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Letter to the Editor: Backbone ^{1}H, ^{13}C and ^{15}N resonance assignments for the 25.8 kDa DNA binding domain of the human p63 protein

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Biological context

The tumor suppressor gene p53 is the most frequent site of genetic alterations found in human cancer cells. The p53 protein acts primarily as a transcriptional activator regulating the expression of genes involved in cell cycle arrest, cellular senescence, anti-angiogenesis and apoptosis (Levine, 1997). Recently, two homologues of the p53 gene, namely p63 and p73, were discovered that code for a variety of different isoforms. This discovery defined a whole family of p53 proteins with remarkable similarities, which play important but different roles in cell differentiation, development and tumor suppression (Yang et al., 2000). With the exception of the highly variable N- and C-terminal domains, all members of the p53 family possess a highly conserved core DNA binding domain (DBD) (∼60% homology), which, for p53, has been shown to contain almost all cancer-associated mutations (Levine, 1997). The crystal structure of the p53 DBD has already been determined and shows that almost all mutations affect residues that either directly contact DNA or stabilize the tertiary structure (Cho et al., 1994). In addition, the p53 DBD has been subjected to several NMR spectroscopic studies in the past (Wong et al., 1999; Klein et al., 2001a; Mulder et al., 2000; Ayed et al., 2001).

Recent phylogenetic analysis of the corresponding genes suggested that p63 might be an evolutionary predecessor of both p53 and p73. In experimental systems p63 shows many p53-like biological properties, but little is known about its structure-function relationships so far. Despite the high sequence homology of the p63 DBD with the p53 DBD (55.4% identity), the p63 DBD has been recently shown to exhibit characteristic differences, namely, a significantly higher thermal stability and, in contrast to p53 DBD, a lack of cooperative binding to specific p53 DNA consensus sites (Klein et al., 2001b).

In order to understand these results on a molecular basis and to gain further insight into the DNA binding specificity, selectivity and regulation as well as into structural and functional properties of the DNA binding domains of the p53 family members, we therefore focused our attention on the p63 DBD with the aim of obtaining its three-dimensional structure in solution. As a basis for these structural investigations, we here report its ^{1}H, ^{13}C and ^{15}N backbone resonance assignment.

Methods and experiments

Sample preparation

[U-^{15}N] and [U-^{13}C, ^{15}N, 75% ^{2}H] p63 DBD (residues 113–345) was expressed and purified as previously described (Klein et al., 2001b). All samples were concentrated using 5 K Ultrafree 4 Centrifugal Filter Devices (Millipore) to final concentrations of 0.4–1.0 mM, supplemented with 0.1% sodium azide and 5% D_2O, flash frozen in liquid nitrogen and stored at −80 °C. For NMR studies, p63 DBD samples of 500–700 μM in 50 mM potassium phosphate pH 6.8, 150 mM KCl and 5 mM DTT were prepared.

NMR Spectroscopy

All NMR spectra were acquired at 303 K on Avance spectrometers (Bruker, Karlsruhe) operating at nominal ^{1}H frequencies of 600, 750 and 900 MHz, equipped with triple (^{1}H, ^{13}C, ^{15}N) and quadruple (^{1}H, ^{13}C, ^{15}N, ^{31}P) probes including triple axis pulse field gradients and lock switch units for ^{2}H decoupling. The following 3D triple resonance experiments were carried out with gradient selection and sensitivity enhancement: [U-^{15}N] labeled sample; 2D ^{1}H,^{15}N-HSQC, 3D HNHA, 3D HNHB, 3D ^{15}N-edited TOCSY-HSQC (50 ms mixing time), 3D ^{15}N-edited

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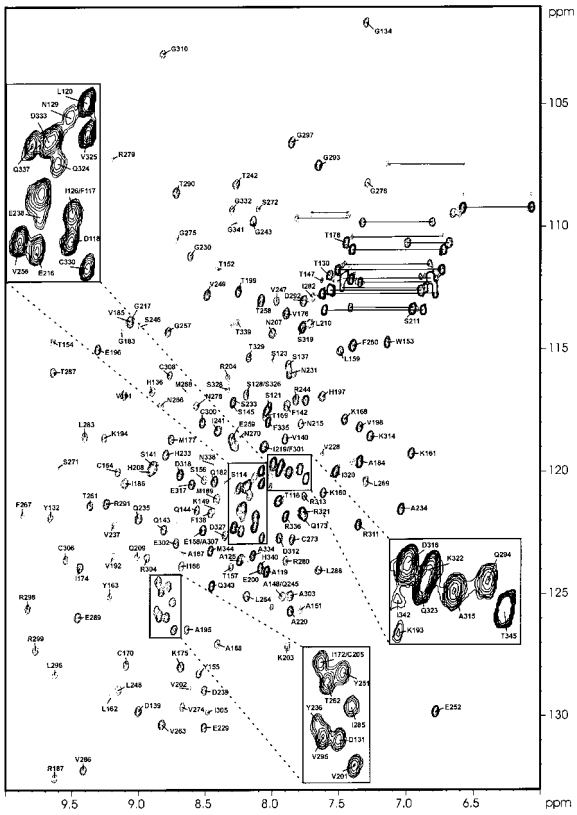

Figure 1. Assigned 2D $^{1}H,^{15}N$ HSQC spectrum with three expanded views of ^{15}N, ^{13}C-labelled p63 in 90% H_2O, 10% D_2O at pH = 6.8, recorded on a 900 MHz Bruker spectrometer at 303 K. The peaks are labelled according to their original position in the complete p63 sequence (113–345). Sidechain amide proton resonances are connected with horizontal lines.

NOESY-HSQC (80 ms mixing time) and 3D $^{15}N,^{15}N$-edited HSQC-NOESY-HSQC (100 ms mixing time).

[U-^{15}N, ^{13}C, 75% ^{2}H] labeled sample (with ^{2}H decoupling): 3D HNCO, 3D TROSY-HNCA, 3D TROSY-HN(CA)CO, 3D TROSY-HN(CO)CACB, 3D TROSY-HNCACB. All the experiments were processed and analyzed using the XWinNMR and Aurelia software (Bruker, Karlsruhe), respectively. The sequence specific resonance assignments were obtained with the help of our automatic assignment program PASTA (Leutner et al., 1998). The backbone assignments could be confirmed through the observation of sequential NH_i-NH_{i+1} and $H\alpha_i$-NH_{i+1} interproton NOEs. Chemical shifts were indirectly referenced via the Ξ ratios (Wishart et al., 1995).

Extent of assignment and data deposition

The 2D $^{1}H,^{15}N$ HSQC spectrum of the p63 DBD (Figure 1) exhibits a good dispersion of the proton and nitrogen resonances. From the above mentioned triple-resonance experiments, an almost complete backbone assignment (94% of the 214 non-Pro residues) could be achieved. Definitive assignments have not been obtained for residues Gly113, Ser114 and the short segment His224-Arg227, whereas Ser115, Arg212, Ser232, Tyr265, Met277, Ile284 and Asn331 could only be sequentially assigned.

Overall, the backbone chemical shifts of the p63 DBD were found to be very similar to that of the p53 DBD for most sequence positions (Wong et al.; 1999, Mulder et al., 2000). In combination with the high sequence homology, this is consistent with a similar global fold for both proteins. Overall, the secondary structure elements revealed by both chemical shifts and interproton NOEs of the p63 DBD are essentially identical to those found previously for p53, which is also consistent with a p53-like fold. The chemical shifts for backbone resonances of p63 DBD have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu/) under the accession number BMRB-5700.

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