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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C resonance assignments of the Tetratricopeptide Repeat (TPR) Domain of hSGT

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Biological context

TPR-containing proteins are involved in a diverse spectrum of cellular functions including cell cycle control, splicing and transcription, protein transport, regulating protein phosphorylation, and protein folding (Blatch and Lassle, 1999; Lamb et al., 1995). TPR motifs often exist as an array with 3 to 25 repeated units to form a characteristic structure in different proteins, and there is no preferential positioning of the motifs along the primary structure of the proteins. Each TPR motif contains 34 residues and forms two α-helices of equal length, known as A- and B-helix, associated with a packing angle of approximately 24° between the helix axes (Das et al., 1998). Adjacent TPR motifs are packed together in a parallel arrangement, such that sequentially adjacent α-helices are antiparallel. The consequence of this uniform angular and spatial arrangement of neighboring α helices creates a right-handed superhelical structure featuring an amphipathic channel that might accommodate the complementary region of a target protein (Das et al., 1998).

Cziepluch and coworkers have identified a small glutamine-rich TPR-containing protein (SGT) that interacts with the major nonstructural protein NS1 of parvovirus H-1 (Cziepluch et al., 1998). Subsequently, the human homologue of SGT (hSGT) was identified (Kordes et al., 1998), and hSGT was ubiquitously present in all human tissues tested. So far, the only known structural domain identified in SGT is the three tandem-arrayed TPR motifs located in the central part of the protein (Cziepluch et al., 1998). Moreover, it was demonstrated that the TPR domain of hSGT interacts with the C-terminal 30-kDa domain of hsc70 and appears to be a negative regulator of hsc70 dependent chaperone function based on *in vitro* luciferase activity assay (Liu et al., 1999). This negative effect of hSGT on hsc70 is similar in magnitude to that observed for the cochaperone CHIP. In order to understand the structural properties of the TPR domain of hSGT and its roles in protein-protein interactions, here we report the ^{1}H, ^{15}N and ^{13}C resonance assignments of the TPR domain of hSGT.

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Methods and experiments

The cDNA encoding the TPR domain of hSGT (residues 88-208) was subcloned into the pET15b (Novagen) vector and expressed in *E. coli* BL21 (DE3). Uniformly ^{15}N or ^{15}N/^{13}C labeled proteins was purified from bacteria in M9 minimal medium supplemented with ^{13}C glucose, $^{15}NH_4Cl$ and trace vital vitamins. The TPR domain of hSGT is a difficult protein to study by NMR because of its narrow chemical shift dispersions, aggregation and repeated amino acid sequences. Samples with ^{15}N labeling or reverse labeling at specific amino acids are needed to help the NMR assignments. The samples were purified by Ni-NTA column (Qiagen) and then the fractions containing hSGT-TPR were pooled and concentrated by Amicon Concentrator (Millipore). The purity of the protein was check by SDS-PAGE to be better than 95%. The molecular mass of the protein was verified by electrospray ionization mass spectroscopy. Sample concentrations for NMR experiments were typically 0.5 to 1.0 mM in phosphate buffer (50 mM K_2HPO_4, 100 mM NaCl, 10 mM NaN_3, and 0.1 mM EDTA,

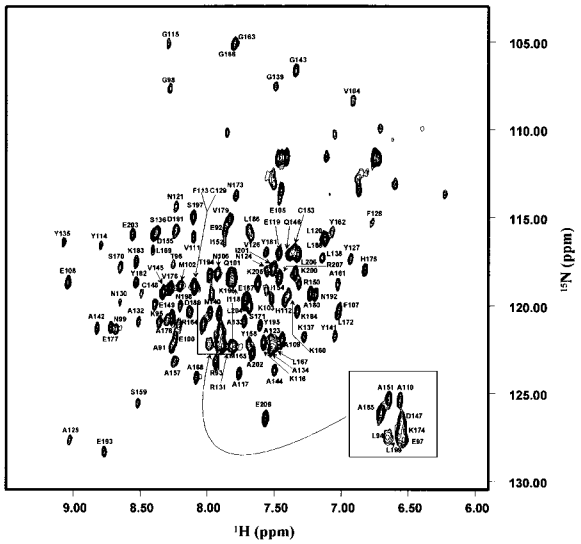

Figure 1. A 600-MHz 2D ^{1}H-^{15}N HSQC spectrum of the TPR domain of hSGT recorded at 25 °C, pH 6.5. The assignments are indicated with the one-letter amino acid code and residue number.

pH 6.5) in either 90% H_2O/10% D_2O or 100% D_2O. All NMR data were acquired with Bruker Avance 600 spectrometer equipped with triple-resonance probe at 25 °C. ^{1}H NMR data were referenced to the ^{1}H resonance frequency of DSS; ^{13}C and ^{15}N resonances were referenced indirectly by multiplying the proton frequency by 0.25144953 for ^{13}C and 0.101329118 for ^{15}N (Markley et al., 1998; Wishart et al., 1995). The NMR experiments performed included 2D ^{1}H-^{15}N HSQC, ^{1}H-^{13}C HSQC, 3D ^{15}N-NOESY-HSQC, HNCO, HN(CA)CO, HN(CO)CA, HNCA, CBCA(CO)NH, and HNCACB for backbone assignments, ^{15}N-TOCSY-HSQC, HCC(CO)NH-TOCSY, HCCH-TOCSY, HCCH-COSY, HBHA(CO)NH for side chain assignments (Ferentz and Wagner, 2000). All spectra were processed with the program XWIN-NMR 2.6 and analyzed using Aurelia Amix 2.1.3 (Bruker).

Extent of assignments and data deposition

The backbone ^{1}H, ^{15}N, ^{13}C, and ^{13}CO assignments of the 121 residue TPR domain of hSGT are essentially complete. A 2D ^{1}H-^{15}N HSQC spectrum is shown in Figure 1. Residues with missing assignments are Ser88, Ala89, and Glu90 all located in the N-terminus. For all of the three Proline residues, assignments were obtained from HNCACB, CBCA(CO)NH, and HBHA(CO)NH spectra. In total, 96% of $^{1}H_{\alpha}$, 53% of $^{1}H_{\beta}$, 98% of $^{13}C_{\alpha}$, 97% of ^{1}HN and ^{15}N, 94% of $^{13}C_{\beta}$, and 97% of ^{13}CO were obtained. Analysis of H_{α}, C_{α}, C_{β}, and ^{13}CO chemical shifts has established that the TPR domain of hSGT is composed of seven α-helices located at Glu92-Lys103 (helix 1), Phe107-Glu119 (helix 2), Ala125-Lys137 (helix 3), Tyr141-Ile154 (helix 4), Ser159-Ser171 (helix 5), His175-Leu186 (helix 6) and Glu193-Lys205 (helix 7). The assigned ^{1}H, ^{13}C and ^{15}N chemical shift values have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 5709.

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