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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N backbone resonance assignments of the hyaluronan-binding domain of CD44

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Biological context

CD44 is the main cell surface receptor for hyaluronic acid (HA), and the binding of CD44 to HA has been implicated in both cell adhesion to the extracellular matrix (ECM) components and cellular signaling cascades (Lesley et al., 1993; Naor et al., 1997).

CD44 contains a functional HA-binding domain (HABD) composed of a Link module with N- and C-terminal extensions. Link modules are found in several ECM molecules and the protein product of tumor necrosis factor-stimulated gene-6 (TSG-6) (Day et al., 2002). The three-dimensional structure and ligand-binding site of the TSG-6 Link module have already been determined by NMR (Kohda et al., 1996; Kahmann et al., 2000). In addition to the Link module, CD44 requires N- and C- terminal extensions for its proper folding and the functional activity, but no structural information about CD44 is available.

Here we report the ^{1}H, ^{13}C and ^{15}N backbone resonance assignments for the CD44 HABD. The assignments obtained from the present study will be used to elucidate the HA-recognition mode of CD44 HABD by cross-saturation and chemical shift perturbation experiments.

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Methods and experiments

The human CD44 HABD gene (residues 21–178) was amplified by PCR from a cDNA encoding a human CD44-IgG fusion protein, and was cloned into the pET11a vector (Novagen). CD44 HABD was expressed in *E. coli* BL21 (DE3) codon Plus RP (Stratagene) without affinity tags. For the unlabeled protein, the transformed cells were grown in Luria-Bertani broth. The uniformly ^{13}C/^{15}N-labeled CD44 HABD was prepared by growing cells in M9 media containing $^{15}NH_4Cl$ (1 g l^{-1}) and [U-^{13}C] glucose (2 g l^{-1}) supplemented with Celtone-CN powder (1 g l^{-1}; Martek), and then was purified as previously described (Banerji et al., 1998). The purified and lyophilized protein was dissolved in 6M guanidine, 100 mM Tris pH 8.0, and 150 mM NaCl, and was dialyzed against $H_2O/^2H_2O$ (9/1) buffer containing 50 mM potassium phosphate pH 6.7 and 150 mM NaCl. To evaluate the HA-binding activity of the CD44 HABD, surface plasmon resonance (SPR) measurements were employed. The results showed that CD44 HABD has HA-binding activity, and the dissociation constant (K_d) for CD44 HABD toward the HA 250-mer is 2.7×10^{-5} M (the K_d for the monomeric CD44Rg protein, expressed in ldl-D cells and deglycosylated, toward the HA 60-mer is 5×10^{-6} M (Skelton, 1998)). For NMR experiments, the sample of 2.0 mM ^{13}C/^{15}N-labeled CD44 HABD was prepared.

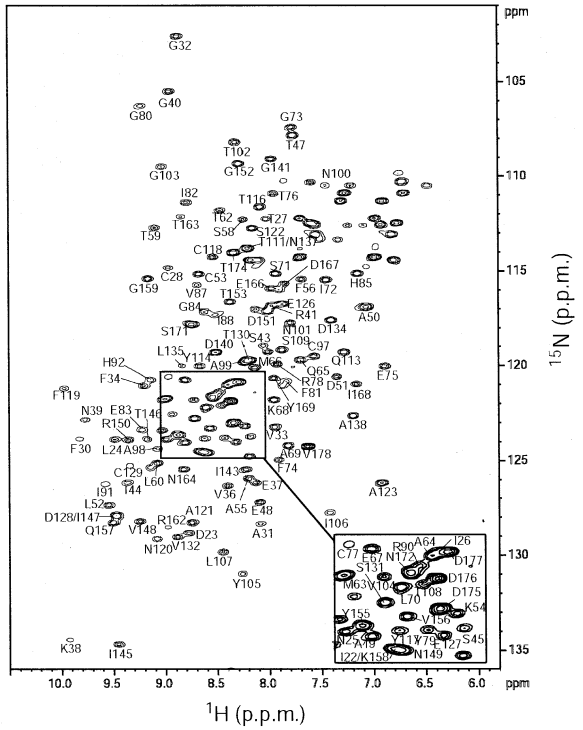

Figure 1. A 500 MHz ^{1}H-^{15}N HSQC spectrum of 0.3 mM uniformly ^{15}N-labeled CD44 HABD. The cross peaks are labeled with the one-letter codes for amino acids and the residue numbers.

All NMR experiments were performed at 25 °C on Bruker DRX 400, Avance 500 or Avance 600 spectrometers. Proton chemical shifts are referenced to the internal methyl signal of DSS (0 ppm). ^{13}C and ^{15}N chemical shifts are referenced indirectly to DSS, using the absolute frequency ratios. The sequential assignments of the ^{1}HN, ^{15}N, $^{13}C_{\alpha}$ and $^{13}C_{\beta}$chemical shifts of CD44 HABD were achieved by the sets of three-dimensional triple resonance experiments (HNCA, HN(CO)CA, CBCA(CO)NH, and HNCACB). All spectra were processed by NMRPipe (Delaglio et al., 1995) and data analysis was assisted by ANSIG (Kraulis, 1989). To facilitate the sequential assignments, several selective ^{15}N labelings of one type of amino acid were carried out to provide starting points for the sequential assignments.

On the basis of both the NOE connectivities observed among the main chain $C_{\alpha}H$ and H_N resonances and the chemical shift index analysis, we determined the secondary structure and the topology of the free state of CD44 HABD. CD44 HABD was identified as a structural domain, comprising two α-helices (α1, 46–56; α2, 63–71) and two β-sheets. Sheet I is composed of five strands: βa (residues 22–27); βb (34–39); βe (116–118); βf (144–148); and βg (155–158). Sheet II is a two-stranded antiparallel β-sheet: βc (79–82) and βd (85–88). A comparison of the topology with that of TSG-6 indicated that the CD44 Link module adopts the consensus fold for the Link module superfamily (Kohda et al., 1996). It should be noted that the N- and C-terminal extensions form three additional β-strands (βa, βf and βg) to sheet I of the consensus fold.

Extents of assignments and data deposition

The 2D ^{1}H-^{15}N HSQC spectrum of the CD44 HABD (Figure 1) exhibits a good dispersion for the proton and nitrogen resonances. From the above mentioned triple resonance experiments, an almost complete backbone assignment (97% of the 148 non-Pro residues) was achieved. The assignments of Gln21, Asn94, Ser95, and Asn100 could not be made due to line broadening. The ^{1}H, ^{13}C and ^{15}N chemical shifts of the backbone resonances have been deposited in the BioMagResBank (http:/www.bmrb.wisc.edu) under the BMRB accession number 5903.

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