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Letter to the Editor: NMR assignments of the DNA-bound human Csx/Nkx2.5 homeodomain and NK2-specific domain

Hyun Sook Lee, James M. Gruschus, Tao Zhang & James A. Ferretti*
Laboratory of Biophysical Chemistry, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892-8013, U.S.A.

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Biological context

The transcription factors of the NK-2 class are known to play important roles during development. One of these factors, Csx/Nkx2.5, is required for proper heart formation (Lints et al., 1993). The transcription factors of this class contain three highly conserved regions: the homeodomain (HD), the tinman (TN) domain (or eh1 motif), and the NK2-specific domain (NK2-SD; also called the NK2 domain) (Harvey, 1996). The homeodomain is a highly conserved DNA-binding domain; the homeodomains of the NK-2 class are believed to prefer the DNA sequence 5′-CAAG-3′, whereas most other homeodomain transcription factors prefer 5′-TAAT-3′. No structure of any NK2-SD has been determined. It is unique to NK-2 class proteins and is separated from the C-terminal end of the homeodomain by a short linker. The NK2-SD is composed of a hydrophobic core with valine or isoleucine in every second position ((V/I)A(V/I) PVLV) and is flanked by basic amino acids. *In vitro*, this domain is not required for high-affinity sequence-specific DNA binding (Damante et al., 1994; Watada et al., 2000). Mapping studies of potential functional domains in Nkx2.1, Nkx2.2 and Nkx2.5 revealed that NK2-SD or a region encompassing the NK2-SD functionally masks the transcriptional activation function present in the activation domains within the N-terminus and/or C-terminus (Chen and Schwartz, 1995; De Felice et al., 1995; Watada et al., 2000). The stretch of hydrophobic residues within the NK2-SD is reminiscent of motifs that are known to be interfaces for protein–protein interactions in other DNA-binding factors. Mapping of the GATA-4 interaction domain on Nkx2.5 revealed that both the homeodomain and its C-terminal region are required for strong physical interaction (Durocher et al., 1997; Lee et al., 1998).

Methods and experiments

Human Nkx2.5 HD-SD (residues 130-236) was subcloned into *Nco*I/*Bam*HI of pET-30a(+) (Novagen), introduced into *E. coli* BL21-CodonPlus (DE3)-RIL (Stratagene), grown in $^{15}NH_4Cl$/[$^{13}C_6$]-D-glucose minimal media, purified by ion exchange (HiTrap Q HP), Ni-NTA affinity (HisTrap HP), and size exclusion chromatography (Superdex-75) to >95% purity (SDS-page, mass spec.), and concentrated in 20 mM sodium acetate, pH 5.0, 80 mM NaCl, 1 mM TCEP, 10% D_2O, mixed with equimolar DNA duplex (+-strand 5′-TGTGTCAAGTGGCTGT-3′, Midland) to 0.3 mM. 2D ^{15}N HSQC, 3D ^{15}N NOESY-HSQC, HNCACB and CBCA(CO)NH experiments were performed on Bruker Avance 800 and DRX 600 spectrometers at 300 K and 308 K, and spectra processed with nmrPipe/nmrDraw (NIH, Bethesda).

*To whom correspondence should be addressed. E-mail: ferrettj@nhlbi.nih.gov

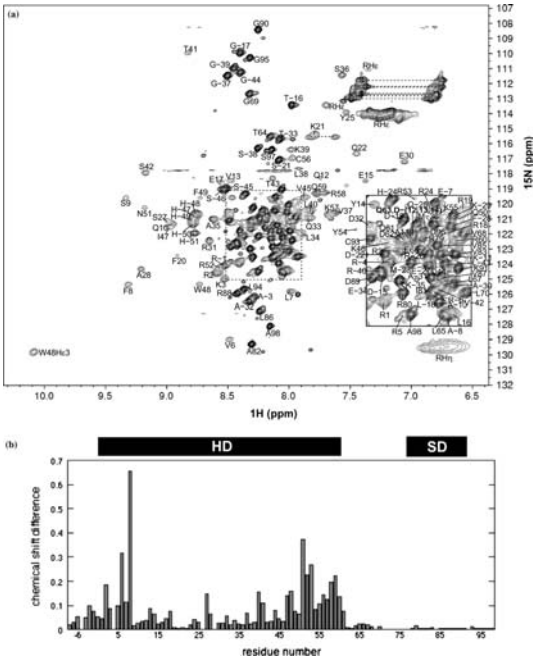

Figure 1. (a) ^{15}N HSQC spectrum with assignments of DNA-bound Nkx2.5 HD-SD at 308 K. Pairs of the side-chain amide protons of N and Q residues are indicated by dotted lines. The inset is an expanded view of the more crowded region in the spectrum. Residues −8 through 99 correspond to 130–236 of human Nkx2.5, residues 1–60 correspond to the HD, residues 77–93 to the NK2-SD, and residues −53 through −9 were added in the cloning process. (b) Comparison between free and DNA-bound Nkx2.5 HD-SD chemical shifts. The combined chemical shift differences, $\Delta\delta_{tot}$, on all residues of Nkx2.5 HD-SD were calculated according to the equation following. $\Delta\delta_{tot} = ((\Delta\delta_{HN}/\Delta D_{HN})^2 + (\Delta\delta_N/\Delta D_N)^2)^{1/2}$. The ΔD_{HN} and ΔD_N are differences between maximum and minimum chemical shifts in the ^{1}H and the ^{15}N dimensions, respectively.

Extent of assignments and data deposition

For the free and DNA-bound Nkx2.5 HD-SD including the vector-derived N-terminal tag residues, 93% of the backbone amides were assigned; nearly all the unassigned residues are prolines, including a seven proline stretch between the HD and the NK2-SD. 93% and 62% of the C^α and C^β resonances were assigned for free and DNA-bound proteins, respectively; no C^α/C^β resonances could be detected for the DNA-bound HD region in the HNCACB and CBCA(CO)NH experiments, presumably due to shorter T_2 times. Only the HD portion showed any tertiary structure, both in the free and DNA-bound proteins, as evinced by much sharper signals (longer T_2 times) and by the lack of any long-range NOE signals for the non-HD regions. The chemical shifts, NOE cross peaks and secondary structure of HD region of Nkx2.5 HD-SD correspond quite closely with those from an Nkx2.5 construct containing the HD alone, both in the free and DNA-bound forms (unpublished results), and because of this the backbone assignment of the bound HD using the 3D ^{15}N NOESY presented no difficulties. No changes were observed in the NK2-SD upon binding DNA (see Figure 1b). Because the HD region showed no obvious structural differences compared with the HD-alone construct, and since the rest of the longer construct, including the NK2-SD, was unstructured, comprehensive side chain assignments were not warranted. Using chemical shift index (CSI) (Wishart and Sykes, 1994) to analyze the secondary structure of non-HD regions suggests a β strand region corresponding to residues 85–87 in the middle of the NK2-SD, though no long-range NOEs were detected for these residues nor were their signal intensities different from the neighboring random coil residues. The assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 6319.

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