論　文　内　容　要　旨

Inhibitory effects of antibiofilm compound 1 against *Staphylococcus aureus* biofilms

(黄色ブドウ球菌のバイオフィルムにおける Antibiofilm compound 1 の抑制効果の研究)

Microbiology and Immunology, 60:148-159, 2016, in press.

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Inhibitory effects of Antibiofilm compound-1 on *Staphylococcus aureus* biofilm

Abstract

A novel benzamidazole molecule, identified in a small-molecule screen, known as antibiofilm compound 1 (ABC-1) was found to prevent bacterial biofilm formation in multiple bacterial pathogens, including *Staphylococcus aureus* without affecting the growth of bacteria. Here, we tested biofilm inhibiting ability of ABC-1 in various biofilm forming strains of *S. aureus*. We demonstrated ABC-1 inhibits biofilm formation in these strains at micro molar concentrations regardless of the strains being Polysaccharide Intercellular Adhesin (PIA) dependent, protein dependent or eDNA dependent. *spa* mRNA expression is significantly decreased upon 156 μM ABC-1 treatment. Also, decreased SpA expression was confirmed by SDS-PAGE and Western blot analyses. *spa* gene disruption mutants showed decreased biofilm formation but still produced more biofilm than ABC-1 treated strains implying that ABC-1 affects not only SpA but also factors other than SpA. Effect of ABC-1 on other factors, PIA production and extracellular DNA (eDNA) release were investigated. Dot blot analyses showed that ABC-1 attenuated PIA production. Quantitative DNA analyses showed decreased eDNA quantity in ABC-1 treated samples suggesting ABC-1 also affects eDNA release. Our results suggested that ABC-1 decreases the expression of SpA, release of PIA and eDNA and thus inhibits the biofilm formation in *S. aureus*.

Introduction

S. aureus biofilm matrix consists of 3 major factors: proteins, polysaccharides, and nucleic acids, which form an extracellular polymeric substance (EPS). Firstly, during the initial phases of *S. aureus* biofilm formation, numerous proteins are found to play an important role in biofilm formation. Protein A (SpA), one of the surface proteins of *S. aureus* is known for its capacity to form biofilms. Bacterial surface proteins that bind human matrix proteins are collectively called MSCRAMMs (microbial surface components recognizing adhesive matrix molecules). The fibronectin binding proteins (FnBA and FnBB) and clumping factor [and clumping factors ClfA and ClfB are MSCRAMMs found to be involved in early stages of biofilm formation. *S. aureus* sortase A (*srtA*) is a protein, located in the cell wall of Gram-positive bacteria, where it catalyzes transpeptidation reactions to form covalent linkage of surface proteins (MSCRAMMs) to pentaglycine bridges of the cell walls. Secondly, during the bacterial accumulation phase, biofilm formation is mediated by polysaccharide intercellular adhesion (PIA) synthesized by *ica* ADBC operon-coded enzymes. A number of surface proteins are known to replace PIA/ PNAG exopolysaccharide in

promoting intercellular adhesion and biofilm development [5]. Thirdly, extracellular DNA (eDNA) has been shown to be an essential matrix molecule produced by many bacterial species during biofilm development.

A benzamidazole molecule (5-methoxy-2-[(4-methylbenzyl)sulfanyl]-1H-benzimidazole), determined by Sambanthamoorthy *et.al*, from a high-throughput screen of 1,039 small molecules, and named as antibiofilm compound 1 (ABC-1), could efficiently inhibit biofilm formation by multiple bacterial pathogens, including methicillin resistant *Staphylococcus aureus* (MRSA) under both static and flow conditions [13]. Here, we tested the effect of ABC-1 inhibits the biofilm matrices of various strains of *S. aureus*.

Methods:

55 biofilm-forming clinical strains were selected from *S. aureus* collection of Department of Bacteriology, Hiroshima University. ABC-1 was synthesized in Department of Synthetic Organic Chemistry. The biofilm forming capacity was analyzed by microtiter plate assay. PIA production was detected by dot-blot using anti-PIA sera. Surface proteins were extracted by lysostaphin treatment in hypertonic condition and analyzed by SDS-PAGE. Expression of protein was analyzed by real-time PCR or by Western blotting. The high biofilm forming HP855 strain which was also sensitive towards ABC-1, was selected as wild type for the gene disruption experiments. The *spa* and *srtA* knockout mutation were constructed by homologous recombination of the target region [14]. eDNA was extracted and quantitated by quantitative PCR using 3 housekeeping genes (*fhuA*, *lysA* and *leuA*).

Results: Biofilm inhibition was observed in 55 biofilm forming MRSA and MSSA strains upon ABC-1 treatment. The dot blot analysis revealed that PIA production was reduced after the treatment of ABC-1 without affecting the transcription of *ica* genes. Real-time PCR and Western blot analysis indicated Protein A (SpA) expression was selectively downregulated among the various major surface proteins. *spa* knockout mutant showed significant but not complete decrease of biofilm production suggesting involvement of other factors. ABC-1 treatment also decreased eDNA release, which was confirmed by qPCR.

Conclusion: Our results demonstrated that ABC-1 inhibited biofilm formation of *S. aureus* through affecting multiple components including SpA, PIA, and eDNA during early phases of biofilm formation without affecting the growth of bacteria. The detailed mechanism of multiple effects on biofilm components remains to be elucidated. However, ABC-1 could be a potential candidate as a new preventive agent to inhibit biofilm formation of *S. aureus*.