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# Letter to the Editor: Assignments of $^{1}$H and $^{15}$N resonances of the *Pseudomonas aeruginosa* K122-4 pilin monomer

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## Biological context

*Pseudomonas aeruginosa* is a common gram-negative bacterium that is of clinical importance due to significant morbidity and mortality, resulting from opportunistic infections, in burns, cancer, cystic fibrosis and immuno-compromised patients (Irvin, 1993). The initial interaction of *P. aeruginosa* with a susceptible host is the adherence of the pathogen to the host's mucosal surface, an event that is mediated by type IV pili. These pili are also essential in other bacterial processes such as twitching motility and bacteriophage absorption. These pili are proteinaceous fibres that are approximately 1000–4000 nm long, 52 Å in outer diameter and with a 1.2 Å central channel. They are formed by the ordered, non-covalent association of thousands of subunits of a protein called pilin. Type IV pili and pilins are produced by many species of pathogenic bacteria including the genera *Pseudomonas*, *Neisseria*, *Moraxella*, *Dichelobacter*, *Vibrio* and *Xanthomonas*.

Due to the early role played by the pilus in the infection process, it is a critical target for development of anti-adhesin vaccines against *P. aeruginosa*. Early studies concentrated on a C-terminal receptor-binding domain for glycosphingolipid receptor asialo-$GM_1$ (Sheth et al., 1995). Antibodies produced against a 17-residue free peptide of this region can provide protection against subsequent *Pseudomonas* infection in animal infection models (Sheth et al., 1995). Extensive NMR structural and relaxation studies were performed on this peptide (Campbell et al., 1995, 2000) that may now be compared to these processes in the intact protein. This study extends that work by reporting the assignment of $^{1}$H and $^{15}$N chemical shifts for a truncated pilin monomer of 129 residues that retains its biological properties. These assignments will allow a detailed structural and motional characterization of the pilin protein from *P. aeruginosa* strain K122-4; providing information leading to an understanding of the interactions that occur during the *P. aeruginosa* infection process which will be useful in the development of vaccines that target pilin for use in a clinical environment.

## Methods and experiments

*P. aeruginosa* pilin from strain K122-4 was truncated at the N-terminus to give a recombinant 129-residue protein. The $^{15}$N-labelled protein was expressed in *E. coli* and purified from periplasmic extracts by cation exchange and size exclusion chromatography. The protein consisted of a seven-residue leader sequence that was required for targeting to the periplasm followed by residues 29 to 150 of the native protein. Residues are numbered as found in the native protein. The final NMR samples contained approximately 0.5 mM K122-4 pilin dissolved in 90% $H_2O$/10% $D_2O$ containing 20 mM deuterated sodium acetate, 1 mM sodium azide and 1 mM DSS, pH 5.0.

NMR spectra were obtained at 30 °C on Varian Unity 600 MHz and INOVA 800 MHz spectrometers. The experiments performed included $^{1}$H{$^{15}$N}-HSQC, TOCSY-HSQC (Zhang et al., 1994), NOESY-

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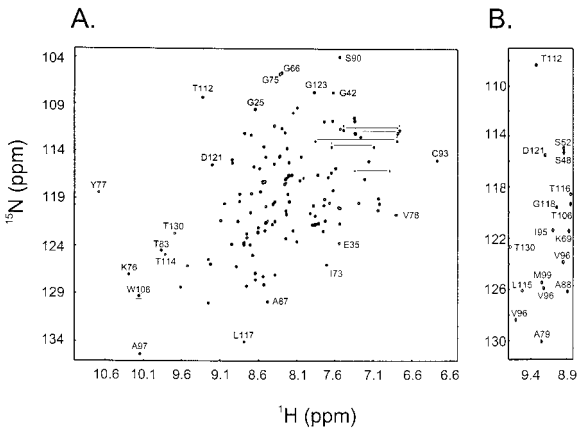


*Figure 1.* (A) 2D $^{1}$H{$^{15}$N}-HSQC NMR spectrum of the $^{15}$N-labelled truncated pilin at 800 MHz and 30 °C. Signals connected by horizontal lines correspond to side chain amide groups of asparagine and glutamine residues whilst the side chain of Trp$^{106}$ is boxed. (B) Expanded region of the spectrum with the assignment for resonances indicated next to the corresponding cross peak.

HSQC and HNHA (Vuister and Bax, 1993). All pulse sequences in water incorporated the WATERGATE sequence (Piotto et al., 1992) to allow significant reduction in the water presaturation powers used at the beginning of the sequences and to improve water suppression. Spectra were processed with NMRPipe (Delaglio et al., 1995) and were then analyzed using NMRView (Johnson and Blevins, 1994).

$^{1}$H and $^{15}$N chemical shifts were assigned using standard techniques. An analysis of the assigned chemical shifts using the CSI method (Wishart and Sykes, 1994) and $^{3}J_{HNHA}$ coupling constants indicated the presence of an α-helical region at the N-terminus (residues 28 to 55) and a four-stranded anti-parallel β-sheet region (residues 78 to 87, 91 to 100, 110 to 119 and 124 to 133). This agrees well with the three-dimensional structure currently being determined (Keizer et al., unpublished).

## Extent of assignments and data deposition

High-quality NMR data for the truncated pilin was obtained as shown by the $^{1}$H{$^{15}$N}-HSQC spectrum collected at 800 MHz in Figure 1. Backbone $^{1}$H and $^{15}$N assignments were complete except for five residues in the disordered leader sequence at the N-terminus, the final four disordered residues at the C-terminus (Thr$^{147}$-Thr$^{148}$-Thr$^{149}$-Pro$^{150}$) and three residues in the C-terminal receptor binding domain (Pro$^{139}$, Lys$^{140}$ and Thr$^{141}$). Resonances were observed for the four threonines but were not assigned due to degeneracy in the chemical shifts, whilst resonances were not observed for Pro$^{139}$, Lys$^{140}$ and Pro$^{150}$. In addition, side chain assignments are nearly complete (>95%) with the exception of some long side chains, particularly belonging to lysine residues. The $^{1}$H and $^{15}$N chemical shifts and $^{3}J_{HNH\alpha}$ coupling constants for the *Pseudomonas aeruginosa* pilin monomer have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under BMRB accession number 4918.

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## References

Campbell, A.P., McInnes, C., Hodges, R.S. and Sykes, B.D. (1995) *Biochemistry*, **34**, 16255–16268.

Campbell, A.P., Spyracopoulos, L., Irvin, R.T. and Sykes, B.D. (2000) *J. Biomol. NMR*, **17**, 239–255.

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

Irvin, R.T. (1993) In *Pseudomonas aeruginosa as an Opportunistic Pathogen* (Campa, M., Ed.), Plenum, New York, NY, pp. 19–42.

Johnson, B.A. and Blevins, R.A. (1994) *J. Biomol. NMR*, **4**, 603–614.

Piotto, M., Saudek, V. and Sklenar, V. (1992) *J. Biomol. NMR*, **2**, 664–665.

Sheth, H.B., Glasier, L.M.G., Willert, N.W., Cachia, P., Kohn, W., Lee, K.K., Paranchych, W., Hodges, R.S. and Irvin, R.T. (1995) *Biomed. Pept. Protein Nucleic Acids*, **1**, 141–148.

Vuister, G.W. and Bax, A. (1993) *J. Am. Chem. Soc.*, **115**, 7772–7777.

Wishart, D.S. and Sykes, B.D. (1994) *Methods Enzymol.*, **239**, 363–392.

Zhang, O., Kay, L.E., Olivier, J.P. and Forman-Kay, J.D. (1994) *J. Biomol. NMR*, **4**, 845–858.