Available online at www.sciencedirect.com
ScienceDirect
International Journal of Medical Microbiology 298 (2008) 619–633
IJMM
www.elsevier.de/ijmm

Mode of action of human β-defensin 3 against *Staphylococcus aureus* and transcriptional analysis of responses to defensin challenge

Vera Sass[a], Ulrike Pag[a], Alessandro Tossi[b], Gabriele Bierbaum[c], Hans-Georg Sahl[a,*]

[a]*Institute of Medical Microbiology, Immunology and Parasitology - Pharmaceutical Microbiology Section, University of Bonn, Bonn, Germany*
[b]*Department of Biochemistry, Biophysics and Macromolecular Chemistry, University of Trieste, Trieste, Italy*
[c]*Institute of Medical Microbiology, Immunology and Parasitology, University of Bonn, Bonn, Germany*

Received 17 August 2007; received in revised form 12 November 2007; accepted 16 January 2008

Abstract

Human β-defensin 3 (hBD3) is a highly charged (+11) cationic defence peptide, which retains activity against staphylococci even at elevated salt concentrations. We studied the antibiotic mode of action of hBD3 against *Staphylococcus aureus* SG511, using whole-cell assays and analysing the transcriptional response to hBD3 treatment. hBD3 caused rapid killing and simultaneously blocked all biosynthetic pathways, however, significant depolarisation was not observed and permeabilisation of the membrane was incomplete. The transcriptional response pattern was in part similar to those of strongly cationic amphiphiles, e.g. in that anaerobic energy production was downregulated. Significantly, part of the staphylococcal cell wall stress stimulon were upregulated. The most prominent microbial counterstrategy appears to be based on the upregulation of ABC transporters possibly functioning in detoxification of the membrane environment; the transporter genes were highly upregulated in both, a short-term response and a long-term adaptation experiment. Knockout of the most highly induced transporter VraDE significantly enhanced hBD3 susceptibility. We propose that the antibiotic activity of hBD3 is based on interference with the organisation over space and time of membrane-bound multienzyme machineries such as the electron transport chain and, in particular, the cell wall biosynthesis complex rather than on formation of defined transmembrane pores.
© 2008 Elsevier GmbH. All rights reserved.

Keywords: *Staphylococcus aureus*; Human defensins; hBD3; Transcriptional analysis; VraDE

Introduction

Antimicrobial peptides (AMPs) serve a vital role in first-line host defence and can be found throughout the animal and plant kingdom (Zasloff, 2002). Although being evolutionary ancient, AMPs still represent an effective weapon against Gram-positive and Gram-negative bacteria, fungi and enveloped viruses as they kill very rapidly and do not easily lead to selection of resistant mutants. In higher organisms, these peptides also have multiple immunomodulatory functions such as induction of chemokine and cytokine production, alteration of gene expression and inhibition of proinflammatory responses to bacterial components (Bowdish et al., 2005; Hancock and Diamond, 2000). The direct antibiotic activity of AMPs is thought to be

*Corresponding author. Tel.: +49 228 73 5272; fax: +49 228 73 5267.
E-mail address: hans-georg.sahl@ukb.uni-bonn.de (H.-G. Sahl).

1438-4221/$ - see front matter © 2008 Elsevier GmbH. All rights reserved.
doi:10.1016/j.ijmm.2008.01.011

based on their cationic and amphiphilic nature, which enables these peptides to interact with negatively charged bacterial surfaces and membranes, subsequently causing membrane leakage. At the molecular level, the mechanisms of membrane interaction and disruption are best studied with model membranes, however, evidence is increasing that mere membrane impairment may not reflect completely the complex processes involved in the killing of microbes (Brogden, 2005; Harder et al., 2001; Sahl et al., 2005; Vylkova et al., 2007; Xiong et al., 2005). Some bacteria have developed mechanisms to reduce the action of AMPs such as the reduction of the net negative charge on the bacterial surface by D-alanylation of teichoic acids (Gram-positives) or incorporation of aminoarabinose in lipid A (Gram-negatives) and the active extrusion of AMPs from the cell (Peschel and Sahl, 2006).

Among the antimicrobial peptides, the defensins represent an important peptide family which consists of two groups, the α- and β-defensins, differing mainly in their disulphide bridge pattern. Human β-defensin 3 (hBD3) carries the highest number of cationic charges of all β-defensins (+11); it is produced by different kinds of epithelial cells and neutrophils, and its expression is inducible by inflammatory stimuli. The peptide displays broad-spectrum activity against many pathogens, including multiresistant *Staphylococcus aureus* (Garcia et al., 2001; Harder et al., 2001; Maisetta et al., 2006), and is a prominent effector molecule of innate immunity. However, little has been reported about the molecular killing mechanisms of hBD3. Harder et al. (2001), when they first described hBD3, observed signs of cell wall perforation in transmission electron micrographs of hBD3-treated *S. aureus* cells. This might indicate that hBD3 primarily interferes with bacterial cell wall biosynthesis rather than forming pores in the cell membrane. However, further experimental evidence was not reported. *S. aureus* is a leading cause of nosocomial as well as community-acquired infections, with a rising number of methicillin-resistant *S. aureus* (MRSA) strains, making it highly important to find alternative substances to treat *S. aureus* infections. In this study, we aim to elucidate mechanisms involved in killing of *S. aureus* by hBD3, applying whole-cell assays as well as microarray experiments to analyse the transcriptional responses of *S. aureus* to hBD3 treatment.

Materials and methods

Bacterial strains, plasmids and growth conditions

Bacterial strains and plasmids used in this study are listed in Table 1. *S. aureus* SG511 was maintained on blood agar (Becton Dickinson, Erembodegem, Belgium), *S.aureus* SG511Δ*vraE* on tryptic soy agar (TSA) containing erythromycin (25 mg/l). Physiological experiments were conducted in half-concentrated Mueller Hinton broth (MH; Oxoid, Basingstoke, United Kingdom) unless stated otherwise. Bacterial cultures used for microarray experiments were grown in B-broth containing 10% casein hydrolysate, 5% yeast extract, 0.5% K_2HPO_4, and 10 mM glucose (pH 7.3).

Table 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Characteristics	Reference or source
Staphylococcus aureus		
SG511	Met^s	Maidhof et al. (1991)
SG511Δ*vraE*	SG511 carrying an EM^r gene in *vraE*	This study
RN4220	Restriction-negative derivative of NCTC8325	Kreiswirth et al. (1983)
Staphylococcus carnosus		
TM300	Cloning host	Schleifer and Fischer (1982)
Plasmids		
pUC19Ery	Amp^r, pUC19, containing a 1.1-kb SmaI/EcoRI fragment including ermB (NCBI AF239772)	This study
pTV0mcs	mcs of pUC18, temperature sensitive, low-copy plasmid, CM^r	Guder et al. (2002)
pVS*vraE*II	pUC19Ery harbouring a HindIII/PstI fragment and a BamHI/EcoRI fragment of *vraE*	This study
pVS*vraE*-Ery	pTV0mcs harbouring the resistance cassette of pVS*vraE*II	This study

Abbreviations: Amp, ampicillin; CM, chloramphenicol; EM, erythromycin; Met, methicillin; r, resistant; s, susceptible.

hBD3 synthesis and folding

Solid-phase peptide synthesis was performed on a PE Biosystems Pioneer™ peptide synthesis system with columns thermostated to 50 °C and loaded with 2-chlorotrityl chloride resin (substitution 0.20–0.25 meq/g). A version of the anti-aggregation magic mixture (dimethylformamide/*N*-methyl pyrrolidone 3:1, 1% Triton X-100, 1 M ethylene carbonate) was used as solvent throughout. The synthesis was interrupted regularly to check the mass of the growing peptides by

Download English Version:

https://daneshyari.com/en/article/2054322

Download Persian Version:

https://daneshyari.com/article/2054322

Daneshyari.com