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Letter to the Editor: Complete ^{1}H, ^{15}N and ^{13}C assignments of the carboxyl terminal domain of the ciliary neurotrophic factor receptor (CNTFR)

David Man[a], Youlin Xia[a], Kong H. Sze[a], David K. Smith[b], Wei He[a], Nancy Y. Ip[a] & Guang Zhu[a,*]

[a]*Department of Biochemistry, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong;* [b]*Department of Biochemistry, The University of Hong Kong, 5 Sassoon Rd, Hong Kong*

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Biological context

The ciliary neurotrophic factor receptor (CNTFR), one of the gp130 family of cytokine receptors (Bravo and Heath, 2000), is essential for motor neuron development and when bound to its specific ligand, CNTF, promotes the survival of neurons after injury (Sleeman et al., 2000). CNTF and CNTF analogues which bind to CNTFR may have a role in the treatment of neurodegenerative disease, obesity and diabetes (Sleeman et al., 2000). Mice that lack CNTFR have severe motor neuron deficits, are unable to feed and die perinatally (DeChiara et al., 1995; Ip and Yancopoulos, 1996). CNTF null mice, however, show that CNTF is not required for development or survival, suggesting that another ligand for CNTFR must exist (DeChiara et al., 1995). Recently, a second ligand for CNTFR was identified (Elson et al., 2000), emphasising the importance of CNTFR in neuronal development. This second ligand is a heterodimer of a gp130 family cytokine, CLC (Elson et al., 2000) and a CNTFR-like molecule, CLF, which if deleted also causes perinatal death in mice.

Like other receptors of the helical cytokines, CNTFR is a modular protein and consists of an N-terminal immunoglobulin (Ig) like domain and two fibronectin type III (FnIII) domains that form the cytokine-binding domain (Bravo and Heath, 2000). These FnIII domains are named the BN and BC domains. The C-terminal BC domain contains the signature WSXWS motif of the helical cytokine receptors, and is involved in receptor-ligand and receptor-receptor interactions (Bravo and Heath, 2000). CNTF activity is induced by the association of CNTF with CNTFR, followed by the recruitment of gp130 and the leukaemia inhibitory factor receptor (LIFR) to form the signal transducing complex (Bravo and Heath, 2000). The CLF/CLC heterodimer also requires CNTFR, gp130 and LIFR to form a functional complex (Elson et al., 2000).

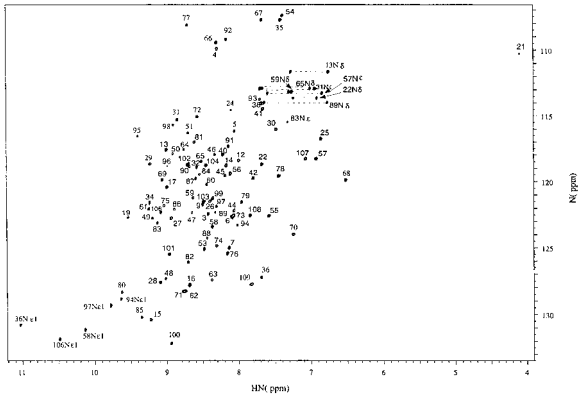

Figure 1. 2D ^{1}H-^{15}N HSQC spectrum of the BC domain of CNTFR. The number labels indicate the assignment at 25 °C and pH 6.5. Side-chain NH_2 resonances of Asn and Gln are connected by bars.

We report here the complete ^{1}H, ^{15}N, and ^{13}C NMR assignments of the BC domain of CNTFR. The structure determination of this molecule will provide insights into the receptor interactions of both CNTF and the newly identified CNTFR ligand CLC/CLF and help to elucidate the functions of these molecules in neuronal development and rescue.

*To whom correspondence should be addressed. E-mail: gzhu@ust.hk

Methods and experiments

A plasmid encoding the BC domain of CNTFR fused with glutathione S-transferase (GST) was transformed into the *E. coli* strain BL21 which was grown in M9 minimal media. To prepare ^{15}N and $^{13}C/^{15}N$-labelled proteins, $^{15}NH_4Cl$ (>99% ^{15}N) and ^{13}C-glucose (>99% U-^{13}C) were used as the sole nitrogen and carbon sources, respectively. The expressed GST-BC fusion protein was first purified using a glutathione sepharose 4B (Amersham Pharmacia) affinity column and then digested by PreScission protease (Amersham Pharmacia). The final BC domain sample was obtained after passing through a Q-sepharose column (Amersham Pharmacia) and concentration with size-exclusion filters (Amicon).

For NMR studies, the ^{15}N and $^{13}C/^{15}N$-enriched BC samples were prepared to concentrations of 2.0 mM and 1.3 mM, respectively, in a solution of 100 mM NaCl, 10 mM sodium phosphate and 0.1 mM EDTA buffered H_2O (10% (v/v) D_2O) at pH 6.5. All NMR spectra were recorded at 25 °C on Varian Inova 500 MHz and 750 MHz NMR spectrometers with triple-resonance (^1H, ^{13}C, ^{15}N) z-gradient probes. All NMR experiments used gradient sensitivity enhancement schemes.

Sequence-specific backbone assignments were made from 3D HNCACB and CBCA(CO)NH experiments. HACAN and HACA(CO)N experiments were used to correlate Proline residues with their preceding residue. 3D HACACO (on a sample dissolved in 100% D_2O) and HNCO experiments provided carbonyl chemical shifts and verified H^{α} and C^{α} assignments. 3D ^{15}N separated TOCSY, HCCH-TOCSY, and HCC-TOCSY-NNH experiments (with mixing times of 64 ms, 14.6 ms, and 14.6 ms, respectively) were used to identify side-chain ^1H and ^{13}C resonances and further confirm the sequence-specific backbone assignment. All NH_2 and NH resonances from Asn, Gln and Trp side chains, except for Trp 30, were assigned with 3D ^{15}N separated TOCSY and 3D ^{15}N separated NOESY (mixing time 150 ms) experiments. The $^1H^{\delta}$and $^1H^{\varepsilon}$ resonances of aromatic residues were assigned with 2D (HB)CB(CGCD)HD and (HB)CB(CGCDCE)HE experiments. Most of the other proton resonant peaks of the aromatic residues could be identified with 2D homonuclear TOCSY (mixing time 70 ms) and NOESY (mixing time 200 ms) experiments.

All data were processed using the nmrPipe software package (Delaglio et al., 1995). Peak-picking and data analysis of the transformed spectra were carried out using Felix and Pipp. Proton chemical shifts were calibrated with respect to the water signal relative to DSS. The ^{15}N and ^{13}C chemical shifts were referenced indirectly to DSS (Wishart et al., 1995). Preliminary chemical shift and NOE analysis reveal that the BC domain has the folded β-sandwich topology of FnIII domains.

Extent of assignments and data deposition

The ^1H-^{15}N HSQC spectrum of the ^{15}N-labelled BC domain of CNTFR is shown in Figure 1. All ^1H, ^{15}N and ^{13}C backbone and side-chain resonances of the protein have been assigned (at pH 6.5 and 25 °C), except for side-chain ^{13}C carbonyl resonances, some ^{13}C aromatic resonances, the NH_2 and NH_3 resonances of Lys and Arg, and some aromatic proton resonances of Trp. The chemical shift data have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) database, under accession number BMRB-4303.

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