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Letter to the Editor: Sequence-specific NMR resonance assignments for Human Interleukin-5

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Biological context

Human interleukin-5 (hIL-5) is a short chain helical cytokine which is uniquely required for eosinophil differentiation and has been found to be important in tissue specific eosinophil responses in humans (Cuss, 2000). Normally, hIL-5 is secreted by T-cell lymphocytes in response to parasite exposure. Degranulation by eosinophils at the site of parasite exposure then results in the degradation of the parasite. However, a process known as immune deviation can result in abnormal immune responses to environmental allergens resulting in hypersensitivity disease states such as childhood asthma (Holgate, 1999). Thus, hIL-5 and its receptor have been targets for the development of asthma drugs for quite some time (Barnes, 1999). Recently, studies employing humanized monoclonal anti-IL-5 antibodies have demonstrated reductions in blood and sputum eosinophils, however, no statistically significant improvements in clinical parameters defining late asthmatic response were observed (Leckie et al., 2000). Thus, hIL-5's role in mediating the asthmatic response has become a matter of some controversy (Hansel et al., 2002).

Native hIL-5 is a homodimeric disulfide cross-linked glycosylated protein with 115 amino acids per subunit. The non-glycosylated protein has a molecular weight of roughly 25 kDa. Despite extensive glycosylation, receptor binding affinity is only slightly reduced upon deglycosylation of the interleukin (Proudfoot et al., 1990). Herein we report the NMR assignments for both backbone and side-chain nuclei for the *E. coli* expressed protein.

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Materials and experiments

Uniformly ^{15}N- and ^{13}C-labeled samples of hIL-5 were obtained using expression and purification procedures previously described (Mehta et al., 1997). Optimum sample conditions used in the acquisition of the majority of the spectra used in assignment were 0.4 mM hIL-5 in 50 mM phosphate buffer (pH 7.0) at 25 °C, 0.1 mM NaN_3 in 90% H_2O, 10% D_2O.

Spectra were recorded on Varian Unity Plus 500 and Bruker DMX 600 NMR instruments. Backbone and side-chain assignments were made based on the interpretation of the following spectra: 3D HNCA, 3D HN(CO)CA, 3D HNCACB, 3D HNCO, 3D HCACO, 3D HCA(CO)N, 3D CBCA(CO)NH, 3D HBHA(CBCACO)NH, 3D HCCH-TOCSY, 3D HNHA, 3D TOCSY-HSQC (τ_m = 70 ms), 3D NOESY-HSQC (τ_m = 150 ms) and 2D HSQC. More or less standard approaches to resonance assignment were used to obtain backbone and side-chain nuclear magnetic resonance frequencies (Grzesiek and Bax, 1993). Figure 1 contains an HSQC spectrum of ^{15}N-labeled hIL-5 with sequence specific assignments associated with all correlation peaks shown. The pET-21b synthetic gene construct results in the expression of the protein from residues 4 to 115. Most side-chain assignments could be made through alignments of CBCA(CO)NH, HNCACB, HBHA(CBCACO)NH and extremely well resolved HCCH-TOCSY spectra. Almost all of the side-chain resonance assignments for aromatic residues were obtained using reasonably well resolved 2D homonuclear NOESY experiments.

Based on the backbone assignments for the protein, chemical shift index predictions of the ranges of secondary structure (Wishart et al., 1997) were performed and compared to the X-ray structure (Milburn et al.,

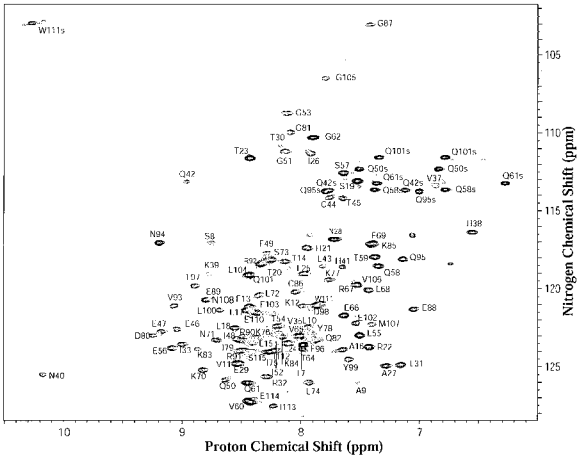

Figure 1. A 600 MHz 2D ^{1}H-^{15}N HSQC spectrum of hIL-5. Correlation peaks are labeled with sequence specific assignments for both backbone and side-chain resonances.

1993). The following ranges for elements of secondary structure were found, X-ray results are indicated in parentheses: Helix I 7–25 (7–27), β-strand 1 32–33 (32–35), helix 2 45–57 (41–58), helix 3 63–85 (64–86), β-strand 2 89–91 (89–92) and helix 4 93–105 (93–105). Good agreement between ϕ angles determined from quantitative analysis of the HNHA spectra and angles determined from the crystal structure, indicate that solution and crystal structures are also very similar.

Extent of assignment and data deposition

Virtually complete assignment of ^{1}H, ^{15}N and ^{13}C resonances have been made for all residues except Glu 4. Solution structure calculations and backbone dynamic analyses have been completed using restraints obtained from 2D homonuclear NOESY, 3D ^{15}N-edited NOESY, 4D ^{13}C, ^{13}C-NOESY and HNHA experiments and will be the subject of a subsequent communication. The sequence specific ^{1}H, ^{15}N and ^{13}C side-chain resonance assignments reported here are located in BMRB-5373 at http://www.bmrb.wisc.edu. While virtually complete assignments have been made based on triple resonance methods there was some limited evidence for low abundance conformers in some limited regions of the protein. For example the W111 side-chain correlation of the protein exhibits some lower intensity features (see Figure 1).

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