

## Human beta-defensin chimeric peptides

### *General*

Antimicrobial peptides have been identified and isolated from a wide variety of organisms, including humans, where they mostly participate in the first-line of defense against pathogens. Among them, defensins are a family of small antimicrobial peptides whose abundance in humans and other vertebrates along with their broad microbicidal activity makes them important effector molecules of neutrophils, mucosal surfaces, skin and other epithelia.

### *State of the art*

Human  $\beta$ -defensins (HBD1-HBD4) are predominantly present in various epithelial cells and tissues. HBD1 was shown to possess antimicrobial activity against Gram-positive and Gram-negative bacteria as well as to protect against adenovirus infection. HBD2 is active against many Gram-negative bacteria, including *E. coli* and *P. aeruginosa*, as well as against *C. albicans* but was found to be bacteriostatic against Gram-positive *S. aureus*. Furthermore, HBD2 is more potent against *E. coli* than HBD1. The antimicrobial activity of both HBD1 and HBD2 is dependent on the presence of NaCl. HBD3, on the other hand, is much more positively charged and possesses bactericidal activity not only against Gram-negative but also against Gram-positive bacteria including *S. aureus* and *E. faecium*. In addition, HBD3 bactericidal activity is not sensitive to physiological salt concentrations.

### *The invention*

This invention exploits the remarkable differences in the antimicrobial properties shown by HBD2 and HBD3 against Gram-positive and Gram-negative bacteria through the generation of chimeric molecules of HBD2 and HBD3 with improved activity against *E. coli* and *S. aureus* strains compared to wild-type HBD2 or HBD3 (Table 1).

The activity of chimeric peptide HBDC3 is surprisingly higher against both tested strains than the activity of HBD2, HBD3 and of all the other tested chimeras (with the exception of HBDC5's MBC against *S. aureus*). HBDC3's higher activity cannot be explained by the total net charge of the peptide alone, since it exceeds the activity of peptides with higher net charge such as HBD3 or HBDC1. The peptide with the highest net charge, HBDC5, is as effective as HBD2 and more effective than HBD3 against *E. coli* and more effective than both wild-type defensins against *S. aureus*. Chimeric defensins HBDC3 and HBDC5 are thus very interesting novel molecules, based on the natural human peptides HBD2 and HBD3, which display a combined and improved biological and therapeutic defensin activity.



Table 1: Antimicrobial activity and net charge at pH 7 of HBD2, HBD3 and HBD2/HBD3-chimeras.

Peptide	<i>E. coli</i> ATCC 35218		<i>S. aureus</i> ATCC 12600		Net Charge (pH 7)
	LD <sub>90</sub> [µg/ml]	MBC [µg/ml]	LD <sub>90</sub> [µg/ml]	MBC [µg/ml]	
<b>HBD2</b>	0,1	0,39	1,56	12,5	+ 5,8
<b>HBD3</b>	0,39	1,56	0,15	1,19	+ 10,7
<b>HBDC1</b>	0,025	0,1	0,39	1,56	+ 10,8
<b>HBDC2</b>	0,39	n.d.	>100	>100	+ 2,7
<b>HBDC3</b>	0,025	0,1	0,1	0,78	+ 8,8
<b>HBDC4</b>	1,56	6,25	0,39	3,125	+ 8,8
<b>HBDC5</b>	0,1	0,39	0,1	0,39	+ 13,8
<b>HBDC6</b>	n.d.	>100	>100	>100	+ 7,7

MBC: minimal bactericidal concentration; LD90: 90% lethal dose; n.d.: not determined.

*Advantages*

- ➔ Antimicrobial peptide based on natural peptides from human skin
- ➔ Combined and improved biological activity compared to natural defensins
- ➔ Pharmaceutical and/or cosmetic use

*Utilisation concept*

Licencing/selling of this invention is sought to a company that will produce, bring to market and distribute the described antimicrobial peptides. If desired PVA SH GmbH will further assist by arranging contact with the inventors.

*IP*

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