Journal of Biomolecular NMR **29:** 551–552, 2004.

Letter to the Editor: ^{1}H, ^{15}N, and ^{13}C chemical shift assignments of the *Vibrio harveyi* histidine phosphotransferase protein LuxU

Dagny L. Ulrich[a,b], Richele Thompson[c], Bonnie Bassler[d], John Cavanagh[c,*] & J. Patrick Loria[a,*]

[a]*Department of Chemistry, Yale University, Sterling Chemistry Laboratory, New Haven, CT 06520, U.S.A.;* [b]*Department of Pharmacology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520, U.S.A.;* [c]*Department of Molecular and Structural Biochemistry, North Carolina State University, Raleigh, NC 27695, U.S.A.;* [d]*Department of Molecular Biology, Princeton University, Princeton, NJ 08544, U.S.A.*

Received 16 March 2004; Accepted 8 April 2004

Biological context

Quorum sensing is a form of bacterial communication, which involves the production and detection of extracellular signaling molecules called autoinducers (AI) (Miller and Bassler, 2001). Quorum sensing is found in both Gram-negative and Gram-positive bacteria, and plays a role in communication within and between bacterial species. While the mechanism and function of the signal transduction system differ in nearly every bacterial species, the end result of quorum sensing is the ability to adapt to complex environmental cues. Thus the process of quorum sensing allows bacteria to employ coordinated survival mechanisms, including the release of virulence factors, formation of biofilms and sporulation. In *Vibrio harveyi*, a quorum-sensing system controls light emission in response to cell density (Bassler, 1999).

The quorum sensing system of *Vibrio harveyi* is unique in that it possesses two autoinducer-response pathways that converge to regulate the cell-density dependent expression of the luciferase operon. Bacterial autoinducers AI-1 and AI-2 are recognized by membrane-bound sensor-kinase response regulator hybrid proteins LuxN and LuxQ. When AI levels are low, LuxQ and LuxN act as kinases and autophosphorylate their response regulator domains. The phosphate is subsequently transferred sequentially to the histidine-58 of the phosphorelay protein LuxU, and then to the aspartic acid of the response regulator protein LuxO (Freeman and Bassler, 1999). The role of LuxU is crucial in this signaling system, as it must function as both a phosphate receiver and a phosphate donor. LuxU has been identified as a member of the Histidine phosphorelay protein family, which includes *Saccharomyces cerevisiae* Ypd1 and *E. coli* ArcB HPt domain. These proteins are known to possess a consensus sequence of semi-conserved residues surrounding the histidine residue, which is involved in phosphate transfer (Freeman and Bassler, 1999). While there is little overall sequence homology among members of this family, the characteristic structure of a phosphorelay protein is a four-helix bundle. It is not known what structural features enable these proteins to recognize and interact with multiple proteins within their signaling pathway or what structural changes may take place upon phosphorylation (Qingping and West, 1999). As an initial step in the structural characterization of LuxU, we report the backbone and side chain ^{1}H, ^{13}C, and ^{15}N resonance assignments of *V. harveyi* LuxU.

Methods and experiments

The full-length gene specifying LuxU was cloned into a modified pRSET A vector (Invitrogen), which allowed for the expression of LuxU containing an N-terminal tag of six histidines. *E. coli* BL21DE3-pLysS were transformed with the vector (pDLU) and grown at 37 °C in LB before being transferred to M9 media supplemented with ^{13}C glucose and/or ^{15}N ammonium chloride (Marley et al., 2001). LuxU was purified by affinity chromatography using a Ni^{2+}-NTA column, with protein purity checked by SDS-PAGE and amino acid analysis. Uniformly labeled

*To whom correspondence should be addressed. E-mails: patrick.loria@yale.edu; john_cavanagh@ncsu.edu

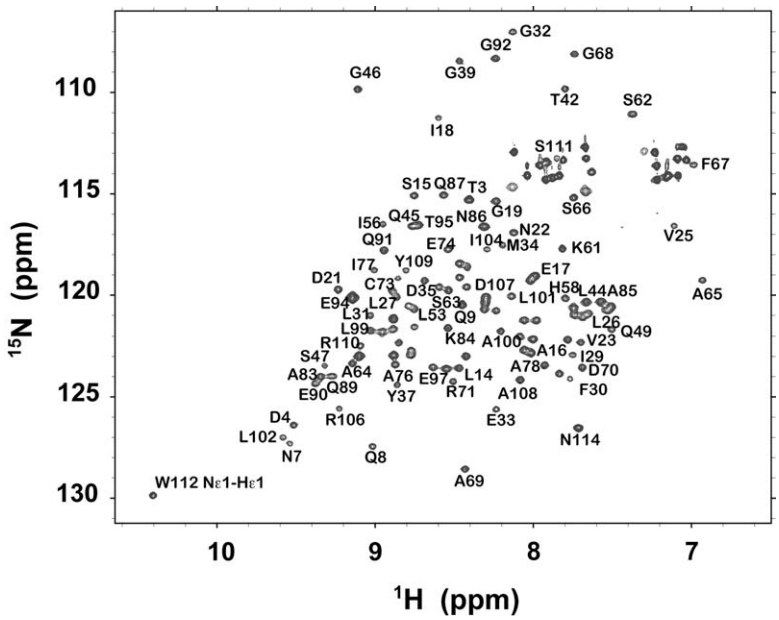

Figure 1. ^{1}H-^{15}N HSQC of LuxU. The spectrum was acquired at 293 K, pH = 6.5, with 128 t1 increments and 16 scans on a Varian Inova600 instrument. Assignments for selected peaks are shown using the one-letter amino acid code.

^{15}N and ^{13}C, ^{15}N NMR samples of 1 mM were prepared using 50 mM phosphate buffer, pH = 6.4, 300 mM NaCl with 10% D2O. All spectra were recorded at 21 °C on a Varian Unity Plus 600 MHz, Inova 600 MHz, Inova 500 MHz, and Inova 800 MHz spectrometers equipped with pulsed field gradients and triple resonance probes. Backbone assignments were obtained using the three-dimensional triple resonance experiments HNCA, HN(CO)CA, HN(CA)CO, CBCA(CO)NH, HNCO, and (HCA)CO(CA)NH. Side chain assignments were obtained from three-dimensional H(CCO)NH, C(CO)NH, HCCH-TOCSY, ^{15}N TOCSY-HSQC, HNHA, HNHB, HNCACB,^{15}N NOESY-HSQC, and ^{13}C NOESY-HSQC. All spectra were processed with NMRPipe (Delaglio et al., 1995) and analyzed using Sparky (Goddard and Kneller).

Extent of assignments and data deposition

The ^{1}H-^{15}N HSQC of LuxU is shown in Figure 1. 99% of the backbone resonances were identified. For the side chains, 92% of all ^{13}C chemical shifts and 88% of the proton chemical shifts have been assigned. A list of ^{1}H, ^{13}C and ^{15}N chemical shifts has been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number (BMRB-10401). Based on the Chemical Shift Index (Wishart and Sykes, 1994) for C^{α}, H^{α}, atoms the secondary structure of LuxU is composed of four alpha helices, which is characteristic of the histidine phosphotransferase family.

References

Bassler, B.L. (1999) *Curr. Opin. Microbiol.*, **2**, 582–587.
Delaglio, F., Grzesiek, S., Vuister, G.W., Zhu, G., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.
Freeman, J.A. and Bassler, B.L. (1999) *J. Bacteriol.*, **181**, 899–906.
Goddard, T.D. and Kneller, D.G. *SPARKY 3*, University of California, San Francisco.
Miller, M.B. and Bassler, B.L. (2001) *Annu. Rev. Microbiol.*, **55**, 165–199.
Marley J., Lu, M., Bracken C. (2001) *J. Biomol. NMR*, **20**, 71–75.
Qingping, X. and West, A.H. (1999) *J. Mol. Biol.*, **292**, 1039–1050.
Wishart, D.S. and Sykes, B.D. (1994) *J. Biomol. NMR*, **4**, 171–180.