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Letter to the Editor: Backbone resonance assignment of the N-terminal 24 kDa fragment of the gyrase B subunit from *S. aureus* complexed with novobiocin

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Biological context

DNA gyrase is a bacterial enzyme that catalyzes the ATP-dependent introduction of negative supercoils into DNA as well as the decatenation and unknotting of DNA. In its active form it consists of two subunits A and B, arranged in a tetrameric A_2B_2 complex. Subunit A (molecular mass 97 kDa) of DNA gyrase is involved in DNA breakage and reunion while subunit B (molecular mass 90 kDa) catalyzes the hydrolysis of ATP (Reece and Maxwell, 1991). As the protein is an essential enzyme in prokaryotes with no direct counterpart in mammals, it has been seen as an attractive target for the development of antibiotics. The cyclothialidines and the coumarins (a representative of which is novobiocin) are two classes of natural products that inhibit bacterial gyrase activity by interfering with the ATP hydrolysis site located on subunit B. However, these substances are only of limited use in clinical applications as they suffer from toxicity problems and from the emergence of bacterial resistance. Despite their shortcomings these molecules have served as starting points for drug development, and a lot of biochemical and structural information has been obtained over the years. An N-terminal domain of gyrase B with a molecular weight of 43 kDa comprises the complete ATP binding site, while a smaller 24 kDa fragment of this domain does no longer interact with ATP, but has retained the ability to bind cyclothialidines and coumarins with an affinity comparable to that of the holo-enzyme. Structural studies of complexes of these fragments with ATP or with natural compounds revealed their mode of inhibition (Wigley et al., 1991; Tsai et al., 1997). The binding sites for the drugs overlap partially with that of the nucleotide, but are distinct from each other. Based on this knowledge a 3D structure based drug design effort led to the identification of novel inhibitors of DNA gyrase (Böhm et al., 2000). In order to validate these substances and to confirm their proposed binding site on the macromolecule, NMR spectroscopic studies of the 24 kDa fragment of gyrase B were initiated. An obvious prerequisite for a detailed analysis of the protein spectrum is the sequence-specific assignment of its resonances, which is reported here.

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Methods and results

The gyrase B fragment of *S. aureus* (residues Gly24–Lys234, carrying the point mutation Ala27 → Val) was overexpressed in *E. coli* cells under conditions of minimal medium for uniform labeling with ^{13}C and ^{15}N and was purified as described (Andersson et al., 1998). NMR samples used to obtain the backbone resonance assignments consisted of a 1:1 complex with novobiocin at a protein concentration of 1.2 mM in 75 mM ammonium acetate, pH 6.5, 250 mM ammonium sulphate, 10% D_2O, 0.02% NaN_3. Measurements were performed at 308 K on a Bruker DMX 600 equipped with a triple-axis TXI probehead. Proton chemical shifts were referenced to internal 3-(trimethyl-silyl)propane-1,1,2,2,3,3-d_6-sulfonic acid, sodium salt (DSS-d_6). ^{13}C and ^{15}N chemical shifts were referenced indirectly according to the absolute frequency ratios (Wishart et al., 1995). Six 3D heteronuclear experiments were recorded on the double

labeled sample: HNCO, CBCA(CO)NH, HNCACB, HNCA, HBHA(CO)NH, and 3D ^{15}N-edited NOESY-HSQC. Their detailed descriptions, along with their original references, have been reviewed elsewhere (Cavanagh et al., 1996). Spectra were processed and peak-picked using the NMRPipe/NMRDraw suite of programs (Delaglio et al., 1995). For further analysis the spectral data and peak lists were converted into the XEASY data format to be compatible for a computer-assisted assignment with version 2.0 of the program GARANT (Bartels et al., 1996).

Based on 200 resolved cross peaks identified in the HNCO experiment (out of an expected number of 206 as deduced from the protein sequence), the HNCACB, CBCA(CO)NH, and HNCA spectra were checked with the 'filter' routine of GARANT to ensure that for each HNCO signal reliable cross peaks were included in the peak lists of the three other experiments. However, it turned out that the information content of these 'through-bond' experiments was insufficient for GARANT to find a unique and unambiguous solution to the problem as only 65% of the backbone resonances could be assigned. Due to the high degree of degeneracies in the unassigned parts of the backbone, attempts failed to complete the task manually in the usual interactive fashion by correlating strips originating from CBCA(CO)NH, HNCA or HNCACB. Therefore, a 3D ^{15}N-edited NOESY-HSQC- and a HBHA(CO)NH-spectrum, together with structural information based on the X-ray structure of a complex of the 24 kDa fragment of gyrase B in complex with novobiocin (Kostrewa, manuscript in preparation), was included into GARANT. This combination of data allowed for 90% of the protein backbone resonances to be assigned in a single run of the program. Subsequently, by visual inspection of the spectra, remaining ambiguities in the GARANT results could be resolved and some more spin systems were mapped onto the sequence, bringing the extent of assigned backbone resonances to 96%.

Extent of assignments and data deposition

The backbone assignments for the 24 kDa fragment of gyrase B are almost complete. No cross peaks are found in the triple resonance spectra for Arg42, Glu92, Gly110, Lys111, and Ala152. In addition, the N-terminal residue Gly24 is unassigned. The ^{15}N/^{1}H resonances for the segment Gly114-Gly115-Gly116 cluster around 108.2 ppm/8.2 ppm and cannot be resolved individually. Out of 186 C_β atoms of the side chains, 179 are assigned. Proton assignments for H_α- and H_β-atoms are obtained from the HBHA(CO)NH experiment and comprise 83% and 49% of the expected signals, respectively. The numbers are based on the assumption that all these protons are non-degenerate. Figure 1 shows the well-resolved ^{1}H-^{15}N HSQC spectrum of the 24 kDa fragment of gyrase B. The assignments have been deposited in the BioMagResBank (accession number 4575).

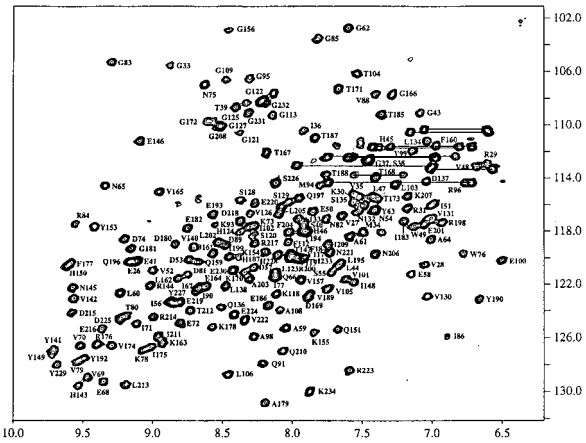

Figure 1. The assigned 2D ^{1}H-^{15}N HSQC spectrum of a 1.2 mM sample of the ^{13}C/^{15}N-labeled 24 kDa fragment of gyrase B (24–234) from *S. aureus*. The experiment was performed at 308 K on a Bruker DMX 600 MHz spectrometer.

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