Journal of Biomolecular NMR, **12:** 357–359, 1998.
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^{1}H, ^{13}C and ^{15}N assignment of the Isl-1 homeodomain

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Received 13 March 1998; Accepted 29 April 1998

Key words: Isl-1, homeodomain, protein, DNA-binding, NMR assignment

Biological context

The insulin gene enhancer binding protein, Isl-1, binds specifically to a transcription activation domain of the insulin gene in rat (Karlsson et al., 1990), acts as a positive regulator of the proglucagon genes (Wang and Drucker, 1995), and is required for embryonic neuron differentiation (Pfaff et al., 1996). Isl-1 has a molecular weight of 42 kDa, and contains two amino terminal zinc-binding LIM domains (Karlsson et al., 1990; McGurie et al., 1992), and a C-terminal region related to homeodomains in other DNA binding proteins. The homeodomain region of Isl-1, Isl-1-HD (66 amino acids), mediates its DNA binding through sequence specific recognition of duplex DNA containing a –TAAT– sequence (Behravan et al., 1997). We have now used NMR to determine the solution structure of the Isl-1-HD (Ippel et al., to be published), while studies on the Isl-1-HD/DNA complex are still in progress. Here we report the assignment of the ^{1}H, ^{13}C, and ^{15}N resonances of the uncomplexed Isl-I-HD.

Methods and results

The Isl-1-HD used in the NMR studies was expressed and purified as described by Behravan et al. (1997), except for the following modifications: (1) the protein sequence does not contain a C-terminal His tag to avoid interference with the DNA binding; (2) the fragment was purified on a Q-sepharose column, followed by a SP-sepharose and a heparin-sepharose column.

Three NMR samples were used: a non-labeled, a [U-^{15}N] and a [U-^{13}C, ^{15}N] sample in phosphate buffer (25 mM Na_2HPO_4/NaH_2PO_4, 25 mM NaCl, pH 6.0, 90% H_2O/10% D_2O) with protein concentrations of 0.8 mM, 0.45 mM and 1.8 mM, respectively. Formation of high molecular weight aggregates during the measurements (invisible in the NMR spectrum, but observed on SDS gels) reduced the effective concentration of the [U-^{13}C, ^{15}N sample to 1.4 mM, as estimated from the decrease in signal intensity seen in the ^{1}H spectrum after the experiment. Isl-I-HD contains two oxidation-sensitive cysteine residues, Cys^{21} and Cys^{56}, which were kept in the reduced state by addition of 10 mM dithiotreitol (DTT), and by regular flushing of the NMR tube with argon. The oxidation state of the protein was monitored during measurements by following the ratio of the ^{1}H signals of reduced and oxidized DTT in a 1D NMR spectrum. NMR samples were stored at 4 °C and remained stable for at least one month.

The NMR experiments performed to obtain the assignments are shown in Table 1. All NMR experiments were performed at 8 °C on a Bruker DRX-600 spectrometer equipped with triple resonance (^{1}H/^{13}C/^{15}N) TXI- or TBI-probes with XYZ-gradient capabilities. Quadrature detection in the indirectly detected dimensions was obtained either by the States-TPPI (Marion et al., 1989) or the echo/anti-echo method (Schleucher et al., 1993). Prior to Fourier transformation, the time domain data were zero-filled (Table 1) and apodized by phase-shifted sine bells; linear prediction was applied for indirect dimensions in the 3D spectra (processing software, UXNMR). Peak-picking and analysis of the transformed spectra was done by the XEASY program (Bartels et al., 1995).

The sequence-specific assignment of Isl-1-HD was initially derived from the ^{15}N-edited 3D NOESY, recorded for the [U-^{15}N] sample, using inter residue $H^N(n)$–$H^N(n-1)$ and $H^N(n)$–$H\alpha(n-1)$ NOE contacts. NH_2 groups of Asn and Gln were assigned sequence

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Table 1. Parameters for NMR experiments used in the ^{1}H/^{13}C/^{15}N assignment of Isl-1-HD

Experiment[a]	Sample[b]	^{1}H		D2			D3			Matrix dimensions[c]	Number of scans[d]	Mixing time[e]
		SW[f]	N*[g]		SW[f]	N*[g]		SW[f]	N*[g]			
[^{1}H,^{1}H]NOESY	1	7184	2048	^{1}H	7184	572				2048 × 1024	64	150
[^{1}H,^{1}H]TOCSY	1	7184	2048	^{1}H	7184	600				2048 × 1024	64	42
[^{15}N,^{1}H]HSQC	2	11062	4096	^{15}N	1216	256				2048 × 256	8	
[^{13}C,^{1}H]CT-HSQC[h]	3	7441	1024	^{13}C	1011	452				1024 × 1024	32	
^{15}N edited 3D NOESY[h]	2	2894	1024	^{1}H	7184	160	^{15}N	1216	64	1024 × 512 × 128	24	150
^{15}N edited 3D TOCSY[h]	2	2894	1024	^{1}H	7184	160	^{15}N	1216	64	1024 × 512 × 128	16	96
^{13}C edited 3D NOESY[h]	3	4006	768	^{1}H	7246	160	^{13}C	8903	140	512 × 256 × 256	8	130
CBCA(CO)NH[h]	3	3472	800	^{13}C	9055	114	^{15}N	1095	48	512 × 256 × 128	16	
HBHA(CC)CO)NH[h]	3	3472	800	^{1}H	3608	64	^{15}N	1095	48	512 × 256 × 128	48	5.8

[a] Experiments recorded at ^{1}H frequency of 600 MHz;
[b] sample used: 1 = non-labeled; 2 = [U-^{15}N]; 3 = [U-^{13}C,^{15}N];
[c] Final processed matrix size in real points;
[d] Number of scans collected per increment;
[e] Mixing time (ms), in case of TOCSY half of the mixing time is taken up by ROE compensation delays;
[f] Sweep width (Hz);
[g] Number of complex points collected in this dimension;
[h] See: Bax and Grzesiek (1993).

specifically by using intra residue N0Es in the ^{15}N-edited 3D NOESY. Assignments of side chain protons in the flexible and α-helical regions of the protein were obtained from the ^{15}N-edited 3D TOCSY/NOESY and the 3D HBHA(CC)(CO)NH, respectively. Assignment of the ring protons of Pro[26,28,44] was achieved using the characteristic chemical shifts of the δCH_2 and Cδ resonances in the ^{13}C-edited 3D NOESY spectrum. Aromatic spin systems were assigned from 2D NOESY/TOCSY spectra together with information derived from chemical shift calculations (Williamson et al., 1995) carried out during different stages of the structure determination. CBCA(CO)NH, ^{13}C-edited 3D NOESY and CT-[^{13}C,^{1}H] HSQC were used to assign Cα, Cβ and methyl carbon resonances. Stereospecific assignment of methyl signals of Val and Leu followed from the ^{13}C-edited 3D NOESY in combination with a floating assignment procedure (Folmer et al., 1997) and chemical shift restraints.

Extent of assignments and data deposition

All ^{1}H and ^{15}N resonances were assigned, except for those of Met[1], and H^N/N of Lys[2], γCH_2/δCH_2 of Arg[55], δCH_2 of Lys[33], εCH_2 of Lys[33,54,57,59], γCH of Leu[36], ε-CH_2 of Met[32,39,64,65], and the exchangeable proton resonances of Ser, Thr, Tyr, His, Lys and Arg sidechains. Chemical shifts of both N and H^N of residues Lys[60] and Arg[61] are near-isochronous, which prevents accurate shift positions for most resonances in these residues. The majority of methylene protons are degenerate and only a single peak is observed. ^{13}C assignments are incomplete, and mainly shifts for Cα, Cβ (except Cβ Asn[25], Arg[27], Met[39] and Lys[66]) and methyl carbons are reported together with stereospecific assignment of several methyl signals of Val and Leu. The ^{1}H, ^{13}C and ^{15}N chemical shifts for Isl-1-HD have been deposited in BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 4121.

Acknowledgements

This work was supported by grants from NFR (to POL, SW), Knut and Alice Wallenberg Stiftelse (to SW), Biotechnology (Umeå) (to SW), Lars Hiertas Minne (to GB), and Magnus Bergvalls Stiftelse (to GB).

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