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ARTICLE

NMR assignments of a hypothetical pseudo-knotted protein HP0242 from *Helicobacter pylori*

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Abstract Many knotted proteins have been discovered recently, but the folding process of which remains elusive. HP0242 is a hypothetical protein from *Helicobacter pylori*, which is a model system for studying the folding pathway of a knotted protein. In this study, we report the ^{1}H, ^{13}C, and ^{15}N chemical shift assignments of HP0242. The results will enable us to further investigate HP0242 by NMR experiments.

Keywords HP0242 · Knotted protein · Protein folding

Biological context

Understanding the mechanism by which a polypeptide chain folds into a topologically knotted configuration has emerged to be a very challenging subject for structural biologists. The three-dimensional (3D) structure of HP0242 has been resolved by X-ray crystallography (PDB codes: 2ouf and 2bo3) (King et al. 2010; Tsai et al. 2006). HP0242 is an all α-helical protein that forms a symmetric homodimer with an intertwined knot-like topology. By concatenating the two monomers into a single chain, the concatenated variant can fold into an identical 3D structure as that of the homodimer. As such, the concatenated form would fulfill the criteria of being a trefoil knotted protein (Mallam 2009; Taylor 2000; Virnau et al. 2011).

HP0242 is an ideal model system for studying the folding pathway of a knotted protein thanks to its small size that is tractable for theoretical simulations (King et al. 2010; Li et al. 2012; Sulkowska et al. 2012). Although the folding stability of HP0242 and its concatenated variant has been reported recently, detailed experimental data regarding their folding pathway are currently lacking. Here, we report ^{1}H, ^{13}C, and ^{15}N chemical shift assignments of the wild-type, homodimeric HP0242 as a first step to study the mechanism of forming knot-like structure and as a model to other knotted proteins.

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Method and experiments

Sample preparation

U-[^{13}C, ^{15}N] HP0242 was expressed in BL21 (DE3) *Escherichia coli* strain in M9 minimal media and subsequently purified using a Ni–NTA column (GE Healthcare) followed by gel filtration chromatography using a Superdex 75 (26/60) column (GE Healthcare) with buffer containing 10 mM potassium phosphate (pH 6.5). The typical yield is more than 25 mg/L and the purified proteins were concentrated into 900 μM, aliquotised, flash-frozen by liquid nitrogen and stored at −20 °C (Figs. 1, 2).

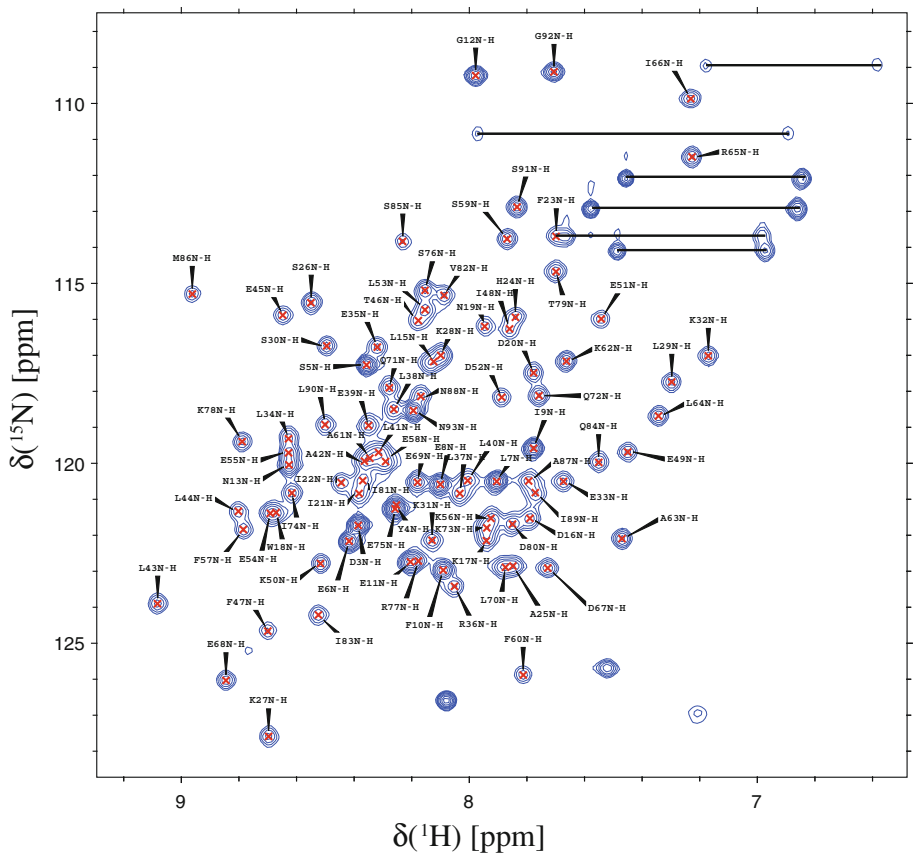

Fig. 1 Assignments of the 2D [^{15}N–^{1}H] HSQC spectrum of HP0242 recorded at 25 °C and 12.1 T. Pairs of side-chain NH_2 resonances are connected by *horizontal lines*

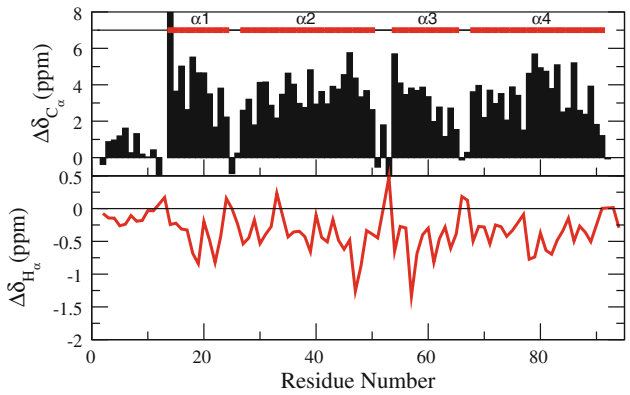

Fig. 2 The secondary chemical shifts of Cα (*upper panel*) and Hα (*lower panel*). The values were derived by subtracting the random coil shifts from the observed chemical shifts ($\Delta\delta = \delta_{observed} - \delta_{random\ coil}$). The residues which are located in the α-helices of the crystal structure (PDB entry 2ouf) are indicated by *red box* at the *top* of the diagram

NMR spectroscopy

The U-[^{13}C, ^{15}N] NMR sample of HP0242 is buffered in 10 mM potassium phosphate (pH 6.5). All NMR data were acquired at 25 °C, using a Bruker Avance III 600 MHz spectrometer equipped with a cryogenic triple resonance probe. [^{15}N–^{1}H] HSQC, HNCO, HN(CA)CO, HNCACB, CBCA(CO)NH experiments were recorded for backbone assignments (H^N, N, C′, Cα, and Hα). Side-chain resonances were assigned using H(CCCO)NH, C(CCO)NH, HBHANH, ^{13}C HSQC experiments(Cavanagh et al. 2007; Sattler et al. 1999). In addition, 2D CBHE and CBHD spectrum were recorded for assigning the resonances of aromatic side chains. All NMR spectra were processed by Topspin (Bruker Biospin) and NMRPipe (Delaglio et al. 1995). Data analysis and assignments were accomplished by Sparky software (Goddard and Kneller) following the procedures as described previously (Hsu et al. 2009; Hsu and Dobson 2009).

Assignments and data deposition

We have assigned all observed cross peaks in the finger print backbone ^{15}N–^{1}H HSQC spectrum (the first two residues at the N-terminus were missing and that of the Pro 14 is not expected). Collectively, we have completed 97 % (543 out of 558) of all backbone resonances (H^N, N, C′, Cα, Cβ, and Hα). Furthermore, the overall completeness of the assignments including side-chains resonances is 92 % (1,149 out of 1,248). Most missing assignments correspond to Cδ of glutamates and Cγ of arginines. These chemical shift assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu/) under accession number

18961. Analysis of the secondary structure by secondary chemical shifts of Cα and Hα as a function of sequence number match well with the X-ray structures (PDB entries 2bo3, 2ouf). The random coil chemical shifts were generated by CamCoil server (De Simone et al. 2009) for the calculation of the secondary chemical shift. The chemical shift assignments of HP0242 will serve as a starting point for detailed characterization of the structure and dynamics of HP0242 at different stages along its folding pathway.

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