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Inhibitory Effects of *ab initio* Antiviral Peptides Efficiently Designed Based on the APD3 Database

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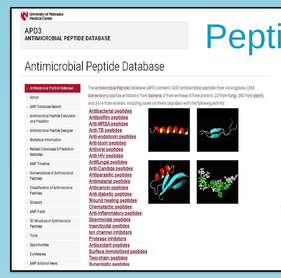
Introduction

Natural antimicrobial peptides (AMPs) aid in many organisms innate immune defense against pathogens. Engineering new therapeutics from natural AMP templates may provide an effective treatment to viral infections such as SARS CoV-2 and Ebola, and drug-resistant bacteria. One way to design antimicrobial peptides is the database filtering technology (DFT). The DFT is an *ab initio* design that selects the most probable parameters for an AMP by statistical analysis in the antimicrobial peptide database (APD3)[1]. To our knowledge, however, the DFT design has never been used to develop an antiviral peptide. The improved DFT proposed provides a faster and simpler design for peptides.

Peptide Design Tool



The APD3 works on classifying natural AMPs so that statistical analysis can occur. The APD3 contains 3273 natural AMPs (193 antiviral peptides) from 3 domains and 6 kingdoms and provides a multitude of search functions and tools to analyze the peptides[2].

Methods

Improved Database Filtering Technology (iDFT)

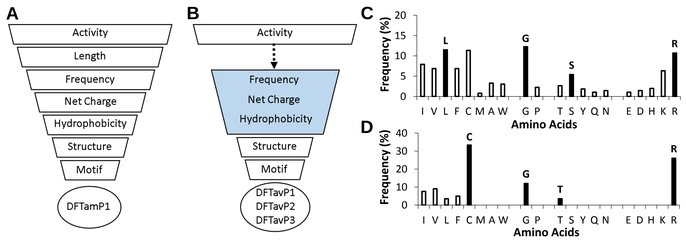


Figure 1: The DFT design (A) compared to the iDFT design (B), the amino acid frequency for 11- 20 residue antiviral peptides (C), and amino acid frequency of 19 Θ defensins in the APD3 (D).

Method for Database Designed and Improved Peptide (DDIP1)

DDIP1 was designed using the database-aided design[3]. It was modified further through a database guided design to improve stability and increase antiviral potency[4].

In Vitro Viral Assay

A prevention and treatment antiviral assay was conducted for DDIP1, iDFT antiviral peptide 3 (DFTavP3), and LL-37 using Ebola pseudoviral system and SARS-CoV-2. LL-37 acted as a positive control.

Peptide Sequences

Peptide	Sequence
DFTavP1	RLLRGLLSGLLR
DFTavP2	RLLRGLLSGLLRRLLS
DFTavP3	RLLRGLLSGLLRRLLSSGLL
DFTavP4	RVVRGVVSGVVRRVVS
DDIP1	GLRCRLGRLLRRLGRCLLR

Cytotoxicity and Stability

Table 1:Cytotoxicity in three different cell lines for DFTavP3 and DDIP1.

Designed Peptide	Human HC_{50}[a] (μM)	Mouse HC_{50}[b] (μM)	TC_{50}[c] (μM)
DFTavP3	<12.5	<10	25
DDIP1	>160	>160	>100

[a]HC50 is the 50% hemolytic concentration in human red blood cells. [b]The animal HC50 was conducted on mouse red blood cells. [c]TC50 is the 50% cytotoxic concentration in a HaCa cell line.

Table 2: Simultaneous antimicrobial and stability assay of designed peptides in trypsin and chymotrypsin solutions at a 40 to 1 molar ratio.

Designed Peptide	Trypsin	Chymotrypsin
DFTavP3	Cleaved	Cleaved
DDIP1	Stable	Stable
17BIphe2	Cleaved	Stable

Viral Prevention

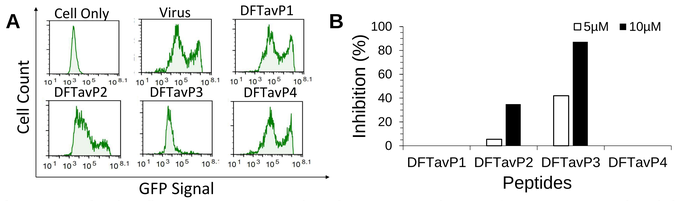


Figure 2: Infection flow cytometer graphs of DFTavP series at 10 μM concentration (A). Inhibition of DFTavP series at 5μM and 10μM concentrations (B).

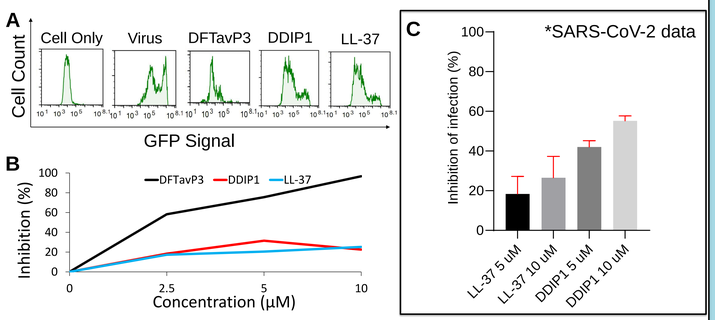


Figure 3: Infection flow cytometer graphs of DFTavP3, DDIP1, LL-37 peptides at 5 μM concentration (A). Inhibition of DFTavP3, DDIP1, LL-37 peptides at 2.5, 5 and 10μM concentrations (B). Prevention of SARS-CoV-2 infection (C).

Viral Treatment

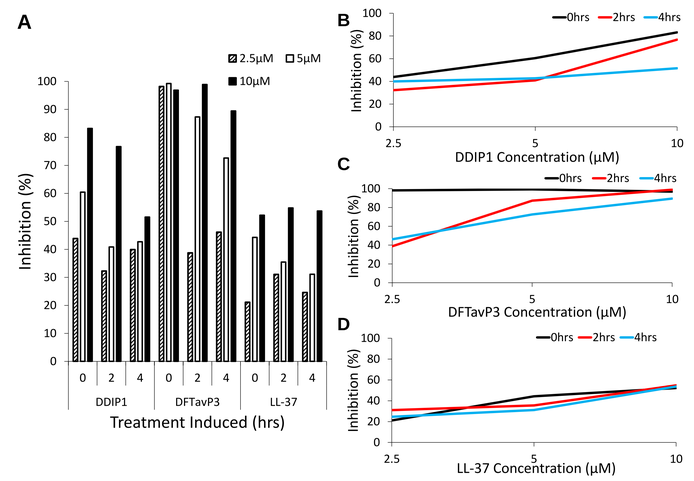


Figure 4: Inhibition of Ebola pseudo-virus after time dependent treatment for all peptides (A), for DDIP1 (B), for DFTavP3 (C), and for LL-37(D).

Conclusion

- ❑The improved DFT resulted in a faster and simpler design method for designing antimicrobial peptides.
- ❑DFTavP3 resulted in viral inhibition but was highly cytotoxic and unstable. DDIP1 was also found to be inhibitory against viral infections including SARS-CoV-2 and was stable and noncytotoxic.
- ❑DFTavP series viral inhibition results demonstrated lengths closer to the most probable length of 20-30 amino acids had better inhibition.

References

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