Point of VIEW

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Making Bacteria Behave: New Agonists and Antagonists of Quorum Sensing

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ABSTRACT Small-molecule agonists and antagonists of bacterial quorum sensing can enhance our understanding of this form of cell–cell communication. A recent effort has discovered effective modulators of the autoinducer-1 circuit for bacterial quorum sensing by the synthesis and evaluation of a small library of aryl-substituted acyl-homoserine lactone analogues. This series highlights the sensitivity to structure of the contrasting responses of agonism and antagonism of the natural signal and identifies an analogue that provokes the same response as the natural signal but at 10-fold lower concentration, a "superagonist".

Quorum sensing is a process through which bacteria perceive their population density by using small-molecule signals called autoinducers (AIs). This phenomenon is implicated in the control of a variety of interesting bacterial phenotypes, both within and among bacterial species and between bacteria and other organisms. Community-wide regulation of gene expression results in changes in a large variety of collective behaviors that are usually associated with the needs of a species inhabiting a particular niche and that are most effective when undertaken as a group (*1–3*). Such phenotypes include bioluminescence, biofilm formation, virulence expression, conjugation, motility, and symbiosis. Because of the integral roles that the secretion and detection of AIs play in this communication system, the identification of non-native small molecules with either antagonist or agonist activity may provide a means of manipulating quorum-sensing circuits and perhaps lead to a new way to control bacterial behavior. In this issue, Geske, O'Neill, and Blackwell (*4*, on p 315) from the University of Wisconsin–Madison report the discovery of both agonists and antagonists of the quorum-sensing circuit of the marine bacterium *Vibrio fischeri*, including most notably a small-molecule agonist (Figure 1, panel a, compound **1b**) that is capable of inducing quorum sensing more efficiently than the natural AI. By employing a library synthesis approach that utilizes microwave technology, they build on their previously reported (*5*) structural type to access a variety of quorum-sensing-active compounds whose structures diverge from that of the natural AI yet retain biological activity. The results address what makes a good agonist or antagonist in bacterial quorum sensing.

Various known mechanisms of quorum sensing exist in the bacterial world, including AI-2-regulated interspecies communication (*6*) and species-specific signals such as the *Pseudomonas* quinolone signal (*7*) and bradyoxetin (*8*). By far, the more widely studied and better understood quorum-sensing mechanism, and the one Geske, O'Neill, and Blackwell deal with in this issue, is system 1 (AI-1) (*9*). In this quorum-sensing circuit, Gram-negative bacteria utilize acyl-homoserine lactones (AHLs; Figure 1) as AIs (*10*). Within multispecies colonies, each bacterial species generally responds to a unique AHL AI; the same general structure is maintained, but the length and the functionality of the acyl tail are varied. For example, the native signal for *V. fischeri* is *N*-(3-oxo-hexanoyl)-L-homoserine lactone (3OC6-HSL or OHHL), whereas for *Pseudomonas aeruginosa*, the side chain carries a longer alkyl chain (3OC12-HSL; Figure 2). At a critical concentration, the signal is detected synchronously across a bacterial population by R-type (receptor) proteins, which are then activated to serve as transcription factors and regulate the expression of gene targets involved in quorum sensing.

Identifying small molecules that mimic or override the complex interactions be-

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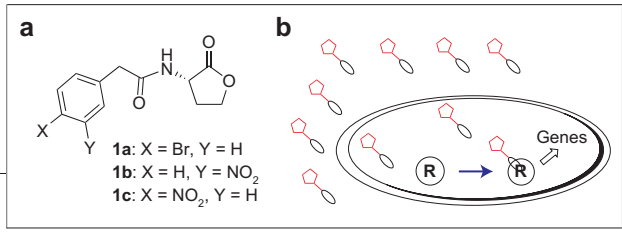

Figure 1. Structures of antagonist/agonist compounds and schematic of quorum sensing. a) Examples of the antagonists (1a and 1c) and agonist (1b) defined by Geske *et al.* (*4*) based on the AHL natural structures. b) In the more common example of quorum sensing in Gram-negative bacteria, the AI (red) diffuses (or is transported) into the cell and binds to and stabilizes the receptor protein, R, and the ligand–protein complex initiates transcription of the quorum-sensing genes.

tween the AI-1 signals and their protein receptors is a challenge. A strong need exists for the isolation and structure determination of the receptor proteins from a variety of bacterial species; to date only TraR from *Agrobacterium tumefaciens* has been fully characterized, including the detailed structure with the signal bound (3OC8-HSL) (*11, 12*). The mechanism of activation of the AI-1/receptor complex and the possible mechanisms of inhibition must be understood. In the TraR system, the protein crystal structure shows the AHL completely enveloped in a long hydrophobic channel, with no solvent interaction; dissociation of the AHL is very slow. The AHL is thought to organize and stabilize a homodimer of TraR, which then becomes the active transcription factor for the quorum-sensing genes (*13*). An agonist for TraR would imply a function like the native ligand, shepherding the protein into the shape of the functioning transcription factor; a close structural mimic of the native ligand is implied. An antagonist of TraR might simply block access to the channel and prevent good ligand binding or subunit dimerization; diverse structures could be imagined. Natural proteins are known that function in this way, such as TraM, an inhibitor of TraR in *A. tumefaciens* (*14*).

In the absence of structural and mechanistic information, potential agonists and antagonists of quorum-sensing circuits can be designed on the basis of the ligand (AHL) structures. At the same time, high-throughput screening can be employed to identify new structural types. A recent example from screening is the AI-1 agonist **2** (Figure 2) for *P. aeruginosa*, which shows no obvious structural connection to the AHL (3OC12-HSL) but was estimated by *in silico* analysis to bind in the same protein pocket as the AHL (*15*). It gives a comparable response (activator) at a concentration one-tenth of that for the natural ligand 3OC12-HSL. Although the natural ligands and analogues retaining the lactone function can be susceptible to deactivation by lactone ring opening, structure **2** avoids that problem. The series of bromofuran natural products (*e.g.*, **3**) exhibit potent quorum-sensing inhibition and appear to function by disturbing the dimerization of the R protein and not by competitive binding at the ligand site (*16*).

Systematic modification of the AHL structure has been actively pursued for many years (*17–19*). Analogues with a modified lactone unit show significant antagonist activity in certain cases. Compare the AHLs with the representative structure **4** (Figure 2) (*20*). It shows agonist activity comparable to 3OC12-HSL with *P. aeruginosa*. Modification of the side chain has also yielded many active compounds. For example, various structures based on **5** with a substituted aryl side chain showed strong inhibition (typically 50% inhibition at 2–10 μM) of quorum sensing in *V. fischeri* (*21*).

In 2005, the Blackwell group reported antagonist activity in *A. tumefaciens* and *P. aeruginosa* from an analogue (Figure 1, panel a, compound **1a**) modified with an aromatic ring on the side chain, an indication of a new direction in analogue design (*5*). In that work, they developed a general solid-phase synthesis strategy, including microwave activation, to speed the process and produce the homoserine lactone derivatives in high purity as a single enantiomer. In this issue, they report the application of that technology in the synthesis of a narrowly directed library of 24 AI-1 analogues based on structure **1a**, which retain the lactone unit and primarily differ in the substitution pattern in the arylacetyl side chain. The use of solid-phase synthesis technology minimizes chemical byproducts and allows the compounds to be obtained in sufficient quantity and purity for biological testing. Using the quorum-sensing circuit of the marine bacterium *V. fischeri*, they show that their analogues affect the downstream expression of quorum-sensing-regulated genes by directly assaying for bioluminescence, a natural phenotype linked to *lux* gene expression in the native *V. fischeri* system. Because bioluminescence is easily quantifiable over a wide dynamic range, it has proven to be a reliable measure of quorum-sensing activity in a variety of systems (*1*). Their use of a *luxR*$^-$ control strain establishes that the most active agonists require the LuxR protein for their activity, an indication of its specificity for interaction of the analogue with LuxR and not at any other point in the quorum-sensing pathway.

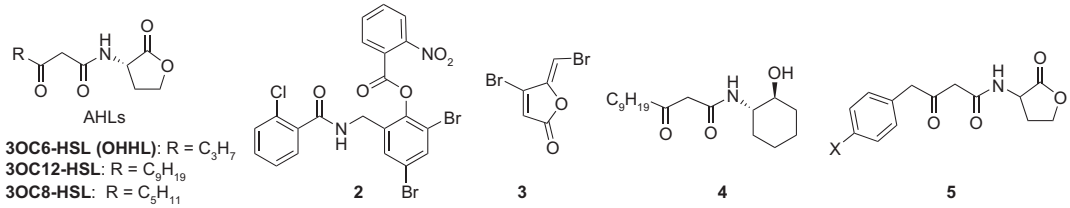

Figure 2. A selection of the diverse structures of known antagonists and agonists of system 1 quorum sensing.

Both agonists and antagonists of AI-1 activity are reported, and the activities show a surprisingly high sensitivity to minor variations in structure, such as moving a halide substituent from the 3 to the 4 position on the aryl ring. Most notably, a small-molecule agonist (**1b**) is identified that is capable of inducing the quorum-sensing response more efficiently (10-fold lower concentration; "superagonist") than the natural AI. The isomer **1c**, with the nitro substituent moved over one position, is a moderate *antagonist*. Indeed, most of the compounds in the library, with one substituent in the 2, 3, or 4 position of the aryl ring (Br, Cl, F, I, NO_2, N_3, Ph, CF_3, CH_3, NHBoc, NH_2, OH, and OMe), show 30–70% inhibition at 5 μM in competition with OHHL at the same concentration.

Geske *et al.* (*4*) are able to draw some conclusions about structure–activity relationships with the set of molecules and the "remarkable and varied" activities that they present in this paper. Interestingly, the original *N*-(4-bromophenylacetyl-)L-homoserine lactone (**1a**) structure upon which all of the AHL analogues in this paper are designed was initially identified as an antagonist of quorum-sensing activity in *A. tumefaciens* and *P. aeruginosa*, and it is now being shown to be a strong inhibitor of *V. fischeri* as well (*4*). This is surprising, because the well-established specificity of bacteria toward their own AHL suggests that each individual R protein has evolved exquisite specificity toward its own native ligand. It is tempting to conclude from these results that this group has come up with a generalized, "broad spectrum" AHL antagonist structure. However, the picture is far from clear. These researchers found a few *agonists*, including superagonist **1b**, based on an *antagonist* structure as the lead. The strong and opposing activity of **1b** containing the same basic framework as **1a** but differing in the substitution on the aryl group emphasizes that only a very uncertain line can be drawn to differentiate agonist from antagonist structures. Geske *et al.* (*4*) report that preliminary *in silico* docking studies suggest that the 3-substituted aryl analogues are accommodated better than the 4-substituted analogues in the binding site of TraR; full understanding will require more analysis of this sort.

The new antagonists may also lead to application as chemical probes of LuxR-type activity and perhaps the beginning of a practical application in bacterial control. The strong superagonist activities inspire more mechanistic investigation, but whether significant applications of this feature will emerge is unclear. The natural signaling molecules are simple, stable, readily available structures that can be applied directly if agonist activity is desired.

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