Journal of Biomolecular NMR, **19:** 379–380, 2001.
KLUWER/ESCOM

Letter to the Editor: Assignment of the ^{1}H, ^{13}C and ^{15}N signals of Sortase

Udayar Ilangovan[a], Junji Iwahara[a], Hung Ton-That[b], Olaf Schneewind[b] & Robert T. Clubb[a,*]
[a]*Department of Chemistry and Biochemistry and UCLA-DOE Laboratory of Structural Biology and Molecular Medicine; and* [b]*Department of Microbiology and Immunology, University of California at Los Angeles, 405 Hilgard Ave., Los Angeles, CA 90095-1570, U.S.A.*

Received 18 October 2000; Accepted 19 December 2000

Key words: resonance assignments, Sortase

Biological context

Surface attached proteins on pathogenic gram-positive bacteria can play a major role in the colonization of an animal host, blocking phagocytosis and opsonization and serving as agglutinins and adhesins (Navarre and Schneewind, 1999). Recently, the protein Sortase was shown to be essential for the attachment of proteins to the peptidoglycan cell wall of *Staphylococcus aureus* (Mazmanian et al., 1999). Sortase anchors proteins by processing an LPXTG sorting signal, which is cleaved between the TG peptide bond and linked to the cell wall by a transpeptidation reaction that presumably involves a thioacyl-enzyme intermediate (Ton-That et al., 1999, 2000). Inhibitors of this reaction may prove to be potent antibiotics, since Sortase is highly conserved, involved in the anchoring of a large number of cell surface proteins and important for the pathogenicity of *Staphylococcus aureus* (Mazmanian et al., 2000). Based on primary sequence homology Sortase is unrelated to any protein of known structure, suggesting that knowledge of its structure may provide unique insights into the mechanism of transpeptidation and its ability to recognize specific sorting signals and peptide regions of the cell wall. We present here the ^{1}H, ^{13}C and ^{15}N resonance assignments of Sortase, which form the foundation for its structure determination.

Methods and experiments

The Sortase protein (residues 60–206; 148 residues; molecular weight 16.7 kDa) was overexpressed in *E. coli* strain BL21(DE3) and purified by a combination of anion-, cation- and gel filtration-chromatography (to be published elsewhere). NMR spectra were acquired at 308 K on Bruker DRX-500 and −600 spectrometers. All measurements were performed in $^{1}H_2O$ on either ^{15}N or ^{15}N and ^{13}C labeled Sortase (2.5 mM Sortase protein, 50 mM Tris-HCl (pH 6.7), 100 mM NaCl, 0.01% NaN_3, 20 mM $CaCl_2$, 3 mM DTT and 7% $^{2}H_2O$). Spectra used for resonance assignments were as follows: 2D ^{1}H-^{13}C HSQC, 2D ^{1}H-^{15}N HSQC, 3D HCACO, 3D HNCO, 3D HNCA, 3D HN(CO)CA, 3D HNCACB, 3D CBCA(CO)NH, 3D double ^{15}N-edited HMQC-NOESY-HSQC, 3D ^{15}N-edited NOESY-HSQC, 3D HNHA, 3D HNHB, 3D ^{13}C-edited NOESY-HSQC, 3D HCCH-COSY, 3D CCH-TOCSY, and 3D HCCH-TOCSY. In addition, 3D ^{15}N-edited TOCSY-HSQC, 3D ^{15}N-edited ROESY-HSQC, {^{15}N-} and {^{13}CO-} ^{13}C spin-echo difference 2D CT ^{1}H-^{13}C HSQC spectra and a long-range ^{13}C-^{13}C correlation spectrum were used for stereospecific assignments. Detailed descriptions of these experiments along with their original references have been reviewed elsewhere (Cavanagh et al., 1996). NMR data were processed and analyzed with the NMRPipe (Delaglio et al., 1995) and NMRView (Johnson and Blevins, 1994) software packages, respectively.

Extent of assignments and data deposition

The NMR spectra of Sortase are of high quality as demonstrated in Figure 1A, which shows a representative 2D cross-section of its 4D ^{13}C,^{15}N NOESY spectrum. Assignments were obtained for ∼96%, ∼89% and ∼86% of the ^{13}C, ^{15}N and ^{1}H resonances, respectively. The assignments are complete enough for

*To whom correspondence should be addressed. E-mail: rclubb@mbi.ucla.edu

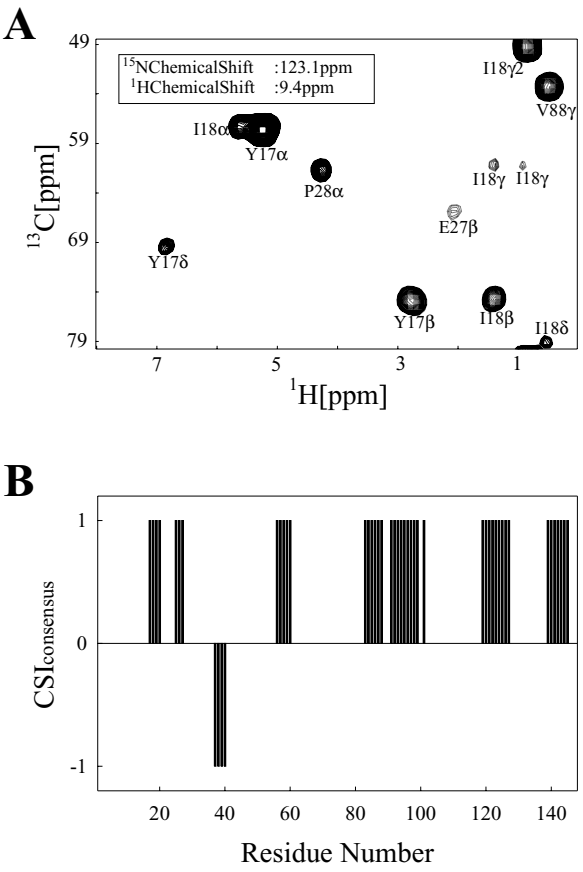

Figure 1. (A) A 2D cross-section through the 4D ^{13}C,^{15}N NOESY spectrum of ^{13}C and ^{15}N labeled Sortase. The cross peaks in this panel correspond to NOEs to the amide of isoleucine-18 and are labeled. The data suggests that the first two strands of the sheet are proximal to one another. (B) Plot of the composite Chemical Shift Index (CSI) (Wishart and Sykes, 1994) as a function of residue number. An index of −1 indicates helical structure, 0 indicates coil, and 1 indicates β-sheet/strand structure.

a structure determination of the protein, which is in progress. Analysis of the secondary chemical shifts indicates that nearly all of the polypeptide is structured and constructed of at least seven beta-strands and a single helix (Figure 1B). The ^{1}H, ^{13}C and ^{15}N chemical shifts have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number BMRB-3407.

Acknowledgements

We thank Dr. Robert Peterson for technical support. This work was supported by a grant from the U.S. Department of Energy (DE-FC-03-87ER60615) to R.T.C.

References

Cavanagh, J., Fairbrother, W.J., Palmer III, A.G. and Skelton, N.J. (1996) *Protein NMR Spectroscopy*, Academic Press, San Diego, CA.

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

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

Mazmanian, S.K., Liu, G., Jensen, E.R., Lenoy, E. and Schneewind, O. (2000) *Proc. Natl. Acad. Sci. USA*, **97**, 5510–5515.

Mazmanian, S.K., Liu, G., Ton-That, H. and Schneewind, O. (1999) *Science*, **285**, 760–763.

Navarre, W.W. and Schneewind, O. (1999) *Microbiol. Mol. Biol. Rev.*, **63**, 174–229.

Ton-That, H., Liu, G., Mazmanian, S.K., Faull, K.F. and Schneewind, O. (1999) *Proc. Natl. Acad. Sci. USA*, **96**, 12424–12429.

Ton-That, H., Mazmanian, S.K., Faull, K.F. and Schneewind, O. (2000) *J. Biol. Chem.*, **275**, 9876–9881.

Wishart, D.S. and Sykes, B.D. (1994) *J. Biomol. NMR*, **4**, 171–180.