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Quorum sensing in *Vibrio harveyi*: probing the specificity of the LuxP binding site

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Abstract—Quorum sensing activity was investigated in the bacterium *Vibrio harveyi* using a series of both natural and nonnatural analogs of DPD, the penultimate precursor to autoinducer AI-2. The progression of molecules that were both synthesized and investigated includes enantiomeric variants, carbon-chain extension, and hydroxyl-functional group addition/deletions of DPD. The compilation of these studies reveals a binding cleft that can accommodate a number of different structural variants of DPD, albeit with invariably lower activities.

Quorum sensing, the cell density dependent process by which bacterial cells communicate, is used by bacterial communities to coordinate function and behavior.[1–3] As a result, colonies of unicellular organisms are able to behave in a manner similar to, and competitive with, multicellular organisms. Important QS-controlled behaviors are biofilm formation, virulence factor expression, antibiotic production, and bioluminescence. These processes are beneficial to a bacterial population only when they are carried out simultaneously. For example, several marine animals are host to *Vibrio fischeri*, a bacterium that produces bioluminescence only upon reaching a critical population density threshold, as regulated by quorum sensing.[1] Thus, the host can use the light for self-defense and feeding, while the bacteria receive the protection and nutrients provided by the host.

Cells regulate quorum sensing through the exchange of signal molecules called autoinducers, which increase in concentration with increased cell populations.[4,5] Autoinducers can be categorized into three classes of compounds: (1) acyl homoserine lactones, used by Gram-negative bacteria; (2) oligopeptides, by Gram-positive bacteria; and AI-2.[6] AI-2 has been labeled a universal signaling molecule, since it is used by both Gram-positive and Gram-negative species. For example, AI-2 signaling is used to control the virulence cascade and biofilm formation in *V. cholerae*, toxin production in *Clostridium perfringens*, antibiotic production in *Pseudomonas luminescence*, and bioluminescence in *Vibrio harveyi*.[7]

Since quorum sensing depends on the concentration of autoinducers, a bacterial population at low cell density can be 'tricked' into an artificial sense of high cell density by addition of synthetic AI-2, causing the synchronous generation of a bioluminescent signal in *V. harveyi*. Although this signaling cascade has been described,[8] and the crystal structure of AI-2 bound to its receptor protein, LuxP, has been solved,[9] the specificity of LuxP remains cryptic.

From the published crystal structure and extensive biochemical evidence,[8,10,11] as well as synthetic verification in our laboratory,[12] it has been shown that (*S*)-4,5-dihydroxy-2,3-pentanedione ((*S*)-DPD), **1**, is the penultimate precursor to AI-2. In this context, it appears that AI-2 is directly derived from (*S*)-DPD. For instance, in *V. harveyi* DPD reacts with boric acid to form a furanosyl-borate diester,[9,13] while in *S. typhimurium* AI-2 has been identified as the noncomplexed hydrated furanone ((2*R*,4*S*)-DHMF).[14]

Keywords: Quorum sensing; Bacterial communication; Quorum sensing analogs.
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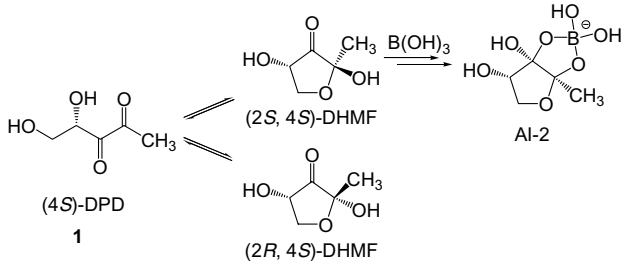


Figure 1. (4*S*)-DPD as a precursor to AI-2.

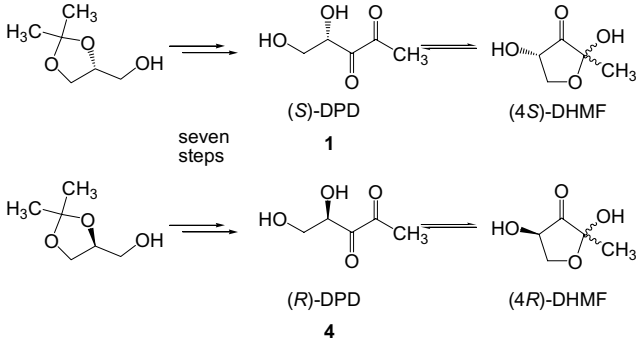
Previous studies have shown that there is some degree of flexibility within the binding pocket of this sensor protein, as both boron-complexed (*S*)-DPD, AI-2 (Fig. 1), as well as the structurally similar natural products laurencione, **2**, and 4-hydroxy-5-methyl-3-(2*H*)-furanone (MHF) **3** (Fig. 2), were able to induce bioluminescence in *V. harveyi*, albeit at higher concentrations.[13,15] Consequently, we became interested in exploring the binding site of the LuxP sensor protein through the use of structural analogs of DPD. Herein, we report our initial studies on investigating the LuxP binding site.

As a starting point for probing the binding pocket of *V. harveyi* LuxP, the chemical synthesis of the opposite enantiomer of DPD, herein designated as (*R*)-DPD, was undertaken (Scheme 1). The short synthesis follows our previously published procedure, differing only in the stereochemistry of the starting material, (*S*)-(−)-2,2-dimethyl-1,3-dioxolane-4-methanol, yielding (*R*)-DPD, **4**, in seven steps. Because DPD is not stable at high concentrations,[12,13] a reliable optical rotation has yet to be obtained. Because of this inherent problem, the optical purity of each enantiomer was assessed indirectly by reaction with 1,2-phenylenediamine;[12,16] this in turn led to a single quinoxaline derivative for each isomer. Verification by optical rotation measurements confirmed the generation of opposite enantiomers. The rotation zobtained for (*R*)-1-(3-methyl-quinoxalin-2-yl)-ethane-1,2-diol (from (*S*)-DPD) was −7.5 (*c* 0.20, $CDCl_3$), whereas the opposite enantiomer was +6.9 (*c* 0.20, $CDCl_3$).

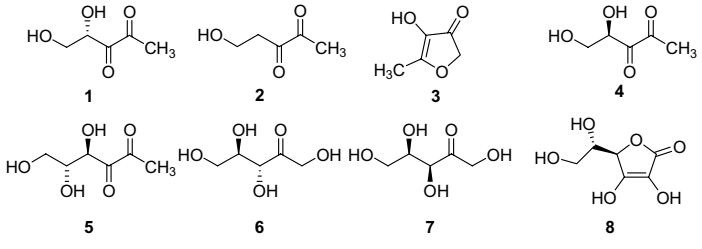
To complement this study, further efforts focused on the extension of the carbon chain of DPD. In pursuit of these efforts, a known synthetic target was chosen to extend the backbone by a hydroxymethyl moiety in the form of 1-deoxy-D-*erythro*-hexo-2,3-diulose (DEHD), **5** (Fig. 2 and Scheme 2). Synthesis proceeded from 2,3-*O*-isopropylidene-D-erythronolactone via the route established by Glomb and Pfahler.[17]

An additional related analog in which the hydroxyl moiety α to the dicarbonyl is removed, laurencione, was prepared in a one step oxidation from 5-hydroxy-2-pentanone (Scheme 2).[18] Also, several commercially available compounds with similar structural features (D-ribulose, **6**, D-xylulose, **7**, and L-ascorbic acid, **8**) were included in our assays for structural comparison to activity (Fig. 2).

The assays were performed following the protocol reported by Schauder et al.[8] Thus, *V. harveyi* strain MM30 (a LuxS mutant that is unable to synthesize

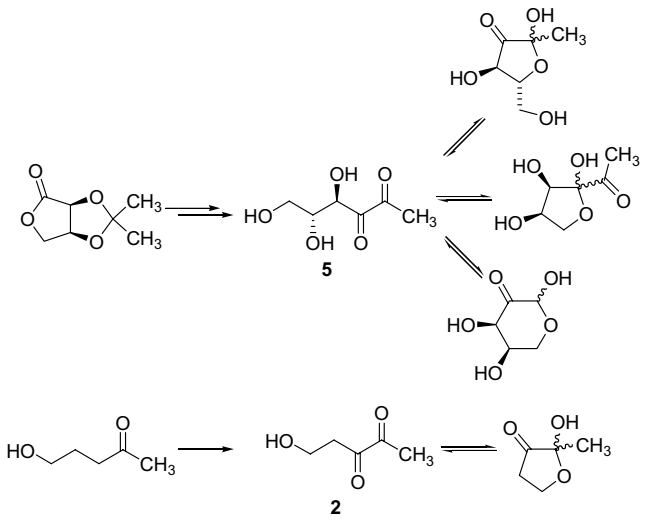


Scheme 1. Synthesis of (*S*)- and (*R*)-DPD.



No.	Compound	EC_{50} (μM) ± SD
1	(4*S*)-DPD	0.044 ± 0.002
2	laurencione	27 ± 9
3	MHF	39 ± 7
4	(4*R*)-DPD	84 ± 3
5	DEHD	429 ± 30
6	D-ribulose	108 ± 17
7	D-xylulose	560 ± 85
8	L-ascorbic acid	737 ± 25

Figure 2. Structure and activity of DPD analogs.



Scheme 2. Preparation of DPD analogs and equilibrium mixtures expected for each compound.

AI-2)[19] was grown for 18 h at 30 °C in AB medium and diluted 1:500 into fresh AB medium. A 96-well microtiter plate was prepared with wells containing test compounds (120 μL/well), serially diluted into AB medium, and 30 μL of the diluted cells was added to each well. Luminescence generally started to increase after 4 h and was measured every 20 min at $T = 30$ °C.

The bioassays revealed a correlation between the stereochemistry and the position of the hydroxyl moieties in DPD and AI-2 binding to LuxP[20]; each DPD analog failed to induce a quorum signal with activity equal to (*S*)-DPD (Fig. 3). Laurencione, lacking a hydroxyl moiety (at C-4), shows an activity reduced about 1000-fold from (*S*)-DPD 1, which roughly corresponds to the activity reported by Semmelhack et al.[13] Analysis of the crystal structure indicates that deletion of the hydroxyl functionality at C-4 leads to a loss of two potential hydrogen bonds; one to the amide side chain of Gln77 and the other to the indole of Trp82. (*R*)-DPD **4**, the nonnatural enantiomer, showed activity similar to laurencione, exhibiting only residual quorum sensing activity. This relationship suggests that inversion of the stereochemistry renders the hydroxyl group inaccessible for hydrogen bonding by the aforementioned residues. Both analogs have activities comparable to MHF **3**, a commercially available material that has been observed as a by-product of *S*-ribosylhomocysteine degradation by the *V. harveyi* enzyme LuxS.[8,10] Unlike laurencione **2** and DPD, MHF **3** does not have the capacity to bind boron, suggesting that neither boron complexation nor the presence of a hydroxyl moiety by itself enhances the affinity of these analogs for the receptor (3).

DEHD **5**, which features the addition of a hydroxymethyl moiety and may bind the LuxP receptor as a five- or six-membered ring,[17] also possesses very low quorum sensing activity. Here, the diminished activity may be a result of ring tautomerization, the acetyl group, or the primary alcohol chain rendering the molecule sterically unfit for productive binding to LuxP.

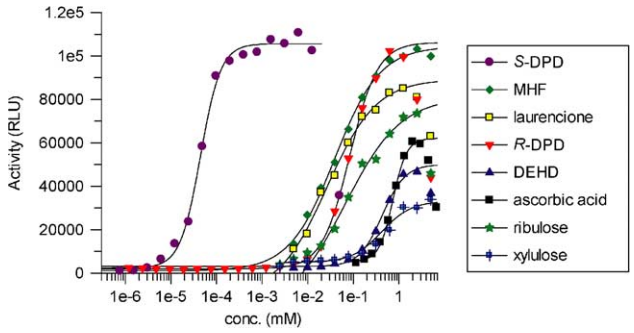


Figure 3. Evaluation of (*S*)-DPD analogs as autoinducers in *Vibrio harveyi*.

From our previous results, it is clear that boron chelation to DPD plays a crucial role in the binding of AI-2 to LuxP, as DPD does not induce bioluminescence in the absence of boron. However, it has been demonstrated that boron complexation to laurencione does not correlate to a difference in activity.[13] This finding, along with the dependence on the position of the hydroxyl moiety, suggests rigidity and the accompanying specificity of the LuxP protein are critical in binding its favored substrate. Even a change as slight as the inversion of stereochemistry of the hydroxyl moiety results in a 2000-fold decrease in bioluminescent activity.

In summary, we have investigated various analogs to act as quorum sensing signals for the LuxP sensor protein in *V. harveyi*. From the obtained activities, both the chelation of boron and the position of the hydroxyl moiety in AI-2 are critical in the binding event. The specificity of the substrate–sensor complex was explored by altering both the stereochemistry and the location of the hydroxyl moiety. Further deviations from the DPD core structure resulted in compounds with even lower activities. We believe our results will aid in the design, synthesis, and evaluation of AI-2 agonists and antagonists for the disruption of cell signaling in bacteria. Future research in this area may provide a new, effective method for combating antibiotic-resistant bacteria.

Acknowledgements

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