Journal of Biomolecular NMR, **20:** 91–92, 2001.
KLUWER/ESCOM

Letter to the Editor: Assignment of ^{1}H, ^{15}N and ^{13}C resonances of the carbohydrate recognition domain of human galectin-3

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Received 19 December 2000; Accepted 17 January 2001

Key words: carbohydrate recognition domain, chemical shift assignment, galectin-3, human, lectin, recombinant protein

Biological context

Galectin-3, a member of a family of β-galactoside-binding animal lectins (Cooper and Barondes, 1999), has been shown to be involved in a number of biological events, including cell adhesion, cell growth regulation, tumor progression and metastasis (Barondes et al., 1994; Perillo et al., 1998). Galectin-3 may bind carbohydrate chains and perhaps cross link cell surface receptors, but other modes of action are also possible. To understand the precise biological role of galectin-3 and explore its possible medical use, it is necessary to learn more about its carbohydrate binding mechanism and the structural basis for the specificity for natural ligands. In order to provide a basis for using high-resolution NMR studies in this regard, we present here the assignment of the ^{1}H, ^{13}C, and ^{15}N backbone and many of the side-chain resonances of an isotopically $^{15}N/^{13}C$-double labeled fragment (residues 108–250) of human galectin-3 containing the CRD (carbohydrate binding domain) complexed with lactose (Seetharaman et al., 1998).

Methods and experiments

For production of ^{15}N,^{13}C-labeled galectin-3, galectin-3 expressing *Escherichia coli* (host BL21 with expression vector pET3C) (Massa et al., 1993) were cultured in M9 minimal medium containing 50 mg/l ampicillin, ^{13}C (99%)-U-glucose and ^{15}N (99%)-NH_4Cl. The C-terminal fragment, aa 108–250, galectin-3C, was produced by collagenase digestion of the intact galectin-3 and repurification on lactosyl-Sepharose (Massa et al., 1993).

NMR spectra were collected at 303 K using a Varian UNITYplus-500 spectrometer equipped with a z-gradient triple-resonance probe. 2D ^{1}H-^{15}N HSQC spectra were recorded using the enhanced sensitivity method (Kay et al., 1992). Several 3D spectra were obtained to correlate backbone chemical shifts, using numbers of complex points acquired in the F1, F2 and F3 dimensions, and spectral widths as follows: 3D HNCACB, 40 × 40 × 512, 7.65 × 1.65 × 8 kHz; 3D CBCA(CO)NH, 40 × 40 × 512, 7.65 × 1.65 × 8 kHz; 3D HCCH-TOCSY, 128 × 32 × 416, 3.5 × 3.0 × 8 kHz; 3D HCC-TOCSY, 64 × 32 × 512, 2.50 × 1.65 × 8 kHz; and 3D CN-NOESY-HSQC (Pascal et al., 1994), 128 × 32 × 416, 3.5 × 3.0 × 8 kHz. A mixing time of 150 ms was employed in the CN-NOESY-HSQC spectra. Usually, 16 scans were accumulated per increment. The FIDs were processed using VNMR software (Varian Associates) or NMRPipe/NMRDraw (Delaglio et al., 1995).

The sequential assignments of the backbone ^{15}N and ^{13}C signals were obtained mainly based on the HNCACB and CBCA(CO)NH spectra for their excellent sensitivities. HCCH-TOCSY and HCC-TOCSY were then used to obtain the assignments of the rest of the backbone and side-chain ^{1}H signals. Also helpful in confirming the sequential connectivity was CN-NOESY-HSQC, using the strong $d_{\alpha N(i,i+1)}$ NOE cross peaks. For seven residues with missing $^{1}H/^{15}N$ cross peaks, ^{13}C and ^{1}H assignments were derived, where possible, from HNCACB and CBCA(CO)NH, followed by HCC- and HCCH-TOCSY connectivity. A similar procedure was used for assigning 11 Pro

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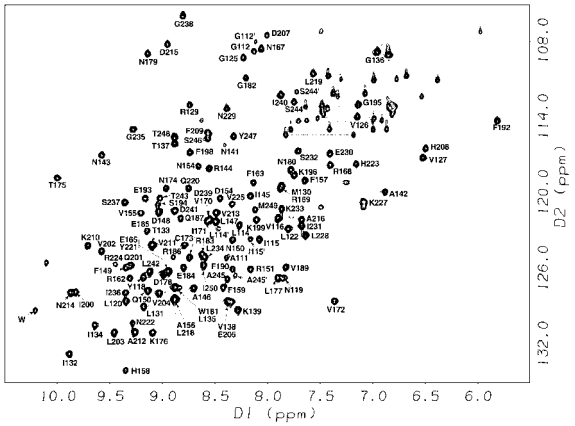

Figure 1. ^{1}H-^{15}N HSQC of the C-terminal fragment (aa 108–250) of human galectin-3, 0.5 mM in phosphate buffer, at 303 K, pH 7.4 with 10 mM lactose. Backbone amide peaks are indicated by residue (one letter code) and number. The Asn and Glu side-chain cross peaks are connected by bars, and that from Trp181 is labeled with W.

residues, and six of them could also be confirmed by the NOE connectivity between their δ-protons and the α-protons of the previous residues.

Extent of assignments and data deposition

Figure 1 shows the ^{1}H-^{15}N HSQC spectrum of galectin-3C. Out of 132 (143 minus 11 prolines) anticipated backbone NH cross peaks, 125 are clearly observed. The residues for which no NH cross peaks could be observed are either in the less ordered part of galectin-3C preceding the CRD (Gly108 and Ala109) or in the loops connecting β-strands within the CRD (Gly125, Asn153, Asn166, Ser188, His 217, and Lys226) (Seetharaman et al., 1998). Hence, rapid exchange of the NH protons in contact with water may account for the missing cross peaks.

The NH of Gly112, Leu114, and Ile115 are represented by double peaks (Figure 1) and the shape of the NH cross peak from Ala111 suggests unresolved double peaks. This indicates that these residues preceding the CRD may have two alternative conformations. Also two residues near the C-terminus, Ser244 and Ala245, produced double NH cross peaks (Figure 1). A possible explanation for their double peaks is influence from residues 109–115, since in the X-ray structure the Val116-Pro117 bond is *cis* and forms a sharp bend that directs the preceding residues to fold back near the CRD surface formed by the first (S1) and last (F1) β-strands. Further evidence for such cross influence is the upfield shift of $^{1}H_{\beta}$ of Ala109 (0.19 ppm), possibly caused by Tyr247.

The complete assignments of the observable ^{1}HN, ^{15}N, $^{13}C_{\alpha}$, $^{13}C_{\beta}$ and $^{1}H_{\alpha}$ signals have been acquired, as well as of many of the side-chain protons. The chemical shift data have been deposited with the BioMagResBank Database (BMRB accession number 4909).

Acknowledgements

We thank the staff at the NMR center of the Faculty of Molecular and Medical Genetics, University of Toronto, for their support during NMR data collection. Grants were from the Swedish Medical Research Council (#12165) and an ICU International Academic Exchange Endowment Grant.

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