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Letter to the Editor: Sequence-specific ^{1}H and ^{15}N assignment and secondary structure of transforming growth factor β3

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Biological context

Transforming growth factors β (TGF-β) are a group of multifunctional cytokines that regulate proliferation and differentiation of many different cell types (Massague, 1990). Three TGF-β isoforms (TGF-β1, -β2 and -β3) have been expressed in mammals. They are 25 kDa disulphide-linked homodimeric proteins composed of 112 amino acids. The TGF-β isoforms are functionally closely related to one another, but show different biological activities. Thus, TGF-β3 activates DNA synthesis in fibroblasts better than TGF-β1 and binds better to the type II receptor of epithelial cells than TGF-β2 (Lyons et al., 1991, and references cited therein). Important functional differences between TGF-β1, TGF-β2 and TGF-β3 have been observed in their ability to alter the deposition, cellular distribution and degradation of amyloid-β (Harris-White et al., 1998). These differences in biological activity are likely to be related to differences in the structures and aqueous properties of the TGF-β isoforms.

The structures of TGF-β2 and TGF-β3 have been determined by X-ray crystallography (Schlunegger and Grütter, 1992; Mittl et al., 1996), whereas the structure of TGF-β1 has been solved by NMR spectroscopy (Archer et al., 1993). No significant differences in the structure of these three isoforms were found. The NMR solution structure of TGF-β3 has not been obtained to date. Moreover, CD spectroscopic structural analysis of TGF-β3 in solution showed the difficulties in structure determination related to the fact that TGF-β3 has a particularly low solubility at physiological pH and easily forms aggregates (Pellaud et al., 1999). The CD studies indicated important differences in the solution state of TGF-β3 with respect to TGF-β1 and TGF-β2.

Here, we present near complete ^{1}H and ^{15}N backbone assignment and the secondary structures of TGF-β3 in solution.

Methods and results

Biologically active, recombinant human TGF-β3 was prepared at Novartis Pharma AG (Basel) by refolding in vitro the monomeric, denatured protein overexpressed in *E. coli* (Cerletti, 1996).

All NMR experiments were acquired on a Varian UNITY-600 NMR spectrometer equipped with a pulsed-field-gradient unit and triple resonance probe with actively shielded Z-gradients. ^{1}H-^{15}N HSQC spectra of 0.5 mM ^{15}N-labeled TGF-β3 were used for optimization of the sample conditions (Figure 1A, B). The best HSQC spectra of TGF-β3 were obtained in the water mixtures of 6–9% dioxane and 3–4% methanol at 40 °C, pH 2.8–3.0. Notably, the quality of the spectra was improved when lyophilized TGF-β3 was dissolved in ready-made solutions containing water, dioxane and methanol. Further, NMR measurements were performed with a sample of 1 mM ^{15}N-labeled TGF-β3 in mixed solvent of 84% H_2O/5% D_2O (or 89% D_2O), 8% dioxane-d_8 and 3% methanol-d_3 (or -d_4), pH = 2.9, at 40 °C.

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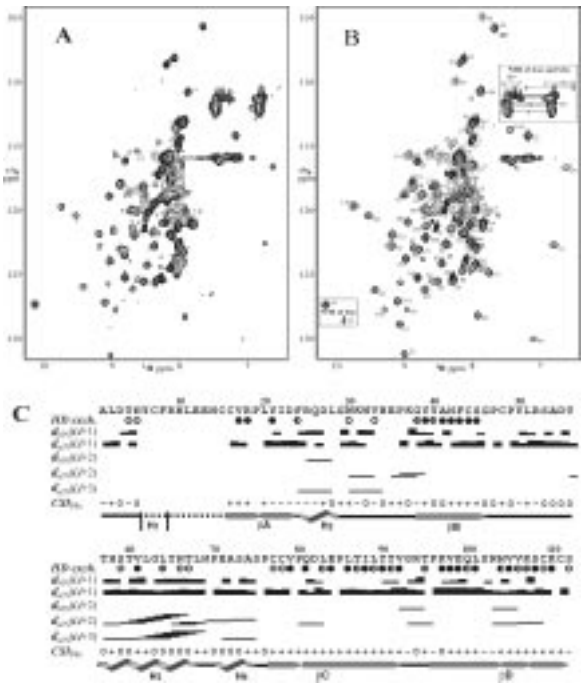

Figure 1. ^{15}N-^{1}H HSQC spectra recorded for uniformly ^{15}N-enriched TGF-β3 at 40 °C and pH = 2.9 dissolved in: (A) water; (B) water, containing 8% dioxane and 3% methanol. Note that new cross peaks appear in spectrum B. Cross peak assignments are indicated by numbers. (C) Amino acid sequence of TGF-β3, amide proton exchange data, short-range NOEs and $^{1}H^{\alpha}$ chemical shift indices (CSI). Sequential and medium-range NOEs observed in the 3D NOESY-HSQC (t_{mix} = 80 ms) spectrum are shown by horizontal lines. The line thickness for the sequential NOEs is inversely proportional to the squared upper distance bound. Circles indicate hydrogen–deuterium exchange rates of TGF-β3 amide groups measured in the mixed solvent at 40 °C and pH = 2.9: open circle, $0.5<t_{1/2}<1$ h; semi-open circle, $1<t_{1/2}<4$ h; filled circle, $t_{1/2}>4$ h. Positions of α- and β-structure suggested by the CSI and the short-range NOEs are shown by a ribbon at the bottom. The unassigned region 6–15 is indicated by a broken line.

Water flip-back, pulsed-field gradient and sensitivity enhanced version of ^{15}N-^{1}H HSQC, 3D ^{15}N-^{1}H TOCSY-HSQC (t_{mix} = 40 ms), 3D ^{15}N-^{1}H NOESY-HSQC (t_{mix} = 60 and 80 ms), ^{1}H TOCSY (t_{mix} = 40 and 70 ms) and ^{1}H NOESY (t_{mix} = 60, 80 and 100 ms) were used for assignments. NMR spectra were processed using the VNMR software (Varian) and analyzed with the program XEASY (Bartels et al., 1995).

Our working model of TGF-β3 secondary structure suggested from NMR data (Figure 1C) is similar to the crystal one (Mittl et al., 1996), but the chemical shift indices (CSI) (Wishart and Sykes, 1994) of ^{1}Hα protons along the helices H3 and H4 are close to random coil values. Also, detected $d_{\alpha N}(i, i+2)$ connectivities along H3 and H4 are unusual for helices. It may suggest that these regions of TGF-β3 are in dynamic equilibrium between helix and an unfolded conformation. These data are in agreement with the CD results, which show that the α-helical content of TGF-β3 is reduced when compared to the crystal structure (Pellaud et al., 1999). The two β-layers of TGF-β3 were evaluated via the long-range NOE connectivities between the β-strands: βA and βB; βC and βD. These NOEs agree well with the crystallographic structure for the TGF-β3 dimer (Mittl et al., 1996) and with the solution structure of the TGF-β1 dimer (Archer et al., 1993). The signal broadening in the N-terminal part of TGF-β3 (NMR unassigned region 6–15 and parts of β-strands βA, 16–20, and βB, 41–45) can be explained by millisecond time scale motions of helix H1 (found in the crystal) interacting with β-strands βA and βB.

Extent of assignments and data deposition

Sequence-specific assignments ($^{1}H^{N}$, ^{15}N, $^{1}H^{\alpha}$) for TGF-β3 have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) database (accession number 4411). 89% of the TGF-β3 residues were identified in the proton and heteronuclear spectra. Sequence-specific assignments were not made for backbone resonances of residues 6–16, 19, 20, 35 and 111. These assignments were hampered by poor chemical shift dispersion of resonances from flexible segments of the molecule and by exchange broadening.

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