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Previews

Molecular Radio Jamming: Autoinducer Analogs

Synthesis of an *N*-acyl-homoserine lactone (*N*-acyl-HSL) analog library yields agonists and antagonists of the *Pseudomonas aeruginosa* quorum-sensing system. Active compounds also reveal heterogeneity in the lactone ring binding pockets of *N*-acyl-HSL-activated transcription factors.

Certain gram-negative bacteria have evolved a way to sense cell density through a process dubbed "quorum sensing" [1]. As individual cells, these bacteria constitutively produce small amounts of a neutral signaling molecule, an *N*-acyl-homoserine lactone (*N*-acyl-HSL), that can freely diffuse out of the cell. As the cells multiply and reach a certain cell density (a quorum), this *N*-acyl-HSL, also called an autoinducer, reaches a certain threshold concentration and binds to its cognate regulatory protein, which can then activate transcription of a variety of genes that often encode virulence factors. In the case of pathogenic bacterial infections, quorum sensing allows the bacterial troops to amass before launching a coordinated assault on the host. Producing virulence factors before a quorum is reached may prematurely alert the host's defense systems, so these bacteria wait for their marching orders: a rising autoinducer concentration akin to a radio signal to attack.

While other chemical classes of autoinducers have been discovered, more than 25 species of gram-negative bacteria use the *N*-acyl-HSL mediated system [1]. All of the known *N*-acyl-HSL autoinducers from these systems have a conserved homoserine lactone ring and differ only in the length and substitutions of their *N*-alkyl chains. Recent crystal structures of a cytoplasmic transcription factor (TraR) show that, when bound, these *N*-acyl-HSL autoinducers are completely enveloped by protein residues [2, 3]. Specific interactions are made between the conserved lactone ring and the binding pocket, and the pocket's shape suggests how specificity may be mediated by the differences found in the alkyl chain.

A quorum-sensing bacterium of particular concern, *Pseudomonas aeruginosa*, is an opportunistic pathogen that forms biofilms on lung surfaces of cystic fibrosis patients [4]. In this system, two specific *N*-acyl-HSL signals, *N*-(butyryl)-L-HSL and *N*-(3-oxododecanoyl)-L-HSL, along with their cognate regulatory proteins, RhlR and LasR, participate in a hierarchical signal transduction pathway that leads to production of virulence factors such as elastase, pyocyanin, and others [5]. Disrupting the quorum-sensing process by genetic knockouts of *N*-acyl-HSL synthases has been shown to arrest biofilm formation at an early stage, resulting in a less robust infection that is much easier to remove [6]. Therefore, strategies to disturb this quorum-sensing pathway are of considerable clinical interest.

Naturally occurring systems capable of disrupting quorum sensing have been reported. Several *Bacillus* species harbor an autoinducer lactonase which can hydrolyze the lactone moiety, thus deactivating the *N*-acyl-HSLs [7]. Another bacteria, *Variovorax paradoxus*, is suspected to harbor enzymes that cleave the *N*-acyl bond of these autoinducers [8]. Interference also comes from the eukaryotic world: the seaweed *Delisea pulchra* produces halogenated autoinducer analogs that can act as *N*-acyl-HSL antagonists [9]. In addition to removing the autoinducers or antagonizing their action, overproduction of *N*-acyl-HSLs can also adversely effect the virulence of various organisms by triggering an attack too soon. In an experimental system, *N*-acyl-HSL-producing transgenic tobacco plants show enhanced resistance to *Erwinia carotovora*, presumably by triggering early release of *Erwinia* virulence factors that in turn activate plant defense responses before a quorum is reached [10]. This suggests that both autoinducer agonists and antagonists will be useful in the manipulation of quorum-sensing systems. If quorum sensing is a form of communication that bacteria use to radio for an attack, then autoinducer analogs can act as a type of radio-jamming system that prevents bacterial coordination.

Taking a cue from nature, synthetic autoinducer analogs might also be used to agonize or antagonize *N*-acyl-HSL-mediated quorum sensing. Previous reports of synthetic autoinducer analogs kept the homoserine lactone ring constant and varied substitutions and the alkyl chain composition but did not yield any strong antagonists [11–13]. In an alternative approach, scientists from Hiroaki Suga's lab at SUNY University at Buffalo synthesized a library of autoinducer analogs (Y. Bu, K.M. Smith, and H. Suga, submitted). Instead of varying the alkyl chain, this was held constant and various amines and alcohols were substituted for the homoserine lactone ring. For *N*-(3-oxododecanoyl)-substituted agonists of LasR, 2-aminocyclohexanol was identified as an allowed ring substitution. Based on this observation and on the recent TraR crystal structure, the hydroxyl group in the cyclohexanol ring was proposed to function as a hydrogen bond acceptor. Subsequently, an additional focused library was synthesized that included both five- and six-membered rings, each with a hydrogen bond acceptor in the same position. In this issue of *Chemistry & Biology*, Smith, Bu, and Suga report several synthetic autoinducer analogs from this focused library that can agonize or antagonize *P. aeruginosa* quorum sensing [14].

Since the specificity of naturally occurring *N*-acyl-HSLs for a particular regulatory protein derives from the differences in their alkyl chains, it would be reasonable to predict that changing the *N*-acyl-chain of the ring analogs would alter their specificity as well. Surprisingly, this is not the case. Synthetic agonists of the LasR system do not become agonists of the RhlR system just by changing the *N*-acyl chain. This suggests that the HSL ring binding pockets in the regulatory proteins are not

absolutely conserved and interact differently with the ring analogs found in synthetic ligands. Smith et al. rightly note that this raises the possibility for design of even more specific ligands for the RhlR and LasR transcription factors [14]. Some of the library compounds that failed to show agonist activity are actually antagonists of RhlR and LasR. One compound in particular, *N*-(2-oxocyclohexyl)-3-oxododecanamide, is a strong antagonist of the quorum-sensing system that significantly reduces production of several virulence factors and prevents biofilm formation by *P. aeruginosa* strains.

The ease of synthesis, chemical stability, and strong agonist and antagonist activity of these autoinducer analogs make them productive leads for future research. The observation of differences in how regulatory proteins bind these homoserine lactone analogs will certainly allow further exploration through focused libraries. Extensive screening of analog libraries will probably yield other types of antagonists as well. The report that long chain *N*-acyl-HSLs can display immunomodulatory effects may also open new avenues of investigation for these compounds [15]. After a long history of bacterial communication, it appears that humans are starting to join the conversation.

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Membrane Proteins: Adapting to Life at the Interface

A recent publication by Cravatt and colleagues which describes the structure of an integral membrane protein (FAAH) highlights that the structural differences between membrane proteins and soluble proteins are not as disparate as is sometimes believed.

Soluble proteins and membrane proteins are sometimes thought of as two completely different classes of biomolecules that inhabit two completely different worlds. Their solubility, the nature of their molecular surfaces, the pathways by which they fold, and the forces that stabilize them are widely considered so different that it is hard to even find a common basis for comparison. But are the distinctions really so clear cut? A new structural study of an integral membrane protein, fatty acid amide hydrolase (FAAH; [1]), has evolutionary implications that highlight the shades of gray between the black and white extremes of soluble versus integral membrane proteins.

Amphitropic membrane proteins have a dual life as soluble and membrane proteins. Under some circumstances, they can be just as soluble as ordinary soluble proteins, yet they are also capable of binding to membranes with high affinity and, in many cases, inserting themselves deeply into the hydrocarbon core of one of the two leaflets of a phospholipid bilayer. The translocation of amphitropic membrane proteins between membrane and soluble phases is often used as a regulatory mechanism in processes such as signaling, cytoskeletal regulation, and membrane trafficking.

The phosphoinositide lipids, which play central roles in all of the above-mentioned cell processes, are turned over by amphitropic enzymes that catalyze their synthesis, hydrolysis, phosphorylation, and dephosphorylation [2]. Phosphoinositide signaling is rich in examples of soluble enzyme folds that have been cannibalized for membrane activity. The kinases that produce polyphosphoinositides have the same catalytic fold as the protein kinase superfamily, but their folds have been drastically modified for action at the interface [3, 4]. Phosphatidylinositol phosphate kinase (PIPK) has a flattened face that interacts electrostatically with phospholipid bilayers through extensive basic patches [5]. Moreover,