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Letter to the Editor: Backbone ^{1}H, ^{13}C and ^{15}N resonance assignment of the N-terminal 24 kDa fragment of the gyrase B subunit from *E. coli*

Massimo Bellanda[a], Evaristo Peggion[a], Gottfried Otting[b], Johan Weigelt[b], Elisabetta Perdonà[c], Enrico Domenici[c], Carla Marchioro[c] & Stefano Mammi[a,*]

[a]*Department of Organic Chemistry, University of Padova, Biopolymer Research Center, C.N.R., I-35131 Padova, Italy;* [b]*Department of Medical Biochemistry and Biophysics, Karolinska Institute, S-171 77, Stockholm, Sweden;* [c]*GlaxoSmithKline Medicines Research Center, I-37135, Verona, Italy*

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Biological context

DNA topoisomerases are ubiquitous enzymes that control the topological state of DNA in cells. There are several classes of topoisomerases, each with distinct properties. Among them, bacterial DNA-gyrase is unique in its function of introducing negative supercoils into closed-circular DNA in a reaction coupled to hydrolysis of ATP. Negative supercoiling prepares DNA for biologically relevant processes that require separation of strands, such as transcription, replication and recombination. Gyrase is essential in all bacteria but it is not found in eukaryotes and is therefore an interesting target for antibiotics. Gyrase consists of 2 subunits, GyrA and GyrB, the active enzyme being an A_2B_2 complex. It is now clear that the protein comprises functional domains associated with distinct biochemical activities. In particular, the N-terminal region of subunit B is responsible for the ATP hydrolysing activity.

The X-ray structure of the dimeric form of the 43 kDa N-terminal domain of gyrase B complexed with ADPNP (Wigley et al., 1991) showed that the ATP binding site is entirely comprised in this domain. It was later proven that the smaller N-terminal 24 kDa fragment (P24) of this domain no longer interacts with ATP while it retains the ability to bind different inhibitors of ATPase activity with affinity comparable to that of the entire domain. Coumarins and cyclothialidines (Lewis et al., 1996) are two classes of natural products isolated from *Streptomyces* that are known to inhibit enzymatic activity of DNA-gyrase by interfering with ATP hydrolysis. These compounds have, however, not enjoyed pharmaceutical success due to poor cell penetration, eukaryotic toxicity, or emerging resistance (Maxwell, 1999).

A backbone resonance assignment of the P24 fragment from *S. aureus*, complexed with novobiocin, was recently published (Klaus et al., 2000). The assignment of fragment P24 in the *apo* form is not available, although this would be important to map the binding site of potential inhibitors. Moreover, all published crystallographic structures of inhibitors complexed with the P24 fragment are of the protein from *E. coli* for which NMR data have not been published. For these reasons, we decided to characterise the *apo* form of the 24 kDa N-terminal fragment of gyrase B from *E. coli* by NMR. Here we report the assignment of backbone ^{1}H, ^{15}N, ^{13}C and $^{13}C^{\beta}$ resonances of this protein. Besides being a useful tool to validate the crystallographic results, this work represents a first step toward an NMR-based procedure to design new antimicrobial agents targeted against DNA-gyrase.

Methods and results

Two samples were utilized for the assignment process, both prepared from *E. coli* XA90 bearing the plasmid pAM24: (i) a doubly labelled [u-$^{15}N/^{13}C$] sample prepared from cells grown at 37 °C in ^{15}N, ^{13}C labelled algal hydrolysate (Celtone-CN from Martek Biosciences), yielding 90 mg of pure protein per litre of culture, and (ii) a partially deuterated [75% ^{2}H, u-$^{15}N/^{13}C$] sample obtained from bacteria grown at 37 °C in minimal medium supplemented with ^{13}C-glucose and $^{15}NH_4Cl$ in 75% D_2O/25% H_2O, yielding about 15 mg of purified protein per litre. The proteins were purified by affinity chromatography on a

*To whom correspondence should be addressed. E-mail: stefano.mammi@unipd.it

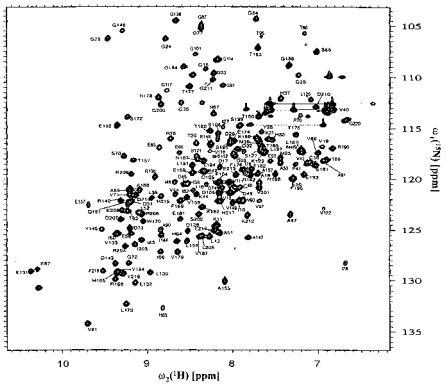

Figure 1. Assigned 2D ^{1}H-^{15}N HSQC spectrum of [75% ^{2}H, u-^{15}N/^{13}C] labelled sample of 24 kDa N-terminal fragment of gyrase B from *E. coli*, acquired on a Varian 800 MHz Unity Plus spectrometer at 298 K.

novobiocin-Sepharose column (Gevi and Domenici, 2002).

A 1.0 mM sample of doubly labelled protein was prepared in 90% H_2O/10% D_2O containing 20 mM phosphate buffer, 40 mM KCl, 40 mM NaCl, 1 mM EDTA, 2 mM DTT and 0.02% (w/v) NaN_3 at pH 7.2. In the second sample, the partially deuterated protein was 1.2 mM in 10 mM phosphate buffer, 20 mM KCl, 1 mM EDTA, 2 mM DTT, 0.02% (w/v) NaN_3 in H_2O/D_2O 9:1 at pH 7.2.

All NMR experiments were acquired on either Bruker 600 MHz DRX or Varian 800 MHz Unity Plus spectrometers at 25 °C.

Sequence specific resonance assignment was obtained by combining data from the following 3D experiments: 3D ^{15}N-edited NOESY-HSQC, HN(CA)HA (recorded with the doubly labelled protein), HNCO, HNCA, HNCACB, HN(CA)CO (recorded with the partially deuterated sample). Triple resonance experiments on the deuterated protein were of the TROSY type (Salzmann et al., 1998, 1999) and deuterium decoupling was applied.

Spectra were processed using the program PROSA (Güntert et al., 1992) and analysed using XEASY (Bartels et al., 1995).

Extent of assignment and data deposition

Spectral analysis allowed the assignment of 196 out of 213 (219 minus 6 prolines) backbone ^{15}N and amide proton resonances. Figure 1 shows the ^{1}H-^{15}N HSQC spectrum of the 24 kDa N-terminal fragment of gyrase B from *E. coli*. Eight of the 17 unassigned residues correspond to the first N-terminal residues. These signals were not observable probably because of the fast exchange rates of the amide protons at the relatively high pH used (pH 7.2). This is consistent with the fact that in all X-ray structures of fragment P24 complexed with different inhibitors, the N-terminus is completely disordered. Unassigned residues I94, M95, L98, H99, G102 are located in the region comprising the loops 80-90 and 99-117 that are also disordered in the co-crystal structures of fragment P24 (Lewis et al., 1996), while they are stabilised by dimer contacts in the complex of the 43 kDa N-terminal gyrase B with ADPNP (Wigley et al., 1991). Other amide resonances remaining to be assigned include those of I134, Q135, H141, E183.

For the residues with assigned amide residues, 96%, 98%, 85%, and 85% of the possible CO, C^{α}, Cβ and Hα chemical shifts were assigned, respectively. For the 6 prolines, assignments were achieved for 6 CO, 4 Cα, 2 Cβ and 5 Hα. With respect to the entire protein, the completeness of the assignment is thus 92%, 90%, 78%, 76% for CO, Cα, Cβ and Hα resonances, respectively.

Using $^{1}H\alpha$, $^{13}C\alpha$, $^{13}C\beta$ and ^{13}CO chemical shift values, the CSI method (Wishart and Sykes, 1994) suggests that the secondary structure of the *apo* protein is essentially equivalent to that found in the co-crystal structures.

The assignment obtained allowed us to map the surface of interaction of the cyclothialidine GR122222 on the fragment P24 and the crystallographic binding site was correctly reproduced (results not shown).

The assignments have been deposited in the BioMagRes Bank (accession number 5218).

References

Bartels, C., Xia, T.-H., Billeter, M., Güntert, P. and Wüthrich, K. (1995) *J. Biomol. NMR*, **5**, 1–10.

Gevi, M. and Domenici, E. (2002) *Anal. Biochem.*, **300**, 34–39.

Güntert, P., Dötsch, V., Wider, G. and Wüthrich, K. (1992) *J. Biomol. NMR*, **2**, 619–629.

Klaus, W., Ross, A., Gsell, B. and Senn, H. (2000) *J. Biomol. NMR*, **16**, 357–358.

Lewis, R.J., Singh, O.M.P., Smith, C.V., Skarzynski, T., Maxwell, A., Wonacott, A.J. and Wigley, D.B. (1996) *EMBO J.*, **15**, 1412–1420.

Maxwell, A. (1999) *Biochem. Soc. Trans.*, **27**, 48–53.

Salzmann, M., Pervushin, K., Wider, G., Senn, H. and Wüthrich, K. (1998) *Proc. Natl. Acad. Sci. USA*, **95**, 13585–13590.

Salzmann, M., Wider, G., Pervushin, K., Senn, H. and Wüthrich, K. (1999) *J. Am. Chem. Soc.*, **121**, 844–848.

Wigley, D.B., Davies, G.J., Dodson, E.J., Maxwell, A. and Dodson, G. (1991) *Nature*, **351**, 624–629.

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