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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N NMR assignments of the C-type lectin TC14

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Introduction

The C-type lectin protein family binds a variety of sugar ligands in a calcium-dependent manner (for review see Drickamer, 1999). TC14 is an early evolutionary form of this family of animal proteins from the tunicate *Polyandrocarpa misakiensis*. It has D-galactose specificity and is involved in the asexual bud development of the organism (Kawamura et al., 1991, 1995). We recently determined the crystal structure of the recombinant TC14 lectin complexed with D-galactose (Poget et al., 1999). TC14 forms a 28 kDa homodimer of two classic α/β C-type lectin domains. The 3 and 4 sugar hydroxyls of the bound D-galactose form direct coordination bonds with a calcium atom at the primary binding site. However, the orientation of the sugar ring is inverted by 180° relative to that observed in the D-galactose specific QPDWG mutant of the C-type lectin mannose binding protein-A (Kolatkar and Weis, 1996). In this paper we present the NMR sequence-specific assignments of TC14 in the presence of bound calcium. These assignments form the basis of ongoing work on the role of the calcium and disulphides in maintaining the C-type lectin fold, oligosaccharide binding and studies on the polypeptide backbone dynamics.

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Methods and results

The recombinant and isotopically labelled TC14 (^{1}H, ^{15}N, ^{15}N/^{13}C) expressed to high levels as inclusion bodies in *Escherichia coli* (approximately 5% of total cell protein by SDS-PAGE). Details of the expression, purification and refolding of the recombinant TC14 have been published elsewhere (Poget et al., 1999). The recombinant protein was based on the primary amino acid sequence of TC14 as determined by Suzuki et al. (1990). The N-terminal methionine is present as shown by electrospray mass spectrometry. Samples were dialysed from calcium containing MES buffer, pH 6.0, into either 10% or 100% $^{2}H_2O$, adjusted with small aliquots of HCl/NaOH to a final pH* of 6.0 and concentrated to 400–500 μL using a centriprep 10000 (Amicon ltd). They were spun at 14000 rpm in a microcentrifuge for 10 min and the supernatant was transferred to a 5 mm Wilmad NMR tube. Under these conditions samples were stable at 328 K for many weeks and at 277 K for months.

NMR data were acquired at both 328 and 310 K on a Bruker AMX 500 spectrometer equipped with a triple-resonance ^{1}H/^{15}N/^{13}C 5 mm probe and z-gradients. Proton (^{1}H) chemical shifts were referenced to the internal standard 3-(trimethylsilyl)-d_4-propionic acid sodium salt at 0.0 ppm. ^{13}C and ^{15}N chemical shifts were referenced indirectly according to the absolute frequency ratios (Wishart et al., 1995). Using the triple resonance approach, main-chain and partial side-chain assignments were obtained by a combination of the following experiments: CBCA(CO)NH, HNCA, HNCACB, HBHA(CBCACO)NH, a 3D ^{1}H/^{15}N TOCSY-HMQC with a 55 ms mixing time and 2D ^{1}H/^{15}N and ^{1}H/^{13}C HSQC spectra. Additional side-chain assignments were obtained from a ^{1}H/^{13}C

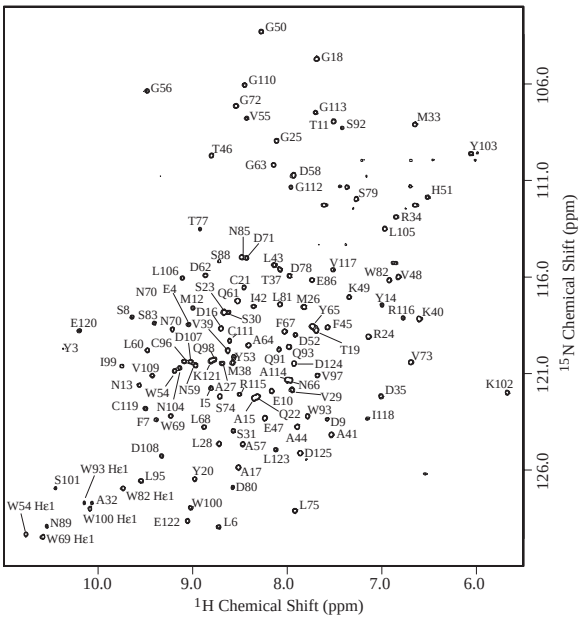

Figure 1. 2D $^{1}H/^{15}N$ HSQC spectrum of holo TC14 at 328 K. Assignments for the backbone amides and tryptophan imines are indicated by residue number. The sequence of the protein is 1MDYEILFSDETMNYADAGTYCQSRGMALVSSAMRDSTMV-KAILAFTEVKGHDYWVGADNLQDGAYNFLWNDGVSLPTD-SDLWSPN**EP**S**N**PQSWQLCVQIWSYNLL**DD**VGCGGARRVIC-EKELDD125 (residues involved in galactose binding are shown in bold).

C-HCCH-TOCSY spectrum with a mixing time of 13 ms acquired in 100% $^{2}H_2O$ (Vuister and Bax, 1992). To obtain assignments for the aromatic ring systems 2D DQF-COSY, 2D TOCSY experiments and a $^{1}H/^{13}C$ HSQC experiment tuned for aromatics were acquired in 100% $^{2}H_2O$. Aromatic ring assignments were connected to side-chain aliphatic protons via Hβ to Hδ NOEs for Tyr and Phe residues and Hβ to $H\delta^{1}$ or $H\varepsilon^{3}$ NOEs for Trp residues. The $^{3}J H_N H_\alpha$ coupling constants for ^{15}N labelled TC-14 lectin were measured from a 2D $^{1}H/^{15}N$ HSQC spectrum by the method of Stonehouse and Keeler (1995). Acquired data were converted to FELIX format using the program 'ux2flx' and processed with FELIX version 2.30 (Biosym Technologies) on an SGI Indigo-2 workstation.

Extent of assignments and data deposition

TC-14 is inherently heat stable (Suzuki et al., 1989), which allowed acquisition of spectra at both 310 and 328 K in order to increase dispersion and reduce intrinsic line width. The ^{15}N HSQC spectrum of holo TC14 at 328 K is shown in Figure 1, which shows the excellent dispersion and peak shape under the conditions used for data acquisition. Complete ^{1}H, ^{15}N, and ^{13}C backbone resonance assignments and most side-chain aliphatic assignments were obtained. Due to the large number of tryptophan residues, many of the aromatic assignments were determined using 2D methods alone. The signal intensity of the amide residue Ser 36 decreased upon increasing the acquisition temperature, most likely from saturation transfer with the bulk solvent, and the assignments for this residue were only obtained in spectra at 310 K. All Cys residues have an oxidized thiol group, with $^{13}C\beta$ chemical shifts similar to values expected for oxidized Cys residues (38.9–42.8 ppm), whereas a reduced Cys $^{13}C\beta$ resonates at approximately 27.6 ppm. The $^{13}C\beta$ chemical shift of Pro 87 (31.55 ppm) is consistent with the cis conformation of the peptide bond observed in the TC14 crystal structure. There are several side-chain resonances that are unusually upfield shifted: −0.77 ppm (Hγ, Lys 49); −0.33 ppm (Hγ, Lys 102); and −0.49 ppm (Hγ2, Leu 75). This is likely to be due to aromatic interactions as in the crystal structure Lys 49 lies close to Phe 45, Lys 102 packs against Trp 100 and Leu 75 is part of a hydrophobic cluster that involves residues Trp 82, Trp 69 and Phe 67.

The ^{1}H, ^{15}N and ^{13}C chemical shifts for holo TC14 at 328 K have been deposited in the BioMagResBank (http:www.bmrb.wisc.edu) under BMRB accession number 4782.

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