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Letter to the Editor: Sequential resonance assignments of the extracellular domain of the human TGFβ type II receptor in complex with monomeric TGFβ3

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Biological context

Isoforms of transforming growth factor-beta (TGFβ1, β2, and β3) are 25 kDa disulfide-linked homodimers that mediate their biological activities by binding and bringing together two related, functionally distinct, single-pass transmembrane receptor kinases, known as the TGFβ type I and type II receptors (TβR1 and TβR2, respectively) (Massagué, 1998). The mechanism by which TGFβ induces the assembly of its signaling receptors is not fully understood, although recently structures of the TβR2 extracellular domain (ecTβR2) (Boesen et al., 2002; Deep et al., 2003) and the TβR2 extracellular domain in complex with dimeric TGFβ3 (ecTβR2$_2$-TGFβ3) (Hart et al., 2002) were reported.

The assignments reported here represent the initial step in our efforts to further explore the role that dynamics plays in ecTβR2-TGFβ binding. The data presented includes backbone and sidechain methyl assignments for the ecTβR2 component of the ∼28 kDa complex formed between ecTβR2 (∼15 kDa) and a monomeric form of TGFβ3 (∼13 kDa), as well as the same set of assignments for the uncomplexed form of ecTβR2 under the same conditions. Monomeric TGFβ3 was used in the present studies, rather than dimeric TGFβ3, because NMR spectra of suitable quality could not be obtained with the dimeric complex, which was soluble only at pH values below which the complex disassociates (pH 4.8), or at pH values above which the instrinsic amide exchange rates become too rapid (pH 9.0). The monomeric complexes on the other hand exhibited improved solubility over much of the pH range and yielded high quality NMR spectra at pH 6.6 in the presence of the non-dentauring detergent 3-[(cholamidopropyl) dimethylammonio]-propanesulfonate (CHAPS).

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Methods and results

The ecTβR2 construct used was 122 residues in length and was prepared by *E. coli* expression and oxidative refolding as previously described (Hinck et al., 2000). Two types of isotope labeled ecTβR2 samples were used for the present study. 'Deuterated' samples were prepared by expressing the ecTβR2 protein on minimal medium containing ^{13}C-glucose, ^{15}N-NH_4Cl, and 99.9% ^2H_2O. 'Methyl-protonated' samples were prepared by expressing the ecTβR2 protein on minimal medium with ^{13}C/^{2}H-glucose, ^{15}N-NH_4Cl, ^{13}C-[3,3′-^{2}H] α-ketobutyrate, ^{13}C-[3-^{2}H] α-ketoisovalerate, and 99.9% ^2H_2O as described (Goto et al., 1999). Monomeric TGFβ3 used in the present study, herein designated TGFβ3$_m$, is 112 residues in length and was also prepared by *E. coli* expression and oxidative refolding. This was accomplished by first generating a T7-based *E. coli* expression construct encoding a variant of the 112-residue human TGFβ3 in which the cysteine residue which normally forms the covalent link between the two TGFβ3 monomers (C77) has been changed to a serine. The protein was then expressed in *E. coli* strain BL21(DE3) and refolded in a CHAPS containing buffer at pH 9.5 as described (Cerletti, 2000).

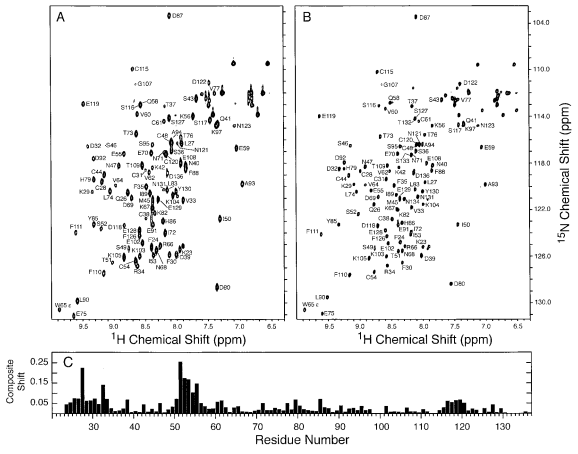

Figure 1. Assigned 2D ^{1}H-^{15}N TROSY spectra of ecTβR2 in complex with TGFβ3$_m$ (panel A) or ecTβR2 alone (panel B). Spectra shown were each recorded in the presence of 20 mM CHAPS at pH 6.6 at a ^{1}H resonance frequency of 700 MHz and 39 °C. Shown in panel C is a composite index indicating the extent of chemical shift differences observed between the free and bound forms of the ecTβR2 domain. The composite index shown was calculated based on differences between the observed H^N, N^H, Cα, Cβ, and C^O shifts and was weighted for the different chemical shift ranges of the different nuclei using the formalism previously described (Grzesiek et al., 1996).

NMR samples of ecTβR2 alone, or ecTβR2 in a 1:1 complex with TGFβ3$_m$, were prepared by reconstituting lyophilized samples of isotope labeled ecTβR2 with buffer alone (25 mM Na_2HPO_4, 20 mM CHAPS, 5% $^{2}H_2O$ at pH 6.6) or buffer containing unlabeled TGFβ3$_m$. The final concentrations of the NMR samples were 0.1 mM for ecTβR2 alone, and 0.7–0.9 mM for the ecTβR2-TGFβ3$_m$ complex. The sequential backbone assignments of the free and bound forms of the ecTβR2 domain were then made at a temperature of 39 °C using 'deuterated' samples and TROSY-based triple-resonance HNCA, HN(CO)CA, HNCACB, HN(CO)CACB, HN(CA)CO, and HNCO sequences (Pervushin et al., 1997; Rance et al., 1999) as implemented on a Bruker 700 MHz NMR spectrometer. Sidechain methyl assignments were made using 'methyl protonated' samples and TROSY-based C(CO)NH and H(CCO)NH sequences.

Extent of assignments and data deposition

The strategy described above resulted in the assignment of the backbone atoms of 112 of the 122 amino acid residues and 26 of the 27 sidechain methyl groups (Ile δ1, Leu δ1/δ2, and Val γ1/γ2) of both the uncomplexed and complexed forms of ecTβR2. The resonances unassigned in the free and bound states were found to be nearly identical to one another, as well as to those previously unassigned in uncomplexed ecTβR2 at pH 5.5 (Hinck et al., 2000). The missing resonances are presumed to be missing due to chemical or conformational exchange since all of the amides that yielded detectable correlations in the triple-resonance experiments were assigned. The largest chemical shift changes between the complexed and uncomplexed forms of ecTβR2 exhibit a 1:1 correspondence with the interfacial contacts identified in the ecTβR2$_2$-TGFβ3 crystal structure (Figure 1, panel C). This indicates that the monomeric and dimeric forms of TGFβ3 bind ecTβR2 in the same overall manner, and that the system and the assignments in hand will enable a detailed analysis of spin relaxation rates so as to further characterize the changes in dynamics which occur upon ecTβR2-TGFβ3 binding. The assignments for the uncomplexed and complexed forms of ecTβR2 have been deposited with BioMagResBank under accession codes 5954 and 5953, respectively.

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