Published in final edited form as:

Biomol NMR Assign. 2014 October ; 8(2): 387–389. doi:10.1007/s12104-013-9524-0.

¹H, ¹⁵N, and ¹³C Backbone Resonance Assignments for the *Yersinia* Protein Tyrosine Phosphatase YopH

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Abstract

YopH is a protein tyrosine phosphatase that functions as a required virulence factor in *Yersinia*. Here we report the backbone resonance assignments for a point mutant of the C-terminal catalytic domain of YopH.

Keywords

YopH; protein tyrosine phosphatase; *Yersinia* outer protein; NMR assignments

Biological context

YopH is a protein tyrosine phosphatase (PTP) and one of several *Yersinia* Outer Proteins (Yops) that are required for *Yersinia* pathogenicity (Hamid et al., 1999). Yops are secreted through the type III secretion system into host macrophages where they act to prevent the cytoskeletal rearrangements necessary for phagocytosis of the infecting cell (Visser et al., 1995). YopH disrupts cytoskeletal dynamics by dephosphorylating proteins involved in adhesion signaling and focal adhesion complexes (Hamid et al., 1999). YopH comprises two domains linked by a proline-rich linker. The C-terminal domain (residues 163–468) is responsible for phosphatase activity, and is perhaps the most powerful PTP yet discovered (Hamid et al., 1999). This catalytic domain has a mixed α -helix/ β -sheet fold remarkably similar to that of the human PTP PTP1B, with the active site formed by two loops (Stuckey et al., 1994). The first of these is the flexible WPD-loop (residues 352–361), named for the consensus WPD sequence conserved among PTPs, contains a critical residue, D356, which functions as a general acid/base during catalysis (Brandao et al., 2009). Residues 403–410 form the second active site loop, the P-loop, which is responsible for binding the phosphate moiety of the substrate. The P-loop also contains a conserved cysteine, C403, which functions as a nucleophile in catalysis (Brandao et al., 2009). Here we report sequential assignments of H^N , N^H , C^α , C^β , and backbone carbonyl resonances in a point mutant of the catalytic domain of YopH, C235R, which is more easily expressed in *E. coli* than wild-type, but maintains wild-type activity (Zhang et al., 1992).

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Methods and experiments

The catalytic domain of YopH (hereafter referred to as YopH, for simplicity) was expressed in *E. coli* BL21(DE3) from pET19b plasmid under control of the T7 promoter. (^2H , ^{15}N , ^{13}C)-labeled samples were prepared by growing cells in M9 minimal media prepared in 100% D_2O and with $^{15}\text{NH}_4\text{Cl}$ and ^{13}C -glucose as the sole nitrogen and carbon sources. Cultures were grown at 37°C until the OD_{600} reached 0.8–0.9, at which point 0.5 mM IPTG was added and the temperature was lowered to 20°C . Cells were grown for an additional 9 hours and then harvested by centrifugation and stored at -80°C . YopH was purified according to published protocol (Brandao et al., 2009) with slight modification. At the completion of the published method, fractions containing YopH were pooled, concentrated to 5 mL, and eluted from a Superdex 200 prep grade column (1.6×100 cm) in 20 mM Bis-Tris propane buffer, pH 6.5, containing 100 mM NaCl, 3 mM DTT, 1 mM EDTA, 0.02% NaN_3 , and 7% D_2O . Fractions containing YopH were then pooled and concentrated to 450 μM .

All NMR experiments were performed at 20°C . Resonance assignments for YopH were made using a suite of triple-resonance TROSY experiments. TROSY HNCA, HN(CO)CA, HN(CA)CB, and HN(COCA)CB experiments (Yamazaki et al., 1994) were performed on 900 MHz spectrometers with cryogenic probes. TROSY HNCO and HN(CA)CO (Loria et al., 1999) experiments were performed on 600 MHz spectrometers with cryogenic probes. NMR spectra were processed using NMRPipe (Delaglio et al., 1995) and assignments made using the program CARA (Keller, 2004).

Assignments and data deposition

Sequential assignments were made for 95.4% of resolvable resonances in a TROSY-HSQC spectrum of YopH (Fig. 1), which corresponds to 92.5% of all non-proline residues in YopH. Assignments were made for 91.8%, 88.2%, and 72.3% of all C^α , C^β , and backbone carbonyl resonances, respectively. These assignments include several resonances in each of the active site loops. In the P-loop, six residues have been assigned, including the nucleophilic cysteine, C403. Four residues (T358, A359, V360, and S361) at the C-terminal end of the flexible WPD-loop have been assigned. Additionally, the sidechain indole N-H resonance of W354, the only tryptophan in YopH, is apparent in the TROSY HSQC spectrum. A plot of $^{13}\text{C}^\alpha$ secondary chemical shifts shows close agreement with the known secondary structure (Wishart and Sykes, 1994) of YopH as revealed by X-ray crystallography (Stuckey et al., 1994) (Fig. 2). The assigned backbone chemical shifts for YopH are deposited in the BioMagResBank (www.BMRB.wisc.edu) with accession number 19272.

Acknowledgments

We thank Jeff Hoch, Dmitry Korzhnev, Mark Maciejewski, Geoffrey Armstrong, and Marco Tonelli for access to, and assistance using, their high-field spectrometers. SKW and JPL acknowledge support from NIH grants T32GM008283 and NSF-MCB1121372.

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Fig. 1. ^1H - ^{15}N TROSY HSQC of the catalytic domain of YopH (residues 163–468) collected at 900 MHz, 20°C, and pH 6.5. Assignments are indicated for 95.4% of all resolvable resonances.

Fig. 2. Assigned $^{13}\text{C}_{\alpha}$ secondary chemical shifts ($\delta\text{C}_{\alpha}^{\text{exp}} - \delta\text{C}_{\alpha}^{\text{coil}}$) are plotted in sequential order. A cartoon representation of the secondary structure of YopH is drawn at top, with helices represented by cylinders, beta sheets by zigzag lines, and loops by straight lines. Helices are correlated with regions of positive secondary shifts, while sheets are correlated to negative secondary shifts. The expression-enhancing C235R mutation is located in an extended loop following the first helix.