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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of the SNT PTB domain in complex with FGFR1 peptide

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Biological context

Suc1-associated neutrophic factor targets (SNTs) are a family of phosphotyrosine binding (PTB) domain-containing adapter proteins that transduce activation of fibroblast growth factor receptors (FGFRs) and neurotrophin receptors (TRKs) to common signaling targets, including SHP-2 tyrosine phosphatase and the Ras/MAPK pathway (Wang et al., 1996; Kouhara et al., 1997). SNTs have been implicated as a functional link between neurotrophic factor signals and the changes in gene expression leading to the development of sympathetic neurons (Hadari et al., 1998). Interestingly, the PTB domain of SNT-1 is capable of interacting with two receptors that share no sequence homology but are both known to bind to growth factors involved in neuronal differentiation. Specifically, the SNT-1 PTB domain can bind to an activated and tyrosine-phosphorylated NPXpY motif in TRKs as well as a non-phosphorylated juxtamembrane region in FGFRs lacking tyrosine or asparagine residues (Xu et al., 1998; Ong et al., 2000). The ability of the SNT PTB domain to recognize two different receptor sequences may play a vital role in regulating differentiating neurons to undergo an observed developmental switch in trophic dependence from FGFs to neurotrophins (Ip et al., 1994; Stemple et al., 1998).

In order to understand the detailed molecular mechanisms of the SNT-1 PTB domain interactions with its biological receptors and the functional implications, we have undertaken NMR structural analysis of the protein in complex with FGFR1. Here we report the nearly complete assignments of ^{1}H, ^{13}C and ^{15}N resonances for the SNT-1 PTB domain complexed to a 22-residue peptide derived from the juxtamembrane region of human FGFR1.

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Methods and results

The PTB domain of human SNT-1 (residues 11–140) was subcloned into a modified bacterial expression vector pET28b (Novagen) and overexpressed in *E. coli* BL21 (DE3) cells as a recombinant protein with a C-terminal cleavable hexa-histidine tag. Uniformly ^{15}N- and ^{15}N/^{13}C-labeled proteins were obtained by growing cells in M9 minimal medium containing $^{15}NH_4Cl$ with or without $^{13}C_6$-glucose (Isotec). Uniformly ^{15}N/^{13}C-labeled and fractionally deuterated protein was prepared using 75% $^{2}H_2O$ in the medium. The protein was expressed mostly in the soluble fraction and purified by affinity chromatography on a nickel-IDA column (Invitrogen). Cleavage of the His_6 tag was achieved by treatment with thrombin, leaving an additional four-residue LVPR sequence at the C-terminus from the engineered thrombin site. A protein refolding procedure was used after thrombin treatment, followed by ion-exchange chromatography to ensure proper folding and high purity. The FGFR1 peptide was chemically synthesized on a MilliGen 900 peptide synthesizer (Perkin Elmer) using Fmoc/HBTU chemistry. NMR samples consisted of the SNT-1 PTB domain/FGFR1 peptide (1/1) of approximately 0.5 mM in 100 mM phosphate buffer of pH 6.5, 5 mM DTT-d_{10} and 0.5 mM EDTA in H_2O/$^{2}H_2O$ (9/1) or $^{2}H_2O$. All NMR experiments were conducted at 30 °C on a Bruker DRX600 or DRX500

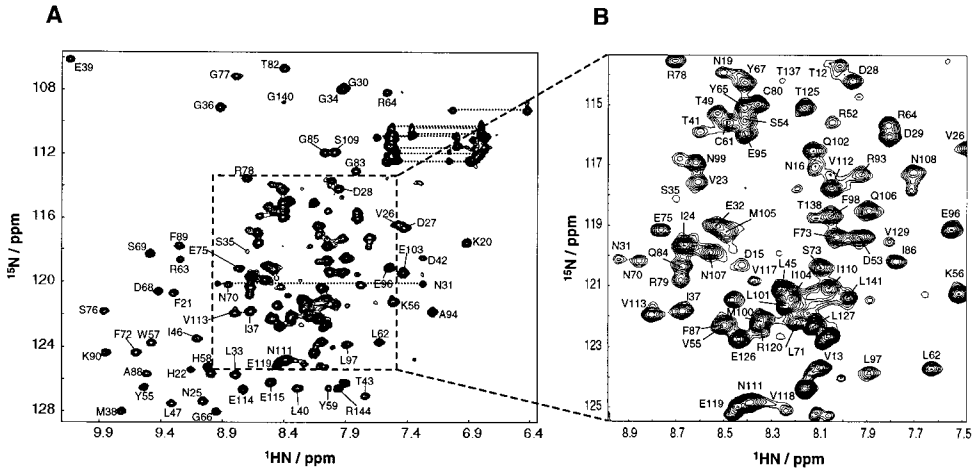

Figure 1. Two-dimensional ^{15}N-HSQC spectrum of the ^{15}N-labeled SNT-1 PTB domain (residues 11–140) in complex with non-labeled FGFR1 peptide, collected at pH 6.5 and 30 °C. (A) The full spectrum of the complex. Peaks of side chain NH atoms of Gln and Asn residues are connected by dashed lines. (B) Expansion of the region enclosed by a dashed box in Figure 1A.

spectrometer equipped with four RF channels and a triple-resonance probe with triple-axis pulsed field gradients. NMR data were processed using the NMRPipe package (Delaglio et al., 1995) and analyzed with the NMRView Program (Johnson and Blevins, 1994). Sequence-specific backbone and side-chain resonance assignments were made using deuterium-decoupled triple-resonance HNCA, HN(CO)CA, HNCACB, HN(CO)CACB and (H)C(CO)NH-TOCSY spectra (Yamazaki et al., 1994) collected with a uniformly ^{15}N/^{13}C-labeled and fractionally deuterated PTB domain in complex with a non-labeled FGFR1 peptide. The remaining side-chain resonances were assigned from a 3D HCCH-TOCSY experiment recorded from a fully protonated and uniformly ^{15}N/^{13}C-labeled PTB domain in complex with non-labeled FGFR1.

Extent of assignments and data deposition

The full ^{15}N-HSQC spectrum for the ^{15}N-labeled protein/non-labeled peptide complex is shown in Figure 1A and the region enclosed by a dashed box is expanded in Figure 1B. Complete backbone assignments were obtained for >98% of the residues comprising the structurally ordered regions of the protein. Side chain ^{1}H and ^{13}C resonances were assigned for >95% of protein residues in these regions. In addition, ^{1}H resonance assignments were made for >90% of the peptide residues. A complete table of ^{1}H, ^{15}N and ^{13}C chemical shift assignments for both the SNT-1 PTB domain and the FGFR1 peptide in the complex has been deposited in the BioMagResBank Database (http://www.bmrb.wisc.edu) under accession number 4790.

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References

Delaglio, F., Grzesiek, S., Vuister, G.W., Zhu, G., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.

Hadari, Y.R., Kouhara, H., Lax, I. and Schlessinger, J. (1998) *Mol. Cell. Biol.*, **18**, 3966–3973.

Ip, N.Y., Boulton, T.G., Li, Y., Verdi, J.M., Birren, S.J., Anderson, D.J. and Yancopoulos, G.D. (1994) *Neuron*, **13**n 443–455.

Johnson, B.A. and Blevins, R.A. (1994) *J. Biomol. NRM*, **4**, 603–614.

Kouhara, H., Hadari, Y.R., Spivak-Kroizman, T., Schilling, J., Bar-Sagi, D., Lax, I. and Schlessinger, J. (1997) *Cell*, **89**, 693–702.

Ong, S.H., Guy, G.R., Hardari, Y.R., Laks, S., Gotoh, N., Schlessinger, J. and Lax, I. (200) *Mol. Cell. Biol.*, **20**, 979–989.

Stemple, D.L., Mahanthappa, N.K. and Anderson, D.J. (1998) *Neuron*, **1**, 517–525.

Wang, J.-K., Xu, H., Li, H.-C. and Goldfarb, M. (1996) *Oncogene*, **13**, 721–729.

Xu, H., Lee, K.W. and Goldfarb, M. (1998) *J. Biol. Chem.*, **273**, 17987–17990.

Yamazaki, T., Lee, W., Arrowsmith, C.H., Muhandiram, D.R. and Kay, L.E. (1994) *J. Am. Chem. Soc.*, **116**, 11655–11666.