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ARTICLE

^{1}H, ^{15}N and ^{13}C backbone resonance assignments of the TPR1 and TPR2A domains of mouse STI1

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Abstract Hop/STI1 (Hsp-organizing protein/stress-induced-phosphoprotein 1) is a molecular co-chaperone, which coordinates Hsp70 and Hsp90 activity during client protein folding through interactions with its TPR1 and TPR2A domains. Hsp90 substrates include a diverse set of proteins, many of which have been implicated in tumorigenesis. Over-expression of Hsp90 in cancer cells stabilizes mutant oncoproteins promoting cancer cell survival. Disruption of Hsp90 and its co-chaperone machinery has become a promising strategy for the treatment of cancer. STI1 has also been described as a neurotrophic signaling molecule through its interactions with the prion protein (PrP^{C}). Here, we report the ^{1}H, ^{13}C and ^{15}N backbone assignments of the TPR1 and TPR2A domains of mouse STI1, which interact with Hsp70 and Hsp90, respectively. ^{1}H-^{15}N HSQC spectra of TPR2A domain in the presence of a peptide encoding the C-terminal Hsp90 binding site revealed significant chemical shift changes indicating complex formation. These results will facilitate the screening of potential molecules that inhibit STI1 complex formation with Hsp70 and/or Hsp90 for the treatment of cancer and detailed structural studies of the STI1-PrP^{C} complex.

Keywords Hop/STI1 · TPR domain · Co-chaperone · Hsp90 · Protein–protein interaction

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Biological context

Heat-shock protein (Hsp)70 and Hsp90 are chaperones which mediate folding of key cellular client proteins involved in processes such as proliferation, differentiation and apoptosis (Taipale et al. 2010; Young et al. 2004). Hsp90 targets numerous proteins implicated in tumor growth and survival including steroid hormone receptors and proto-oncogenic kinases such as Akt (Sato et al. 2000), Raf-1 (Stancato et al. 1993) and Her2/neu (Xu et al. 2001). Hsp90 over-expression correlates with tumor invasiveness and poor prognosis, thus pharmacological inhibition of the chaperone response has become an alluring target for therapeutic intervention in multiple cancer types (Trepel et al. 2010).

Client maturation is facilitated by a number of co-chaperone proteins that regulate Hsp70 and Hsp90 activity. Hop/STI1 (Hsp-organizing protein/stress-induced-phosphoprotein 1) is a key scaffold protein, which mediates client transfer from Hsp70 to Hsp90 in the later stages of client maturation (Smith et al. 1993; Chen and Smith 1998). Initially, client proteins complex with Hsp40 and Hsp70 (Hernandez et al. 2002; Pratt and Toft 2003). Then Hop/STI1 promotes client transfer from Hsp70 to Hsp90 by simultaneously binding the two chaperones through distinct tetratricopeptide repeat (TPR) domains (Scheufler et al. 2000).

Hop/STI1 encodes three TPR domains, TPR1, TPR2A and TPR2B and two aspartate-proline motif regions (DP1 and DP2) (Odunuga et al. 2004). TPR domains are composed of multiple degenerate 34 amino acid repeats forming anti-parallel helix-turn-helix motifs. These serve as protein–protein interaction modules in multi-protein complexes (Cortajarena and Regan 2006). TPR1 and TPR2A preferentially bind the conserved C-terminal

residue 'EEVD' motifs of Hsp70 and Hsp90, respectively. X-ray crystallographic data of human TPR1 and TPR2A domain in complex with Hsp70 and Hsp90 C-terminal peptides indicate specificity is determined by hydrophobic contacts directly N-terminal of the 'EEVD' motif (Scheufler et al. 2000).

Studies have demonstrated that STI1 promotes proliferation and migration in glioblastoma and pancreatic cancer cell lines (Fonseca et al. 2012; Walsh et al. 2011). Down-regulation of STI1 by RNAi resulted in decreased pancreatic cell line invasiveness and down-regulation of numerous oncogenic Hsp90 client proteins (Walsh et al. 2011). Inhibition of Hsp90 interaction with the TPR2A domain of STI1 using a novel hybrid TPR peptide has demonstrated selective cancer-cell cytotoxicity (Horibe et al. 2011). Secreted STI1 also functions as a cell-signaling molecule promoting neuritogenesis and neuroprotection to hippocampal neurons through its interaction with the cellular prion protein (PrP^C) (Zanata et al. 2002; Lopes et al. 2005). The PrP^C binding site of STI1 has been localized to the TPR2A domain of STI1. The neurotrophic signaling induced by PrP^C-STI1 binding may have a role in the pathology of neurodegenerative diseases. Backbone resonance assignment of the TPR1 and TPR2A domains will facilitate screening of further selective inhibitors of Hsp complex formation that may prove effective in cancer treatment and for future studies involving the STI1-PrP^C signaling complex.

Methods and experiments

Plasmid generations

The expression vector pTrcHis containing mouse STI1 cDNA (GenBank# NM_016737) was graciously provided by Dr. Vilma Martins (A.C. Camargo Hospital, Sao Paulo,

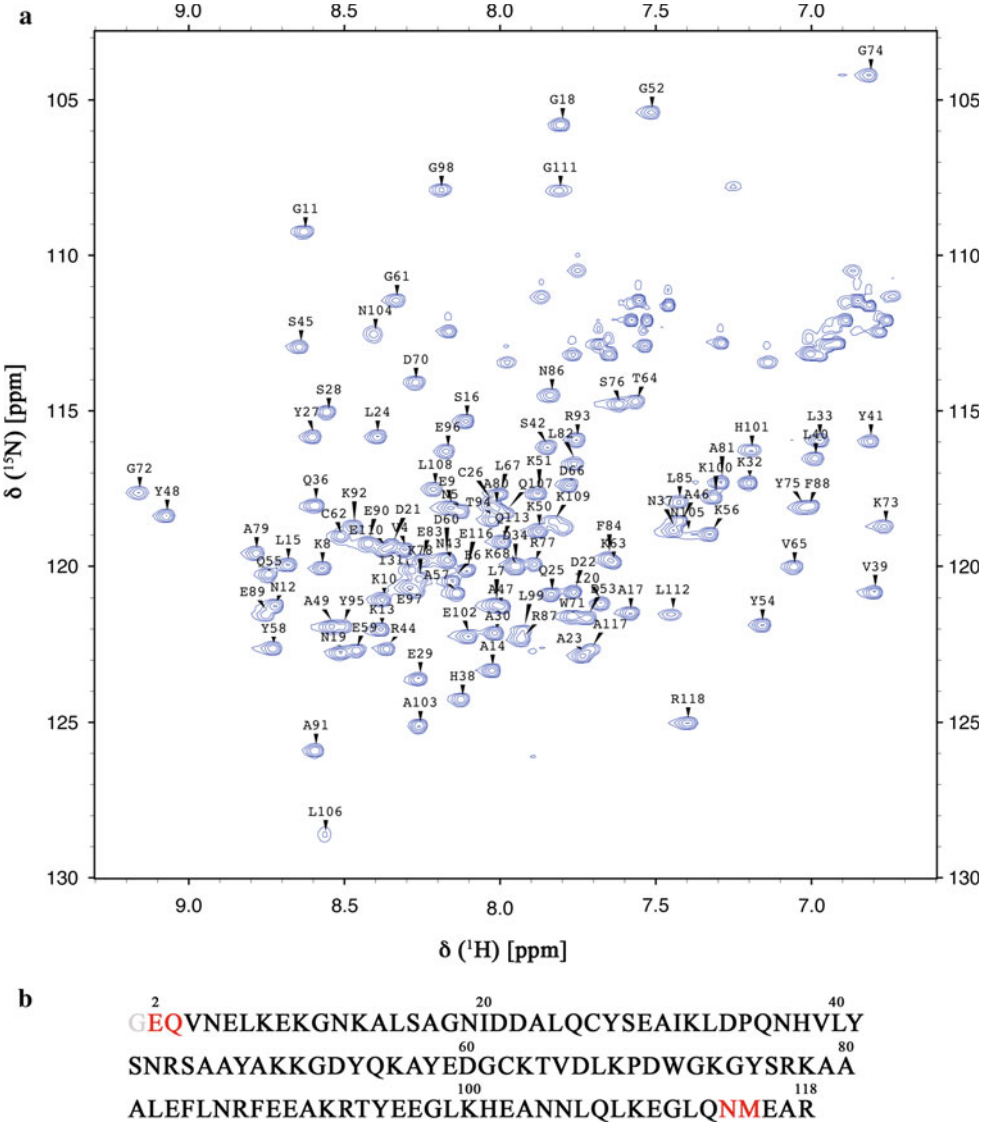
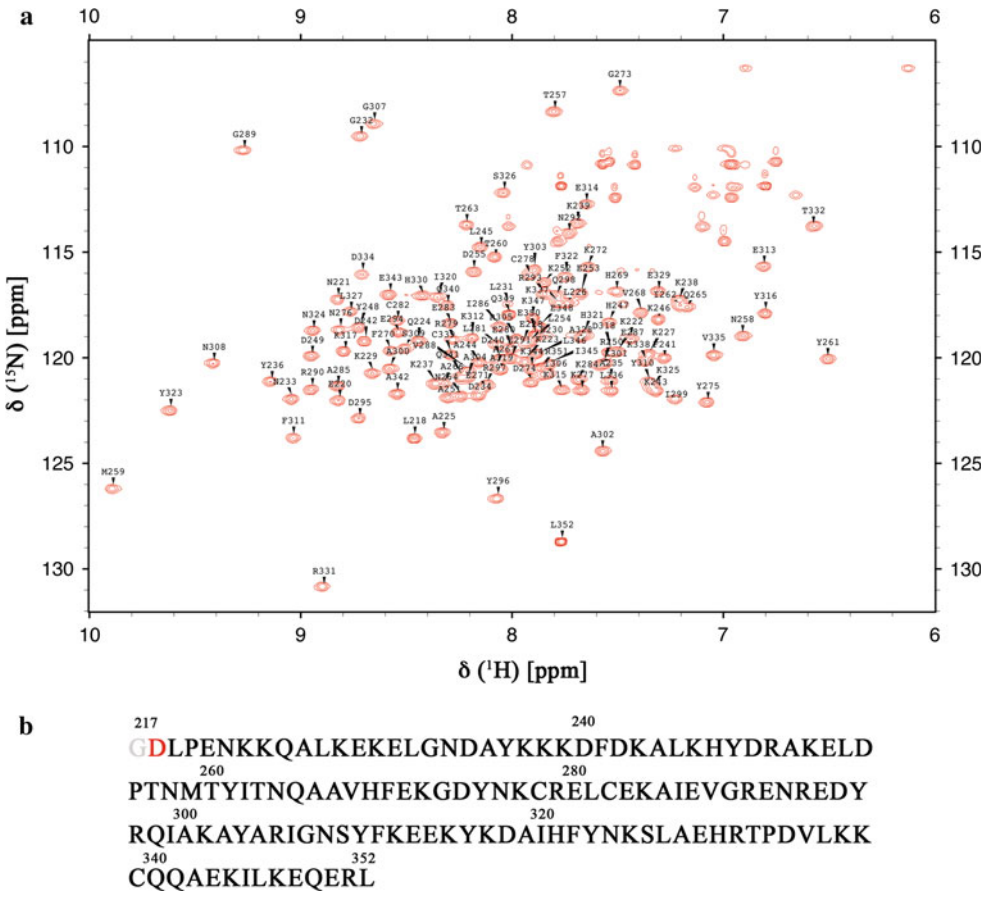


Fig. 1 **a** ^{1}H-^{15}N HSQC spectrum and backbone resonance assignment of $^{15}N/^{13}C$ labeled TPR1 domain of STI1. Spectra were generated using Sparky (Goddard and Kneller). **b** Amino acid sequence of TPR1 domain with unassigned residues *colored red*. The N-terminal glycine is a non-native residue from the TEV protease recognition site



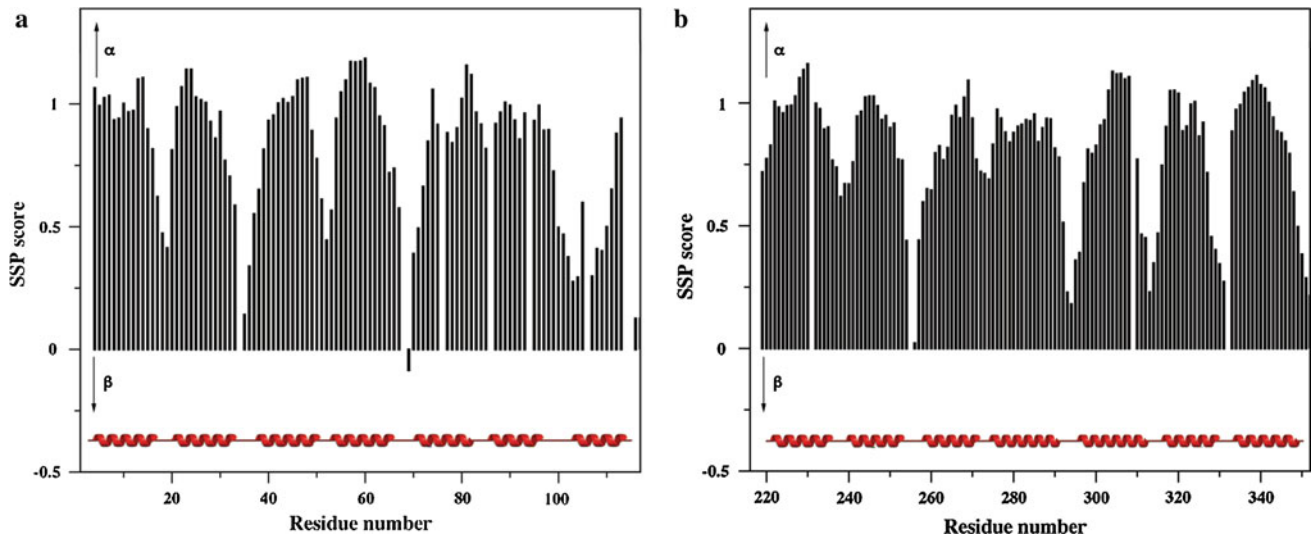


Fig. 3 Secondary structure propensity (SSP) scores and DSSP analysis of **a** the TPR1 and **b** TPR2A domains of STI1. ^{13}C α/β chemical shifts were used to calculate SSP scores for each domain (Marsh et al. 2006). Crystal structures of TPR1 in complex with Hsp70 C-terminal peptide (PDB: 1ELW) and TPR2A in complex with C-terminal Hsp90 peptide (PDB: 1ELR) (Scheufler et al. 2000) were used to generate the secondary structure diagrams of each domain (Laskowski 2009)

Brazil). Sequence encoding the TPR1 domain, residues 2-118, were amplified by PCR using the two primers:

5′-GGGGACAAGTTTGTACAAAAAAGCAGGCTCTG AAAACCTGTATTTTCAGG GAGAGCAGGTGAATGAG CTAAAGG-3′ and 5′-GGGGACCACTTTGTACAAGAAA GCTGGGTCTCACCTGGCCTCCATGTTCTG-3′.

TPR2A domain, residues 217–352, were amplified by two successive rounds of PCR using primers 5′-GAAAA

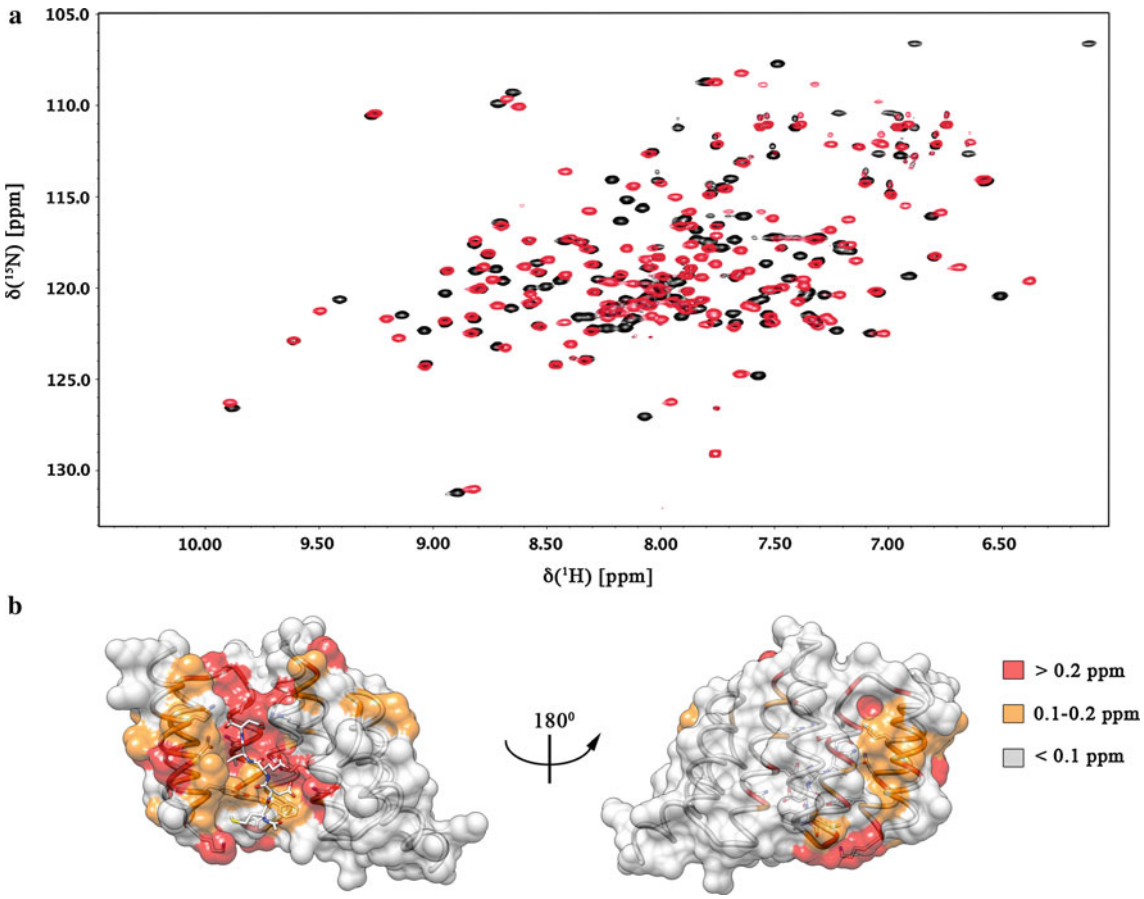


Fig. 4 **a** Overlay of ^{1}H-^{15}N HSQC spectra of TPR2A in the absence (*black*) and presence (*red*) of equimolar concentrations of Hsp90 C-terminal peptide. **b** Crystal structure of TPR2A in complex with Hsp90 peptide (PDB: 1ELR; Scheufler et al. 2000) with traceable chemical shift changes upon addition of Hsp90 peptide colored based on the magnitude of the combined chemical shift changes in ^{1}H and ^{15}N dimensions

CCTGTATTTTCAGGGAGATCTTCCAGAGAATAA-3′ and 5′-AAGAAAGCTGGGTCTCACAAGCGCTCCTG-3′ followed by amplification using 5′-GGGGACAAGTTT GTACAAAAAAGCAGGCTCTGAAAACCTGTATTTTC-3′ and 5′-GGGGACCACTTTGTACAAGAAAGCTGGG-3′. The resultant cDNA was cloned into separate pDEST17 (Invitrogen) expression vectors with an additional tobacco etch virus (TEV) cleavage recognition site 'ENLYFQG' between the 6×His tag and the insert.

Sample preparation

The expression vectors containing mouse TPR1 and TPR2A were transformed in *Escherichia coli* BL21 (DE3) pLysS. Uniformly ^{15}N, ^{13}C- labeled protein was over-expressed by growing *E. coli* in 1 L M9 media supplemented with 1 g of NH_4Cl and 3 g of ^{13}C-glucose at 37 °C to an OD_{600} of 0.9, at which point protein over-expression was induced with 1 mM IPTG. Cultures grew for 6 h at 37 °C and centrifuged pellets were frozen. N-terminally His-tagged fusion proteins were purified by affinity chromatography using Ni Sepharose™ 6 Fast Flow beads (Amersham Biosciences). The N-terminal His-tag was removed by incubation with His-tagged TEV protease overnight at 22 °C. TEV protease was removed by an additional affinity chromatography step using Ni Sepharose™ 6 Fast Flow beads (Amersham Biosciences). Proteins were dialyzed into 50 mM sodium phosphate buffer, 50 mM NaCl, 1 mM DTT at pH 7 and subsequently concentrated to ~500 μM. NMR samples contained 10 % D_2O and 100 μM 2,2-dimethyl-2-sila-pentane-5-sulfonic acid (DSS) for ^{1}H and ^{13}C chemical shift referencing.

NMR spectroscopy

NMR experiments for backbone resonance assignments of TPR1 and TPR2A were conducted at 25 °C on a Varian INOVA 600 MHz spectrometer equipped with either a cryogenic probe or a regular triple resonance probe (UWO Biomolecular NMR Facility). Sequential assignments were obtained for the TPR1 domain using the following experiments: HNCACB and CBCA(CO)NH and C(CO)NH

spectra. The TPR2A domain was assigned using HNCACB and CBCA(CO)NH experiments. All spectra were processed using NMRPipe (Delaglio et al. 1995) and analyzed using CARA (Keller 2004).

Ligand binding experiment

Binding of the TPR2A domain to the peptide encoding the C-terminal Hsp90 residues (Ac-MEEVD-NH_2 ordered from GenScript Inc. USA) was confirmed by ^{1}H-^{15}N HSQC spectra collected in the presence and absence of equimolar concentrations of Hsp90 peptide ($\sim$250 μM). Experiments were collected on a Varian INOVA 600 MHz spectrometer equipped with a cryogenic probe (UWO Biomolecular NMR Facility).

Assignments and data deposition

Backbone resonance assignments for $^{1}H^{N}$, ^{15}N, ^{13}Cα and ^{13}Cβ were obtained for the TPR1 and TPR2A domains of STI1. For the TPR1 domain, 97 % of the $^{1}H^{N}$ and ^{15}N resonances of non-proline residues, 94 % ^{13}Cα and 87 % ^{13}Cβ of all residues were assigned (Fig. 1). For the TPR2A domain, 99 % of $^{1}H^{N}$ and ^{15}N resonances of non-proline residues, 95 % ^{13}Cα and 96 % ^{13}Cβ of all residues were assigned (Fig. 2). Chemical shift assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu), under accession numbers 18691 and 18689 for the TPR1 and TPR2A domains, respectively.

Crystal structures of TPR1 and TPR2A in complex with C-terminal peptides of Hsp70 and Hsp90 indicate each domain to be composed of seven α-helices arranged in antiparallel helix-turn-helix motifs (Scheufler et al. 2000) (PDB: 1ELW for TPR1 and PDB: 1ELR for TPR2A). Secondary structure propensity (SSP) scores (Marsh et al. 2006) using ^{13}C α/β chemical shifts indicate no β-strand propensity in TPR1 or TPR2A domains and agree with the secondary structure diagram (Laskowski 2009) generated from the TPR1 and TPR2A crystal structures (Fig. 3) (Scheufler et al. 2000).

^{1}H-^{15}N HSQC of the TPR2A domain collected in the absence and presence of equimolar concentrations of Hsp90 C-terminal peptide produced large chemical shift changes indicative of binding (Fig. 4a). The magnitude of chemical shift perturbations for traceable residues was calculated from the combined chemical shift changes in ^{1}H and ^{15}N dimensions $\left(\Delta\omega = |0.2 * \Delta^{15}\mathrm{N}| + |\Delta^{1}\mathrm{H}^{\mathrm{N}}|\right)$ in ppm. Residues demonstrating the largest combined chemical shift changes (>0.1 ppm) clustered to the binding interface of the TPR2A-Hsp90 C-terminal peptide complex (Fig. 4b) (Scheufler et al. 2000) further confirming the assignment.

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