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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of the DNA binding domain of the human Forkhead transcription factor AFX

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Biological context

Forkhead transcription factors serve as regulatory keys in embryogenesis, in tumorigenesis and maintenance of differentiated cell states (Kaufmann and Knöchel, 1996). The conserved forkhead DNA binding domain (which belongs to the winged helix superfamily of proteins) encompasses about 100 amino acid residues. Three major alpha helices are packed against each other resting on a small three-stranded anti-parallel beta sheet from which two loops ('wings') protrude. The highest degree of sequence conservation among forkhead domains is found in the three helical segments. Helix 3 has been identified as being responsible for most direct base contacts with DNA, although other contacts with DNA have also been observed (Clark et al., 1993; Jin et al., 1999).

AFX (*human*) belongs to a small subfamily of forkhead transcription factors. Studies performed in *Caenorhabditis elegans* revealed that the orthologous transcription factor DAF-16 is involved in an insulin-like signaling pathway (Ogg et al., 1997). Based on these results it was suggested that some of the metabolic defects caused by declines in insulin signaling, in both type I and type II diabetes, may be due to unregulated activity of AFX or FKHR (another human DAF-16 orthologue) (Ogg et al., 1997).

The transcriptional activity of AFX is regulated by phosphorylation and it has been shown that in response to insulin, AFX is phosphorylated through a phosphatidylinisitol-3-OH-kinase/protein kinase B (PI(3)K/PKB) pathway (Kops et al., 1999). Lack of PKB activity does not fully inhibit phosphorylation of AFX and a second Ras/Ra1 insulin dependent pathway has also been identified (Kops et al., 1999). When phosphorylated, AFX is translocated from the cell nucleus to the cytoplasm (Takaishi et al., 1999). An amino acid sequence comparison of the DNA binding domain of AFX (AFX-DBD) and forkhead domains of known three-dimensional structure (HNF3-γ, Genesis and FREAC-11 (Clark et al., 1993; Jin et al., 1999; van Dongen et al., 2000)) shows that the loop preceding the DNA recognition helix in AFX-DBD contains a five amino acid residue insertion, and that the first wing (i.e. the loop connecting strands 2 and 3 of the beta sheet) is shortened by two amino acid residues. The sequence conservation in helix 3 is very high but still different forkhead domains show different DNA binding specificity. Thus it has been argued that the DNA binding specificity must be attributed to residues outside the recognition helix (Kaufmann and Knöchel, 1996). It is anticipated that a detailed structural investigation of free AFX, as well as AFX in complex with DNA, will reveal further details of the molecular basis of DNA recognition in forkhead proteins.

Methods and results

Isotopically enriched (u-^{15}N labeled, u-^{13}C/^{15}N double labeled and 10%-^{13}C/u-^{15}N partially double labeled) samples of AFX-DBD (residues 82–207) were prepared from transformed *Escherichia coli* strain BL21(DE3) (Novagen) cells. The protein construct contained 24 additional residues at the N-terminus (GSSHHHHHHSSGLVPRGSHMLEDP) included for purification purposes and not removed. Bacteria were grown in minimal medium with $(^{15}NH_4)_2SO_4$ and ^{13}C-glucose as the sole nitrogen and carbon sources. The protein was purified in a single step using Ni^{2+} affinity chromatography.

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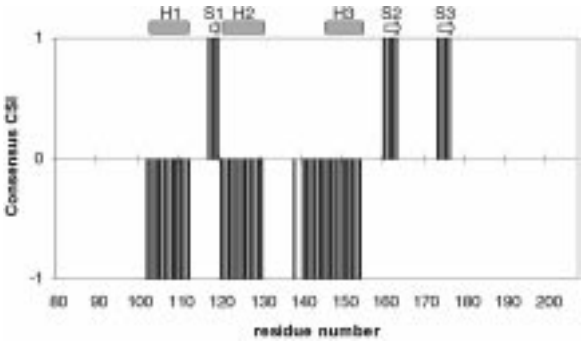

Figure 1. Consensus chemical shift index (CSI) (H^{α}, C^{α}, C^{β} and C'), calculated using the program CSI (Wishart and Sykes, 1994), of AFX-DBD. Indices of −1 and 1 indicate helical structure and beta strand structure, respectively. The drawing at the top shows the secondary structure predicted by homology to other forkhead domains. The only difference between the CSI derived secondary structure as compared to the predicted secondary structure is an N-terminal extension of the DNA recognition helix (H3). This observation could reflect the existence of five additional residues in the loop preceding the helix (see text), and might be of functional importance.

All NMR measurements were performed in either H_2O or D_2O at 31 °C on Varian UNITY Inova 600 MHz and 800 MHz spectrometers. The protein concentration was approximately 1.5 mM and sample buffers contained 20 mM sodium phosphate (pH 6.3), 100 mM NaCl, 0.02% (w/v) NaN_3, 1 mM EDTA and 1 mM Pefabloc protease inhibitor. H_2O samples contained 10% (v/v) D_2O. Resonance assignments were obtained using standard NMR techniques. The data were processed using NMRPipe/NMRDraw (Delaglio et al., 1995) and analyzed using ANSIG (Kraulis et al., 1994).

Figure 1 shows the consensus chemical shift index (CSI) of AFX-DBD (Wishart and Sykes, 1994). The secondary chemical shifts confirm a typical forkhead winged helix fold comprising residues 102–176. The N-terminal and C-terminal parts of the protein show random coil chemical shifts and narrow resonances, indicating a disordered and highly flexible structure. Noteworthy is that no helical tendency is observed for the residues C-terminal to the third beta strand, analogous to the observations made for free Genesis (Jin et al., 1999). The three-dimensional structure of the DNA complex of Genesis, on the other hand, shows the formation of a short helix in this sequence stretch (Jin et al., 1999).

Extent of assignments and data deposition

Backbone resonance assignments (H^N, N, H^{α}, C^{α} and C') were completed for all residues except Gly58*-Ser68*, His76*, (Met77*), Asp80*, Arg88, (Lys89), (Glu115), (His157-Ser158) and Pro187-Arg189. The parentheses indicate residues for which H^N and N resonance assignments are missing. Residues 58–81 (marked with an asterisk) belong to the *his-tag* and are not found in the native protein. Sidechain resonance assignments (^1H and ^{13}C) were more than 96% complete for residues 95 through 181, which encompass the structured core of the domain. For residues outside this region the completeness of sidechain resonance assignments is about 55%, not including residues for which backbone resonance assignments were missing. Further resonance assignments were primarily hampered by extensive resonance overlap of signals at random coil chemical shifts.

The ^1H, ^{13}C and ^{15}N chemical shifts of the DNA binding domain of AFX have been deposited at the BioMagResBank (http://www.bmrb.wisc.edu/) database (accession number 4675).

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