KR-12, the minimal antibacterial peptide of human cathelicidin LL-37: Discovery, engineering and applications

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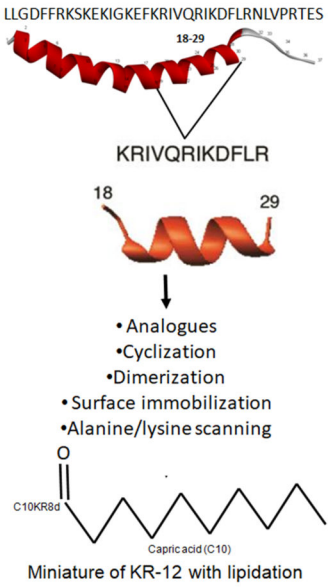
Abstract

This poster will summarize the recent research results on KR-12, a 12-residue cationic antimicrobial peptide derived from human cathelicidin LL-37. KR-12 has been shown to have a selective toxic effect on bacteria but not human cells. The positive charges of KR-12 allow it to interact with negatively charged bacterial membranes. Moreover, KR-12 has been found to possess anti-inflammatory properties beneficial for developing novel wound dressings. KR-12 has been shown to promote the osteogenic differentiation of human bone marrow stem cells by stimulating BMP/SMAD signaling. In addition, different forms of KR-12 have been designed, including conjugated hybrids, lipidated analogs, and cyclic peptides. Finally, KR-12 has been immobilized on various surfaces to prevent biofilm formation. In conclusion, KR-12 has shown promise for multiple applications in medicine, food, animal husbandry, and aquaculture.

Introduction

LL-37 is a human defense peptide with multiple functions, including antimicrobial and anticancer activities [Li et al., 2006]. Through peptide library screening and structure-based design, LL-37 has been transformed into selective, stable, and potent antimicrobial compounds [Wang, 2014; Tripathi et al., 2015]. The antimicrobial activity of LL-37 is achieved through damaging effects on bacterial cytoplasmic membranes and its immunomodulatory action [Kiattiburut et al., 2018]. Other peptides derived from LL-37, such as GI-20 and GF-17, have been found to have antiviral, antibacterial, and spermicidal activities [Ridyard, 2022; Wang, 2008; Zhang et al., 2021]. During the LL-37 structure analysis, Wang G reported the identification and structural studies of the shortest fragment peptide of LL-37, KR-12, corresponding to residues 18–29 of LL-37. The selective toxicity of KR-12 makes it a promising candidate for developing novel and potent antimicrobial agents [Wang, 2008]. KR-12 has been conjugated with nanofibrillated cellulose (CNF) and has been shown to have potential applications in various forms [Blasi-Romero et al., 2023].

Engineering of KR-12 Peptides



Miniature of KR-12 with lipidation

Table 1: Modifications and Sequences of KR-12

Type of Modification	Name	Sequence	Reference
Linear	KR-12	KRIVQRIKDFLR	Wang 2008
	retro-KR-12	RLFDIKRQVRIK	Gunasekara S et al., 2020
Cyclic	cd4(Q5K,D9K)	AGGKRIVKRIKKFLRGAGGKRIVKRIKKFLRG	Gunasekara S et al., 2020
	CD4-PP	CPGGKRIVKRIKAFLRGPGGKRIVKRIKAFLR	White et al., 2022
	CD4-CC	CFLRGAGGKRIVCRIKAFLRGAGGKRIVKRIK	Mohammad T et al., 2023
	CD4-CCPP	CFLRGPGGKRIVCRIKAFLRGPGGKRIVKRIK	
Analogue	KR-12-a6	LRIVKLILKWLR	Lanqing Fu et al., 2020
	KR-12-a5	KRIVKLILKWLR	Hui Li et al., 2019
Amino acid scanning	Alanine ScanKR-12	KRIVQRIKDFLR AAAAAAAA*AAA	Gunasekara S et al., 2008
	Lysine Scan KR-12	KRIVQRIKDFLR -KKKK*KK-K*KKK	
Surface immobilization	Egg Shell Membrane-CKR-12	ESM-CysKRIVKRIKKWLR	Menglong Liu et al., 2019
	Ti-KR-12 Covalent immobilization onto titanium surface	Ti- KRIVQRIKDFLR	Bin'en et al., 2016
Lipidation and D form	C10KR8d Miniature KR-12 with Capric acid conjugated	C10-KRIWQRIK	Jayaram L N et al., 2021

Therapeutic potential of KR-12

• Many peptide derivatives have been produced both in Wang lab (unpublished) and in other labs. In addition to antimicrobial and antibiofilm activities, KR-12-a6 and KR-12-a5 analogues promote the osteogenic differentiation of human bone marrow mesenchymal stem cells via BMP/SMAD signaling, low cytotoxicity, LPS-binding activity, and inhibition of TNF-α production.

• KR-12 has also been cyclized to increase peptide stability (Gunasekera, S., et al., 2020, *White, J.K., et al., 2022*).

• Conjugates of KR-12 segments with fatty acid with varying chain lengths have also been arrayed. The D-form C10-KR8d peptide effectively prevents MRSA biofilm formation in a mouse model with catheter-associated infections, presenting new therapeutic possibilities.

• KR-12 has been immobilized on the titanium surface to prevent the colonization of pathogens.

Conclusion

KR-12 has been designed into different molecular forms. They have demonstrated antimicrobial activity against pathogenic bacteria, fungi, and viruses. In Osteogenesis, it possesses anti-inflammatory properties. Overall, antimicrobial peptides, including KR-12, offer potential therapeutic applications. These results will be reviewed in a manuscript to be submitted.

References

1. *Wang, G. (2008). doi:10.1074/jbc.M805533200*
2. *Mishra, B., et al., (2013) doi:10.1039/C3RA42599A*
3. *Wang,G. et al., (2014) doi: 10.1021/cb500475y*
4. *Mishra, B., & Wang, G. (2017). doi:10.1080/08927014.2017.1332186*
5. *Lakshmaiah Narayana, J., et al., (2021). doi:10.1021/acsinfecdis.1c00101*
6. *White, J. K., et al., (2022). doi:10.1007/s00018-022-04440-w*
7. *Muhammad, T., et al.,(2023). doi:10.3390/biomedicines11020504*
8. *White, J. K., et al., (2023). doi:10.1021/acsinfecdis.2c00598*