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Letter to the Editor: Secondary structure and backbone resonance assignments for human interleukin-13

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Biological context

Interleukin-13 (IL-13) is a T helper 2 (Th2) cytokine that elicits both pro-inflammatory and anti-inflammatory immune responses. At least two receptors exist for IL-13, one of which is shared with interleukin-4 (IL-4). The second receptor is overexpressed in several cancers, including high-grade gliomas, and has been characterized as a Cancer Testes Antigen (CTA) (Mintz and Debinski, 2000). Recent studies suggest that IL-13 plays a role in a number of other diseases such as AIDS, asthma, leishmaniasis, and helminthic infections (Brombacher, 2000). Thus, the IL-13/IL-13R complex provides attractive targets for structure-based drug design. Because IL-13 shares a 25% sequence identity with IL-4, as well as a high sequence similarity to other structurally characterized cytokines, it has been presumed to adopt a four-helix-bundle motif with an up-up-down-down topology. Furthermore, IL-13 contains four of the six cysteines present in IL-4 with analogous disulfide bonds formed (Eisenmesser et al., 2000; Tsarbopoulos et al., 2000). Circular dichroism and NMR studies have supported this high helical content; yet, thus far, no direct structural evidence has been reported (Cannon-Carlson et al., 1998; Eisenmesser et al., 2000). Here we report the backbone chemical shift assignments and secondary structure of recombinant human IL-13 (hIL-13).

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Methods and experiments

hIL-13 was expressed as a fusion protein with maltose binding protein and cleaved in vivo by constitutively expressed tobacco etch virus (TEV) protease. The cleaved protein formed inclusion bodies, which were processed to yield pure, refolded protein as described previously (Eisenmesser et al., 2000). Samples for NMR experiments were prepared in 25 mM Na_2HPO_4, pH 6.1, 50 mM NaCl, 0.01% NaN_3, and 1 mM EDTA, and the solvent was either 95% H_2O/5% D_2O or 99% D_2O as appropriate. Spectra were collected on either a Varian Unity-plus 500 or 600 spectrometer (Varian, Palo Alto, CA).

^{1}H, ^{13}C, and ^{15}N assignments were made using a combination of 3D experiments (for review and primary references see Cavanagh et al., 1996; Sattler et al., 1999), acquired using a ^{13}C,^{15}N- and a ^{15}N-labeled sample. Backbone sequential assignments were made using CBCA(CO)NH, HNCACB, HNCO, and 3D ^{15}N-edited NOESY-HSQC with τ_{mix} = 150 ms, run with PFG-coherence selection and sensitivity enhancement. Aliphatic assignments that included $^{13}C^{\alpha}$, $^{13}C^{\beta}$, $^1H^{\alpha}$ and $^1H^{\beta}$ resonances were made using HCCH-TOCSY, TOCSY-HSQC with τ_{mix} = 70 ms, 3D HNHA and 3D HNHB. Short range NOEs ($i \leq i+4$) were derived from the 3D ^{15}N-edited NOESY-HSQC, a simultaneous 3D ^{13}C- and ^{15}N-edited NOESY-HSQC with τ_{mix} = 120 ms, and a 3D ^{13}C-edited NOESY-HSQC in D_2O with τ_{mix} = 100 ms. Spectra were processed using NMRPipe (Delaglio et al., 1995) and analyzed using Ansig 3.3 (Kraulis, 1989).

The secondary structural elements of hIL-13 were determined by analysis of chemical shifts and short range NOEs (Figure 1). The chemical shift index

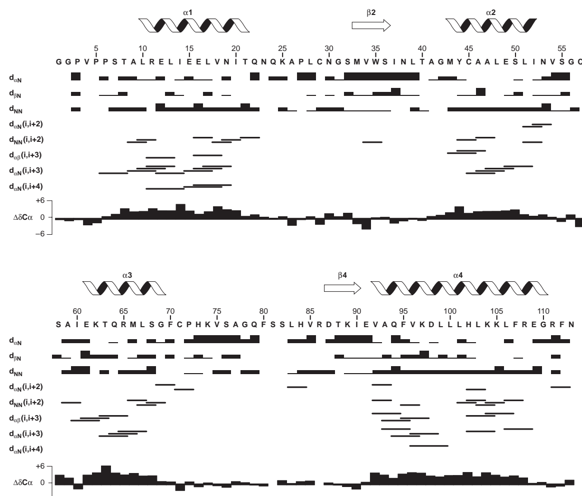

Figure 1. Secondary structure and supporting data for hIL-13. NOE connectivities and secondary $^{13}C^{\alpha}$ chemical shifts are shown as a function of sequence, along with the determined secondary structural elements.

(CSI) analysis combined with $^{13}C^{\alpha}$, $^{13}C^{\beta}$, ^{13}CO, and $^{1}H^{\alpha}$ shifts identified four helical segments spanning residues 10–21, 43–52, 61–69, and 92–110. The definition of these helical segments was further supported by N-terminal helix capping, $^{3}J_{H^{N}H^{\alpha}}$ coupling constants, i-i+3 NOEs, and predicted ϕ, ψ torsion angles using TALOS. Although earlier computational methods failed to produce a reasonable model of these helices, the experimentally determined helices correspond very closely to the lengths predicted in a recent sequence alignment with IL-4 (Tsarbopoulos et al., 2000). In addition to the four helices, two short β-strands are identified spanning residues 33–36 (β1) and 87–90 (β2) that form an anti-parallel β-sheet, analogous to the one formed in IL-4 between residues 32–34 and 110–112. These results, along with the recent structural determination of the shared receptor bound to IL-4 (Hage et al., 1999), may help elucidate IL-13/receptor interactions critical for its receptor engagement.

Extent of assignments and data deposition

Nearly complete backbone assignments were made for $^{1}H^{N}$, ^{15}N, $^{1}H^{\alpha}$, $^{1}H^{\beta}$, $^{13}C^{\alpha}$, and $^{13}C^{\beta}$ (over 98%). Residues with missing assignments included Gly^{1}, Ser^{58}, Ser^{81}, Ser^{82}, and Arg^{86}, none of which were found within elements of secondary structure (Figure 1). Most ^{13}CO assignments were determined (90%), excluding those of the aforementioned residues, residues preceding prolines, and Gln^{24}, Arg^{65}, Gln^{79}, Asp^{87}, and Phe^{112}, due to overlapping $^{1}H^{N}$ and ^{15}N resonances. Chemical shift assignments have been deposited in the BioMagResBank database (accession number 4843).

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