Journal of Biomolecular NMR (2005) 31: 373–374
DOI 10.1007/s10858-005-2470-0

Letter to the Editor: Backbone and side chain ^{1}H, ^{13}C and ^{15}N assignments for *Escherichia coli* SdiA1-171, the autoinducer-binding domain of a quorum sensing protein

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Received 29 November 2004; Accepted 31 January 2005

Biological context

Bacteria use external pheromone-like chemical factors to communicate. This phenomenon, termed "quorum-sensing" (Nealson and Hastings, 1979) was first noted in a bioluminescent marine bacterium, *Vibrio fischeri*. Many other bacteria appear to utilize quorum-sensing for regulation of gene expression in response to fluctuations in cell population density. Processes regulated in this way include symbiosis, virulence, competence, conjugation, antibiotic production, motility, sporulation and biofilm formation (reviewed in Miller and Bassler, 2001).

Proteins with sequence homology to the LuxR protein of *Vibrio fischeri* and the TraR protein of *Agrobacterium tumefaciens* are found in a wide variety of bacteria. A single analog protein termed SdiA is found in commensal and pathogenic bacteria of the intestine, such as *E. coli* and *Salmonella enterica* serovar Typhimurium. Enteric bacteria apparently utilize an autoinducer system that may be geared towards detection of signals from other species of bacteria (Michael et al., 2001). Quorum sensing mediated by SdiA is directly implicated in the virulence of enteropathogenic and enterohemorrhagic strains of *E. coli*, among other pathogens (reviewed in Gruenheid and Finlay, 2000).

We report the backbone resonance assignments of the full length autoinducer-binding domain SdiAl-171

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Methods and results

Protein expression and purification

The gene for SdiAl-171 (the autoinducer-binding domain), was cloned into a pET21a vector using standard methods. Uniformly $^{13}C/^{15}N$ labeled proteins were prepared by growing transformed *E. coli* EL21(DE3) in minimal medium with $^{15}NH_4Cl/(^{15}NH_4)_2SO_4$ and $^{13}C_6$-glucose as the sole nitrogen and carbon sources. Cells were grown at 37 ° C, induced with 0.5 mM IPTG and 400 μM HSL at an OD_{600} of ~0.8, and left at 15 °C for overnight expression. Perdeuterated proteins were overexpressed essentially the same as described except that bacterial cell were grown in M9 medium in 99.99% D_2O. Cells were harvested, suspended in lysis buffer (25 mM Tris pH 8, 10 mM DTT, 1 mM EDTA), and lysed by sonication. The lysed cells were centrifuged and the supernatant was loaded onto a 10 ml Q-Sepharose column. Protein was eluted from the column with a linear gradient of buffer (25 mM Tris pH 8, 2 mM DTT, 1 mM EDTA, 0.6 M NaCl). Fractions containing SdiA1-171 were verified by SDS-PAGE and concentrated and further purified through a 345 ml Sephacryl S-100 column. The buffer for gel filtration was 25 mM Tris pH 8, 2 mM DTT, 1 mM EDTA and 50 mM NaCl.

NMR sample preparation

Since SdiA1-171 is easy to aggregate and precipitate, extensive tests were carried out to improve the protein solubility and stability. The final NMR buffer was 50 mM sodium acetate, pH 4.2,

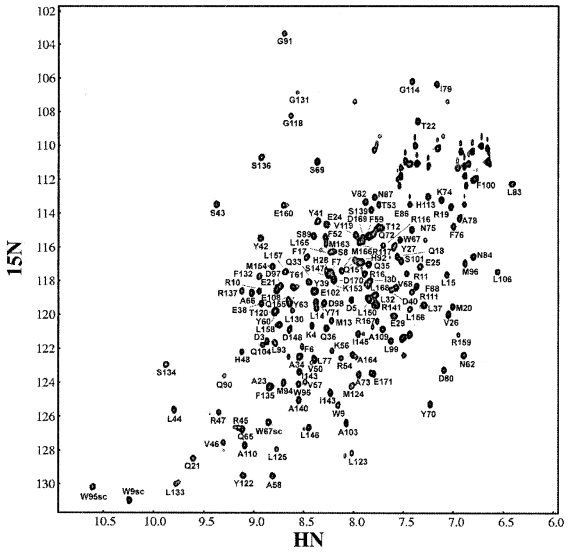

Figure 1. 600 MHz ^{1}H–^{15}N HSQC spectrum of SdiA1–171 showing backbone resonance assignments.

2 mM DTT, 1 mM EDTA and 0.2 M urea. Fractions containing SdiA1-171 from the Sephacryl S-100 column were combined, concentrated and dialyzed against the NMR buffer. NMR samples were at a concentration of approximately 0.5 mM in 0.5 ml of NMR buffer containing 95% H_2O/5% D_2O.

NMR spectroscopy

2D ^{15}N–^{1}H HSQC, 3D ^{15}N resolved NOESY, 3D TROSY based HNCO, HNCA, HN(CO)CA, HN(CA)CO, HNCACB, HN(CA)CB were recorded to obtain sequence specific backbone assignments. Side chain assignments were achieved by a combination of 3D ^{13}C resolved NOESY, HCCH-COSY/TOCSY, (H)CCH-COSY/TOCSY. All spectra were acquired at 20 °C on Bruker DRX600, AVANCE 800 and AVANCE900 spectrometers. Spectra were processed with NMRPipe, and analyzed with NMRView. Proton chemical shifts were referenced to external DSS at 0 ppm. ^{13}C and ^{15}N chemical shifts were referenced indirectly using absolute frequency ratios (Wishart et al., 1995).

Extent of assignments and data deposition

The 2D ^{15}N–^{1}H HSQC spectrum of SdiA 1-171 is shown in Figure 1. The good dispersion of chemical shifts indicates the autoinducer binding domain has ordered structure. At present, out of 162 non-Pro residues, 155 have been assigned in the ^{15}N–^{1}H HSQC spectrum, together with 96%, 94% and 96% of the CA, CB and C′ resonances respectively. Approximately 87% of the side chain ^{1}H and ^{13}C resonances have been assigned. The assigned backbone and side chain chemical shifts have been submitted to the BMRB databank (BMRB-6454).

Acknowledgements

We thank John Chung and Gerard Kroon for help with NMR experiments and Linda Tennant for expert technical assistance. This work was funded by grant GM57374 from the National Institutes of Health.

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