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Computational design of ultrashort peptide inhibitors for the RBD of the SARS-CoV-2 S-protein

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Abstract

Targeting the interaction between severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) receptor-binding domain (RBD) and angiotensin-converting enzyme 2 (ACE2) is believed to be an effective strategy for drug design to inhibit the infection of SARS-CoV-2. Herein, several ultrashort peptidase inhibitors against the RBD-ACE2 interaction were obtained by a computer-aided approach based on the RBD-binding residues on the protease domain (PD) of ACE2. The designed peptides were tested on a model coronavirus GX_P2V, which has 92.2 and 86% amino acid identity to the SARS-CoV-2 spike protein and RBD, respectively. Molecular dynamics simulations and binding free energy analysis predicted a potential binding pocket on the RBD of the spike protein, and this was confirmed by the specifically designed peptides SI5alpha and SI5alpha-b. They have only seven residues, showing potent antiviral activity and low cytotoxicity. Enzyme-linked immunosorbent assay result also confirmed their inhibitory ability against the RBD-ACE2 interaction. The ultrashort peptides are promising precursor molecules for the drug development of SARS-CoV-2, and the novel binding pocket on the RBD may be helpful for the design of RBD inhibitors or antibodies against SARS-CoV-2.

Results and Discussion

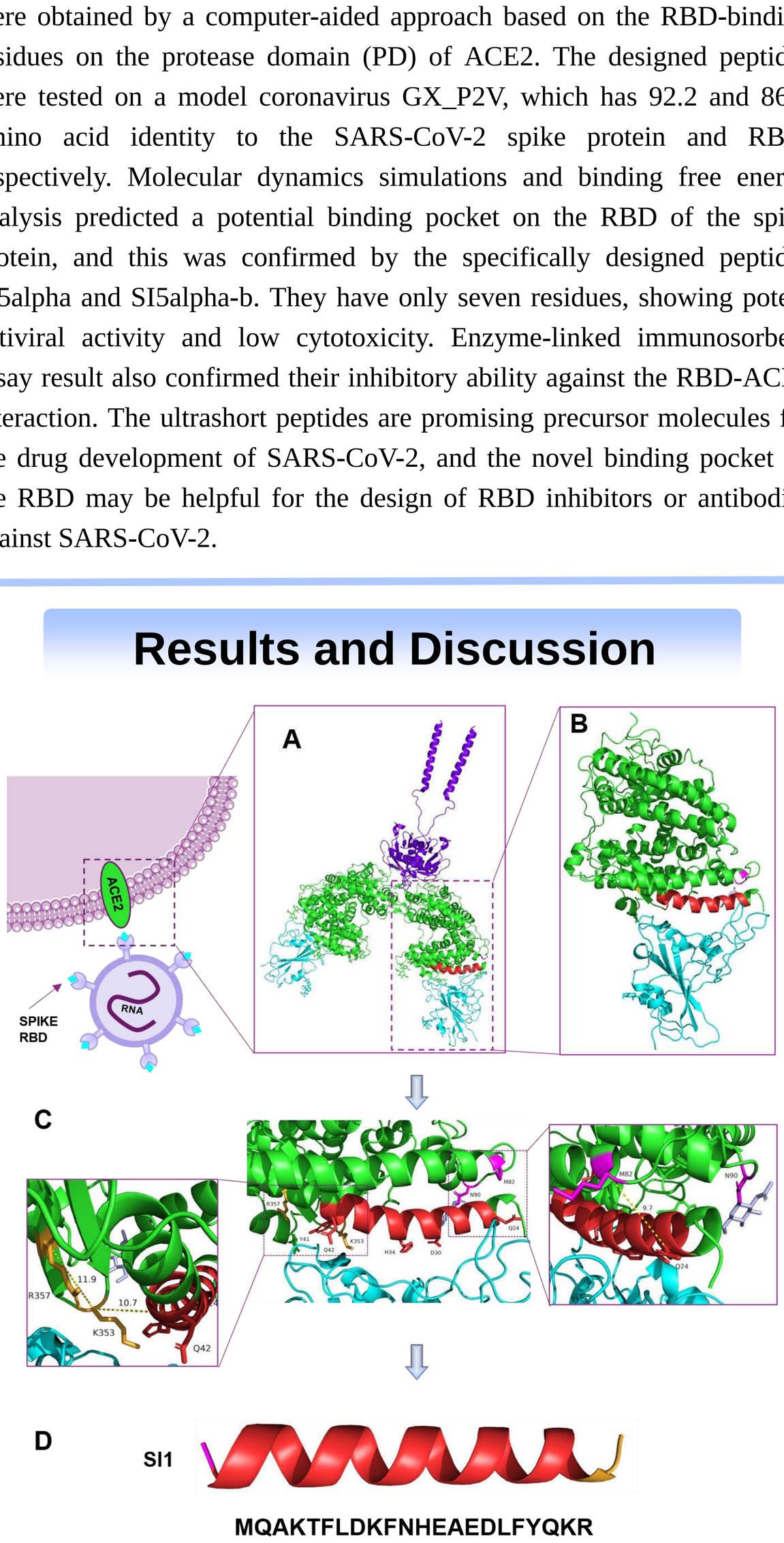

Figure 1. Design of RBD inhibitor SI1. (A) The structure of ACE2–RBD complex (split from RBD–ACE2–B0AT1 complex, PDB ID: 6M17). The PD is colored in green, the C-terminal collectrin-like domain (CLD) is colored in purple and the RBD of SARS-CoV-2 is colored in cyan. (B) The structure of the PD–RBD complex that separated from the ACE2–RBD complex. (C) The key residues on ACE2 that bound by the RBD, which were derived from the crystal structure analysis7. The distance between residues was measured by PyMol. (D) Designed sequence of the starter peptide inhibitor SI1. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; D, Asp;E, Glu; F, Phe; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; S, Ser; T, Thr; V, Val; W, Trp and Y, Tyr.

The first peptide inhibitor was designed in a structure-based manner, by analyzing the RBD-ACE2 complex (PDB ID: 6M17), and extract the critical residues in ACE2. Then the molecular docking and simulation technologies were applied for the sequence evolution, and several ultrashort peptides were obtained, namely SI4, SI4-b, SI5α and SI5α-b.

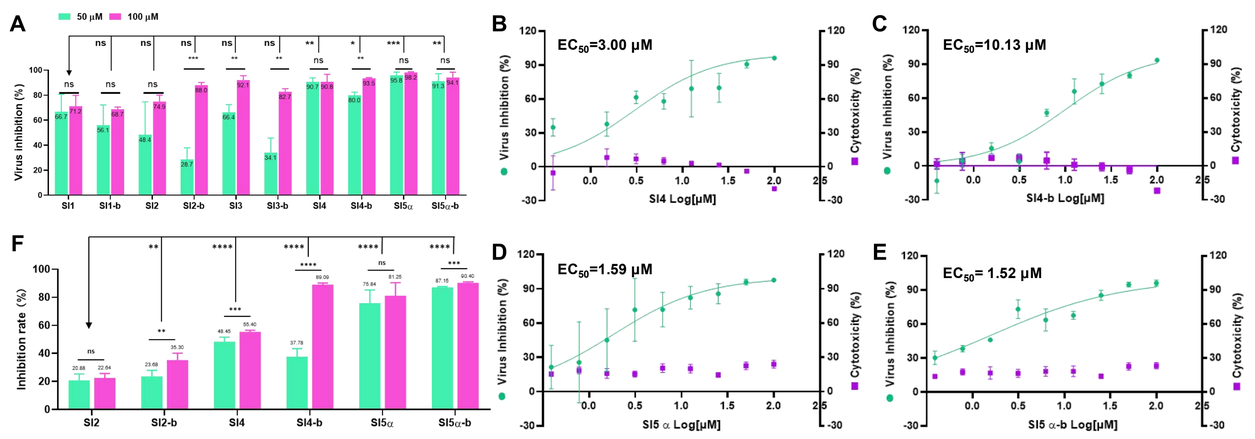

Figure 2. Antiviral activity and cytotoxicity of the peptide inhibitors. (A) Antiviral tests of the peptide inhibitors at concentrations of 100 and 50 μM using the model coronavirus GX_P2V. (B–E) EC50 and cytotoxicity of the high potency peptide inhibitors on GX_P2V virus and Vero E6 cells were determined. (B) SI4; (C) SI4-b; (D) SI5α and (E) SI5α-b. (F) ELISA for the determination on the ability of the peptides to inhibit the binding between RBD and ACE2. The data are shown as mean ± SD. Two-way ANOVA was used for the comparisons between the shorter peptides with SI1. Student's t-test was used to compare between two concentrations of a peptide (∗P < 0.05, ∗∗P < 0.01, ∗∗∗P < 0.001, ∗∗∗∗P < 0.0001).

The antiviral activity of the designed peptides was firstly tested on GX_P2V, a model coronavirus of SARS-CoV-2. The ultrashort peptides showed high antiviral activity, with the EC50 = 3.00, 1.59, and 1.52 μM for SI4, SI5α and SI5α-b (Figure 2 A-E). The ability of the peptides to inhibit the RBD-ACE2 binding was confirmed by a competitive ELISA kit (Figure 2 F). Moreover, MD simulation and binding energy calculation indicated an important binding pocket that located between E484 and Y505 on the RBD, which is critical for the activity of RBD inhibitors.

Conclusion

In conclusion, several ultra-short peptide inhibitors against the interaction between the RBD and ACE2 were designed by a computer-aided method, and confirmed to be potent in antiviral test using a model coronavirus of SARS-CoV-2 and an ELISA kit. MD simulation and binding energy calculation on the interaction between the peptides and the RBD indicated an important binding pocket on the RBD, which may be helpful for the design of RBD inhibitors or antibodies in future.

Acknowledgements

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Reference

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