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Letter to the Editor: Assignment of ^{1}H, ^{13}C and ^{15}N resonances of the I-domain of human leukocyte function associated antigen-1

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Biological context

The interaction between the integrin leukocyte function associated antigen 1 (LFA-1) and intercellular adhesion molecule 1 (ICAM-1) is primarily mediated via an inserted domain (I-domain) of approximately 190 amino acids (for review see Gahmberg, 1997), which is a common feature of all leukocyte integrins. X-ray structures of the LFA-1 and Mac-1 I-domains in the presence of EDTA or divalent cations are available (see, for example, Qu and Leahy, 1995), and we have recently completed an NMR structure determination (Legge et al., 2000) which was based on the sequence-specific assignments of the LFA-1 I-domain reported in the present paper. These assignments provide the basis for further studies on LFA-1 I-domain/ICAM-1 interactions.

Methods and results

The recombinant protein ^{1}H, ^{15}N and $^{15}N/^{13}C$ isotopically labeled protein was obtained by over-expression in *Escherichia coli.* Details of the expression, purification and reconstitution with Mg^{2+} have been described elsewhere (Legge et al., 2000). The recombinant material used for these studies corresponds to residues 125–311 of the αL domain of LFA-1 and electrospray mass spectrometry data indicated that a single species was obtained which lacked the N-terminal methionine. Residue 189 (tryptophan) differs between the present construct and that used for the X-ray crystal structures (arginine). NMR samples (1.2 mM) of purified unlabeled, ^{15}N and uniformly $^{15}N/^{13}C$ isotopically labeled LFA-1 I-domain were prepared in 10 mM sodium phosphate, pH 7.2, 10 mM $MgSO_4$, 150 mM NaCl, 0.05% w/v NaN_3 and 5% v/v $^{2}H_2O$. Samples containing 2 mM $MgCl_2$, pH 7.3, 0.02% NaN_3 and 5% v/v $^{2}H_2O$ gave spectra with narrower linewidths.

NMR data were acquired at 22 °C using Bruker DMX-750, DRX-600 and AMX-500 NMR spectrometers fitted with triple resonance probes and triple axis gradients. Quadrature detection was achieved in the phase-sensitive mode by TPPI, States-TPPI, or pulsed field gradient coherence order selection and sensitivity enhancement (Kay et al., 1992). The software package Felix97 (Molecular Simulations, San Diego, CA) was used for all NMR data processing. A shifted squared sine-bell function was applied to the time domain data before zero filling. Mirror-image linear prediction was applied to all constant-time evolution periods. Spectra were referenced indirectly to TSP at 0.00 ppm and the heteronuclear dimensions were referenced using the appropriate gyromagnetic ratios.

Backbone sequential assignments were obtained using HNCACB, CBCA(CO)NH, CONH and HNCO experiments. The aliphatic side chain assignments were obtained by using HBHA(CO)NH, ^{15}N TOCSY-HSQC, ^{13}C HCCH-COSY and HCCH-TOCSY spectra. The quality of the NMR spectra allowed assignment of the protein resonances without the necessity for extensive deuterium labeling. Assignment of

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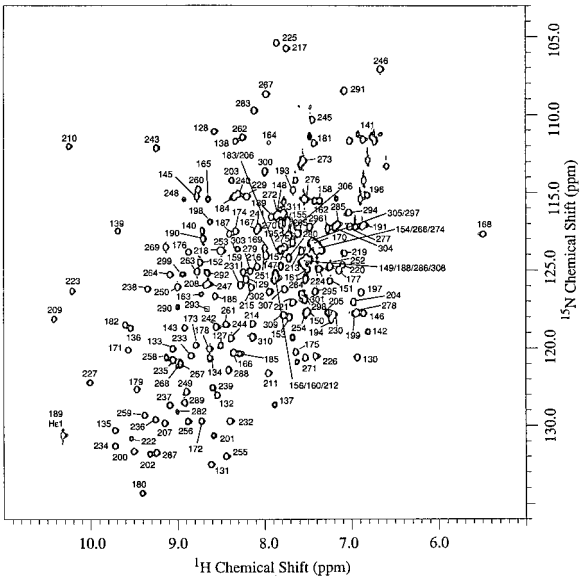

Figure 1. 2D ^{1}H{^{15}N} HSQC spectrum of the Mg^{2+} complex of ^{15}N-labeled LFA-1 I-domain at 22 °C. Assignments for the backbone and tryptophan side-chain amides are indicated by residue number. The cross peak for residue 293, which is represented by a box, lies below the contour level of the rest of the spectrum.

the aromatic resonances will be reported elsewhere (R. Kriwacki, manuscript in preparation).

Extent of assignments and data deposition

All ^{1}H, ^{15}N and ^{13}C polypeptide backbone resonances were assigned, except Ala 125 Hα and Hβ and the Ser 126 amide nitrogen and proton, which are affected by rapid amide exchange at the N-terminus. As an indication of the quality of the NMR data, the ^{15}N HSQC spectrum is shown in Figure 1. Almost all of the aromatic side-chain assignments were obtained, in spite of the large number of phenylalanine residues and associated resonance degeneracy. Only the side chains of Phe 173 and His 198, 201, 264 and 275 were unassigned. The Hε methyls of all methionine residues were assigned via intraresidue NOE connectivities and a number of slowly exchanging hydroxyl groups were assigned (7 serines and 4 threonines). A total of 9 Val, 8 Ile and 4 Thr residues had their side-chain methyl groups stereospecifically assigned using the three-bond coupling experiments CGCO and CGCN (Grzesiek et al., 1993; Vuister et al., 1993). In addition, a total of 19 Hβ methylene groups were stereospecifically assigned by analysis of the 2D COSY and the 3D NOESY spectra. The hydroxyl protons of the Mg^{2+}-binding residues Ser 16 and Ser 18 show large downfield shifts from random coil chemical shift values (8.51 and 10.9 ppm, respectively). Of the other labile side-chain protons, 3 of the 7 glutamines and 4 of the 5 asparagines, the three Arg Hε and the Trp $N^{\varepsilon}H$ were assigned.

The ^{1}H, ^{15}N and ^{13}C chemical shifts for the LFA-1 I-domain have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 4553.

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