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Letter to the Editor: ^{1}H and ^{15}N chemical shift assignments for domain 4 of the common β-chain of the IL-3, IL-5 and GM-CSF receptors

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Biological context

The cytokines interleukin (IL) -3, IL-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF) are extracellular signalling molecules that bind to a specific class of cell surface receptors. They elicit cellular responses such as proliferation, differentiation or protection from programmed cell death. Typically, cytokine–receptor complexes are oligomeric, involving the ligand and two or more (identical or different) receptor molecules. In the case of IL-3, IL-5 and GM-CSF the receptor complex involves an α subunit, which is specific to the ligand involved (IL-3Rα, IL-5Rα and GMRα), and a β subunit (β_c) common to all three, which confers high affinity binding of cytokines and tranduces signals (Bagley et al., 1997).

The human β_c molecule (SWISS-PROT: P32927) is a protein of 897 residues composed of 16 signal residues, four ∼100-residue extracellular regions predicted to be fibronectin type-III (Fn3) domains, a single membrane spanning region and 437 intracellular residues (Hayashida et al., 1990). Fn3 domains are made up of seven β-strands (denoted A–G) arranged in a three-stranded antiparallel β-sheet (strands A, B and E) and a four-stranded antiparallel β-sheet (strands D, C, F and G). The four Fn3 domains comprise two cytokine receptor modules (CRMs) each of which, on the basis of structural data for several cytokine receptors, is expected to be a pair of loosely related Fn3 domains oriented roughly at right angles to each other. Mutational analyses of β_c have mapped sites that confer high affinity binding of ligand to the second CRM and specifically to the putative B–C (Lock et al., 1994; Woodcock et al., 1994) and the F–G loops (Woodcock et al., 1996) of the membrane proximal Fn3 module, domain 4. Domain 4 contains two sequence motifs that are well conserved across CRMs: an alternating pattern of hydrophobic residues YXVVRVR expected to lie in β-strand F and the WSXWS motif expected to lie at the beginning of β-strand G. We are investigating the structure of domain 4 and the structural effect of several mutations that lead to ligand independent signalling. We report here the ^{1}H and ^{15}N chemical shift assignments for the wild-type sequence domain 4 of the human common β chain of the IL-3, IL-5 and GM-CSF receptors.

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Methods and results

Domain 4 of β_c (residues 338–438 with an additional N-terminal Met, denoted Met^{-1}) was expressed in *E. coli* strain BO4 in minimal medium containing $^{15}NH_4Cl$ (0.8 g/L) as the sole nitrogen source. The protein was purified by RP-HPLC using a gradient of acetonitrile from 25% to 50% in the presence of 0.1% trifluoroacetic acid. The presence of the correct protein, and the incorporation of ^{15}N, were confirmed by ion-spray mass spectrometry which also detected a minor form (approx. 10%) that lacked the N-terminal Met residue. Samples contained 10 mM sodium phosphate, pH 6.1 and a protein concentration of approximately 0.4 mM.

All NMR experiments were performed at 20 °C on a Varian Inova 600 spectrometer equipped with a 5 mm inverse triple resonance ^{1}H/^{13}C/^{15}N pfg probe. 2D [^{15}N-^{1}H] HSQC experiments were recorded in

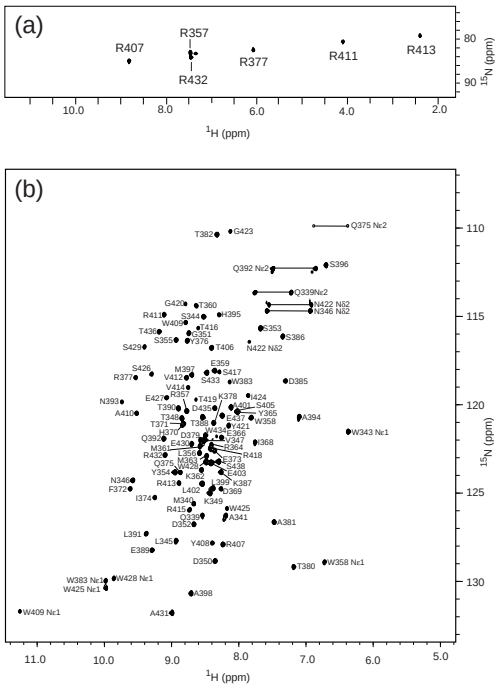

Figure 1. Regions of 2D [^{15}N-^{1}H] HSQC spectra of [U-^{15}N]domain 4 at 20 °C. (a) The region showing only the Arg N$^{\epsilon}$H resonances. (b) Backbone NH resonances are labelled with their single letter code and residue number. Side chain resonances are identified with an additional qualifier and the Asn and Gln side chain amide signals are connected by horizontal lines. The sample contained 0.4 mM protein and 10 mM sodium phosphate buffer, pH 6.1, in 90% H_2O/10% D_2O.

90% H_2O using 128 × 2048 complex points with 1640 Hz in F1 and 8000 Hz in F2. 3D-TOCSY-HSQC and 3D-NOESY-HSQC experiments were recorded in 90% H_2O using 106 × 32 × 1024 complex points and spectral widths of 1640 Hz in F2 and 8000 Hz in F1 and F3. 2D-NOESY, TOCSY and DQF-COSY data were recorded in 90% H_2O and 100% D_2O using 512 × 2048 complex points and spectral widths of 8000 Hz in F1 and F2. 3D and 2D NOESY data were recorded with mixing times of 150 ms and TOCSY data were recorded with spin-lock times of 50 ms.

Preliminary analysis of NOESY data and the deviations of C$^{\alpha}$H chemical shifts from random coil values (Wishart et al., 1995) confirm that the protein is folded and the putative positions of the seven β-strands agree well with those predicted in a model of domain 4 (Jenkins et al., 1996) based on the structure of the growth hormone receptor complex (De Vos et al., 1992). A striking feature of the [^{15}N-^{1}H] HSQC spectrum is a series of seven resonances that are roughly co-linear at ∼85 ppm in the ^{15}N dimension (Figure 1a). These resonances are from the N$^{\epsilon}$H groups of the protein's Arg residues and vary in ^{1}H-chemical shift from 8.68 ppm (N$^{\epsilon}$H Arg^{407}) to 2.28 ppm (N$^{\epsilon}$H Arg^{413}). The random coil ^{1}H-chemical shift values for Arg N$^{\epsilon}$H groups is 8.07 ppm (Wishart et al., 1995). Interestingly, the two most upfield-shifted resonances belong to Arg^{411} (3.92 ppm) and Arg^{413} (2.28 ppm) in the YXVVRVR motif. These chemical shift values alone provide circumstantial evidence that their side chains are interdigitated with the side chains of Trp^{425} and Trp^{428} in the WSXWS motif as predicted in the model of domain 4 (Jenkins et al., 1996). This novel spectral feature may serve as an indicator of the structural integrity of these sequence motifs in other CRMs.

Extent of assignments and data deposition

We have assigned the ^{15}N and ^{1}H chemical shifts of all backbone NH groups with the exception of the two N-terminal residues (Met^{-1} and Ile^{338}) and residues Lys^{384} and Asn^{422} (Figure 1b). In addition, >99% of the non-exchangeable hydrogens were assigned ^{1}H-chemical shifts. With regard to exchangeable side chain groups: all Trp N$^{\epsilon 1}$H, Asn N$^{\delta 2}$H and Gln N$^{\epsilon 2}$H groups were assigned and six of the nine Arg N$^{\epsilon}$H groups were assigned (the exceptions being Arg^{364}, Arg^{415} and Arg^{418}). No Arg N$^{\eta}$H, His N$^{\delta 1}$H, Ser O$^{\gamma}$H or Tyr O$^{\eta}$H groups could be assigned chemical shifts. However, the O$^{\gamma 1}$H group of Thr^{436} was in slow exchange and could be assigned. The chemical shift data have been deposited in the BioMagResBank, accession number BMRB-4308.

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