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Engineered Control of Bacterial Cell-to-Cell Signaling Using Cyclodextrin

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Abstract : Quorum sensing (QS) is a cell-to-cell communication system in bacteria to regulate expression of target genes. In gram-negative bacteria, activation on QS is controlled by a concentration increase of N-acylhomoserine lactone (AHL), which can diffuse in and out of the cell. Effective control of QS is expected to avoid virulence factor production in infectious pathogens, biofilm formation, and antibiotic production because various cell functions in gram-negative bacteria are controlled by AHL-mediated QS. In this research, we applied cyclodextrins (CDs) as artificial hosts for the AHL signal to reduce the AHL concentration in the culture broth below its threshold for QS activation. The AHL-receptor complex induced under the high AHL concentration activates transcription of the QS-target gene. Accordingly, artificial reduction of the AHL concentration is one of the effective strategies to inhibit the QS. A hydrophobic cavity of the CD can interact with the acyl-chain of the AHL due to hydrophobic interaction in aqueous media. We studied N-hexanoylhomoserine lactone (C6HSL)-mediated QS in Serratia marcescens; accumulation of C6HSL is responsible for regulation of the expression of pig cluster. Inhibitory effects of added CDs on QS were demonstrated by determination of prodigiosin amount inside cells after reaching stationary phase, because production of prodigiosin depends on the C6HSL-mediated QS. By adding approximately 6 wt% hydroxypropyl-β-CD (HP-β-CD) in Luria-Bertani (LB) medium prior to inoculation of S. maecescens AS-1, the intracellularly accumulated prodigiosin was drastically reduced to 7-10%, which was determined after the extraction of prodigiosin in acidified ethanol. The AHL retention ability of HP-β-CD was also demonstrated by Chromobacterium violacuem CV026 bioassay. The CV026 strain is an AHL-synthase defective mutant that activates QS solely by adding AHLs from outside of cells. A purple pigment violacein is induced by activation of the AHL-mediated QS. We demonstrated that the violacein production was effectively suppressed when the C6HSL standard solution was spotted on a LB agar plate dispersing CV026 cells and HP-β-CD. Physico-chemical analysis was performed to study the affinity between the immobilized CD and added C6HSL using a quartz crystal microbalance (QCM) sensor. The COOH-terminated self-assembled monolayer was prepared on a gold electrode of 27-MHz AT-cut quartz crystal. Mono(6-deoxy-6-N, N-diethylamino)-β-CD was immobilized on the electrode using water-soluble carbodiimide. The C6HSL interaction with the β-CD cavity was studied by injecting the C6HSL solution to a cup-type sensor cell filled with buffer solution. A decrement of resonant frequency (ΔFs) clearly showed the effective C6HSL complexation with immobilized β-CD and its stability constant for MBP-SpnR-C6HSL complex was on the order of 102 M-1. The CD has high potential for engineered control of QS because it is safe for human use.

Keywords : acylhomoserine lactone, cyclodextrin, intracellular signaling, quorum sensing
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