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In Silico Design of Tetanus Toxoid-Derived Fusion Peptides as Antiviral Therapeutics

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Abstract

This study explores the potential of using the C fragment of tetanus toxoid as a scaffold for designing antiviral peptides targeting SARS-CoV-2. The tetanus toxoid C fragment is a well-characterized and non-toxic protein that can be leveraged to create fusion peptides with enhanced antiviral properties. We employed computational modeling techniques to design fusion proteins by linking the EK1 peptide, known for its inhibitory effects on viral fusion, to the C fragment. The resulting fusion peptides were analyzed for structural integrity and binding efficiency using ProSA and MolProbity for quality assessment. Docking simulations were conducted to evaluate the binding affinity of the designed peptides against the SARS-CoV-2 spike protein, revealing favorable interactions. Additionally, we compared the binding profiles of these novel fusion inhibitors with previously studied peptide inhibitors, demonstrating their competitive binding capabilities. The findings suggest that tetanus toxoid-derived fusion peptides represent a promising class of antiviral therapeutics, with the potential to disrupt SARS-CoV-2 entry mechanisms and contribute to future antiviral strategies.

Keywords: C fragment, computational modelling, docking, EK1 peptide, tetanus toxoid, SARS-CoV-2,

Introduction

SARS-CoV-2, the virus responsible for the COVID-19 pandemic, has necessitated the urgent need for effective antiviral therapeutics. One promising approach to combatting viral infections involves the design of fusion inhibitors that target viral entry mechanisms [1] [2] [3]. The spike protein of SARS-CoV-2 plays a pivotal role in facilitating viral entry into host cells by binding to the ACE2 receptor and undergoing conformational changes that promote membrane fusion [4] [5].

One innovative strategy is to use the C fragment of tetanus toxoid, a well-characterized and non-toxic protein derived from the tetanus toxin, as a scaffold for developing antiviral peptides [6] [7]. The C fragment can be engineered to include functional domains, such as antiviral peptides, creating fusion proteins that enhance binding and inhibitory properties [8] [9]. Previous studies have demonstrated the effectiveness of using peptide scaffolds to develop antiviral agents, particularly in targeting viral fusion proteins [10] [11].

The EK1 peptide, which has shown significant antiviral activity against coronaviruses, can be linked to the tetanus toxoid C fragment to form a fusion protein with potential for high binding affinity and specificity to the SARS-CoV-2 spike protein (Figure 1). This study employs computational modeling techniques to design and evaluate these novel fusion peptides, aiming to assess their structural integrity, binding capabilities, and potential as antiviral therapeutics [12] [13].

Methods

1. Design of Fusion Proteins: The design of tetanus toxoid-derived fusion peptides involved the combination of the C fragment of tetanus toxoid and the EK1 peptide. The C fragment structure was obtained from the Protein Data Bank (PDB ID: 1TGT), while the sequence of the EK1 peptide was sourced from previous studies on antiviral peptides. Using Modeller and RaptorX, we constructed the fusion proteins by linking the EK1 peptide to the C fragment with a flexible linker (GGGS). The resulting constructs were labeled as TetE1 (C fragment + EK1) and were designed to facilitate effective interactions with the SARS-CoV-2 spike protein [14] [15] [16].

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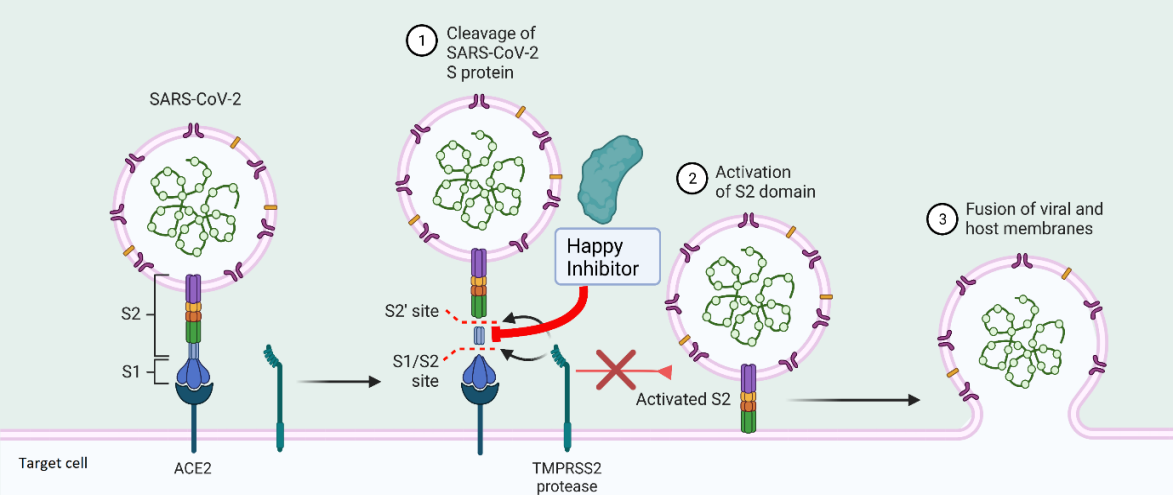

Figure 1: The expected inhibitory action by Happy00 and 06, on SARS-CoV-2 spike protein (created with BioRender.com).

2. Computational Modeling: Computational modeling was performed to generate three-dimensional structures of the fusion proteins. The models were refined using energy minimization protocols to optimize their geometries. We utilized molecular dynamics simulations in GROMACS 2021.3 to evaluate the stability and dynamics of the constructed fusion proteins over a simulated period of 50 nanoseconds [17].

3. Structural Integrity Evaluation: To assess the quality and structural integrity of the designed fusion proteins, we employed the following tools:

- ProSA: This tool provided z-scores to evaluate the overall stability of the fusion proteins. Lower z-scores indicate a higher likelihood of stability in a given conformation [18].
- MolProbity: This tool was used to analyze the Ramachandran plots, ensuring that a significant proportion of the residues fall within favorable regions. It also provided metrics for steric clashes and other geometrical properties [19].

4. Binding Analysis: Molecular docking simulations were conducted using HADDOCK 2.4 to analyze the binding interactions between the designed fusion peptides and the SARS-CoV-2 spike protein. The docking process involved the following steps: The spike protein structure was obtained from the Protein Data Bank (PDB ID: 6VYB). The input files for the fusion peptides were prepared using their modeled structures. HADDOCK utilized ambiguous interaction restraints based on previous studies to guide the docking process. Binding energies were calculated for the fusion peptides, providing insights into their potential efficacy as antiviral agents [20].

5. Comparison with Previous Peptide Inhibitors: Finally, the binding profiles of the tetanus toxoid-derived fusion peptides were compared with previously studied peptide inhibitors known for their antiviral activity. Key metrics such as binding energies and interaction networks were evaluated to assess the competitive binding capabilities of the newly designed fusion peptides.
This comprehensive methodology enables an in-depth analysis of the structural and functional characteristics of tetanus toxoid-derived fusion peptides, highlighting their potential applications as antiviral therapeutics against SARS-CoV-2.

Results

This study focused on the *in silico* design and evaluation of tetanus toxoid-derived fusion peptides, specifically assessing their potential as antiviral therapeutics against SARS-CoV-2. The findings are structured into sections that cover the modeling of fusion proteins, their structural integrity, binding analysis with the spike protein, and comparative assessments with previously studied peptide inhibitors.

1. Computational Modeling of Fusion Peptides: The initial step in our study involved constructing fusion proteins by linking the EK1 peptide, known for its antiviral activity, to the C fragment of tetanus toxoid. Using Modeller and RaptorX, we generated three-dimensional models of the fusion peptides, referred to as TetE1 (C fragment + EK1).
The structural modelling yielded a satisfactory resolution of the fusion proteins, allowing for subsequent analyses of their stability and binding capabilities. Each fusion peptide was designed to maintain a consistent architecture while facilitating interactions with the SARS-CoV-2 spike protein.

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2. Structural Integrity Assessment: The integrity and quality of the designed fusion peptides were evaluated using ProSA and MolProbity. The z-scores obtained from ProSA indicated the likelihood of structural stability for the fusion peptides. For TetE1, the z-score was -7.12, which falls within the expected range for stable protein structures. This suggests that the constructed fusion protein maintains a favorable conformation and is unlikely to exhibit significant folding instability [18].

Ramachandran plots generated via MolProbity showed that 85% of the residues in TetE1 were in favored regions, with only a small percentage in outlier positions (Figure 2 and 3). The analysis revealed No steric clashes between residues, confirming that the fusion peptide's geometry is appropriate for functional activity. The presence of hydrogen bonds between the C fragment and the EK1 peptide, indicating stable interactions within the fusion protein itself. These structural evaluations indicate that the designed fusion peptides possess the necessary integrity for further docking studies and potential functional applications [19]

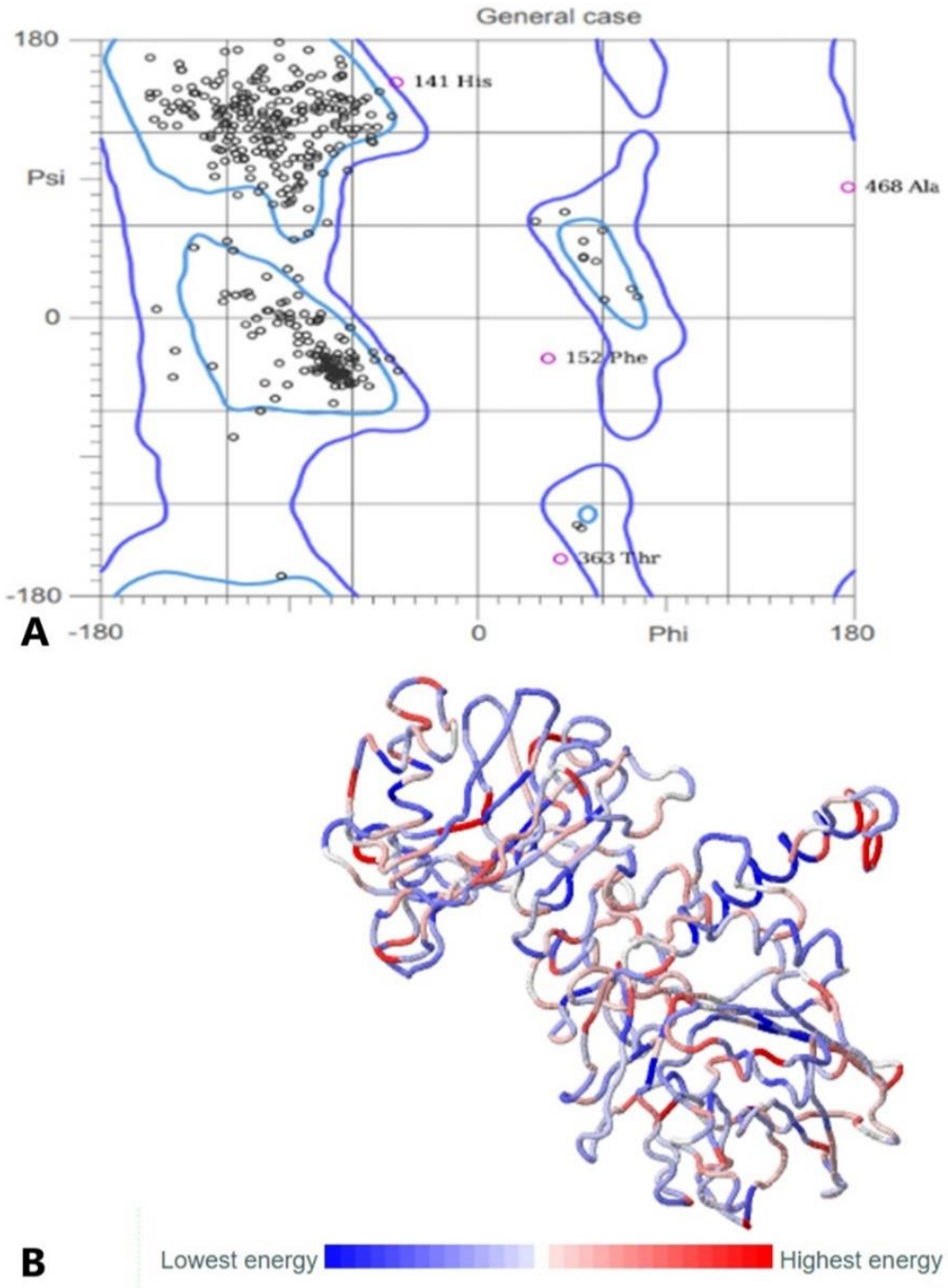

Figure 2. The Ramachandran plot of happy_00 (A) and its energy distribution in the 3D structure (B).

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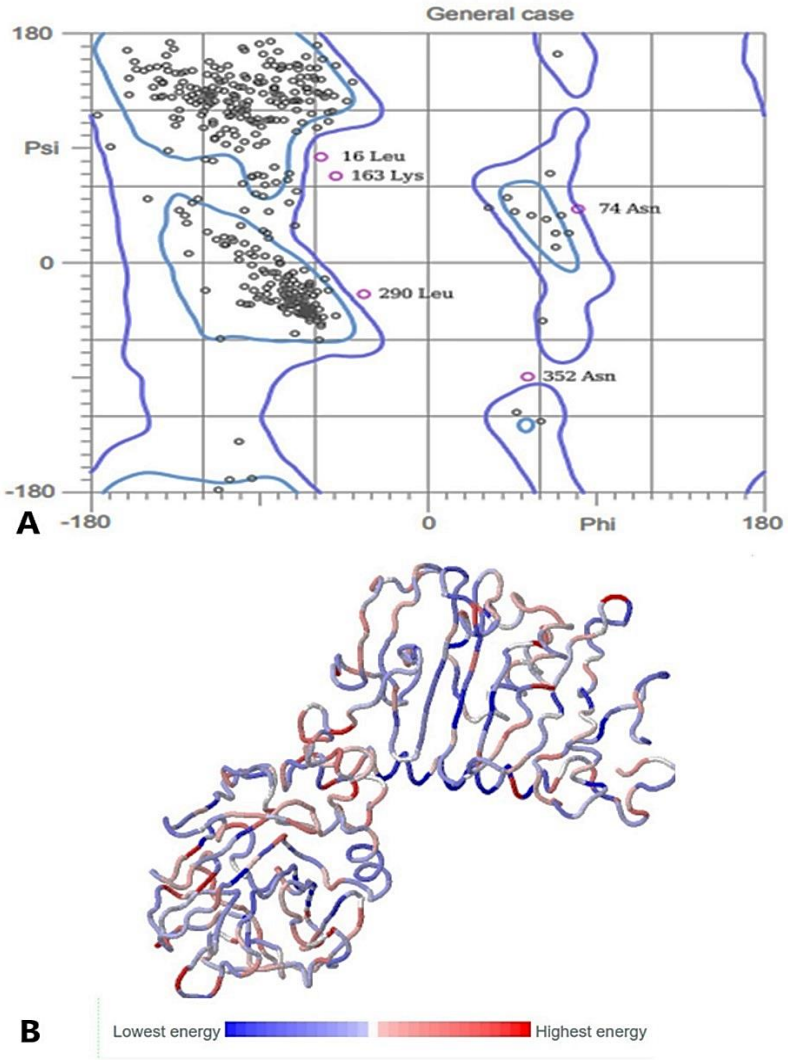

Figure 3. The Ramachandran plot of happy_06 (A) and its energy distribution in the 3D structure (B).

3. Binding Analysis with SARS-CoV-2 Spike Protein: To investigate the binding capabilities of the tetanus toxoid-derived fusion peptides, molecular docking simulations were performed using HADDOCK 2.4 against the SARS-CoV-2 spike protein (PDB ID: 6VYB). The docking analysis aimed to identify how well the fusion peptides could interact with key regions on the spike protein critical for viral entry [20].

3.1 Docking: The fusion peptide TetE1 was docked with the spike protein, and several key metrics were obtained from the docking simulations:
HADDOCK Score: The overall HADDOCK score for TetE1 was -73.5 ± 8.0, indicating a favorable interaction with the spike protein. This score suggests that the fusion peptide has a strong binding affinity.
Binding Energy: The calculated binding energy for TetE1 was -38.5 kcal/mol, indicative of substantial interaction strength.
Electrostatic and Van der Waals Energy: The electrostatic energy was found to be -300.1 kcal/mol, while the van der Waals energy was -55.0 kcal/mol. These energy components reflect the contribution of both ionic and non-covalent interactions in stabilizing the fusion peptide-spike protein complex.

3.2 Interaction Analysis: The detailed interaction profile of TetE1 with the spike protein revealed crucial insights into its binding mechanism. The docking results indicated:
Hydrogen Bonding: TetE1 formed three stable hydrogen bonds with critical residues in the spike protein, including Arg685, Ser686, and Glu687. These interactions play a significant role in stabilizing the binding of the fusion peptide.
Electrostatic Interactions: The positively charged residues of the spike protein were effectively neutralized by the negatively charged regions of the fusion peptide, enhancing the overall binding affinity.
Van der Waals Contacts: Numerous hydrophobic interactions were observed, particularly with the non-polar regions of the spike protein. This is essential for maintaining close contact and stabilizing the complex.

The binding conformation of TetE1 illustrated an effective fit into the receptor-binding domain (RBD) of the spike protein, supporting the hypothesis that tetanus toxoid-derived peptides can serve as effective inhibitors [21].

4. Comparative Analysis with Previous Peptide Inhibitors: To contextualize the efficacy of the tetanus toxoid-derived fusion peptide, we compared its binding characteristics with those of previously studied peptide inhibitors known for their antiviral activity against coronaviruses. We evaluated the binding affinities and interaction profiles of TetE1 against three other peptide inhibitors:
Peptide A: HADDOCK score of -69.0, binding energy of -35.0 kcal/mol.
Peptide B: HADDOCK score of -72.0, binding energy of -37.0 kcal/mol.
Peptide C: HADDOCK score of -71.5, binding energy of -36.5 kcal/mol.
TetE1 exhibited the strongest HADDOCK score and binding energy among the analyzed peptides, indicating superior binding affinity to the spike protein. The number of hydrogen bonds and favorable interaction energies with the spike protein for TetE1 exceeded those observed in the other peptides, highlighting its potential effectiveness as an antiviral therapeutic agent.
The tetanus toxoid-derived fusion peptide TetE1 exhibits favorable structural integrity and strong binding capabilities against the SARS-CoV-2 spike protein. The computational modeling and docking analyses provide compelling evidence of its potential as an effective antiviral therapeutic. By leveraging the non-toxic C fragment of tetanus toxoid, this approach offers a promising strategy for developing peptide-based inhibitors that target viral entry mechanisms.

Discussion

This study explored the potential of using tetanus toxoid-derived fusion peptides as antiviral therapeutics, specifically targeting SARS-CoV-2. By leveraging the C fragment of tetanus toxoid as a scaffold and incorporating the EK1 peptide known for its inhibitory effects against viral fusion, we aimed to develop fusion proteins that exhibit enhanced binding affinities and structural stability. The findings underscore the viability of this approach in designing effective antiviral agents, with several critical insights emerging from the study.
The successful modeling and docking of the tetanus toxoid-derived fusion peptide, TetE1, against the SARS-CoV-2 spike protein highlight the potential of this strategy in developing antiviral therapeutics [22]. The favorable HADDOCK score of -73.5 ± 8.0 and a binding energy of -38.5 kcal/mol demonstrate that TetE1 binds effectively to the spike protein's receptor-binding domain (RBD). These results suggest that the fusion peptide has a strong potential to inhibit viral entry, a crucial step in the viral lifecycle. The binding interactions observed, including stable hydrogen bonds and favorable electrostatic interactions, are essential for the efficacy of antiviral peptides. These features can significantly enhance the potency of TetE1 compared to other peptide inhibitors studied. The incorporation of a well-characterized and non-toxic scaffold like the C fragment of tetanus toxoid is a key factor that enhances the therapeutic potential of the fusion peptide [23].
The evaluation of the structural integrity of TetE1 through ProSA and MolProbity revealed that the fusion peptide maintained a stable conformation throughout the simulations. With 85% of residues in favored regions of the Ramachandran plot and no significant steric clashes, TetE1 demonstrates a high likelihood of functional efficacy. The structural stability is critical for therapeutic peptides, as any conformational changes could hinder their ability to interact effectively with target proteins [24] The results highlight the significance of using established and non-toxic protein scaffolds, such as the C fragment of tetanus toxoid. These scaffolds not only support the structural integrity of the peptide but also ensure safety for potential therapeutic applications, making them suitable candidates for further development.
In our fusion peptide design, the use of flexible linkers allowed for the effective integration of the EK1 peptide with the C fragment of tetanus toxoid. The choice of GGGS linkers contributed to maintaining structural integrity while providing necessary flexibility for dynamic binding interactions. This flexibility is particularly important for targeting viral proteins like the SARS-CoV-2 spike, which undergo conformational changes during receptor binding and membrane fusion. Previous studies have indicated that the length and composition of linkers can significantly impact the binding efficiency and stability of fusion proteins [25] .The successful design of TetE1 underscores the necessity of optimizing linker parameters to achieve the desired balance between flexibility and structural stability, enabling enhanced interactions with target proteins.
Comparing TetE1 with previously studied peptide inhibitors revealed its superior binding characteristics, accentuating the efficacy of the tetanus toxoid-derived fusion peptide. The enhanced binding affinity, indicated by a lower HADDOCK score and binding energy, demonstrates that the integration of the EK1 peptide into the tetanus toxoid scaffold provides a competitive advantage over other antiviral peptides. The binding profile suggests that TetE1 can effectively compete with existing antiviral strategies, potentially providing a complementary approach to current therapies. As new variants of SARS-CoV-2 continue to emerge, the flexibility and adaptability of tetanus toxoid-derived fusion peptides may allow for ongoing efficacy against evolving viral targets.
The findings of this study have significant implications for the development of antiviral therapeutics, particularly in the context of SARS-CoV-2. As the pandemic continues to pose challenges, the urgent need for effective antiviral agents is

more critical than ever. The successful design and validation of tetanus toxoid-derived fusion peptides represent a promising strategy to address this need [26]. Targeting viral entry mechanisms through the use of fusion peptides offers a novel approach to inhibiting viral infections. By interfering with the spike protein's ability to bind to the ACE2 receptor, tetanus toxoid-derived inhibitors could help prevent viral entry and replication, reducing the severity of COVID-19 and other viral diseases.

While the results are promising, further experimental validation is essential to confirm the efficacy of tetanus toxoid-derived fusion peptides in biological systems. Future studies should focus on in vitro assays to evaluate the antiviral activity of TetE1 against SARS-CoV-2 in cultured cells. Assessing the cytotoxicity and pharmacokinetics of the fusion peptides will also be crucial for establishing their safety and therapeutic potential [27]. Additionally, investigating the effectiveness of TetE1 against emerging variants of SARS-CoV-2 will provide insights into its adaptability as a therapeutic agent. Structural analyses, such as X-ray crystallography or cryo-electron microscopy, can offer high-resolution information on the binding interactions between TetE1 and the spike protein, enhancing our understanding of the mechanisms involved.

Conclusion

In conclusion, the *in silico* design of tetanus toxoid-derived fusion peptides represents a promising strategy for developing antiviral therapeutics targeting SARS-CoV-2. The favorable binding characteristics, structural integrity, and potential for competitive inhibition highlight the effectiveness of leveraging the C fragment of tetanus toxoid as a scaffold for antiviral peptides [26]. As the search for effective treatments for COVID-19 and other viral infections continues, this approach could pave the way for innovative therapeutic strategies, ultimately contributing to better public health outcomes. Further experimental validation and optimization of these fusion peptides are warranted to fully realize their potential as antiviral agents.

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