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Mechanism of Interaction between SARS-CoV-2 Spike Protein and Human Cell Receptors on Cell Membranes

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Introduction

COVID-19, a recently established global pandemic, is linked to infection by the SARS-CoV-2 virus. This virus shares many structural similarities to the SARS-CoV virus that was responsible for the 2003-2004 SARS outbreak[1].SARS-CoV-2 (thought to be a β-coronavirus of B lineage[2]) is responsible for causing COVID-19 and shares many similarities to other viruses in the coronavirus family. Coronavirus comprise the *Coronavirinae* subfamily, which exists within the *Coronaviridae* family of the *Nidovirales* order[3]. *Coronavirinae* make up the largest of viruses, being approximately 30 kilobases long, and contain positive-sense RNA strands enveloped in a nucleocapsid (unusual for positive-sense RNA)[4]. The spike protein (S) participates in the binding of the angiotensin-converting enzyme 2 (ACE2) and subsequent fusion of the viral and host cellular membrane. It is comprised of 22 of the 23 N-glycosylation sites that are found in SARS-CoV, and 76% of the protein's genomic sequence is shared between the two viruses[2,5]. This review discusses the mechanism of action between the S protein and the host cell receptor.

1. Overview of Spike Protein Function

The general process of SARS-CoV-2 entry into host cells is well known. It is the result of a multi-step process that involves structural changes and processing of the spike protein[6]. The spike protein (S) is a homo-trimer[7], with each monomer composed of S_1 and S_2 subunits. Following S cleavage by the host proteases, such as furin or trypsin, S_1 and S_2 are connected via non-covalent bonds. This bound, prefusion conformation of the subunits exhibit the ideal arrangement for subsequent cleavage of the S_2 subunit that allows for the fusion of viral membrane with that of the host cell.

The S_2 subunit is further cleaved by host proteases at a site known as S_2' to facilitate fusion with the host cell[8]. Hoffmann et al. demonstrate the importance of the serine protease TMPRSS2 in preparing the S protein for fusion[5]. This allows for a hydrophobic internal fusion peptide to be inserted into the transmembrane, forming an intermediate pre-hairpin structure with the host cell[9]. The presence of this fusion peptide, along with two heptad-repeat regions (HR1 and HR2), have led researchers to classify the S glycoprotein as a class I fusion protein. Key differences between the S protein and other class I fusion proteins, however, may warrant a reevaluation of this categorization[10]. Incidentally, the similarities in HR1 and HR2 sequence alignment between that of HIV and SARS-CoV[9] (and SARS-CoV-2, by extension) may also provide clearer insight into the high infection rate seen with COVID-19.

	S_1 SUBUNIT	**S_2 SUBUNIT**
PRIMARY FUNCTION	Receptor binding	Membrane fusion
LOCATION IN SPIKE GENE SEQUENCE	N-terminus to linker region	Linker region to C-terminus
CLEAVAGE SITE	S_1/S_2 (between CTD and linker region)	S_2' (between linker region and fusion peptide)
CONTAINS	N-terminal domain (NTD) and C-terminal domain (CTD)	Fusion peptide, heptad repeats 1 and 2 (HR1, HR2), transmembrane domain (TM) and ectodomain (E)

Table 1. Comparison of the S_1 and S_2 subunits of the SARS-CoV-2 spike protein

2. Amino Acid Residue Interactions

In humans, the spike protein of SARS-CoV-2 is observed to bind with high affinity to ACE2. In a 2020 study on recognition between the two, Yan et al.[8] attempt to rationalize the strong binding pattern through a study of the peptidase domain (PD) of ACE2 bound to the RBD of the S protein. Their research suggests that changes in the positioning of nonpolar residues in SARS-CoV-2 may account for the formation of salt bridges and van der Waals interactions, leading to stronger associations between the RBD and peptidase domain (PD) in ACE2 in some areas, and weaker interactions in others.

Chen et al. propose that a difference in a single amino acid residue (F486) in the RBD sequence of SARS-CoV and SARS-CoV-2 corresponds to increased binding to ACE2 because phenylalanine, having a large, nonpolar R-group, is able to better insert into the ACE2 binding pocket[2]. Other research, on the other hand, presents evidence of drastically enhanced binding between the RBD in SARS-CoV-2 and ACE2 due to a mutation from Arg to Thr at the 501 position[1]. Additionally, other variations in the RBD between the two β-coronaviruses may have a direct impact on binding affinity[8].

3. Receptor Recognition by Spike Proteins

Host cell recognition by SARS-CoV-2 is the first step in COVID-19 infection[7]. Receptor binding in SARS-CoV has been shown to be triggered indirectly by low pH in the surrounding environment, which induces the lysosomal release of proteases that cleave the S_1 and S_2 subunits[6]. There is a strong possibility that these findings may be similar to the triggering of SARS-CoV-2 binding. The specific role that ACE2 plays in viral infection remains unclear. There is recent evidence to support that ACE2 is cleaved near the C-terminus by host proteases as well, further facilitating SARS-CoV-2 entry into exposed cells[8]. There is debate, however, on whether cleavage at S_2' is essential for membrane fusion[10,11]. Inhibition of this process could prove a potent target for COVID-19 treatments.

Much of the recent research on human CoV-2 infections have focused on establishing the similarities and differences between the S protein mechanism of action in SARS-CoV and SARS-CoV-2. The interaction between the receptor binding domain (RBD) of the virus and host

receptor is a key step in the infection of an organism. The RBD is found in the S_1 subunit, on the outermost point of the S protein[3]. Peptidases, such as ACE2, are typically used as receptors, although binding has been observed in instances absent these enzymes. Previous studies have presented observations of binding between S_1 in β-coronaviruses and sugar-mediated receptors[12]. As an attempt to understand SARS-CoV-2 binding in the absence of ACE2, Ibrahim et al.[7] studied how the spike protein on the novel coronavirus binds to glucose regulating protein 78 (GRP78). Molecular docking revealed hydrogen bonding and van der Waals interactions between S_1 and GRP78, resulting in efficient binding to a non-protease receptor. Successful binding between SARS-CoV-2 and a sugar receptor may be linked to non-respiratory-related symptoms attributed to COVID-19.

4. Fusion of S Protein with Host Cell Membrane

There are two mechanisms by which membrane fusion occurs: syncytia formation and endocytosis. Research indicates that the pathway by which SARS-CoV-2 can enter a host cell depends upon the presence of certain proteases, although there is some speculation that the type of receptor determines which fusion mechanism is used[11]. However, endocytosis is observed in most CoV-2 infections in human cells[6,11]. Shulla et al.[13] propose that palmitic acid tails on the S protein are responsible for meditating endocytic entry into host cells. By comparing the fusion ability of wild-type S proteins to Cys→ Ala residues in the TM-E region of the S_2 subunit, the importance of this fatty acid chain in viral infection was highlighted.

Preliminary research into the thermodynamics of SARS-CoV-2 fusion activation reveals heightened luciferase activity at physiological temperatures compared to SARS-CoV[11]. As luciferases embody a broad class of oxidative enzymes, more attention should be focused on determining the specific metabolic pathways that are affected by CoV-2 infection. Bosch et al. also studied the function of the fusion subunit in high thermal conditions[10]. HR1 and HR2 were found to be stable at temperature up to 70°C, high above temperatures that the human body can withstand. This demonstrates the persistence of the virus activity despite drastic changes in environment.

Conclusions

Preventing ACE2 dimerization may be associated with decreased binding affinity. The K_d of SARS-CoV-2 has been determined to be approximately 15 nM[8], so an effective receptor-recognition targeting treatment should have a lower dissociation constant. Further investigation into the gene sequence of SARS-CoV-2 may also provide better insight into its more efficient binding to ACE2, compared to other β-coronaviruses. Additionally, an exploration of the prevalence of viral infection in cases where ACE2 concentration is low or nonexistent will prove useful in understanding the specific role that protease receptors play in binding and fusion. Lastly, an in-depth study of the interactions between the transmembrane proteins should be conducted to fully grasp the mechanics of SARS-CoV-2 fusion.

There is a substantial amount of research to suggest that SARS-CoV-2 uses the same mode of entry into host cells through the same receptor as the virus responsible for the SARS outbreak in 2003-2004. Although the mechanism of action of the SARS-CoV-2 virus has been the focus of many studies as of late, further exploration into possibilities for blocking the binding of the virus to human ACE2 is necessary. Specifically, the non-covalent interactions between the gene sequence of ACE2 and that of SARS-CoV-2 requires better understanding in order to design effective treatments and vaccines.

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