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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N assignments of single-stranded DNA binding domains from the 70 kDa subunit of Human Replication Protein A

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Biological context

Human Replication Protein A (RPA) plays a crucial role in organizing and protecting single-stranded DNA against endonuclease activity during the assembly and function of DNA replication, recombination and repair complexes (Wold, 1997). RPA is a heterotrimer composed of 70 kDa, 32 kDa and 14 kDa subunits. The individual subunits each contain structured domains, which are functionally linked through physical interactions in the heterotrimer or manifest by ssDNA binding and protein-protein interactions in DNA processing assemblies (Bochkareva et al., 2002). RPA contains four ssDNA binding domains, two of which are located in the central region of the large RPA70 subunit, RPA70A ($RPA70_{181-291}$) and RPA70B ($RPA70_{299-422}$). RPA70AB ($RPA70_{181-422}$) has also been implicated in stimulating the activities of several replication and recombination proteins including SV40 large T-Antigen, DNA Polymerase-α, and RAD51 during DNA processing [reviewed in (Wold, 1997)].

A short flexible tether connects RPA70A and RPA70B, but their structural independence is evident from both solution NMR studies (Arunkumar et al., 2003) and X-ray crystallography (Bochkareva et al., 2001). NMR chemical shift perturbation assays are being used to locate the multiple protein and ssDNA interaction sites of RPA domains and characterize the structural and dynamic changes induced by these binding events. Here we report the ^{1}H, ^{13}Cand ^{15}N backbone and side-chain assignments of the isolated RPA70A and RPA70B domains, as well as intact RPA70AB.

Methods and experiments

The genes for human RPA70A, RPA70B and RPA70AB were cloned into the pet15b vector (Invitrogen) which contains a thrombin cleavable His-tag. Proteins were expressed in *E. coli* and purified as described in detail elsewhere (Arunkumar et al., 2003). NMR samples of 0.5–1.0 mM uniformly ^{15}N- and ^{15}N,^{13}C-labeled protein were prepared in 20 mM tris-d_{11} buffer, 50 mM KCl, 10 mM $MgCl_2$, 2 mM DTT, 0.01% NaN_3, 90%H_2O/10%D_2O at pH 7.2. The data sets for RPA70A and RPA70B were acquired at 298 K on Bruker *AVANCE* 600 MHz and 800 MHz spectrometers equipped with triple axis gradient probes. Sequential backbone assignments for RPA70A and RPA70B were made using 3D-CBCA(CO)NH, 3D-HNCO (Muhandiram and Kay, 1994), 3D-CBCANH (Grzesiek and Bax, 1992), 3D-HNCA and 3D-HN(CO)CA (Yamazaki et al., 1994) experiments. Aliphatic side-chain proton and carbon assignments were made using 3D-H(CCO)NH-TOCSY, 3D-(H)C(CO)NH-TOCSY (Grzesiek et al., 1993) and 3D HCCH-TOCSY (12–15 ms mixing) (Kay et al., 1993). Additional assignments were obtained from ^{15}N-edited 3D NOESY-HSQC (100 ms mixing) and ^{15}N-edited 3D TOCSY-HSQC (52 ms mixing) spectra acquired on a 800 MHz spectrometer.

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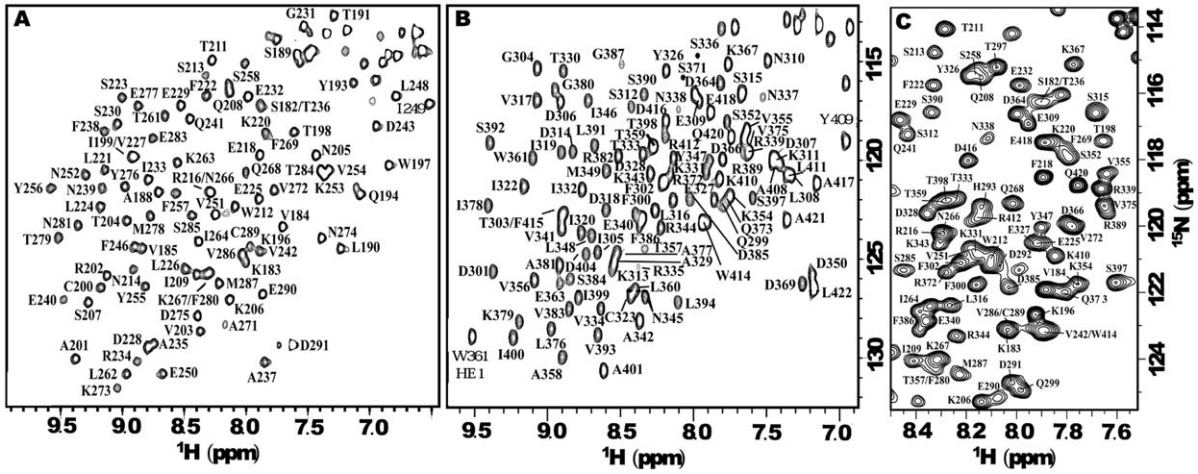

Figure 1. Annotated ^{1}H-^{15}N HSQC spectra of the central region of (A) RPA70A (111 total, 107 non-pro, 103 assigned residues), (B) RPA70B (124 total, 121 non-pro, 119 assigned), (C) RPA70AB spectra (242 total, 234 non-pro, 225 assigned residues) acquired under similar conditions of sample pH 7.2 and temperature 298 K. To increase legibility of the figure, only the central region of the spectrum is shown. The peaks of I187, R210, G217, G219, F245, G260, A265, W197HE1, W212HE1 in RPA70A and G321, K324, S325, T351, G353, G362, A365, F368, G388, G413, N402, I405, E407, G419, W414HE1 in RPA70B appear outside the regions shown in the figure.

Aromatic ring proton and carbon assignments were obtained from 3D HCCH-TOCSY (12 ms mixing) and 3D ^{13}C-edited NOESY-HSQC (100 ms mixing) spectra optimized for the aromatic region. A 3D-CBCA(CO)NH spectrum on intact RPA70AB was acquired at 310 K on the 600 MHz spectrometer equipped with a single axis z-gradient CryoProbe (Bruker). All data sets were processed and analyzed in Felix-2000 (Accelrys) with standard parameter sets reviewed elsewhere (Cavanagh et al., 1996).

Extent of assignments and data deposition

Figure 1 displays the annotated spectra of the isolated domains, RPA70A and RPA70B, and a region of intact RPA70AB. Backbone resonances of non-proline residues were observed and assigned including 103 out of 107 in RPA70A (lacking Q181, S195, S215, N282) and 119 out of 121 in RPA70B (lacking S395, S396). Side-chain proton-carbon assignments including the aromatics are complete for all residues with the exception of Q181, S195, F257, K259, K267 in RPA70A and K311, E327, R344, R372, S395 in RPA70B. All of the residues with incomplete chemical shift assignments are located in flexible loops of the two domains. The complete structural independence of the isolated domains, RPA70A and RPA70B, was confirmed by comparing H^N, N, Cα, Cβ chemical shifts with those of the same residues in RPA70AB. The only differences observed were shifts in the HN resonances of the residues at the C-terminus of RPA70A (D291) and N-terminus of RPA70B (Q299). Backbone assignments of D292, H293 and T297 were also made for residues located in the linker region of RPA70AB. The chemical shift index of the backbone resonances analyzed using TALOS agrees with the β-barrel structure of the oligonucleotide binding (OB) folds observed in the crystal structure. The chemical shift assignments have been deposited with BioMagResBank under accession code BMRB-5821 (RPA70A), BMRB-5822 (RPA70B) and BMRB-5823 (RPA70AB).

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