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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C resonance assignments of the SH2 domain of Bruton's tyrosine kinase

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Biological context

X-linked agammaglobulinemia (XLA) is caused by mutations in the Bruton's tyrosine kinase (Btk) (Vetrie et al., 1993). The kinase is expressed in most haematopoietic cells, but is selectively down regulated in plasma cells and T-lymphocytes. Mutations or deletions in the Btk gene were detected in unrelated XLA patients (Tsukada et al., 1993), and it strongly suggests that the kinase is directly involved in the disease and, therefore, in the process of B cell development. Like many other cytoplasmic tyrosine kinases involved in signaling pathways, Btk contains an N-terminal pleckstrin homology (PH) domain, a proline-rich Tec homology (TH) domain, a Src homology 3 (SH3) domain, a Src homology 2 (SH2) domain, and a catalytic tyrosine kinase domain (Cohen et al., 1995; Pawson, 1995). The SH3 and SH2 domains are small protein modules that mediate protein–protein interactions and occur in many proteins involved in intracellular signal transduction. It has been shown that Btk SH3 domain can bind to proline-rich peptides from its own TH domain and from p120 and Btk SH2 domain binds with phosphopeptides in the order pYEEI > pYDEP > pYMEM > pYLDL > pYIIP (Tzeng et al., 2000).

Recently, the B-cell linker protein (BLNK) was found to interact with the SH2 domain of Btk and this association is required for the activation of phospholipase C-γ (Su et al., 1999). In order to understand the structure of Btk SH2 domain and the molecular basis for the interaction between Btk SH2 domain and BLNK and the cause of XLA, here we report the ^{1}H, ^{15}N and ^{13}C resonance assignments of Btk SH2 domain.

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Methods and experiments

The human Btk cDNA was kindly provided by Dr. Shi-Han Chen (University of Washington, Seattle). The Btk cDNA encoding the SH2 domain (residues 270–384) was subcloned into the pET15b (Novagen) vector and expressed in *E. coli* BL21 (DE3). Uniformly ^{15}N or ^{15}N/^{13}C labeled proteins were purified from bacteria in M9 minimal medium supplemented with ^{13}C glucose, $^{15}NH_4Cl$ and trace vital vitamins. The samples were purified by Ni-NTA column (Qiagen) and then the fractions containing Btk-SH2 were pooled and concentrated by Amicon Concentrator (Millipore). The purity of recombinant SH2 domain from Btk 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 0.6 to 2.0 mM in phosphate buffer (50 mM K_2HPO_4, 100 mM NaCl, 10 mM NaN_3, and 0.1 mM EDTA, 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,

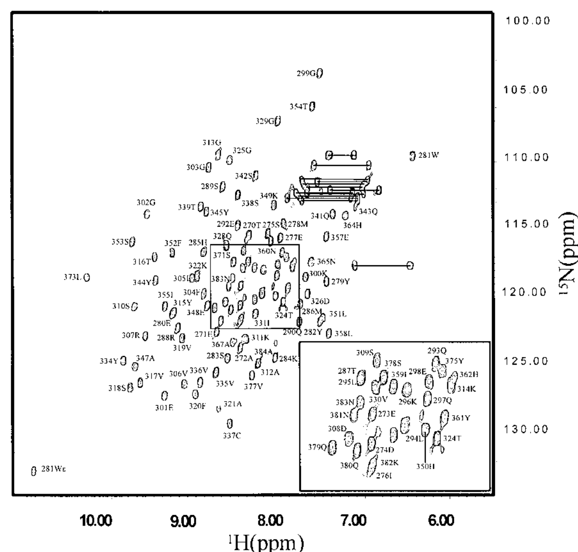

Figure 1. A 600-MHz 2D ^{1}H-^{15}N HSQC spectrum of Btk SH2 domain recorded at 25 °C, pH 6.5. The assignments are indicated with the one-letter amino acid code and residue number. Side chains of NH_2 resonances of asparagine and glutamine are connected by horizontal lines.

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 115 residue Btk SH2 domain are essentially complete. A well-resolved 2D ^{1}H-^{15}N HSQC spectrum is shown in Figure 1. Residues with missing assignments are Arg332, Ser366, and Arg372. Examination of the spectrum under a variety of sample conditions (temperature and pH) has failed to reveal these 'missing peaks'. For all of the Proline residues, assignments were obtained from HNCO, HN(CA)CO, HN(CO)CA, HNCA, CBCA(CO)NH and HCCH-TOCSY spectra. In total, 95% of $^{1}H_{\alpha}$ and $^{13}C_{\alpha}$, 91% of ^{1}HN and ^{15}N, 92% of $^{13}C_{\beta}$ and ^{13}CO were obtained. As for the side chain resonances, 91% of the $^{1}H_{\beta}$ and 83% of the overall side chain ^{1}H resonances were assigned. Analysis of C_{α}, C_{β}, NH and H_{α} chemical shifts has established that the Btk SH2 domain has the mixed α/β topology of typical SH2 domain (Kuriyan and Cowburn, 1993). 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 6422.

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