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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C resonance assignments of the BRCT region of the large subunit of human Replication Factor C**

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Abbreviations: RFC – replication factor C; PCNA – proliferating cell nuclear antigen.

Biological context

Replication factor C (RFC) is a complex of five proteins required for the replication and repair of chromosomal DNA (Waga & Stillman, 1998). The primary function of RFC appears to be to open the toroidally shaped, 'sliding clamp' protein PCNA and 'load' it onto DNA where it serves as a binding platform for a multitude of enzymes and regulatory proteins involved in the replication and repair of DNA. RFC consists of five subunits, four homologous, low molecular weight proteins and a fifth, which has a molecular mass of 140 kDa in mammals (referred to as p140). The N-terminal half of RFC p140 contains sequences unique to RFC, including a region shown to have DNA binding activity (Tsurimoto and Stillman, 1991; Burbelo et al., 1993; Fotedar et al., 1996; Allen et al., 1998), but that is not required for the clamp loading activity (Uhlmann et al., 1997; Gomes et al., 2000).

The DNA binding region, which is homologous to bacterial DNA ligases (Burbelo et al., 1993), has an unusual specificity for the 5′ phosphorylated terminus of dsDNA (Allen et al., 1998). Interestingly, binding is independent of the sequence of the double-stranded region, so long as there are at least 10 fully paired bases (manuscript in preparation). Since the ligase homology was initially noted, it has been recognized that sequences between residues 403 and 486 of human RFC p140 form part of a distinct class of BRCT domains (Bork et al., 1997; Callebaut and Mornon, 1997). However, it has been shown that residues outside of this region are additionally required for DNA binding (Allen et al., 1998 and manuscript in preparation) thus bringing into question whether the residues 403–486 actually fold similarly to known BRCT domains. As part of a project to determine the biological role of the N-terminal half of RFC p140 and to further understand the molecular mechanism of the unusual DNA recognition, we are determining the solution structure of a protein-DNA complex that consists of residues 375–480 and a 10 bp, hairpin oligonucleotide with a recessed, 5′ phosphorylated terminus. Due to, presumably, dynamic behaviour whose frequency is intermediate on the NMR timescale, the spectra of some residues of the protein are of poor quality. In particular, the majority of Hα–Cα and Hβ–Cβ correlations are absent or weak in the 3D [^{13}C,^{1}H] NOESY-HSQC (Figure 1). Despite this fact, we report here essentially complete ^{1}H, ^{15}N and ^{13}C resonances of the 19 kDa protein moiety of the protein-DNA complex.

Methods and experiments

The gene coding for human RFC p140 residues 375–480 was cloned into pET-20b (Novagen) to allow its expression in fusion with a C-terminal His_6-tag. The final plasmid codes for Met and Asn

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These data have been deposited in BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number **6353.

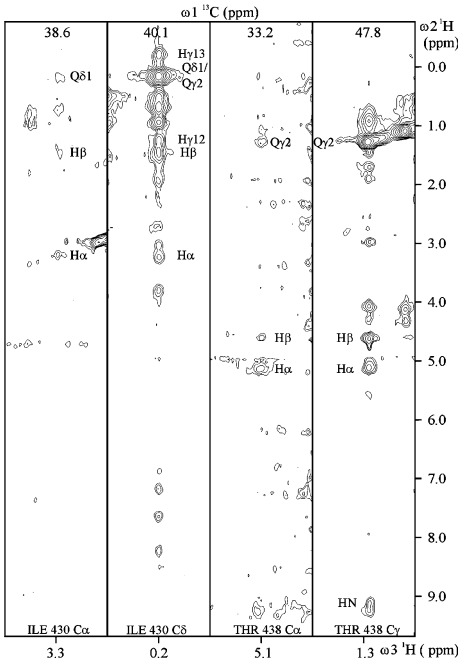

Figure 1. Selected strips from the 3D [^{13}C,^{1}H]-NOESY HSQC of the RFC p140(375–480)-DNA complex. Strips from the Hα–Cα and Qδ1–Cδ1 correlation of IIe 430 and the Hα–Cα and Qγ2–Cγ2 correlation of Thr 438 are shown. The ^{13}C chemical shift is shown above each strip.

prior to residue 375 and Asn, Leu and Glu before the C-terminal His_6-tag. Recombinant protein was produced in *Escherichia coli* BL21(DE3), purified by immobilized metal affinity chromatography on HisBind resin (Novagen) charged with Ni^{2+} ions and subsequently gel filtration using Superose 12 resin (1.6 × 75 cm, Amersham Biosciences). Isotopically labeled proteins were prepared from cells grown in M9-based minimal medium supplemented with $^{15}NH_4Cl$ as the sole nitrogen source, and either $^{13}C_6$-glucose or unlabeled glucose. To form the complex, RFC p140(375–480) was diluted to 10 μM in 25 mM Tris-HCl pH 7.5, 5 mM NaCl, 1 mM DTT and 1.2 equivalents of the oligonucleotide (pCTCGAGGTCGTCATC-GACCTCGAGATCA) were added. The complex was concentrated to 0.5 mM using vacuum dialysis (Spectrum Labs) and the buffer was exchanged to 25 mM D_{11}-Tris-HCl pH 7.5, 5 mM NaCl in 95/5 H_2O/D_2O.

All NMR data were acquired at 25 °C on a Bruker DMX600 spectrometer. Most of the sequential assignments for the backbone were obtained using 3D HNCACB, CBCA(CO)NH and HBHA(CO)NH spectra. Aliphatic side-chain resonances were derived from 3D HCCH-TOCSY and CCH-TOCSY spectra. Additional data provided by 2D [^{1}H,^{1}H] NOESY, 3D [^{15}N,^{1}H] NOESY-HSQC and [^{13}C,^{1}H] NOESY-HSQC experiments were used for further assignment as well as confirmation of the through-bond data.

Extent of assignments and data deposition

In general, the spectra of the protein-DNA complex are of moderate quality (c.f. Figure 1) displaying linewidths that are broader than expected for a 19 kDa complex and with missing correlations. However, we have been able to assign over 95% of the non-labile aliphatic ^{1}H's and 96% of the protonated aliphatic ^{13}C resonances. More than 99% of the amide ^{1}H and ^{15}N resonances of the backbone have been assigned with chemical shifts missing only for Tyr 379. ^{1}H-^{15}N correlations have been found for all glutamine and asparagine side chains. RFC p140(375–480) has a rather low aromatic content (5 Tyr and 1 Phe). Of these, we have unambiguously assigned the ^{1}H aromatic resonances of Tyr 382, 385 433 and 447, and Phe 412 using a combination of homonuclear and heteronuclear edited NOESY spectra. The chemical shift values have been deposited in the BioMagResBank database under the accession number 6353.

Secondary structure identification was performed based on the chemical shift index using $^{13}C\alpha$, $^{13}C\beta$ and $^{1}H\alpha$ nuclei (Wishart and Sykes, 1994). The result (manuscript in preparation) indicates that secondary structure of RFC p140(375–480) is consistent with the known structures of BRCT domains with an extra α-helix at the N-terminus.

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References

Allen, B.L. et al. (1998) *Nucl. Acids Res.*, **26**, 3877–3882.
Bork, P. et al. (1997) *FASEB J.*, **11**, 68–76.
Burbelo, P.D. et al. (1993) *Proc. Natl. Acad. Sci. USA*, **90**, 11543–11547.
Callebaut, I. and Mornon, J.P. (1997) *FEBS Lett.*, **400**, 25–30.
Fotedar, R. et al. (1996) *EMBO J.*, **15**, 4423–4433.
Gomes, X.V. et al. (2000) *J. Biol. Chem.*, **275**, 14541–14549.
Tsurimoto, T. and Stillman, B. (1991) *J. Biol. Chem.*, **266**, 1950–1960.
Uhlmann, F. et al. (1997) *J. Biol. Chem.*, **272**, 10058–10064.
Waga, S. and Stillman, B. (1998) *Annu. Rev. Biochem.*, **67**, 721–751.
Wishart, D.S. and Sykes, B.D. (1994) *J. Biomol. NMR*, **4**, 171–180.