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Letter to the Editor: ^{1}H, ^{13}C, and ^{15}N backbone assignments of the ligand binding domain of TGFβ type II receptor

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Biological context

The Transforming Growth Factor Beta (TGFβ) cell signaling system exemplifies a large superfamily of growth factors involved in many aspects of normal growth, development, and homeostasis for a large variety of metazoan species (reviewed in Massague, 1998). Other family members include bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs), and activins/inhibins. Disruption of the signaling pathway by mutation of TGFβ receptors or signaling molecules has been implicated in several types of cancer, fibrotic disorders, and inflammatory diseases (reviewed in Massague, 1990).

The steps required for formation of the TGFβ signaling complex and its exact stoichiometry are still being investigated; however, the postulated mode of activation differs from the receptor tyrosine kinase paradigm (Yamashita et al., 1994; Wells et al., 1999). Briefly, two type II TGFβ receptors bind free ligand and form a non-covalent complex. The subsequent binding of type I receptors establishes a heterotetrameric receptor complex capable of initiating intracellular signaling. The 3D structure of the receptor ligand binding domains complexed with ligand will provide molecular verification of this model. To this end, we have initiated solution structure studies on the TGFβ type II receptor extracellular domain (exTβRII).

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Methods and results

DNA corresponding to amino acids 27–147 of chick exTβRII was amplified from previously cloned cDNA coding for the extracellular domain, residues 22–152, of the receptor (Barnett et al., 1994). This DNA was sub-cloned into the pET-32 LIC vector (Novagen) and the 31 kDa fusion protein (thioredoxin-6xHis tag-cleavage site-exTβRII) was expressed in *E. coli* BL21 (DE3). The fusion protein was isolated with nickel affinity chromatography and cleaved with recombinant enterokinase (Novagen). Anion exchange chromatography was used to purify the 14 kDa protein of interest. Native and denaturing gel electrophoresis and MALDI-TOF mass spectrometry were used to assess the folding and purity of the samples. All six disulfides remained in the oxidized state throughout purification. In vitro and in vivo binding assays were used to confirm activity. Uniformly ^{15}N or ^{13}C/^{15}N labeled protein was obtained with the same procedure from cells grown on M9 media with the appropriate supplement of glucose and/or ammonium chloride. NMR samples were 1–2 mM in 90% H_2O/10% D_2O, pH 6.5. All spectra were recorded at 27 °C on Bruker 500, 600, and 750 MHz spectrometers equipped with triple-resonance gradient probes. The ^{1}H chemical shifts were referenced directly to internal DSS and the ^{15}N and ^{13}C references were determined with the appropriate gyromagnetic ratios (Markley et al., 1998). Spectra were processed with Felix97 (MSI Inc.) and analyzed with XEASY (Bartels et al., 1995). The backbone assignments (Figure 1) were determined primarily with the combination of ^{15}N-HSQC, ^{15}N edited ^{1}H-TOCSY, CBCANH/CBCA(CO)NH, and

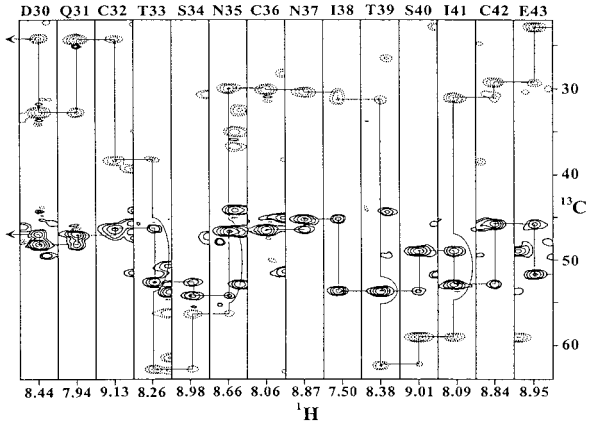

Figure 1. Strips of ^{1}H-^{13}C planes from a 3D CBCANH spectrum. Lines indicate sequential connectivities of residues D30-E43 C^{α} atoms (solid contours) and C^{β} atoms (dashed contours).

HNCO/HN(CA)CO experiments (for review and primary references see Sattler et al. 1999).

The H^{α}, C^{α}, C^{β}, and C′ chemical shift values were analyzed for secondary structure propensities utilizing chemical shift indices (Wishart and Sykes, 1994). Six consensus β-strands were identified with a complete absence of predicted helical structure. Five exTβRII β-strands aligned to β-strands identified in the activin type II receptor crystal structure (Greenwald et al., 1999). The remaining β-strand (residues Q31–S34) was in a region corresponding to a gap in the sequence alignment. Given the observed dispersion of chemical shifts and the difficulty in predicting isolated, solvent exposed β-strands by CSI analysis, we expect to identify more secondary structural elements in the final 3D structure.

Extent of assignments and data deposition

The first six N-terminal residues (MHDRSK) and the C-terminal proline are not assigned. Four residues centered around position 86 are only partially assigned. The Ns and H^{α}s of three prolines and S82 C′ also remain unassigned. Over 90% of all backbone atoms are assigned. The assignments have been deposited in the BioMagResBank under accession number 4698.

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