend beyond protein modification and folding. They may play an important role in viral receptor binding, virus-cell and cell-cell fusion. Mutagenesis analyses of SARS-CoV S protein have identified seven glycosylation sites critical for DC/L-SIGN-mediated entry, an alternative site for SARS-CoV entry (Han et al., 2007). Interestingly, mutation of multiple sites together did not have a synergistic effect on entry (Han et al., 2007). On the other hand, mutation of a single N-linked glycosylation site (N330) abolishes the specific interaction between SARS-CoV S protein and mannose-binding lectin, a serum protein that serves an important function in host defenses during opsonization and complement activation (Zhou et al., 2010).

Mass spectrometry analysis has been used to determine the structure of the N-glycan on SARS-CoV S protein, and major glycans were shown to be high-mannose ($Man_{5-9}GlcNAc_2$), hybrid and bi-, tri- and tetra-antennary complex with and without bisecting GlcNAc and core fucose (Ritchie et al., 2010). Moreover, treatment with glucosidase inhibitor inhibits N-glycan processing and replication of SARS-CoV in Vero E6 cells (Ritchie et al., 2010).

Carbohydrate binding agents found in plants are able to inhibit coronavirus infections by targeting the S and membrane glycans of the mouse hepatitis virus (MHV) and feline infectious peritonitis virus (van der Meer et al., 2007). These results and those from other viruses show that the glycans on the S protein provide great avenues and potential for further exploration and understanding, particularly in the area of protein mediated fusion. Carbohydrate binding agents may be a useful tool in combating viral pathogenesis in future. As described by Balzarini in 2007, these agents may act via a dual mechanism of antiviral action, by firstly binding to the glycans and subsequently blocking virus entry, or by creating deletions in the “glycan shield” of a particular viral surface, inducing the immune system to act against otherwise hidden immunogenic epitopes. Before this becomes a full-fledged antiviral method, more work needs to be done to characterize the function of carbohydrates in these viruses, in particular, the S protein here.

In this study, 29 N-linked glycosylation sites on IBV S protein were predicted by NetNGlyc server 1.0 software. Among them, 8 sites were confidently confirmed by proteomics approaches. The functional importance of N-linked glycosylation in IBV S protein was further studied by mutagenesis of 13 predicted N-linked glycosylation sites in Cluster I. The impact of these mutations on S protein-mediated cell-cell fusion and viral infectivity was investigated.

2. Materials and methods

2.1. Cells and viruses

African Green Monkey kidney Vero cell line and a Vero-adapted IBV Beaudette strain (IBV p65) were used in this study (Ng and Liu, 1998). Confluent Vero cells were inoculated with IBV at a multiplicity of infection (moi) of approximately 1 plaque forming unit (PFU)/cell. Cells were then incubated for 24 h at 37 °C in 5% humidified CO_2, until cytopathic effect (CPE) was observed. Both culture medium and cells were harvested and aliquot in appropriate amounts.

The recombinant vaccinia/virus (vTF7-3) was prepared by infection of confluent Vero cells with 0.1 PFU/cell of the virus for 24 h, before fresh medium was used to replace the inoculum. After 48 h, when cytopathic effects were observed, the infected cells were harvested. The viruses were released from cells via three rounds of freeze-thaw, before aliquot in 1.5 ml screw-cap vials and stored at –80 °C.

2.2. Construction and expression of plasmids containing mutant S genes

Forward and reverse primers were designed for the predicted N–linked glycosylation sites. Conventional PCR reactions were carried out. PCR products were treated with *Dpn*I restriction endonuclease digestion for 2 h at 37 °C. For this study, overlapping PCR was done on PKT0-S plasmids (PKT0 vector containing S gene) to create the mutant constructs (Ng and Liu, 2002).

Confluent monolayers of Vero cells were infected with vTF7-3 for

Download English Version:

https://daneshyari.com/en/article/5674818

Download Persian Version:

https://daneshyari.com/article/5674818

Daneshyari.com