# Effects of spike proteins on angiotensin converting enzyme 2 (ACE2)

Jennyfer Bejoy , Charlye I. Williams , Hattie J. Cole , Shavaiz Manzoor , Parsa Davoodi , Jacqueline I. Battaile , Arjun Kaushik , Sofia I. Nikolaienko , Tinatin I. Brelidze , Sergiy G. Gychka , Yuichiro J. Suzuki

## Abstract

The Coronavirus Disease 2019 (COVID-19) pandemic was caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which enters host cells through interactions of its spike protein to Angiotensin-Converting Enzyme 2 (ACE2). ACE2 is a peptidase that cleaves Angiotensin II, a critical pathological mediator. This study investigated if the spike protein binding to ACE2 compromises its peptidase activity. Spike/ACE2 Binding Assays suggested that spike proteins of SARS-CoV-2, SARS-CoV and MERS-CoV, but not HKU1, bind to ACE2. S1 and receptor-binding domain (RBD), but not S2, extracellular domain (ECD) or CendR domain, bind to ACE2. While glycosylated spike proteins prepared in HEK293 cells bind to ACE2, non-glycosylated proteins produced in E. coli do not. Cysteine residues of the spike protein expressed in HEK293 cells are fully oxidized, while those of the protein expressed in E. coli are reduced. The deglycosylation of HEK cell-produced protein attenuates the ACE2 binding, while the oxidation of the E. coli protein does not promote the binding. The S1 protein of SARS-CoV-2 enhances the ACE2 peptidase activity, while SARS-CoV, MERS-CoV or HKU1 does not. The ACE2 activity is enhanced by RBD, but not ECD or CendR. In contrast to distinct ACE2 binding capacities of proteins expressed in HEK293 cells and in E. coli, spike proteins expressed in both systems enhance the ACE2 activity. Thus, the spike protein of SARS-CoV-2, but not other coronaviruses, enhances the ACE2 peptidase activity through its RBD in a glycosylation-independent manner.

## Introduction

The Coronavirus Disease 2019 (COVID-19) pandemic was caused by a positive sense single stranded RNA virus, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) [1,2]. This virus has similarities to Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) that caused the SARS outbreak between 2002 and 2004 [3]. Both SARS-CoV and SARS-CoV-2 use the viral fusion protein, spike protein, to enter the host cells by binding to their host cell receptor, Angiotensin-Converting Enzyme 2 (ACE2) [[4], [5], [6]]. ACE2 is a peptidase that cleaves Angiotensin II (Ang II) into Ang (1–7), thus regulating the levels of Ang II, a critical mediator of pathogenesis, especially in the cardiovascular system.

The S1 subunit of the spike protein contains the Receptor Binding Domain (RBD) that interacts with ACE2. In addition to ACE2 binding to the spike protein RBD of the intact virus to facilitate the viral entry, the S1 subunit of the spike protein can be cleaved off from the virus by proteases such as Furin and may affect various organs. Widely used mRNA COVID-19 vaccines encode for the full-length spike protein S1+S2 and may produce circulating S1 protein

The binding of the SARS-CoV-2 spike protein to ACE2 downregulates ACE2 protein expression, resulting in the reduction of available ACE2 to degrade Ang II and thus the accumulation of this pathologic mediator, which may cause cardiovascular diseases associated with COVID-19. As the S1 protein binds to ACE2, it may interfere with the enzymatic activity of ACE2, thereby enhancing the Ang II-mediated pathology, which is worrisome. However, previous studies showed that the S1 protein does not inhibit, but rather slightly enhances, the peptidase activity of ACE2. Thus, S1 is capable of binding to ACE2 as well as enhancing the ACE2 enzymatic activity.

Currently, the relationships between the ability of RBD to bind to ACE2 and the enhancing action of S1 protein on the ACE2 peptidase activity have not been well defined. Therefore, the present study further investigates these events in order to provide mechanistic insights into the action of the SARS-CoV-2 spike protein.

## Section snippets

### Recombinant proteins

Recombinant SARS-CoV-2 Spike S1 (Cat# 40591-V08H), SARS-CoV-2 Spike RBD (Cat# 40592-V08H), SARS-CoV-2 Spike S1 Delta (Cat# 40591-V08H23), SARS-CoV-2 Spike S1 Omicron (Cat# 40591-V08H41), Middle East respiratory syndrome coronavirus (MERS-CoV) Spike S1 (Cat# 40069-V08H), HCoV-HKU1 (HKU1) Spike S1 (Cat# 40021-V08H), HIV-1 gp120 subtype A (Cat# 40403-V08H), HIV-1 gp120 subtype B (Cat# 40404-V08H), and human ACE2 (Cat# 10108-H08H) produced in HEK293 cells were purchased from Sino Biological Inc.

### The use of spike protein/ACE2 binding assays to study the biology of spike protein

The Spike protein/ACE2 Binding Assay Kits have been used to screen for inhibitors for the purpose of developing therapeutic agents to treat COVID-19. In this study, these assay kits were used to understand the mechanisms of spike protein biology. The RayBio COVID-19 Spike-ACE2 Binding Assay Kit I has the RBD protein coated at the bottom of the microplates to which ACE2 proteins are added. After

washing unbound components, the antibody against ACE2 is added and the RBD/ACE2 binding is detected

## Discussion

The spike protein plays a crucial role in the infection of human host cells with SARS-CoV-2 [5,6]. In addition, it appears that the shedding of the S1 subunit occurs, resulting in circulating S1 proteins and ACE2-dependent complications [8,9]. A number of people have been administered with COVID-19 vaccines that also produce the SARS-CoV-2 spike protein [10]. While the end of the COVID-19 pandemic has officially been declared by the World Health Organization, people still get infected with

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