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Letter to the Editor: Resonance assignments for the hypothetical protein yggU from *Escherichia coli*

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Biological context

In recent years, several structural genomics initiatives have been established world-wide aimed at rapidly elucidating protein structures of functional and biological interest, as well as providing a more comprehensive picture of protein conformational space. The Northeast Structural Genomics Consortium (NESG) is particularly focused on clusters of eukaryotic domain families from several model organisms, including humans, and homologous proteins from bacteria and archaea (www.nesg.org).

NESG target ER14 is a 100-residue hypothetical protein, yggU, from *Escherichia coli* [Swiss-Pro ID, P52060]. ER14 is a highly soluble, basic (pI = 9.1) protein of unknown function. This protein shows some sequence identity (∼26%) to a second NESG target from *Methanobacterium autotrophicum*, TT135, whose recent solution structure revealed a novel protein fold (Pineda-Lucena et al., 2002). In this note we report the nearly complete backbone and side chain ^{1}H, ^{13}C, and ^{15}N resonance assignments of ER14, determined by a combination of conventional triple-resonance and novel reduced-dimensionality NMR techniques (Szyperski et al., 2002).

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Methods and experiments

Uniformly ^{13}C,^{15}N-enriched ER14 was cloned, expressed and purified following standard protocols used in our consortium. Briefly, the full-length gene (*yggU*) from *E. coli* was cloned into a pET21d (Novagen) derivative, yielding the plasmid pER14-21. The resulting ER14 open reading frame contains eight non-native residues at the C-terminus (LEHHHHHH) of the protein. *E. coli* BL21 (DE3) pMGK cells were transformed with pER14-21, and cultured in MJ minimal medium (Jansson et al., 1996) containing $(^{15}NH_4)_2SO_4$ and *U*-^{13}C-glucose. The final yield of pure *U*-^{13}C,^{15}N ER14 (>97% homogeneity by SDS-PAGE; 12.5 kDa by MALDI-TOF mass spectrometry) was ∼10 mg l^{-1}.

Samples of *U*-^{13}C,^{15}N ER14 for NMR spectroscopy were prepared at a concentration of 1.0 mM in 95% H_2O/5% D_2O solution containing 20 mM MES, 50 mM NaCl, 5 mM DTT at pH 6.5. All NMR data were collected at 20 °C on four-channel Varian INOVA 500, 600, and 750 MHz NMR spectrometers, processed with NMRPipe 2.1 (Delaglio et al., 1995), and analyzed using SPARKY 3.106 (Goddard and Kneller, Univ. Calif., San Francisco). Proton chemical shifts were referenced to DSS, while ^{13}C and ^{15}N chemical shifts were referenced indirectly using the gyromagnetic ratios of ^{13}C:^{1}H (0.251449530) and ^{15}N:^{1}H (0.101329118), respectively. Backbone (H^N, H^α, N, C′, C^α) and C^β resonance assignments were made with AutoAssign

1.9 (Zimmerman et al., 1997; Moseley et al., 2001), using peak lists from 2D ^{1}H-^{15}N HSQC and 3D HNCO, HN(CO)CACB, HNCACB, HN(CO)CA, HNCA, HA(CA)NH, and HA(CACO)NH spectra, along with glycine-specific spectral information derived from a 2D (H^N-N plane) Gly-phased HA(CACO)NH (Montelione et al., 1999). Backbone assignments were also determined by manual analysis of backbone reduced-dimensionality (RD) experiments, $H^{\alpha/\beta}C^{\alpha/\beta}$(CO)NHN, HACA(CO)NHN, HNNCAHA, $H^{\alpha/\beta}$ $C^{\alpha/\beta}$COHA, and HNN<CO,CA> (Szyperski et al., 2002). Side chain aliphatic assignments were determined using 3D (H)CC(CO)NH-TOCSY, H(CCCO)NH-TOCSY, HCCH-COSY, and RD HCCH-COSY experiments. Side chain aromatics were assigned using 2D HBCB(CGCD)HD and H-TOCSY-HCH-COSY RD experiments and 3D ^{13}C-edited NOESY spectra. Individual Asn/Gln side chain amide protons (H(*E*) and H(*Z*)) of were assigned on the basis of NOE intensity in both 3D ^{15}N- and aliphatic ^{13}C-edited NOESY spectra as described previously (Montelione et al., 1984).

Extent of assignments and data deposition

Using the assignment strategy outlined above and neglecting the C-terminal tag, we obtained nearly complete backbone (C′: 98/100; C^{α}: 99/100; H^N-N: 92/93; H^{α}: 107/108) and side chain (C^{β}: 91/92; C^{γ}: 84/89; C^{δ}: 52/59; C^{ε}: 14/19; H^{β}: 150/152; H^{γ}: 123/125; H^{δ}: 78/81; H^{ε}: 37/48; N^{ε}: 1/4) assignments for ^{1}H, ^{13}C, and ^{15}N signals. The assigned ^{1}H-^{15}N HSQC spectrum of ER14 and AutoAssign connectivity map are shown in Figures 1A and B, respectively. Our Assignment Validation Suite (AVS) software (H.N.B. Moseley and G.T. Montelione, in preparation) revealed three unusually upfield shifted ^{1}H resonances, H^N of A44 (5.62 ppm; Figure 1A), an H^{β} of D28 (1.21 ppm), and an H^{γ} of Q62 (1.06 ppm). The ^{1}H, ^{13}C and ^{15}N chemical shift data have been deposited in the BioMagResBank (accession number 5596). These chemical shift data corroborate our initial tertiary structure of ER14, which demonstrates that ER14 is an α/β protein, featuring a long helix and a number of short β-stretches in a ββββαββα topology. We are currently refining the solution structure of ER14 using residual dipolar coupling and stereospecific assignment strategies.

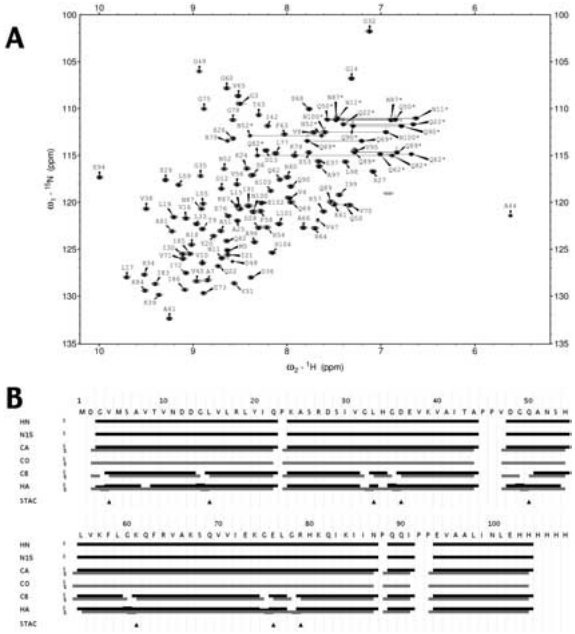

Figure 1. (A) Assigned ^{1}H-^{15}N HSQC spectrum of *E. coli* ER14, pH 6.5, 20 °C. Lines denote pairs of Asn/Gln side chain amide protons, each labeled with asterisks. (B) AutoAssign connectivity map for ER14. Intraresidue and sequential connectivities for the three-rung assignment strategy matching intraresidue and sequential H^{α}, C^{α}, and C^{β} resonances (Moseley et al., 2001) are shown as horizontal black and grey lines, respectively. Sequential spin-system typing assignment constraints (STACs), obtained from the Gly-phased HA(CACO)NH and used as overrides in AutoAssign, are represented by filled triangles.

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