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# Short peptides derived from the interaction domain of SARS coronavirus nonstructural protein nsp10 can suppress the 2′-O-methyltransferase activity of nsp10/nsp16 complex

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ABSTRACT

Coronaviruses are the etiological agents of respiratory and enteric diseases in humans and livestock, exemplified by the life-threatening severe acute respiratory syndrome (SARS) caused by SARS coronavirus (SARS-CoV). However, effective means for combating coronaviruses are still lacking. The interaction between nonstructural protein (nsp) 10 and nsp16 has been demonstrated and the crystal structure of SARS-CoV nsp16/10 complex has been revealed. As nsp10 acts as an essential trigger to activate the 2′-O-methyltransferase activity of nsp16, short peptides derived from nsp10 may have inhibitory effect on viral 2′-O-methyltransferase activity. In this study, we revealed that the domain of aa 65–107 of nsp10 was sufficient for its interaction with nsp16 and the region of aa 42–120 in nsp10, which is larger than the interaction domain, was needed for stimulating the nsp16 2′-O-methyltransferase activity. We further showed that two short peptides derived from the interaction domain of nsp10 could inhibit the 2′-O-methyltransferase activity of SARS-CoV nsp16/10 complex, thus providing a novel strategy and proof-of-principle study for developing peptide inhibitors against SARS-CoV.



## 1. Introduction

Coronaviruses are the largest RNA viruses, which are enveloped and contain a single-stranded, positive-sense RNA genome ranging from 27 to 31.5 kb in length. They have long been recognized as pathogens of humans and animals such as feline, bovine, mouse and swine. The genome of severe acute respiratory syndrome coronavirus (SARS-CoV) is ~29.7 kb long and contains 14 open reading frames (ORFs) flanked by 5′ and 3′-untranslated regions of 265 and 342 nucleotides in length, respectively (Snijder et al., 2003). The 5′-proximal two third of the genome encodes 2 large overlapping ORFs (1a and 1b), which encode the proteins making the replication and transcription complex (RTC). While ORF1a is translated from the genome RNA to produce the polyprotein 1a, ORF1b is expressed by a −1 ribosomal frameshifting at the end of ORF1a to generate a large polyprotein (pp1ab) covering both ORF1a and 1b (Dos Ramos et al., 2004). These two polyproteins are cleaved into 16 mature replicase proteins, named as nonstructural proteins nsp1-16, which assemble the RTC located on a dedicated membrane surface (van Hemert et al., 2008). Of the 16 nsps, nsp14 was shown as exoribonuclease and (guanine-N7)-methyltransferase (N7-MTase) (Chen et al., 2007, 2009; Minskaia et al., 2006) and nsp16 as a 2′-O-methyltransferase (2′-O-MTase) (Chen et al., 2011; Decroly et al., 2008).

In previous studies, we and others have performed screenings to explore the interactions between SARS-CoV-encoded proteins by yeast two-hybrid (Y2H) or mammalian two-hybrid (M2H) systems (Imbert et al., 2008; Pan et al., 2008; von Brunn et al., 2007). It was demonstrated that nsp10 of SARS-CoV could interact with nsp14 and nsp16, respectively (Pan et al., 2008; von Brunn et al., 2007). The crystal structure revealed that nsp10 belongs to a new class of zinc finger proteins and was proposed to be a viral transcription factor (Joseph et al., 2006; Su et al., 2006). It was also shown that SARS-CoV nsp16 acts as a 2′-O-MTase in complex with nsp10 to selectively 2′-O-methylate the cap-0 structure to cap-1 structure (m7GpppAm-RNA) (Bouvet et al., 2010; Chen et al., 2011). Recently, we and Bruno Canard's group independently resolved the crystal structure of nsp10/nsp16 complex (Chen et al., 2011; Debarnot et al., 2011). We further demonstrated that nsp10 acts as the active partner of nsp16 by stabilizing the SAM-binding pocket and extending the substrate RNA-binding groove of nsp16 (Chen et al., 2011;

*Abbreviations:* SARS-CoV, severe acute respiratory syndrome coronavirus; nsp, nonstructural protein; RTC, replication and transcription complex; aa, amino acid.

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Debarnot et al., 2011). Also it was reported that 2′-O-methylation of viral mRNA cap provides a molecular signature for the distinction of self and non-self mRNA, indicating that nsp16 may help the virus to evade host innate immunity (Daffis et al., 2010; Zust et al., 2011). Altogether, nsp16 plays a key role in the life-cycle of coronaviruses and the interaction between nsp10 and nsp16 is crucial for the functions of nsp16. Thus, inhibition of viral MTase activity should be able to suppress viral replication and attenuate viral virulence in infection and pathogenesis. The MTase active site was suggested as a drug target for developing antiviral drugs (Dong et al., 2008; Moheshwarnath Issur, 2011; Shuman, 2001). However, the MTase fold is structurally conserved between viral and cellular MTases, and it is thus difficult to obtain antiviral compounds with high specificity targeting MTase active sites. For this reason, it looks more promising to target the interface of nsp16 and nsp10 complex, which is unique to coronaviruses (Chen et al., 2011).

Although the crystal structure of nsp10/nsp16 complex is available, it is of importance to map the minimal domain of SARS-CoV nsp10 needed for its interaction with nsp16 and define the functional domain of nsp10 that is essential for stimulating nsp16 MTase activity. Such knowledge would be useful for designing and developing small molecules as protein–protein interaction inhibitors that could specially inhibit the enzymatic activity of nsp16 and the replication of SARS-CoV. Lugari and colleagues identified a number of key nsp10 residues involved in the interaction with nsp16 and in regulating nsp16 RNA cap 2′-O-MTase activity by site-directed mutagenesis (Lugari et al., 2010). In this study we mapped the linear domain consisting of aa 65–107 of nsp10 as the core area for the interaction with nsp16, which is located inside the interface of the nsp10/nsp16 complex as revealed from the crystal structure (Chen et al., 2011; Decroly et al., 2011). The biochemical assays between truncated proteins of nsp10 and full-length nsp16 showed that the core 2′-O-MTase activation domain of nsp10 was aa 42–120, which is overlapping with but larger than the domain for interaction with nsp16. Based on the interaction domain sequence of nsp10, we identified two short peptides which could significantly inhibit the 2′-O-MTase activity of nsp16/nsp10 complex. These peptides could be potentially developed into specific drug candidates against coronavirus.

## 2. Materials and methods

### 2.1. Construction of plasmids

The primers for nsp10 and nsp16 were designed according to the genomic sequence of SARS-CoV strain WHU (GenBank accession number: AY394850) and the templates of PCR were the cDNA of the SARS-CoV WHU strain (Pan et al., 2008). The coding sequence of SARS-CoV nsp16 was cloned into the yeast expression vector pGBKT7 (Clontech), fusing with the GAL4 DNA-binding domain, resulting in plasmid pBD16. The cDNAs of full-length and truncated nsp10 of SARS-CoV were cloned into the yeast expression vector pGADT7 (Clontech), fusing with the GAL4 activation domain, giving rise to plasmids pAD10 (aa 1–139), pAD10ΔN41 (aa 42–139), pAD10ΔN64 (aa 65–139), pAD10ΔN90 (aa 91–139), pAD10ΔC19 (aa 1–120), pAD10ΔC32 (aa 1–107), pAD10ΔC53 (aa 1–86), pAD10ΔN55&ΔC32 (aa 56–107) and pAD10ΔN64&ΔC32 (aa 65–107), respectively (Fig. 1B). For protein expression of SARS-CoV nsp16, nsp10 and its truncation mutants (nsp10ΔN41, nsp10ΔN64, nsp10ΔC19, nsp10ΔC32), the corresponding sequenceses were amplified from yeast expression plasmids by PCR and cloned into the protein expression vector pET30a(+) (Novagen). All of the clones were confirmed by DNA sequencing.

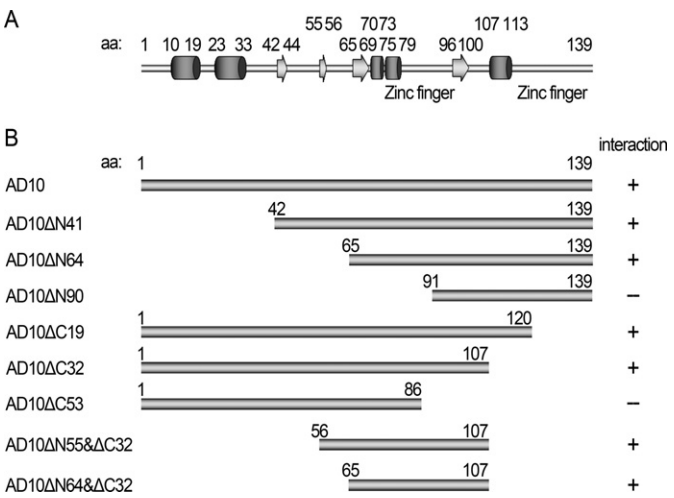


**Fig. 1.** Mapping of the domain of SARS-CoV nsp10 involved in the interaction with nsp16. (A) The secondary structure of nsp10 based on its crystal structure (PDB entry: 2FYG). Cylinder: alpha helix; arrow: beta sheet. (B) Summary of the interactions between nsp16 and nsp10 or its truncation mutants. The results of Y2H are shown at the right: (+) indicates positive interaction; (−) indicates negative interaction.

### 2.2. Yeast two-hybrid assays

Yeast strain AH109 was used to co-transform BD16 and AD10 or its truncation mutants. The transformation of yeast cells was conducted according to the manufacturer's protocol (Clontech PT3247-1). The co-transformed yeast cells were inoculated on the QDO (SD/-Ade/-His/-Trp/-Leu) screening plates at 30 °C and then performed with $\beta$-galactosidase assay according to the protocol (Clontech PT3247-1).

### 2.3. Expression and purification of recombinant proteins

For all recombinant protein expression plasmids, transformed *E. coli* BL21 (DE3) cells were grown at 37 °C in Luria–Bertani (LB) broth with 50 μg ml$^{-1}$ kanamycin and induced with 0.4 mM isopropyl β-D-thiogalactopyranoside (IPTG) at 16 °C for 12–16 h. Then the cells were collected by centrifugation and resuspended in buffer A [50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 5 mM $MgSO_4$, 5% glycerol] supplemented with 10 mM imidazole. After sonication and centrifugation, cleared lysates were applied to nickel–nitrilotriacetic acid (Ni–NTA) resin (Genesript) and washed with buffer A supplemented with an imidazole gradient of 20 mM, 50 mM, and 80 mM. Protein was eluted with buffer A supplemented with 250 mM imidazole. At last, the elution buffer was changed to reaction buffer [50 mM Tris–HCl, pH 7.5, 50 mM NaCl, 2 mM DTT, 10% glycerol] and fractions were frozen at −80 °C. The expression and purification of SARS-CoV nsp14 and other proteins are described previously (Chen et al., 2009).

### 2.4. Preparation of capped RNA substrates

RNA substrates representing the 5′-terminal 259 nucleotides of the SARS-CoV genome were in vitro transcribed, $^{32}$P-labeled at cap structures (m7G*pppA-RNA or G*pppA-RNA, where the * indicates that the following phosphate was $^{32}$P labeled), and purified as described previously (Chen et al., 2009, 2011). RNAs containing $^{32}$P-labeled cap-1 structure (m7G*pppAm-RNA) as positive control were converted from cap-0 structure m7G*pppA-RNA by a vaccinia virus 2′-O-methyltransferase VP39 by following the manufacturer's protocol (Epicentre). RNAs containing unlabeled cap structures (m7GpppA-RNA) were in vitro transcribed and prepared