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Previews

Mastering the Chemical Language of Bacteria

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The publication of the crystal structures of a bacterial quorum sensing receptor complexed with a variety of ligands (Zou and Nair, 2009) provides a much-needed molecular rationale for the modulation of this intercellular signaling process, which may facilitate the development of new therapeutic agents.

Many species of bacteria employ a mechanism of cell-cell communication known as quorum sensing. This signaling process, mediated by small molecules (termed autoinducers) is used by bacterial colonies to coordinate gene expression in a cell density-dependent manner (Fuqua et al., 1994). Several clinically relevant pathogens use quorum-sensing systems to regulate processes associated with virulence. However, quorum sensing is not directly involved in biological processes that are essential for bacterial survival (Rasmussen and Givskov, 2006). Thus, selective disruption of quorum sensing using nonnative small molecule entities represents a strategy to attenuate bacterial pathogenicity without imposing an intense selective pressure for the development of resistant mutants (Amer et al., 2008; Kapadnis et al., 2009). Zou and Nair (2009) now describe crystal structures of a *Pseudomonas aeruginosa* quorum-sensing receptor protein LasR bound to various small molecule quorum-sensing agonists. This work marks a significant advancement in our understanding of the molecular basis of quorum sensing in this bacterium. It reveals the fundamental information about bonding interactions between the receptor and small molecules that are necessary for modulation of this communication system. Such insights may allow the rational design of inhibitors to target the quorum-sensing pathway in this clinically relevant pathogen, with possible therapeutic applications in the treatment of human bacterial infections.

P. aeruginosa is an opportunistic human pathogen that is involved in a range of life-threatening nosocomial infections and is also the primary cause of mortality in cystic fibrosis sufferers (Rasmussen and Givskov, 2006). The virulence of this bacterium is regulated by a complex quorum-sensing signaling cascade that utilizes numerous signaling molecules, most notably *N*-3-oxo-dodecanoyl homoserine lactone (OdDHL) (Figure 1). The binding of OdDHL to the bacterial signaling receptor LasR results in the transcription of most genes that are associated with the progression of infection and resistance to the host immune system (Zou and Nair, 2009; Welch et al., 2005). It is therefore unsurprising that intense research efforts have been directed toward the discovery of small molecules that can bind to LasR and thereby modulate this quorum-sensing pathway (Geske et al., 2008; Lee et al., 2008; Glansdorp et al., 2004).

Small molecule agents capable of modulating the LasR quorum-sensing system have typically been discovered through a design and synthesis process, using the structure of the known agonist OdDHL as a template. However, such a rational design approach is complicated by the fact that LasR shows a very high specificity for its cognate autoinducer OdDHL. For example, the autoinducer OOHL (*N*-3-oxo-octanoyl homoserine lactone) with a related signaling receptor TraR is structurally identical to OdDHL, except that the acyl chain is four carbon atoms shorter. However, despite the structural similarity, OOHL does not interact with the LasR receptor (Zou and Nair, 2009). Thus, there are significant structural constraints placed upon any nonnative small molecule modulators of LasR, which are based on the homoserine lactone scaffold, with little deviation from the parent framework apparently tolerated. In addition, there are problems associated with any pharmaceutical agent that incorporates a homoserine lactone moiety; this structural feature is unstable at alkaline pH and is readily degraded by mammalian lactonases, thus limiting the efficacy of any synthetic derivatives (Glansdorp et al., 2004). Consequently, the identification of new classes of small molecule modulators of LasR that are structurally distinct from the homoserine lactone autoinducer is an area of significant interest.

The high-throughput screening of small molecule libraries has proven to be a valuable method for the discovery of quorum-sensing modulators. Such an approach was recently employed by Muh et al. (2006a). This lead to the identification of a triphenyl scaffold based compound (TP-1), which was shown to be a potent activator of LasR-dependent signaling, despite the lack of any appreciable structural similarities to the LasR autoinducer OdDHL (Figure 1). Further research established that TP-1 and its derivatives TP-2, TP-3, and TP-4 act directly through the LasR receptor in a highly selective fashion (Muh et al., 2006b). Zou and Nair (2009) now report the high-resolution crystal structures of the ligand-binding domain of the LasR receptor in complex with the triphenyl compounds TP-1, TP-3, and TP-4. Analysis of these crystal structures allows delineation of the process of recognition of these novel compounds by LasR at a molecular level, which may allow a determination of the ensemble of steric and electronic features required in a ligand to ensure optimal and specific interaction with the LasR receptor (Hajduk and Greer, 2007). These results thus provide a template for the rational design of novel inhibitors, based around the triphenyl scaffold (and perhaps other frameworks) that target the quorum-sensing

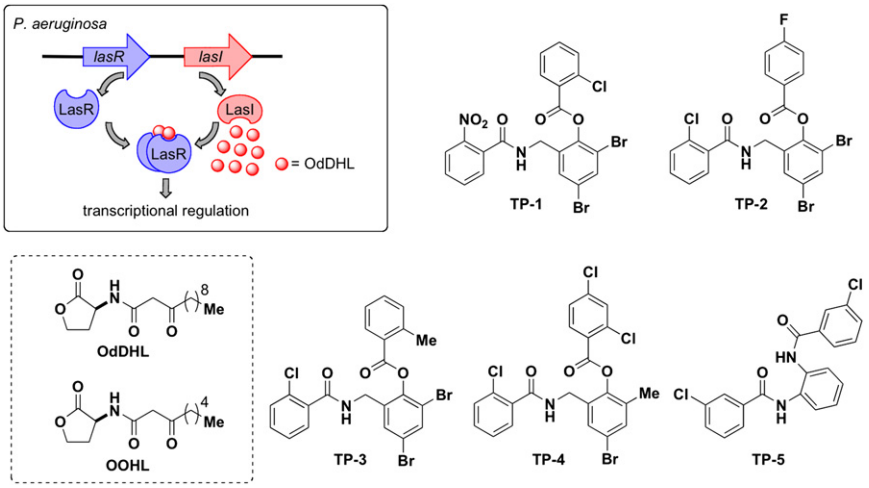

Figure 1. An Outline of LasR-Mediated Quorum Sensing in *P.aeruginosa*
Binding of OdDHL to the LasR receptor promotes receptor dimerization and the resultant homodimer complex binds target DNA to activate gene transcription. The chemical structure of OdDHL is given, together with OOHL, that acts via a different receptor protein. The structure of the nonnative TP-ligands developed by Muh et al. (2006b) are also shown.

signal pathway in pathogenic *P. aeruginosa*. Furthermore, such information could potentially be applied in fragment-based approaches toward the discovery of new LasR modulators, whereby libraries of small fragment units each containing structural motifs that are known to be capable of high-affinity interaction with the LasR receptor can be screened for activity, thus providing good, low molecular-weight starting points for a lead optimization drug discovery program (Hajduk and Greer, 2007).

In addition, the work of Zou and Nair (2009) allows a molecular rationale for understanding how structurally distinct classes of compounds can interact with the same highly selective receptor. This should provide a framework for understanding more precisely the molecular basis behind the activity of previously developed small molecule modulators of LasR-dependent signaling. Such knowledge may facilitate the deliberate rational structural modification of such agents in order to improve various molecular properties (e.g., efficacy and selectivity) with the ultimate goal of developing an efficient inhibitor of LasR that shows low toxicity to humans. However, in this context, it is worth noting that out of the five triphenyl compounds that were identified, only TP-5 was found to function as an antagonist, rather than an agonist, of LasR function. Unfortunately, Zou and Nair (2009) report that the crystal structure of the LasR-TP-5 complex could not be determined. Thus, while the binding mode of TP-5 to LasR is expected to be similar to that of the other triphenyl ligands due to their structural similarities, there nevertheless remains the possibility that TP-5 may interact with LasR in an entirely different manner. This raises the option that the dictates of molecular recognition of a LasR antagonist may be very different to those of an angonist, which could have significant implications in terms of rational antagonist design; there thus remains significant scope for further insights in this area.

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