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SCIENCE DIRECT
Virus Research 120 (2006) 146–155
Virus Research
www.elsevier.com/locate/virusres

Inhibition of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) infectivity by peptides analogous to the viral spike protein

Bruno Sainz Jr.[a,*], Eric C. Mossel[b,1], William R. Gallaher[c], William C. Wimley[d], C.J. Peters[b,e], Russell B. Wilson[f], Robert F. Garry[a]

[a] *Department of Microbiology and Immunology, Tulane University Health Sciences Center, New Orleans, LA 70112, USA*
[b] *Department of Microbiology and Immunology,, University of Texas Medical Branch, Galveston, TX 77555, USA*
[c] *Department of Microbiology, Immunology and Parasitology, Louisiana State University Health Sciences Center, New Orleans, LA 70112, USA*
[d] *Department of Biochemistry, Tulane University Health Sciences Center, New Orleans, LA 70112, USA*
[e] *Department of Pathology, University of Texas Medical Branch, Galveston, TX 77555, USA*
[f] *Autoimmune Technologies, LLC, New Orleans, LA 70112, USA*

Received 20 July 2005; received in revised form 9 February 2006; accepted 1 March 2006
Available online 17 April 2006

Abstract

Severe acute respiratory syndrome-associated coronavirus (SARS-CoV) is the cause of an atypical pneumonia that affected Asia, North America and Europe in 2002–2003. The viral spike (S) glycoprotein is responsible for mediating receptor binding and membrane fusion. Recent studies have proposed that the carboxyl terminal portion (S2 subunit) of the S protein is a class I viral fusion protein. The Wimley and White interfacial hydrophobicity scale was used to identify regions within the CoV S2 subunit that may preferentially associate with lipid membranes with the premise that peptides analogous to these regions may function as inhibitors of viral infectivity. Five regions of high interfacial hydrophobicity spanning the length of the S2 subunit of SARS-CoV and murine hepatitis virus (MHV) were identified. Peptides analogous to regions of the N-terminus or the pre-transmembrane domain of the S2 subunit inhibited SARS-CoV plaque formation by 40–70% at concentrations of 15–30 μM. Interestingly, peptides analogous to the SARS-CoV or MHV loop region inhibited viral plaque formation by >80% at similar concentrations. The observed effects were dose-dependent (IC50 values of 2–4 μM) and not a result of peptide-mediated cell cytotoxicity. The antiviral activity of the CoV peptides tested provides an attractive basis for the development of new fusion peptide inhibitors corresponding to regions outside the fusion protein heptad repeat regions.

Keywords: Severe acute respiratory syndrome-associated coronavirus; Class I viral fusion protein; Peptide inhibitor; Murine hepatitis virus; Six-helix bundle; Anti-viral

1. Introduction

Severe acute respiratory syndrome (SARS) is an atypical pneumonia characterized by influenza-like symptoms including fever, cough, dyspnea and headache. The epidemic of 2002–2003 produced an overall mortality of approximately 10%, resulting in 774 deaths in 29 countries world-wide (Goldsmith et al., 2004; Holmes, 2005; Peiris et al., 2003b). The etiological agent of SARS (SARS-CoV) was quickly identified as belonging to the family *Coronaviridae*, a group of large enveloped RNA viruses exhibiting a broad host range and capable of causing respiratory, hepatic and enteric diseases (Lai and Holmes, 2001; Siddell, 1995). Initial phylogenetic analyses and sequence comparisons revealed significant differences between SARS-CoV and other CoVs, distinguishing it as a unique group (group 4), unrelated to previously characterized CoV groups 1–3 (Drosten et al., 2003; Ksiazek et al., 2003; Peiris et al., 2003a). More recent analyses, however, report that SARS-CoV more closely resembles group 2 CoV and should therefore be classified as a subgroup within group 2 (Gorbalenya et al., 2004; Magiorkinis et al., 2004; Song et al., 2005). Further phylogenic

* Corresponding author at: The Scripps Research Institute, Molecular and Experimental Medicine, 10550 N. Torrey Pines Rd, SBR-10, La Jolla, CA 92037, USA. Tel.: +1 858 784 2051; fax: +1 858 784 2960.
E-mail address: bsainz@scripps.edu (B. Sainz Jr.).
[1] Present address: Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO 80523-1619, USA.

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doi:10.1016/j.virusres.2006.03.001

analyses and studies investigating the ancestral origins of SARS-CoV should yield a consensus regarding its proper grouping. Nonetheless, as animal reservoirs of SARS-CoV appear to exist, reemergence of this unique CoV is highly probable and therefore the threat of a new SARS outbreak remains a constant public health concern.

Like other enveloped viruses, CoVs enter target cells by inducing fusion between the viral and cellular membranes, a process mediated by the viral spike (S) glycoprotein (Gallagher and Buchmeier, 2001). The S1 subunit of the S protein mediates receptor binding (Cavanagh and Davis, 1986; Taguchi, 1995), while the S2 subunit is responsible for driving viral and target cell membrane fusion (Taguchi and Shimazaki, 2000). In the case of SARS-CoV, the S1 subunit binds to the mammalian receptor angiotensin-converting enzyme 2 (ACE2) (Huang et al., 2006; Li et al., 2003, 2005; Wang et al., 2004; Wong et al., 2004) and/or CD209L (Jeffers et al., 2004), initiating the entry process via a cathepsin L-dependent pathway (Huang et al., 2006). Analysis of the murine hepatitis virus (MHV) and SARS-CoV S proteins (Li et al., 2005; Xu et al., 2004a,b,c) reveal common structural features shared with class I viral fusion proteins (Gallaher, 1996; Gallaher et al., 1989), including a hydrophobic fusion peptide (Sainz et al., 2005b), a pair of extended α helices (N-helix and C-helix) (Tripet et al., 2004), a loop domain separating the two α helices and a cluster of aromatic amino acids (Sainz et al., 2005a) proximal to a hydrophobic transmembrane anchoring domain. Class I viral fusion proteins mediate viral and target cell membrane fusion through a series of conformational changes involving insertion of a fusion peptide into the target cell membrane, trimerization and extension of the heptad repeat (HR) helices toward the target membrane and the subsequent formation of a six-helix coiled-coil bundle (Gallaher, 1987). In this structure, a trimer of HR1 helices forms a central coiled-coil surrounded by three HR2 helices in an anti-parallel mode (Xu et al., 2004b). The loop region is believed to act as a hinge, facilitating six-helix coiled-coil bundle formation and mediating membrane juxtaposition.

While current models of viral:cell membrane fusion are hypothetical in most aspects, the importance of several of the structural/functional motifs of class I viral fusion proteins as drug development targets has been established. For example, analogs of the orthomyxovirus, paramyxovirus (Richardson et al., 1980) and HIV-1 fusion peptide domains (Gallaher et al., 1992; Owens et al., 1990; Silburn et al., 1998) block viral infection, presumably by forming inactive heteroaggregates. Likewise, peptides analogous to the HR regions of the HIV-1 (Gallaher et al., 1992; Qureshi et al., 1990; Wild et al., 1992, 1993), paramyxovirus (Lambert et al., 1996; Young et al., 1999) or Ebola virus (EboV) (Watanabe et al., 2000) class I viral fusion proteins block virion infectivity by preventing the transition of the fusion protein into the six-helix bundle state. The anti-HIV-1 peptidic drug Fuzeon™ (DP178, T-20 or enfuvirtide), which overlaps HR2 and the aromatic domain of gp41, was the first of a new class of fusion inhibitor antivirals to gain FDA approval (2003). This peptide has been shown to potently inhibit HIV-1 virion:cell fusion at very low concentrations (50% inhibition at 1.7 nM) in vitro (Wild et al., 1993). Likewise, in clinical trials, 100 mg/day of Fuzeon™ caused an ∼100-fold reduction in plasma HIV-1 load of infected individuals (Kilby et al., 1998; Lalezari et al., 2003a,b). These results substantially validate the efficacy of this new class of antivirals and have greatly motivated the search for peptide fusion inhibitors designed to target other viruses, such as SARS-CoV (Gallaher and Garry, 2003; Kliger and Levanon, 2003).

To date, studies examining peptide fusion inhibitors of CoV have only examined the efficacy of peptides analogous to the HR regions of the viral fusion protein. Bosch et al. (2003) first demonstrated that a peptide analogous to the HR2 helix of the MHV S2 subunit could block viral entry at concentrations of 10–50 μM. Several other groups later showed that HR1 and/or HR2 peptides could likewise inhibit SARS-CoV entry and replication (Bosch et al., 2004; Liu et al., 2004; Yuan et al., 2004; Zhu et al., 2004). The antiviral capacity of these peptides is attributed to the inherent interactions between HR1 and HR2 (Tripet et al., 2004; Xu et al., 2004b,c). Peptides analogous to either one of the two HR regions are believed to directly inhibit endogenous HR1 and HR2 interactions, preventing formation of the six-helix bundle.

Although the HR analogs appear to be effective inhibitors of SARS-CoV entry in vitro, development of other peptide fusion inhibitors, based on non-HR regions of the viral fusion protein, has yet to be explored. Here we employed a novel approach to identify new peptide inhibitors of SARS-CoV. Using the Wimley and White (WW) interfacial hydrophobicity scale (IHS) (Wimley and White, 1996), we identified five regions within the SARS-CoV and MHV S2 subunit with a high propensity to interact with the lipid interface of membranes. These regions, spanning the entire S2 subunit, may play an important role in viral fusion protein:target cell membrane interactions and may therefore represent possible targets for therapeutic interference. Our results demonstrate that peptides analogous to these regions of high interfacial hydrophobicity are effective inhibitors of SARS-CoV infectivity. In particular, peptides analogous to the loop region and/or the N-terminal region of the S2 subunit potently inhibit CoV plaque formation by 70–90% at concentrations of 30 μM or less (∼100 μg/ml). These findings provide an alternative approach to the development of viral peptide inhibitors, allowing for the identification of other possible therapeutic peptides outside of the HR regions of class I viral fusion proteins.

2. Materials and methods

2.1. Peptide synthesis

The MHV and SARS-CoV synthetic peptides were synthesized by solid-phase methodology using a semi-automated peptide synthesizer and conventional N-alpha-9-fluorenylmethyloxycarbonyl chemistry by Genemed Synthesis Inc. (San Francisco, CA). Peptides were purified by reversed-phase high performance liquid chromatography, and their purity confirmed by amino acid analysis and electrospray mass spectrometry. Peptide stock solutions were prepared in 10% dimethyl sulfoxide (DMSO, spectroscopy grade): 90%