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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C backbone assignment of the carboxyl terminal domain of the cytokine binding module of the interleukin-6 receptor

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Biological context

Cytokines are key molecules in intercellular communication and signal transduction (Arai et al., 1997). The family of interleukin-6 (IL-6)-type cytokines comprises IL-6, IL-11, leukemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1) and cardiotrophin-like cytokine (CLC). All of these proteins share the characteristic four-helix bundle fold (Grötzinger, 2002).

IL-6, which gave the name to this group of cytokines, is involved in haematopoiesis, regulation of immune responses, and the acute phase reaction (Heinrich et al., 2003). Due to its pleiotropy, the dysregulation of both IL-6 and its receptor has been implicated in the pathogenesis of several diseases, e.g., multiple myeloma, postmenopausal osteoporosis, autoimmune diseases and prostate cancer (for a review see Kallen (2002)).

IL-6-type cytokines act on target cells by binding to their specific cell surface receptors and induce receptor oligomerization for signal transduction into the cell (Grötzinger, 2002). The receptor of interleukin-6 (IL-6R) is a modular built protein. The extracellular part of IL-6R consists of an N-terminal immunoglobulin (Ig)-like domain and two fibronectin type III (FN)-like domains, which form the cytokine recognition site (Grötzinger, 2002). Since the cytoplasmic part of this α-receptor lacks intrinsic protein kinase activity it is not involved in the intracellular signal transduction cascade and only upon association with gp130, the common signal transducing protein of the IL-6-type family of cytokines, initiates the signalling cascade.

Only after forming a high-affinity complex with IL-6R, can IL-6 efficiently recruit its signalling receptor subunit gp130 (Grötzinger, 2002; Heinrich et al., 2003).

The N-terminal immunoglobulin-like domain of IL-6R is thereby not involved in IL-6 binding, whereas the C-terminal FN-III-like domain, which contains the signature tryptophan-serine-X-tryptophan-serine (WSXWS) sequence motif, is responsible for receptor-ligand and receptor-receptor interactions (Özbek et al., 1998). The third membrane proximal domain (IL-6R-D3) alone accounts for more than 90% of the binding energy to IL-6 (Özbek et al., 1998). Interestingly, it has been shown recently that another member of the IL-6 type family, CNTF, can associate with the IL-6R (Schuster et al., 2003).

In order to identify the key residues that constitute the binding site of IL-6R to IL-6 we have carried out the sequence-specific backbone assignment of the recombinant third domain of the IL-6R and delineate its secondary structure.

Methods and experiments

The third domain of IL-6R (IL-6R-D3) constitutes residues 192 to 317 (14.7 kDa) of IL-6R. The cDNA of IL-6R-D3 was cloned into the pRSet expression vector and transformed into BL21(DE3) pLysS *E. coli*

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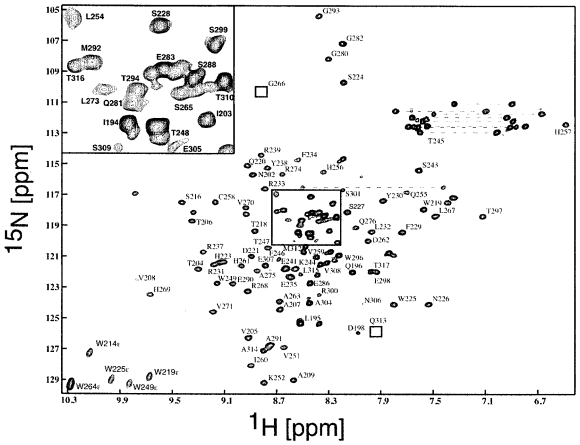

Figure 1. Sequential assignment of the third extracellular domain of the interleukin-6 receptor. 2D ^{1}H-^{15}N HSQC spectrum of the third extracellular domain of IL-6R acquired at 20 °C. Sequential assignments obtained in this study are indicated. Although most cross peaks were assigned, some are not labeled for clarity. Side-chain amide resonances of Asn and Gln residues are connected by horizontal lines. Due to the low peak intensities, the resonances of Gly-266 and Gln-313 are not contoured and marked by rectangles.

cells as described previously (Özbek et al., 1998). Production of uniform ^{13}C and ^{15}N double labeled recombinant IL-6R-D3 was achieved by growing the bacteria in M9 minimal medium containing 1 g l^{-1} ^{15}N-ammonuim chloride and 2 g l^{-1} $^{13}C_6$-labeled glucose as sole nitrogen and carbon sources, respectively. Purification and refolding of the recombinant protein was performed as described previously (Özbek et al., 1998).

After purification, the protein concentration was determined by UV-spectroscopy. Using an Amicon stirring cell (MILLIPORE, Eschborn, Germany) NMR samples were concentrated to 1.0 mM in 20 mM phosphate buffer at pH 5.0 containing 95% H_2O/5% D_2O or 100% D_2O.

All NMR experiments were recorded at 293 K on Bruker DRX600 and Varian INOVA 600 MHz NMR spectrometers equipped with pulsed-field gradient ^{1}H/^{15}N/^{13}C probes optimized for ^{1}H detection. Sequence-specific backbone assignments of IL-6R-D3 were accomplished using ^{1}H-^{15}N HSQC, CBCA(CO)NH, CBCANH, HNCA, HNCO, HBHA(CBCACO)NH and C(CO)NH spectra. The sequential assignment data were finally confirmed by 3D ^{15}N-edited NOESY-HSQC and ^{13}C-edited NOESY-HSQC experiments. Partial side-chain assignments were achieved from C(CO)NH and HCCH-TOCSY data. NMR data were processed using the NMRPipe software package (Delaglio et al., 1995) and analyzed with the NMRView program (Johnson and Blevins, 1994). Proton chemical shifts were referenced to TSP, while ^{15}N and ^{13}C chemical shifts were indirectly referenced (Markley et al., 1998).

Extent of assignments and data deposition

Almost all of the protein backbone resonances have been assigned. Even the Cα and Hα resonances of the prolines and the protons of the indole side chain of tryptophan residues are assigned. A couple of residues could only be partially assigned: the HN and N resonances of Arg-210 to Leu-215, Leu-236, Glu-278, Phe-279, Trp-284, Ser-285 and Trp-287 were missing from the spectra, this is probably due to local conformational exchange on an intermediate timescale. Figure 1 shows the ^{1}H-^{15}N-HSQC-spectrum of a uniformly ^{15}N labeled sample of the IL-6R-D3. Analysing the chemical shifts revealed that the third domian of the IL-6R is predominantly in β-sheet conformation (Wishart et al., 1992) (data not shown). With the help of this assignment it is now possible to determine the epitope of the interleukin-6 receptor which is responsible for the binding to IL-6 and CNTF. A full 3D structure determination is in progress. The chemical shift data have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) database, under accession number BMRB-5940.

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