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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N backbone resonance assignment of the Integrin α2 I-domain

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Biological context

Integrins constitute a major class of proteins mediating cell-cell and cell-extracellular matrix (ECM) interactions. These interactions are crucial for physiological processes such as development, differentiation, migration, wound healing, and immune system function. Cell-cell interactions mediated by integrins are mainly confined to cells of the immune system while integrins are used by nearly all mammalian cell types for cell-ECM interactions. Binding to an extracellular ligand triggers intracellular signaling mechanisms including protein phosphorylation events and cytoskeletal reorganisation (Hynes, 2002). Integrins are composed of two subunits, a 120–150 kDa α subunit and a 90–110 kDa β subunit, each comprising a single transmembrane helix. Both subunits interact noncovalently. Currently, there are 19 different α subunits and eight different β subunits known in vertebrates forming at least 25 different heterodimers. Integrin α2 and additional six integrin α subunits contain an inserted 200 amino acid domain, the so called I-domain (Shimaoka et al., 2002). The integrin α2β1 is expressed on a variety of different cell types serving as a receptor for collagen and laminin. Recombinantly expressed integrin α2 I-domain retains many of the ligand binding properties of the complete integrin (Tuckwell et al., 1995). The integrin α2 I-domain contains a single Mg^{2+} ion which is necessary for ligand binding. This metal-ion is coordinated by conserved amino acid residues which form the metal ion-dependent adhesion site (MIDAS). Crystal structures of the integrin α2 I-domain alone and in complex with collagen have already been determined (Emsley et al., 1997, 2000). Here, we present the backbone and Cβ assignment of the human integrin α2 I-domain as a step towards a better understanding of conformational changes encompassing collagen binding.

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Methods and experiments

Expression and purification of integrin α2 I-domain

DNA coding for amino acids 142-337 from human integrin α2 encompassing the I-domain was cloned into the NcoI/BamHI-site of the pKM263 expression vector (Melcher, 2000). In the resulting expression vector, pKM263Iα2_142-337, the coding region for the integrin α2 I-domain is preceded by six consecutive histidine residues, the glutathione S-transferase (GST)-gene and a Tobacco etch virus (Tev) cleavage site. The vector was transformed in *Escherichia coli* BL21 (DE3) (Novagen) for protein expression. As a result of cloning and Tev-cleavage four additional amino acids precede the integrin I-domain sequence. These residues were annotated, with negative numerical characters (-4 GAMG -1). For producing deuterated and ^{13}C, ^{15}N labeled protein cells were adapted to grow in 90% D_2O (Silantes GmbH, München, Germany) in M9 minimal medium, containing $^{15}NH_4Cl$ (Cambridge Isotope Labs, Andover, MA) and ^{13}C-glucose (Campro Scientific, Veenendal, The Netherlands) as the sole nitrogen and carbon sources, respectively. Bacteria were grown to an $OD_{600} = 0.7$ and protein expression was induced with 1 mM IPTG for 5 h at 25 °C. Protein labeled only with ^{15}N was produced accordingly. Purification and cleavage of the

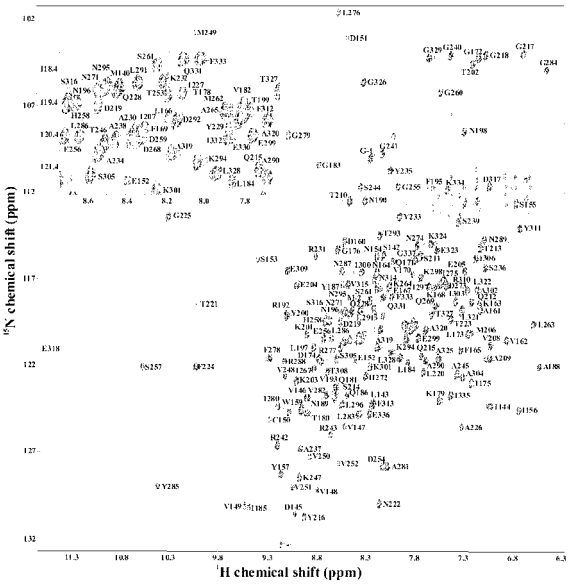

Figure 1. ^{1}H, ^{15}N-TROSY-HSQC spectrum of integrin α2 I-domain (142-337) at 298K. Peaks of D151, M249 and L276 are negative due to folding. The region with overlapping peaks is shown seperately in enlargement.

fusion protein was done with minor modifications as described by Melcher (2000).

NMR-spectroscopy

The backbone resonances of integrin α2 I-domain (142-337) was assigned using a sample containing 1 mM (^{2}D, ^{13}C, ^{15}N) protein in 50 mM NaP_i, 150 mM NaCl, 5 mM DTT, 5 mM $MgCl_2$, 0.01% NaN_3 and 10% D_2O at pH 6.5. Spectra were acquired at 298 K on a Bruker DRX-800 spectrometer with z-axis gradient. All chemical shifts were referenced to internal DSS (2,2-dimethyl-2-silapentane-5-sulfonate), either directly (^{1}H) or indirectly (^{13}C, ^{15}N) (Markley et al. 1998). The sequence-specific backbone resonance assignments were derived from TROSY versions of ^{1}H, ^{15}N HSQC (Figure 1), CBCA(CO)NH, HNCACB, HNCO and HN(CA)CO experiments (Salzmann et al. 1998). NMR data were processed using the program XWINNMR (Bruker). Analysis of the spectra were performed using the program XEASY (Bartels et al., 1995).

With the assignment of the backbone resonances (HN, N, C′, C^{α} and C^{β}) the program TALOS (Cornilescu et al., 1999) was used to predict ϕ, ψ torsion angles. Comparing the predicted angles with those of the crystal structure, the values agree within the error margin. Therefore we conclude that the secondary structure elements in solution coincide with those in the crystal structure. 8 α-helices and 6 β-strands were identified: βA (Ile144-Asp151), α1 (Trp159-Val170), βB (Thr180-Tyr187), βC (Pro191-Phe195), α2 (Lys203-Ser211), α3 (Thr223-Tyr233), βD (Thr246-Thr253), α4 (Leu263-His272), βE (Leu276-Val282), α5 (Gly284-Arg288), α6 (Lys294-Lys301), α7 (Thr308-Tyr311), βF (Phe312-Val315), α8 (Glu318-Phe333).

Extent of assignment and data deposition

All amide backbone resonances of integrin α2 I-domain were assigned except for two residues at the N-terminus (G-4, A-3). The assignment comprises 99% of all HN, N, C′, C^{α} and C^{β} resonances covering 194 of the 196 non-proline residues. Chemical shifts have been deposited in the BioMagResBank database under accession number BMRB 5752 (http://www.bmrb.wisc.edu).

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