Quorum Sensing Autoinducer-3 Finally Yields to Structural Elucidation

A novel quorum sensing autoinducer, which regulates virulence in enterohemorrhagic *Escherichia coli*, had eluded structural characterization—until now.

In the last two decades, our understandings of microbial sociology and physiology have been vastly expanded, and it is now appreciated that akin to higher organisms, unicellular organisms and even biological particles (viruses) scan their environments, listen to social cues, conserve their resources, and make deliberate decisions using hierarchical signaling systems that involve quorum sensing, various response regulators associated with two-component systems, second messengers, etc.\(^1\) Most often, production of virulence factors does not occur by chance, but virulence factors are only deployed when permissive conditions exist and/or after kin recognition.\(^1\) Fungi, bacteria, and viral particles all harbor signaling components that facilitate the integration of kingdom or interkingdom signals as well as environmental factors to guide precision behaviors and optimize their survival. For example, viral particles, once thought of as devoid of social interactions, are now known to make decisions about lysis and lysogeny via small molecule communication systems.\(^2\) Bacteria integrate quorum sensing autoinducers and nutrient availability to "decide" whether to form biofilms or release toxins.\(^1\) Crawford and co-workers have now increased our knowledge of bacterial quorum sensing by elucidating the structures of the AI-3 family of compounds (Figure 1),\(^3\) initially reported as bacterial signaling molecules that regulate the virulence of the foodborne pathogen enterohemorrhagic *Escherichia coli* (EHEC),\(^4\) but have eluded structural characterization for more than 15 years until now.

Bacterial pathogens, which infect complex hosts and especially those that infect the gut, have various challenges to overcome. In addition to transiting various compartments (mouth, stomach, intestines, rectum) with different pH values, such microbes have to contend with diverse environmental conditions (including different oxygen levels and nutrient availability), competing bacteria, and host-released microbicides. For the adventurous or voyeur pathogen that seeks systemic exposure, a battle with the innate and adaptive immune cells also awaits.

*E. coli*, a regular member of commensal bacteria in the gut, colonizes humans soon after birth, but several pathotypes, such as EHEC, also exist. EHEC causes foodborne diseases in humans such as hemorrhagic colitis and acute bloody diarrhea, which can also lead to hemolytic uremic syndrome, a serious complication that can include renal failure.\(^6\) Antibiotic treatment of EHEC infection is discouraged since that could possibly lead to this syndrome,\(^5\) so there has been enormous interest in understanding the molecular details of EHEC colonization behavior with the hope that such insights could lead to new treatment strategies.\(^6\) Foundational studies by the Sperandio group in the early 2000s revealed that EHEC is a sophisticated pathogen that senses various factors to regulate the locus of enterocyte effacement (LEE), a pathogenicity island.\(^4\) The expression of LEE leads to the production of toxins, such as Shiga toxin. EHEC also senses host-derived stress hormones, epinephrine and norepinephrine, as well as the bacteria-derived quorum sensing molecule AI-3 using the QseBC two-component system.\(^1\) Despite attempts to characterize AI-3,\(^4\) structural elucidation and/or biosynthetic origins of this quorum sensing autoinducer had been intractable. Just as AI-3 was about to attain mythical and "dark molecule" status, Crawford and co-workers (in collaboration with the Sperandio team) presented not only the structural elucidation of this often
anticipated molecule but also a biosynthetic proposal for how AI-3 is formed.³

Since previous attempts to obtain sufficient quantities of AI-3 from bacterial cultures for characterization failed, Crawford and co-workers employed a clever trick of stressing E. coli with sublethal levels of an antibiotic (erythromycin) to increase the production of secondary metabolites. The structures of AI-3 and some analogues are shown in Figure 1.

Figure 1. (A) (i) Proposed biosynthetic pathway for phevalin synthesis.⁵ A: adenylation domain; C: condensation domain; T: thiolation domain; R: reductase domain. Adapted from Figure 1 in Zimmermann et al. Chem. Biol. 2010, 17, 925−930. Used by permission, copyright 2010 Elsevier. (ii) Various reactions of aminoacetone or 1-amino-3-methylbutan-2-one (AMB) in bacteria. (B) EHEC uses complex signaling cascades to coordinate the expression of genes related to fucose utilization and virulence factors production. (C) Microbiota, such as Bacteroides thetaiotaomicron, cleave fucose from the mucus layer, which is sensed by EHEC as a cue that it has arrived in the intestinal lumen. Later, as the disease progresses and mucinases from EHEC have obliterated the mucus layer, EHEC forms an attaching and effacing lesion. Adapted from Sperandio et al.¹ Used by permission, copyright 2010 Elsevier.
Using $^{13}$C-labeled amino acids, the team was able to show that the most potent inducer of LEE expression among isolated metabolites is 3,6-dimethylpyrazin-2-one, and hence was designated as AI-3. Additional $^{13}$C-labeled amino acid feeding experiments and subsequent structural analyses revealed that AI-3 is formed from two molecules of threonine. A biosynthetic proposal for the formation of AI-3 from threonine involves threonine dehydrogenase-catalyzed oxidation of threonine into 2-amino-3-ketobutyric acid, followed by its spontaneous decarboxylation into aminoacetone. The next step includes dimerization of aminoacetone, followed by oxidation into AI-3. Clardy and Pupo proposed a similar biosynthetic scheme for the formation of 2,5-dimethylpyrazine, DMP, (a component of the ant pheromone trail) from the synthetic scheme for the formation of 2,5-dimethylpyrazine, oxidation into AI-3. Clardy and Pupo proposed a similar biosynthetic scheme for the formation of 2,5-dimethylpyrazine, DMP, (a component of the ant pheromone trail) from the dimerization of aminoacetone, whereas Bassler proposed that 3,5-dimethylpyrazin-2-one (DPO), a quorum sensing autoinducer, originates from the condensation of aminoacetone and alanine, followed by cyclization and oxidation. Thus, it is emerging that the regulation of aminoacetone concentration shapes microbial—microbial and microbial—host interactions.

Crawford and co-workers also showed that upon antibiotic stress in vitro, various 3,5-disubstituted pyrazin-2-ones were also formed in *E. coli* and many other bacteria (such as *Vibrio cholerae, Klebsiella pneumonia, Staphylococcus aureus, Pseudomonas aeruginosa*). A biosynthetic proposal for the formation of the 3,5-disubstituted pyrazin-2-ones postulates that upon stress abortive tRNA synthetase products react with aminoacetone or 1-amino-3-methylbutan-2-one to form dipeptide ketones, which can undergo cyclizations and oxidations to form the 3,5-disubstituted pyrazin-2-ones (AI-3 analogues). This proposal is consistent with the fact that the AI-3 analogues could be synthesized in vitro using only tRNA synthases, ATP, amino acids and aminoacetone, or 1-amino-3-methylbutan-2-one without using ribosomes or tRNA.

Dipeptide carbonyl compounds are known to cyclize into pyrazinones. Indeed, it has long been established that other bacteria-derived pyrazinones such as phevalin (see Figure 1 for structure), some of which are known to regulate bacterial virulence, originate from dipeptide aldehydes, produced by nonribosomal peptide synthases. In addition to literature precedents, Crawford and co-workers also chemically synthesized the presumed dipeptide ketones and showed that such molecules cyclized into the respective pyrazinones.

As mentioned earlier, the QseBC and QseEF two-component systems sense host’s catecholamines, epinephrine and norepinephrine, and can be inhibited with adrenergic antagonists. Crawford and co-workers showed that on the contrary, the bacteria-derived pyrazinones do not inhibit adrenergic receptors, but they do modulate various host immune responses. For example, compound 2 (see Figure 1) increased secretion of a chemokine by macrophages differentiated from THP-1 cells via uncharacterized host receptors.

Often, genetic approaches are limited in uncovering details of biological processes that arise from nonenzymatic reaction of intermediates that serve other purposes or compounds that are made from diverted/abortive processes. This landmark study beautifully shows how the power of chemical biology can be used to uncover biological insights that had resisted illumination using traditional genetic studies. After savoring the news that AI-3 has finally been conquered, new perplexing questions that have emerged from this study will need to be answered. For example, Crawford and co-workers show that only AI-3 and DPO molecules (Figure 1), but not the other AI-3 analogues that could be generated in stressed bacteria in vitro, could be detected in vivo. Could this mean that dipeptide ketones do not readily cyclize in vivo? If so, then how did the detected DPO form in vivo? Indeed, recent work by the Bassler group has revealed that when administered to cells, the putative dipeptide ketone precursor to DPO did not cyclize into DPO, hinting that perhaps DPO is synthesized via an alternative mechanism. Second, aminoacetone is the precursor to AI-3, DPO, and insect trail pheromones, suggesting that aminoacetone is not quickly oxidized into methylglyoxal and that high enough concentrations could be reached inside cells to support bimolecular homodimerization. Do some bacteria use an enzyme to aid the dimerization of aminoacetone into AI-3? Indeed, Molla and co-workers have suggested that an aminoacetone oxidase from *Streptococcus oligofermentans* catalyzes the dimerization of aminoacetone into 2,5-dimethylpyrazine as the main product via 3,6-dimethyl-2,5-dihydropyrazine. Since both AI-3 and 2,5-dimethylpyrazine originate from aminoacetone via the same precursor, future work should help clarify which in vivo conditions favor pyrazine over pyrazine formation. Other organisms, including mammals, can also produce aminoacetone, which begs the question: can mammals also produce AI-3? What are the host receptors that recognize...
AI-3 family compounds as pathogen-associated molecular patterns? We anticipate that flurries of follow-up studies are going to be initiated by other researchers now that AI-3 has finally been uncloaked.

Author Information

Herman O. Sintim — Department of Chemistry and Institute for Drug Discovery, Purdue University, West Lafayette, Indiana 47907, United States; Purdue Institute of Inflammation, Immunology and Infectious Disease, West Lafayette, Indiana 47907, United States; orcid.org/0000-0002-2280-9359; Email: hsintim@purdue.edu

Delmis E. Hernandez — Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, United States

Complete contact information is available at: https://pubs.acs.org/10.1021/acscentsci.0c00033

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Kendall, M. M.; Sperandio, V. What a Dinner Party! Mechanisms and Functions of Interkingdom Signaling in Host-Pathogen Associations. mBio 2016, 7 (2), e01748–15.
(2) Erez, Z.; Steinberger-Levy, I.; Shamir, M.; Doron, S.; Stokar-Avihai, A.; Peleg, Y.; Melamed, S.; Leavitt, A.; Savidor, A.; Albeck, S.; et al. Communication between Viruses Guides Lysis–Lysogeny Decisions. Nature 2017, 541 (7638), 488–493.
(3) Kim, C. S.; Gatsios, A.; Cuesta, S.; Lam, Y.; Wei, Z.; Chen, H.; Ressell, R.; Shine, E.; Wang, R.; Wyche, T.; et al. Characterization of Autoinducer-3 Structure and Biosynthesis in E. Coli. ACS Cent. Sci. 2020, DOI: 10.1021/acscentsci.9b01076.
(4) Sperandio, V.; Torres, A. G.; Jarvis, B.; Nataro, J. P.; Kaper, J. B. Bacteria–Host Communication: The Language of Hormones. Proc. Natl. Acad. Sci. U. S. A. 2003, 100 (15), 8951–8956.
(5) Wyatt, M. A.; Wang, W.; Roux, C. M.; Beasley, F. C.; Heinrichs, D. E.; Dunman, P. M.; Magarvey, N. A. Staphylococcus Aureus Nonribosomal Peptide Secondary Metabolites Regulate Virulence. Science 2010, 329 (5989), 294–296.
(6) García, A.; Fox, J. G.; Besser, T. E. Zoonotic Enterohemorrhagic Escherichia Coli: A One Health Perspective. ILAR J. 2010, 51 (3), 221–232.
(7) Silva-Junior, E. A.; Razzini, A. C.; Paludo, C. R.; Nascimento, F. S.; Currie, C. R.; Clardy, J.; Pupo, M. T. Pyrazines from Bacteria and Ants: Convergent Chemistry within an Ecological Niche. Sci. Rep. 2018, 8 (1), 2595–2595.
(8) Papenfort, K.; Silpe, J. E.; Schramma, K. R.; Cong, J.-P.; Seyedosayyamdst, M. R.; Bassler, B. L. A Vibrio Cholerae Autoinducer–Receptor Pair That Controls Biofilm Formation. Nat. Chem. Biol. 2017, 13 (5), 551–557.
(9) Huang, X.; Duddy, O. P.; Silpe, J. E.; Paczkowski, J. E.; Cong, J.; Henke, B. R.; Bassler, B. L. Mechanism Underlying Autoinducer Recognition in the Vibrio Cholerae DPO-VqmA Quorum-Sensing Pathway. bioRxiv 2019, 12.19.881847.
(10) Molla, G.; Nardini, M.; Motta, P.; D’Arrigo, P.; Panzeri, W.; Pollegioni, L. Aminocacetone Oxidase from Streptococcus Oligofermentans Belongs to a New Three-Domain Family of Bacterial Flavoproteins. Biochem. J. 2014, 464 (3), 387–399.