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

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Supplementary Material

Backbone ^{1}H, ^{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/Xl 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 ^{1}H, ^{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 $^{15}N/^{13}C$-labeled hexahistidine-tagged FKBP38 samples were expressed in media containing either $^{15}NH_4Cl$, $^{15}NH_4Cl$ plus [U-^{13}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 ^{1}H, ^{15}N, ^{13}C resonances were assigned using data from 2D ^{1}H-^{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 ^{1}H and ^{13}C resonances were obtained from 3D HCC(CO)NH-TOCSY, 3D HCCH-TOCSY, and 3D ^{15}N-^{1}H NOESY-HSQC, and 3D ^{13}C-^{1}H 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 ^{1}H-^{15}N correlations spanning residues 5-151 (which includes 17 prolines), 129 (99.2%) residues have been assigned. For carbon, 144 out of 147 $^{13}C^{\alpha}$ (97.9%) and 133 out of 136 $^{13}C^{\beta}$ (97.8%, 11 glycines with no $^{13}C^{\beta}$) resonances have been assigned. The unassigned residues are M1, G2, Q3, P4, Pro54, Arg128, Pro133 and the C-terminal His_6-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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List of Figure

Figure 1. 2D ^{1}H-^{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.

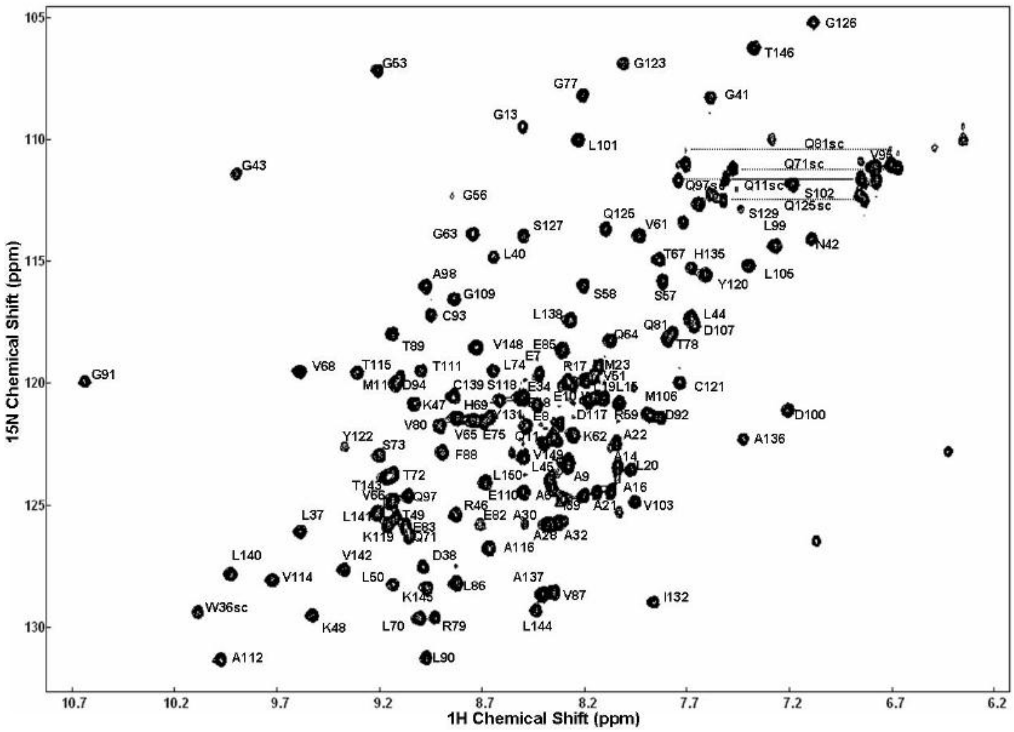
15N Chemical Shift (ppm)
1H Chemical Shift (ppm)
105
110
115
120
125
130
10.7
10.2
9.7
9.2
8.7
8.2
7.7
7.2
6.7
6.2
G126
T146
G53
G123
G77
G41
G13
L101
Q81sc
V95
G43
Q71sc
Q97sc
Q11sc
S102
Q125sc
G56
S129
L99
Q125
V61
G63
S127
N42
L40
T67
H135
L105
A98
Y120
S58
S57
G109
L138
L44
C93
Q81
D107
T89
V148
Q64
T78
E85
E7
L74
T115
T111
R17
M23
V68
G91
V51
M110
D94
C139
S118
E34
L15
C121
E10
M106
K47
H69
D117
R59
D92
D100
V80
Y131
E8
V65
E75
K62
A22
A136
Y122
S73
V149
A14
F88
L20
T72
L160
L45
A9
T143
V66
E110
A6
A16
Q97
R46
V103
A21
L37
L141
T49
E82
A30
E83
A28
A32
K119
Q71
A116
L140
V142
D38
V114
L50
L86
A137
K145
V87
I132
W36sc
K48
L70
R79
L144
A112
L90

Figure 1.