Structural Analysis of the \( \alpha_2 \) Integrin I Domain/Procollagenase-1 (Matrix Metalloproteinase-1) Interaction*

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Previous studies have established that ligation of keratinocyte \( \alpha_2 \beta_1 \) integrin by type I collagen induces expression of matrix metalloproteinase-1 (MMP-1) and that MMP-1 activity is required for the \( \alpha_2 \beta_1 \) integrin-dependent migration of primary keratinocytes across collagenous matrices. We now present evidence that MMP-1 binds the \( \alpha_2 \beta_1 \) integrin via the I domain of the \( \alpha_2 \) integrin subunit. Using an enzyme-linked immunosorbent assay with purified human MMP-1 and recombinant \( \alpha_2 \) integrin I domain, we showed that the \( \alpha_2 \) integrin I domain specifically bound in a divalent cation-dependent manner to both the pro and active forms of MMP-1, but not to MMP-3 or MMP-13. Although both the I domain and MMP-1 bound divalent cations, MMP-1 bound, in a divalent cation-dependent manner, to \( \alpha_2 \) integrin I domains containing metal ion-dependent adhesion sites motif mutations that prevent divalent cation binding to the I domain, demonstrating that the metal ion dependence is a function of MMP-1. Using a series of MMP-1-MMP-3 and MMP-1-MMP-13 chimeras, we determined that both the linker domain and the hemopexin-like domain of MMP-1 were required for optimal binding to the I domain. The \( \alpha_2 \) integrin/MMP-1 interaction described here extends an emerging paradigm in matrix biology involving anchoring of proteinases to the cell surface to regulate their biological activities.

The extracellular matrix is not a static environment. Remodeling and degradation of the extracellular matrix is a vital component of physiological and pathophysiologically processes, such as development and differentiation, cell migration, angiogenesis, wound healing, and metastasis. Matrix metalloproteinases (MMPs)1 play a central role in the turnover of extracellular matrix components. MMPs constitute a large family of metal-dependent endopeptidases with varying substrate specificities for many extracellular proteins. The structure of native triple helical type I collagen makes it resistant to proteolysis, and only six MMPs, MMP-1, MMP-8, MMP-13, MMP-14 (MT1-MMP), MMP-18, and MMP-2, exhibit an ability to cleave native fibrillar collagen within its triple helical domain (2–8). Similar to most MMPs, the collagenses (MMP-1, MMP-8, and MMP-13) have several structural features in common, including an N-terminal prodomain, a catalytic domain, and a short proline-rich linker connected to a hemopexin-like domain at the C terminus (9). The catalytic domain contains a Zn\(^{2+}\)-binding site that is conserved in all MMPs and is required for catalytic activity (10, 11). The catalytic domain of the collagenses contains an additional structural Zn\(^{2+}\), as well as three structural Ca\(^{2+}\) ions (12). The hemopexin-like domain contains a Ca\(^{2+}\) and a Ca\(^{2+}\)–Cl\(^{-}\) ion pair (12).

The three collagenses differ in patterns of tissue expression. In humans, MMP-1, which is expressed by epithelium, endothelium, fibroblasts, chondrocytes, and macrophages, seems to be the enzyme principally responsible for collagen turnover in most tissues (13–18). During cutaneous wound healing, human keratinocytes express MMP-1 while migrating over the type I collagen-rich dermis. This spatially and temporally confined expression of MMP-1 is induced by ligation of the \( \alpha_2 \beta_1 \) integrin by dermal type I collagen (19). Both migration and MMP-1 expression induced by type I collagen are inhibited by function blocking antibodies against the \( \alpha_2 \beta_1 \) integrin, but not by antibodies directed against the \( \alpha_1 \beta_1 \) or \( \alpha_3 \beta_1 \) integrins (19).

Previous studies have established the role of the \( \alpha_2 \beta_1 \) integrin as a receptor for type I collagen, as well as for type IV collagen, laminins, echovirus 1 and 8, and rotaviruses (20–22).

Multiple lines of evidence have revealed that the I domain of the \( \alpha_2 \) integrin subunit mediates the binding of the \( \alpha_2 \beta_1 \) integrin to its ligands (20). For example, the \( \alpha_2 \beta_1 \) integrin-binding site for collagen and the epitopes recognized by antibodies that block type I collagen binding to the integrin map to the I domain of the \( \alpha_2 \) integrin subunit (20). In addition, recombinant \( \alpha_2 \) integrin I domain specifically binds all known ligands of the \( \alpha_2 \beta_1 \) integrin, including collagens, laminin, the C-terminal propeptide of type I collagen, and echovirus 1 and 8 (20, 22).

In the accompanying paper (23), we demonstrated that, in addition to inducing expression of MMP-1, the \( \alpha_2 \beta_1 \) integrin also directly binds MMP-1. In this paper, we show that MMP-1 binds to the I domain of the \( \alpha_2 \) integrin subunit, and we define the structural basis of the interaction.

**EXPERIMENTAL PROCEDURES**

Cloning and Expression of the \( \alpha_2 \) Integrin I Domain—The cloning and expression of the \( \alpha_2 \) integrin subunit I domain has been described elsewhere (24). Briefly, the cDNA of full-length \( \alpha_2 \) integrin was used as a template in the polymerase chain reaction to produce a fragment that encodes Ser\(^{124}\) through Met\(^{349}\) of the published \( \alpha_2 \) sequence (25). The PCR primers were designed such that the amplification product would contain a BglII site at the 5’-end and a XhoI site at the 3’-end. The
products were digested, purified, by agarose gel electrophoresis, and ligated into a BgII-XhoI-digested pGEX-5x-1 vector (Amersham Pharmacia Biotech), which is a glutathione S-transferase fusion protein expression vector. The cDNA of the full-length α1 integrin (Dr. Eugene M. Marcatonio, Columbia University) was used as a template to amplify the I domain. As with the β1 integrin, this cDNA fragment, Ser superscript 1 through Met superscript 149 of the published α1 sequence (26). Like the α2 integrin I domain, the PCR primers were designed with a 5’ BgII site and a 3’ XhoI site, and the resulting product was cloned into the pGEX-5x-1 vector.

Expression and Purification of the GST-I Domain Fusion Proteins—The GST-α1, α2, and α3 integrin I domains have been described elsewhere (24). Briefly, DH5α Escherichia coli containing the appropriate plasmid were grown at 37 °C in 500 ml of 2 x yeast extract/tryptone buffer until A500 reached 0.3–0.4. The cultures were induced with 1 mM isopropyl-β-D-thiogalactopyranoside and returned to the incubator for 3 h. The cells were recovered by centrifugation at 2,600 × g for 10 min, washed twice with 10 ml of ice-cold phosphate-buffered saline, and stored at −70 °C until needed. The GST-I fusion proteins were expressed, purified, and characterized exactly as recently described (24).

Construction of the MIDAS Motif Mutant—The glutamate at position 254 or 151 of the α1 integrin I domain sequence was replaced with an alanine in a single mutagenesis reaction using pBluescript/Iα1. The 969-base pair GST BI-XhoI fragment of pBluescript and the 4669-base pair XhoI/XhoI fragment of pGEX-5x-1α1 were isolated and ligated to create pGEX-5x-1/D151A or D254A. The sequences of the cDNAs used in this study, including all of the chimeras, were determined using the BigDye terminator cycle sequencing method (PerkinElmer Applied Biosystems, Foster City, CA) and were compared with the published α2 sequence (25, 26).

Purification and Activation of MMP-1—Human pro-MMP-1 was purified by two-step chromatography from conditioned medium of interleukin-1-treated dermal fibroblasts cultured as described (27). To isolate prodomain and activated MMP-1, autoactivation of pro-MMP-1 was induced with 1 mM 4-aminophenylmercuric acetate (Sigma) for 1 h at room temperature. The wells were again washed three times, and 0.1 ml of the appropriate wash buffer, and 0.1 ml of a 1:8000 dilution of anti-GST antibody in the appropriate wash buffer was added for 1 h at room temperature. Again the wells were washed three times. The plates were read at 450 nm.

RESULTS

In our accompanying paper (23), we used co-immunoprecipitation and platelet adhesion to demonstrate that MMP-1 binds to the α2β1 integrin. The 226-amino acid I domain of the α2 subunit mediates binding of all identified ligands of the α2β1 integrin. As a GST fusion protein, recombinant I domain provides a reliable reporter in a solid phase binding assay. Wells were coated with collagen or with pro or activated forms of MMP-1, MMP-3, or MMP-9, enzymes that are expressed by keratinocytes in wounded skin (13, 29). In the presence of Mn superscript 2+, which shifts α2β1 to a more active state (20), soluble I domain bound to both pro and active MMP-1 (Fig. 1). The level of binding to MMP-1 was equivalent to that detected for collagen, used as a positive control. Of note, we found that the I domain still bound appreciable levels of pro and active MMP-1 in the presence of EDTA, which abolished binding to collagen (Fig. 1).

Next, we sought to establish whether the pro or active forms of MMP-1 were ligands for the α2 integrin I domain and whether the prodomain bound directly. Fig. 2A demonstrates that the α2 integrin I domain binds to both pro-MMP-1 and activated MMP-1 in a concentration-dependent manner. No binding to BSA was observed. To determine whether the slight difference observed between the binding of α2 integrin I domain to pro-MMP-1 and active MMP-1 was a result of a binding interaction between the I domain and the propeptide, we also investigated the binding of I domain to the propeptide of MMP-1. Although activated MMP-1, which lacks the propep-
tide, bound to of the α2 integrin I domain, the purified propeptide failed to bind (Fig. 2B). Thus the propeptide of MMP-1 is not directly involved in the interaction between the α2 integrin I domain and pro-MMP-1. This conclusion is further supported by the data from experiments with MMP chimeras shown in Fig. 6. The small difference observed between the binding of pro-MMP-1 and activated MMP-1 is most likely due to differences in coating efficiency, because the difference is in the amount of α2 integrin I domain bound at saturation. Additionally, the binding data were best described by a single binding site with half-maximal binding for both active and proMMP-1 occurring between 10 and 40 nM, depending upon the experiment. There was no significant difference between the half-maximal binding of α2 integrin I domain to pro or active MMP-1. These data indicate that both the pro and active forms of MMP-1 are ligands for the α2 integrin I domain, but, as suggested in our accompanying paper (23), the physiologic ligand may be limited to pro-MMP-1.

To further characterize MMP-1 binding to the α2 integrin I domain, we studied the divalent cation dependence of this interaction. The different ligands for the α2β1 integrin differ in their dependence upon divalent cations for binding (20). Both Mn²⁺ and Mg²⁺ support I domain binding to collagen, whereas Ca²⁺ does not (20). Predictably, EDTA completely inhibits the binding of collagens to the I domain (20). On the other hand, binding of echovirus 1 to the α2 integrin I domain is divalent cation-independent (30). The metal dependence of I domain interaction with ligands is mediated by the divalent cation-binding site called the MIDAS motif (31). The catalytic and hemopexin domains of MMP-1 also bind divalent cations (12). Because both MMP-1 and the α2 integrin I domain contain divalent cation-binding sites, any divalent cation dependence of MMP-1 binding to the α2 integrin I domain could arise from either or both the MMP and the I domain. Fig. 3 demonstrates that both 2 mM Mn²⁺ and 2 mM Mg²⁺ support binding of α2 integrin I domain to pro-MMP-1 and to collagen. As shown previously (24), 2 mM EDTA abolishes the binding of the α2 integrin I domain to type I collagen; however, a significant component (about 50–60%) of the binding of α2 integrin I domain to pro-MMP-1 was retained in 2 mM EDTA, indicating a fundamental difference in the binding of the α2 integrin I domain to MMP-1 as compared with collagen type I. These results suggest that there are two components to the α2 integrin I domain MMP-1 interaction: a metal-dependent component and a metal-independent component. As expected, 2 mM
Ca\(^{2+}\) did not support binding of the \(\alpha_2\) integrin I domain to collagen. In contrast, 2 mM Ca\(^{2+}\) effectively supported binding of the \(\alpha_2\) integrin I domain to pro-MMP-1. Because Ca\(^{2+}\) does not bind to the I domain MIDAS motif (24), the metal enhancement of I domain binding to MMP-1 observed in the presence of Ca\(^{2+}\) and the significant difference in metal cation dependence between the \(\alpha_2\) integrin I domain-collagen interaction and the \(\alpha_2\) integrin I domain/MMP-1 interaction suggest that the metal dependence of the MMP-1/\(\alpha_2\) integrin I domain interaction may arise from the MMP-1 rather than from the I domain. These results indicate that there are critical differences between the binding of \(\alpha_2\) integrin I domain to collagen as opposed to pro-MMP-1.

To investigate further the metal dependence of the MMP-1/\(\alpha_2\) integrin I domain interaction, we utilized I domains with either of two point mutations in the MIDAS motif of the \(\alpha_2\) integrin I domain. D256A and D151A both result in loss of essential metal coordinating side chains within the MIDAS motif of the \(\alpha_2\) integrin I domain (31). As expected, these mutants do not bind collagen in either the presence or absence of divalent cations (Fig. 4A). However, the mutant I domains retained the ability to bind to pro-MMP-1 in the presence of either Ca\(^{2+}\) or Mn\(^{2+}\). Divalent cation-dependent binding of the MIDAS motif mutants to pro-MMP-1 was comparable with that of wild-type I domain, even though the divalent cation chelation site of the I domain had been disrupted (Fig. 4B). Both the wild-type and mutant \(\alpha_2\) integrin I domains bound to pro-MMP-1 to a lesser extent in the presence of EDTA, again indicating both a metal-dependent, as well as a metal-independent, component of binding. These results further demonstrate that the divalent cation requirement for the optimal binding of MMP-1 by the \(\alpha_2\) integrin I domain derives from the MMP and not the I domain.

Like the \(\alpha_\beta_1\) integrin, the \(\alpha_\beta_1\) integrin is a cell surface collagen receptor that contains a structurally similar I domain (20). As with the \(\alpha_2\) I domain, recombinantly expressed \(\alpha_2\) I domain recapitulates the binding properties of the full-length \(\alpha_\beta_1\) integrin (20). Therefore, we also tested the binding of the \(\alpha_2\) integrin I domain to pro-MMP-1. As observed with the \(\alpha_2\) integrin I domain, Ca\(^{2+}\) and Mn\(^{2+}\) supported binding of pro-MMP-1 by the \(\alpha_2\) I domain. In 2 mM EDTA, both I domains showed a significant degree of metal-independent binding to pro-MMP-1, again suggesting the presence of both metal-dependent and metal-independent interactions (Fig. 5A). The binding of pro-MMP-1 to the \(\alpha_\beta_1\) integrin was confirmed in intact cells by co-immunoprecipitation experiments. Monocytic U937 cells were differentiated to macrophages in culture, and then MMP-1 and \(\alpha_2\) were immunoprecipitated from cell lysates. Immunoprecipitation with both anti-\(\alpha_2\) integrin and anti-MMP-1 antibodies precipitated nearly equivalent amounts of MMP-1 derived from the cell layer (Fig. 5B), suggesting that most of the MMP-1 was bound to the cell surface via \(\alpha_\beta_1\).

As described above, pro-MMP-1 contains an N-terminal propeptide, a catalytic domain, and a short proline-rich linker connected to a hemopexin-like domain (12). Although the data shown in Fig. 2 suggest that the propeptide sequence of MMP-1 does not mediate the binding by the \(\alpha_2\) integrin I domain, we were interested in determining in greater detail which domain(s) of MMP-1 are required for binding to \(\alpha_2\) integrin I domain. To address this question, we used MMP chimeras in which domains of MMP-1 were replaced with the equivalent domains of either MMP-3 or MMP-13, neither of which binds the \(\alpha_2\) integrin I domain (Fig. 6B). To name the chimeras, the principal domains (namely, the propeptide and catalytic domain, the linker, and the hemopexin-like domain) were assigned a number corresponding to the MMP from which they originated. Thus, pro-MMP-1 was designated 1-1-1, and pro-MMP-3 was 3-3-3 (Fig. 6A). Consequently, 3-1-1 was composed of the propeptide and catalytic domain from MMP-3, and the linker and hemopexin-like domain of MMP-1 (Fig. 6A). Fig. 6B depicts the binding of chimeras 3-1-1, 3-3-1, 1-1-13, and 13-13-1 to the \(\alpha_2\) integrin I domain. 1-1-1 (pro-MMP-1) effectively supported binding of the to \(\alpha_2\) integrin I domain; 3-3-3 (pro-MMP-3) and 13-13-13 (pro-MMP-13) did not. 3-1-1 bound the \(\alpha_2\) integrin I domain as effectively as 1-1-1, indicating that the propeptide and the catalytic domain of MMP-1 were not
required for interaction of MMP-1 with \( \alpha_2 \) integrin I domain. This conclusion agrees with the results shown in Fig. 2. 3-3-1 and 13-13-1 bound less effectively to the \( \alpha_2 \) integrin I domain than did 1-1-1, indicating that the hemopexin-like domain of MMP-1 alone was not sufficient to support optimal binding of the \( \alpha_2 \) integrin I domain. Similarly, 1-1-13 did not support optimal binding of the \( \alpha_2 \) integrin I domain, indicating that the linker alone was also insufficient for optimal binding. Thus, both the linker and hemopexin-like domains were necessary to reconstitute efficient binding of \( \alpha_2 \) integrin I domain to MMP-1.

However, the degree of binding of 1-1-13, 13-13-1, and 3-3-1 were intermediate between the positive control (1-1-1) and the negative control (3-3-3 or 13-13-13). Thus, the presence of either the linker or the hemopexin-like domain of MMP-1 conferred readily detectable but suboptimal binding of the \( \alpha_2 \) integrin I domain. These findings suggest that two contact points are formed between the I domain and MMP-1: one with the linker domain and one with the hemopexin-like domain.

**DISCUSSION**

In this paper, we demonstrate that MMP-1, in both its active and pro forms, binds specifically to both the \( \alpha_1 \) and \( \alpha_2 \) integrin I domains. We also demonstrate that the interaction between the \( \alpha_2 \) integrin I domain and MMP-1 has both a divalent cation-dependent component and a divalent cation-independent component. In addition to \( \text{Mn}^{2+} \) and \( \text{Mg}^{2+} \), \( \text{Ca}^{2+} \) also supported binding of the \( \alpha_2 \) integrin I domain to MMP-1. However, because \( \text{Ca}^{2+} \) has not been shown to support binding of the \( \alpha_2 \) integrin I domain to any other ligand (20), the metal dependence seemed to be a requirement of MMP-1, not of the I domain. This conclusion was confirmed with the use of \( \alpha_2 \) integrin I domain MIDAS motif mutants. As expected, these mutants failed to bind to collagen, but they did bind to MMP-1 in a divalent cation-dependent manner. These results indicate...
that the metal dependence of the α₂/MMP interaction is conferred by the proteinase, whereas the metal dependence of the α₂-collagen binding is conferred by the integrin.

We used chimeric MMPs to begin to dissect the structural basis of the interaction between MMP-1 and the α₂ integrin I domain. Although MMP-3 and MMP-13 have significant structural homology to MMP-1 (9), neither enzyme bound to the α₂ integrin I domain. Thus, they provide suitable partners for domain swaps in chimera construction. These chimeras demonstrated that neither the propeptide nor the catalytic domains of MMP-1 are required for binding to the integrin I domains but that both the linker and hemopexin-like domain must be present for optimal binding. The presence of either the linker domain or the hemopexin-like domain of MMP-1 in a chimera is sufficient to confer detectable, albeit suboptimal binding. These findings suggest that there are at least two points of contact between the α₂ integrin I domain and MMP-1, one with the linker and one with the hemopexin domain. It is interesting to note that the divergent cation experiments described above suggest that the interaction between MMP-1 and the α₂ integrin I domain has both cation-dependent and cation-independent components. Perhaps the cation-dependent component of the interaction emanates from the hemopexin-like domain, which in the crystal structure has been shown to bind Ca²⁺, whereas the divergent cation-independent component of the interaction is derived from the linker domain, which does not bind any divalent cation in the crystal structure (12).

Localization of matrix metalloproteinases and related enzymes to the cell surface is not without precedent. Proteinases localized to the cell surface include MMP-2 by TIMP-2 (32, 33), MMP-2 by α₂β₁ integrin (34, 35), seprase by α₂β₁ integrin (36), MMP-9 by CD44 (37, 38), and MMP-7 by heparan sulfate proteoglycans (39). In this work, we add to the growing list of cell surface-associated proteinases by demonstrating that MMP-1 binds to the α₂ integrin I domain, and our accompanying paper (23) demonstrates that pro-MMP-1 is bound to the cell surface by interaction with the α₂ integrin. Anchoring proteinases to the cell membrane may afford the cell precise control over both proteinase activity and location. Binding of pro-MMP-1 to a collagen receptor could result in an enzyme that is properly positioned for cleavage of its substrate. In addition, binding of pro-MMP-1 to the α₂β₁ integrin could result in activation of the enzyme, either by a second proteinase or by a conformational change of MMP-1 itself.

In addition to regulating enzyme activity, binding of MMP-1 to the α₂β₁ integrin may allow the cells to carefully control proteinase expression levels in their microenvironment. Ligation of the α₂β₁ integrin by collagen induces expression of pro-MMP-1 in migrating keratinocytes (19). When MMP-1 cleaves type I collagen at physiological temperature, the triple helical molecule denatures. Denatured collagen is no longer a high affinity ligand for the α₂β₁ integrin; consequently, migration and signaling are turned off (40, 41). Thus cells have a mechanism to coordinate and integrate the expression of pro-MMP-1 and the α₂β₁ integrin, the amount of type I collagen in the matrix, and the activity of MMP-1. Because the enzyme is surface-bound, diffusion of the active enzyme within the extracellular matrix may be low, facilitating focal cleavage of collagen, as well as allowing each cell to sense its microenvironment and adjust its MMP-1 levels independently of its neighbors. Expression of MMP-1 may also be sensitive to α₂β₁ integrin levels, in terms both of ligated integrin available to signal and of integrin available to orient/activate the enzyme.

Functionally, localization of MMP-1 to the cell surface may have important effects upon cell migration. The α₂β₁ integrin cannot bind to MMP-1-cleaved collagen fragments, which would slow migration across a modified surface (40). In wound healing, keratinocytes require activity of MMP-1 for migration across a collagen surface (19). It was suggested that MMP-1 may be required for direction finding to ensure that the cells progress across to the wound face toward full-length collagen (19). Reverse migration to the wound edge is prevented by turning a pro-migratory substrate into a poor migratory substrate (19). Interestingly, a recent report suggested that MMP-1 bound to the surface of smooth muscle cells is important for stimulating migration by enhancing rear release of the integrin from the degraded collagen (42). Although this work did not determine whether the MMP-1 was bound to the α₂β₁ or α₂β₃ integrins, the finding is entirely consistent with the model we have presented in this report. Indeed, it has been shown that an intermediate level of integrin expression is required for optimal migration (43). If levels are too low, the cell cannot gain traction; if levels are too high, presumably the cell becomes too firmly attached to the matrix. Similar considerations apply to ligand density within an adhesive substrate. Perhaps MMP-1 associates with the α₂β₁ integrin to carefully regulate the concentration of ligand available for the integrin and thus regulate its avidity.

There are interesting parallels in our structure-function analysis and the studies carried out on the MMP-2/α₂β₁ integrin interaction (34, 35). Specifically, both studies have highlighted the hemopexin-like domain as a point of interaction between the integrin and the MMP. In addition, we found that the linker domain in between the catalytic domain and the hemopexin-like domain was also important for binding to the α₂ integrin I domain. At least a portion of the linker domain may also be important for interaction of MMP-2 with α₂β₃; the hemopexin-like domain used in those experiments was generated by MMP-2 autocatalysis and may contain a piece of the linker in addition to the hemopexin-like domain. It is interesting to speculate that the hemopexin domain of other MMPs may also be important for their localization to the cell surface; mediated, perhaps, by binding other integrins.

In summary, we have shown that MMP-1 interacts with the α₂β₁ integrin via the I domain of the α₂ integrin subunit. We demonstrated that both the linker and hemopexin-like domains were required for binding but that the propeptide and catalytic domains were not. Our results indicate that there are two separate contact points between the I domain and MMP-1: one on the linker and one on the hemopexin domain. We also showed that the MMP-1/α₂ integrin I domain interaction had both a metal-dependent and a metal-independent component, and through the use of I domain mutants, we showed that the metal dependence was a function of the MMP.

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