Journal of Biomolecular NMR, **13:** 393–394, 1999.
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Letter to the Editor: ^{1}H, ^{15}N, and ^{13}C resonance assignments for the N-terminal 20 kDa domain of the DNA single-strand break repair protein XRCC1

Assen Marintchev, Mark W. Maciejewski & Gregory P. Mullen*
Department of Biochemistry, University of Connecticut Health Center, Farmington, CT 06032, U.S.A.

Received 15 December 1998; Accepted 6 January 1999

Key words: DNA repair, 3D NMR, X-ray induced DNA damage

Biological context

XRCC1 is a 633-residue protein necessary for the repair of single-strand DNA breaks in mammalian cells. The XRCC1 protein has three apparent domains that include the N-terminal 20 kDa domain, a central BRCT domain, and a C-terminal BRCT domain. Two intervening segments of the XRCC1 protein have not been classified. Chinese hamster ovary cell lines with mutations in the XRCC1 gene have characteristic DNA repair defects that include sensitivity to ionizing radiation, elevated levels of single-strand DNA breaks, and increased sister chromatid exchange (Thompson et al., 1990; Shen et al., 1998).

Using methods that included affinity chromatography and yeast two-hybrid screening, the XRCC1 N-terminal domain and intact XRCC1 were shown to interact with mammalian β-Pol (Kubota et al., 1996). The C-terminal BRCT domain interacts with a BRCT domain in DNA ligase III. The central BRCT domain of XRCC1 has been shown to interact with a BRCT domain on PARP (Masson et al., 1998). Thus, the XRCC1 protein has been considered to be a scaffolding protein, necessary for formation of a β-Pol-XRCC1-DNA ligase III repair complex, and this complex may also interact with PARP. No structural information is available for the XRCC1 N-terminal domain. Here we report on the ^{1}H, ^{15}N, and ^{13}C resonance assignments for the backbone and essentially all side chains within the XRCC1 20 kDa N-terminal domain.

*To whom correspondence should be addressed. E-mail: gmullen@panda.uchc.edu

Abbreviations: XRCC1, X-ray cross-complementing group 1 protein; β-Pol, DNA polymerase β; BER, base excision repair; BRCT, BRCA1 (breast cancer susceptibility protein) C-terminal domain; PARP, poly(ADP-ribose) polymerase; IPTG, isopropyl β-D-thiogalactopyranoside; AEBSF, 4-(2-aminoethyl)benzenesulfonylfluoride; DTT, dithiothreitol.

Methods and results

The XRCC1 N-terminal domain was overexpressed from plasmid X-183-pET23a in the BL21(DE3) cell line containing the pLysS vector. X-183-pET23a was constructed using the pET23a expression vector (Novagen) and the DNA coding sequence for residues 1–183 of the human gene for XRCC1. The ^{15}N- or ^{15}N/^{13}C-labeled XRCC1 N-terminal domain was produced from a cell culture grown in a minimal media supplemented with $^{15}NH_4Cl$, $^{13}C_6$-glucose, and vitamins (Weber et al., 1992) by induction with IPTG (1 mM) at an OD_{600} of 0.9. Incubation after induction was continued for 3 h. Purification was performed by selective ammonium sulfate fractionation, collecting the supernatant at 50% saturation and retaining the pellet at 77% saturation. Further purification was done using gel filtration chromatography on a Sephadex G50 column (43 × 1.4 cm) in 25 mM Tris, pH 7.5, 400 mM NaCl, 1 mM EDTA, 2 mM β-mercaptoethanol, and 1 mM phenylmethanesulfonyl fluoride. The final NMR sample contained XRCC1 N-terminal domain at a concentration of 2 mM in 2.5 mM Tris-d_{11}, pH 6.8, 400 mM NaCl, 5 mM DTT, and 0.1 mM AEBSF in 90% H_2O/10% D_2O. The sample was exchanged into D_2O for the HCCH-TOCSY and 2D TOCSY experiments.

NMR experiments were performed at 25 °C on a Varian INOVA 600 MHz spectrometer equipped with a z-gradient triple resonance probe. Water suppression was achieved by the use of pulsed field gradients.

The data were processed using Felix97 (MSI, Inc.) and analyzed using XEASY (Bartels et al., 1995). Squared sine bell window functions with shifts of 60–70° were applied in all dimensions. Zero filling and/or linear prediction were performed in the indirect dimensions. ^{1}HN, ^{15}N, $^{13}C_{\alpha}$, and $^{13}C_{\beta}$ assignments were made through use of through-bond sequential connectivities in 3D HNCACB and CBCA(CO)NH spectra and 3D HNCA and HN(CO)CA spectra. $^{1}H_{\alpha}$ resonance assignments were obtained from 3D ^{15}N-edited TOCSY and HCC(CO)NH spectra. Side chain ^{1}H and ^{13}C assignments were made from 3D HCC(CO)NH, CC(CO)NH, and HCCH-TOCSY spectra. Aromatic resonance assignments were made using 2D (HB)CB(CGCD)HD, 2D TOCSY, aromatic ^{1}H-^{13}C HSQC, 3D ^{15}N-edited NOESY, and 3D ^{13}C-edited NOESY. A 3D HNCO experiment was used for assigning ^{13}C carbonyl resonances.

Extent of assignments and data deposition

Backbone ^{1}H and ^{15}N assignments are shown in Figure 1. ^{1}HN, ^{15}N, $^{1}H_{\alpha}$, $^{13}C_{\alpha}$, and $^{13}C_{\beta}$ resonances for all amino acid residues were assigned except for all resonances in M1 and P2, for which no assignments were obtained. The $^{1}H_{\beta}$ resonances were assigned for all residues except for P96, R107, and Q134. Excluding the M94 and M110 methyls, all aliphatic hydrocarbon ^{1}H and ^{13}C side chain resonances were assigned except those in P96, P105, R107, Q134, and K164 for which partial assignments were made. All ^{13}CO resonances were assigned except those for 9 residues and the C-terminus. Aromatic proton assignments for 7 Phe, 3 Tyr, and 2 Trp are complete except for the H_{ε} and H_{ζ} resonances of F93, F11, and F142. Partial ^{13}C resonance assignments were made for the aromatic rings. The 4 His $^{1}H_{\delta 2}$, $^{1}H_{\varepsilon 1}$, $^{13}C_{\delta 2}$ resonances, and for two of these, the $^{13}C_{\varepsilon 1}$ resonances were assigned. Side chain amide ^{1}H, ^{15}N, and ^{13}CO resonance assignments were made for 4/5 Asn and 6/7 Gln residues and for the εNH of two Arg residues. The ^{1}H, ^{15}N, and ^{13}C chemical shifts have been deposited at the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4282.

Acknowledgements

This work was supported by NIH grant GM52738 (G.P.M.) and by an NIH postdoctoral fellowship GM18956 (M.W.M.).

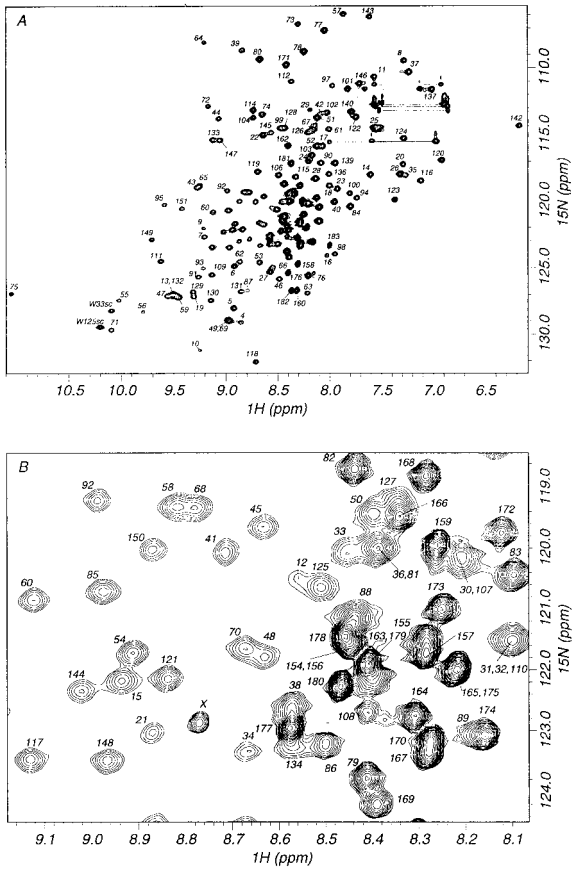

Figure 1. The ^{1}H-^{15}N HSQC spectrum of the XRCC1 N-terminal domain showing the assignments of backbone ^{1}H-^{15}N cross peaks. (A) Complete spectrum. (B) Expansion of the crowded central region. The cross peaks for residues 3 and 138 are below the contour threshold. An amide marked with an X is not assigned.

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