Bacterial Communication

DOI: 10.1002/anie.201202440

Deconvoluting Interspecies Bacterial Communication**

*Roberta J. Worthington and Christian Melander**

autoinducer-2 · bacterial communication · 4,5-dihydroxy-2,3-pentanedione · NMR spectroscopy · quorum sensing

Our understanding of the complexity of bacterial behavior has deepened considerably over the last few decades. Once thought to be simple, single-celled organisms, bacteria have shown an uncanny ability to make community-based decisions in response to diffusible signals. One of the most well known of these behaviors is a process termed quorum sensing (QS), in which bacteria alter their gene expression based upon population density. There are two types of QS that can occur: intraspecies and interspecies communication. In Gram-negative bacteria, intraspecies communication is typically achieved through the use of acyl homoserine lactone derivatives, while in Gram-positive bacteria intraspecies communication is achieved using autoinducing peptides. Given that bacteria rarely exist as single species communities, one fundamental question is the identity of the signal that bacteria employ to sense and respond to the presence of other bacterial species (interspecies communication).

One such putative universal signal is termed autoinducer-2 (AI-2) and is derived from 4,5-dihydroxy-2,3-pentanedione (DPD).[1] The enzyme LuxS catalyzes the synthesis of DPD, and the LuxS/AI-2 system has been identified in more than 55 species of bacteria.[2] DPD on first inspection is a rather simple molecule (Scheme 1 a); however it is able to exist in multiple tautomeric forms, conformations, hydration states, and in the case of *Vibrio harveyi* undergo borate ester formation to achieve a number of distinct molecular architectures, potentially allowing this molecule to serve as a universal signal amongst bacteria. To date, only two AI-2 chemical signals have been definitively characterized: the cyclic hydrate **C2** that is recognized by *Salmonella typhimurium*, *Sinorhizobium melitoti*, *and Yersinia pestis*,[3] and the borate ester **D** that is recognized by *V. harveyi*.[4] It has been documented that DPD can also undergo phosphorylation by the kinase LsrK and affect gene expression in some enterobacteriaceae,[5] thus significantly increasing the potential signaling diversity that can be achieved by DPD.

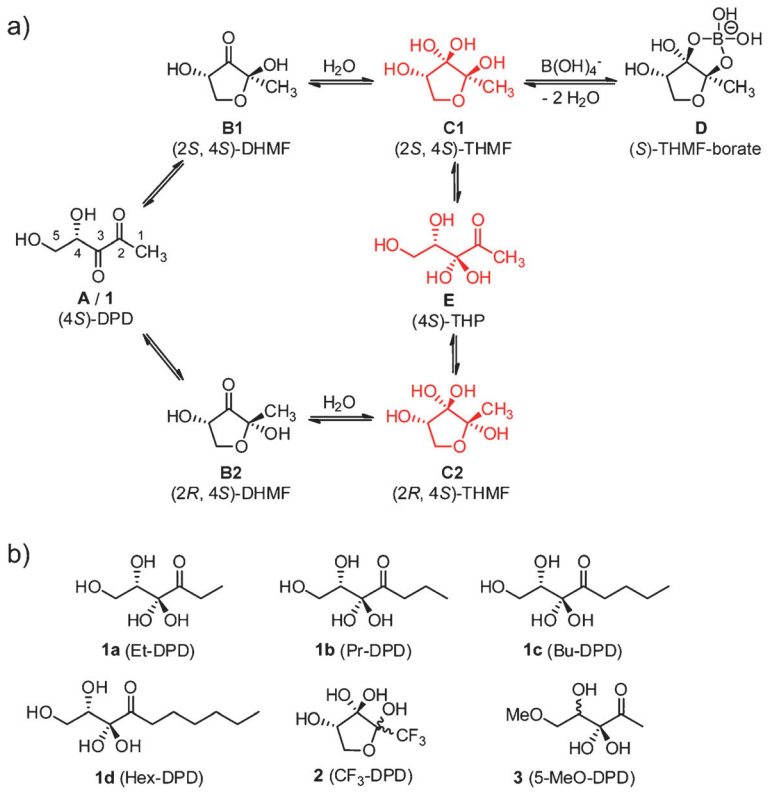
In a current Communication,[6] Janda and co-workers have applied NMR spectroscopy to establish that the structural diversity that exists for DPD at various pH is even greater than previously posited. Previous studies have established that DPD exists in a 1:4 ratio of linear/cyclic forms under strongly acidic conditions.[7] However, upon studying the equilibrium states at physiological pH by ^{1}H NMR spectroscopy, the authors observed a complex spectrum of overlapping methyl and DPD core peaks that could not be fully assigned. Furthermore, an additional signal was noted after 24 h that is subscribed to the final stage of equilibrium. Next, the authors performed a pH study (1–10) and noted that the most significant changes occurred around pH 4–5 while at pH 10 the dominant peaks that are observed at pH 1 disappear completely. Importantly, when the solution is acidified to pH 1 the same spectra initially observed at this pH was obtained, indicating that the observed changes are reversible and that DPD, unlike other quorum sensing signals, is a relatively stable molecule.

Given the complexity of the ^{1}H NMR spectrum, the authors next turned to studying the equilibrium of DPD using ^{13}C NMR spectroscopy, employing both single and double ^{13}C-labeled DPD. At physiological pH, it was determined that the singly labeled DPD exists as eight or nine different species, whose assignment is made possible by the increased resolution of the ^{13}C NMR peaks. Using the relaxation times associated with the ^{13}C NMR nuclei in the doubly labeled DPD sample, it was shown that although the concentration of the original species becomes reduced at higher pH, the ratio of the linear/cyclic equilibrium remains unchanged over the entire pH range studied. Furthermore, by following the chemical shifts associated with the C2 position, the authors note that three major species are present and that there is one major signal at $\delta = 211$ ppm. The consistency of the signal at $\delta = 211$ ppm allows the authors to exclude the occurrence of any hydration/dehydration processes at C3. Based upon the documented tendency of α-diketones to undergo monohydration, the authors posit that the actual structure of linear DPD in solution is in fact hydrate **E**, while based upon additional NOESY and ROESY spectra, the cyclic forms of DPD are hydrates **C1** and **C2**. Although they cannot rule out entirely the presence of **A**, **B1**, or **B2**, no definitive spectroscopic evidence is observed for the existence of these species in aqueous solution.

Finally, the equilibrium is further probed by the synthesis of several DPD analogues and the study of their behavior both in solution and in quorum sensing reporter assays (Scheme 1 b). A 5-methoxy DPD analogue (**3**) that is unable to undergo intramolecular cyclization was synthesized to

[*] Dr. R. J. Worthington, Prof. Dr. C. Melander
Department of Chemistry, North Carolina State University
Raleigh, 27695 NC (USA)
E-mail: ccmeland@ncsu.edu

[**] The authors would like to thank the NIH, the V Foundation, and the DOD for research support.



Scheme 1. a) The equilibrium of autoinducer-2 (AI-2/1) as typically described in the literature (black color) and the equilibrium identified in the study of Janda et al. (red color).[6] The diketone species **A** is in equilibrium with **B1** and **B2**, which are hydrated to grant **C1** and **C2**. The borate species **D** was discovered as the active species in *V. harveyi*. Species **E** is proven as the major linear structure. DHMF = 2,4-dihydroxy-2-methyldihydrofuran-3-one, THMF = 2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran, THP = tetrahydroxypentan-2-one. b) DPD analogues described in Ref. [6].

probe the hydration state of C3 in the linear form of DPD, while trifluoromethyl DPD **2** was synthesized as an analogue that essentially exists exclusively in the cyclic form. As predicted, both **3** and **2** display predominant hydration at C3. The authors then extend this analysis one step further and relate how the equilibrium of DPD analogues explains the agonist/antagonist activity in *S. typhimurium*. Specifically, since *S. typhimurium* recognizes the linear form of DPD as its quorum signal, molecules that are less prone to intramolecular cyclization could (assuming they are not sterically precluded from binding the receptor) act as potent modulators of QS. Propyl-DPD (**1b**), butyl-DPD (**1c**), and hexyl-DPD (**1d**) all have ratios of less than 1:1 cyclic/linear forms (in comparison to DPD itself which is 4.3:1) and are all potent antagonists of AI-2 QS in *S. typhimurium*, while ethyl-DPD (**1a**), which has a ratio of greater than 1:1 cyclic/linear form is a weak agonist.[8]

There is no doubt that new and innovative approaches to combating bacterial infections are sorely needed. This situation is so dire that the World Health Organization has recently named multi-drug resistant bacteria as one of the top three global threats to human health.[9] By providing a detailed behavior of DPD, Janda and co-workers have opened the door to many new possibilities by establishing that a single molecule is able to accomplish interspecies communication. This will surely lay the groundwork to allow the medicinal chemistry community to design new, more potent analogues of DPD with antivirulence activity as an innovative strategy for the control of bacterial infections in vivo.

Received: March 28, 2012
Published online: May 29, 2012

[1] M. G. Surette, M. B. Miller, B. L. Bassler, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 1639 – 1644.
[2] K. R. Hardie, K. Heurlier, *Nat. Rev. Microbiol.* **2008**, *6*, 635 – 643.
[3] J. S. Kavanaugh, L. Gakhar, A. R. Horswill, *Acta Crystallogr. Sect. F* **2011**, *67*, 1501 – 1505.
[4] X. Chen, S. Schauder, N. Potier, A. Van Dorsselaer, I. Pelczer, B. L. Bassler, F. M. Hughson, *Nature* **2002**, *415*, 545 – 549.
[5] K. B. Xavier, B. L. Bassler, *J. Bacteriol.* **2005**, *187*, 238 – 248.
[6] D. Globisch, C. A. Lowery, K. C. McCague, K. D. Janda, *Angew. Chem.* **2012**, *124*, 4280 – 4284; *Angew. Chem. Int. Ed.* **2012**, *51*, 4204 – 4208.
[7] M. F. Semmelhack, S. R. Campagna, M. J. Federle, B. L. Bassler, *Org. Lett.* **2005**, *7*, 569 – 572.
[8] C. A. Lowery, J. Park, G. F. Kaufmann, K. D. Janda, *J. Am. Chem. Soc.* **2008**, *130*, 9200 – 9201.
[9] Infectious Diseases Society of America, *Clin. Infect. Dis.* **2010**, *50*, 1081 – 1083.

