Creative Education, 2023, 14, 103-110
https://www.scirp.org/journal/ce
ISSN Online: 2151-4771
ISSN Print: 2151-4755

Use of the D614G Mutation of the SARS-CoV-2 Spike Protein as a Biophysical Model to Explain Coulomb's Law Function

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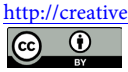
How to cite this paper: Fuentes-Acosta, M., Mulia-Rodríguez, J., & Osorio-González, D. (2023). Use of the D614G Mutation of the SARS-CoV-2 Spike Protein as a Biophysical Model to Explain Coulomb's Law Function. *Creative Education, 14,* 103-110.
https://doi.org/10.4236/ce.2023.141008

Received: December 15, 2022
Accepted: January 17, 2023
Published: January 20, 2023



Abstract

The scientific work of Charles-Augustin de Coulomb has transcended as a significant contribution to explain phenomena in nature and generate crucial technological development in recent daily activities. We used Coulomb's law to calculate the changes generated in the electrostatic interactions of residue 614 of SARS-CoV-2 Spike protein when the D614G mutation occurs. We made a physical analysis of the transformation and the biological implications in the whole molecule's structural stability, obtaining that a greater electronegativity of the mutation stage favors the open state of Spike, which is manifested as a greater efficiency to bind to the ACE2 human receptor.

Keywords

Spike, Coulomb's Law, Electrostatic Interactions

1. Introduction

Electrostatic interactions are essential for biomolecule function, e.g., changes in these interactions on residues can influence phosphorylation and dephosphorylation, thereby inducing significant structural effects, such as protein denaturation and thus disruption of metabolic processes in living organisms (Zhou & Pang, 2018). In fact, to fully understand their activity, it is necessary to comprehend the relationship between their structure and energy, where the most relevant factors are associated with the electrostatics interactions (Warshel & Russell, 1984).

In proteins, electrostatic forces have a central role in their structure and stability, as well as enzymatic activity, ligand binding, and allosteric control (Ha-

noian et al., 2015; Laskowski et al., 2009; Nakamura, 1996). Protein electrostatics is composed of intramolecular interactions and interactions with the medium. Such electrostatic properties are mainly due to the different types of amino acids that can be combined to make a protein (each one characterized by unique physicochemical properties) interacting with each other through salt bridges, hydrogen bonds, and charge-dipole interactions to stabilize the molecule. On the other hand, electrostatic interactions are long-range and highly dependent on the environment and the ions surrounding the biomolecule (Bremer et al., 2004; Nakamura, 1996).

The recognition and binding of a protein with other biomolecules (ligand, receptor, or antibody) depends on different chemical and physical factors, mainly electrostatic energy—whose contribution is the result of Coulomb interactions between the molecules—(Voet et al., 2013). The electric field in the active site of a protein regulates its catalytic activity and determines the relative binding orientations; in addition, the surface of the proteins and the interface generated after the interaction have many polar and charged residues (Sheinerman et al., 2000). For example, antibodies with a predominantly positive surface charge interact electrostatically with anionic cell membranes or tissues as long as the ionic strength and pH of the protein solution is adequate (Boswell et al., 2010; Nguyen et al., 2021); this is known as electrostatic complementarity: regions of positive electrostatic potential in one biomolecule interact with regions of negative electrostatic potential in the other. Complementarity has been observed in many biological complexes; in proteins, the influence of charged amino acids in the structure generates charged surfaces that promote interactions with other biomolecules, and a single change in their sequence—for example, a mutation—could modify the electrostatic interactions by increasing or decreasing the attraction of the complex (Goher et al., 2022; Ishikawa et al., 2021).

The SARS-CoV-2 virus is responsible for causing the COVID-19 disease that has led to one of the most severe health emergencies in recent years, and as a result, the virus has been the subject of extensive research and open access information and therefore represents an excellent model for teaching. SARS-CoV-2 is made up of five structural proteins, of which the Spike protein is fundamental since it interacts with the human receptor—Angiotensin Converting Enzyme 2 (ACE2)—to begin the infection process. The D614G mutation in Spike quickly became a dominant variant of SARS-CoV-2 worldwide (Fernández, 2020; Gobeil et al., 2021); hence, structures with and without the mutation are available in each conformational state that distinguishes the Spike protein (open or 1-RBD up and closed or 3-RBD down) (Gobeil et al., 2021; Walls et al., 2020). Since mutation D614G is present in the predominant variants worldwide, it has been suggested that it could be a candidate for antigen design and future vaccines, mainly because G614 Spike is prone to maintain the open state (Koenig & Schmidt, 2021). However, D614G is not considered to affect current vaccines, in fact, by promoting the open state, the mutation increases the exposure of neutra-

lizing epitopes, and it has been shown that G614 variant is therefore better neutralized (Plante et al., 2021; Weissman et al., 2021).

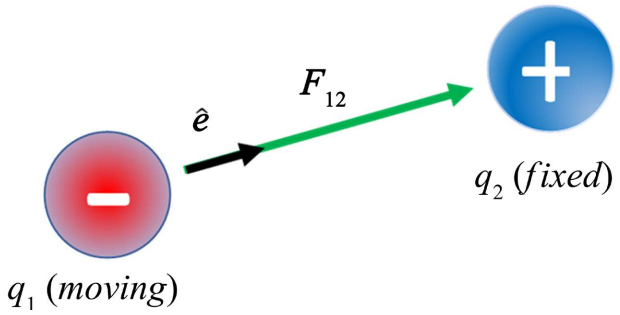
In this work, we use open-source technological and scientific resources to calculate the electrostatic interactions of residue 614 of the SARS-CoV-2 Spike protein with and without the D614G mutation. These computational tools are essential in various areas of biology and are fundamental for the study of biomolecules where the calculation of electrostatic interactions is a necessary procedure to identify the regions of chemical affinity in different viral proteins (Pena-Negrete et al., 2020; Osorio-González et al., 2021). We compare the open and close Spike conformational states to highlight the biophysical implications of Coulomb's law.

2. Materials and Methods

Coulomb's law is also known as the law of electrostatics; it was published for the first time in 1785 (Coulomb, 1785), and it justified—with an experimental foundation—the nature of the forces of attraction or repulsion between two charges q_1 and q_2 separated by a distance r

$$\boldsymbol{F} = \frac{1}{4\pi\varepsilon_0}\frac{q_1 q_2}{r^2}\hat{\boldsymbol{e}}$$

where ε_0 is the constant of electric permittivity in a vacuum and $\hat{\boldsymbol{e}}$ is a unit vector in the direction of $\boldsymbol{F}$. Considering that the q_1 charge is mobile and the q_2 a charge is fixed:



Coulomb's law has applications in many areas of knowledge; however, this paper will highlight its usefulness in describing the biological problem related to a mutation of the SARS-CoV-2 virus. This mutation allows a change in electrostatic interactions that surprisingly makes the virus more efficient for binding to the human ACE2 receptor.

As a starting point, we use the differential relationship between force and potential energy V given by

$$\boldsymbol{F} = -\frac{\mathrm{d}V}{\mathrm{d}r}$$

Likewise, using Coulomb's law, it can be easily verified that the electric force has a potential energy associated with it in the following way:

$$V = \frac{1}{4\pi\varepsilon_0}\frac{q_1 q_2}{r}$$

The superposition principle applies to a set of n individual charges such that the total potential energy would be:

$$V = \frac{1}{4\pi\varepsilon_0}\sum_{pairs\ i,j}\frac{q_i q_j}{r_{ij}}$$

We used this last expression to calculate the difference in the electrostatic interactions of the atoms that make up residues D614 and G614 of the SARS-CoV-2 Spike protein. The Cartesian coordinates of these residues were taken from the Protein Data Bank using the codes of identification 6VXX, 6VYB, 7KDL, and 7KDK. We used the charges defined by the GROMOS 53a force field (Oostenbrink et al., 2004), and the distances between the atoms of each residue were calculated using the PyMOL 2.3.4 molecular viewer, the results of which are presented in the next section.

3. Results

When the SARS-CoV-2 virion approaches the human receptor, conformational changes occur in the Spike protein that allows the opening of an RBD—that is, the transition from the closed to the open state—for the recognition and binding with ACE2 (Takeda, 2022). D614 is a surface residue located in the Spike subunit (S1) that contains the receptor-binding domain (RBD), and although it is not a residue that has a direct interaction with ACE2, the D614G mutation disrupts critical hydrogen bonds, causing a change in the observed balance between the open and closed state of Spike (Gobeil et al., 2021). By applying Coulomb's law to all non-covalently bonded pairs of atoms in the 614 residue, it was shown that the D614 residue in the closed state (6VXX) is more electronegative and therefore more attractive than in the open state (6VYB) (**Table 1**), that attraction is necessary to maintain conformation and balance between open and closed states.

On the other hand, the electrostatic interactions between the open and closed state of G614 did not show differences (**Table 1**), which could be because the mutation favors the open state of Spike and the conformational changes from the closed to the open state does not imply an energetic change (Gobeil et al., 2021; Weissman et al., 2021; Yurkovetskiy et al., 2020). A recent investigation found that the Spike models with G and D lead to an endless number of contacts that are similar between the three protomers that make up the protein; however, in the D model, the contacts can be described as symmetrical which are lost when Spike switches to the open state; while in the model with the D614G mutation, Spike maintains the symmetry in the number of persistent contacts when switching to the open state, which implies that the energy of the protomers between the different conformational states is similar (Mansbach et al., 2022), as can be seen when comparing the distances in D614 and G614 in both states (**Figure 1**). This allows us to understand the impact of a mutation in a single

Table 1. Electrostatic interactions of residue 614 of the Spike protein in the open and closed models, with and without mutation.

	Spike Open State	Spike Closed State	Energy difference between conformational states
D614	−223489467.11	−246015170.5	**22525703.40**
G614	−92225067.57	−92225067.57	**0**
Energy difference between D614 and G614	**131264399.54**	**153790102.94**	

D614 open state (PDB ID: 6VYB), G614 open state (PDB ID: 7KDL), D614 closed state (PDB ID: 6VXX), and G614 closed state (PDB ID: 7KDK).

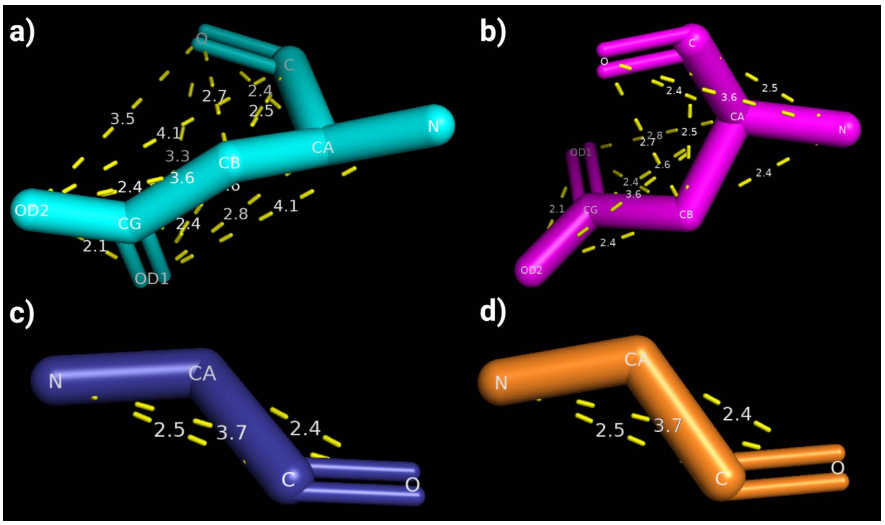


Figure 1. The distances between atoms are shown in residue 614 of the different Spike models. (a) Open state without mutation (PDB ID: 6BYV), (b) Closed state without mutation (PDB ID: 6VXX), (c) Open state with D614G mutation (PDB ID: 7KDL), and (d) Closed state with D614G mutation (PDB ID: 7KDK).

residue that can facilitate conformational changes towards the open state in Spike, thus improving viral infectivity.

Both the open and closed states are more electronegative in the D614 form of Spike than with G614, which is 58.7% and 62.5% less electronegative in the open and closed state, respectively, this difference could be related to the fact that the Spike structure with D614 is less stable than the G614 variant and, in general, Spike with G614 naturally favors a prefusion state (1RBD-up) and presents the 3RBD-down and 1RBD-up conformations with better stability (Zhang et al., 2021).

4. Conclusion

In the present work, we verify that Coulomb's law allows us to explain simply and directly why a single mutation of the SARS-CoV-2 virus enables high effectiveness in binding to human receptors and that the electrostatic nature of bio-

molecules is essential for biological interactions. Specifically, changes in electrostatic potential make it possible to understand the biophysical and structural consequences of the D614G mutation in Spike. The biophysical analysis of said transformation shows that under this situation, a greater electronegativity prevails in the vicinity of residue 614, and this confers more structural stability to the whole molecule in its open state, which is manifested as a greater efficiency in binding to the human receptor ACE2 and, consequently, facilitates the spread of COVID-19.

From a scientific and academic perspective, it is gratifying that such a complex biological process can be modeled with a physical law that was proposed more than 270 years ago and that its simple form makes it possible to understand a phenomenon that history will record as the pandemic of the 21st century.

Acknowledgements

The authors wish to express gratitude to the Center for Research, Technology Transfer, and Business Innovation (CITTIES) for supporting the development of new teaching techniques.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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