Journal of Biomolecular NMR, **12:** 461–462, 1998.
KLUWER/ESCOM

Assignment of ^{1}H and ^{15}N resonances of murine Tec SH3 domain

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Received 3 June 1998; Accepted 25 June 1998

Key words: assignment, protein tyrosine kinase, Src homology 3 domain, Tec kinase

Biological context

Tec is a widely expressed intracellular tyrosine kinase thought to be involved in the control of cell growth and differentiation of haematopoietic cells. Although the precise function of Tec remains unclear, it has been implicated in signalling pathways downstream of a variety of receptor proteins, including c-Kit and gp130 (Matsuda et al., 1995; Tang et al., 1994). Tec, like other family members, Btk, Txk, Bmx and Itk, is composed of multiple domains, which include pleckstrin homology (PH), Tec homology (TH), Src homology 2 (SH2), Src homology 3 (SH3) and kinase domains, as well as a proline-rich region (PRR) (Mano et al., 1990). Alternative splicing of exon 8 in specific tissues is responsible for producing the two major Tec isoforms, III and IV, with Tec III lacking the C-terminal 22 residues of the SH3 domain. An equivalent deletion has been seen in Btk and has been implicated in X-linked agammaglobulinemia (Zhu et al., 1994).

In the Tec family, the SH3 domain is likely to play an important regulatory role through intra- as well as inter-molecular interactions (Andreotti et al., 1997). In the case of Tec, the intramolecular mode of association is complicated by the presence in the PRR of four putative SH3 binding motifs (PXXP). We are investigating the structure and molecular interactions of Tec regulatory domains and report here the ^{1}H and ^{15}N chemical shifts for the isolated Tec SH3 domain.

Methods and results

Purified samples of the Tec SH3 domain protein, representing residues 181–245 of the Tec IV sequence, were obtained after thrombin cleavage of GST-fusion protein expressed in *Eschericia coli* strain BL21–DE3. Figure 1a shows the primary sequence of the Tec SH3 domain protein used in this study and includes a GlySer N-terminal extension (numbered -2 and -1, respectively) derived from the fusion partner. Uniformly ^{15}N-labelled protein was grown in Minimal A medium supplemented with 0.81 g/L $^{15}NH_4Cl$ (Miller, 1972). Mass spectrometric analysis of all samples agreed with the expected values of M_r 7899 and M_r 7994 for the unlabelled and ^{15}N labelled samples respectively. Spectra were recorded on 2.0 mM (unlabelled) and 1.25 mM (^{15}N labelled) samples, in 10 mM phosphate, 0.1% (w/v) NaN_3, pH 6.0.

NMR experiments were performed on Bruker AMX-500 and DRX-600 and Varian Inova 600 spectrometers. All data sets were recorded at 25 °C using 5 mM inverse triple resonance $^{1}H/^{13}C/^{15}N$ pfg probes. 3D-NOESY HSQC and 3D-TOCSY HMQC data were recorded using 1024 × 32 × 72 complex points and sweep widths of 1824 Hz in F1 and 7507 Hz in F2 and F3. NOESY and TOCSY mixing times of 200 ms and 50 ms, respectively, were used for both the 3D and 2D experiments. Cosine-squared functions were applied to the data prior to Fourier transformation. The final matrix size was 896 × 64 × 256. 2D ^{1}H–^{15}N HSQC data were recorded with spectral widths of 2500 Hz in F2 and 6250 Hz in F1, using 400 t_1 increments. 2D NOESY, TOCSY and DQF-COSY experiments were recorded with a sweep width of 8000 Hz in both dimensions and a minimum of 2048 × 512 complex points. A cosine-squared function was applied to the data prior to Fourier transformation. These data were also zero-filled to a final matrix size of 2048 × 2048. Processed data was analysed using XEASY (Bartels et al., 1995).

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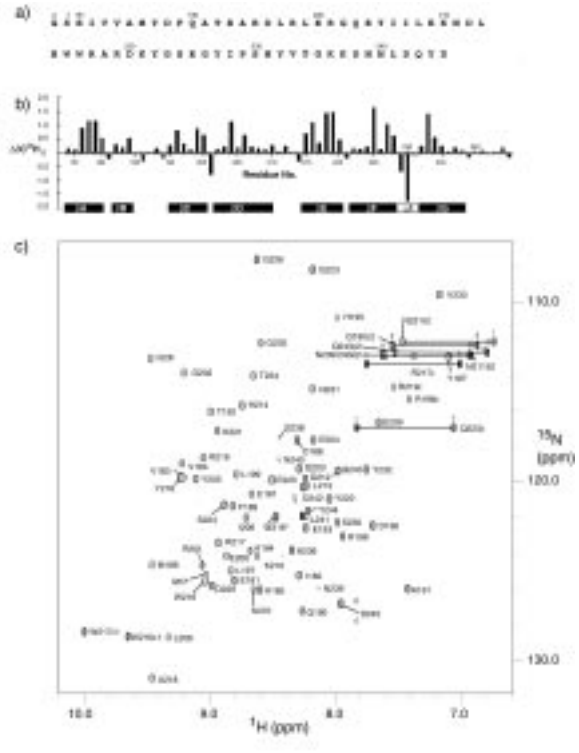

Figure 1. (a) Primary sequence of the Tec SH3 domain representing amino acids 181–245 of the Tec IV sequence. The N-terminal residues Gly^{-2} and $Ser^{-1}$are derived from the fusion partner after thrombin digestion. (b) Deviation of $C^{\alpha}H$ chemical shifts from random coil values (Wishart et al., 1995), plotted against residue number. Sections of three or more with values greater than 0.1 ppm are indicative of β-sheet, whereas values of less than −0.1 ppm indicate α-helix. Regions of β-strands predicted from this analysis are shown in black boxes and are labelled βA-βG. A region of helix is predicted and is shown as an open box labelled α1. (c). Contour plot of a fully assigned 600 MHz 2D ^{1}H-^{15}N HSQC spectrum recorded on a 1.25 mM solution of uniformly ^{15}N-labelled Tec SH3 domain in 90% H_2O/10% D_2O at pH 6.0 and 25 °C. Spectral widths of 2500 Hz in F2 and 6250 Hz in F1 were used, along with 400 t_1 increments. All residues are shown, except for Pro^{229} and the N-terminal dipeptide. All Arg, Asn and Gln side-chain NH resonances have been highlighted and connecting lines drawn between the two NH resonances for Asn and Gln side chains.

Extent of assignments and data deposition

A contour plot of a ^{15}N–^{1}H HSQC spectrum recorded on uniformly ^{15}N-labelled Tec SH3 domain is shown in Figure 1c. This HSQC shows good dispersion in both the ^{15}N and ^{1}H dimensions, although one section (∼118–122 ppm ^{15}N) displays some overlap. All backbone amide resonances except those from Gly^{-2} and Ser^{-1} are labelled, as are the Arg $N^{\epsilon}H$, Gln $N^{\epsilon 2}H$, Asp $N^{\delta 2}H$ and Trp $N^{\epsilon 1}H$ resonances.

Backbone assignments have been obtained for 65 of 67 residues, the exceptions being Gly^{-2}, for which no resonances could be assigned, and Ser^{-1} for which no backbone NH resonance could be assigned. Complete side chain assignments of non-exchangeable protons were obtained, with the exception of the $C^{\epsilon 1}H$ resonances of His^{195} and His^{214}. With regard to exchangeable side chain groups, all Trp $N^{\epsilon 1}H$, Asn $N^{\delta 2}H$, Gln $N^{\epsilon 2}H$ and Arg $N^{\epsilon}H$ were assigned, but no Arg $N^{\eta}H$ or Ser and Thr hydroxyl protons could be assigned.

The chemical shift deviation from random coil, plotted against residue number (Figure 1b) indicates that the secondary structure of Tec SH3 domain is predominantly β-strand, with a small helix (α1) present between amino acids 229–231. This pattern of secondary structure, is similar to that of other published SH3 domains, suggesting that α1 is likely to be a 3_{10}-helix.

Structure calculations are underway for the isolated SH3 domain in parallel with studies of the binding of Tec SH3 domain to intramolecular and intermolecular ligands. All chemical shift information has been deposited in BioMagResBank, accession number BMRB-4145.

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