Journal of Biomolecular NMR, **21:** 69–70, 2001.
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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C assignments of the N-terminal domain of *Yersinia* outer protein H in its apo form and in complex with a phosphotyrosine peptide

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Received 2 April 2001; Accepted 24 June 2001

Key words: heteronuclear NMR, peptide complex, sequential assignment, YopH

Biological context

Pathogenic species of the genus *Yersinia* cause the plague and a range of other gastrointestinal syndromes (Cornelis et al., 1997). The 51 kDa *Yersinia* outer membrane protein H (YopH) is injected into host cells by a type III secretion system, and defeats their non-specific immune response by selective dephosphorylation of essential signaling proteins (Black et al., 2000). YopH contains a tyrosine phosphatase domain (residues 190–468) while the signals for secretion and translocation are contained in the first 71 residues.

The host cell focal adhesion proteins $p130^{Cas}$ and SKAP-HOM are cellular substrates of YopH (Black et al., 2000). These proteins have multiple tyrosine phosphorylation sites in their substrate binding regions that conform to the YopH recognition motif DE(pY)XXP. Moreover, it was demonstrated that residues 1–129 of YopH (YopH-NT) bind to $p130^{Cas}$ in a phosphotyrosine-dependent manner (Black et al., 1998). Besides secretion, the N-terminal region has therefore the additional function of targetting the catalytic domain to substrates in the infected cell.

The Yop virulon comprised of YopH and about 40 other proteins is a well-characterized archetype of type III secretion systems present in many pathogenic Gram-negative bacteria. Study of the structure and function of YopH-NT is thus not only of interest for the understanding of the pathogenic action of YopH itself, but also for insight into the mechanism of infection by a large class of organisms. Elucidation of YopH structure and function might thus contribute to development of anti-bacterial agents designed to render bacteria avirulent by inhibiting deployment of the type III secretion system, rather than killing the bacteria (Galan et al., 1996).

Here we report the ^{1}H, ^{15}N and ^{13}C resonance assignments for YopH-NT as well as its complex with the peptide Ac-DE(pY)DDPF-NH_2 derived from the murine host protein target SKAP-HOM.

Methods and results

YopH-NT (YopH 1-130 with a C-terminal His_6 tag; 14.8 kDa), encoded on a plasmid kindly provided by Dr. J. Bliska (SUNY, Stony Brook), was expressed in *E. coli* DH5α Two samples were prepared for the assignment process. A [^{2}H, ^{15}N, ^{13}C]-containing sample was purified from bacteria grown at 37 °C in M9 minimal medium supplemented with ^{13}C-glucose, $^{15}NH_4Cl$ and $^{2}H_2O$. A [^{15}N, ^{13}C] sample was prepared using ^{13}C glucose and $^{15}NH_4Cl$. Both samples were purified in a single step on a Ni-NTA column (Qiagen) and then dialyzed into the sample buffer (50 mM Na phosphate pH 6.5, 0.05% NaN_3, 5% $^{2}H_20$ in water), and concentrated as appropriate.

Peptide titration experiments: The peptide with the sequence Ac-DEpYDDPF-NH_2 was synthesized at the University of Michigan Protein and Carbohydrate Structure Facility. The optimal concentration of 1.2 peptide:protein required to form a stable complex, was determined from ^{1}H-^{15}N HSQC experiments at increasing concentrations of the peptide.

NMR spectra were recorded on either Bruker 500 MHz AMX, 600MHz AMX, or Varian 800 MHz Inova spectrometers, at 25 °C. All data were processed using NMRPipe (Delaglio et al., 1995). Resolution

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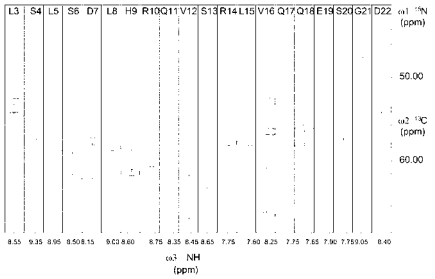

Figure 1. HNCA connectivity pathway for residues L3 to D22 in the complex of YopH-NT with the peptide Ac-DE(pY)DDPF -NH_2 derived from the murine host protein target SKAP-HOM.

was enhanced using forward linear prediction where feasible. Apodization with Lorentzian to Gaussian transformation in the acquisition dimension, and a cosine bell in the indirect dimensions was applied. Time domain data was zero filled to the next power of two.

The [^{2}H,^{15}N,^{13}C] sample was used for the sequential assignment of Cα, Cβ, C′, N, and HN for the apo-protein. The pertinent experiments were: HNCA, HN(CO)CA, HNCO, HN(CA)CO, and HNCACB. All these experiments were based on those described in the literature, with minor modifications (see Yamazaki et al., 1994). Deuterium decoupling was applied in early experiments, but was not found to improve data much for this particular system possibly because the coherence did not reside very long on any of the ^{13}C nuclei, and therefore decoupling was omitted in later experiments. Further verification of sequential assignments was obtained from ^{13}C, ^{15}N resolved- NOESY experiments with mixing times of 100, 400, 800 ms.

The [^{15}N,^{13}C] sample was used for the assignment of aliphatic protons and side-chain carbons. The pertinent experiments were: HN(CA)HA, (H)CCH TOCSY, and HCCH TOCSY (Bax et al., 1993). This sample was later titrated with the phosphopeptide and the resulting sample was used for sequential assignments on the complex. Analysis of strips from the appropriate pairs of $i/i-1$ spectra was carried out using NMRView (Johnson et al., 1994) in the case of the apo-protein and XEASY (Bartels et al. 1995) in the case of complex. Some sequential connectivities for the complex are shown in Figure 1 which also highlights the quality of data.

Extent of assignments and data deposition

The assignments of backbone atoms and Cβ are 93% complete for the apo-protein and 95% for the complex. The amides of Met1, Asn2, Gly29, Gly41, Lys82, His83, Asn84, Leu85 and Asn86 in the case of the apo-protein and Met1, Thr80, Asn84, Leu85 and Asn86 for the complex are missing from all spectra and consequently there are no assignments for these residues. In addition, the carbonyls of Glu50 and Ser51 for the apo-protein and Lys35, Gln40, Ser79 and Val81 for the complex are unassigned. We observed severe exchange broadening of the ^{15}N resonances of several residues in the loop 82–88 (results not shown) and therefore assume that the missing resonances 84–86 are broadened beyond detection. Using the ^{1}Hα, ^{13}Cα, ^{13}Cβ and ^{13}CO chemical shift values, it was predicted by the CSI method (Wishart and Sykes, 1994) that YopH-NT in the complex form contains four α-helices and five β-strands.

Of those resonances for which backbone atoms and Cβ were assigned, approximately 70% of residues for the apo-protein and 90% for the complex have complete side-chain assignments, excluding labile side-chain protons. The assignments have been deposited at the BioMagRes Bank, accession number 4558 for the apo-protein and 4961 for the complex.

Acknowledgements

This work was supported by NIH grants GM-52406 and GM-52421 (E.R.P.Z, P.K. and K.K.) and AI-34095 (M.A.S. and C.S.). We thank Dr J.B. Bliska (SUNY Buffalo) for inspiring this project, and Yuxi Pang and Shawn Y. Stevens for stimulating discussions. The Keck Foundation, NIH and NSF are acknowledged for support of the 800 MHz system.

References

Bartels, C., Xia, T., Billeter, M., Guntert, P. and Wüthrich K. (1995) *J. Biomol. NMR*, **6**, 1–10.
Bax, A. and Grzesiek, S. (1993) *Acc. Chem. Res.*, **26**, 131–138.
Black, D.S., Marie-Cardine, A., Schraven, B. and Bliska, J.B. (2000) *Cell Microbiol.*, **2**, 401–414.
Black, D.S., Montagna, L.G., Zitsmann, S. and Bliska, J.B. (1998) *Molec. Microbiol.* **29**, 1263–1274.
Cornelis, G.R. and Wolf-Watz, H. (1997) *Molec. Micro.,* **23**, 861–867.
Delaglio , F., Grzesiek, S., Vuister, G.W. et al. (1995) *J. Biomol. NMR*, **6**, 277–293.
Galan, J.E. and Bliska, J.B. (1996) *Ann. Rev. Cell Dev.*, **12**, 221–255.
Johnson, B.A. and Blevins, R.A. (1994) *J. Biomol. NMR*, **4**, 603–614.
Wishart, D.S. and Sykes, B.D. (1994) *J. Biomol. NMR*, **4**, 171–180.
Yamazaki, T., Lee, W., Arrowsmith, C.H. et al. (1994) *J. Am. Chem. Soc.*, **116**, 11655–11666.