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Dissection and identification of regions required to form pseudoparticles by the interaction between the nucleocapsid (N) and membrane (M) proteins of SARS coronavirus

Seisuke Hatakeyama [a], Yusuke Matsuoka [a], Hidehiro Ueshiba [b], Nobukazu Komatsu [c], Kyogo Itoh [c], Shigeki Shichijo [c], Takao Kanai [b], Masaya Fukushi [a], Isao Ishida [d], Teruo Kirikae [a], Takehiko Sasazuki [e], Tohru Miyoshi-Akiyama [a,*]

[a] Department of Infectious Diseases, Research Institute, International Medical Center of Japan, 1-21-1, Toyama, Shinjuku-ku, Tokyo 162-8655, Japan
[b] Institute of Laboratory Animals, Tokyo Women's Medical University, Japan. 8-1, Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan
[c] Department of Immunology, Kurume University School of Medicine, Asahi-machi, Kurume, Fukuoka 830-0011, Japan
[d] Frontier Laboratory, Kirin Pharma Co., Ltd., 26-1, Jingumae 6-chome, Shibuya-ku, Tokyo 150-8011, Japan
[e] International Medical Center of Japan, 1-21-1, Toyama, Shinjuku-ku, Tokyo 162-8655, Japan

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ABSTRACT

When expressed in mammalian cells, the nucleocapsid (N) and membrane (M) proteins of the severe acute respiratory syndrome coronavirus (SARS-CoV) are sufficient to form pseudoparticles. To identify region(s) of the N molecule required for pseudoparticle formation, we performed biochemical analysis of the interaction of N mutants and M in HEK293 cells. Using a peptide library derived from N, we found that amino acids 101–115 constituted a novel binding site for M. We examined the ability of N mutants to interact with M and form pseudoparticles, and our observations indicated that M bound to NΔ(101–115), N1–150, N151–300, and N301–422, but not to N1–150Δ(101–115). However, pseudoparticles were formed when NΔ(101–115) or N301–422, but not N1–150 or N151–300, were expressed with M in HEK293 cells. These results indicated that the minimum portion of N required for the interaction with M and pseudoparticle formation consists of amino acids 301–422.

Introduction

Severe acute respiratory syndrome (SARS), caused by a newly identified coronavirus (SARS-CoV), has been observed in about 30 countries, affecting more than 8,000 people and resulting in death in about 10% of cases (Guan et al., 2003). SARS-CoV was identified as a novel type of coronavirus, differing from known coronaviruses (Guan et al., 2003; Marra et al., 2003; Rota et al., 2003). The genome and functional receptor of SARS-CoV have been identified (Li et al., 2003), but no vaccine or drug treatment has yet been approved. Although bats carry viruses similar to SARS-CoV (Wang et al., 2006), its natural host has not yet been identified.

Coronaviruses have a positive single-stranded RNA genome of approximately 30 kb that encodes structural proteins, such as the spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins. The S protein encoded by SARS-CoV, a large type-I transmembrane glycoprotein, is necessary for binding and fusion to host cells. S protein consists of the N-terminal S1 domain, which binds human angiotensin-converting enzyme 2 (ACE2), a functional receptor for SARS-CoV (Li et al., 2003), and a C-terminal S2 domain that mediates the fusion of viral and host cell membranes (Tripet et al., 2004; Xu et al., 2004).

The E protein, a type-II transmembrane glycoprotein, is the smallest structural protein in SARS-CoV (Wu et al., 2003). Although required for the formation of pseudoparticles in insect cells (Mortola and Roy, 2004), E is not necessary to form pseudoparticles in mammalian cells (Huang et al., 2004). E acts as a cation-selective ion channel (Wilson et al., 2004). rSARS-CoV-ΔE has been shown to replicate to titers 100- to 1000-fold lower than recombinant wild-type virus *in vivo*, although both viruses show the same morphology and stability at severe pH and temperature (DeDiego et al., 2007).

The M protein consists of a short N-terminal ectodomain harboring an *N*-glycosylation site, three transmembrane domains and a long cytoplasmic tail (Hu et al., 2003), and a C-terminal domain (residues 194–205 or 197–221) that binds to N (Fang et al., 2005; Luo et al., 2006b). M is essential for the formation of pseudoparticles (Huang et al., 2004). In the case of murine coronavirus, only M and E are required for formation of pseudoparticles (Bos et al., 1996; Vennema et al., 1996), in marked contrast with the case of SARS-CoV requiring M and N only to form pseudoparticles (Huang et al., 2004).

* Corresponding author. Fax: +81 3 3202 7364.
E-mail address: takiyam@ri.imcj.go.jp (T. Miyoshi-Akiyama).

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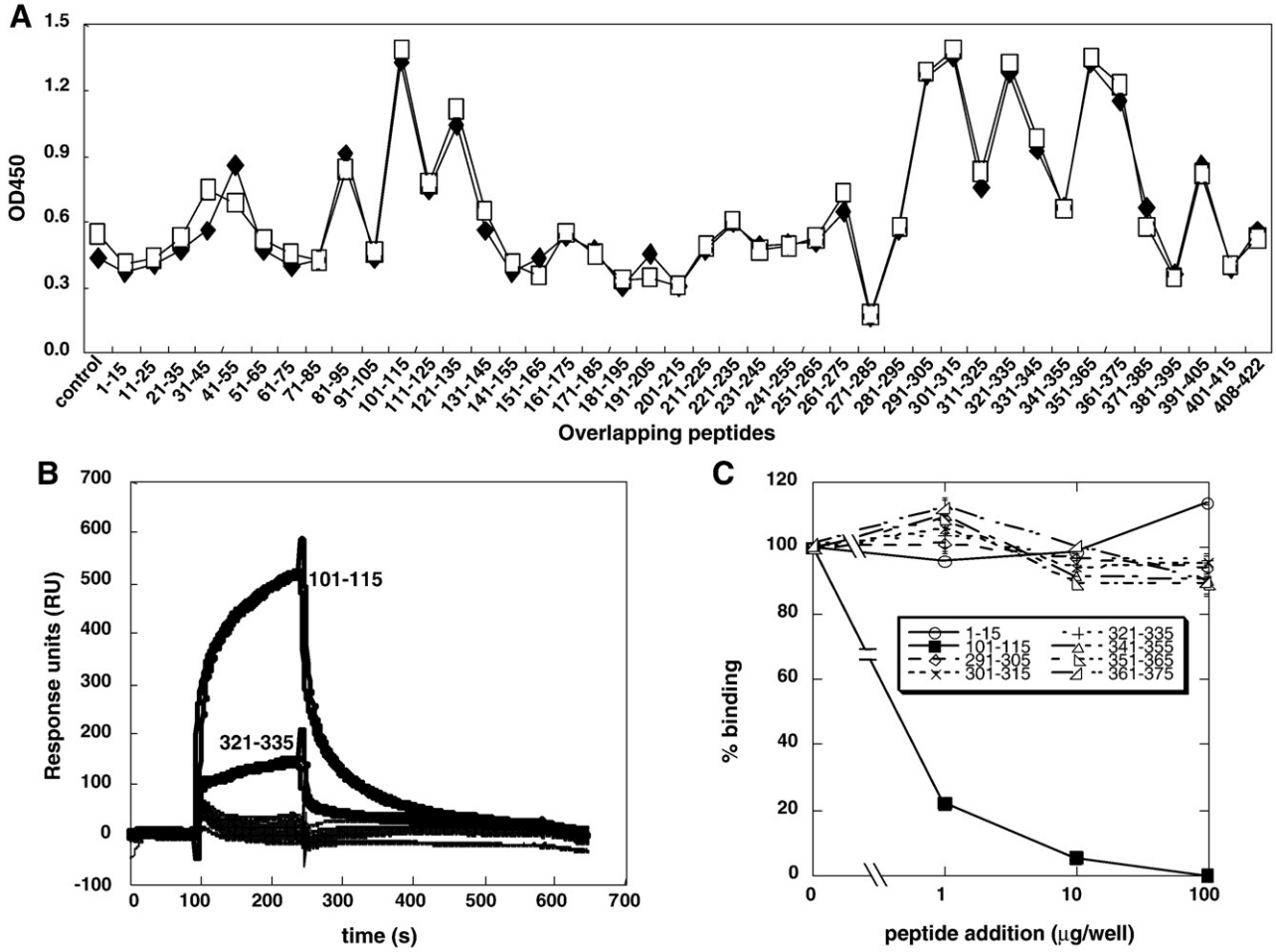

Fig. 1. Interaction of N-derived peptides with M protein. (A) Each peptide (10 μg/mL) was immobilized on ELISA plates. After blocking, the plates were incubated with M63 (final 1 μg/mL), and binding of M63 to N protein was detected with rabbit anti-M63 plus HRP-goat anti-rabbit IgG. The two data points for each peptide are the results of two independent experiments. (B) Each peptide (10 μg/mL) was injected into a BIAcore flow cell containing immobilized M116. The injection was started at 100 s, stopped at 240 s, and dissociated for 300 s. (C) Competitive inhibition of binding between N and M63 proteins by N-derived peptides. M63 was preincubated with each N peptide, and each mixture was added to a plate coated with N protein. Binding of M63 to N protein was detected as in (A). Binding in the absence of peptide was set at 100%. Data are presented as means±SD.

The N protein of coronaviruses is a major structural component that plays a role in virion assembly through its interactions with the viral genome and M protein (Hurst et al., 2005; Masters, 1992; Nelson and Stohlman, 1993; Narayanan et al., 2000; Narayanan et al., 2003). The N protein is also considered essential for viral RNA synthesis, although the cellular events affected by N are not yet known (Almazan et al., 2004). Crystal structures of C-terminal domain (amino acids 248–365) (Chen et al., 2007), N-terminal domain (amino acids 1–174) (Saikatendu et al., 2007) and dimerization domain (amino acids 270–370) (Yu et al., 2006) have been reported. N contains a disordered region in the middle of the molecule (Chang et al., 2006; Zuniga et al., 2007), which does not form a particular structure otherwise it binds adequate binding partners.

Virion formation is the fundamental process in viral replication, and results from the interactions between viral proteins under physiological conditions. The expression of M, E, and S in Sf9 insect

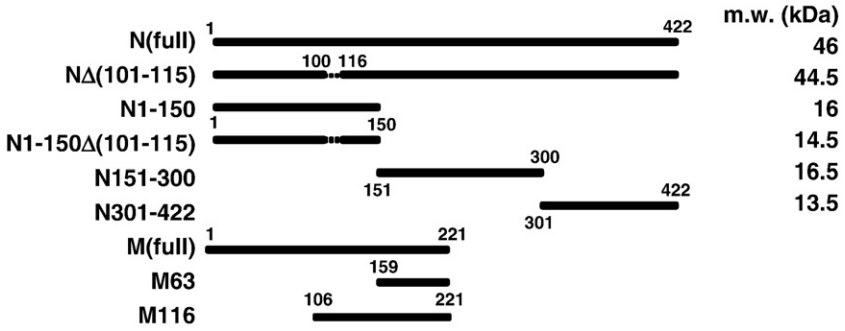

Fig. 2. Schematic diagram of the recombinant N and M proteins used in this study. N mutants are numbered according to the amino acid positions in the full-length N. M116 and M63 consist of the C-terminal 116 and 63 amino acids of M, respectively.