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# Letter to the Editor: $^{1}$H, $^{13}$C and $^{15}$N chemical shift assignments of the D2 domain of the fibroblast growth factor receptor

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## Biological context

Fibroblast growth factors (FGFs) are the largest family of heparin binding growth factors that control key cellular processes such as angiogenesis, embryogenesis, differentiation and wound healing (Friesel and Maciag, 1999; Arunkumar et al., 2002a, 2002b). The diverse activities of FGFs are mediated by receptor tyrosine kinases, consisting of three immunoglobulin-like domains (D1 domain, D2 domain and D3 domain), single transmembrane domain, a cytoplasmic domain with protein kinase activity (Ibrahimi et al., 2001). Precise regulation of FGF-mediated signaling is governed by the mode of binding of FGFs to their receptor (FGFR, Schelessinger et al., 2000). Therefore investigation of the structural interactions that govern FGF-receptor specificity are crucial not only for understanding the molecular mechanism(s) underlying the multitude of activities exhibited by FGFs, but also for the development of rational design of both agonists and antagonists for the treatment of FGF-induced pathogenesis. The D2-D3 segment of the extracellular ligand binding Ig-like domain of FGFR is shown to be minimal unit sufficient for specific ligand binding (Plotnikov et al., 2000). In addition, most of the residues that govern the specificity of the ligand (FGF) – receptor binding are shown to be located in the D2 domain (Plotnikov et al., 2000; Spivak-Kroizman et al., 1994). It is in this context, we embarked on the task of determination of the three-dimensional structure of the D2 domain of FGFR using multidimensional NMR techniques. The first step towards achievement of this objective is the assignment of the $^{1}$H, $^{13}$C and $^{15}$N resonances in the D2 domain, on which we report here. No NMR structure of the D2 domain of FGFR is available and, to our knowledge this is the first report of the resonance assignment of the protein (D2 domain).

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## Methods and experiments

The cDNA encoding the D2 domain (103 aa, residues 147 to 249) was amplified by the polymerase chain reaction (PCR). An amplified fragment was subcloned into an expression vector (pET20b+). The recombinant protein (D2 domain) was overexpressed ($\sim$ 10 mg/L) as a c-terminal $His_6$-tagged fusion protein in *Escherichia coli* strain, BL21(DE3). Uniformly labeled $^{13}$C/$^{15}$N- and $^{15}$N-isotopically enriched protein samples were prepared by growing the cells in M9 minimal media containing $^{15}NH_4Cl$, either with $^{13}C_6$-D-glucose or $^{12}C_6$-D-glucose. The protein was purified (to $\sim$ 90% purity) by affinity chromatography on a Ni-NTA resin (Qiagen). Further purification of the protein was achieved by heparin-sepharose affinity chromatography using a stepwise gradient of NaCl.

All NMR experiments were performed at 298 K on a BRUKER Avance 600 MHz and 700 MHz spectrometers equipped with a triple resonance probe head. Sample concentration for NMR experiments were typically in the range of 1.0 mM. The samples were prepared in 20 mM phosphate buffer (pH 6.5, in 90% $H_2O$ and 10% $D_2O$) containing 50 mM NaCl, 50 mM ammonium sulfate and 10 mM sodium azide. All NMR data were referenced to the $^{1}$H resonance frequency of DSS. Sequence specific assignments of the polypeptide backbone were made from $^{1}$H–$^{15}$N HSQC, HNCA, HNCO, HN(CO)CA,

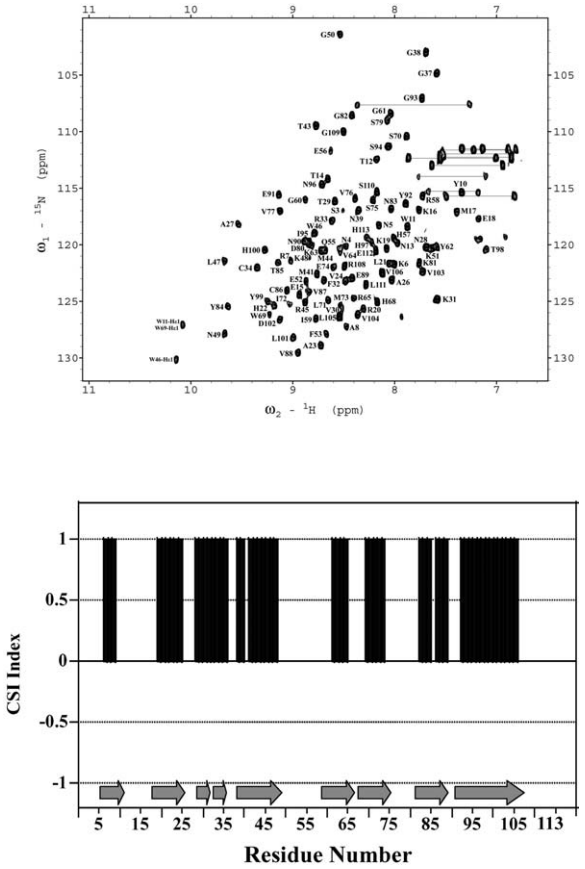


*Figure 1.* $^{1}$H-$^{15}$N HSQC spectrum of the D2 domain of the fibroblast growth factor receptor obtained at pH 6.5 and 298 K. Side chains of $NH_2$ resonances of asparagines and glutamines are connected by horizontal lines. The consensus CSI plot of the D2 domain is shown in the lower panel. The secondary structural elements in the protein include nine β-strands (indicated by arrows at the bottom of the figure).

CBCA(CO)NH and HNCACB spectra. Ambiguities in the assignment were resolved by selective $^{15}$N labeling of the protein. Side chain resonances were assigned from the combined information content of the $^{15}$N-edited TOCSY-HQSC, $^{15}$N-edited NOESY-HSQC, $^{13}$C-edited NOESY-HSQC, HCCH-TOCSY and HBHACONH. The backbone dihedral angles were predicted using the TALOS software (Cornilescu et al., 1999) and HNHA analysis. NMR data were processed using XWIN-NMR (version 3.5) and analyzed by Sparky (T.D. Goddard and D.G. Koeller, Sparky 3.0, University of California, San Francisco).

## Extent of assignments and data deposition

$^{1}$H-$^{15}$N HSQC spectra of the D2 domain is well-dispersed and 95% of the $^{1}$H-$^{15}$N crosspeaks in the spectrum (excluding the 6 proline residues) have been assigned (Figure 1). The backbone $^{1}$H-$^{15}$N resonances of Met1, Asn2, Ala36, Lys54, Asn66 and Gln67 could not be assigned. Regions near these sites exhibit weakened and broadened signals in all spectra collected. 95% of the $C^{\alpha}$, 90% of the $C^{\beta}$, 95% of the $C^{\alpha}H$ and 92% of the $C^{\beta}H$ resonances in the protein have been unambiguously assigned. In total, 93% of all the $^{1}$H and $^{13}$C resonances have been assigned. Secondary structure prediction using the CSI method ($^{1}$Hα, $^{13}$Cα, $^{13}$Cβ and $^{13}$CO) (Wishart and Sykes, 1994) and TALOS (Cornilescu et al., 1999) revealed that the D2 domain of FGFR contains 9 β-strands.

The chemical shifts for backbone and side chain assignments have been deposited in the BioMagResBank (http:/www.bmrb.wisc.edu) under BMRB accession number 5943.

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