Journal of Biomolecular NMR, **24:** 75–76, 2002.
KLUWER/ESCOM

Letter to the Editor: ^{1}H, ^{15}N and ^{13}C resonance assignments for the DNA-binding domain of myocyte nuclear factor (Foxk1)

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Received 17 June 2002; Accepted 30 July 2002

Key words: DNA-binding domain, myocyte nuclear factor, resonance assignment

Biological context

Members of the forkhead family of transcription factors are expressed in many eukaryotic organisms and are known to play important roles in development, inflammation, and neoplasia (Hormas et al., 1995). Members of this family are characterized by a conserved 100-amino acid DNA-binding domain that contains three α helices, three β strands, and two loops. The core derived from α-helical and β-sheet elements is flanked by two wing-like loops. Therefore, the DNA-binding domain of forkhead proteins has been named the winged helix/forkhead domain (Kaufmann et al., 1996). Since the discovery of the winged helix/forkhead motif, a large number of topologically related proteins with diverse biological functions have been characterized by both X-ray crystallography and NMR spectroscopy (Gajiwala et al., 2000a). Despite the fact that 3D structures of winged helix/forkhead proteins exhibit a conserved fold, these proteins have diversity in biological function and versatility in DNA recognition (Gajiwala et al., 2000b; Jin et al., 1999).

Myocyte nuclear factors (MNFs) are transcription factors that are selectively expressed in myogenic stem cells. MNFs regulate the genes that coordinate the proliferation and differentiation of myogenic stem cells after muscle injury (Garry et al., 2000). Two MNF isoforms were found: MNF-α and MNF-β contain 617 and 414 amino acids, respectively (Bassel-Duby et al., 1994; Yang et al., 1997). They are derived from a single *mnf* gene by means of alternative splicing. However, the expression of MNF-α and MNF-β is differentially regulated, and they possess distinctive functional properties with respect to DNA binding in vitro and transcriptional regulatory activity in transient-transfection assays. Deletion studies have shown that these properties are coupled to the regions outside of the DNA-binding domain (Yang et al., 1997). MNF-β is identical to MNF-α within the N-terminal and DNA-binding domains (residues 1–409), but the sequence diverges in the C-terminus (Yang et al., 1997). The DNA-binding domain of MNFs belongs to the winged helix/forkhead family because residues 289–389 of MNF-α and MNF-β share 35–89% similarity with other known members of this family. Based on the unified nomenclature for winged helix/forkhead transcription factors, the DNA-binding domains of MNF-α and MNF-β have been designated as Fox (Forkhead box) k1 (Kaestner et al., 2000). To study the structure and function relationships of the DNA-binding domain of MNFs, we expressed the protein in *Escherichia coli* with a yield of 40–60 mg/l. We assigned ^{1}H, ^{13}C, and ^{15}N resonances and deduced the secondary structures of the DNA-binding domain of MNF by using multidimensional NMR spectroscopy. In contrast to other proteins of this family, secondary structure analysis of the DNA-binding domain of MNF revealed that an eight-residue α helix is in place of the typical wing 2 region. This atypical helical structure may modulate the DNA-binding specificity of MNF. This result suggests a possible structural diversity in the winged helix/forkhead proteins.

Methods and experiments

Total RNA was isolated from mouse skeletal muscle myoblasts. The structural gene of the 101-residue DNA-binding domain of MNF-α was amplified by using a polymerase chain reaction (PCR) with the sense primer 5′-GGATCCGGATCCGAATCAAAGCCGCC TTAC-3′ with *Bam*HI recognition and the antisense primer 5′-GGATCCGGATCCGAATCAAAGCCGCC TTAC-3′ with *Hind*III recognition. The PCR prod-

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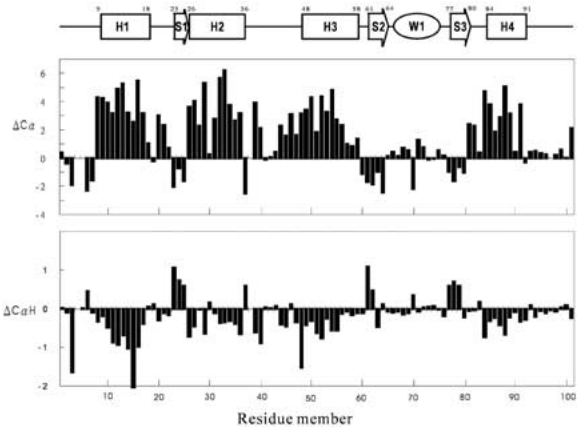

Figure 1. The secondary structures for the DNA-binding domain of MNF are deduced from the secondary shifts ($\Delta^{13}C\alpha$ and $\Delta C\alpha H$).

uct was purified and then cloned into the *Bam*HI and *Hind*III sites of pET-21a vector. The recombinant plasmid was transformed into the *E. coli* BL21(DE3)pLyS strain, and the system was inducibly expressed under the control of strong T7 promoter. The recombinant protein contained 13 extra residues (ASMTGGQQMGRGS) at the N-terminus. Protein was purified by means of Sulphopropyl (SP) Sepharose cation chromatography at pH 9.5 and further purified with C18 reverse-phase high-performance liquid chromatography.

M9 minimal media was used and 1 g/l $^{15}NH_4Cl$ (99% ^{15}N) and/or 2 g/l [^{13}C]-glucose (99% ^{13}C) were substituted for the unlabeled compounds in the growth media. NMR samples were lyophilized and made to 10% or 100% D_2O at pH 6.0 in 25 mM phosphate buffer and 100 mM NaCl. NMR experiments were performed by using a Bruker Avance 600 spectrometer at 27 °C. Experiments of ^{15}N-edited TOCSY and NOESY, HCCH-TOCSY, HNCA, HN(CO)CA, HNCACB, CBCA(CO)NH, HBHANH, HBHA(CO)NH, and HCC(CO)NH-TOCSY were performed for the purpose of proton, carbon, and nitrogen resonance assignments. Selective [^{15}N]-Ala– and [^{15}N]-Gln–labeled proteins were prepared to confirm the assignments. 2D COSY, TOCSY, and NOESY experiments with an unlabeled sample in D_2O provided the basis for the aromatic proton assignments. Data was processed and analyzed by using the XWINNMR and Aurelia programs (Bruker) on an O2 Silicon Graphics workstation.

Extent of assignments and data deposition

The ^1H, ^{15}N, and ^{13}C resonance assignments for the DNA-binding domain of MNF were obtained by analyzing triple resonance spectra. The 13 extra residues at the N terminus showed random coiled chemical shifts and $^3J\alpha N$ coupling constants, a similar magnitude of $d\alpha N$ (i, i + 1) NOE, and an absence of dNN NOEs; all of these findings indicated the formation of a random coiled structure in this region. We therefore summarized only the chemical shifts from the DNA-binding domain of MNF. Of 101 residues, four NH groups of the residues T42, A43, D44, and Q48 were not observed; they were most likely absent due to the rapid exchange with the solvent. The resonance assignments of the aromatic protons were complete, whereas 96% of the resonances of other aliphatic proton and carbon side chains have been assigned. The analysis of the secondary shifts ($\Delta^{13}C\alpha$ and $\Delta C\alpha H$) are shown in Figure 1. Judging by the shifts, the DNA-binding domain of MNF consists of four α helices, three β strands, and one wing. Interestingly, in contrast to other winged helix/forkhead proteins, the DNA-binding domain of MNF contains a C-terminal α helix in place of the typical wing 2 region. ^1H, ^{15}N, and ^{13}C resonance assignments have been deposited in the BioMagResBank databank under accession number BMRB-5365.

Acknowledgements

This work was supported by grants (NSC-90-2311-B-006-006 and the Program for Promoting University Academic Excellence 91-B-FA09-1-4) from the National Science Council of ROC.

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