Journal of Biomolecular NMR, **17:** 279–280, 2000.
KLUWER/ESCOM

Letter to the Editor: ^{1}H, ^{13}C and ^{15}N chemical shift assignments of the acidic fibroblast growth factor from *Notopthalmus viridescens*

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Received 19 April 2000; Accepted 18 May 2000

Key words: assignment, growth factor, newt, triple resonance

Biological context

Fibroblast growth factors play a vital role in a wide array of cellular mechanisms related to vertebrate development (Plotnikov et al., 1999). The amphibian newt (*Notophthalmus viridescens*) is a popular model to understand the biology of the limb regeneration process mediated by acidic fibroblast growth factor(s) (Patrie et al., 1997). Amino acid sequence comparisons reveal that the acidic fibroblast growth factor from newt (nFGF-1) exhibits about 75% homology with its counterpart(s) from mammalian species. Interestingly, despite the high degree of sequence homology, polyclonal antibodies raised against mammalian FGF-1 fail to cross-react with nFGF-1. Thus, it is possible that significant differences exist in the three-dimensional structures of FGF-1 isolated from newt and mammalian sources. It is in this context that we embarked on the determination of the solution structure of nFGF-1 using triple resonance NMR experiments.

Methods and results

nFGF-1 (154 amino acids long) DNA construct was cloned into the prokaryotic vector pET21b (+) and expressed in *E. coli* BL21 (DE3) pLysS after IPTG induction. The expressed protein was purified on a heparin sepharose affinity column over a NaCl gradient of 0–1.5 M. ^{15}N enriched nFGF-1 samples were made by growing *E. coli* on a minimal medium containing 1 g/L of $^{15}NH_4Cl$. The ^{15}N–^{13}C double labeled nFGF-1 was obtained using 1 g/L of $^{15}NH_4Cl$ and 2 g/L ($^{13}C_6$)-glucose. The purified protein was found to undergo specific autocatalytic cleavage at the carboxyl end of Tyr22. To avoid non-homogeneity of the protein sample, the peptide segment containing the first 22 residues at the N-terminal end of the full form of nFGF-1 was cleaved specifically by chymotrypsin. Chymotrypsin digestion was carried out by incubating the column material (heparin sepharose containing the bound protein) with the enzyme in 0.85 M NaCl. The truncated nFGF-1 bound to the heparin sepharose column was eluted with 10 mM phosphate buffer (pH 7.2) containing 1.5 M NaCl. The authenticity of the truncated nFGF-1 sample was verified by ES-mass analysis. The mitogenic activity of the full and truncated forms of nFGF-1 was almost identical.

NMR samples were prepared in 10 mM phosphate buffer (pH 6.0), H_2O:D_2O = 90:10, containing 100 mM NaCl, 0.1 mM EDTA, 0.1 mM mercaptoethanol and 1.5 mM sucrose octasulfate. The concentration of the nFGF-1 ranged between 1.5–2.0 mM. All the NMR experiments were recorded at 308 K on a 600 MHz Bruker DMX spectrometer equipped with a triple resonance (^{1}H, ^{15}N and ^{13}C) probe including shielded z-gradients. Linear prediction was used in the ^{13}C and ^{15}N dimensions to improve the digital resolution. All data were processed by XWIN-NMR (Bruker AG, Germany) and analyzed using AURELIA on SGI computers.

Sequence-specific assignment of the backbone atoms was achieved by the following independent

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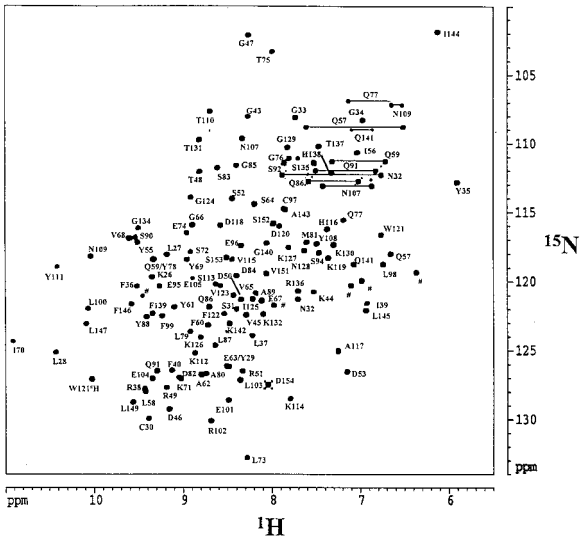

Figure 1. ^{1}H–^{15}N HSQC spectrum of nFGF–1. Side chains of NH_2 resonances of asparagine and glutamine are connected by bars. Crosspeaks labeled as '#' in the spectrum represent arginine side chains.

connectivity analysis. The assignments of the amide atoms and α-carbons were achieved using the HNCA and the HN(CO)CA experiments (Bax and Grzesiek, 1993; Cavanagh et al., 1996). The few ambiguities encountered were resolved by the HNCACB experiment (Bax and Grzesiek, 1993; Cavanagh et al., 1996). The HNCO and HN(CA)CO experiments provided the carbonyl chemical shifts (Bax and Grzesiek, 1993; Cavanagh et al., 1996). The HNCACB experiment was useful for obtaining the chemical shift values of the backbone atoms and the C^{β}atoms were used as anchor points for the aliphatic side chain resonance assignment (Bax and Grzesiek, 1993; Cavanagh et al., 1996). The aliphatic carbon and hydrogen atoms in the amino acid side chains were identified using the information in the CC(CO)NH-TOCSY and HCCH-TOCSY experiments (Bax and Grzesiek, 1993; Cavanagh et al., 1996).

Aromatic spin systems were assigned using ^{1}H-^{1}H NOESY spectra and a ^{1}H-^{13}C HSQC experiment optimized for the aromatic resonances. Combined information from the HNCACB and ^{15}N-edited NOESY experiments yielded assignments for side chain amide resonances of the Asn and Gln residues. The $C^{\epsilon}H_3$ groups of the methionine residues in the protein were assigned using the ^{1}H-^{13}C HSQC and ^{13}C-edited NOESY-HSQC spectra (Bax and Grzesiek, 1993; Cavanagh et al., 1996).

Extent of assignment and data deposition

The ^{1}H-^{15}N HSQC spectrum is shown in Figure 1. We have assigned all the protonated ^{15}N resonances of the backbone and asparagine and glutamine side chains. With the exception of the first two residues at the N-terminus, the assignments of all H^{N}, H^{α}, H^{β}, C^{α} and C^{β} have been accomplished. In total, $>$ 95% of the ^{1}H and ^{13}C resonances of the side chains have been assigned. More than 95% assignment of the carbonyl carbons has been achieved. The ^{1}H, ^{15}N and ^{13}C chemical shifts described here have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number: BMRB-4712.

Acknowledgements

We thank the National Science Council, Taiwan and the C.S. Tsou Memorial Research Foundation for financial support.

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