Exploring the Mechanism of the Envelope Protein of SARS-CoV-2: A Molecular Dynamics Study

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Abstract

According to the World Health Organization, the number of people infected with COVID-19 continues to increase globally, highlighting the urgent need for effective treatments. Research has shown that certain antibodies targeting the envelope (E) protein of the virus can prevent viral entry. This study aimed to investigate the ion transport process mechanism in the E protein of COVID-19 and to identify potential inhibitors using virtual screening. The study utilized molecular docking and molecular dynamics simulations to understand the ion transport mechanism and proposed possible inhibitors that can bind strongly to the opening of the ion channel. The simulations revealed significant conformational changes during the ion transport process. These findings demonstrate the potential of simulation techniques for understanding the structural dynamics in developing inhibitors that can bind to the E protein of COVID-19.

Keywords: MD simulation, envelope protein, virtual screening, COVID-19, drug discovery.

Introduction

The COVID-19 pandemic has had a profound impact on the world, affecting nearly every aspect of our daily lives.[1] Since its emergence in late 2019, the virus has spread rapidly across the globe, infecting millions and leading to significant morbidity and mortality.[2-4] In addition to

its toll on human health, the pandemic has also had extensive social and economic consequences, disrupting businesses, straining healthcare systems, and triggering widespread unemployment.[5] The pandemic has underscored the importance of effective disease control measures, including testing, contact tracing, and vaccination campaigns.[4] While significant progress has been made in developing and deploying vaccines, the emergence of new variants has highlighted the need for continued vigilance and adaptation in our response efforts.[6-8] Ultimately, the COVID-19 pandemic has emphasized the interconnectedness of our global community and the critical importance of collaboration, scientific innovation, and public health preparedness in addressing global health crises.

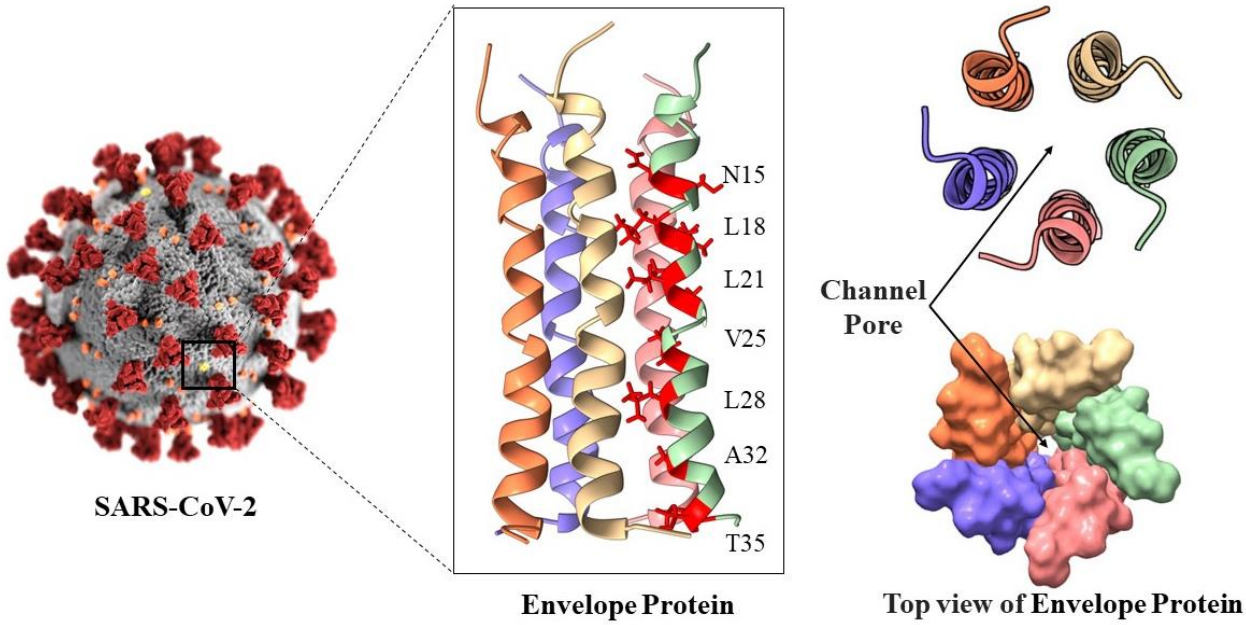

Figure 1: Envelope protein is shown in a ribbon format. The top view of the E protein is displayed on the right side. The surface map of the protein is shown in the bottom right, revealing the narrow pore of the channel.

The envelope protein is one of the four structural proteins encoded by the RNA genome of the novel coronavirus, SARS-CoV-2, responsible for the COVID-19 pandemic.[1, 9, 10] This protein plays a crucial role in the viral life cycle, especially in the assembly and release of new

virions from infected cells.[11] As a transmembrane protein anchored in the lipid bilayer of the viral envelope, it is abundant on the virus surface, marking it as a significant target for developing antiviral therapies and vaccines.

Recent studies have shed light on the structural and functional properties of the envelope protein. It has been found to be involved in viral morphogenesis, budding, and assembly, and is thought to interact with other viral proteins and host cell factors during the viral life cycle.[1, 9, 10] The envelope protein is also known to play a role in modulating the host immune response to the virus, particularly by interfering with the host's innate immune signaling pathways. Furthermore, the envelope protein has been shown to induce an antibody response in infected individuals, and is therefore a potential target for serological diagnostics. Given the central role of the envelope protein in the viral life cycle and the immune response to SARS-CoV-2, there is a growing interest in understanding the molecular mechanisms underlying its function and exploring its potential as a therapeutic target. Ongoing research efforts are focused on elucidating the structure of the envelope protein, characterizing its interactions with other viral and host factors, and identifying compounds that can inhibit its function.

Membrane proteins constitute an important class of proteins that play crucial roles in various cellular processes. Because of this, they have emerged as attractive targets for drug development. Inhibition of these proteins can modulate their function, potentially leading to therapeutic effects. For example, statins are a class of drugs that inhibit HMG-CoA reductase, a membrane protein critical for cholesterol synthesis. This inhibition lowers cholesterol levels in the blood. Another class of drugs, angiotensin-converting enzyme inhibitors, target the membrane protein ACE, a regulator of blood pressure. Beta blockers work by inhibiting beta-adrenergic receptors, essential membrane proteins in the regulation of heart rate and blood pressure.

Antihistamines act by inhibiting histamine receptors, membrane proteins implicated in allergic reactions. Furthermore, SGLT2 inhibitors target the membrane protein SGLT2, responsible for glucose reabsorption in the kidneys. These examples underscore the significant potential of targeting membrane proteins in the development of therapeutic agents.

In this study, we utilized molecular dynamics simulations to investigate the behavior and functioning of the envelope protein. This powerful computational technique allowed us to analyze the movement and interactions of atoms and molecules in a system over time. By conducting these simulations on the envelope protein, we aimed to understand its structural dynamics and identify potential drug binding sites. Furthermore, we employed virtual screening to pinpoint drugs that could bind to and inhibit the envelope protein. This computational method was used to screen large databases of compounds to identify potential drug candidates. Utilizing this technique enabled us to identify a set of compounds with a high probability of binding to the envelope protein and inhibiting its function. Overall, our study offers valuable insights into the behavior and functioning of the envelope protein and identifies potential drug candidates for developing therapeutic agents to combat COVID-19.

Computational methods

The monomeric form of the E protein structure was obtained from the hexameric 1.9 Å resolution X-ray structure (PDB ID: 7K3G).[9] The binding poses of ligands to the E protein opening were explored using Autodock Vina 1.5.6 software.[12] For each protocol, 10 poses were generated for all four enzyme-substrate complexes, with an exhaustiveness value of 20. To perform the MD simulations, the GROMACS-4.5.6 program[13] was utilized, along with the AMBER03[14] force field. The simulations were performed in a cubic box filled with TIP3P[15] water molecules, and the system was neutralized by replacing some water molecules with

potassium and chloride ions to simulate a physiological ion concentration of 0.154 M. The MD simulations were performed for 200 ns. The SETTLE algorithm was used to constrain the bond lengths and angles of the water molecules, and the LINCS algorithm was employed to constrain the remaining bond lengths. The simulations were carried out in NPT ensembles with constant N, P, and T.

Results and discussion

This study investigated the functioning of the E protein using molecular docking and molecular dynamics techniques. Furthermore, five inhibitors were also proposed using computational simulations. The root-mean-square deviations (RMSDs) analysis confirmed the equilibration of all five complexes within the simulation time. The study compared the electrostatic potentials (ESPs), secondary structures, non-covalent interactions, root-mean-square fluctuations (RMSF), and inhibitors binding free energies to discuss the preferential binding of these inhibitors and the coordination flexibility of the enzyme.

The examination of molecular dynamics sheds light on the behavior of ion channels, particularly focusing on the alignment and subsequent twisting of the 5 alpha helical domains. Initially, these domains are parallel when the channel is in a closed state, maintaining a structured configuration. However, the scenario changes post a 200ns molecular dynamics (MD) simulation, where a noticeable twist in the domains is observed as shown in Figure 2. This twist is indicative of structural transformations happening within the ion transport process of the channel, embodying a dynamic mechanism. The structural metamorphosis doesn't just stop at domain twisting; it further manifests in the outward displacement of the residues E8 and N15. This outward migration is not arbitrary but is in line with the findings of experimental studies.[16] These studies conjecture a

significant role for E8 and N15 residues when the ion channel is in a closed state. They seem to bind to the metal ion, setting the stage for the ion's accommodation and subsequent transport.

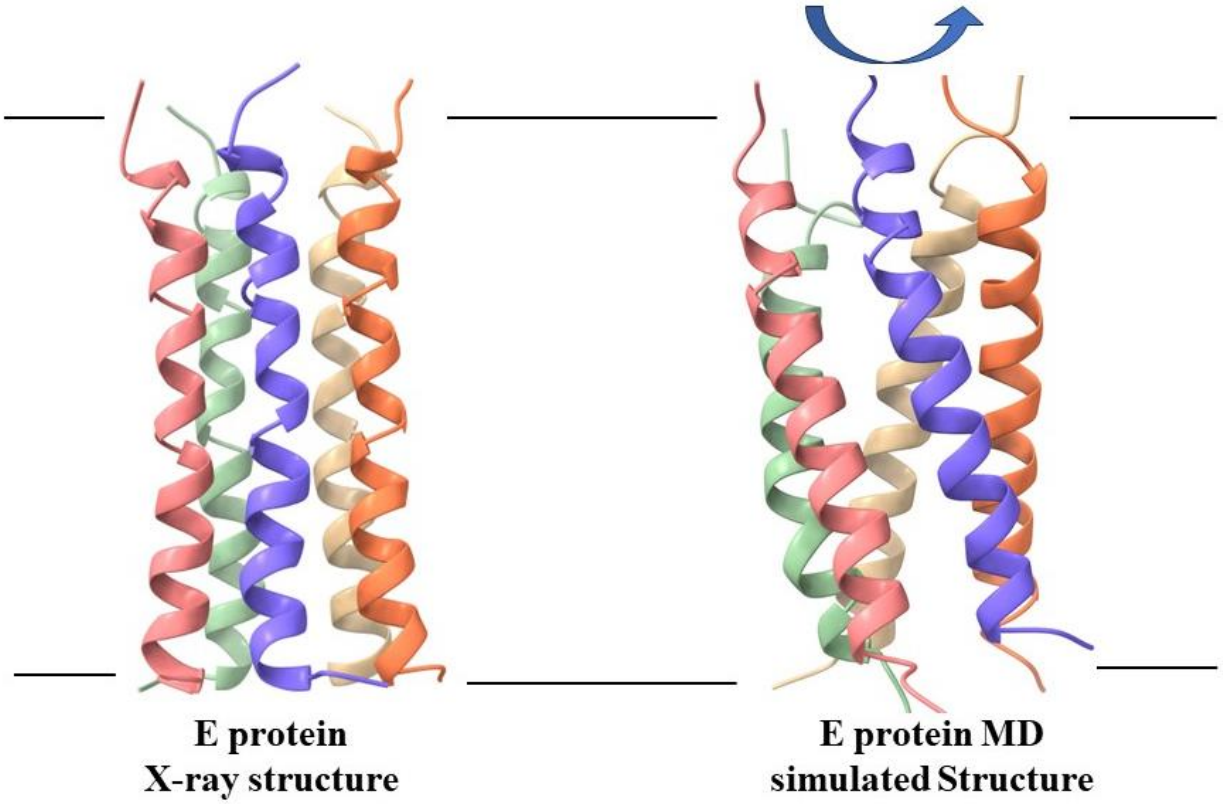

Figure 2: Closed-state X-ray structure of the envelope protein (left) and open-state simulated structure obtained from molecular dynamics simulation. The simulated structure twists and opens the pore of the ion channel.

This preparation is crucial for the ion transport process, ensuring the ions are in the right position before the actual transport occurs. The once accommodating E8 and N15 residues distance themselves, letting go of the ion. This release is not haphazard but seems to be a calculated move, dropping the ion precisely towards the channel opening. This mechanism underscores a well-coordinated sequence of events that regulate ion transport through the channel, showcasing a blend of structural and functional intricacies inherent in the ion channel dynamics.

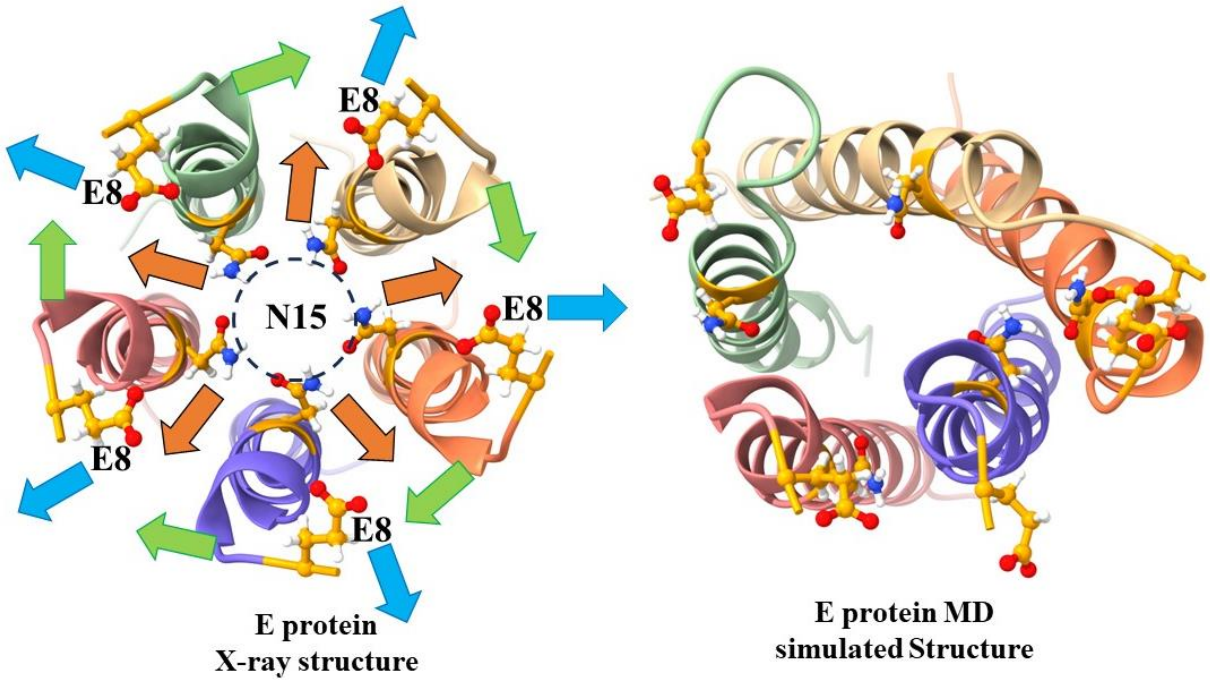

Figure 3: In the closed state, residues E8 and N15 are close to each other, allowing them to function in accommodating the metal ion by binding to it. In the simulated E protein, residues E8 and N15 move outward, releasing the metal ion into the opening of the ion channel.

The twisting motion led to the outward movement of residues F20, F23, and F26, resulting in a change in hydrogen bonding among these residues as shown in Figure 4. This phenomenon has been confirmed in experimental studies.[16] As a result, it can be concluded that Phe residues in the open state do not obstruct the channel pore. Silva et al. have proposed that the structural changes in the N and C termini facilitate ion permeation through a specific mechanism.[16] According to them, the "Phe" motif located between amino acid residues 20 and 26 serves as a signaling relay system for conformational changes by influencing the orientations of the aromatic side chains. Furthermore, 19F NMR studies have shown that both F20 and F26 can adopt two distinct side-chain conformations, which affect their exposure to lipids and water.[17] These findings imply that the equilibrium between the conformations of F20 and F26 may impact the channel's pore diameter and the overall structural stability of the helical bundle.

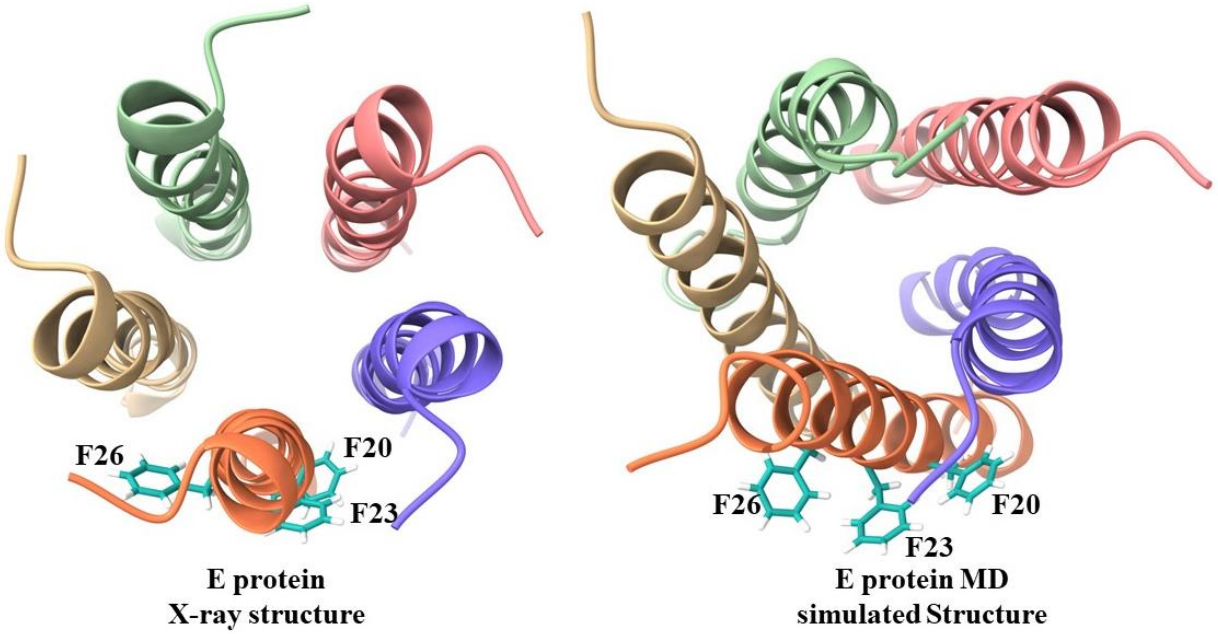

Figure 4: In the closed-state X-ray structure of the envelope protein (left), the F20, F23, and F26 residues are inward facing, while in the open-state simulated structure (right), the three residues are outward facing, showing that fluctuations in these three residues result in the opening and closing of the ion channel.

The proposed inhibitors obtained from molecular docking and MD simulations are shown in Figure 5. All of these inhibitors bind strongly to the ion channel's opening and remain in that location throughout the entire MD simulations.

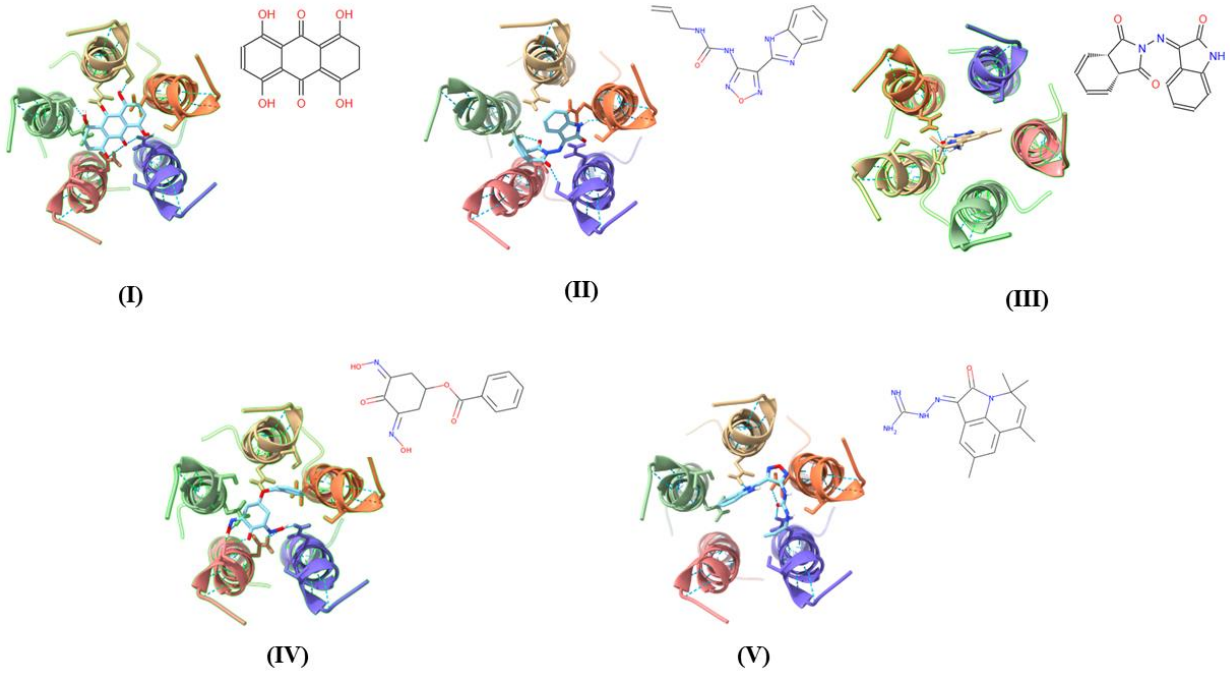

Figure 5: Inhibitors (I-V) obtained from molecular docking and molecular dynamics simulations.

Based on our simulation we have proposed the following ion transport mechanism. The ion transport mechanism involves a pH-dependent conformational change in the channel. At neutral pH, the alpha-helical domains are closed and aligned. However, when the pH becomes acidic, specific residues such as F20, F23, and F26 shift outward, leading to a twist in the pentameric domains. This twisting motion causes the E8 and N15 amino acids to move outward as well, thereby widening the channel pore. In this open state, ion transport takes place. Proposed inhibitors target E8 and N15 amino acids, effectively restricting ion transport by binding to these critical residues in the channel.

Summary and conclusion

The current study focuses on exploring the mechanism of ion transport in the Covid envelope protein. The study elucidates the intricate roles that metal ions play in the ion transport mechanism of the envelope protein. Based on our simulations, it is evident that the envelope

protein exhibits distinct conformational dynamics and coordination flexibility. The orientation of the F20, F23, and F26 residues plays a major role in these structural changes. On the other hand, E8 and N15 accommodate the ions in the closed state and move outward in the opening state, releasing the metal ion to the opening of the ion channel. Our simulations also propose the ion transport and structural modifications involved in ion transport. Finally, using molecular docking techniques, we have also identified potential inhibitors that can bind to the ion channel opening and inhibit its activity, thereby hindering coronavirus maturation. The compounds predicted by this study will undergo experimental validation through the application of Isothermal Calorimetry (ITC), followed by subsequent in vitro investigations to ascertain their biological activity and interaction mechanisms.

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Notes: The authors declare no competing financial interests.

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