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Letter to the Editor: Backbone ^{1}H, ^{13}C and ^{15}N resonance assignments of the response regulator HP1043 from *Helicobacter pylori*

Eunmi Hong[a], Jin-Won Jung[a], Joon Shin[a], Jin Hwan Kim[b], Young-Ho Jeon[b], Toshio Yamazaki[c] & Weontae Lee[a,*]

[a]*Department of Biochemistry and HTSD-NMR Laboratory, Yonsei University, Seoul 120-749, Korea;* [b]*Crystalgenomics, Inc. Daeduk, Taejon 305-390 Korea;* [c]*RIKEN Genomic Science Center, Tsurumi 230-0045, Yokohama, Japan*

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Biological context

A bacterium *Helicobacter pylori* survives in the harsh acidic environment of the stomach and it causes the duodenal ulcer, gastric cancer and peptic ulcer disease. HP1043, together with HP166 and HP1021, has been identified as a gene encoding an essential cell cycle regulator. HP1043, two-component regulatory protein is the predominant signal transduction device used by prokaryotes, which is frequently involved in the regulation of cellular functions in response to environmental conditions. In addition, it is closely related to the phosphorelay process from a sensor histidine kinase (HK) to an intracellular response regulator protein (RR) that typically acts as a transcription regulator. Previous study suggested that HP1043 formed a dimer *in vivo* and it was localized by the dimerization to the N-terminal regulatory domain (Delany et al., 2002). HP1043 was purified as a symmetric dimer with two functional domains, an N-terminal regulatory domain (∼14 kDa) and C-terminal DNA-binding/transactivation domain (∼11 kDa). Here, we present the backbone resonance assignments of the response regulator HP1043 using TROSY-based triple resonance NMR experiments.

Methods and experiments

The cDNA segment of the encoding two domains of HP1043 from strain 26695 (residues 1-223) was amplified using a primer pair by PCR from the full-length HP1043 cDNA and cloned into the *Bam*HI-*XhoI* sites of pET21b3-2 (Pharmacia Biotech), *E. coli* expression vector. The intact DNA binding domain contained a cleavable His-Tag in its N-terminal coded region from the vector sequence. The vector was used to transform the *E. coli* strain BL21 (DE3) for fusion protein expression. Uniformly [90% ^{2}H/^{13}C/^{15}N]- and [90% ^{2}H/^{15}N]-labeled protein samples were prepared by growing cells in the D_2O M9 minimal media containing $^{15}NH_4Cl$, either with or without $^{13}C_6$-D-glucose as the sole source of nitrogen and carbon. The purified protein was concentrated to 0.8 mM in 50 mM potassium phosphate, 300 mM KCl and 2 mM DTT solution at pH 7.2 with Centricon-10 concentrators (Millipore). Finally, protein was transferred to a 5 mm symmetrical micro cell (Shigemi).

All NMR experiments were performed at 303K on Bruker DRX500, DRX600 and DRX800 spectrometers, each equipped with a triple resonance probe head with gradients. All spectra were collected using deuterium-decoupled TROSY-based triple-resonance pulse sequences (Pervushin et al., 1997) and processed using NMRPipe/NMRDraw software (Delaglio et al., 1995). Processed spectra were further analyzed with the program Sparky. The assignment was started by two-dimensional ^{1}H-^{15}N HSQC and 3D HNCO spectra, to obtain HN(*i*) and ^{15}N(*i*) resonance frequencies and to resolve resonance overlap. The backbone assignments were accomplished using the HNCA, HN(CO)CA, HNCACB and HN(CO)CACB spectra. Backbone carbonyl assignments were mainly derived from the HNCO spectrum. Proton chemical shifts were referenced directly to internal DSS, while ^{15}N

*To whom correspondence should be addressed, E-mail: wlee@spin.yonsei.ac.kr

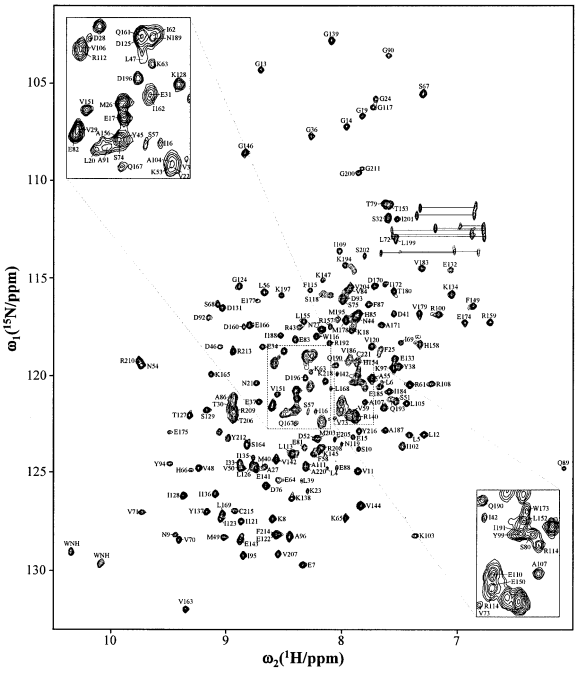

Figure 1. 2D ^{1}H-^{15}N HSQC spectrum of uniformly [^{2}H/^{13}C/^{15}N]-labeled HP1043 recorded on a Bruker DRX800 spectrometer at 303K, pH 7.2.

and ^{13}C shifts were indirectly referenced (Markley et al., 1998).

Extent of assignments and data deposition

Automatic resonance assignment based on the program AUTOASSIGN (Zimmerman et al., 1997) was performed during initial assignment stage (∼30% assignment) and manual assignment was further used to complete backbone assignment. 99% of C^{α}, 97% of ^{13}CO, NH and ^{15}N, and 98% of C^{β} resonances were assigned except for nine proline residues. Based on backbone resonance assignments, we tried to perform sidechain assignments using conventional HCCH-TOCSY and ^{15}N-edited HSQC-TOCSY. However, we were able to assign only 23% of the sidechain proton resonances unambiguously due to poor sensitivity and severe resonance overlap. Figure 1 shows the 2D TROSY ^{1}H-^{15}N HSQC spectrum for the HP1043 domain with assignments. Based on data of consensus chemical shift indices (Wishart and Sykes, 1994) and NOEs, the secondary structure of HP1043 consists of 12 β-strands and 8 α-helices. The backbone and sidechain chemical shifts of HP1043 have been deposited at BioMagResBank (http://www.bmrb.wisc.edu) under accession number 5686.

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