

HYBRID HBD4 DEFENSIN DESIGN

Bernat Granell Sabaté (bernatgranell@gmail.com)



Universitat Autònoma de Barcelona
June 2015

INTRODUCTION

Defensins are small cationic antimicrobial peptides involved in innate immunity and highly conserved in vertebrates. In humans, defensins are classified into two subgroups (α and β) based on both unique amino acid sequences and disulfide bonds. α -defensins are mostly expressed by neutrophils and β -defensins are secreted by epithelial cells of the skin and mucosae. Despite their antimicrobial activity against bacteria, viruses, fungi and protozoa, they are modulators of the adaptive immunity. The most studied human β -defensins are HBD1, HBD2 and HBD3 but the predicted peptide sequence is available for all human β -defensins known.

STRUCTURE

The different subfamilies of defensins are thought to share a common ancestry. Human α - and β -defensins gene family is in the main locus on chromosome 8p22-23. The tertiary structures of human β -defensins are very similar despite low sequence conservation in their amino acids. Their cationic structure is composed by 3 β -strands arranged in an antiparallel sheet stabilized by 3 intramolecular disulfide bonds between 6 cysteine and flanked by an α -helix.

Human β -defensin paralogues	Peptide	Gene	Net Charge	Antimicrobial activity
DHYNYSGGGGLYSAPLPTTICRNYE-BA-KCC	HBD1	DEFB1	+5	More potent against Gram-ve
GISGDPVTCISGAIHPVPSFRYYOITRGLPOT-KCCNRP	HBD2	DEFB4	+6	More potent against Gram-ve
GIINTLQRYRYSVYSGRAVLSLPLFEEQIPIKSTK-GR-KCCSRKRP	HBD3	DEFB03	+11	Broad spectrum
KETLHLLKQGTANRSLRPFSSQKSRVIRPNTY-ARLRRNDELLN-+H	HBD4	DEFB04	+6	Strong against some Gram+ve and -ve
RALISRRMCRQEAFLTFEVLIRLAELAPPTK-GRKSK	HBD7	DEFB07	+4	Poor against both Gram+ve and -ve

Figure 1. β -defensin sequences and properties. Cysteine residues highlighted in red, basic residues in blue and conserved glycine in green.

ANTIMICROBIAL ACTIVITY

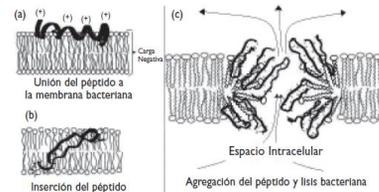


Figure 2. The attachment of peptides is initiated by electrostatic interactions with the negatively charged membranes of pathogens. The hydrophobic residues enable their internalization and pore formation until pathogen is killed by osmotic shock. High salt concentrations inhibit the attachment of β -defensins except HBD3.

AIMS

Despite HBD4 has not been isolated in vivo, this peptide exhibits the strongest antimicrobial activity but only at low ionic strength. Moreover, HBD3 has a broad spectrum activity but weaker than HBD4 and it is not inhibited at high salt concentrations. My aim is to modify the HBD4 amino acid sequence mimicking the HBD3 sequence to introduce its features in a synthetic HBD4.

DESIGN OF PEPTIDES

β -defensins are a rapidly evolving peptide family with low sequence similarity between paralogs. However, some gene regions evolve under positive selection and they are directly involved in mature peptide function.

According to my aim, I have changed some HBD4 residues subject to positive selection mimicking the HBD3 homologues sites and increasing the net charge of own peptide.

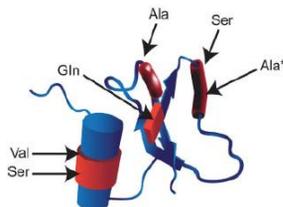


Figure 3. Sites subject to positive selection are mapped to the structure of HBD4 mature peptide. Sites subject to selection are colored red and the particular residues are named and arrowed.

Figure 4 (right site). Native HBD4 followed by designed peptides named with a roman number (I-XX), modified residues are highlighted in yellow.

PEPTIDES	SEQUENCES	NET CHARGE
HBD4	ELDRICGGYGTARCRKKCRSQEYRIGRCPNTYACCLRK	+7
HBD4-I	ELDRICRYGTARCRKKCRSQEYRIGRCPNTYACCLRK	+8
HBD4-II	ELDRICRYRTARCRKKCRSQEYRIGRCPNTYACCLRK	+9
HBD4-III	ELDRICRVRTARCRKKCRSQEYRIGRCPNTYACCLRK	+9
HBD4-IV	ELDRICRYRTARCRKKCRSQEYRIGRCPNTYACCLRK	+9
HBD4-V	ELDRICGGYGTARCRKCSRSQEYRIGRCPNTYACCLRK	+6
HBD4-VI	ELDRICGGYGTARCRKKCRSQEYRIGRCPRTYACCLRK	+8
HBD4-VII	ELDRICGGYGTARCRKKCRSQEYRIGRCPNTRACCLRK	+8
HBD4-VIII	ELDRICGGYGTARCRKKCRSQEYRIGRCPTRACCLRK	+9
HBD4-IX	ELDRICGGYGTARCRKKCRSQEYRIGRCPNTRACCLRK	+9
HBD4-X	ELDRICGGYGTARCRKKCRSQEYRIGRCPTRRACCLRK	+10
HBD4-XI	ELDRICGGYGTARCRKKCRSQEYRIGRCPTRRCCRK	+11
HBD4-XII	ELDRICRYGTARCRKKCRSQEYRIGRCPNTRCCLRK	+9
HBD4-XIII	ELDRICRYRTARCRKKCRSQEYRIGRCPNTRCCLRK	+10
HBD4-XIV	ELDRICRYRTARCRKKCRSQEYRIGRCPNTRCCLRK	+11
HBD4-XV	ELDRICRYRTARCRKKCRSQEYRIGRCPTRRCCRK	+12
HBD4-XVI	ELDRICRYRTARCRKKCRSQEYRIGRCPTRRCCRK	+13
HBD4-XVII	ELDRICRYGTARCRKKCRSQEYRIGRCPNTRCCLRK	+10
HBD4-XVIII	ELDRICRYRTARCRKKCRSQEYRIGRCPNTRCCLRK	+11
HBD4-XIX	ELDRICRYRTARCRKKCRSQEYRIGRCPTRRCCRK	+12
HBD4-XX	ELDRICRYRTARCRKKCRSQEYRIGRCPTRRCCRK	+13

EXPECTED RESULTS

If the results of antimicrobial assay are good, we can expect an improvement of own peptides functions. The first four peptides possess a α -helix-like HBD3 which is responsible for the attachment to pathogens membrane, therefore we could expect a wider spectrum activity. The second group of peptides have a higher number of basic residues which increase the net charge and allegedly their antimicrobial activity and salt concentrations resistance. This assumption is supported by the fact that HBD3 possess the highest net charge value and it is not inhibited at high salt concentrations. Finally, the last group of peptides have been designed to incorporate the previous features into one peptide.

The analysis of the structural and functional characteristics of β -defensins opens the way to the engineering of these peptides with therapeutic applications. This study could show that the rational design of these hybrid peptides is possible.

REFERENCES

Figures 1 and 3. Taylor K, Barran P.E, Dorin J.R. *Structure-activity relationships in β -defensin peptides*. Wiley InterScience, 2007.

Figure 2. Rivas-Santiago B, Sada E, Hernández-Pando R, Tsutsumi V. *Péptidos antimicrobianos en la inmunidad innata de enfermedades infecciosas*. Salud Pública Mex, 2006.