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^{1}H, ^{15}N, and ^{13}C chemical shift assignments of mouse HOXA13 DNA binding domain

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Abstract The homeobox gene (*HOXA13*) codes for a transcription factor protein that binds to AT-rich DNA sequences and controls expression of many important proteins during embryonic morphogenesis. We report complete NMR chemical shift assignments of the mouse HOXA13 DNA binding domain (A13DBD; BMRB no. 16252).

Keywords HOXA13 · Homeodomain · Hox · DNA binding domain · NMR

Biological context

Homeobox (Hox) genes encode a conserved family of transcription factor proteins that are critically important in vertebrate development (Krumlauf 1994). In humans, the Hox genes are distributed into four linkage groups (HOXA, B, C, D) comprising 39 genes located on chromosomes 7, 17, 12, and 2. Hoxa13 has been linked to syndromes affecting genitourinary development (Innis et al. 2002; Mortlock and Innis 1997), and particular mutations in *HOXA13* cause hand-foot-genital syndrome, an autosomal dominant disorder that causes preaxial digit loss and a loss of interdigital programmed cell death (Mortlock and Innis 1997). *HOXA13* functions in the cell by binding to specific DNA sequences (Knosp et al. 2007) that regulate transcription of many downstream genes. The atomic-resolution structure of HOXA13 bound to duplex DNA is needed to understand the mechanism of sequence specific DNA binding. We report here NMR assignments of the human HOXA13 DNA binding domain (A13DBD) at pH 7.0, as an important first step toward elucidating the structural basis of sequence specific DNA recognition.

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Methods and experiments

Expression and Purification of HOXA13 DNA Binding Domain (A13DBD)

Uniformly ^{15}N-labeled and ^{13}C,^{15}N-labeled A13DBD was expressed in *E. coli* stain, BL21(DE3) using the high cell density method (Marley et al. 2001; Sivashanmugam et al. 2009). Recombinant protein was purified by Ni-NTA affinity column and gel-filtration size-exclusion chromatography after Thrombin cleavage to remove the N-terminal His-tag. Typically, about 10 mg of purified protein was obtained from a 1-liter culture. The identity and integrity of the final protein sample was confirmed by SDS–PAGE.

NMR spectroscopy

Samples for NMR analysis were prepared by dissolving ^{15}N, or $^{15}N/^{13}C$ -labeled A13DBD protein (0.5–1 mM) in 0.3 mL of a 95% H_2O/5% D_2O solution containing 20 mM phosphate at pH 7.0 with 1 mM EDTA-d_{12}. All NMR experiments were performed at 285 K on a Bruker Avance 800 MHz spectrometer equipped with a four channel

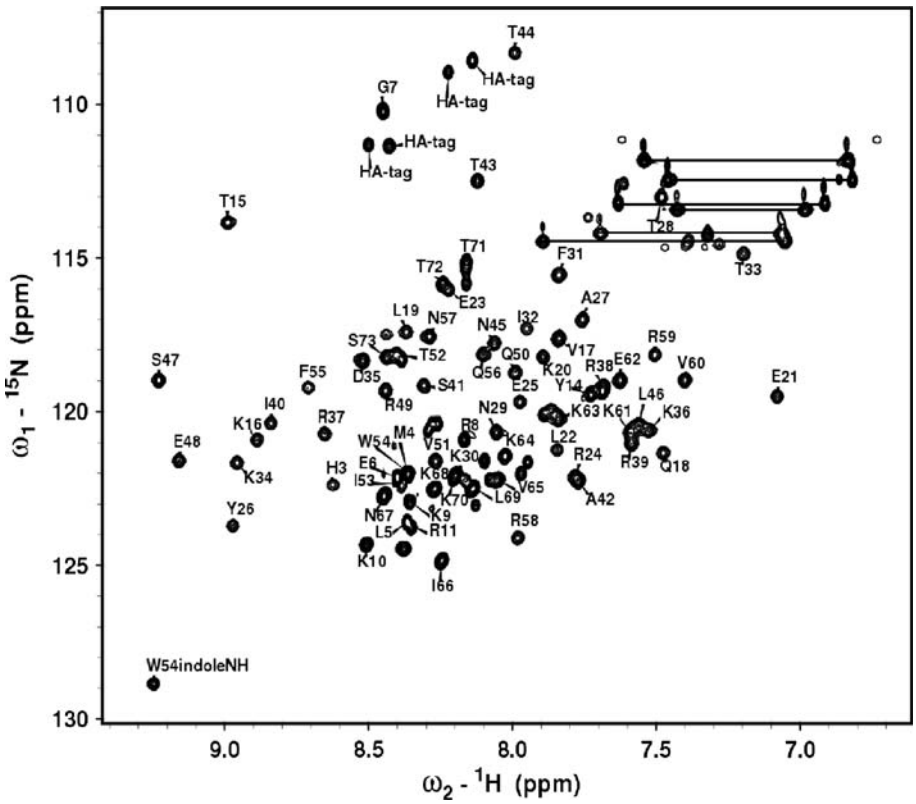

Fig. 1 Two-dimensional ^{15}N-^{1}H HSQC spectrum of A13DBD at pH 7.0 recorded at 800-MHz ^{1}H frequency. The protein sample was uniformly labeled with nitrogen-15. Amide side-chain resonances are connected by solid lines. Resonance assignments are indicated and reported in BMRB accession no. 16252

interface and triple resonance cryogenic (TCI) probe. The ^{15}N-^{1}H HSQC spectrum (Fig. 1) was recorded with the following parameters: the number of complex points and acquisition times were 256, 52.6 ms for ^{15}N (F1), and 2,048, 106 ms for ^{1}H(F2). Assignment of backbone and side-chain resonances were obtained by analyzing the following spectra: HNCACB, CBCA(CO)NH, HNCO, HBHA(CO)NH, C(CO)NH–TOCSY, H(CCO)NH–TOCSY, HCCH–TOCSY. The NMR data were processed using NMRPipe and analyzed using Sparky.

Assignments and data deposition

Figure 1 presents ^{1}H/^{15}N HSQC spectrum of A13DBD at pH 7.0 to illustrate representative backbone resonance assignments. NMR assignments were based on 3D heteronuclear NMR experiments performed on ^{13}C/^{15}N-labeled A13DBD (residues 1-73). The protein sample in this study consists of 73 native residues including 6 extra residues from a HA-tag attached at the C-terminus. All non-proline residues exhibited strong backbone amide resonances with uniform intensities, indicative of a well-defined three-dimensional protein structure. More than 95% of the backbone resonances (^{1}HN, ^{15}N, ^{13}Cα, ^{13}Cβ, and ^{13}CO) and ~80% of aliphatic side chain resonances were assigned, including stereospecific assignment of valine and leucine

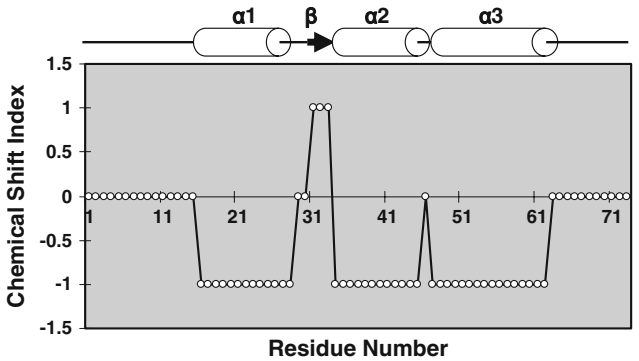

Fig. 2 Chemical shift index (*CSI*) plot of A13DBD. The values of *CSI* for β-strand, α-helix and random coil are +1, −1 and 0, respectively, as defined by the program *CSI* (Wishart and Sykes 1994). The assigned secondary structure (*top*) closely resembles that of canonical homeobox proteins

methyl groups. The chemical shift assignments (^{1}H, ^{15}N, ^{13}C) of A13DBD have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 16252.

The chemical shift index of each amino acid residue (Fig. 2) reveals three α-helices (α1: T15–T28; α2: K34–N45; α3: S47–E62) and a short β-strand (F31 – T33). The protein secondary structure closely resembles the canonical secondary structure and topology seen in other homeobox proteins. The amino acid sequence of the DNA binding domain

of HOXA13 (A13DBD) is most similar to that of HOXC13 (85% identity) and also very similar to that of HOXD13 (84%) and HOXB13 (77%). The NMR assignments reported here for HOXA13 are overall similar to that reported previously for HOXB13 (BMRB4357). Interesting differences in chemical shifts are seen for non-conserved residues in the N-terminal region and C-terminal helix that might play a role in conferring sequence specific DNA binding.

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