Journal of Biomolecular NMR **30:** 103–104, 2004.

Letter to the Editor: Assignment of ^{1}H, ^{13}C and ^{15}N resonances of the *Escherichia coli* YojN Histidine-Phosphotransferase (HPt) domain

Vladimir V. Rogov, Frank Löhr, Frank Bernhard & Volker Dötsch
Institute of Biophysical Chemistry, Centre for Biomolecular Magnetic Resonance, J.W. Goethe-University, Frankfurt-am-Main, Germany

Received 10 March 2004; Accepted 2 June 2004

Key words: histidine phosphotransferase, phosphorelay system, RcsC, sensor kinase, YojN

Biological context

Prokaryotic organisms sense changing environmental conditions by signalling machineries which use phosphorylation cascades to initiate adaptive responses. The functional units of those systems consist of membrane bound sensor kinases and cytoplasmic response regulator proteins. These two-component systems composed of those basic elements are ubiquitous in bacteria (Hoch and Silhavy, 1995; Appleby et al., 1996). Signal processing is initiated by autophosphorylation of a conserved histidine kinase domain of the sensor protein by using ATP as a donor and the phosphate group is then transferred directly or through a multiple phosphorelay system to a conserved aspartic acid residue in the receiver domain of a cognate response regulator. The phosphorylation acts as a molecular switch and controls the functional state of the response regulator by modulating its DNA-binding or enzymatic activities. The less abundant phosphorelay systems employ multimodular hybrid sensor kinases, and out of a total of 28 sensor kinases encoded by the *E. coli* genome, only six belong to that group (Mizuno, 1997). Classical hybrid kinases like the anaerobic sensor ArcB contain an additional receiver domain followed by a histidine phosphotransferase (HPt) domain and thus allow a more complex His→Asp→His→Asp phosphotransfer scheme. The two hybrid kinases RcsC and YojN of *E. coli* are unusual as they consist of only three domains. RcsC contains a C-terminal receiver domain that is replaced in YojN by an HPt domain. In addition, the central HK domain in YojN seems to be unique as it lacks the highly conserved H-box motif (Takeda et al., 2001). RcsC and YojN are thus assumed to associate as a heterodimer in order to form a functional His→Asp→His→Asp phosphorelay system that controls the phosphorylation state of the global regulator RcsB (Hagiwara et al., 2003). The YojN-HPt domain, therefore, plays a central role in the Rcs signal transfer as it specifically mediates the directed phosphotransfer between the two RcsC and RcsB receiver domains. In order to analyse the signalling mechanism of the Rcs phosphorelay system, we have assigned the resonances of the YojN-HPt domain (residues 775-890). These assignments will be important for direct mapping the binding sites of proteins involved in the Rcs signal transfer and for monitoring the conformational changes via YojN-HPt phosphorylation.

*To whom correspondence should be addressed. E-mail: vdoetsch@em.uni-frankfurt.de

Methods and experiments

The DNA fragment encoding the YojN-HPt domain (residues 775-890) was cloned into the expression vector pQE30 for protein overproduction with an N-terminal poly-$[His]_6$-tag. The method of YojN-HPt expression and purification was designed and adopted for the stable-isotope enrichment in M9 minimal media. The NMR samples contained 0.9–1.1 mM protein in 15 mM Bis-Tris (pH 7.00), 15 mM NaCl, 0.03% NaN_3 in 5%:95% D_2O:H_2O or 100% D_2O.

NMR data were collected at 295 K on Bruker DMX 500, 600 and Avance 700 MHz NMR spectrometers. The spectrometers were equipped with 5 mm triple-resonance ($^{1}H/^{13}C/^{15}N$) probes with XYZ- or Z-gradient capability. Proton chemical shifts were referenced relative to internal DSS. The ^{15}N and ^{13}C chemical shifts were referenced indirectly using the consensus Ξ ratios (Wishart et al., 1995).

A set of 3D triple-resonance [^{15}N,^{1}H]-TROSY-HNCO, (HCA)CO(CA)NH and [^{15}N,^{1}H]-TROSY-HNCACB spectra were collected for the sequen-

tial backbone assignment. Side-chain assignment was achieved using the following 3D experiments: HBHA(CBCA)(CO)NH, H(CC)(CO)NH-TOCSY, (H)CC)(CO)NH-TOCSY, ^{15}N-edited TOCSY-HSQC. The resonances of aromatic ring protons were assigned using 2D ^{1}H-^{1}H TOCSY in D_2O. The assignment was confirmed and completed with 3D NOESY-[^{15}N,^{1}H]-TROSY, 3D NOESY-[^{13}C,^{1}H]-HSQC, 3D NOESY-ct-[^{13}C,^{1}H]-HSQC and 3D NOESY-ct-[^{13}C,^{1}H]-TROSY experiments.

Extent of assignments and data deposition

Nearly complete backbone and side-chain resonance assignments were achieved for YojN-HPt, except for the acidic carboxyl groups (D, E), hydroxyl protons and ^{1}H,^{15}N resonances of the sidechains of Arg and Lys. The assigned ^{1}H,^{15}N resonances are presented in figure 1A. For simplicity, the protein residues are counted taking YojN Met775 as the starting Met1. The secondary structure of YojN-HPt was derived from the consensus chemical shift index (Wishart et al., 1994) and ^{1}H-^{2}H exchange experiments, indicating the presence of six α-helices in the protein structure (data not shown). The N-terminal tail of YojN-HPt (residues 1-26) appears to contain neither regular secondary nor stable tertiary structure.

In the course of resonance assignment a few unusual chemical shifts were observed for the protein nuclei, mostly occurring in the protein regions between predicted elements of the secondary structure, suggesting a participation of these amino acids in tight turns between α-helices.

The puzzling backbone correlations involving resonances of Thr66 were found to be noteworthy. In the (HCA)CO(CA)NH spectra, the resonances of intraresidual carbonyls usually have a higher intensity than sequential correlations, reflecting the relative magnitudes of $^{1}J_{C^{\alpha}_{N}}$ and $^{2}J_{C^{\alpha}_{N}}$ coupling (Löhr and Rüterjans, 1995). In contrast, the intraresidual ^{13}C(O) resonance observed at Thr66 ^{1}H and ^{15}N frequencies in the (HCA)CO(CA)NH spectrum (i.e., the $^{13}C(OO^{-})$ at 176.4 ppm, Figure 1B, upper panel, strip Thr66) is much less intense than the sequential one. It could be interpreted as a missassignment, but analysis of all recorded spectra clearly indicates that the observed resonance belongs to the Thr66. The rational explanation of this discrepancy can be done taking in account the degeneracy of C^{α} and C^{β} resonances of the Thr66 identified in the [^{15}N,^{1}H]-TROSY-HNCACB spectrum (i.e., the $^{13}C^{\alpha}$, $^{13}C^{\beta}$ at 66.3 ppm; Figure 1B, bottom panel, strips Thr66 and Ala67). In this particular case, the strong coupling between C^{α} and C^{β} during the long (≈28 ms) C^{α}–C(O) HMQC-like transfer distorts the intensity of the intraresidual correlation.

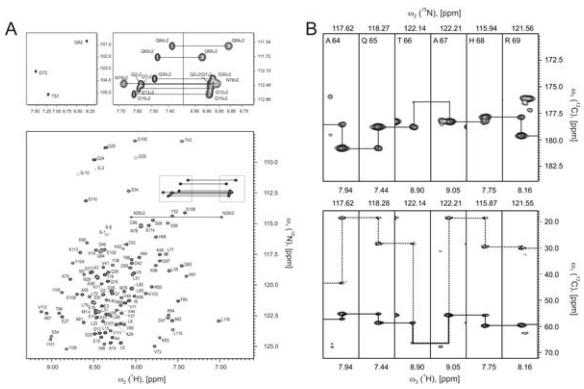

Figure 1. (A) ^{1}H,^{15}N–HSQC spectrum and assignments of YojN-HPt. For the more detailed presentation, the highfield part of the spectrum (containing residues Thr57, Gly72 and Gly82 ^{1}H,^{15}N resonances) is plotted separately (left top square). The Asn and Gln side-chain amide region (thin boxes in the main plot) is expanded (right top squares). (B) Representative strips from (HCA)CO(CA)NH (top panel) and HNCACB (bottom panel) spectra taken at ^{1}H and ^{15}N frequencies of YojN-HPt residues Ala64-Arg69.

The resonance assignments and the chemical shift values for the YojN-HPT protein have been deposited in the BioMagResBank under the accession number 6133.

Acknowledgements

We would like to thank Soma Sengupta and Birgit Schäfer for the help in sample preparation. Financial support was provided by Centre for Biomolecular Magnetic Resonance, Frankfurt, Germany.

References

Appleby, J.L., Parkinson, J.S. and Bourret, R.B. (1996) *Cell*, **86**, 845–848.

Hagiwara, D., Sugiura, M., Oshima, T., Mori, H., Aiba, H., Yamashino, T. and Mizuno, T. (2003) *J. Bacteriol.*, **185**, 5735–5746.

Hoch, J.A. and Silhavy, T.J. eds. (1995) *Two-Component Signal Transduction*, American Society for Microbiology Press, Washington, DC.

Löhr, F. and Rüterjans, H. (1995) *J. Biomol. NMR*, **6**, 189–197.

Mizuno, T. (1997) *DNA Res.*, **4**, 161–168.

Takeda, S.I., Fujisawa, Y., Matsubara, M., Aiba, H. and Mizuno, T. (2001) *Mol. Microbiol.*, **40**, 440–450.

Wishart, D.S. and Sykes, B.D. (1994). *J. Biomol. NMR*, **4**, 171–180.

Wishart, D.S., Bigam, C.G., Yao, J., Abildgaard, F., Dyson, H.J., Oldfield, E., Markley, J.L. and Sykes, B.D. (1995). *J. Biomol. NMR*, **6**, 135–140.