Viewpoint

Allosteric Site of ACE-2 as a Drug Target for COVID-19

Kunal Dutta*

Cite This: ACS Pharmacol. Transl. Sci. 2022, 5, 179–182

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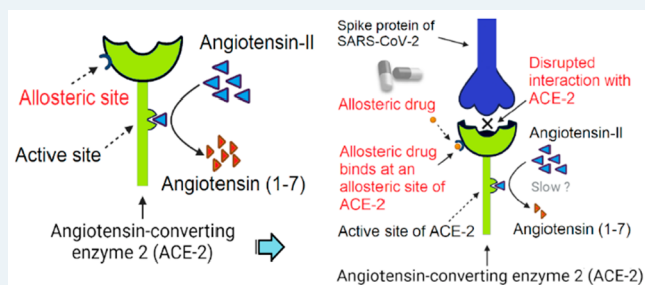
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ABSTRACT: The coronavirus disease 2019 (COVID-19) pandemic has a significant impact on healthcare systems and our lives. Vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) provide protection against SARS-CoV-2. However, mutations in the viral genome are common, raising concerns about the effectiveness of existing vaccines for SARS-CoV-2. The receptor-binding domain (RBD) of SARS-CoV-2 uses angiotensin-converting enzyme-2 (ACE-2) as a gateway to enter host cells. Therefore, the ACE-2-RBD interaction may be targeted by antiviral drugs. In this context, allosteric modulation of ACE-2 may offer a promising approach. It may lead to allosteric inhibition of the interaction between ACE-2 and SARS-CoV-2.

KEYWORDS: COVID-19, SARS-CoV-2, ACE-2, renin-angiotensin system, allosteric site, allosteric drug

The World Health Organization (WHO) had declared the COVID-19 pandemic on March 12, 2020. We are currently observing COVID-19 infections primarily with new variants of SARS-CoV-2. For example, recently, a new variant of SARS-CoV-2 (B.1.1.529) was identified in South Africa, and on November 26, 2021, the WHO named it Omicron, a variant of concern.[1] Many countries have meanwhile reported the presence of Omicron infection cases. Fortunately, a large portion of the human population in many countries has already been vaccinated. But the worrying fact is that the Omicron variant has many mutations in the spike protein and other areas of its genome[2] and the vaccines are less effective against this variant.

SARS-CoV-2 that caused COVID-19 uses membrane-bound angiotensin-converting enzyme-2 (ACE-2) together with an auxiliary receptor, transmembrane protease serine 2 (TMPRSS2), to enter into the host cells.[3] At present, most targets against SARS-CoV-2 are focused on the viral RNA-dependent RNA polymerase (RdRp), the main viral protease (M^{pro}), the spike protein, and the receptor-binding domain (RBD, e.g., fusion inhibitors, ACE-2 contact inhibitors).[4] Vaccines designed using the spike protein of SARS-CoV-2 offer a proactive immune option that produces monoclonal antibodies against the spike protein of SARS-CoV-2. However, mutations are present in the spike protein, for instance, in the delta and omicron variants of SARS-CoV-2.[5] The mutations in the spike protein raise questions about the effectiveness of the SARS-CoV-2 vaccination program. On the other hand, medications administered after a confirmed SARS-CoV-2 infection are usually administrated after a few days of illness.

At this stage of the COVID-19 disease, inhibiting viral communication with the ACE-2 receptor may still be beneficial.

ACE-2 is a hydrolase, and it is classed as a carboxypeptidase. There are two types of ACE-2: soluble ACE-2 and membrane-bound ACE-2, which SARS-CoV-2 utilizes as a gateway of the host cell.[6] The physiological role of ACE-2 is mainly to convert angiotensin-I into angiotensin (1−9) and to convert angiotensin-II to angiotensin (1−7). Both angiotensin (1−7) and angiotensin (1−9) bind with Mas receptors and prevent vasoconstriction, inflammation, oxidation, proliferation, and fibrosis. In the absence of ACE-2, angiotensin-I and angiotensin-II interact with the angiotensin II type 1 (AT1) receptor and increase vasoconstriction, inflammation, oxidation, proliferation, and fibrosis.[7] Likewise, SARS-CoV-2 infection also compromises endothelial function through the downregulation of ACE-2, which leads to lung injury.[8] Moreover, among other pathophysiological aspects of COVID-19, stress on the renin-angiotensin system also plays a role in disease development and severity.[9] But, it is not clear at which stage of COVID-19 disease ACE-2 gets downregulated (e.g., early, late-early, mid or midlate, etc.). It is also imperative to keep in mind the downregulation fact, probably because an allosteric drug may be beneficial at the early stage.

In general, there are two types of pharmaceutical targets available when an enzyme plays a critical role in a particular disease, i.e., an orthosteric site and an allosteric site of an enzyme.[10] Pharmaceuticals that are designed for the orthosteric site usually bind to the active site of the enzyme via competitive, uncompetitive, or noncompetitive inhibitions.[11] Drugs for allosteric sites bind to sites other than the active site of the enzyme and often alter the shape of the active site;[10] that is, they can allosterically alter the conformation of the protein. Evidence indicates that the known ACE-2 orthosteric (active site) blockers fail to stop the progression of the SARS-CoV-2 infection.[12] A previous study showed that critical amino acid residues in the orthosteric site of ACE-2 are H345, H505, and R273[13] located in the center of ACE-2.

However, the allosteric property of the human ACE-2 is not well-studied. But nevertheless, research indicates allosteric sites can be identified by using AlloFinder, CAVER Analyst, etc. Therefore, allosteric sites 1−3 of ACE-2 were identified using AlloFinder[14] and CAVER Analyst.[15] The allosteric site 1 (AS1) of ACE-2 is located just below its orthosteric site. Allosteric site-2 (AS2) and -3 (AS3) are found in close proximity to the interacting amino acid residues,[16] that are usually participating in hydrogen bonding with the receptor-binding domain (RBD) of SARS-CoV-2 (Figure 1). Recent work by Wang et al. also indicates that an allosteric site of ACE-2 is located in close proximity to the active site.[17] The surrounding amino acid residues of the AS1 are F428, P289, R288, N290, E430, L418, P415, I291, T434, E435, N437, K541, T414, M366, F438, L439, K441, Y279, A413, H540, C542, A412, L539, Y587, Q442, L410, L370, and Q526 with a cavity volume of 448.4 Å^3 (Table 1). Furthermore, according to the AlloFinder algorithm, AS1 has an AlloScore of 8.18, suggesting it could be druggable.

The biophysical properties of ACE-2 play an important role because they are essential for interactions between ACE-2 and the viral RBD.[4] Altering the biophysical properties of ACE-2 has a strong effect on the biophysical interactions between ACE-2 and the viral RBD. In other words, due to altered biophysical properties of the ACE-2 receptor, the viral RBD may lose or improve the degree of affinity toward the ACE-2 receptor. For example, a recent study indicates that an ACE-2 mutant (with altered biophysical properties) has a 100-fold greater binding affinity for the RBD due to improved hydrophobic packing and hydrogen-bonding geometry at the interface.[18] Recently, the allosteric interactions and communication pathways in the SARS-CoV-2 spike protein with ACE-2 have been highlighted.[19] The allosteric sites of ACE-2 have been overlooked. Allosteric drugs can alter the biophysical properties of an enzyme.[20] In addition, some allosteric drugs act as molecular switches, whereby a slight structural change (altered biophysical proper-ties) disturbs the mechanism of protein−protein interaction.[21]

Similarly, alteration of the biophysical properties of the ACE-2 receptor could be possible upon binding of an allosteric drug, which may disrupt the interactions between ACE-2 and the RBD of SARS-CoV-2. In particular, binding of a drug at the allosteric site of the ACE-2 receptor may decrease biophysical interactions (e.g., electrostatic, hydrogen bonding) between ACE-2 and the viral RBD. A recent study by Wang et al. showed that dexamethasone (DEX), chloroquine (CQ), and telmisartan (TLS) disrupt the interactions between the SARS-CoV-2 spike protein and human ACE-2 through binding to an allosteric site by a conformational shift of the ACE-2.[17] And modulating the conformation of ACE2 may limit SARS-CoV-2 invasion owing to unfavored poses for spike protein binding.[17] However, the
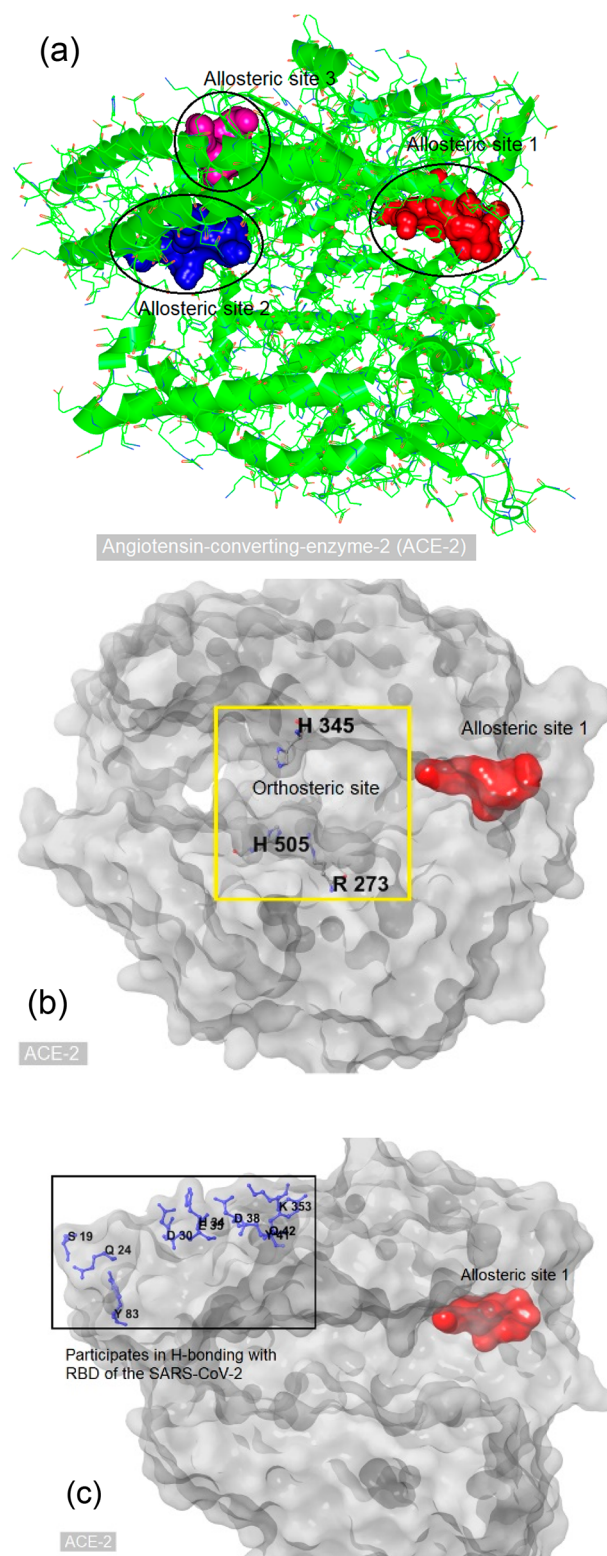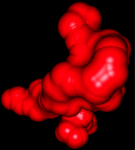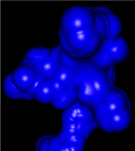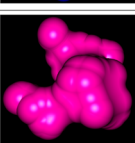

Figure 1. Allosteric sites of angiotensin-converting enzyme 2. (a) Cartoon presentation of the human angiotensin-converting enzyme 2 (3SCJ). Potential allosteric areas are highlighted by different black shapes. (b) Orthosteric site of ACE-2 is highlighted by a yellow square. Important amino acid residues are H345, H505, and R273. (c) Allosteric site 1 (red), and amino acid residues (blue) of ACE-2 participating in hydrogen bonding (H-bond) with the receptor-binding domain (RBD) of SARS-CoV-2.

Table 1. Characteristics of the Potential Allosteric Sites of Angiotensin-Converting Enzyme 2

Allosteric sites	Surrounding amino acid residues	Shape	Volume (Å³)
Allosteric site 1	F428, P289, R288, N290, E430, L418, P415, I291, T434, E435, N437, K541, T414, M366, F438, L439, K441, Y279, A413, H540, C542, A412, L539, Y587, Q442, L410, L370, Q526		448.4
Allosteric site 2	A396, N397, L392, S563, E564, K562, W566, P565, L391, D206, V212, V209, E208, L91, Q96, L95, N210, A99, Q98, K94, L97, I88, L85		941.5
Allosteric site 3	F356, D355, D382, G354, D350, L351, Y385, A386, G352, K353, R393, F390, F40, E37		208.6

binding of a drug at an allosteric site of ACE-2 may also reduce the enzymatic substrate conversion of angiotensin-I and -II. In addition, an allosteric drug for the ACE-2 receptor may probably be beneficial to reduce the stress on the renin-angiotensin system and to inhibit SARS-CoV-2 induced impaired endothelial function. This would have to be explored by detailed future research.

It is hypothesized that SARS-CoV-2 may lose the ability to infect new host cells due to allosterically impaired interaction between the ACE-2 receptor and the viral RBD. Thus, altering the biophysical properties of the ACE-2 receptor by modulating an allosteric site of ACE-2 may be a promising strategy against COVID-19.

AUTHOR INFORMATION

Corresponding Author

Kunal Dutta − *Department of Human Physiology, Vidyasagar University, Midnapore 721102 West Bengal, India;* orcid.org/0000-0002-0818-8787; Email: Kunal_lifesc@mail.vidyasagar.ac.in

Complete contact information is available at:
https://pubs.acs.org/10.1021/acsptsci.2c00003

Notes
The author declares no competing financial interest.

ACKNOWLEDGMENTS

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