Journal of Biomolecular NMR, **18:** 179–180, 2000.
KLUWER/ESCOM

Letter to the Editor: Assignment of the ^{1}H, ^{15}N and ^{13}C resonances of the C-terminal domain of the TolA protein of *Escherichia coli*, involved in cell envelope integrity

Christophe Deprez[a], Laurence Blanchard[a,*], Jean-Pierre Simorre[a], Marthe Gavioli[b], Françoise Guerlesquin[b], Claude Lazdunski[b], Roland Lloubès[b] & Dominique Marion[a]
[a]*Institut de Biologie Structurale 'Jean-Pierre Ebel' (CEA-CNRS), 41 rue Jules Horowitz, F-38027 Grenoble Cedex 1, France;* [b]*Institut de Biologie Structurale et Microbiologie (CNRS), 31 chemin Joseph Aiguier, F-13402 Marseille Cedex 20, France*

Received 10 July 2000; Accepted 31 July 2000

Key words: colicin translocation, heteronuclear NMR assignment, TolA protein, Tol-Pal system

Biological context

The Tol-Pal system of *Escherichia coli* is composed of two protein complexes located in the cell envelope. One is located in the cytoplasmic membrane and involves the TolA, TolQ and TolR proteins interacting with each other by their transmembrane segments (Derouiche et al., 1995; Lazzaroni et al., 1995). The other complex is associated with the outer membrane (Bouveret et al., 1995) and is composed of the outer membrane anchored Pal lipoprotein and the periplasmic TolB protein. *tol-pal* genes have been found in many other Gram negative bacteria in addition to *E. coli*. The precise function of the Tol-Pal system remains unknown apart from its role in cell envelope integrity. A pleiotropic phenotype linked to the loss of outer membrane integrity has been observed for *tol* and *pal* mutants which are leaky for periplasmic proteins, hypersensitive to drugs and detergents (Levengood-Freyermuth et al., 1993), and release outer membrane vesicles (Bernadac et al., 1998). The Tol-Pal system is parasitized by colicins (bacterial toxins produced by and active against *E. coli* and closely related bacteria) and filamentous single-stranded DNA bacteriophages (Webster, 1991). The TolA protein plays a central role in these infection processes. This protein consists of three domains. The N-terminal domain which anchors the protein in the cytoplasmic membrane, the central domain which is assumed to be an α-helical structure long enough to span the periplasmic space, and the C-terminal domain involved in the interaction with colicins (for a review see Lazdunski et al., 1998).

In order to understand the interaction process between colicins and the TolA protein, and to further investigate the Tol-Pal system, we have initiated NMR structural studies of the C-terminal domain of the TolA protein. In this note we report the assignment of the ^{1}H, ^{15}N and ^{13}C resonances of a 106 residue C-terminal domain of the TolA protein.

Methods and results

Isotopically ^{15}N-labeled and $^{15}N/^{13}C$ double-labeled samples of TolA (accession number swiss-prot P19934) spanning amino acids 325 to 421 (corresponding respectively to residue 4 and 100 in our numbering scheme) were obtained from bacteria grown in M9 minimal medium containing respectively 1 g/l $^{15}NH_4Cl$, and 1 g/l $^{15}NH_4Cl$ and 2 g/l $^{13}C_6$-glucose as sole nitrogen and carbon sources. The protein was overexpressed in the periplasm of *E. coli* strain W3110. The construct contained 3 additional residues (AEF) at the N-terminus of the mature protein and 6 additional histidine residues at the C-terminus for purification purposes, which were not removed. Details on the expression and purification will be published elsewhere. Samples used for resonance assignments were prepared in potassium phosphate buffer (50 mM pH 6.8) with protease inhibitor cocktail (Complete, Boehringer Mannheim), 0.5 M NaCl and 10% D_2O.

*To whom correspondence should be addressed. E-mail: laurence@rmn.ibs.fr

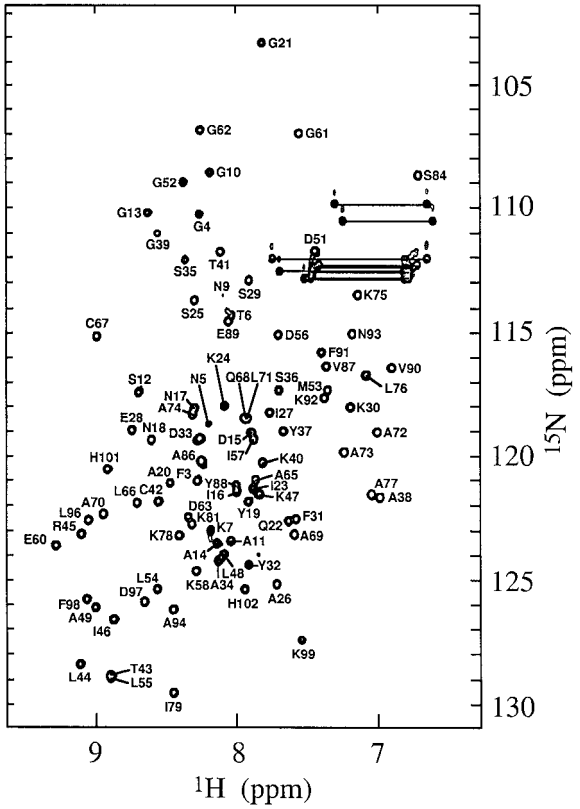

Figure 1. 2D ^{1}H-^{15}N HSQC spectrum of 0.2 mM ^{15}N-labeled C-terminal domain of the TolA protein recorded on a 800 MHz Varian Inova spectrometer at 300 K. The side chain NH_2 resonances of asparagine and glutamine residues are connected by horizontal bars.

The final protein concentrations were respectively 0.2 mM, 0.7 mM (^{15}N samples) and 0.5 mM (^{15}N/^{13}C sample).

NMR spectra were acquired at 300 K on two Varian Inova spectrometers (600 MHz and 800 MHz) equipped with a triple resonance (^{1}H, ^{13}C, ^{15}N) probe including shielded z-gradients. Spectra used for resonance assignments were as follows: 2D ^{1}H-^{15}N HSQC, 3D ^{15}N-edited TOCSY-HSQC, 3D ^{15}N-edited NOESY-HSQC, 3D HNCA, 3D CBCA(CO)NH, 3D HNCO, 3D HC(C)H-TOCSY, 3D (H)CC(CO)NH-TOCSY and 3D H(CC)(CO)NH-TOCSY. All triple resonance experiments used the pulse sequences provided by the Varian protein pack (available at ftp site: ftp.nmr.varian.com). Parameters for all experiments can be obtained from the authors. All data were processed with Felix (Molecular Simulations Inc.) using 90° shifted sine-bell squared apodizing functions. Residual water suppression was achieved using a sinebell convolution. Proton chemical shifts were reported with respect to the H_2O signal relative to DSS. The ^{15}N and ^{13}C chemical shifts were referenced indirectly using the ^{1}H/X frequency ratios of the zero-point: 0.101329118 (^{15}N) and 0.251449530 (^{13}C) (Wishart et al., 1995).

Extent of assignments and data deposition

With the exception of residues N9 and H103 to H106 (poly-histidine tag), 95.7% of nonprolyl backbone ^{15}N and backbone amide ^{1}H, 90.6% of non-labile ^{1}H, 99.7% of protonated ^{13}C (the aromatic are not included) and 86.1% of backbone carbonyl ^{13}C have been assigned. The chemical shift values of ^{1}H, ^{15}N and ^{13}C of the C-terminal domain of the TolA protein have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under accession number BMRB-4771.

Acknowledgements

This work was supported by a grant from PCV (Physique et Chimie du Vivant), the C.N.R.S, the C.E.A. (France) and MSI (Molecular Simulations Inc., San Diego). C.D. is a recipient of a MENRT fellowship.

References

Bernadac, A., Gavioli, M., Lazzaroni, J.C., Raina, S. and Lloubès, R. (1998) *J. Bacteriol.*, **180**, 4872–4878.

Bouveret, E., Derouiche, R., Rigal, A., Lloubès, R., Lazdunski, C. and Bénédetti, H. (1995) *J. Biol. Chem.*, **270**, 11071–11077.

Derouiche, R., Bénédetti, H., Lazzaroni, J.C., Lazdunski, C. and Lloubès, R. (1995) *J. Biol. Chem.*, **270**, 11078–11084.

Lazdunski, C.J., Bouveret, E., Rigal, A., Journet, L., Lloubès, R. and Bénédetti, H. (1998*) J. Bacteriol.*, **180**, 4993–5002.

Lazzaroni, J.-C., Vianney, A., Popot, J.-L., Bénédetti, H., Samatey, F., Lazdunski, C., Portalier, R. and Geli, V. (1995) *J. Mol. Biol.*, **246**, 1–7.

Levengood-Freyermuth, S.K., Click, E.M. and Webster, R.E. (1993) *J. Bacteriol.*, **175**, 222–228.

Webster, R. (1991) *Mol. Microbiol.*, **5**, 1005–1011.

Wishart, D.S., Bigam, C.G., Yao, J., Abildgaard, F., Dyson, H.J., Oldfield, E., Markley, J.L. and Sykes, B. D. (1995) *J. Biomol. NMR*, **6**, 135–140.