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Letter to the Editor: Sequence-specific ^{1}H, ^{15}N, and ^{13}C assignment of the N-terminal domain of the human oncoprotein MDM2 that binds to p53

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Biological context

One of the most important functions of the oncoprotein MDM2 is negative regulation of p53 (Lozano and Montes de Oca Luna, 1998). By binding to the transactivation domain at the N-terminus of p53, MDM2 inhibits the ability of p53 to activate transcription (Oliner et al., 1993; Kussie et al.,1996) and promotes the rapid degradation of p53 (Haupt et al., 1997). The crystal structure of a 109-residue N-terminal domain of MDM2 in complex with a 15-residue transactivation domain peptide of p53 has been determined (Kussie et al., 1996). The p53 α-helical peptide binds to a deep hydrophobic cleft present in the MDM2 domain. The interaction is primarily dependent on van der Waals forces, with only two hydrogen bonds existing between MDM2 and the p53 peptide. The hydrophobic interface of MDM2 and p53 is sterically complementary and dominated by a triad of p53 amino acids (comprising F19, W23, and L26) which insert into the MDM2 cleft. This cleft is composed of two helices (M50–R65 and E95–Y104) forming the sides of the cleft, two shorter helices (P32–G42 and L81–G87) that make up the bottom, and a pair of three-stranded β-sheets (T26–P30, Y48–T49, N106–V109 and L66–D68, H73–C77, P89–V93) that cap each end. The MDM2–p53 interface is defined by M50, L54, L57, G58, I61, M62, Y67, H73, V75, F91, V93, H96, I99, and Y100 (Kussie et al., 1996).

In normal cells, the MDM2/p53 interaction forms a negative feedback loop that limits the growth-suppressing activity of p53. Increasing MDM2 levels raise the signal threshold necessary for p53-induced apoptosis (Midgley and Lane, 1997) and retard the rate of the p53-induced expression of the cell cycle inhibitor p21 (Chen et al., 1993). In tumours, elevated levels of MDM2 can lead to the constitutive inhibition of p53 and failure of the genome integrity checkpoint. It has been recently shown that a peptide homolog of p53 is sufficient to induce p53-dependent cell death in cells overexpressing MDM2 (Wasylyk et al., 1999). This result provides clear evidence that disruption of the p53/MDM2 complex might be effective in cancer therapy.

Here we report the ^{1}H, ^{13}C, and ^{15}N NMR backbone assignments of the N-terminal domain of the human MDM2 that binds to p53.

Methods and results

The recombinant human MDM2 protein was obtained from an *E. coli* BL21(DE3) expression system and contained the first 118 N-terminal residues of human MDM2 cloned in a pQE-40 vector (Qiagen), C-terminally extended by an additional serine residue. The protein was renatured from *E. coli* inclusion bodies as previously published (Jaenicke and Rudolph, 1986). Refolded MDM2 was applied first to a Butyl Sepharose 4 Fast Flow (Pharmacia) and then to a HiLoad 16/60 Superdex75 gel filtration column (Pharmacia). The uniformly $^{13}C/^{15}N$, and ^{15}N isotopically enriched protein samples were prepared by growing the bacteria in minimal media containing $^{15}NH_4Cl$, either with or without $^{13}C_6$-glucose. For selectively enriched samples (^{15}N-Val, ^{15}N-Leu, and ^{15}N-Phe),

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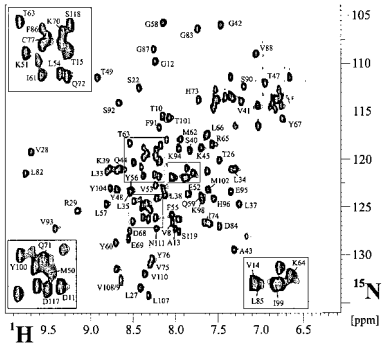

Figure 1. 500 MHz 2D ^{1}H-^{15}N-HSQC spectrum of the N-terminal domain of human MDM2 at 300 K and pH 7.4. Residue-specific assignment of the backbone ^{1}H and ^{15}N frequencies is indicated.

the minimal medium consisted of 300 to 1000 mg l^{-1} of the isotopically enriched amino acids and all other amino acids. A reverse ^{14}N-His sample of human MDM2 was prepared by adding 300 to 1000 mg l^{-1} of ^{14}N-His to the minimal medium containing $^{15}NH_4Cl$.

All NMR spectra were acquired at 290 K and 300 K on Bruker AMX500, DRX500, DRX600, and DMX750 spectrometers. Typically, NMR samples contained up to 0.5 mM of protein in 50 mM KH_2PO_4, 50 mM Na_2HPO_4, 150 mM NaCl, pH 7.4, 5 mM DTT, 0.02% NaN_3, and protease inhibitors. The quality of the spectra for MDM2 was dramatically reduced by aggregation and fast precipitation, especially at concentrations higher than 0.5 mM at pH 7.4 and 300 K. Especially the short lifetime of the samples prevented recording of high quality 2D- and 3D-NOESY spectra. Backbone and side chain sequential resonances were assigned with CT-HNCA, CBCA(CO)NH, and in part with 2D TOCSY ($\tau_m = 42$ ms) and 2D NOESY ($\tau_m = 120$ ms), 3D ^{15}N-TOCSY-HSQC ($\tau_m = 36$ ms), and 3D ^{15}N-NOESY-HSQC ($\tau_m = 120$ ms) experiments, by selective enrichment using ^{15}N-Leu, Phe, Val samples, and by a reverse ^{14}N-His sample of MDM2. All 2D and 3D NMR experiments were performed as described previously (Kalus et al., 1998). Assignment was performed using our software NMRXplorer, which is based on CC-NMR (Cieslar et al., 1993; Kalus et al., 1998).

Extent of assignments and data deposition

Figure 1 shows the ^{1}H-^{15}N-HSQC of human MDM2. The N-terminal domain of human MDM2 extensively aggregates at concentrations required for NMR measurements. Nonetheless, a basically complete assignment of the backbone $^{1}H^{N}$, ^{15}N, and the $^{13}C^{\alpha}$ and $^{13}C^{\beta}$ NMR resonances was obtained for the folded core of human MDM2. Only the signals for K31, K36, D46, S78–L81, R97, I103, R105, and N106 could not be detected in the spectra of human MDM2. In the unstructured N- and C-terminal regions, M1–S7, T16–I19, A21, E23–E25, and Q112–S116 could not be assigned. Many side chain resonances were missing or could not be assigned unambiguously due to the poor quality of 2D and 3D TOCSY and NOESY spectra resulting from the intrinsic tendency of the N-terminal domain of human MDM2 to aggregate. Nevertheless, the extent of assignments is sufficient to perform ligand binding studies for this protein on the basis of ^{1}H-^{15}N HSQC spectra, because all residues of human MDM2 involved in binding to p53 have been assigned (Kussie et al., 1996). In fact, the binding site of the peptide derived from p53 on human MDM2 was successfully mapped by multidimensional NMR (data not shown and Kussie et al., 1996).

A table of the ^{1}H, ^{15}N, and ^{13}C chemical shift assignments of the N-terminal domain of human MDM2 has been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) database under accession number 2410.

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