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Letter to the Editor: Backbone ^{1}H, ^{13}C, and ^{15}N assignments of a 42 kDa RecR homodimer

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Biological context

DNA replication is a key step in the proliferation of living cells. Defects in replication are one of the main causes of genomic instability that leads to ageing and cancer. Recently, DNA homologous recombination has gained increased attention owing to its indispensable involvement in post-replicational repair processes. RecR is one of the key components of the RecFOR dependent replicational repair pathway, which is widely conserved in eubacteria. In *Escherichia coli*, RecR forms a complex with other RecF pathway proteins such as RecO and RecF, in a competitive manner (Bork et al., 2001). The RecR-RecO complex facilitates RecA protein to load onto SSB coated single stranded DNA (Umezu and Kolodner, 1994), while the RecR-RecF complex limits the extension of RecA filaments onto the double stranded DNA adjoining a single-stranded gap (Webb et al., 1997). These concerted actions of RecFOR proteins results in the loading of RecA protein specifically onto SSB-coated gapped DNA (Morimatsu and Kowalczykowski, 2003). Sequence comparison suggested that RecR possesses three major motifs, an N-terminal helix-hairpin-helix motif, a central C4-type zinc finger motif and a C-terminal Toprim domain (Aravind et al., 1998). The presence of the Toprim-domain, a conserved domain found in topoisomerase, DnaG primase and others, and the C4-type zinc finger domain, also conserved in DNA maintenance proteins such as UvrA and Fpg, raises the possibility that RecR might be involved in the recognition of specific DNA structure at the gaps. No detailed structural information exists in the literature for RecF, RecO and RecR. The backbone assignment of RecR is a key step towards the clear understanding of the role of RecR in post-replicational DNA repair by providing a framework for the analyses of the molecular basis of interaction between RecR and RecF, RecO, as well as DNA using NMR methods. Here we report the backbone NMR assignment for the full-length 42.4 kDa *Thermus thermophilus* HB8 RecR homodimer. The *T. thermophilus* RecR shares 45% sequence identity and 57% homology with *E. coli* RecR.

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Methods and experiments

The structural gene for the 194-residue RecR was amplified by PCR using the *T. thermophilus* HB8 genomic DNA as a template. The amplified NdeI-BglII fragment was inserted into the NdeI-BamHI site of a pET11a vector for overexpression in the *E. coli* BL21(DE3) strain. To produce ^{2}H/^{13}C/^{15}N-labelled RecR, the bacteria expressing recombinant RecR were grown in M9 medium (95% $^{2}H_2O$) containing $^{15}NH_4Cl$ and ^{13}C-labelled glucose as the sole nitrogen and carbon sources, respectively. Protein expression was induced with 1 mM IPTG at an OD_{600}

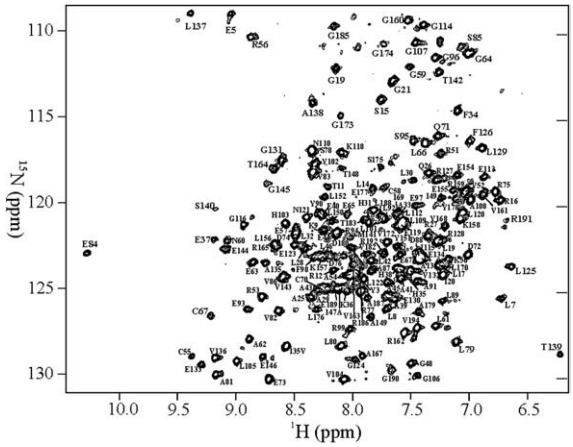

Figure 1. The assigned ^{1}H-^{15}N TROSY-HSQC spectrum of the *Thermus thermophilus* RecR at 45 °C, pH 7.2. Resonances are labelled according to the backbone assignments.

of 0.6, followed by growth for another 7 h. The purification of the protein involved heat treatment at 80 °C for 10 min to remove host proteins, followed by hydrophobic interaction and anion exchange column chromatography. Analytical gel-filtration chromatography of the purified sample showed that *T. thermophilus* RecR exists as a stable homodimer. Samples for NMR-spectroscopy were concentrated to 0.6 mM (0.3 mM in dimer) and dissolved in 20 mM Tris-HCl buffer (pH 7.2) containing 150 mM KCl, 1 mM EDTA and 10 % $^{2}H_2O$.

All NMR experiments were recorded at 45 °C on a Bruker DRX600 spectrometer equipped with an x, y, z-pulsed field-gradient triple resonance probe. Spectra were processed on Linux PCs with the AZARA version 2.7 software package (Boucher, W., unpublished). All of the spectra were analysed on Linux PCs using the OpenGL version of the ANSIG software version 3.3 (Kraulis, 1989) together with customised macro-programs. The sequence specific backbone $^{1}H^{N}$, $^{13}C^{\alpha}$, $^{13}C'$, ^{15}N and side chain $^{13}C^{\beta}$ resonance assignments for $^{2}H/^{13}C/^{15}N$-labelled RecR were obtained from the six TROSY-based (Pervushin et al., 1997) 3D triple-resonance *J*-correlation experiments, HNCA, HN(CO)CA, HN(CA)CB, HN(COCA)CB, HN(CA)CO, and HNCO.

Extent of assignments and data deposition

Nearly complete (97%) assignments of the backbone $^{1}H^{N}$, $^{13}C^{\alpha}$, $^{13}C'$, ^{15}N and side chain $^{13}C^{\beta}$ resonances were obtained for almost all non-proline residues, except for the first two residues of RecR and residues S6, K23, G169, E181 and L184, for which ^{1}H-^{15}N HSQC resonances could not be observed. Figure 1 shows ^{1}H-^{15}N TROSY-HSQC spectrum of $^{2}H/^{13}C/^{15}N$-RecR, in which the assignments are indicated for individual cross peaks. The secondary structure of RecR, predicted using the Chemical Shift Index (CSI) method (Wishart and Sykes, 1994), is in good agreement with the predicted domain patterns of strands and helices: a helix-hairpin-helix motif with αα fold, followed by a zinc-finger motif with ββ fold, and a Toprim domain with βαβαβαβ fold. The $^{1}H^{N}$, $^{13}C^{\alpha}$, ^{13}C,$^{13}C'$, and ^{15}N chemical shifts have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) database under the accession number BMRB-5793. The backbone resonance assignments would now be utilised to map the binding of RecR with RecO, RecF, and DNA, through NMR titration experiments.

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