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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C Backbone resonance assignments of the 40 kDa LicT-CAT-PRD1 protein

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Biological context

LicT from *Bacillus subtilis* is a phosphorylatable regulatory protein that belongs to the BglG/SacY family of transcriptional antiterminators. LicT, like all the proteins of this family, is composed of three domains: the RNA-binding domain, CAT, containing the 56 N-terminal residues, and two consecutive homologous regulatory domains called PRD1 and PRD2. Each PRD is composed of about 100 residues and contains two highly conserved histidine residues which are subject to reversible phosphorylation. Phosphorylations of the PRDs are believed to modify the tertiary and quaternary structure of the protein and thereby to modulate the RNA-binding activity of the CAT domain (van Tilbeurgh and Declerck, 2001). CAT and PRDs form dimers in the full-length protein as well as in the isolated domains (Manival et al., 1997; Declerck et al., 2001). The structure of the LicT-CAT domain alone or in complex with its RNA target has been previously solved by NMR and/or crystallography in our laboratory (Declerck et al., 1999; Yang et al., 2002). Recently, the structure of an activated mutant form of the LicT-PRD1-PRD2 regulatory domain has been elucidated by crystallography (van Tilbeurgh et al., 2001). However, the structural basis of signal transduction and interaction between the PRDs and the RNA-binding domain remains unknown. We have prepared ^{15}N/^{13}C/^{2}H -labelled LicT-CAT-PRD1 in order to analyse its three-dimensional structure by NMR spectroscopy. We report here on the ^{1}H, ^{13}C and ^{15}N resonance assignments for the 40 kDa LicT-CAT-PRD1 homodimer with triple resonance 3D-NMR experiments. The chemical shift assignments will constitute the basis for the characterisation of the solution structure of the interacting domains.

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Methods and experiments

The *licT*(1–167) gene fragment encoding the 167 N-terminal residues of LicT was cloned into a pET15 derivative allowing expression of LicT-CAT-PRD1 fused to a C-terminal His tag (Leu-Glu-6xHis). The fusion protein was produced in *E. coli* BL21(DE3) and purified by immobilised metal affinity chromatography on a Ni-NTA Superflow resin (Qiagen, Germany) followed by gel filtration on a 180 ml Superdex 75 (Pharmacia) column. Isotopically labelled LicT was prepared from cells grown on minimal M9 media containing ^{15}N ammonium chloride, ^{13}C glucose (Eurisotop, St Aubin, France) in pure $^{2}H_20$ (SDS, Marseille, France). NMR samples contained 1 mM LicT-CAT-PRD1, 10 mM sodium phosphate (pH 6.4), 50 mM Na_2SO_4, 0.1 mM benzamidine, 2 mM DTT and 0.1 mM EDTA. All NMR experiments were performed at 20 °C on Bruker DMX-600, DRX-500 and DMX-800 MHz and on Varian Inova 800 MHz spectrometers. The following spectra were used for the ^{1}H, ^{15}N, ^{13}Cα, ^{13}Cβ and ^{13}CO resonance assignments: TROSY-type (Pervushin et al., 1997) ^{1}H-^{15}N HSQC, HNCA, HNCACB, HN(CO)CACB, HN(CO)CA, HN(CA)CO and HNCO. An additional ^{15}N-edited NOESY experiment was recorded (mixing time 175 ms) and proved to be helpful by providing NH_i/NH_{i+1} correlations for helices. ^{1}H chemical shifts were directly referenced to the resonance of 3 (trimethylsilyl) propionic acid-d4 (TSP), while ^{13}C and ^{15}N chemical shifts were

indirectly referenced using internal TSP with the absolute frequency ratios $\Xi(^{13}C/^{1}H) = 0.251449530$ and $\Xi(^{15}N/^{1}H) = 0.101329118$. All the NMR experiments were processed with Gifa (Pons et al., 1996). Assignments were performed using NMRView 5.0 (Johnson and Blevins, 1994).

Extent of assignments and data deposition

The ^{1}H-^{15}N TROSY-HSQC spectrum of triple labelled LicT-CAT-PRD1 is shown in Figure 1. More than 90% of the backbone resonances have been assigned. LicT-CAT-PRD1 is composed of 169 residues (including 2 prolines) plus a six histidine tag. The first histidine of the C-terminal tag has been fully assigned, whereas the five others appear to share degenerate frequencies. Of the 167 remaining NH expected resonances, 155 have been assigned (93%). Backbone and Cβ atoms that could not be definitely assigned included : the NH and ^{15}N resonances for M1 and T49, the NH, ^{15}N and Cβ resonances of F59 and K53, the Cβ and CO resonances of F48, the CO resonances of N52 and L62, all resonances of the segments L63-I66 and V95-L97, the Cβ resonance of S56, S74, S79, S92, D99, W120, T122 and the NH, ^{15}N and Cβ resonances of T144. The missing resonances belong mostly to Thr and Ser rich segments, in particular the linker region between the CAT and PRD1 domains (residue 55-67). As the protein undergoes both precipitation and hydrolysis in the NMR tube, the study presented here that required one month spectrometer time and 3 NMR samples of 1 mM triple-labelled LicT-CAT-PRD1 was stopped at this point.

The assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number BRMB-5362.

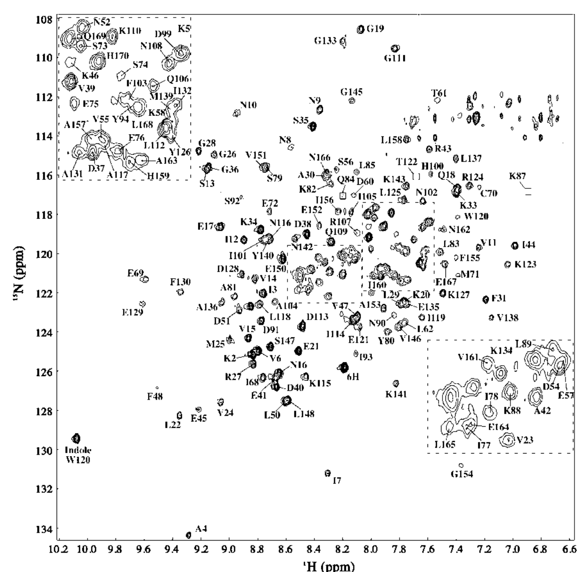

Figure 1. ^{1}H-^{15}N TROSY HSQC spectrum of the CAT-PRD1 domain of LicT recorded at 293 K. The assignments of peaks is indicated with their one-letter amino acid and number. The correlation peak of G154 is folded into the spectrum. The correlation peaks of Q84, K87 and T122 are visible only at higher levels of noise, but their position is however indicated on the spectrum.

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