Invited Review

Quorum Sensing Regulation of Biofilm Formation by Periodontal Pathogens

Bong-Kyu Choi[*]

Department of Oral Microbiology and Immunology, School of Dentistry, Seoul National University, Seoul 03080, Republic of Korea

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Quorum sensing (QS) is a cell density-dependent communication mechanism between bacteria through small signaling molecules. When the number of QS signaling molecules reaches a threshold, they are transported back into the cells or recognized by membrane-bound receptors, triggering gene expression which affects various phenotypes including bioluminescence, virulence, adhesion, and biofilm formation. These phenotypes are beneficial for bacterial survival in harsh environments. This review summarizes the application of QS inhibitors for control of biofilm formation and virulence expression of periodontal pathogens.

Quorum sensing

Quorum sensing (QS) is mediated by a cell-to-cell signaling system composed of small signaling molecules secreted by bacteria. It is dependent on cell density and was first discovered in *Vibrio fischeri*, a Gram-negative bacterium [1,2]. Bacteria secrete signaling molecules that, at an extracellular threshold level, reenter the cells or are sensed by the membrane-bound receptors, resulting in signal transduction to induce a variety of genes for virulence factors, bioluminescence, and biofilm formation [3]. There are several types of QS molecules. Acyl homoserine lactones are produced from Gram-negative bacteria and oligopeptides are produced from Gram-positive bacteria. Autoinducer-2 (AI-2) can be produced by both Gram-negative and Gram-positive bacteria [3]. Quinolones, hydroxyl ketones, bradyoxetin, and AI-3 have been identified in several specific bacterial species as QS signaling molecules [4-7]. QS can be a target to combat bacterial virulence. Therefore, QS inhibition has been suggested as an attractive alternative for antimicrobial strategies [8].

AI-2-dependent QS in periodontal pathogens

Periodontitis is initiated by bacteria in subgingival biofilms composed of mostly Gram-negative anaerobes. AI-2 is involved in universal intergeneric signaling and plays an important role in biofilm formation involving multiple bacterial species, such as dental biofilms [9]. A high level of AI-2 was detected in culture supernatants of *Fusobacterium nucleatum* (*F. nucleatum*), *Porphyromonas gingivalis* (*P. gingivalis*), and *Prevotella intermedia* (*P. intermedia*) [10,11]. In *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*), the AI-2 level was maximal in the mid-exponential phase and decreased significantly at the late log phase [12]. AI-2-dependent QS was

*Correspondence to: Bong-Kyu Choi, Department of Oral Microbiology and Immunology, School of Dentistry, Seoul National University, 101 Daehak-ro, Jongno-gu, Seoul 03080, Republic of Korea
Tel: +82-2-740-8640, Fax: +82-2-743-0311
E-mail: bongchoi@snu.ac.kr
ORCID : 0000-0003-3743-7209

observed in mutualistic dual species biofilm formation between *Streptococcus oralis* (*S. oralis*) and *Actinomyces naeslundii*, oral commensals [13]. This dual-species biofilm formation was dependent on the concentration of AI-2. Optimal concentration was 100-fold lower than that required to induce bioluminescence from *Vibrio harveyi*(*V. harveyi*), suggesting that the bacteria can respond to different concentrations of AI-2. Oral commensals secrete and respond to very low AI-2 concentrations (10 - 100 pM), whereas periodontal pathogens secrete and respond to high concentrations of AI-2 (1-10 nM) [9]. In developing subgingival biofilm, accumulation of AI-2 enhances the communication among transition bacterial species including *F. nucleatum*, leading to biofilm growth of periodontal pathogens. *F. nucleatum* plays a critical role in the development of subgingival biofilm by connecting early colonizing commensals and late pathogenic colonizers including *P. gingivalis*, *Treponema denticola* (*T. denticola*), and *Tannerella forsythia* (*T. forsythia*) [9]. Although these periodontal pathogens are Gram-negative bacteria, they do not produce acyl homoserine lactones.

AI-2 is formed from spontaneous cyclization of 4,5-dihydroxy-2,3-pentanedione, which is the product of the catabolism of S-adenosylhomocysteine with the LuxS enzyme. This type of QS was first discovered in the marine bacterium *V. harveyi* [14]. Even in bacteria that do not produce AI-2 synthase, AI-2 signal transduction can occur, suggesting both intergenic and intragenic signaling. A *luxS* homolog gene was identified in *P. gingivalis* [10,15], *A. actinomycetemcomitans* [12], *Campylobacter jejuni* [16], *Eikenella corrodens* [17], and oral streptococci including *Streptococcus gordonii* (*S. gordonii*) [18] and *Streptococcus mutans* [19,20].

AI-2 is recognized by the two-component signal transduction system [21]. Three types of AI-2 receptors have been identified: LuxP in *V. harveyi* [14,22], LsrB in *Salmonella typhimurium* and *A. actinomycetemcomitans* [23-25], and RbsB in *A. actinomycetemcomitans* [26]. The periplasmic proteins LsrB and RbsB in *A. actinomycetemcomitans* are similar to LuxP [24,25]. RbsB exhibited a higher affinity to AI-2 than did LsrB [24,26]. In *A. actinomycetemcomitans*, QseBC homolog genes have been identified [27] and QseBC genes are known to be involved in a two-component system in *E. coli* to regulate biofilm formation [28]. In *A. actinomycetemcomitans*, AI-2 induced the QseBC two-component system and inactivation of QseC, a sensor histidine kinase, resulting in reduced biofilm formation and significantly less bone resorption in a mouse model compared to the wild type [27]. QseC resides

downstream of LsrB and RbsB, AI-2 receptor proteins. Recently, D-galactose binding protein that exhibited high sequence similarity with RbsB has been identified in *F. nucleatum* as a putative AI-2 receptor [29]. Since *F. nucleatum* can coaggregate with both early and late colonizers, its AI-2 plays a critical role in the pathogenic biofilm development. Semi-purified AI-2 of *F. nucleatum* induced the biofilm growth of single and dual species and coaggregation between *F. nucleatum* and each species of the 'red complex' composed of *P. gingivalis*, *T. denticola*, and *T. forsythia* [11]. It induced gene expression of the adhesion molecules of the bacteria: *fadA* of *F. nucleatum*, gingipain *rgpA* of *P. gingivalis*, *msp* of *T. denticola*, and *bspA* of *T. forsythia* [11]. AI-2 of *A. actinomycetemcomitans* induced leukotoxin and iron transport protein AfuA expression [12]. LuxS- and autoinducer-2 receptor deficient strains of *A. actinomycetemcomitans* formed a mature biofilm with significantly lower total biomass and biofilm depth compared with the wild-type strain [24,25]. Complementation experiments showed that *A. actinomycetemcomitans* AI-2 was able to complement the *luxS* mutation of *P. gingivalis* by increasing *uvrB* expression and blocking *hasF* expression. *P. gingivalis uvrB* and *hasF* have been demonstrated to be differently regulated by *P. gingivalis* LuxS system [12]. LuxS inactivation in *P. gingivalis* led to upregulation of a hemin acquisition protein and arginine-specific protease, whereas it resulted in reduced expression of a hemin-regulated protein and an excinuclease [15]. A *P. gingivalis luxS* mutant produced 45% less Rgp and 30% Kgp activity relative to the wild type [30].

QS inhibitors for control of biofilm formation of periodontal pathogens

QS inhibition can be a way to prevent bacterial biofilm formation and virulence expression without affecting bacterial growth or death. This inhibition is accomplished by disruption of AI synthase, inactivation of QS signaling molecules, and antagonization of QS receptors [31-34]. Since AI-2 plays a critical role in biofilm development of periodontal pathogens and virulence expression, AI-2 antagonists have the potential to prevent periodontitis. A furanone compound, (5Z)-4-bromo-5-(bromomethylene)-2(5H)-furanone and D-ribose exhibited an inhibitory effect on biofilm formation, coaggregation, and the expression of the adhesion molecules of *F. nucleatum, P. gingivalis, T. denticola*, and *T. forsythia* mediated by *F.*

nucleatum AI-2 [11]. AI-2 and D-ribose bind to the same site of RbsB [26], suggesting that D-ribose is a QSI inhibitor. D-Ribose reduced biofilm growth of *A. actinomycetemcomitans* [25]. *F. nucleatum* AI-2 enhanced biofilm growth of *S. gordonii* and attachment of *F. nucleatum* on preformed biofilms of *S. gordonii* [35]. However, it reduced biofilm growth of *S. oralis* and attachment of *F. nucleatum* on preformed biofilms of *S. oralis*. These results indicate that early colonizing streptococci may affect the accumulation of *F. nucleatum* to accelerate the binding of periodontal pathogens. D-Galactose has been shown to inhibit biofilm formation of periodontal pathogens. D-Galactose inhibited *F. nucleatum* AI-2 activity and biofilm growth of *F. nucleatum*, *P. gingivalis*, and *T. forsythia* induced by *F. nucleatum* AI-2 [29]. New synthetic compounds bicyclic brominated furanones, 3-(dibromomethylene)isobenzofuran-1 (3H)-one derivatives, inhibited *F. nucleatum* AI-2 activity and biofilm formation of *F. nucleatum*, *P. gingivalis*, and *T. forsythia* induced by *F. nucleatum* AI-2 without affecting bacterial growth [36]. These bicyclic brominated furanones significantly decreased biomass and depth of biofilms of the bacteria at concentrations between 0.002 and 2 μM. The compounds did not affect the cell viability of a human monocytic cell line THP-1 cells, human gingival fibroblasts, or human keratinocyte cell line HOK-16B cells. Moreover, it did not induce an inflammatory response in these cells. In vivo experiments using mice showed that treatment with the furanone compound and D-ribose significantly reduced the distance from the alveolar bone crest to the cement-enamel junction, when mice were orally inoculated with *P. gingivalis* [37]. In mice models coinfected with *P. gingivalis* and *F. nucleatum*, the furanone compound and D-ribose significantly reduced alveolar bone loss and increased bone volume compared to the control group [38]. Taken together, these findings indicate that AI-2 QS inhibitors have the potential to inhibit biofilm formation and virulence of periodontal pathogens.

Conclusions

AI-2 is a universal QS signaling molecule that mediates intra- and intercellular communication in bacteria and plays an important role in biofilm development of periodontal pathogens. Limited studies showed that AI-2 QS inhibitors are able to reduce biofilm formation and virulence of periodontal pathogens, suggesting that they may be used as preventive agents of periodontitis without concerns about drug resistance. More intensive studies can accelerate the development and application of AI-2 QS inhibitors to prevent periodontitis in early phases.

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Conflict of interest

The author declares no conflict of interest.

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