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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C resonance assignments for the PTB domain of the signaling protein Shc

Amjad Farooq*, Lei Zeng & Ming-Ming Zhou*
Structural Biology Program, Department of Physiology and Biophysics, Mount Sinai School of Medicine, New York, NY 10029-6574, U.S.A.

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Biological context

Protein-protein interaction via phosphotyrosine is an essential mechanism in cell signal transduction (Pawson and Nash, 2000). One of the key players in orchestrating cell signaling via tyrosine phosphorylation is the phosphotyrosine binding (PTB) domain of the signaling protein Shc (Kavanaugh and Williams, 1994). The PTB domain of Shc binds in a highly specific manner to the cytoplasmic domain of activated tyrosine-phosphorylated receptors and relays the signal to downstream proteins for cellular activities such as cell growth, cell differentiation and apoptosis. In order to understand the structural basis of the action of the PTB domain, we have already solved a high resolution solution structure of the Shc PTB domain complexed to a tyrosine-phosphorylated peptide (Zhou et al., 1995). However, the notion that proteins lack intrinsic globular structure under physiological conditions and that the attainment of fully folded structure only occurs upon ligand binding is rapidly gaining popularity (Dyson and Wright, 2002). It has been argued that such a behavior of proteins accounts for their diverse structural and functional versatility. Given that the Shc PTB domain is functionally promiscuous in its ability to recognize receptors as diverse as growth factor, antigen, cytokine, and G-protein-coupled and hormone receptors, it is possible that such a mechanism of protein structural and functional versatility may also be operative in this important protein module. In an effort to understand the extent to which ligand binding controls the formation of the intrinsic globular fold in the case of the Shc PTB domain, we have undertaken studies to obtain a high resolution solution structure of this PTB domain in the absence of its peptide ligand. Here, we report the nearly complete sequence-specific backbone and side-chain ^{1}H, ^{15}N, and ^{13}C resonance assignments of the free form of the PTB domain of the signaling protein Shc.

*To whom correspondence should be addressed. E-mails: amjad.farooq@mssm.edu; zhoum@inka.mssm.edu

Methods and results

The Shc PTB domain construct (residues 1–207) was subcloned into the pET15b vector and expressed in E. Coli BL21(DE3) cells. Uniformly ^{15}N-labeled or $^{13}C/^{15}N$-labeled protein samples were prepared by growing the bacteria in minimal media containing $^{15}NH_4Cl$, with or without [U-^{13}C]-glucose (Cambridge Isotope Labs). Uniformly $^{13}C/^{15}N$-labeled, fractionally deuterated proteins were prepared in a similar fashion by using 75% $^{2}H_2O$. The proteins were purified to apparent homogeneity by affinity chromatography on a nickel-IDA column (Invitrogen) followed by the removal of poly-histidine tag by thrombin cleavage. NMR samples of the protein (typically $\sim$ 0.5 mM) were prepared in 50 mM Sodium phosphate buffer of pH 6.5, containing 20 mM perdeuterated DTT, in $H_2O/^{2}H_2O$ (90%/10%) or $^{2}H_2O$. All NMR experiments were carried out at 35 °C on Bruker DRX500 and DRX600 spectrometers equipped with four rf channels, and a triple-resonance probe with 3-axis pulsed field gradients. The NMR data were processed and analyzed using programs of NMRPipe (Delaglio et al., 1995) and NMRView (Johnson and Blevins, 1994). Deuterium-decoupled triple-resonance experiments HNCACB, and HN(CO)CACB (Yamazaki et al., 1994) which

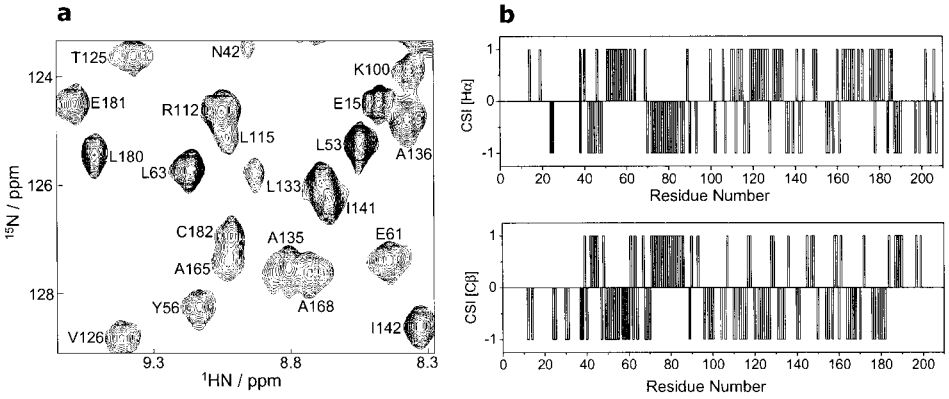

Figure 1. PTB domain of the signaling protein Shc (residues 1–207). (a) A region of $^{1}H/^{15}N$ HSQC showing amide resonances of select residues. (b) CSI plots for the Hα and Cα atoms as a function of protein chain length.

were recorded with a uniformly $^{13}C/^{15}N$-labeled and fractionally (75%) deuterated sample, were used to obtain the backbone resonance assignments. The side chain ^{1}H and ^{13}C atoms were assigned using a 3D (H)C(CO)NH-TOCSY (Logan et al., 1993) experiment recorded on the $^{2}H(75\%)/^{13}C/^{15}N$-labeled sample. Side chain ^{1}H resonances were assigned with a 3D HCCH-TOCSY spectrum (Clore and Gronenborn, 1994) using a fully protonated $^{13}C/^{15}N$-labeled sample in $^{2}H_2O$, and were confirmed with a 3D ^{15}N-dispersed TOCSY-HSQC. The side chain ^{1}H and ^{13}C resonances for the aromatic residues were assigned using a combination of experiments, including ^{13}C HSQC, CT-^{13}C HSQC, 3D HCCH-TOCSY recorded in the aromatic carbon region with the double labeled sample.

Extent of assignment and data deposition

Apart from the unstructured N-terminal region (residues 1–39), backbone assignment of ^{1}HN, ^{15}N, $^{13}C\alpha$, and $^{13}C\beta$ atoms for nearly 95% residues was obtained. The assignment for the residues 1–39 proved extremely difficult due largely to poor chemical shift dispersion and line broadening in the NMR spectra. Figure 1a shows a region of the $^{1}H/^{15}N$ HSQC spectrum for the Shc PTB domain at pH 6.5 and 35 °C. Excluding the N-terminal unstructured region (residues 1–39), the side chain ^{1}H and ^{13}C resonance assignments were obtained for about 90% of the residues. A total of 51 slowly exchanging amide protons were identified with a series of ^{15}N-HSQC spectra recorded on an uniformly ^{15}N-labeled sample after the H_2O buffer was changed to $^{2}H_2O$ buffer. A total of 69 $^{3}J_{NH\text{-}H\alpha}$ coupling constants were measured with a 3D HNHA spectrum (Vuister and Bax, 1993). The chemical shift index (CSI) of the Hα and Cα atoms (Figure 1b), characteristic sequential and medium range NOEs, and $^{3}J_{NH\text{-}H\alpha}$ coupling constants indicate that like the complex form, the free form of the Shc PTB domain is also comprised of a mixed αβ fold. A table of the ^{1}H, ^{15}N, and ^{13}C chemical shift assignments of the Shc PTB domain has been deposited in the BioMagResBank Database (http://www.bmrb.wisc.edu) under the accession number 5566. The ^{13}C chemical shifts reported are referenced against the protonated sample.

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