Bacterial Communication (‘Quorum Sensing’) via Ligands and Receptors: a Novel Pharmacologic Target for the Design of Antibiotic Drugs

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Abbreviations: AHL (acyl-HSL, or HSL), acylated homoserine lactones; AI-1, N-3-oxohexanoyl-L-homoserine lactone; AI-2, N-octanoyl-L-homoserine lactone; AIP, autoinducing peptides; Lux, luminescence (lux) gene.
ABSTRACT

The purpose of the present Perspective is to present a synopsis of the literature on bacterial ‘quorum sensing’ as background for the proposal that interference with this communication system offers potential targets for the design of novel antibiotic drugs. Quorum sensing is the recently discovered chemical communication system among bacteria (both gram-positive and gram-negative). It is vital for intra- and inter-bacterial gene-regulation and for keeping bacterial colonies (‘biofilms’) intact, allowing resident bacteria to assume specialized roles that contribute to enhanced survival of the group. There are several processes involved in quorum sensing that are familiar to pharmacologists: viz., specific signaling molecules bind to and activate receptors that transduce the quorum-sensing signal into intracellular 2nd-messenger responses. We highlight here the similarity between quorum sensing communication to ligand-receptor interactions, suggesting that inhibitor drugs could be designed using current standard pharmacologic principles. Such drugs would have novel mechanisms of action and might therefore be more effective against antibiotic-resistant strains of bacteria.
Introduction

When admitted to a hospital, one expects to be treated for the presenting condition, but not incur a new one. Yet, every year an estimated two million people acquire nosocomial infections (Weinstein, 1998) that may be more difficult to treat because many bacteria are resistant to at least one antibiotic, and some are resistant to all commonly used antibiotics. For many years vancomycin provided a last resort against treating resistant gram positive infections, but there are now reports of vancomycin-resistant strains (Lowy, 2003). Unfortunately, the development of antibiotic resistance continues to outpace the development of new antibiotics (Walsh, 2003).

Multiple factors contribute to resistance, including overuse, infections in immune-compromised patients, and increased use of indwelling medical devices, which provide a fostering environment (Donlan, 2002). The prevalence of biofilms (a strongly adherent assemblage of differentiated microbial cells enclosed in a matrix of polysaccharides) (Stoodley et al., 2003) in infections and on surfaces of medical implant devices has focused attention on the increased antibiotic resistance (1,000-fold) of biofilm-resident bacteria vs. the more commonly studied planktonic (free-floating) form. It has recently been suggested that biofilm-resident bacteria ‘communicate’ by a process termed ‘quorum sensing’ and that this contributes to their competitive advantage and their enhanced antibiotic resistance. Quorum sensing – detection of the surrounding cell density and activation of appropriate compensatory regulation of cell function – utilizes chemical signaling and ‘sensor’ molecules. Described in an early review by Fuqua et al. (1994), quorum sensing
has subsequently been found to be widespread in gram-positive and gram-negative bacteria (Sturme et al., 2002). In this process, compounds diffuse from, or are secreted from, bacteria as the population grows. These compounds – such as γ-butyrolactones and ‘auto-inducing peptides’ (autoinducers) in gram-positive bacteria and N-acyl homoserine lactones, quinolones, or cyclic dipeptides in gram-negative bacteria (Dunn and Handelsman, 2002; Hastings and Greenberg, 1999) – diffuse away from the cell and interact with the same or other cells by attaching to and activating specific cell-surface associated or intracellular receptors. Once sufficient signal is detected, transduction leads to induction of genes that control a variety of survival functions, including production of antimicrobial substances, and protection against the host’s defense mechanisms (Salmond et al., 1995). The recent discovery of an autoinducer produced by the luxS gene found in gram-positive and gram-negative bacteria suggests the possibility of ‘cross-talk’ between the two bacteria types (Dunn and Handelsman, 2002).

Several organisms appear to have evolved the ability to interrupt this process. Examples include plants (e.g., tomato, rice, and pea) and soil bacteria that secrete compounds that alter homoserine lactone activity, and D. pulchra, which secretes a halogenated furanone that inhibits quorum-sensing signaling (Bauer and Robinson, 2002). This suggests that synthetic analogs of such substances, or novel compounds from drug-discovery efforts, could interrupt quorum sensing in one or more (Stewart, 2003) ways.

It is the purpose of this review to highlight the similarity of quorum sensing processes to ligand-receptor binding and the use of this construct as a guide to
direct novel antibiotic drug design efforts based on standard pharmacologic
principles and drug-discovery processes. The unique nature of their mechanism
should provide these new antibiotics with greater activity against currently resistant
bacteria.

**Biofilms**

*Description*

Biofilms have been perhaps most succinctly described as "an assemblage of
microbial cells that is irreversibly associated (not removed by gentle rinsing) with a
surface and enclosed in a matrix of primarily polysaccharide material" (Donlan,
2002). Images of biofilms can be found at www.MicrobeLibrary.org (search for
‘biofilm’) (Figure 1). Bacteria can adhere to surfaces and form biofilms in places
as seemingly diverse as teeth, lungs, intestines, contact lenses, and water pipes.
Biofilm formation is considered the major contributor to the virulence of
*Staphylococcus epidermidis*, the most frequent cause of nosocomial sepsis and
catheter-related infections (Vuong et al., 2003).

Many bacterial species are now known to form biofilms (Donlan, 2002) and
gram-positive bacteria, gram-negative bacteria, and yeasts can coexist within the
same biofilm. The bacteria in biofilms can be differentiated from free floating
planktonic forms by an extracellular polymeric substance, slower growth rate, and
the up- or down-regulation of certain genes. The extracellular polymeric substance
acts as a filter and conduit for nutrients and minerals that are channeled to interior
cells and protects cells from potentially harmful agents, including antibiotics
(Donlan, 2002). Further, plasmid exchange occurs faster in biofilms because of greater cell-to-cell contact (Donlan, 2002). It is postulated that biofilms contribute to antibiotic resistance by at least three mechanisms: reduced antibiotic penetration across the extracellular polymeric substance; a favorable (e.g., anaerobic) environment within the inner layers; and bacteria cell differentiation and specialization of function that provides increased protection (Stewart and Costerton, 2001).

Biofilm-growing bacteria can become resistant to antibiotics sooner than their planktonic counterparts due to protective features of the film such as impedance against diffusion and favorable environment within the film (Stewart and Costerton, 2001). Even if antibiotic therapy is effective against some of the colony, surviving bacteria can feed off nutrients left behind (Costerton and Stewart, 2001). Natural defenses against bacteria, such as mucosal secretions, are less effective once biofilms have formed (Singh et al., 2002). As a result, bacteria in biofilms survive exposure to concentrations of antibiotics 1000-fold greater than are lethal when the cells are in suspension (Stewart and Costerton, 2001).

**Locations**

Biofilms develop on insides of distribution pipes and rogue bacteria eventually break off and enter the water supply. Conventional disinfectant techniques such as chlorination often are ineffective. In medical settings, biofilms occur in a myriad of places, such as the intestinal brush border (e.g., *V. cholera*), urethra lining (e.g., *N. gonorrhoeae*), lymphoid patches in the intestine (e.g., *S.*
typhimurium) (Costerton et al., 1978), antibiotic-recalcitrant acne (Coates et al., 2003), chronically infected tonsils (Chole and Faddis, 2003), cystic fibrosis (lungs) (Prince, 2002), urinary and central venous catheters, and mechanical heart valves (Donlan, 2001).

Quorum Sensing

Description and overview

Quorum sensing is a form of bacterial communication that helps regulate group behavior. Bacteria release chemical substances called 'autoinducers' into their surroundings. As the population density increases, so does autoinducer concentration. When the population density is sufficiently high (i.e., a 'quorum' is achieved), autoinducer concentrations become high enough to bind to receptors on/within the source or nearby bacteria. The signal is then transduced into an intracellular biochemical signal or into altered gene expression in the target bacteria. This induces a variety of adaptive physiological changes such as bioluminescence, production of antibiotics, and activation of biofilm formation.

Quorum sensing is widespread. For example, it occurs in squid light organs (e.g., V. fischeri) (Visick and McFall-Ngai, 2000), soil and plant roots (Dunn and Handelsman, 2002), and human infections (e.g., P. aeruginosa in cystic fibrosis, ocular infections, and burns) (Rumbaugh et al., 2000). It occurs in gram-positive (e.g., S. pneumoniae, B. subtilis, S. aureus) and gram-negative (e.g., V. fischeri, P. aeruginosa, A. tumefaciens, E. carotovora) bacteria, and is a mechanism of 'cross-talk' between gram positive and gram-negative bacteria (Miller and Bassler, 2001;
Dunn and Handelsman, 2002). One autoinducer, N-octanoyl-L-homoserine lactone (termed 'AI-2'), is proposed to be a signaling molecule in all bacteria (Xavier and Bassler, 2003), essential for the formation of mixed-species biofilms containing \textit{P. gingivalis} and \textit{S. gordonii} (McNab \textit{et al.}, 2003).

\textit{Autoinducers and their receptors}

Three types of autoinducers have been identified to date: acylated homoserine lactones (AHL, acyl-HSL, or HSL), such as \textit{N}-3-oxohexanoyl-L-homoserine lactone (‘AI-1’), which are found in gram-negative bacteria; autoinducing peptides (AIP), which are found in gram-positive bacteria; and autoinducer-2 compounds (AI-2’s), which are found in gram-negative and gram-positive bacteria.

At least twenty-five species of gram-negative bacteria (excluding \textit{V. harveyi} and \textit{M. xanthus}) utilize ‘LuxI/LuxR-type’ quorum sensing similar to that used by \textit{V. fischeri}. LuxI, an AHL synthase, is involved in the biosynthesis of AHL from fatty acids. LuxR is an AHL-dependent transcriptional regulatory protein. AHL binds in a dose-related manner to LuxR ‘receptor’ localized to the cytoplasm and cytoplasmic face of the inner bacterial cell membrane. The AHL-LuxR complex activates target gene transcription through intracellular biochemical pathways (Miller and Bassler, 2001; Whitehead \textit{et al.}, 2001; Kolibachuk and Greenberg, 1993) (\textbf{Figure 2A}). Many other species of gram-negative bacteria utilize a similar chemical signaling system.

AIP’s are amino acids or short peptides synthesized in gram-positive bacteria and are processed, modified, and exported by the ATP binding cassette export
systems (Kolibachuk and Greenberg, 1993; Sturme et al., 2002). AIPs bind to cell surface-bound histidine protein kinase, which autophosphorylates and in turn phosphorylates, a response regulator which activates transcription of one or more target genes (Miller and Bassler, 2001; Sturme et al., 2002) (Figure 2B).

AI-2's, common to both bacteria types, are derived from furanones. The specific structures are yet to be determined. An exception is a furanosyl cyclic borate diester recently identified as the AI-2 secreted by *V. harveyi* (Chen et al., 2002). This AI-2 is encoded by the *luxS* gene. It binds to a LuxP protein (a LuxR homolog) (Coulthurst et al., 2002). The AI-2/LuxP complex then binds to membrane-bound histidine protein kinase, and signal transduction occurs by multi-step phosphorylation similar to that of AIP’s (Miller and Bassler, 2001; Xavier and Bassler, 2003). In other bacteria, extracellular AI-2 is transported back into the cell through a Lsr (LuxS regulated) transporter (Taga and Bassler, 2003) (Figure 2C).

**Intracellular signal transduction**

Autoinducer-induced transcriptional changes include protective bioluminescence, increased virulence, biofilm formation, antibiotic production, increased competence, and sporulation. For example: *V. fischeri* produces a mutually-beneficial camouflaging bioluminescence inside squid (Whitehead et al., 2001); quorum sensing in *P. aeruginosa* contributes to biofilm formation in persistent lung infections of cystic fibrosis patients and eye infections caused by wearing contact lenses (Costerton et al., 1999); deletion of one or more quorum-sensing genes in *P. aeruginosa* decreases the bacterium's destructiveness and
mortality rate in burn wound infections (Rumbaugh et al., 1999); a mutant strain of *P. aeruginosa* has a quorum-sensing gene that produces a biofilm sensitive to biocide, a treatment to which biofilms are normally resistant (Davies et al., 1998); autoinducers are found in *P. aeruginosa* biofilms growing on urethral catheters (Stickler et al., 1998); AI-2 is required for the mixed-species biofilms of *P. gingivalis* and *S. gordonii* in dental plaque (McNab et al., 2003); and quorum sensing at high cell densities enhances the competence of soil *B. subtilis* by enabling the bacteria to take in exogenous DNA from cell lysis and using it to form dormant spores during nutrient-deprived periods or to repair damaged or mutant chromosomes (Miller and Bassler, 2001). A particularly intriguing example is the transcriptional change in *E. carotovora* (potato-rotting bacteria) resulting in production of carbapenem, a beta-lactam antibiotic, which kills competing bacteria (Axelrod et al., 1988).

**Biofilms and Quorum Sensing as Antibiotic Targets**

Multiple approaches, including NSAIDs (Alem and Douglas, 2004), are being investigated for use to attack biofilm structure or integrity, or to optimize treatment using currently available antimicrobial agents. The present review focuses on interference with quorum sensing.

The premise of the present *Perspective* is that quorum-sensing signaling can be interrupted in similar manners to ligand-receptor pathways – *i.e.*, by inhibiting ligand synthesis, transport, or release; inhibiting receptor synthesis and processing;
and perhaps most analogous to current pharmacotherapy, inhibiting enzyme activity or ligand-receptor binding.

**Targets for drug design: 1. receptors**

The currently identified receptor targets are those for AHL’s, AIP’s, and AI-2:

**AHL’s** The receptors for AHL’s are the LuxR family of transcriptional regulators (Hanzelka and Greenberg, 1995), localized to the cytoplasm and cytoplasmic face of the inner membrane. Interaction of AHL with the N-terminal region of LuxR unmasks LuxR’s C-terminal DNA-binding domain (which is blocked/inhibited by the N-terminal domain). The AHL–LuxR complex binds to specific promoters and activates transcription (Fuqua et al., 2001).

**AIP’s** The receptors for AIP’s are located on the cell membrane and are comprised of five to eight transmembrane segments in their N-terminal domain and a common histidine protein kinase-type C-terminal domain (Kleerebezem et al., 1997). Upon AIP binding, the receptor kinase is activated leading to its autophosphorylation. The activated receptor then phosphorylates the response regulator, which in turn activates several genes, including the genes for AIP, the receptor, the ABC exporter and the response regulator.

**AI-2** The target receptor for AI-2 is species-dependent (Taga and Bassler, 2003). For example, in *V. harveyi* it is LuxP, a homolog
of LuxR. In *S. typhimurium*, extracellular AI-2 binds to the Lsr transporter and is internalized into the cell, where it acts on AI-2-regulated genes.

**Targets for drug design: 2. signaling molecules**

The quorum-sensing signaling molecules thus far identified fall into distinct chemical families, broadly classified as acylated homoserine lactones (found in gram-negative bacteria), oligopeptides (found in gram-positive bacteria), and AI-2’s (found in both types) (Taga and Bassler, 2003). Each of these could be used as chemical template starting points for drug-discovery efforts using standard high-throughput screening or molecular modeling approaches. Some of these templates include the following.

**AHL’s**

*N*-acyl-homoserine lactones (*Figure 3A-E*), first identified in *V. fischeri* (Whitehead *et al.*, 2001), vary in the size (4 - 14 carbon atoms) and composition (double bonds or hydroxyl groups) of the acyl chain. Over 50 species of bacteria are known to use AHL’s for quorum sensing (Fuqua *et al.*, 2001). AHL’s are also called AI-1’s and appear to be used exclusively for intra-species communication in gram-negative bacteria. 2-Heptyl-3-hydroxy-4-quinolone is an autoinducer found so far only in *P. aeruginosa*, which uses two quorum-sensing pathways. It is a regulatory link between the two pathways (Miller and Bassler, 2001).
AIP’s

AIP autoinducers are peptides, post-translationally modified to yield a variety of diverse structures (Sturme et al., 2002) (Figure 3F) that provide binding selectivity and signal specificity. Unlike the AHL’s, which freely diffuse out of the cell, AIP’s are actively secreted. AHL’s can be linear, have dehydrated amino acids, have an N-terminal extension that is removed during or after secretion (Kleerebezem et al., 1997), or have specific features like the cyclic lactone in E. faecalis or the cyclic thiolactone in S. aureus (Sturme et al., 2002) (Figure 3G). Interestingly, an AHL recently identified in V. haveyi produces a signal similar to that in gram-negative bacteria, but the response is similar to that in gram-positive bacteria (Xavier and Bassler, 2003).

Targets for drug design: 3. disruption of quorum sensing by plants and bacteria

Some species of plants and bacteria have evolved chemicals that disrupt the quorum sensing of other species. The marine red alga D. pulchra secretes halogenated furanones and enones that structurally resemble the AHL autoinducer of S. liquefaciens, antagonize the binding of AHL’s at the receptor, and successfully ward off S. liquefaciens infestation by interrupting quorum-sensing mediated swarming motility (Miller and Bassler, 2001). Tomato, pea, soybean, rice, crown vetch and M. truncatula (a legume closely related to alfalfa), secrete chemical compounds (not yet isolated) that stimulate specific AHL receptors and compounds
from alfalfa root stimulate the production of the antibiotic zwittermicin A by *B. cereus*, which inhibits the more pathogenic *P. torulosum* (Dunn and Handelsman, 2002). Strains of *S. aureus* gain competitive advantage when their AIP’s inhibit the quorum sensing of other strains (Miller and Bassler, 2001). This is an example of intra-species interference. An example of inter-species interference occurs in the case of the soil bacteria *B. subtilis* and *E. carotovora*. *B. subtilis* secretes the enzyme AiiA (*aiiA* gene product), a homologue of zinc-binding metallohydrolases. AiiA inactivates *E. carotovora*’s AHL autoinducer (by hydrolyzing either the amide bond between the acyl side chain and the lactone ring of the AHL or the ester bond within the lactone ring) (Dong *et al.*, 2000), rendering it inactive. There is even a strain of bacteria (*V. paradoxus*) that disrupts the quorum sensing of other bacteria by ingesting and using their quorum sensing compounds as a nutrient source of carbon and nitrogen (Leadbetter and Greenberg, 2000). Interruption of quorum sensing impedes biofilm formation of *E. coli* (Ren *et al.*, 2001). These natural molecules could serve as chemical templates for drug-discovery efforts using standard high-throughput screening or molecular modeling methodology.

**Design of quorum sensing antagonists**

Quorum sensing pathways bear a striking similarity to mammalian signaling pathways and, therefore, should be amenable to pharmacologic manipulation in ways similar to those used to design drugs. Autoinducer ligands interact with quorum-sensing receptors in a concentration-dependent manner – consistent with traditional concepts of receptor-binding (*e.g.*, Hanzelka and Greenberg, 1995). Also consistent with traditional concepts of receptor-binding, autoinducer ligands
that act at the same quorum-sensing receptor compete with each other. For example, a series of structural analogs of the \textit{Pseudomonas aeruginosa} autoinducer \textit{N}-3-oxo-dodecanoyl homoserine lactone compete with autoinducer binding to LasR and have varying degrees of agonist activity (Passador \textit{et al.}, 1996) and analogs of the autoinducer 3-oxohexanoyl HSL competitively antagonize the binding of the autoinducer in \textit{Vibrio fischeri} (Schaefer \textit{et al.}, 1996).

Reference to Figure 2 reveals multiple sites to target for the design of anti-quorum-sensing, anti-biofilm antibiotics. Some of these include LuxI, LuxR, the AIP receptor, LuxS, and the Lsr transporter. For gram-negative bacteria, enzyme inhibitors of LuxI or antagonists of the LuxR receptor-binding site should be sought (Figure 2A). For gram-positive bacteria, antagonists of AIP processing or the AIP receptor should be sought (Figure 2B). Enzyme inhibitors of LuxS or inhibitors of the Lsr transporter (Figure 2C) could have application to both gram-negative and gram-positive bacteria. Combinations of mechanisms would be expected to be more effective than single-mechanism approaches. Examples of some early efforts toward the design and development of such agents are summarized below.

- Based on the observation that the fronds of red alga growing in Botany Bay Australia are rarely covered with biofilms despite thousands of bacterial species in the waters, Rice and colleagues (Rice \textit{et al.}, 1999) determined that the alga secretes halogenated furanone compounds that prevent biofilm formation and help disrupt existing biofilms (Costerton and Stewart, 2001).
- Hentzer \textit{et al.} (2003) used gene microarray technology and transcriptome analysis to show that the targets of a synthetic furanone were genes of the
quorum-sensing system of *P. aeruginosa* and that the compound inhibited bacterial activity *in vitro* and *in vivo*. That the effect was specific to biofilms was shown by the lack of activity against planktonic cultures of the same bacteria.

- *P. aeruginosa*, a common cause of nosocomial infections and responsible for chronic lung infection in an estimated more than 90% of cystic fibrosis patients, uses a quinolone signal as part of its quorum-sensing system. Calfee *et al.* (2001) determined that anthranilic acid is a precursor to the quinolone signal and that an anthranilate analog (methyl anthranilate) inhibits the quinolone signal production and decreases the expression of cellular virulence factors in a dose-dependent fashion.

There is recent evidence that joint use of a ligand that disrupts quorum sensing and a more traditional antibiotic results in a synergistic antibacterial effect. Balaban *et al.* (2004) report that RNA III-inhibiting protein, a heptapeptide that inhibits staphylococcal biofilm formation by obstructing quorum-sensing mechanisms, and DD(13), K(4)-S4(1-13)(a) a 13-residue dermaseptin derivative believed to kill bacteria by disrupting membranes, act in synergy by attacking bacteria simultaneously *via* two different mechanisms.

**SUMMARY**

Quorum sensing is a bacterial chemical communication system that involves signals, signal sensors, and signal transduction mechanisms. The viability of biofilms, coordinated colonies protected by an outer matrix that confers increased
antibiotic resistance over planktonic bacteria, is an example of an antibiotic-resistant system that depends on the fidelity of quorum sensing and other communication systems. Interference with quorum sensing should disrupt biofilm protective measures and greatly enhance susceptibility of bacteria to antibiotic drugs. The nature of quorum sensing communication – involving enzymes, transporters, and ligand-receptor interactions – presents classic pharmacologic targets for drug discovery efforts using standard techniques. The novel nature of their mechanisms of action might allow these new ‘quorum-sensing inhibitors’ to be effective against bacterial strains that are currently antibiotic-resistant.

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REFERENCES


Figure legends

**Fig. 1** Image of a biofilm composed of *P. aeruginosa*, *P. fluorescens*, and *K. pneumoniae*. The image shows cell clusters – discrete aggregates of microbial cells in a slime matrix (teal; nucleic acid stain propidium iodide), separated by interstitial voids or water channels (red; fluorescein stain). Scale bar = 100 µm. Reproduced with permission (deBeer et al., 1994) (linked to: [http://www.MicrobeLibrary.org](http://www.MicrobeLibrary.org)).

**Fig. 2** Schematic representations of the three known quorum sensing signaling pathways: (**A**) LuxI/LuxR in a gram-negative bacillus. Autoinducer (*e.g.*, homoserine lactones; *spheres*) is synthesized through pathways involving LuxI, released and reenter bacteria, then bind to receptors (LuxR) that alter cellular response elements. (**B**) AIP in a gram-positive bacillus. Amino acids or short peptides (*linked shapes*) are exported, then bind to cell surface-bound sites, which activate phosphorylation cascades leading to transcriptional changes. (**C**) LuxS/Lsr transporter in both gram-negative and gram-positive bacteria. Autoinducer (*e.g.*, furanones; *spheres*) is synthesized (*e.g.*, through pathways involving LuxS), released and reenter bacteria (*e.g.*, through a Lsr transporter), then act on regulated genes. Based on Xavier and Bassler (2003).
Fig. 3  Chemical structures of some quorum-sensing ligands: N-acyl-homoserine lactones (A–E), where R is typically an aliphatic chain; AIP autoinducers (F); AI-2 (G). Based on Whitehead et al. (2001) and Dunn and Handelsman (2002). Amino acids are represented by their standard single-letter abbreviations.
Fig 3