Rationally Designing the Most Potent Agonists and Antagonists for the Enterococcus faecalis for Circuit: Improving Attenuation of Quorum Sensing-Dependent Pathogenicity

Dominic N. McBrayer, Crissye D. Cameron, Brooke K. Gantman, Yftah Tal-Gan
Department of Chemistry, University of Nevada, Reno, 1664 N. Virginia Street, Reno, NV, 89557, USA

Introduction

Resistance to antibiotics by bacteria remains a critical problem, particularly in cases of multi-drug resistance. Enterococcus faecalis is a common opportunistic commensal gut bacterium, and is the leading cause of clinical enterococci infections. E. faecalis has exhibited both intrinsic and acquired antibiotic resistances and has been demonstrated to be capable of sharing antibiotic resistance with other bacterial species, such as Methicillin-resistant Staphylococcus Aureus (MRSA), increasing the prevalence of multi-drug resistant bacteria. Attenuating E. faecalis infections can thus not only decrease the costs associated with the initial infection, but can also help to prevent the spread of antibiotic resistance. An alternative approach to conventional antibiotics is to attenuate the pathogenicity of an organism, aiding the patient’s immune system in clearing the infection while reducing the selective pressure for development of resistance to the treatment.

E. faecalis pathogenicity has been shown to be enhanced by activation of its quorum sensing (QS) circuit. The auto-inducing peptide (AIP) used by E. faecalis to activate this circuit is termed gelatinase autoinducing peptide (GBAP). Structure-activity relationship studies between GBAP and the fur receptor conducted by us and others have established a basic understanding of components crucial for agonist and antagonist activity. In the presented study, we have rationally designed the most potent agonistic and antagonistic peptide analogs known to date while further increasing our understanding of the structural components necessary for interaction between GBAP and the fur receptor. Our results have also suggested avenues for further improvement.

Synthetic Approaches:

Most couplings used: HATU-facilitated Fmoc-based SPPS. A) Entirely on-resin method: after attachment of protected glutamic acid via its side chain to rink amide MBHA resin, a) sequence is extended to the tail. b) selective deprotection of Ser3 side chain, on-resin ester formation, extension of remaining peptide sequence, and on-resin cyclization, c) final cleavage from the resin yields the desired peptide. B) In-solution ester cyclization using Ala-loaded Wang resin, extend the sequence to tail, d) cleave the peptide from the resin, e) cyclize in solution via ester formation for final peptide. C) In-solution amide cyclization: using Ala-loaded Wang resin, extend the sequence to tail, f) selective deprotection of Ser3, on-resin ester formation, extension of remaining peptide sequence, g) cleavage from the resin and subsequent cyclization in solution via amide formation.

Results

Effect of Addition of Z-group Demonstrates Modification Compatibility Differences:

Red shading indicates loss of potency while Green indicates the analog resulted in improved potency. (A) relative to GBAP or (B) relative to the most potent analog prior to capping the N-terminus with the Z-group.

GBAP-Based Antagonists

Sequence

IC50 (95% CI) (nM)

Z-QNSP-YbeI-FgGAW

>10,000

Ac-QNSP-YbeI-FgGAW

>10,000

QNSP-YbeI-FgGW

139 [61.8 – 213]**

[Z-QNSP-YbeI-FgGW]

227 [172 – 295]**; 38.7 [26.8 – 55.9]**

[PyGlu]-QNSP-YbeI-FgGW

295 [232 – 375]**

Ac-QNSP-YbeI-FgGW

468 [25 – 916]**

Ac-QNSP-YbeI-FgGW

872 [20 – 1463]**

QNSP-YbeI-FgGW

438 [225 – 853]**

Designed Inhibitors

Z-qNSP-YbeI-FgGW

488 [183 – 826]**

Z-CA-NSP-YbeI-FgGW

>10,000

qNSP-YbeI-FgGW

967 [729 – 1281]

QnsA-SbI-FgGW

>10,000

QnsA-SbI-FgGW

>10,000

QnsA-CAb-FgGW

>10,000

GBAP Competitor Conc.: (150 nM) *5 A

Most Potent Inhibitor Attenuates Biofilm Formation:

The wild type biofilm production is compared with a deletion mutant that cannot quorate. Cells were treated with QNSP-YbeI-FgGW: at a concentration that is 5-fold its IC50, 50 nM of exogenous GBAP was used as a competitor. All results are normalized relative to the untreated wild type biofilm production.

Conclusions

• Rational design proved effective for development of more potent agonists, although several modifications were found to be incompatible with each other.
• The modifications that improved agonist activity were unable to work with the modifications required to produce an antagonist.
• This study has identified the most potent agonists and antagonists for the E. faecalis for QS circuit known to date.
• The peptides developed are potent enough to allow more in depth bioactivity studies to be conducted – such as being tested in vivo model organisms.

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