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Research Article

Ascorbic Acid as an Inhibitor for SARS-CoV-2 Virus Reproduction: A Theoretical Approach

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A B S T R A C T

In the present study, ascorbic acid's or Vitamin C's influence (VC) in inhibition of SARS-CoV-2 virus reproduction was investigated. Gaussian 09 with a basis set of 6-311G (d, p), MGL tools, DSV, and LigPlus software were used. According to the Total Electron Density (TED) and Millikan charges, the active sites for adsorption were determined. Further, the docking study had clearly revealed the role of VC in inhibition of the virus reproduction in accordance with binding energy (Eb) and ligand efficiency (LE). The vitamin's interaction with the virus's spikes may limit its replication or provide the immune system sufficient time to recognize the infection, which enhances the possibility of producing appropriate antibodies.

Keywords: COVID-19, Vitamin C, DFT, Docking, TED

Introduction

Ascorbic acid is a water-soluble vitamin that is required by the body. It is naturally found in fresh vegetables and fruits and is found in organs like kidneys and liver. This vitamin is required for the survival of a broad range of multicellular animals, most notably humans.[1] Numerous researchers, most notably Linus Pauling, have found that high doses of vitamin C are directly antiviral.[2] In vitro studies have demonstrated that extremely high doses of vitamin C are effective in the presence of free copper and/or iron, most likely via the production of hydrogen peroxide and other radical species.[3,4]

Low pH may also have improved vitamin C's antiviral activity in vitro. Vitamin C, on the other hand, is extremely unlikely to be directly veridical in vivo. Vitamin C is an effective antioxidant; it has been shown that at therapeutic quantities, it may display paradoxical pro-oxidant characteristics (the formation of reactive oxygen species) through transition metal reduction.[5] Nonetheless, one study found that it reduced viral load in Epstein-Barr virus (EBV)-infected cells,[6] which means the presence of a second mechanism. Cinatl and colleagues reported that pre-treatment of human foreskin fibroblast and endothelial cells with vitamin C before cytomegalovirus (CMV) infection dramatically decreased viral antigen expression and viral load in the cells.[7] This finding was not replicated after viral infection with vitamin C. The researchers discovered that this effect was most likely caused by vitamin C's immunomodulatory qualities rather than direct antiviral action. Vitamin C concentrations are notably high in leucocytes, lymphocytes, and macrophages. Vitamin C enhances the Chemotaxis, neutrophil phagocytosis, and oxidative death, as well as lymphocyte proliferation.[8,9]

Following the emergence of the COVID-19 pandemic in 2019, many treatment regimens have been researched for compassionate use, except for remdesivir, which had promising outcomes and was granted emergency

permission by the Food and Drug Administration (FDA) for the treatment of this pandemic.[10] As a result, supportive care, which may include micronutrient supplements such as vitamin C, has become an essential component of COVID-19 therapy. The vitamin levels in serum and leukocytes are reduced during the acute stage of illness.[11,12] Clinical trials have established that supplementing with a high dosage of vitamin C decreases the severity and duration of respiratory viral infections.[13] In light of these results, vitamin C may be utilized to treat COVID-19, since it may boost the immune response to the new virus (SARS-CoV-2).

While the virus causes some of the pathological damage produced by SARS-CoV-2 infection, the majority is produced by a huge host immunological response and oxidative stress-mediated by free radical production.[13] Infection with SARS-CoV-2 causes an increase in proinflammatory cytokines, termed cytokines storm, and increased generation of reactive oxygen species, both of which cause significant lung infection and the development of adult respiratory distress syndrome (ARDS).[14] Septic shock, which is a primary cause of ICU admission and death in patients over 60 years old, may develop from ARDS.[15,16] Vitamin C is a powerful antioxidant as well as an immunomodulatory,[17-19] so it could be utilized to treat and prevent COVID-19-related problems.

Since vitamin C is often utilized as a supportive supplement in the treatment of the virus. In the present work, we reported a potential mechanism for vitamin C's action on the protein's membrane of the SARS-CoV-2 virus. This suggests that one of vitamin C's effects is mediated by the reduction of coronavirus viral spikes, which may assist in decreasing viral adsorption or could inhibit the virus reproduction.

Computational Approach

The structure of the protein is downloaded from RCSB (Research Collaboratory for Structural Bioinformatics).[20] Auto docking was carried out utilizing tools from the Molecular Graphics Laboratory (MGL). Autodock technique were used to separate the coordinates of the spike structure and the ligand (Vitamin C) (ADT, version 1.5.6). By adding all hydrogen atoms, Gasteiger charges, the SARS-CoV-2 spike glycoprotein-S1 and ligand structures were converted to a format accepted by the ADT (*.pdbqt files).

Unless the user indicates otherwise, the Autodock will frequently attempt to determine the molecule's root, so the Autodock chose the root on its own. Auto grid (version 4.2.6) was used to calculate atom-specific affinity maps, electrostatic and desolation potentials for all ligand atoms.23 Before docking calculations, VC was optimized in the gas phase with no symmetry constraints, Figure 1 depicts the optimal structure of VC, and Table 1 contains their coordinates. Using the unrestricted DFT approach and the 6-311G (d,p) basis set, the molecular electrostatic potential (MEP) was also explored.[24,25]

Table 1.Cartesian Coordinates of VC Structure

Center number	Atomic	Coordinates (Angstroms)		
		X	Y	Z
1.	6	0.508700	0.563300	-0.420800
2.	6	0.910500	-0.856000	-0.101300
3.	6	2.180000	-0.872600	0.303700
4.	6	2.614900	0.405500	0.264800
5.	8	1.686800	1.315300	-0.154000
6.	8	3.755800	0.664500	0.585600
7.	8	2.934100	-1.928800	0.675600
8.	8	0.139500	-1.957800	-0.225000
9.	6	-0.643100	1.086900	0.424000
10.	6	-1.960700	0.370300	0.195400
11.	8	-2.974400	1.117800	0.836700
12.	1	0.274000	0.648800	-1.507200
13.	8	-0.859200	2.439900	0.096200
14.	1	3.821000	-1.584100	0.880100
15.	1	0.692200	-2.719600	0.022300
16.	1	-0.404848	1.678960	1.282837
17.	1	-2.219900	0.313200	-0.884900
18.	1	-1.973000	-0.649400	0.637100
19.	1	-3.821000	0.678100	0.739900
20.	1	-1.678100	2.719600	0.515800

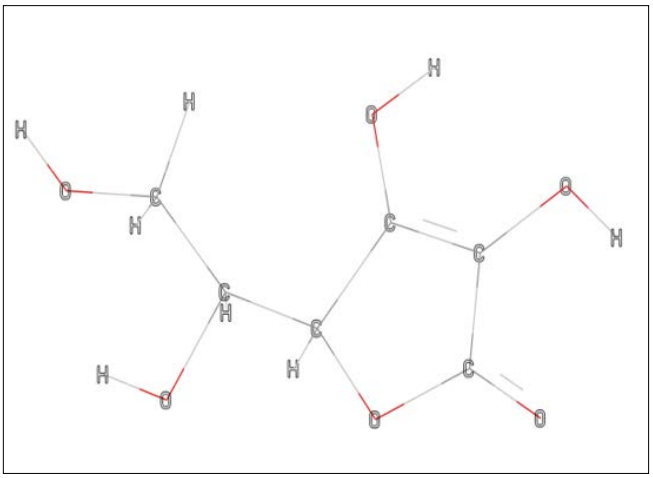

Figure 1.VC Structure

Results and Discussion

Interaction of VC with the Spikes

The suggested mechanism of SARS-CoV-2 virus inhibition is by interaction of VC with the spikes, which could enhance

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the role of immunity system. The active functional groups in VC are OH and C=O and there are planarity in some sections, according to geometry optimization (depending on the cis and trans of atoms in the molecule, the dihedral angle of each four cis atoms is about zero, while in trans is about 180º).

Figure 2 shows the PDB structural code (6acd) and the interaction of the protein with VC. Measuring the binding energy, the affinity of vitamin C to the receptor, enabled us to compare the optimum sites for linking with the protein.[26,27] The binding energy range was ranging from -0.94 to -2.17 Kcal/mol. So, the best-bounded site (BS) on the protein is the one that has the highest value of the binding energy 2.17 kcal/mol.

Additionally, the Ligand Efficiency (LE) of the ligand per atom attached to the receptor protein is -0.18 kcal/mol.[28,29] Thus, this process may give the body an additional time to produce suitable antibodies.

Table 2.Binding Energy Value and Ligand Efficiency of the Studied Inhibitor

Comp.	Eb (kcal/ mol)	LE	-Eb range	BS
VC	2.17	-0.18	-0.94 to -2.17	6

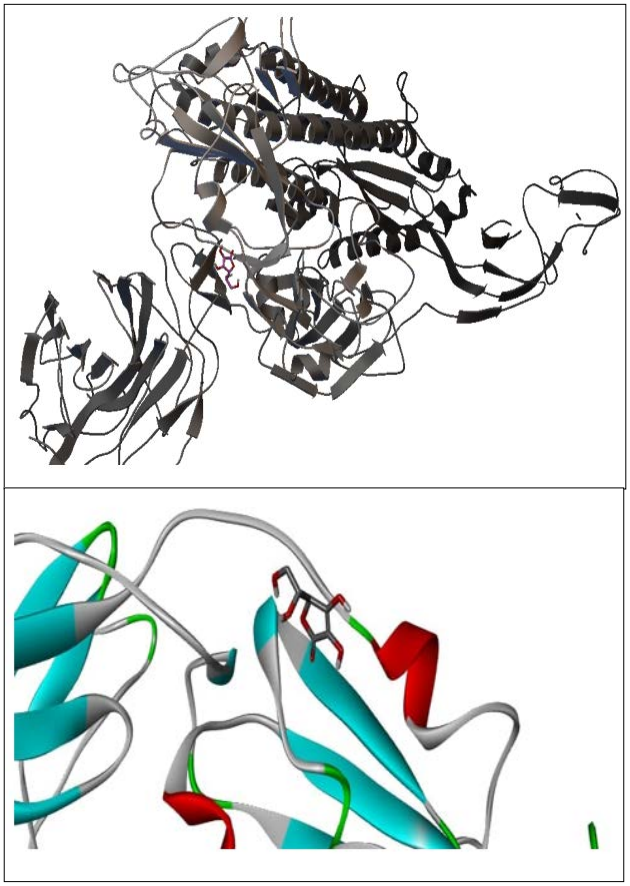

Figure 2.Interaction of VC with the Protein

The Bonds of VC with Virus's Amino Acids

By analysis of the results obtained from the DSV, LigPlus, and MGL tools, the interaction between the amino acid of the virus spike and VC is clearly revealed. The OH and C=O groups form hydrogen bonds with the VAL and ASP amino acids, respectively. The bond length of VAL-HO(vc) and ASP-CO(vc) are of 3.19 and 3.11 Å, respectively. In addition, the amino acids viz ASN, LEU, CYC, ALA, VAL, and PHE showed low effect but cannot be bypassed (Figure 3). The electron density of the donor atom determines the strength of the adsorption bond. The electron density on the molecule was visualized using total electron density (TED).

As shown in Figure 4, the red color represents the sites with high electronegativity, such as the O atoms of a hydroxyl groups (OH). While the atoms with moderate electronegativity are represented by yellow color. In the same context, the blue region is the most favorable positive area for accepting electrons from donor atoms [30-32], as it is the lowest electronegativity.

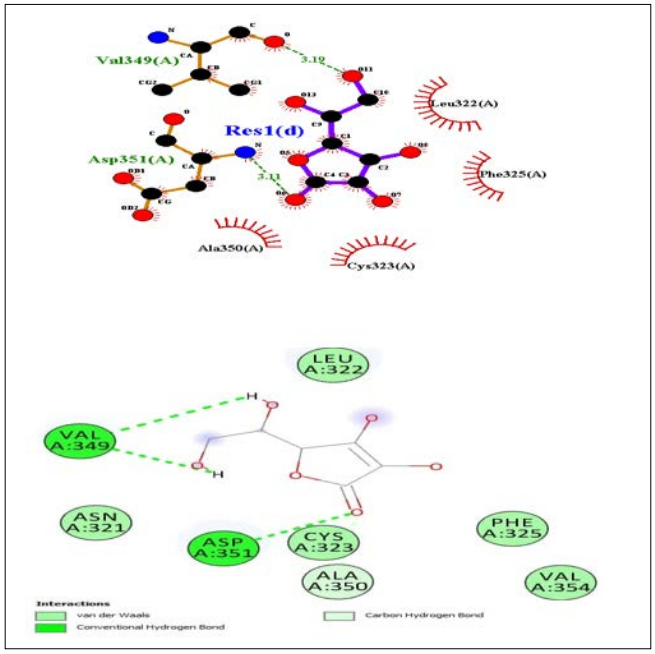

Figure 3.2D Interactions of VC with COVID-19 Amino Acids

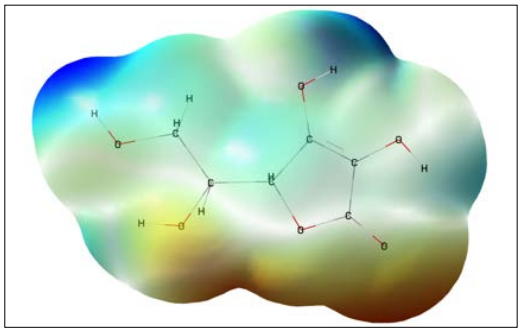

Figure 4.TED Map of VC

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Local Reactivity of VC

To investigate the localized reactivity of the studied inhibitor (nucleophilic and electrophilic centers), The DFT method used for Mulliken charges population analysis to determine the reactive centers of molecules. As a result, the molecule regions with a large electronic charge are chemically softer than those with a small electronic charge, so the electron density is significant when calculating chemical reactivity.

Interactions in chemical adsorption could be electrostatic or orbital in nature. Electrostatic interactions are driven by the electrical charges in the molecule. The physicochemical properties of compound reactions are influenced by proven charges.33, 34 As a result, the nucleophilic attack will be on the site that has the highest negative charge value. In turn, the positive charge value determines the electrophilic attack site. Therefore, the favored sites for electrophilic attack and the most reactive of VC are C and H atoms. The green color represents the high positive charge, so the decrease in color intensity indicates a decrease in positivity. It is found that the most reactive sites that can accept electrons for VC in the nucleophilic attack are O atoms (Figure 5).

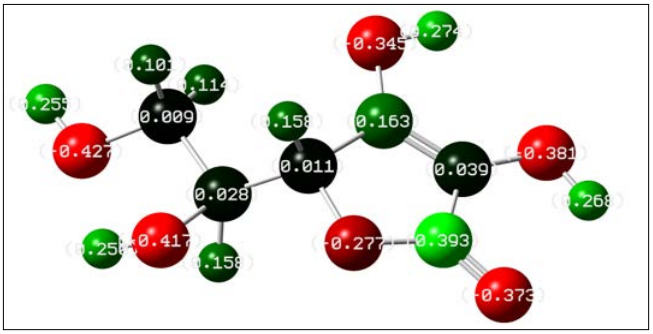

Figure 5.Millikan Charges of VC

Conclusion

A theoretical research has been undertaken to evaluate the effectiveness of ascorbic (vitamin C) as an antiviral agent against the SARS-CoV-2 virus. Computational calculations were used to investigate the binding of the vitamin with the virus's spikes. The TED revealed that the OH and C=O groups in VC are the interaction sites with the virus. The binding energy reflected the high affinity of the spike proteins to the VC. The calculations showed that the VC interacts with the spikes via hydrogen bonding.

Docking study refers to the ability of VC to reduce the activity of SARS-CoV-2, depending on Eb (-2.17 kcal/mol)) and LE (-0.18 kcal/mol) values. Depending on Millikan's descriptions, the electrophilic and nucleophilic sites are (C, H) and (O) atoms, respectively.

Conflict of Interest: None

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