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Letter to the Editor: Backbone ^{1}H, ^{13}C, and ^{15}N resonance assignments for the C-terminal region of Ku86 (Ku86CTR)

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Biological context

DNA double strand breaks (DSB) are caused by free-radicals, ionizing radiation, and occur as intermediates in V(D)J recombination. Un-repaired or improperly repaired DSB can cause cell death, cell cycle arrest and chromosome translocations, resulting in increased rates of mutation and ultimately carcinogenesis. Eukaryotic cells have evolved systems to recognize and repair DSB, one of which is the non-homologous end joining (NHEJ) process (for a recent review see Khanna and Jackson, 2001). The NHEJ process, in which the broken ends are rejoined to each other, is dependent upon the XRCC4/DNA ligase IV protein complex, and a multi-protein complex, DNA-dependent protein kinase (DNA-PK) that consists of the DNA end-joining protein, Ku, and a catalytic subunit, DNA-PKcs. Ku, a heterodimer of proteins Ku70 and Ku86, binds to DNA ends regardless of the sequence or structure (i.e., blunt, recessed or hairpinned) and its main function is the primary recognition of DSB. In mammalian cells, the heterodimeric Ku, bound to the broken DNA ends, recruits the catalytic subunit of DNA-PK, which requires the association of Ku and the presence of DNA for its protein kinase activity (Gottlieb and Jackson, 1993) and could assist in promoting internal translocation of Ku.

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Recently the structure of the Ku heterodimer free and bound to DNA has been solved (Walker et al., 2001). However this complex does not contain the C-terminal domain of Ku86 (545–732). Although low in sequence identity (∼15%), there is a high structural identity between Ku70 and Ku86, except in the C-terminal regions that are not required for binding to DSB. The roles of the C-terminal region in Ku70 and Ku86 are different. Whilst the C-terminal domain of Ku70 comprises a DNA-binding SAP domain and is potentially involved in pausing Ku at specific DNA sequences, genetic and biochemical experiments suggest that the C-terminal region of Ku86 is necessary for recruiting DNA-PKcs (Singleton et al., 1999). In this note we report the backbone NMR assignments and secondary structure of a 167 residue C-terminal region (543–709) of the Ku86 protein (Ku86CTR).

Methods and results

Sample preparation

Uniformly ^{15}N- and $^{13}C/^{15}N$-labelled hexahistidine-tagged recombinant Ku86CTR was over-expressed in *E. coli* BL21 Star (DE3) grown in M9 minimal medium with 0.5 g l^{-1} $(^{15}NH_4)_2SO_4$ and 2 g l^{-1} $^{13}C_6$-glucose, with subsequent purification by immobilized nickel ion affinity chromatography and size exclusion chromatography. For NMR studies, a sample of 1.0 mM $^{13}C/^{15}N$-labelled Ku86CTR in 20 mM TRIS buffer (pH 8.0), 100 mM NaCl and H_2O/D_2O (9:1), and a sample of 1.0 mM ^{15}N-labelled Ku86CTR in

20 mM phosphate buffer (pH 7.0), 100 mM NaCl and H_2O/D_2O (9:1) were prepared.

NMR spectroscopy

NMR spectra were acquired at 298 K on Varian UNITYplus spectrometers (operating at nominal ^1H frequencies of 500 MHz and 600 MHz) equipped with a triple resonance (^1H, ^{13}C, ^{15}N) probe including Z-axis pulse field gradients. Sequence-specific resonance assignments were obtained by combining the data from the following 3D gradient sensitivity enhanced triple resonance experiments: HNCO, HNCA, HN(CO)CA, HN(CA)CO, HNCACB (Yamazaki et al., 1994), and CBCA(CO)NH (Muhandiram and Kay, 1994). All spectra were processed using NMRpipe/NMRDraw (Delaglio et al., 1995) and analyzed using ANSIG v3.3 (Kraulis et al., 1994). Chemical shifts were indirectly referenced to DSS.

Extent of assignments and data deposition

Analysis of the triple resonance experiments allowed identification and sequential assignments for 107 out of the 163 Ku86CTR (167 less 4 prolines) backbone ^{15}N and amide proton resonances (i.e., not including the hexahistidine tag). Definitive assignments have not been obtained for residues Lys543-His558, Thr563-Asn593, Lys606, Phe609, Thr629, His673, Glu689, Ser692, Ser695, Ala698, and Ala706. The absence of unambiguously identifiable NH crosspeak for 56 residues at pH 8.0 and the observation of a number of additional NH crosspeaks, observed at pH 7.0, are attributed to high exchange rate for these residues. Relaxation measurements for Ku86CTR at pH 7.0 reveal that these extra NH crosspeaks have longer T_2 values (average of ~250 ms) as compared to those NH crosspeaks observed at pH 8.0 (average of ~66 ms), and small heteronuclear NOE values (<0.4), indicating that they are in unstructured/random coil regions. Figure 1 shows an assigned 2D ^{13}C-decoupled ^1H-^{15}N HSQC spectrum of $^{13}C/^{15}N$-labelled Ku86CTR at pH 8.0, recorded at a ^1H frequency of 600 MHz.

From the assigned amide resonances, we were able to obtain 97% (67% of the total possible resonances), 96% (66%),96% (66%) and 82% (56%) of the possible Cα, Cβ, CO, and Hα chemical shifts, respectively. Using the $^{13}C\alpha$, $^{13}C\beta$, ^{13}CO, and $^1H^\alpha$ chemical shift values, the globular domain of Ku86CTR is predicted to contain six α-helices by the Chemical Shift Index method (Wishart and Sykes, 1994).

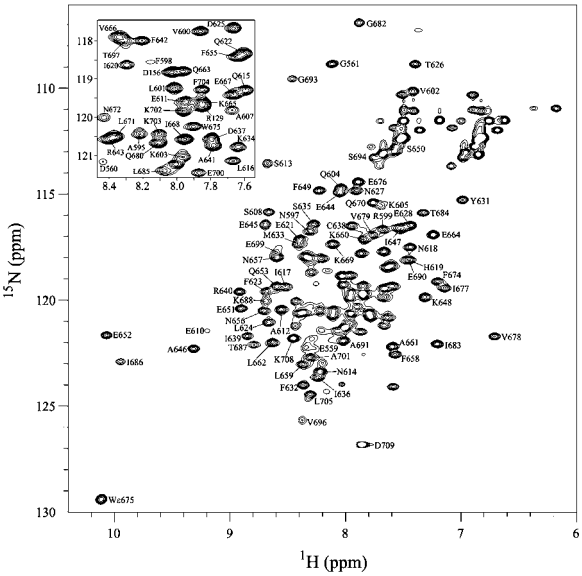

Figure 1. Assigned 2D ^{13}C-decoupled ^1H-^{15}N HSQC spectrum of $^{13}C/^{15}N$-labelled Ku86CTR recorded on a 600 MHz Varian UNITYplus spectrometer at 298 K.

In summary, the Ku86CTR contains a globular domain (residues Pro594-Asp709) with a flexible, unstructured linkage (residues Lys543-Asn593). The full analysis of the three dimensional solution structure of Ku86CTR is in progress. The chemical shifts for backbone resonances of Ku86CTR have been deposited in the BioMagResBank (accession number 5221).

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