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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C resonance assignments for the C-terminal protein interaction region of the 32 kDa subunit of human replication protein A

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Biological context

Replication protein A (RPA), the nuclear single-stranded DNA-binding protein in eukaryotes, is required in DNA replication, homologous recombination, nucleotide excision repair and possibly base excision repair, suggesting that it has multiple functions in DNA metabolism (Wold, 1997; Iftode et al., 1999). RPA is composed of three subunits (RPA70, RPA32 and RPA14), each of which is conserved in all eukaryotes. Four similarly folded DNA-binding domains have been identified; three in the central and C-terminal portions of RPA70 and one in the middle part of RPA32. RPA also contains two multi-protein recognition domains; one located at the N-terminus of RPA70 and the other in the C-terminal region of RPA32. We recently initiated structural and binding studies providing direct evidence that the C-terminus of human RPA32 ($RPA32_{172-270}$) interacts with the xeroderma pigmentosum damage-recognition protein XPA, uracil DNA glycosylase, and the DNA recombination protein RAD52. We report here the ^{1}H, ^{15}N and ^{13}C chemical shift assignments for $RPA32_{172-270}$.

Methods and results

Human $RPA32_{172-270}$ was subcloned into pET15b vector (Novagen) and overexpressed in *E. coli* strain BL21(DE3) pLysS as a fusion to an N-terminal hexahistidine tag. Unlabeled, uniformly ^{15}N-, ^{15}N/^{13}C-, and ^{15}N/10% ^{13}C-isotope labeled $RPA_{172-270}$ was obtained by growing the cells in LB broth or M9-minimal media containing $^{15}NH_4Cl$ and $^{13}C_6$-glucose (Cambridge Isotope Laboratories, Inc.). The protein was purified by affinity chromatography on a nickel His-bind column (Novagen) and, after removal of the histidine tag by digestion with thrombin, anion exchange high-performance liquid chromatography on a Mono-Q column (Pharmacia Biotech. Inc.). NMR samples of $RPA32_{172-270}$ ($\sim$1 to 2 mM) were prepared in 25 mM sodium phosphate buffer (pH 7.0), 50 mM NaCl, 5 or 10 mM DTT in either 7% or 99.99% D_2O. NMR experiments were recorded at 25 °C on Bruker AMX-500, AMX-600, DRX-600, DMX-750 and DRX-800 spectrometers. The NMR spectra were processed and analyzed using Felix97 (Molecular Simulations Inc.).

Sequence-specific assignments for the backbone were obtained, using HBHA(CBCACO)NH (Grzesiek et al., 1993), ^{15}N-TOCSY-HSQC, and ^{15}N-NOESY-HSQC spectra with identification of aliphatic carbon side chain spin systems provided by HNCACB (Wittekind and Mueller, 1993), CBCA(CO)NH, and C(CO)NH-TOCSY (Grzesiek and Bax, 1992) spectra. Side chain proton resonances were assigned from H(CCO)NH-TOCSY, ^{15}N-TOCSY-HSQC and ^{13}C-NOESY-HSQC. Aromatic protons were assigned using 2D ^{1}H-NOESY, TOCSY and 2Q (Braunschweiler et al., 1983) spectra. Sixty methylene protons were stereospecifically assigned from the analysis of intra-

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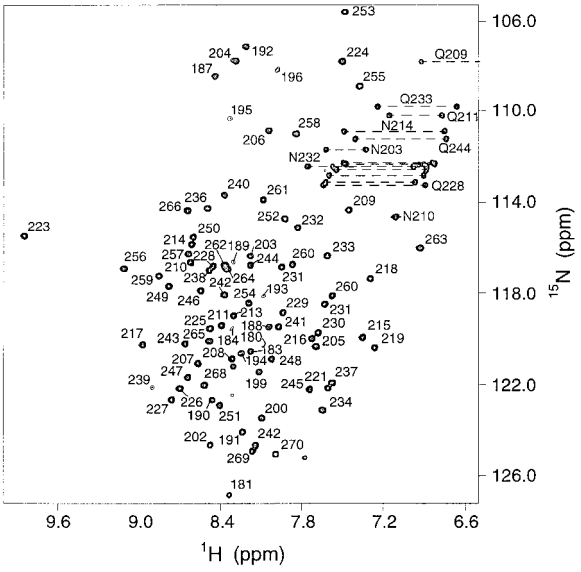

Figure 1. ^{15}N-HSQC NMR spectrum of RPA recorded at pH 7.0 and 25 °C.

residue NOEs in a short mixing time ($\tau_m = 30$ ms) 2D ^{1}H-NOESY combined with information on $^{3}J_{HN-H\beta}$, and $^{3}J_{H\alpha-H\beta}$ coupling constants obtained from 3D HNHB (Madsen et al., 1993) and HACAHB-COSY (Grzesiek et al., 1995), respectively. Stereospecific assignments of valine and leucine methyl groups were obtained from a ^{13}C-HSQC spectrum of 10% ^{13}C-labeled protein (Neri et al., 1989).

Extent of assignments and data deposition

Spectra of RPA32$_{172-270}$ were of good quality and allowed the assignment of 90% of all protons, 97% of all ^{1}H, ^{15}N and ^{13}Cα backbone nuclei, and 74% of all side chain ^{1}H, ^{15}N and ^{13}C nuclei. Figure 1 shows the ^{15}N-HSQC of RPA32$_{172-270}$. Most of the missing resonances are located in the N-terminal part of the protein which appears to be unfolded. The patterns of NOEs, $^{3}J_{HNH\alpha}$ coupling constants (Vuister and Bax, 1993), and deviations of ^{13}Cα chemical shifts from random coil values indicate that the C-terminus of RPA32$_{172-270}$ consists of three α-helices, from residues 207–217, 227–233 and 239-252, respectively, and a small three-stranded antiparallel β-sheet involving residues 225–226 (strand I), 255–258 (strand II), and 263-266 (strand III). The ^{1}H, ^{13}C and ^{15}N chemical shift assignments for RPA32$_{172-270}$ have been deposited with the BioMagResBank database (http://www.bmrb.wisc.edu) under accession number 4460.

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