Dynamic Structural Changes Are Observed upon Collagen and Metal Ion Binding to the Integrin α1 I Domain*

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Background: Integrin α1 I domain undergoes conformational changes upon collagen binding. Results: Deuterium exchange was used to measure the effects of cations, collagen, or an antibody on the α1I solution structure. Conclusion: Full-length collagen and metal ions induce changes that differ in key aspects from previously proposed models for α1I activation. Significance: These studies support a new model for integrin I domain activation.

We have applied hydrogen-deuterium exchange mass spectrometry, in conjunction with differential scanning calorimetry and protein stability analysis, to examine solution dynamics of the integrin α1 I domain induced by the binding of divalent cations, full-length type IV collagen, or a function-blocking monoclonal antibody. These studies revealed features of integrin activation and α1I-ligand complexes that were not detected by static crystallographic data. Mg²⁺ and Mn²⁺ stabilized α1I but differed in their effects on exchange rates in the αC helix. Ca²⁺ impacted α1I conformational dynamics without altering its gross thermal stability. Interaction with collagen affected the exchange rates in just one of three metal ion-dependent adhesion site (MIDAS) loops, suggesting that MIDAS loop 2 plays a primary role in mediating ligand binding. Collagen also induced changes consistent with increased unfolding in both the αC and allosteric C-terminal helices of α1I. The antibody AQC2, which binds to α1I in a ligand-mimetic manner, also reduced exchange in MIDAS loop 2 and increased exchange in αC, but it did not impact the C-terminal region. This is the first study to directly demonstrate the conformational changes induced upon binding of an integrin I domain to a full-length collagen ligand, and it demonstrates the utility of the deuterium exchange mass spectrometry method to study the solution dynamics of integrin/ligand and integrin/metal ion interactions. Based on the ligand and metal ion binding data, we propose a model for collagen-binding integrin activation that explains the differing abilities of Mg²⁺, Mn²⁺, and Ca²⁺ to activate I domain-containing integrins.

Because of their critical role in mediating cell/cell and cell/matrix interactions, integrins are among the most studied families of transmembrane receptors. In particular, great progress has been made in understanding the general structural features of these heterodimeric cell-surface receptors and the conformational changes that regulate affinity modulation, ligand binding, and signaling (1, 2). Crystal structures of both intact integrins and functional domains have been critical to this process. Of particular interest is the α subunit I domain that is primarily responsible for mediating ligand binding by integrins containing this domain. Among others, the co-crystal structures of disulfide-“locked” αI I domains with ICAM-1 (3), ICAM-3 (4), and ICAM-5 (5) and the α2 I domain (α2I) with a collagen triple-helical peptide (THP)⁴ (6) have provided snapshots of the closed (nonligand bound), open (ligand-bound), and intermediate conformations of the ligand-binding regions of these integrins. In many cases, direct structural comparisons between free and ligand-bound integrins (or integrin I domains) cannot be made on the basis of existing data. Even less data are available on the nature of the conformational changes that occur upon integrin-ligand binding in solution. Thus, it is important to develop solution-based methods that allow the direct assessment of the conformational dynamics of integrin/ligand interactions.

In humans, the 18 α and 8 β integrin subunits pair to form at least 24 known heterodimers (7). Nine of the α subunits contain I domains, which play a central role in ligand binding and selectivity. The four collagen-binding receptors (α1β1, α2β1, α10β1, and α11β1) fall into this subset of I domain-containing integrins. Of these, α1β1 (VLA1) and α2β1 (VLA2) have been the most thoroughly studied. The crystal structure of α2I has been determined in the presence of a heterotrimeric THP corresponding to its primary ligand, type I collagen (6). These studies, along with structural studies of the α I domains of the β2

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*The abbreviations used are: THP, collagen triple-helical peptide; VLA1, very late antigen-1 (α1β1 integrin); VLA2, very late antigen-2 (α2β1 integrin); MIDAS, metal ion-dependent adhesion site; DXMS, hydrogen-deuterium exchange mass spectrometry; DSC, differential scanning calorimetry; GdnHCl, guanidine hydrochloride; ND, nondeuterated; D/D, hydrogen-deuterium.

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integrins LFA-1 (4, 5, 8–11), Mac-1 (12, 13), and αXβ2 (14), have helped to establish and refine a model for the ligand-induced conformational changes that lead to “outside-in” signaling in I domain-containing integrins (1). In this model, ligand binding to the divalent cation within the I domain metal ion-dependent adhesion site (MIDAS) induces a shift from the so-called “closed” (unliganded) to the “open” (liganded) conformation and induces allosteric changes in the C-terminal α7 helix of the I domain, triggering a large scale rearrangement of the global topology of the receptor. This model was refined to include the concept of an “intrinsic ligand,” in which a glutamate from the C terminus of the I domain binds to the MIDAS site within a similar domain within the β subunit (the I domain or “I-like” domain) (15, 16).

VLA1 is a major receptor for basement membrane-type IV collagen and is expressed on hematopoietic, neuronal, and mesenchymal cell types (17). Inhibition of VLA1 function, through the use of genetic knock-outs or pharmacological agents, blocks cell retention and activation in pathogenic sites and leads to reduced inflammation and amelioration of disease in a variety of animal models (for examples, see Refs. 18–25). Despite the wealth of biological and pharmacological data on VLA1, limited structural information is available for this integrin. Crystal structures of isolated α1 I domains (α1I) have been reported (26–28), as well as the structure of α1I in complex with the function-blocking antibody AQC2 (29, 30). Although computational models have been proposed for the α1I open conformation (27) and for the α1I/collagen interaction site (31), no structures of α1I in complex with any type of ligand or ligand-mimetic peptide have been reported (2). Recently, the structure of α1I containing an activating mutation (E317A) was determined (32). This protein adopted a novel conformation that differed from both the open and closed structures previously published for other integrins, indicating that the activation mechanism of collagen-binding integrins may differ from that of other integrins.

Peptide amide hydrogen-deuterium exchange mass spectrometry (DXMS) has been used to analyze protein/protein, protein/substrate, protein/inhibitor, protein/solvent, and protein/DNA interactions, as well as protein dynamics and protein conformational changes (33–40). Here, we utilized DXMS to directly study the solution binding and conformational dynamics of the interaction between α1I and full-length type IV collagen. DXMS allowed us to investigate the conformational changes that occur when α1I binds to collagen under conditions closely resembling the native physiological environment and to test the current structural models for integrin I domain/ligand interactions that are largely based on static crystallographic data. We also examined conformational changes induced upon binding of the function-blocking α1 monoclonal antibody AQC2 and compared them with those mediated by the native ligand. Our results reveal important differences from existing models for integrin I domain/ligand interactions and ligand-induced allosteric changes, and they suggest novel dynamic features of the α1I/collagen and α1I/metal ion interactions. The methods we developed to characterize the α1I domain should be easily adapted to the study of other integrins.

**EXPERIMENTAL PROCEDURES**

**Protein Preparation**—These studies utilized a chimeric α1I comprising elements of both the rat and human sequences (Fig. 1). The sequence numbering is based on the full-length mature human α1 integrin sequence (NCBI Reference Sequence NP_852478.1). Four human residues (Val-213, Gln-214, Arg-215, and Arg-218) were substituted into the MIDAS region of the corresponding rat α1I sequence. This form of α1I was used to establish the binding sites for anti-human α1I integrin antibodies using biochemical methods (41) and was crystallized in complex with a Fab fragment of the function-blocking anti-human α1I integrin antibody AQC2 (29, 30). The bacterially expressed chimeric α1I is significantly more soluble than its fully human GST-α1I fusion counterpart, making it more suitable for structural studies. Recombinant α1I was expressed in Escherichia coli as a glutathione S-transferase (GST) fusion protein and was purified as described previously (41). Samples were analyzed using size exclusion chromatography on a Superdex 200 10/300 GL column using phosphate-buffered saline (PBS) as the mobile phase and compared with a reference standard mixture (Bio-Rad). For studies requiring removal of the GST tag, the protein was cleaved using thrombin and purified as described previously (41). The anti-α1β1 monoclonal antibody AQC2 was prepared as described previously (41).

**Ligand Binding Studies**—The binding of GST-α1I to collagen was measured using an electrochemiluminescence assay (42). Type IV collagen from human placenta (Sigma) was coated onto Tosyl M-280 Dynabeads (Dynal, A.S., Oslo, Norway) at a ratio of 20 μg of collagen/mg of beads, as described previously (42). Beads were blocked by incubation with 8% rat plasma in assay buffer (50 mM Hepes, pH 7.5, 150 mM NaCl, 0.1% Triton X-100) for 15 min at 25 °C prior to use. A goat polyclonal antibody against GST (GE Healthcare) was labeled with ruthenium(II) tris(bipyridine)-NHS ester (TAG-NHS Ester; IGEN International, Inc., Gaithersburg, MD) as per the manufacturer’s instructions. Serial dilutions of GST-α1I in assay buffer were incubated with 30 μg/ml labeled anti-GST antibody, 0.2 mg/ml collagen-coated beads, and 0.5 mM MnCl2 in a final volume of 120 μl. After gentle agitation at 25 °C for 60 min, 200 μl of assay buffer was added, and samples were read in an ORIGEN 5.1 detector according to the manufacturer’s instructions (IGEN). To assess the binding ability of AQC2, serial dilutions of AQC2 were incubated with 5 ng/ml GST-α1I, 2 μg/ml labeled anti-GST antibody, 0.2 mg/ml collagen-coated beads, and 1 mM MnCl2 in a final volume of 100 μl and processed as described above.

**Differential Scanning Calorimetry**—DSC was performed using an automated capillary DSC (capDSC, GE Healthcare). Protein and reference solutions were sampled automatically from 96-well plates using the robotic attachment. Prior to each sample measurement, two buffer scans were performed to define the base line for subtraction. All 96-well plates containing protein were stored within the instrument at 5 °C. Each I domain sample was diluted to 1 mg/ml in relevant buffer. Scans were performed from 10 to 100 °C at 2 °C/min using the high feedback mode for enhanced peak resolution. Scans were analyzed using the Origin software package supplied by the manu-
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Hydrogen-Deuterium Exchange Experiments—Samples were prepared with three states of hydrogen-deuterium exchange as follows: nondeuterated (ND), partially deuterated, and equilibrium deuterated. All samples were processed as described above. Equilibrium deuterated samples were prepared by incubating protein at room temperature in D₂O buffer (1% (v/v) formic acid) overnight. Procedures for individual experiments are described below. The hydrogen-deuterium exchange experiments performed with our automated apparatus produces measurements of deuterium incorporation where the standard deviation is less than 2% of the mean of triplicate determinations (49, 50).

α1 I Domain—α1 I domain solutions (8 mg/ml) were mixed with solutions of CaCl₂, MnCl₂, or EDTA to final concentrations of 1, 0.5, or 5 mM, respectively, or with MgCl₂ at either 1 or 10 mM. The solutions were preincubated at room temperature for 10 min and then chilled to 0 °C. The ND sample was processed exactly as described under “Optimization of Fragmentation Conditions.” Hydrogen-deuterium exchange experiments were initiated by mixing 6 μl of preincubated samples with 18 μl of D₂O buffer (8.3 mM Tris, 150 mM NaCl, pH 7.2, in D₂O). The samples with 10 mM Mg²⁺ were incubated at 0 °C for 10, 30, or 100 s or at 25 °C for 30, 100, or 300 s. All other samples were incubated at 0 °C for 10, 30, or 300 s or at 25 °C for 30, 100, 300, 1000, 30,000, 100,000, or 300,000 s. At the indicated times, the H/D exchange was quenched by adding 36 μl of ice-cold quench solution (0.8% formic acid, 16.6% glycerol, and 0.05 mM GdnHCl). The samples were then transferred to ice-cooled autosampler vials, frozen on dry ice, and stored at −80 °C. As shown in previous studies (51–54), incubation at 0 °C for 10, 30, or 100 s is equivalent to incubation at 25 °C for 1, 3, or 10 s, respectively. For all ribbon diagrams, the data generated at 0 °C were converted to the data equivalent at 25 °C.

AQ2-C-a1 I Domain Complex—The complex between antibody AQ2 and α1I was prepared by mixing solutions of α1I (7 mg/ml) and antibody (6.4 mg/ml) at a 2:1 molar ratio at room temperature for 30 min and then chilling to 0 °C. The ND sample was processed by diluting 25 μl of the complex with 75 μl of H₂O Buffer (8.3 mM Tris, 150 mM NaCl, pH 7.2) and then mixed with 100 μl of quench solution (0.8% formic acid, 16.6% glycerol, 0.5 mM GdnHCl). For deuterated samples, 25 μl of preincubated sample was mixed with 75 μl of D₂O buffer and incubated at various time intervals (10, 30, and 100 s at 0 °C and 30, 100, 300, 1000, 3000, 10,000, 30,000, 100,000, or 300,000 s at 25 °C) before being added to 100 μl of quench solution. All samples were frozen on dry ice. Incubation at 0 °C for 10, 30, or 100 s is equivalent to incubation at 25 °C for 1, 3, or 10 s, respectively.

GST-a1 I Domain—For the ND sample, 5 μl of GST-α1I (12.7 mg/ml with 10 mM MgCl₂) was diluted with 64 μl of H₂O buffer and quenched with 96 μl of quench solution (0.8% formic acid, 16.6% glycerol, 0.5 mM GdnHCl). Deuterated samples were prepared by diluting 5 μl of GST-α1I with 11 μl of H₂O buffer, followed by mixing with 48 μl of D₂O buffer. Samples were incubated for 10, 100, and 1000 s at 0 °C and 300, 1000, 3000, 10,000, 30,000, and 100,000 s at 25 °C. At the indicated times, the samples were added to vials containing the 96 μl of quench solution. The samples were transferred to autosampler vials...
and frozen on dry ice. Incubation at 0 °C for 10, 100, or 300 s is equivalent to incubation at 25 °C for 1, 10, or 100 s, respectively.

**GST-α1 I Domain-Collagen Complex**—An on-column exchange process was used to eliminate the interference of collagen peptides during data acquisition. The collagen column was created by the following procedure: 5 mg of collagen (type IV collagen from human placenta, Sigma) was dissolved in 2 ml of 50 mM sodium citrate, pH 4.4. 140 μg of POROS AL 20 media (Applied Biosystems), 330 μl of 1 M sodium cyanoborohydride, and 3.45 ml of 1.5 M sodium sulfate were added, and the reaction was mixed continuously using a tube shaker at 25 °C for 16 h. The beads were washed with 50 mM sodium citrate, pH 4.4, and incubated with 6 ml of 0.1 M ethanolamine, 50 mM sodium citrate, and 330 μl of 1 M sodium cyanoborohydride, pH 4.4, at 25 °C for 2 h. The beads were further washed using H2O Buffer and packed into a 150-μl bed volume column at 10 ml/min with H2O Buffer. For ND samples, 20 μl of 12.7 mg/ml GST-α11 was loaded onto the collagen column following pre-equilibration with H2O Buffer and incubated at 0 °C for 5 min. To initiate the H/D exchange reaction, 500 μl of D2O buffer was loaded onto the column and incubated for various times (10, 100, and 1000 s at 0 °C and 300, 1000, 30,000, and 100,000 s at 25 °C). 500 μl of 1% formic acid was applied to the column to quench the reaction and elute the GST-α11. 150 μl of eluent was added to 12.7 μl of quench solution. Samples were stored at −80 °C prior to analysis. Incubation at 0 °C for 10, 100, or 1000 s is equivalent to incubation at 25 °C for 1, 10, or 100 s, respectively. The collagen column was regenerated between runs by washing it with 1% formic acid until the acid-eluted material measured by absorbance at 214 nm (A214) reached base line, followed by equilibration of the column with neutral pH buffer (8.3 mM Tris, 150 mM NaCl, pH 7.2, with appropriate divalent cations). The efficiency of regeneration was confirmed by observation of complete restoration of ligand binding capacity assessed by both saturable binding capacity and the magnitude of material eluted after binding (measured by A214). In control studies, when the acid-washed, re-equilibrated column was again eluted without protein re-loading, less than 2% of the maximum protein binding capacity of the column was eluted, demonstrating that the column had been cleared of previously bound GST-α11 by the regeneration procedure.

**Calculated**—DXMS Explorer was used for the data processing and reduction of hydrogen-deuterium exchange experiments. Back-exchange occurs immediately after addition of the quench solution, and corrections for it were determined by previously described methods (48) and as shown in Equation 1,

\[
\text{deuteration level (\%)} = \frac{m(P) - m(N)}{m(F) - m(N)} \times 100 \quad \text{(Eq. 1)}
\]

where \(m(P)\), \(m(N)\), and \(m(F)\) are the centroid value of the partially deuterated, ND, and equilibrium deuterated peptide, respectively.

**Generation of Ribbon Diagrams**—The deuteration levels of peptides were further sublocalized using overlapping peptides (44). First, fragments \(f_i\) within each peptide were delineated in a set of overlapping peptides according to overlapping regions. At least five residues were included within each fragment to avoid overinterpreting the experimental data. Second, a variable \(s_i\) was defined to represent the number of deuterium of fragment \(f_i\) values at the on-exchange time point \(t\). Thus, a set of linear equations was established such that the sum of \(s_i\), in a specific peptide was equal to the total number of deuterons observed in DXMS experiments. The best solutions were determined by linear least square fitting, with the following stipulations: (a) that \(s_i = 0\); and (b) for a given fragment \(f_i\), mass shift at the longer on-exchange time point cannot be smaller than the mass shift at the shorter on-exchange time point. These fitted values were colored according to the degree of deuterium exchange (ribbon diagrams).

**Generation of α11-Collagen Model**—A predicted structure of the α11-collagen complex was created by homology modeling with the program MODELLER (55, 56). The crystal structure of the α2 I-domain in complex with collagen (Protein Data Bank code 1DZ1) (6) and a structure of the α1 I domain (Protein Data Bank code 1MHP) (30) were used as templates. Type IV collagen was modeled as a trimeric TSP (31). Restraints for the MIDAS site and the αC helix were taken exclusively from the α2-collagen template structure so that the modeled I domain would remain in the open conformation. The model was refined by simulated annealing under MODELLER spatial restraints from the template structures.

**RESULTS**

**Purity and Bioactivity of α1 Integrin I Domain Proteins**—The α1 I domain was expressed as a GST fusion protein comprising elements of both the rat and human α1I sequences (Fig. 1) (41). To fully characterize the properties of this chimeric form of α1I, both the GST fusion (GST-α11) and α1I generated by cleavage of the GST tag were purified (Fig. 2A). Under reducing conditions, GST-α11 migrated at ∼45 kDa (Fig. 2A, lane 1) and α1I migrated at ∼24 kDa (lane 2), consistent with their predicted molecular masses (51.1 and 24.9 kDa, respectively). Using size-exclusion chromatography, α1I migrated as a monomer (apparent mass ∼20 kDa), whereas GST-α11 eluted with an apparent molecular mass corresponding to a trimer (∼135 kDa), consistent with the native trimeric structure of GST (57). The purified GST-α11 protein bound to type IV collagen with an EC50 of 31 nM (Fig. 2B), and this interaction could be completely blocked by incubation with AQC2 (IC50 = 64 pm, Fig. 2C). The apparent affinity of chimeric GST-α11 for collagen...
Ca\(^{2+}\) does not support \(\alpha 1\beta 1\) collagen binding, and it has been shown to bind with poor (mM) affinity to integrin I domains (61–63). Unlike Mn\(^{2+}\) and Mg\(^{2+}\), Ca\(^{2+}\) did not stabilize \(\alpha 1\) toward thermal denaturation at concentrations up to 100 mM (Fig. 3B). However, at equimolar concentrations (10 mM), Ca\(^{2+}\) completely reversed the stabilizing effect of Mg\(^{2+}\), indicating that Ca\(^{2+}\) is capable of binding to \(\alpha 1\) and displacing Mg\(^{2+}\) from the MIDAS site. Interestingly, in urea denaturation studies, addition of 10 mM Ca\(^{2+}\) increased the urea concentration required for half-maximal denaturation to \(\sim 7\) M, similar to the amount of stabilization observed with Mn\(^{2+}\) (Fig. 3D). Similar results were observed with urea denaturation studies using the rat \(\alpha 1\) I domain (Ref. 41 and data not shown). These results clearly indicate that Ca\(^{2+}\) is capable of binding to the \(\alpha 1\) MIDAS, even though it does not effectively support collagen ligand binding. The difference between the thermal and chemical denaturation results suggests that Ca\(^{2+}\) binding affects the local environment around the MIDAS such that it can be detected by chemically induced unfolding but not by calorimetry, which is a more global measure of stability.

**DXMS of \(\alpha 1\)**—DXMS was utilized to evaluate the structural properties of unliganded \(\alpha 1\) in solution and to provide a reference for subsequent experiments incorporating bound ligands. The DXMS method measures the solvent accessibility of main-chain amides in defined segments of a protein through a combination of time-dependent deuterium exchange, limited proteolysis, and mass spectrometry (33–40). Percent deuteration is operationally defined as the number of deuterium ions incorporated into a given peptide at a fixed time, divided by the maximum level incorporated at equilibrium. The DXMS profile of \(\alpha 1\) in the presence of 10 mM MgCl\(_2\) is shown in Fig. 4. Briefly, a sample of \(\alpha 1\) was diluted 1:3 into a deuterated buffer and incubated at 25 °C for defined time intervals from 1 to 1000 s. The exchange reaction was quenched by lowering the temperature and pH, and samples were digested with pepsin and analyzed using LC-MS. The sequence coverage of \(\alpha 1\) was 93%. The data generated from 77 overlapping peptides was used to gen-
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A, thermal denaturation curves. Purified α1I was incubated for 1 h with either 10 mM EDTA (blue), 10 mM CaCl2 (light green), 1 mM MgCl2 + 1 mM CaCl2 (pink), 10 mM MgCl2 (dark green), or 1 mM MnCl2 (red) and analyzed using DSC. **B**, effect of varying concentrations of MnCl2, MgCl2, or CaCl2 on the melting temperature (Tm) in °C of α1I measured by DSC. The results were plotted as change in Tm (ΔTm) relative to a reference sample containing 10 mM EDTA. **C**, reversal of Mn2+-induced stabilization of α1I by Ca2+, as measured by DSC. The Tm values for samples of α1I in the presence of 10 mM EDTA, 10 mM MgCl2, or 10 mM MnCl2 at 10 mM CaCl2 were determined using DSC. **D**, effect of metal ions on the chemical denaturation of α1I. Purified α1I in buffer containing 10 mM EDTA (circles), 1 mM MnCl2 (triangles), or 10 mM CaCl2 (diamonds) was incubated with urea at the indicated concentrations, and the fraction of unfolded protein was determined by monitoring tryptophan fluorescence (excitation 280 nm and emission 350 nm). Representative data confirmed by three independent experiments are shown.

Effect of Divalent Cations on α1I Domain Solution Structure—We next evaluated the impact of MIDAS site occupancy with different divalent cations on the DXMS exchange properties of α1I. The incorporation of deuterium into α1I was assessed in the presence of 5 mM EDTA (apo-α1I) or in 10 mM Mg2+, 0.5 mM Mn2+, or 1 mM Ca2+ (Fig. 5). Relative to apo-α1I, all three cations significantly decreased the exchange rates in multiple peptides, including residues 157–171, 195–206, and 250–264, as shown in Fig. 5A. No regions showed increased exchange rates in the presence of cation, suggesting an overall decrease in flexibility throughout the α1I structure. Difference maps comparing each of the cation-bound states versus apo-α1I (Fig. 5B) show that, at 100 s, slower exchange rates were observed over the majority of the α1I sequence. Larger decreases in exchange rates were observed with Mg2+ and Mn2+, consistent with the stabilizing effects measured using DSC and tryptophan fluorescence. Ca2+ led to smaller but still significant effects on exchange rates, further demonstrating that Ca2+ is competent to bind the MIDAS site and partially stabilize α1I.

Generally, the exchange rates of α1I in Mg2+ and Mn2+ were very similar (Fig. 5). This was confirmed by generating difference maps comparing these two conditions over a wide range of exchange times (1–300 s) (Fig. 6A). One set of peptides covering the αC helix region, however, exchanged much faster in Mn2+ than in Mg2+. This was exemplified by the 283–290-residue peptide, which incorporated notably higher levels of deuterium at exchange times <100 s in the presence of MnCl2 than it did in MgCl2 (Fig. 6B). This result was observed even when the protein was first treated with EDTA and reconstituted with excess MgCl2 or MnCl2, indicating that the result was not due to the effect of metals introduced during protein production or purification (data not shown). The observed differences in
exchange rates in this region were surprising, because Mg\(^{2+}\) and Mn\(^{2+}\) were shown to bind integrin I domains with similar geometries (11, 62, 64) and showed essentially identical abilities, at saturating concentrations, to stabilize integrin structure (Fig. 3) and promote ligand binding (42, 60). The faster exchange rate in the presence of Mn\(^{2+}\) has led us to generate a new model to explain the differences in the relative abilities of various metal ions to activate integrin I domains, in which...
FIGURE 5. Divalent cations decrease the rate of H/D exchange in α1I. A–C, time courses of deuterium incorporation into peptides 157–171, 195–206, and 250–264, in the presence of 10 mM MgCl₂, 0.5 mM MnCl₂, 1 mM CaCl₂, or 5 mM EDTA. Data are plotted as the number of deuterium ions (#D) incorporated into each peptide at a given time. D, difference map, indicating the change in #D in the presence of each metal ion relative to EDTA, after 100 s of incubation. The peptides described in A–C are indicated with boxes. The threshold for this plot was defined to resolve changes of up to ± 3 deuterium ions/peptide.

FIGURE 6. Mg²⁺ and Mn²⁺ induce different structures in the αC helix. A, difference map depicting deuterium ion incorporation into α1I in the presence of 0.5 mM MnCl₂ or 10 mM MgCl₂. The number of deuterium ions (#D) incorporated into each peptide at a given time was determined, and the value of #D in Mg²⁺ was subtracted from that in Mn²⁺. The threshold for this plot was defined to resolve changes of up to ± 4 deuterium ions/peptide. Residues 283–290, corresponding to one of the peptides that exchanged notably faster in Mn²⁺ than in Mg²⁺, are indicated by a box. B, representative deuterium buildup curve for peptide 283–290 in the αC helix.
Mn\(^{2+}\) preferentially unfolds the αC helix by assuming a pentacoordinated geometry (discussed below). In any case, the ability of Mn\(^{2+}\) and Mg\(^{2+}\) to induce detectably different conformational changes in α1I indicates that, unlike previous suggestions (65), the choice of divalent cation impacts the local structure around the ligand-binding site.

**DXMS Profile of the Complex between α1I and Full-length Collagen**—A crystal structure of α2I in complex with a triple-helical collagen-based peptide, but not a corresponding α1I-collagen peptide structure, has been reported (6). Because intact fibrillar collagen cannot be crystallized, no structure has been reported for full-length collagen bound to an integrin. To explore the nature of these interactions under native conditions, we determined the DXMS exchange pattern of the α1I protein bound to full-length type IV collagen. For these studies, a complex was formed by incubating the GST-α1I fusion protein with collagen IV that was immobilized on a bead-based support in the presence of 10 mM MgCl\(_2\). Deuterium exchange was then performed on the immobilized GST-α1I. The DXMS exchange rates in the presence of collagen were subtracted from those determined for GST-α1I alone to generate a series of difference maps (Fig. 7A). The exchange data at early (1 s) and late (10,000 s) time points were superimposed on a model of α1I bound to collagen IV (Fig. 7B), generated by docking α1I, in an open conformation, with a collagen THP as described previously (31).

Regions at the interface of protein-protein binding surfaces are typically protected from amide exchange with water (66). Using DXMS, only residues in MIDAS loop 2 of α1I, exemplified by peptide 207–221, showed significant protection against deuterium exchange when bound to collagen (Fig. 7A). The structure of the α2I-collagen peptide complex suggested interactions between all three MIDAS loops (151–156, 212–220, and 253–260) and the collagen peptide (6). Similar interactions were proposed for the α1I-collagen IV structure, including stabilizing interactions with collagen involving Arg-218 in loop 2 and Glu-255 in loop 3 (31). The DXMS results indicate that little change in solvent accessibility occurs in either loop 1 or loop 3 upon binding to collagen. A closer examination of the model (Fig. 7B) suggested an explanation for this result. Loops 1 and 2 both make significant contacts with the collagen. Loop 2 has exposed backbone amides, particularly in residues 215–219, which are apparently protected upon collagen binding. Contacts of loop 1 with collagen are mediated mainly by the side chains of Asn-153 and Tyr-156, but the backbone amides of loop 1 are largely buried by folding within α1I itself. As a result, exchange is substantially slower in loop 1 even in the absence of collagen (see Fig. 4). Consequently, it is not surprising that collagen binding has a negligible effect on the loop 1 amides. In contrast, loop 3 has exposed backbone amides, but the model suggests it has very limited contact with collagen, which explains the limited protection observed in loop 3. This result does not support the proposal that Glu-255 forms a salt bridge with collagen (31) but is instead consistent with data showing that the corresponding glutamic acid in α2 (Glu-256) is not critical for ligand binding (67).

Several regions of α1I displayed significantly increased rates of exchange when complexed with collagen (Fig. 7). These increased rates of exchange reflect greater solvent exposure of the affected amide protons and suggest an increase in flexibility in these regions. The changes were clustered in the C-terminal region of the molecule. Residues 283–290, which include the αC helix, displayed very rapid exchange, with essentially complete deuteration incorporation within 1 s of solvent exposure, suggestive of extensive structural changes in this region. Residues 318–332, in the C-terminal α7 helix, and other intervening structural elements between αC and α7 (residues 298–317) also showed notable increases in the rates of deuteration incorporation, although exchange was not as fast as in the αC helix. These results provide direct evidence that α1I undergoes an allosteric conformational change in the C-terminal helix upon ligand binding, as has been postulated for other I domain-containing integrins on the basis of static crystal structures (3, 4, 6, 12). In addition, the striking increase in conformational flexibility of the α7 helix in the presence of ligand provides direct support for the proposal that the I domain C-terminal helix is unusually mobile (5), although this mobility appears to be induced by ligand binding rather than an intrinsic property of the unliganded I domain structure (discussed below).

**Effect of the “Pseudo-ligand Mimetic” Antibody AQC2 on α1I Exchange Rates**—The α1 I domain was previously crystallized with a Fab fragment of the anti-α1β1 function-blocking antibody AQC2 (30). AQC2 can be classified as a pseudo-ligand mimetic antibody, because it binds to α1I in a ligand-mimetic fashion via direct interaction of an antibody aspartate residue with the MIDAS metal ion, but it does not induce a change to the open conformation (30, 68). The AQC2-α1I complex was generated in the presence of Mg\(^{2+}\), and DXMS studies were carried out in a similar manner as described above for the collagen-α1I complex (Fig. 8). A difference map is shown in Fig. 8A, with blue and red regions indicating decreased and increased exchange, respectively, upon antibody binding. The data at 1 and 1000 s were mapped onto the co-crystal structure of the AQC2-α1I complex (Fig. 8B) (30).

The most dramatic decreases in exchange rates were in residues 214–226, corresponding to MIDAS loop 2. This is consistent with the inability of AQC2 to bind to wild-type rat α1I (41), which has four amino acid differences in the 213–218 residue region, and with direct evidence from the crystal structure showing Arg-218 located inside of a cleft within the antibody (30). As in the collagen-α1I complex, no significant effects on MIDAS loops 1 or 3 were observed. The correlation between the collagen-α1I and AQC2-α1I exchange patterns within the MIDAS region indicates that the local interactions of AQC2 with α1I at the MIDAS site are very similar to those of the native ligand. This result is in striking contrast to the crystal structure of AQC2-α1I, which showed interactions of the antibody with all three loops, and suggests that the loop 2 region of α1I is predominantly responsible for the interaction of α1I with the antibody.

Similar to the collagen complex, the exchange rate in the αC helix (residues 283–290) increased upon AQC2 binding, indicating that the antibody induces a significant loss of secondary structure in this region. However, unlike collagen binding, the structural changes in the α7 C-terminal helix (residues 318–332) were modest. The crystal structure of α1I bound to AQC2
Dynamics of Ligand Binding to α1 Integrin I Domain

A

Peptide 207-221

GST-α1

GST-α1 + collagen

Number of deuterons

-3 0 3

B

Peptide 281-290

Peptide 296-318

Peptide 321-332

1s 10,000s
(30) adopted a closed conformation very similar to that of the unbound α1I (26, 28), with no evidence for a shift in the C-terminal helix. Thus, even though AQC2 binding to α1I leads to similar effects as the native ligand within the local region surrounding the MIDAS, it does not induce allosteric changes that would indicate a shift from the closed to the open conformation, characteristic of ligand binding.

**DISCUSSION**

We have successfully used hydrogen-deuterium exchange mass spectrometry (DXMS) to explore the conformational dynamics of cation, ligand, and antibody binding to the I domain of integrin α1 in aqueous solution. Most detailed structural information on integrin I domains is based on crystallographic data, so DXMS provided a unique opportunity to study the solution dynamics of the α1I domain. The DXMS mapping method provided coverage of 93% of the α1I sequence with over 200 exchangeable hydrogens. The average peptide length was 15 amino acids. The observed H/D exchange rates for unliganded α1I were remarkably consistent with the structural elements observed in crystallographic studies of this domain. Regions at the N and C termini of the protein that did not show measurable density in the crystal structures incorporated deuterium rapidly, establishing the limits of the globular domain as comprising residues Asp-144 to Phe-332. Slow exchanging regions throughout the sequence largely correlated with defined secondary structural elements, although the unstructured loop regions, including the MIDAS loops involved in ligand binding, exchanged more rapidly.

In I domain-containing integrins, the I domain MIDAS coordinates a divalent cation such as Mg2+, which interacts directly with an acidic residue (Asp or Glu) in the ligand. Some non-physiological cations, such as Mn2+, are capable of activating integrins much more strongly than Mg2+, whereas Ca2+ is incapable of mediating ligand binding, but the structural basis for this phenomenon is not well understood. Previous studies established that Mg2+ and Mn2+ at millimolar concentrations stabilize the I domain from α1 (41). Using DSC, we observed up to 8–10°C increases in the Tm values for α1I at saturating concentrations of either Mn2+ or Mg2+. This overall stabilization by Mn2+ or Mg2+ was further established using DXMS, which revealed decreased H/D exchange rates throughout the α1I sequence in the presence of these cations.

Interpretation of the role of Ca2+ in integrin/ligand interactions is complicated (69). In α I domains, Ca2+ affinity for MIDAS, and its ability to regulate ligand binding, is both concentration- and activation-state dependent (61, 70, 71). The affinity of the αM I domain for Ca2+ was shown to be low (millimolar) (61–63), and quantum calculations on the αL I domain predicted that the Ca2+-bound state would have lower affinity than either the Mg2+- or Mn2+-bound state for ligand (71). However, some integrin I domains have shown differences in their relative abilities to bind calcium (70, 72), suggesting that the affinity of each I domain MIDAS site for Ca2+ may be different. Interestingly, even though we observed no effect of millimolar levels of Ca2+ by DSC, several other measures provided clear evidence for a stabilizing effect of Ca2+ on α1. DXMS showed decreases in exchange rates throughout the α1I sequence. The concentration of urea required to denature α1I was increased by 2 m in the presence of 10 mM Ca2+, which was comparable in magnitude to the effect of Mn2+. Finally, Ca2+ at an equimolar concentration reversed the stabilizing effect of 10 mM Mg2+ ions. Thus, our results demonstrate that Ca2+ is capable of binding to the α1I MIDAS and that even though Ca2+ binding does not stabilize the I domain to thermal denaturation or support ligand binding, it significantly impacts the solution dynamics of the protein.

The DXMS patterns of α1I in Mg2+, Ca2+, and Mn2+ were very similar, except for a striking difference in the αC helix, a structural element unique to collagen-binding integrins that is believed to be critical for binding and collagen subtype selectivity (6, 59, 73). Relative to apo-α1I, saturating concentrations of Mg2+ stabilized the αC helix toward deuterium exchange to a much greater extent than either Mn2+ or Ca2+. This result was surprising, because both Mg2+ and Mn2+ support binding of collagen IV to α1β1 at these concentrations (42, 58), and crystallographic data on integrin I domains support the concept that Mg2+- and Mn2+-bound I domains form essentially identical structures (65). These data, in conjunction with the recently reported crystal structure of α1I with the activating mutation E317A (32), led us to propose a model for collagen-binding integrin I domain activation that provides a structural basis for the enhanced activating ability of Mn2+ and the inability of Ca2+ to mediate ligand binding (Fig. 9). The α1I(E317A) structure, which was the first to display an unwound αC helix in an I domain in the absence of a bound collagen peptide, contained a unique penta-coordinated Mg2+ in the MIDAS, suggesting that a change from penta- to hexa-coordinated metal ion upon ligand binding drives allosteric conformational changes within α1I. In our model, the closed conformation, with a hexa-coordinate metal ion and tightly wound αC helix, is in equilibrium with the activated conformation, containing a penta-coordinate metal and unwind αC helix. Collagen ligand binding leads to conversion back to the more stable hexa-coordinate geometry and induces allosteric changes in the C-terminal helices. Importantly, Mg2+ ions pay a significant energetic penalty for deviating from a hexacoordinate (octahedral) geometry, whereas Mn2+ is capable of adopting lower coordination states (32). Thus, Mn2+, with a lower energetic barrier to adopting the activated conformation, is more effective at driving the opening of the αC helix and enabling ligand binding. Ca2+, which is unable to adopt a pentavalent conformation, is incapable of mediating ligand binding.

**FIGURE 7. Effect of collagen binding on GST-α1I domain hydrogen-deuterium exchange rates.** A, difference map indicating exchange rates in free versus collagen-bound GST-α1I in the presence of 10 mM MgCl2. Blue regions indicate decreased exchange (#D), and red regions indicate increased exchange, in the presence of collagen. The threshold for this plot was defined to resolve changes of up to ±3 deuterium ions/peptide. Exchange curves for representative peptides 207–221, 281–290, 296–318, and 321–332 are shown and indicated with boxes on the map. B, exchange rate differences at 1 and 10,000 s were mapped onto a model of the α1I-collagen complex. The THP used to generate the model is shown in cyan (at top of figure). Color coding on α1I is identical to that shown in A.
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A

B
The observation of such striking metal ion-mediated differences in the αC helix conformation demonstrates the importance of studying the dynamics of integrins in solution as a complement to crystallographic studies. Because there are no crystal structures of free (not bound to ligand or antibody) α1 or α2 I domains in Mn²⁺, we cannot rule out the possibility that Mg²⁺ and Mn²⁺ would produce different conformations of αC even in a crystalline state. More likely, however, this difference is only detectable in solution, where crystal packing effects are eliminated. The concept that Mg²⁺ and Mn²⁺ induce different conformations of αC in solution is supported by tryptophan fluorescence studies on α1I, which demonstrated that binding of Mg²⁺ led to conformational changes in the vicinity of Trp-158, which is located at the end of MIDAS loop 1, very near the αC helix in the three-dimensional structure of α1I, and that these changes differed from the effects of added Mn²⁺ (72).

DXMS provided an ideal tool to characterize the conformational changes in α1I induced upon collagen binding and to test the existing models for integrin I domain/ligand interactions. The DXMS pattern of α1I bound to collagen showed significantly reduced exchange only in MIDAS loop 2, suggesting that this region of α1I integrin is a critical element mediating the interaction with collagen. Collagen also induced faster exchange in the αC, α6, and α7 helices, consistent with models in which ligand binding causes a downward shift of the C-terminal α7 helix of the I domain, leading to engagement of Glu-335 (in the case of α1 integrin) with the MIDAS site of the β subunit I-like domain (1). It has been proposed that the α7 helix is intrinsically mobile, allowing it to trigger this process with minimal energetic penalty (5, 10). Our DXMS data on α1I indicate that the flexibility of α7 is greatly increased upon ligand binding. We therefore propose a modification to this model in which ligand binding is responsible for relaxing the α7 helical structure and facilitating the conformational changes necessary for effective intersubunit communication, e.g. in outside-in signaling.

One of the key differences between the studies reported here and those previously described is the use of full-length collagen instead of a THP fragment. Solution binding studies on α2 I domain binding to a collagen THP have been reported using NMR (74). A number of residues located near the α7 helix showed changes in chemical shift pattern, although only one residue (Gly-329, equivalent to Gly-328 in α1I) within α7 itself was noted. The relatively small number of chemical shift changes observed in this region compared with our observations using DXMS on α1I-collagen could be due to limitations of the NMR method, such as the inherently reduced peak intensity observed upon peptide binding. Alternatively, the use of a THP instead of a full-length collagen ligand may have led to less robust conformational changes in the integrin. It is important to note that a single molecule of collagen contains multiple integrin-binding sites with differing affinities (75). This has the
potential to lead to dispersion in the DXMS signal. It is unclear whether different residues on α11 are involved in binding to different collagen GXGXR motifs. If so, one could imagine that DXMS studies using α11 bound to individual THP units might be informative.

In contrast to collagen, binding of the function-blocking α1 antibody AQC2 led to much more limited changes in the DXMS pattern of α11. In the crystal structure of the AQC2-α11 complex, Asp-101 from the antibody heavy chain bound directly to the metal ion in the α11 MIDAS, but the remainder of the I domain, including the C terminus, retained the closed conformation (30). We found that AQC2 binding selectively protected MIDAS loop 2, the same region protected from exchange by collagen. The similarity of AQC2 and collagen in their interactions with the metal ion and MIDAS loop 2, coupled with the stark differences in their abilities to transmit allosteric changes to the C terminus of α11, is remarkable.

Two other examples of ligand-mimetic I domain antibodies have been reported, both of which also present Asp residues that interact with the MIDAS metal ion. One of these is the α1 antibody AL-57 (76). Unlike AQC2, AL-57 binds to the open form of αL (68). It was suggested that a key salt bridge formed with Glu-241 of αL by either the native ligand ICAM-1 or AL-57 is necessary for the allosteric rearrangement to occur and that the reason AQC2 binds to the open form of α11 is that it does not form a similar bridge with the corresponding residue in α11, Glu-255 (68). Unlike in the αL-ICAM interaction, this conserved glutamic acid (Glu-255 in αL or Glu-256 in α2) is not critical for collagen binding by integrins (6, 67). Nonetheless, the finding that collagen but not AQC2 can induce long range conformational changes in α11 is consistent with the conclusion that AL-57 is a true ligand mimic, whereas AQC2 co-opts a key element of the ligand-binding site (i.e. the MIDAS cation) without engaging other regions necessary for induction of signaling. One would predict that solution DXMS studies using AL-57 and the αL I domain should demonstrate pronounced antibody-induced conformational changes in the α7 helix. A true ligand-mimetic antibody would be less desirable as a therapeutic, however, because it could lead to agonistic effects through outside-in integrin signaling. Another related antibody, mAb107, preferentially binds to the αM I domain in Ca²⁺⁺ via a bidentate interaction with Asp-107 (61, 77). Similarly to AQC2, ligation of mAb107 did not induce conversion to the open conformation. Interestingly, the bidentate mode of binding led to a heptavalent Ca²⁺⁺ ion in the mAb107-I domain complex, highlighting the range of different geometries accessible by the MIDAS.

In summary, we have explored the dynamic conformational changes in the α1 integrin I domain induced by divalent cations and by ligand binding, and we tested several elements of the existing models for collagen-induced conformational changes and allosteric regulation, using DXMS. The data validated some elements of the models derived from crystallographic studies but importantly identified novel features of ligand and metal ion binding that were not detected using static methods. The application of DXMS to other integrin/ligand interactions should be very powerful, as it provides a method to explore the interaction of isolated I domains or even complete integrin heterodimers with native ligands, and it does not require the use of either conformationally restricted integrins (e.g. locked-open I domains) or engineered fragments of ligands (e.g. collagen THPs or RGD peptides).

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REFERENCES

1. Luo, B. H., Carman, C. V., and Springer, T. A. (2007) Structural basis of integrin regulation and signaling. Annu. Rev. Immunol. 25, 619–647
2. Takaqi, J. (2007) Structural basis for ligand recognition by integrins. Curr. Opin. Cell Biol. 19, 557–564
3. Shimaoa, M., Xiao, T., Liu, J. H., Yang, Y., Dong, Y., Jun, C. D., McCormack, A., Zhang, R., Joachimiak, A., Takagi, J., Wang, J. H., and Springer, T. A. (2003) Structures of the αL I domain and its complex with ICAM-1 reveal a shape-shifting pathway for integrin regulation. Cell 112, 99–111
4. Song, G., Yang, Y., Liu, J. H., Casasnovas, J. M., Shimaoa, M., Springer, T. A., and Wang, J. H. (2005) An atomic resolution view of ICAM recognition in a complex between the binding domains of ICAM-3 and integrin αLβ2. Proc. Natl. Acad. Sci. U.S.A. 102, 3366–3371
5. Zhang, H., Casasnovas, J. M., Jin, M., Liu, J. H., Gahmberg, C. G., Springer, T. A., and Wang, J. H. (2008) An unusual allosteric mobility of the C-terminal helix of a high affinity αL integrin I domain variant bound to ICAM-5. Mol. Cell 31, 432–437
6. Emsley, J., Knight, C. G., Farndale, R. W., Barnes, M. J., and Liddington, R. C. (2000) Structural basis of collagen recognition by integrin α2β1. Cell 101, 47–56
7. Hynes, R. O. (2002) Integrins. Bidirectional, allosteric signaling machines. Cell 110, 673–687
8. Huth, I. R., Olejniczak, E. T., Mendoza, R., Liang, H., Harris, E. A., Lusher, M. L., Jr., Wilson, A. E., Fesik, S. W., and Staunton, D. E. (2000) NMR and mutagenesis evidence for an I domain allosteric site that regulates lymphocyte function-associated antigen 1 ligand binding. Proc. Natl. Acad. Sci. U.S.A. 97, 5231–5236
9. Shimaoa, M., Salas, A., Yang, W., Weitz-Schmidt, G., and Springer, T. A. (2003) Small molecule integrin antagonists that bind to the β2 subunit I-like domain and activate signals in one direction and block them in the other. Immunity 19, 391–402
10. Legge, G. B., Kriwacki, R. W., Chung, J., Hommel, U., Ramage, P., Case, D. A., Dyson, H. J., and Wright, P. E. (2000) NMR solution structure of the inserted domain of human leukocyte function-associated antigen 1 ligand binding. J. Mol. Biol. 295, 1251–1264
11. Qu, A., and Leahy, D. J. (1996) The role of the divalent cation in the structure of the I domain from the CD11a/CD18 integrin. Structure 4, 931–942
12. Lee, J. O., Rieu, P., Arnaout, M. A., and Liddington, R. (1995) Crystal structure of the α1 domain, complement receptor type 4. EMBO J. 29, 666–679
13. Alonso, J. L., Essafi, M., Xiong, J. P., Stehle, T., and Arnaout, M. A. (2002) Does the integrin αα domain act as a ligand for its βA domain? Curr. Biol. 12, R340–R342
14. Li, R., Rieu, P., Griffith, D. L., Scott, D., and Arnaout, M. A. (1998) Two functional states of the CD11b A-domain. Correlations with key features of two Mn²⁺-complexed crystal structures. J. Cell Biol. 145, 1523–1534
15. Xie, C., Zhu, J., Chen, X., Mi, L., Nishida, N., and Springer, T. A. (2010) Structure of an integrin with an αδ domain, complement receptor type 4. EMBO J. 29, 666–679
16. Duband, J. L., Belkin, A. M., Syfrig, J., Thiery, J. P., and Koteliansky, V. E. (1992) Expression of α1 integrin, a laminin-collagen receptor, during...
myogenesis and neurogenesis in the avian embryo. *Development* **116**, 585–600.
25. Abraham, W. M., Ahmed, A., Serebiakov, I., Carmillo, A. N., Ferrant, J., de Fougerolles, A. R., Garber, E. A., Gotwals, P. J., Koteliansky, V. E., Taylor, F., and Lobb, R. R. (2004) A monoclonal antibody to α1β1 blocks antigen-induced airway responses in sheep. *Am. J. Respir. Crit. Care Med.* **169**, 97–104.
26. Chen, L., Huq, S., Gardner, H., de Fougerolles, A. R., Barabino, S., and Taylor, F., and Lobb, R. R. (2004) A monoclonal antibody modulates the development of secondary lymphoid tissue mediates opening of the lid region in group IVA cytosolic phospholipase A2. *J. Biol. Chem.* **279**, 31227–31236.
27. Burns, J. E., Huq, S., Mayer, D., Taylor, F., and Lobb, R. R. (2004) A monoclonal antibody to c- Rel inhibits inflammation in experimental colitis. *J. Clin. Invest.* **114**, 749–759.
28. Burns-Hamuro, L. L., Hamuro, Y., Kim, J. S., Sigala, P., Fayos, R., Stranz, D. D., Jennings, P. A., Taylor, S. S., and Woods, V. L., Jr. (2005) Distinct interaction modes of an AKAP bound to two regulatory subunit isoforms of protein kinase A revealed by amide hydrogen/deuterium exchange. *Protein Sci.* **14**, 2982–2992.
29. Camilleri, G. A., Boriack-Sjodin, P. A., Eldredge, J., Fitch, C., Friedman, B., Krieglstein, C. F., Cerwinka, W. H., Sprague, A. G., Doherty, P. C., de Fougerolles, A. R., and Topham, D. J. (2002) Collagen-binding integrin α1β1 regulates intestinal inflammation in experimental colitis. *J. Clin. Invest.* **110**, 1773–1782.
30. Chang, M. H., Kato, T., and Sherman, W.,(2005) Divalent cations stabilize the α1β1 integrin I domain. *Biochemistry* **38**, 8280–8288.
31. Chang, Y. H., Matsuura, T., and Fredman, P. (2005) Resolution of the α1β1 integrin intermediate structure using amide hydrogen/deuterium exchange mass spectrometry. *J. Biol. Chem.* **280**, 340–345.
32. Chang, M. H., Kato, T., and Sherman, W.,(2005) Divalent cations stabilize the α1β1 integrin I domain. *Biochemistry* **38**, 8280–8288.
33. Chang, Y. H., Matsuura, T., and Fredman, P. (2005) Resolution of the α1β1 integrin intermediate structure using amide hydrogen/deuterium exchange mass spectrometry. *J. Biol. Chem.* **280**, 340–345.
34. Chang, M. H., Kato, T., and Sherman, W.,(2005) Divalent cations stabilize the α1β1 integrin I domain. *Biochemistry* **38**, 8280–8288.
35. Chang, Y. H., Matsuura, T., and Fredman, P. (2005) Resolution of the α1β1 integrin intermediate structure using amide hydrogen/deuterium exchange mass spectrometry. *J. Biol. Chem.* **280**, 340–345.
36. Chang, M. H., Kato, T., and Sherman, W.,(2005) Divalent cations stabilize the α1β1 integrin I domain. *Biochemistry* **38**, 8280–8288.
37. Chang, Y. H., Matsuura, T., and Fredman, P. (2005) Resolution of the α1β1 integrin intermediate structure using amide hydrogen/deuterium exchange mass spectrometry. *J. Biol. Chem.* **280**, 340–345.
38. Chang, M. H., Kato, T., and Sherman, W.,(2005) Divalent cations stabilize the α1β1 integrin I domain. *Biochemistry* **38**, 8280–8288.
Dynamics of Ligand Binding to α1 Integrin I Domain

Kliman, J. P. (2010) Temperature dependence of protein motions in a thermophilic dihydrofolate reductase and its relationship to catalytic efficiency. Proc. Natl. Acad. Sci. U.S.A. 107, 10074–10079

Wilderman, P. R., Shah, M. B., Liu, T., Li, S., Hsu, S., Roberts, A. G., Goodlett, D. R., Zhang, Q., Woods, V. L., Jr., Stout, C. D., and Halpert, J. R. (2010) Plasticity of cytochrome P450 2B4 as investigated by hydrogen-deuterium exchange mass spectrometry and x-ray crystallography. J. Biol. Chem. 285, 38602–38611

Sali, A., and Blundell, T. L. (1993) Comparative protein modeling by satisfaction of spatial restraints. J. Mol. Biol. 234, 779–815

Eswar, N., Webb, B., Marti-Renom, M. A., Madhusudhan, M. S., Eramian, D., Shen, M. Y., Pieper, U., and Sali, A. (2007) in Current Protocols in Protein Science (Colligan, J. E., Dunn, B. M., Speicher, D. W., and Wingfield, P. T., eds) pp. 2.9.1–2.9.31, Wiley Interscience, Hoboken, NJ

Chasseaud, L. F. (1979) The role of glutathione and glutathione S-transferases in the metabolism of chemical carcinogens and other electrophilic agents. Adv. Cancer Res. 29, 175–274

Ayrolle, T., Sugimori, T., Goldmann, W. H., Fatallah, D. M., Xiong, J. P., and Arnaout, M. A. (2004) Binding affinity of metal ions to the CD11b I domain of integrin α4β7. J. Biol. Chem. 279, 25483–25488

Ajroyd, K., Milner, A. M., Moon, J. B., Mott, J. E., Forty, D. J., and Teisinger, J. (1999) Plasticity of cytochrome P450 2B4 as investigated by hydrogen-deuterium exchange mass spectrometry and x-ray crystallography. J. Biol. Chem. 285, 38602–38611

Lambert, L. J., Bobkowski, A. A., Smith, J. W., and Marassi, F. M. (2008) Competitive interactions of collagen and jararhagin-derived disintegrin peptide with the integrin α2β1 domain. J. Biol. Chem. 283, 16665–16672

Siljander, P. R., Hamaia, S., Peachey, A. R., Slatter, D. A., Smethurst, P. A., Ouwendag, W. H., Knight, C. G., and Farndale, R. W. (2004) Integrin activation state determines selectivity for novel recognition sites in fibrillar collagens. J. Biol. Chem. 279, 47763–47772

Shimaoka, M., Kim, M., Cohen, E. H., Yang, W., Astrof, N., Peer, D., Salas, A., Ferrand, A., and Springer, T. A. (2006) AL-57, a ligand-mimetic antibody to integrin LFA-1, reveals chemokine-induced affinity up-regulation in lymphocytes. Proc. Natl. Acad. Sci. U.S.A. 103, 13991–13996

Mahalingam, B., Ajroyd, K., Alonso, J. L., Anand, S., Adams, B. D., Horeinstein, A. L., Malavasi, F., Xiong, J. P., and Arnaout, M. A. (2011) Stable coordination of the inhibitory Ca2+ ion at the metal ion-dependent adhesion site in integrin CD11b/CD18 by an antibody-derived ligand aspartate. Implications for integrin regulation and structure-based drug design. J. Immunol. 187, 6393–6401