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Supplementary Material

Backbone ^1H , ^{13}C , and ^{15}N resonance assignments of the N-terminal domain of FKBP38 (FKBP38NTD)

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Biological Context

Bcl-2 family proteins play important roles in the mitochondria-mediated apoptotic regulation (Vander Heiden and Thompson, 1999). Recently, the immunosuppressant FK-506 binding protein 38 (FKBP38) was shown to interact with Bcl-2/XI and help them localize at the mitochondrial membrane (Shirane and Nakayama, 2003). The down-regulation of FKBP38 appears to exhibit an effect on the stability of Bcl-2 and consequently induce apoptosis (Shirane and Nakayama, 2003; Kang et al., 2005). FKBP38 is a unique protein among the FKBP family. It contains the FKBP domain which is conserved among FKBP family proteins and important for the peptidyl prolyl *cis-trans* isomerase (PPIase) and FK-506 binding activity, three tetracopeptide repeats (TPR), calmodulin-binding (CaM), and transmembrane (TM) domains. Unlike other proteins in the FKBP family, FKBP38 appears to have no FK-506 binding activity (Shirane and Nakayama, 2003). Recently, it has been also demonstrated that FKBP38 may play important role in neuronal signaling (Blulgakov et al., 2004; Rosner et al., 2003; Wang et al., 2005). The biological function of FKBP38 is apparently diverse and remains to be further studied. Thus, to understand the function of FKBP38 at molecular level, as the first step, we performed NMR studies on FKBP38 and here we report ^1H , ^{13}C , and ^{15}N resonance assignments of the N-terminal domain of FKBP38 (FKBP38NTD), which includes the FK-506 binding domain and flanking sequences in its N-terminus.

Methods and Experiments

Protein Preparation. The cDNA encoding M1-D149 of FKBP38 (FKBP38NTD) was cloned into the expression vector pET29b with Nde I and Xho I restriction enzyme sites. The Uniformly ^{15}N - and $^{15}\text{N}/^{13}\text{C}$ -labeled hexahistidine-tagged FKBP38 samples were expressed in media containing either $^{15}\text{NH}_4\text{Cl}$, $^{15}\text{NH}_4\text{Cl}$ plus $[\text{U-}^{13}\text{C}]$ -glucose and subsequently purified by Ni^{2+} -NTA affinity chromatography and Sephacryl S-200 size exclusion chromatography as previously described (Kang et al., 2005^b). For NMR studies, the samples (0.5 - 1 mM) in 20 mM sodium phosphate (pH 7.0), 1 mM DTT, 50 mM NaCl, 0.1 mM EDTA were used.

NMR Spectroscopy. All NMR spectra were recorded at 303 K on Bruker AV700 spectrometers equipped with a cryoprobe accessory. Backbone ^1H , ^{15}N , ^{13}C resonances were assigned using data from 2D ^1H - ^{15}N HSQC, 3D HNCA, HN(CO)CA, 3D HNCO, 3D HNCACB, and 3D CBCA(CO)NH spectra (Sattler et al. 1999). The side chain ^1H and ^{13}C resonances were obtained from 3D HCC(CO)NH-TOCSY, 3D HCCH-TOCSY, and 3D ^{15}N - ^1H NOESY-HSQC, and 3D ^{13}C - ^1H NOESY-HSQC. All spectra were processed using Topspin version 1.3 (Bruker) and NMRPipe (Delaglio et al., 1995), and analyzed using Felix (Accelrys) and NMRView (Johnson and Blevins, 1994).

Extent of Assignments and Data Deposition

The amide backbone assignments are summarized in Figure 1. Out of a total of 130 observable backbone ^1H - ^{15}N correlations spanning residues 5-151 (which includes 17 prolines), 129 (99.2%) residues have been assigned. For carbon, 144 out of 147 $^{13}\text{C}^\alpha$ (97.9%) and 133 out of 136 $^{13}\text{C}^\beta$ (97.8%, 11 glycines with no $^{13}\text{C}^\beta$) resonances have been assigned. The unassigned residues are M1, G2, Q3, P4, Pro54, Arg128, Pro133 and the C-terminal His₆-tag. Excluding the C-terminal His-tag residues, assignments of the side chains are about 80% complete. An analysis of its backbone chemical shifts using the program CSI (Wishart and Sykes, 1994) suggests that FKBP38NTD contains at least six β -strands and two α -helices. We believe that the quality of the NMR data is sufficient for the structure determination of FKBP38NTD, which is currently in progress. The assignments of FKBP38NTD have been deposited in the BioMagResBank (accession number: 6923).

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- Figure 1. 2D ^1H - ^{15}N -HSQC spectrum of the N-terminal domain of FKBP38 (FKBP38NTD). Spectra were recorded at 303K on a Bruker Avance 700 MHz spectrometer. The assignments for resolved backbone residues are labeled with one letter amino acid code and residue number.

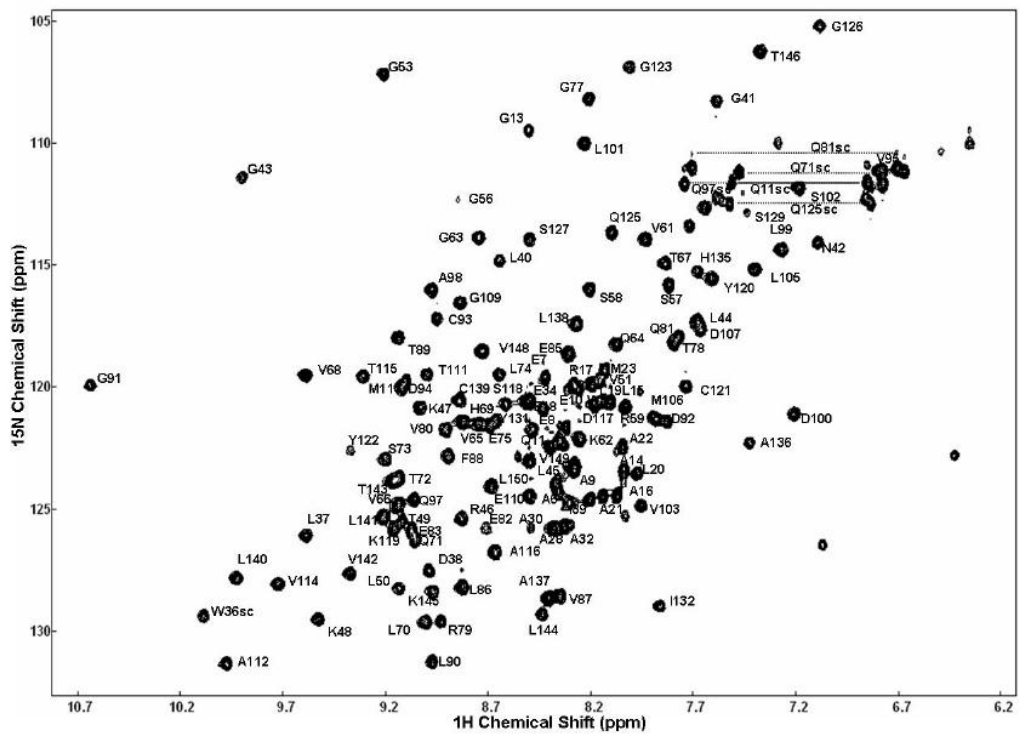


Figure 1.