Journal of Biomolecular NMR **27:** 401–402, 2003.
KLUWER/ESCOM
© 2003 *Kluwer Academic Publishers. Printed in the Netherlands.*

Letter to the Editor: ^{1}H, ^{15}N, and ^{13}C chemical shift assignments of the *Escherichia coli* nitrogen regulatory phosphocarrier IIA^{Ntr}

Xia Li[a], Alan Peterkofsky[b] & Guangshun Wang [a,*]
[a]*Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, 986805 Nebraska Medical Center, Omaha, NE 68198-6805, U.S.A.;* [b]*Laboratory of Cell Biology, Building 50, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD 20892, U.S.A.*

Received 1 May 2003; Accepted 20 May 2003

Key words: Escherichia coli, IIA^{Ntr}, nitrogen metabolism, PTS, sigma factor

Biological context

In the bacterial phosphoenolpyruvate (PEP):sugar phosphotransferase system (PTS), the glucose protein cascade plays a central role. The phosphoryl transfer from PEP to glucose is mediated by four proteins, from enzyme I (EI), HPr, IIA^{Glc}, to the glucose transporter IICB^{Glc}. Recent NMR structural studies of the protein-protein complexes in the glucose cascade have significantly enriched our knowledge in this field and also contributed to the structural genomics initiatives of *E. coli* (Peterkofsky et al., 2001). Interestingly, paralogous proteins (EI^{Ntr}, NPr, and IIA^{Ntr}) have been suggested to regulate nitrogen assimilation. It was proposed that these proteins function to link carbon and nitrogen metabolism in bacteria. Indeed, HPr was shown to phosphorylate nitrogen-related enzyme IIA (IIA^{Ntr}), which negatively regulates the transcription of sigma factor σ^{54}-regulated genes (Powell et al., 1995). Subsequently, Bordo et al (1998) solved the x-ray structure of IIA^{Ntr}. To further understand this carbon-nitrogen link at the atomic level, especially the mechanisms of intra- and inter-cascade protein-protein recognition, we commenced NMR studies of the proteins in the nitrogen cascade. Here we report the chemical shifts of ^{1}H, ^{15}N, and ^{13}C resonances of IIA^{Ntr} in aqueous solution.

Methods and results

E. coli chromosomal DNA was used as a template to amplify by PCR the coding sequence for IIA^{Ntr}. The purified PCR product was cloned into the *Nde*I and *Xba*I sites of pRE-His-Tag (Zhu et al., 1997). The recombinant vector, encoding IIA^{Ntr} preceded by a Met followed by six His residues, was transformed into strain GI698. Transformed bacteria were grown at 30 °C in a modified M9 medium to A_{600} of ∼0.5, then induced for production of IIA^{Ntr} by addition of tryptophan (100 μg/ml). For isotope-labeled proteins, $^{15}NH_4Cl$ (1 g l^{-1}) and/or ^{13}C-glucose (2 g l^{-1}) were employed to substitute for corresponding natural abundance compounds. After overnight growth, induced cells were harvested by centrifugation. Cell pellets were resuspended in buffer (25 mM Tris, pH 8.0/200 mM NaCl/1 mM PMSF). The suspension was passed twice through a French press (10 000 psi) and then centrifuged for 1 h at 37 000 rpm. The supernatant solution was purified by affinity chromatography using Talon metal affinity resin (Clontech). After washing out unbound proteins, the His-Tag-IIA^{Ntr} was eluted with 25 mM Tris, pH 8.0/200 mM NaCl/100 mM imidazole. The purified IIA^{Ntr} was sequentially dialyzed against 25 mM Tris, pH 8.0/200 mM NaCl, then 25 mM Tris, pH 8.0, then 25 mM phosphate buffer, pH 7.0, then 2 mM phosphate buffer, pH 7.0 and used for NMR analysis (see Figure 1 legend).

All NMR spectra were collected at 35 °C on a 14.1 Tesla four-channel Varian INOVA600 instrument with waveform generators and triple-axis pulsed field gradient accessories. A suite of double- and triple-resonance NMR experiments in the Varian Proteinpack were performed, including HNCACB, CBCA(CO)NH, HNCA, HN(CO)CA, HNCO, C(CO)NH, H(CCO)NH, HNHA and HNHB

*To whom correspondence should be addressed. E-mail: gwang@unmc.edu

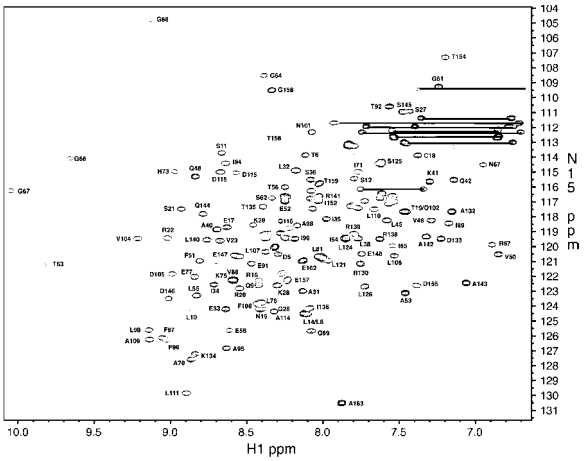

Figure 1. The HSQC spectrum of ∼1 mM ^{15}N,^{13}C-labeled IIA^{Ntr} in 10 mM potassium phosphate buffer, pH 7.3, at 35 °C. Peaks are selectively labeled for clarity. Pairs of the side-chain amide protons of Asn and Gln are connected by solid lines.

(Bax and Grzesiek, 1993; Muhandiram and Kay, 1994). HCCH-TOCSY and ^{15}N- or ^{13}C-separated 3D NOESY spectra were also collected. A DIPSI-3 isotropic mixing time of 12 ms was used for the H^N-detected TOCSY unit and 100 ms was employed in the NOESY case. Typically, 3D experiments were recorded with sweep widths (increments) of 2200 Hz (30) for ^{15}N and 12066 Hz (64) for ^{13}C in the indirect dimensions and 8510.6 and 512 complex points in the ^{1}H-detected dimension, respectively. Data were processed using NMRPipe (Delaglio et al., 1995) and analyzed by PIPP (Garrett et al., 1991). ^{1}H chemical shifts were referenced to DSS and ^{13}C and ^{15}N chemical shifts were referenced according to the IUPAC recommendation (Markley et al., 1998).

Extent of assignments and data deposition

Figure 1 shows the HSQC spectrum of IIA^{Ntr}. The backbone chemical shifts of ^{1}H^N, ^{15}N and ^{13}C resonances for IIA^{Ntr} were assigned based on 3D HNCACB, CBCA(CO)NH, HNCA, HN(CO)CA and HNCO experiments. The H$^\alpha$ and H$^\beta$proton resonances were obtained from 3D HNHA and HNHB experiments. These assignments were subsequently extended to the side chains of the protein via complementary C(CO)NH and H(CCO)NH spectra, yielding ^{13}C and ^{1}H chemical shifts, respectively. The missing or ambiguous resonances in these two spectra were further assigned or corroborated on the 3D HCCH-TOCSY and ^{15}N-edited NOESY- HSQC spectra. The chemical shifts of the aromatic side chains of Y and F were assigned based on a 3D ^{13}C-separated aromatic-only NOESY spectrum. Assignments for side-chain amide H^N and carbonyl resonances were achieved using X(CO)NH type experiments (e.g., X = CBCA) and HNCO, respectively. The assignments were complete except for amide protons of H24, S27, E37, K118, T119, and H122, presumably due to fast hydrogen exchange. Residue P46 was not assigned since the residue which follows it is also a proline. The secondary structure of the protein was deduced based on an analysis of the backbone chemical shifts using the program TALOS (Cornilescu et al., 1999). Helices were identified for residues 10–12, 16–18, 28–43, 47–60, 114–115, 117–132, 134–142 and 146–154 while β-strands were found for residues 14–15, 19–21, 69–75, 83–90, 92–95 and 106–113. Similar secondary structures have been found in the 2.35 Å crystal structure of IIA^{Ntr}(PDB entry: 1abj; Bordo et al., 1995). The chemical shift assignments of IIA^{Ntr} have been deposited with the BioMagResBank in Madison, WI (accession code BMRB 5789).

Acknowledgements

We thank Paul Keifer (Eppley Institute, UNMC) for maintaining the 600 MHz Varian spectrometer and Frank Delaglio and Dan Garrett (NIDDK, NIH) for NMR software and assistance.

References

Bax, A. and Grzesiek, S. (1993) *Acc. Chem. Res.*, **26**, 131–138.

Bordo, D., van Monfort, R.L.M., Pijning, T., Kalk, K.H., Reizer, J., Saier, Jr., M.H. and Dijkstra, B.W. (1998) *J. Mol. Biol.*, **279**, 245–255.

Cornilescu, G., Delaglio, F., and Bax, A. (1999) *J. Biomol. NMR*, **13**, 289–302.

Delaglio, F., Grzesiek, S., Vuister, G.W., Zhu, G., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.

Garrett, D.S., Powers, R., Gronenborn, A.M. and Clore, G.M. (1991) *J. Magn. Reson.*, **95**, 214–220.

Markley, J.L., Bax, A., Arata, Y., Hilbers, C.W., Kaptein, R., Sykes, B.D., Wright, P.E. and Wüthrich, K. (1998) *J. Biomol. NMR*, **12**, 1–23.

Muhandiram, D.R. and Kay, L.E. (1994) *J. Magn. Reson.*, **103B**, 203–216.

Peterkofsky, A., Wang, G., Garrett, D.S., Lee, B.R., Seok, Y.-J. and Clore, G.M. (2001) *J. Mol. Microbiol. Biotechnol.*, **3**, 347–354.

Powell, B.S., Court, D.L., Inada, T., Nakamura, Y., Michotey, V., Cui, X., Reizer, A., Saier, Jr., M.H. and Reizer, J. (1995) *J. Biol. Chem.*, **270**, 4822–4839.

Zhu, P.-P., Nosworthy, N., Ginsburg, A., Miyata, M., Seok, Y.-J. and Peterkofsky, A. (1997) *Biochemistry* **36**, 6947–6953.