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Resonance assignment and secondary structure of the cold shock domain of the human YB-1 protein

Cathelijne P.A.M. Kloks[a,*], Astrid Hoffmann[b,*], James G. Omichinski[b], Geerten W. Vuister[a], Cornelis W. Hilbers[a,**] and Stephan Grzesiek[b,c,**]

[a] *NSR Center for Molecular Structure, Design and Synthesis, Laboratory of Biophysical Chemistry, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands;* [b] *IBI-2, Forschungszentrum Jülich, D-52425 Jülich, Germany;* [c] *Institute of Physical Biology, Heinrich-Heine-Universität, D-40225 Düsseldorf, Germany*

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Biological context

The human protein YB-1 is a member of the family of Y-box proteins, which are highly conserved amongst eukaryotes and ubiquitous in nature. They have been found to interact with single- and double-stranded DNA and RNA, and are predicted to play a role in translational and transcriptional regulation (Wolffe, 1994) as well as germ cell-specific developmental regulation. The Y-box proteins consist of a Cold Shock Domain (CSD) flanked by an N- and a C-terminal domain. The CSD is thought to be responsible for selective binding of the protein to single- and double-stranded DNA containing the Y-box sequence, CTGATTGGCCAA, which is present in promoters of a variety of eukaryotic genes (Didier et al., 1988).

The Cold Shock Domain of Y-box proteins is extremely conserved; it is 90% homologous within the Y-box protein family and has a 43% homology with the prokaryotic Cold Shock proteins (Csps) (Schnuchel et al., 1993). The Y-box CSD contains the RNA-binding RNP-1 and RNP-2 motifs that are also found in the Csps and the RNP-protein family. Here, we report the ^{1}H, ^{13}C and ^{15}N resonance assignment and the secondary structure of the CSD of human YB-1.

Methods and results

A DNA fragment encoding an N-terminal methionine followed by residue Lys^{52} to Gly^{129} of the cold shock domain of the human Y-box protein YB-1 was derived from a human cDNA library and amplified by polymerase chain reaction. The DNA product was subcloned into the NdeI and BamHI site of the expression vector pET11a (Novagene, USA). Protein expression from the pET construct in *E. coli* strain BL21(DE3) was induced by 0.5 mm IPTG for 3 h. ^{15}N and ^{13}C labeling was carried out by growing the bacteria on minimal medium using $^{15}NH_4Cl$, and $^{13}C_6$-glucose as the sole nitrogen and carbon sources, respectively. Cells were lysed using a French press, and proteins were purified from the soluble extract by ion exchange and reverse phase chromatography. Three samples (U-^{15}N 95% H_2O/5% D_2O; U-^{13}C, U-^{15}N 95% H_2O/5% D_2O; U-^{13}C, U-^{15}N 100% D_2O)

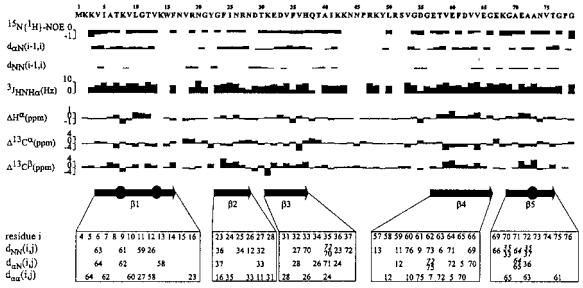

Figure 1. Summary of heteronuclear, sequential, and cross-strand NOEs, $^{3}J_{HNH\alpha}$-couplings, and deviations from random-coil values of the $^{1}H^{\alpha}$, $^{13}C^{\alpha}$, $^{13}C^{\beta}$ chemical shifts as obtained for the cold shock domain of YB1 as a function of residue number. The secondary structure derived from these data is consistent with 5 β-strands, as indicated. Filled ellipses in the β-strands indicate the positions of the bulges. Cross-strand NOE connectivities are indicated by residue numbers. Italic numbering indicates potential NOEs obscured by overlap.

*Both authors contributed equally.
**To whom correspondence should be addressed.

were prepared as 1.5 mM solutions at pH 6.7 without any further addition of salt.

The following NMR experiments, described in detail by Grzesiek et al. (1997), were recorded: ^{15}N-HSQC, CBCA(CO)NH, CBCANH, HBHA(CCO)NH, C(CO)NH-TOCSY, H(CCO)NH-TOCSY, ^{15}N-NOESY-HMQC, HNHA, HNCO, and ^{13}C-NOESY-HMQC. All experiments were recorded at 298 K, on a Bruker DMX 600 MHz spectrometer equipped with a 5 mm triple-resonance, triple-gradient probe. The data were processed using established protocols (Grzesiek et al., 1997) and the software package NMRPipe (Delaglio et al, 1995). Assignments were carried out with the peak-picking program PIPP (Garrett et al., 1991).

The sequential walk was made using data from the CBCA(CO)NH, CBCANH, HBHA(CCO)NH, HNHA, and ^{15}N-NOESY-HMQC. The side chain assignment was completed using C(CO)NH-TOCSY and H(CCO)NH-TOCSY. The $^{3}J_{HNHA}$ coupling constants were calculated from the HNHA-spectrum using a 1.05 correction factor to account for the faster relaxation of the antiphase magnetization.

The data allowed the evaluation of secondary structural elements. Five β-strands could be identified based upon the sequential NOEs, deviations of the $^{1}H^{\alpha}$, $^{13}C^{\alpha}$ and $^{13}C^{\beta}$ chemical shifts from random coil values and $^{3}J_{HNHA}$ coupling constants. The first β-strand encompasses V4 to F16, the second G23 to N28, the third K31 to H37, the fourth E57 to G66, and the fifth G69 to T76. RNP-1 (residues N20 to R27) is located partly in the second β-strand and RNP-2 (residues V34 to H37) in the third strand. In addition, analysis of cross-strand H^{α}-H^{N} and H^{N}-H^{N} NOEs yielded the topology of the β-strands; a 5-stranded anti-parallel β-barrel. The barrel is closed by a parallel line-up of strands 3 and 5. The β-strands are connected by tight turns, except strands 3 and 4, which are connected by a long, apparently unstructured loop formed by residues Q38 to E57. Figure 1 shows a summary of the NMR-data and the secondary structure.

Extent of assignments and data deposition

Most residues were fully assigned, with exception of the aromatic and amide side chain resonances. For the residues M1, K14, and N17 no assignments could be obtained. In addition assignments of the H^{N} and N are missing for K2, W15, Y22, and N44, whereas side chain assignments for V13 and K43 are lacking. At the moment 91% of all ^{1}H, ^{15}N, and ^{13}C backbone nuclei and 60% of all side chain nuclei are assigned.

These ^{1}H, ^{15}N, and ^{13}C chemical shifts of the Cold Shock Domain of human YB-1 (corresponding to residues 52 to 129 in the original YB-1) recorded at 298 K, pH 6.7 have been deposited at BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 4147.

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