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Interstitial Collagen Catabolism*

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Interstitial collagen mechanical and biological properties are altered by proteases that catalyze the hydrolysis of the collagen triple-helical structure. Collagenolysis is critical in development and homeostasis but also contributes to numerous pathologies. Mammalian collagenolytic enzymes include matrix metalloproteinases, cathepsin K, and neutrophil elastase, and a variety of invertebrates and pathogens possess collagenolytic enzymes. Components of the mechanism of action for the collagenolytic enzyme MMP-1 have been defined experimentally, and insights into other collagenolytic mechanisms have been provided. Ancillary biomolecules may modulate the action of collagenolytic enzymes.

Enzymes That Catalyze Interstitial Collagen Catabolism

Collagens are composed of three α chains of primarily repeating Gly-Xaa-Yaa triplets, which induce each α chain to adopt a left-handed poly-Pro II helix. Three chains then intertwine, staggered by one residue and coiled, to form a right-handed triple helix. Triple helices assemble to form semicrystalline aggregates referred to as fibrils, and bundles of fibrils form fibers. The proteolysis of interstitial collagen (types I–III) is integral for numerous physiological functions, including morphogenesis, tissue remodeling, and wound healing, and has been recognized as a contributing factor to multiple pathologies, including tumor cell spreading (metastasis), arthritis, glomerulonephritis, periodontal disease, tissue ulcerations, cardiovascular disease, and neurodegenerative diseases. Identifying proteases capable of processing triple helices provides a starting point for defining the roles of collagen catabolism in health and disease.

Members of the matrix metalloproteinase (MMP)[2] family of zinc-dependent endopeptidases possess collagenolytic activity (1). Interstitial collagens are hydrolyzed by the "classic" collagenases, MMP-1, MMP-8, and MMP-13 (Fig. 1), into 1/4 and 3/4 length fragments (Table 1) (1, 2). MMP-2 (Fig. 1) cleaves type I collagen (3), although how robust the collagenolytic activity is has been brought into question (4). MMP-9 (Fig. 1) cleaves type I and III collagens (5). Hydrolysis of type I collagen was monitored at 37 °C, at which some denatured triple helices might exist. For MMP-2 and MMP-9, the cleavage site is the same as for the classic collagenases (Table 1).

Two membrane-type (MT) MMPs (MT-MMPs), MT1-MMP and MT2-MMP, allow invasion-incompetent cells to penetrate type I collagen matrices (6). MT1-MMP (Fig. 1) processes type I-III collagens at the same site as the classic collagenases (Table 1) (3). MT3-MMP also cleaves type III collagen at the classic site (Table 1) (3). MT6-MMP was initially reported to have little or no collagenolytic activity (7, 8) but was subsequently found to cleave type I and II collagens (albeit at 37 °C) (9) and a triple-helical peptide (THP) model of the classic collagenase cleavage site (10). The catalytic (CAT) domain of MMP-12 processes type I and III collagens, where hydrolysis occurs at the classic cleavage site and numerous other sites (11). The classic collagenase cleavage site seems to be the most sensitive to MMP-12 (Table 1). *Xenopus laevis* MMP-18 and chicken MMP-22/MMP-27 cleave type I collagen at the same site as the classic collagenases (3).

The interstitial collagen triple helix is cleaved by the Cys protease cathepsin K under acidic conditions (optimum pH 5.0). Five distinct sites of cathepsin K hydrolysis type I collagen have been identified, as well as one in type II collagen (Table 1) (12, 13).

An extracellular Ser protease contributes to collagenolysis by temporomandibular joint fibroblasts (14). Several Ser proteases possess interstitial collagenolytic activity, including human neutrophil elastase, *Uca pugilator* (fiddler crab) collagenase 1, *Hypoderma lineatum* (insect) collagenase, *Penaeus vanameii* (shrimp) chymotrypsin, and *Pseudoalteromonas* sp. SM9913 deseasin MCP-01 (3, 15–18). However, neutrophil elastase is ineffective toward fibrillar type III collagen (19). For a number of collagenolytic Ser proteases, the site of collagen cleavage is close to the site of MMP action (Table 1). Although initially reported as being collagenolytic, the Ser proteases fibroblast activation protein/separase and trypsin-2 do not cleave interstitial collagens within their triple helices (20).[3]

Additional interstitial collagenases include several that act under acidic conditions, such as *Cynara cardunculus* Asp protease cardosin A (22) and *Alicyclobacillus sendaiensis* Ser-carboxyl protease kumamolisin-As/ScpA (23), as well as the Cys proteases ginger (*Zingiber officinale*) GP2 and GP3 (24) and *Fasciola hepatica* FhCL2 and FhCL3 (25). GP2 hydrolyzes type I collagen at three distinct sites (Table 1) (24). FhCL2 cleaves the α1(I) chain at 43 sites and the α2(I) chain at 26 sites, whereas FhCL3 cleaves the α1(I) chain at 24 sites and the α2(I) chain at 24 sites, with only three sites shared by the two proteases (25).

Clostridium histolyticum possesses two zinc proteases with collagenolytic activity: class I (ColG) and class II (ColH) (Fig. 1). ColG cleaves interstitial collagens initially near the N termini, whereas ColH cleaves interstitial collagens near the middle to produce 35- and 62-kDa fragments (Table 1) (26). *Clostridium perfringens* produces a collagenase that is highly similar to ColG, whereas *Vibrio alginolyticus* collagenase is a zinc prote-

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[2] The abbreviations used are: MMP, matrix metalloproteinase; MT, membrane-type; THP, triple-helical peptide; CAT, catalytic; HPX, hemopexin-like; MO, maximum occurrence; CBD, collagen-binding domain.

[3] L. S. Mirigian, E. Makareeva, H. Koistinen, O. Itkonen, T. Sorsa, U.-H. Stenman, T. Salo, and S. Leikin, manuscript submitted for publication.

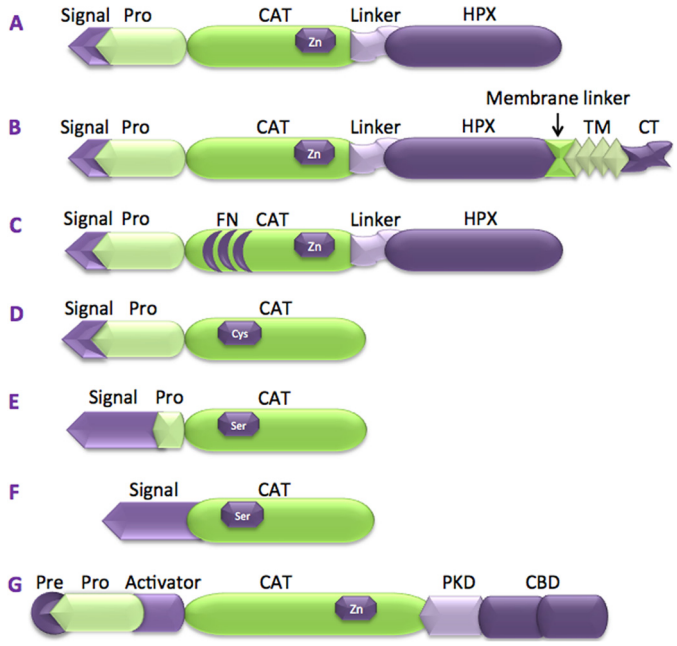

FIGURE 1. **Domain structures of collagenolytic proteases.** *A*, MMP-1, MMP-8, and MMP-13. *B*, MT1-MMP. *C*, MMP-2 and MMP-9. *D*, cathepsin K. *E*, neutrophil elastase. *F*, fiddler crab collagenase. *G*, *C. histolyticum* class I collagenase (ColG). *Signal*, secretory signaling peptide; *Pro*, prodomain; *TM*, transmembrane domain; *CT*, cytoplasmic tail; *FN*, fibronectin domain; *Pre*, predomain; *PKD*, polycystic kidney disease-like domain.

ase that initially processes collagen at a similar site as collagenolytic MMPs (3).

Pathways of Collagen Catabolism

There are presently four pathways that have been considered for mammalian collagen catabolism: 1) phagocytosis mediated by the $\alpha 2\beta 1$ integrin, where internalized insoluble collagen is transported to lysosomes and degraded by cathepsins (27); 2) cathepsin K collagenolysis in osteoclast-mediated bone resorption (28); 3) extracellular MMP hydrolysis, followed by gelatinolytic MMPs laterally diffusing on collagen extracellularly, finding "tails" from the cleaved sites, denaturing the triple helix, and further proteolyzing the α chains (29, 30); and 4) extracellular MMP hydrolysis, followed by the resulting collagen fragments undergoing endocytosis (mediated by urokinase plasminogen activator receptor-associated protein/Endo180 on mesenchymal cells and mannose receptor on macrophages), lysosomal delivery, and cathepsin-catalyzed degradation (31). Collagen can also be degraded intracellularly by autophagy-mediated lysosomal processes, which may be a form of collagen regulation (32).

In vivo processing of collagen for pathways 1) and 2) above initially involves MMP interaction with fibrils. Hydrolysis of collagen proceeds at the outer edge of the fibril (33, 34). MMP-1 is a diffusion-based "burnt bridge" Brownian ratchet capable of biased diffusion on the surface of collagen fibrils, where the bias is driven by proteolysis (35). Surface-bound MT1-MMP movement is via a similar diffusion mechanism (4). While on collagen fibrils, MMP-1 spends ~90% of its time in one of two distinct pause classes (36). Class I occurs randomly along the fibril, whereas class II occurs periodically at 1.3 and 1.5 μm along the fibril and exhibits multistep escape kinetics (36). Five percent of the class II pauses result in initiation of processive collagen degradation for ~15 consecutive cleavage events (36). The temperature dependence of the pauses suggests local unfolding, but the low probability of hydrolysis (~5%) indicates that local unfolding is not sufficient for hydrolysis (36).

Unique Features of Interstitial Collagen Cleavage Sites

MMPs bind to multiple sites in collagen (37), but hydrolysis ultimately occurs at a single site (Table 1). Collagen primary structure is not the only basis for discriminatory MMP collagenolytic behavior (1). A model of the cleavage sites in interstitial collagens suggested that all of the information necessary for efficient hydrolysis of collagen is contained in a 24-residue stretch (subsites P_{13}–P_{12}') (2). Cleavage site regions were distinguished by <10% charged residues, being "tightly" triple-helical (high Pro/Hyp content) prior to the cleavage site and being "loosely" triple-helical (low Pro/Hyp content) following the cleavage site (2). Arg residues in the P_5' or P_8' subsite have been proposed to stabilize the triple helix through electrostatic interactions, and these interactions may need to be disrupted for hydrolysis to occur (38).

Soluble collagens are thermally unstable at physiological temperatures, slowly melting unless incorporated into fibrils (39). This instability may lead to local flexibility/microunfolding that is needed for protease processing of collagen. Molecular dynamics simulations indicate microunfolding of interstitial collagens at the MMP cleavage site (40–42). Based on enzyme susceptibility, the type I collagen MMP cleavage site undergoes local reversible relaxation (43), whereas the cleavage site in type III collagen has been proposed to be more flexible than the one in type I collagen (19, 44).

Homotrimeric type I collagen ($\alpha 1(I)_3$) is much less susceptible to MMP-1 hydrolysis than heterotrimeric type I collagen, and the effect is not due to binding (45). The homotrimer is more thermally stable than the heterotrimer by ~2.5 °C and melts 100 times slower (46, 47). The microunfolding patterns of the two collagen subtypes are different (47). Thus, the difference between MMP-1 activity toward homotrimeric *versus* heterotrimeric type I collagen is due to local triple-helical unwinding at the cleavage site (45). The $\alpha 2(I)$ chain also increases hydrophobicity compared with the $\alpha 1(I)$ chain, driving out structured water and facilitating hydrolysis (46). Homotrimeric type I collagen is produced by a variety of tumor cells and enhances tumor cell proliferation and migration compared with heterotrimeric type I collagen (48).

The Ile residue in one of the three chains at the site of MMP hydrolysis has a distinct chemical shift, a higher *J* coupling value, increased dynamics, and decreased local stability (49). This suggests that a single locally dynamic chain, rather than a labile region with three comparably dynamic chains, is a determining factor for collagen to be cleaved by MMPs (49). Also, a Pro residue at the P_3 subsite influences the P_1' subsite Ile residue, enhancing its accessibility to collagenolytic MMPs (49).

The collagen cleavage site model would be valid only if collagenases had extended active or substrate-binding sites. Modulation of MMP-1, MMP-8, MMP-13, and MT1-MMP activities was observed in THP substrates spanning subsites P_{13}–P_{17}' (50–53). Utilizing interstitial collagen sequences inserted into

TABLE 1
Representative protease cleavage sites within interstitial collagen triple-helical domains

Enzyme	Collagen chain	Sequence[a]
MMP-1/2/8/9/12/13 and MT1-MMP	α1(I)	Pro-Gln-Gly^{775}∼Ile^{776}-Ala-Gly
MMP-1/2/8/9/12/13 and MT1-MMP	α2(I)	Pro-Gln-Gly^{775}∼Leu^{776}-Leu-Gly
MMP-1/8/13 and MT1-MMP	α1(II)	Pro-Gln-Gly^{775}∼Leu^{776}-Ala-Gly
MMP-1/8/9/12/13, MT1-MMP, and MT3-MMP	α1(III)	Pro-Leu-Gly^{775}∼Ile^{776}-Ala-Gly
Cathepsin K	α1(I)	Gly-Pro-Arg^{9}∼Gly^{10}-Leu-Pro
Cathepsin K	α1(I)	Gly-Pro-Gln^{21}∼Gly^{22}-Phe-Gln
Cathepsin K	α1(I)	Gly-Leu-Asp^{96}∼Gly^{97}-Ala-Lys
Cathepsin K	α1(I)	Gly-Pro-Gln^{189}∼Gly^{190}-Val-Arg
Cathepsin K	α1(I)	Gly-Pro-Ser^{810}∼Gly^{811}-Ala-Ser
Cathepsin K	α2(I)	Gly-Pro-Arg^{9}∼Gly^{10}-Pro-Pro
Cathepsin K	α2(I)	Gly-Pro-Gln^{21}∼Gly^{22}-Phe-Gln
Cathepsin K	α2(I)	Gly-Leu-Lys^{99}∼Gly^{100}-Pro-Gln
Cathepsin K	α2(I)	Gly-Ala-Arg^{144}∼Gly^{145}-Ser-Asp
Cathepsin K	α2(I)	Pro-Pro-Gly^{814}∼Ala^{815}-Arg-Gly
Cathepsin K	α1(II)	Lys-Pro-Gly^{61}∼Lys^{62}-Ser-Gly
Elastase	α1(III)	Ala-Gly-Ile^{779}∼Thr^{780}-Gly-Arg
Crab collagenase 1	α1(I)	Ala-Gly-Gln^{779}∼Arg^{780}-Gly-Val
Crab collagenase 1	α1(I)	Gly-Gln-Arg^{780}∼Gly^{781}-Val-Val
Crab collagenase 1	α1(I)	Gly-Glu-Arg^{792}∼Gly^{793}-Phe-Hyp
Crab collagenase 1	α1(I)	Arg-Gly-Leu^{587}∼Thr^{588}-Gly-Pro
Crab collagenase 1	α2(I)	Gly-Phe-Leu^{783}∼Gly^{784}-Leu-Pro
Cardosin A	α2(I)	Pro-Gly-Phe^{464}∼Asn^{465}-Gly-Leu
Kumamolisin-As/ScpA	α1(I)	Gly-Pro-Lys^{108}∼Gly^{109}-Glu-Hyp
Kumamolisin-As/ScpA	α1(I)	Gly-Pro-Arg^{183}∼Gly^{184}-Ser-Glu
Kumamolisin-As/ScpA	α1(I)	Gly-Ala-Arg^{396}∼Gly^{397}-Gln-Ala
Kumamolisin-As/ScpA	α1(I)	Gly-Asp-Ala^{489}∼Gly^{490}-Ala-Hyp
Kumamolisin-As/ScpA	α2(I)	Gly-Pro-Arg^{42}∼Gly^{43}-Pro-Ala
GP2	α1(I)	Gly-Pro-Ala^{285}∼Gly^{286}-Glu-Glu
GP2	α1(I)	Gly-Ala-Arg^{498}∼Gly^{499}-Glu-Arg
GP2	α1(I)	Gly-Pro-Ser^{711}∼Gly^{712}-Asn-Ala
GP2	α2(I)	Gly-Pro-Ser^{285}∼Gly^{286}-Glu-Glu
GP2	α2(I)	Gly-Ala-Arg^{498}∼Gly^{499}-Glu-Arg
GP2	α2(I)	Gly-Pro-Ser^{711}∼Gly^{712}-Ile-Ser
ColG	α1(II)	Gly-Phe-Gln^{24}∼Gly^{25}-Asn-Pro
ColG	α1(III)	Gly-Glu-Arg^{69}∼Gly^{70}-Leu-Hyp
ColH	α1(I)	Gly-Ala-Arg^{396}∼Gly^{397}-Gln-Ala
ColH	α2(I)	Gly-Ala-Arg^{396}∼Gly^{397}-Glu-Pro
ColH	α1(II)	Gly-Phe-Pro^{405}∼Gly^{406}-Pro-Lys
ColH	α1(III)	Gly-Pro-Arg^{399}∼Gly^{400}-Gln-Hyp

[a] Numbering begins at the N terminus of the triple-helical region of each collagen.

bacterial collagen, the minimum type III collagen sequence necessary for MMP-1 or MMP-13 hydrolysis was found to be 15 residues (subsites P_4–P_{11}'), whereas a similar rate of hydrolysis to type III collagen was obtained with a sequence spanning subsites P_7–P_{11}' (54). Thus, both the THP and bacterial collagen studies confirm that the collagenolytic MMPs interact with a significant span of the collagen triple helix.

Molecular Mechanisms of Collagen Catabolism

The 15 Å collagen triple helix does not fit into the 5 Å MMP CAT domain active site cavity (55). Models have generally accounted for this steric clash by (*a*) requiring active unwinding of the triple helix by an MMP (55–57) and/or (*b*) considering that the site of hydrolysis within collagen has a distinct conformation or conformational flexibility, rendering it more susceptible to proteolysis than other regions in collagen (2). The "vulnerable" site hypothesis proposes that the distinct cleavage site region within collagen is alone responsible for collagenolysis (58).

A detailed mechanism of initial collagenolysis was developed from examination of structures of MMP-1 and MMP-1·THP complexes (59). MMP-1 is in equilibrium between open/extended and closed structures (Fig. 2*A*) (60). An open form of MMP-1 is favored in solution (see below). The hemopexin-like (HPX) domain binds the leading chain (1T) and the middle chain (2T) of the THP, and due to the flexibility of the linker, the CAT domain is guided toward the Gly∼Ile bond of chain 1T (Fig. 2*B*). This structure would thus correspond to the first event of collagen recognition by MMP-1. The exposure of the MMP cleavage site by removal of the collagen C-terminal telopeptide (34) permits interactions of the MMP-1 HPX and CAT domains with triple helices on the outer edge of the fibril. Visual inspection of the complex at this point suggests that a back-rotation of the CAT and HPX domains would need to occur to achieve the x-ray crystallographic closed MMP-1 conformation. To approximate this action, the residues at the interface between the HPX and CAT domains in the x-ray structure of MMP-1 in the closed form (Protein Data Bank code 1SU3) were imposed as constraints in a docking calculation. In the resulting structure, with the CAT and HPX domains arranged in the x-ray crystallographic closed conformation, the THP is unwound (Fig. 2*C*). The domain movement drives chain 1T into the active site, allowing the polypeptide to establish a number of H-bonding interactions and the carbonyl oxygen of the cleavage site amide bond to coordinate the metal ion. This result is consistent with the experimentally observed weakening in NOEs for the interaction of chain 1T with chains 2T and 3T (the trailing chain) at the cleavage site. It has been proposed that when released from the triple-helical conformation, the cleavage site sequence in type I collagen has the propensity to form β-bend and β-strand structures (61). Protease active sites

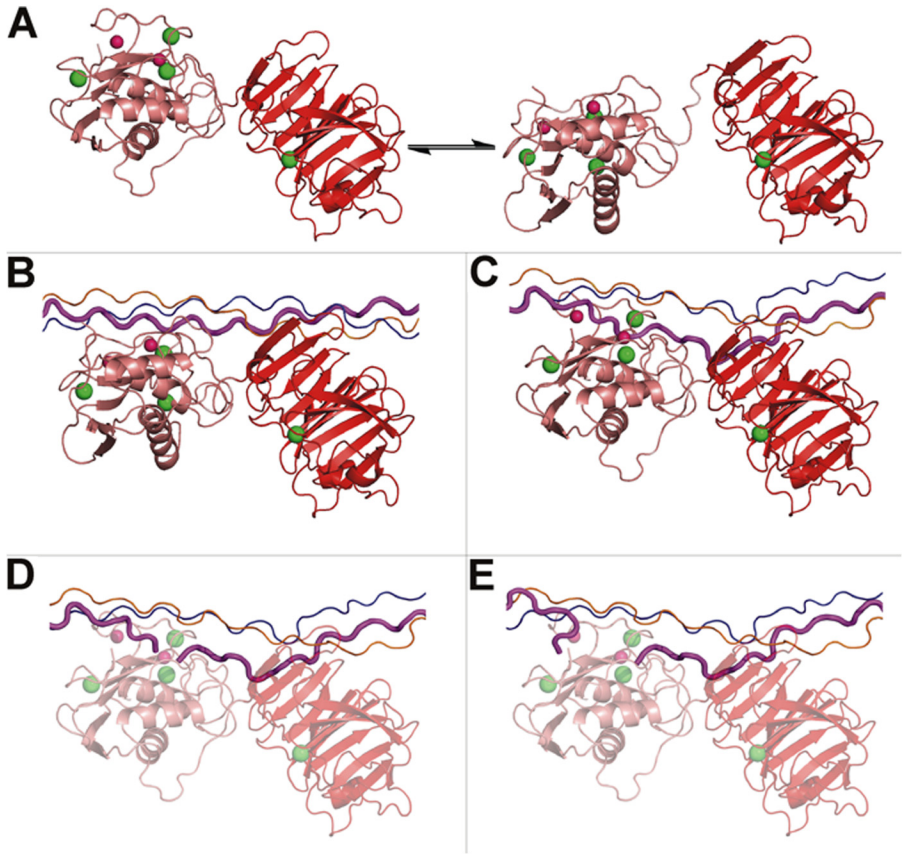

FIGURE 2. **Initial steps of collagenolysis (59).** *A*, closed (*left*) and open/extended (*right*) forms of full-length MMP-1 in equilibrium. *B*, the extended protein binds α1(I)-(772–786) THP chains 1T-2T at Val^{23}–Leu^{26} with the HPX domain and the residues around the cleavage site with the CAT domain. The THP is still in a compact conformation. *C*, closed full-length MMP-1 interacting with the released 1T chain (*magenta*). *D*, after hydrolysis, both peptide fragments (C- and N-terminal) are initially bound to the active site. *E*, the C-terminal region of the N-terminal peptide fragment is released. This figure has been reprinted with permission from the *Journal of the American Chemical Society*.

appear to accommodate β-strand structures universally (62). Besides liberating the N-terminal region of chain 1T for hydrolysis, the destabilization of the THP also causes a partial detachment of chain 3T near the THP C terminus (Fig. 2*C*), consistent with the observed lengthening in NOEs of chain 3T. The position that the two peptide fragments assume after cleavage (Fig. 2, *D* and *E*) is almost superimposable on the x-ray crystallographic structure of the complex between the MMP-12 CAT domain and the two fragments obtained by enzymatic cleavage of the α1(I) collagen model Pro-Gln-Gly-Ile-Ala-Gly hexapeptide at the Gly∼Ile bond (Protein Data Bank code 2OXZ).

The MMP-1·THP complex, in which one strand of the triple helix is displaced from the other two (Fig. 2*C*), is an energetically and mechanistically feasible route between the complexes in Fig. 2 (*B* and *D*) (59). MMP-1 may not actively unwind the triple helix (45). Rather, MMP-1 shifts the equilibrium between native helical and locally unwound states, destabilizing the helical state and/or stabilizing the unwound state (45).

The maximum occurrence (MO) of MMP-1 conformations in solution has been recently calculated by paramagnetic NMR and small angle x-ray scattering.[4] Many of the MMP-1 conformations with the highest MO value (>35%) were found to have interdomain orientations and positions that could be grouped into a cluster.[4] Within this cluster, the collagen-binding residues of the HPX domain are solvent-exposed, and the CAT domain is correctly positioned for its subsequent interaction with the collagen. A ∼50° rotation around a single axis of the CAT domain with respect to the HPX domain positions the CAT domain right in front of the interstitial collagen cleavage site.

Binding sites for the triple helix within the HPX domain have been identified (52, 59, 64, 65). Initially, in MMP-1, Ile^{290} and Arg^{291} in the blade I A-B loop were identified as key residues in collagenolysis (52). Subsequently, Phe^{301}, Val^{319}, and Asp^{338} were implicated in collagen binding (64). Phe^{320} was found to be an important contributor, along with Ile^{290} and Arg^{291}, to the S_{10}' binding pocket (65). The S_{10}' binding pocket binds the P_{10}' subsite of collagen, which possesses a conserved Leu residue important for interaction of triple helices with MMP-1 (53, 64, 65). The lack of this Leu residue explains why an earlier study did not observe binding of a THP to the HPX domain (66). Other residues within the HPX domain may also participate in collagen binding (52, 64, 65).

There is some controversy over MMP-1 Phe^{301}, which was identified as a binding site by NMR spectroscopy but was deemed as buried in the CAT domain/HPX domain interface by x-ray crystallography (64, 65). All available x-ray structures

[4] I. Bertini, L. Cerofolini, G. B. Fields, M. Fragai, C. F. G. C. Geraldes, C. Luchinat, G. Parigi, E. Ravera, D. I. Svergun, and J. M. C. Teixeira, manuscript submitted for publication.

of human full-length MMP-1 (Protein Data Bank codes 1SU3, 2CLT, and 4AUO) display relatively closed conformations. The MO values obtained for the x-ray structures for pro-MMP-1 (code 1SU3) and active MMP-1 (2CLT) were 20 and 19%, respectively.[4] The x-ray crystallographic structure of an MMP-1·THP complex (code 4AUO) has a more closed structure compared with code 2CLT (65) and has a MO of 18%. Thus, these structures are not the dominant ones sampled by the protein in solution. The radii of gyration (R_g) of the crystallographic structures range from 25.5 to 25.7 Å, whereas the structures with the highest MO (>35%) have R_g of 28.9 ± 1.3 Å. This range of R_g is in better agreement with values from small angle x-ray scattering data, indicating that the x-ray structures are more compact than the average solution conformation. Furthermore, the relative orientations of the HPX and CAT domains in the structures with the highest MO are different from those in the x-ray crystallographic structures. It was reported that the x-ray crystallographic structure of the MMP-1·THP complex is a nonproductive complex (65). Thus, Phe^{301} probably interacts with the triple helix initially but is then utilized for domain interaction during collagenolysis (64).

Based on chimeric studies using MMP-3 sequences, the active site cleft of MMP-1 is a significant determinant for collagenolytic activity (67, 68). Tyr^{210} is specifically involved in collagenolytic (but not general peptidase) activity (50, 69).

MMP-3 binds to type I collagen but does not cleave the native triple helix (70). However, the MMP-3 CAT domain can cleave collagen when the triple helix is unwound by catalytically inactive MMP-1 (55). Thus, MMP-3 is entirely competent to cleave type I collagen but does not. Based on the MMP collagenolysis mechanism, the linker needs to be able to properly orient the CAT and HPX domains (59). Gly^{272} is critical for the collagenolytic activity of MMP-1, with its role proposed to be the linker-bending motion that allows the HPX domain to present collagen to the CAT domain (71). The MMP-1 and MMP-8 linkers are considerably shorter than the MMP-3 linker, whereas the MT1-MMP linker is very long (33 residues), with significant and heterogeneous *O*-glycosylation (72). An MMP-8 chimera with the linker region (16 residues) replaced with the corresponding MMP-3 sequence (25 residues) loses activity toward collagen (73). In a similar fashion, MMP-1/MMP-3 chimeras possessing the MMP-3 linker are not active toward collagen (67, 70). The linker appears to be critical for proper alignment of the CAT and HPX domains during collagenolysis. Ultimately, there may be negative regulation of collagenolytic activity due to (mis)alignment of the CAT and HPX domains in the case of MMP-3 and other non-collagenolytic MMPs.

The experimentally determined mechanism is not consistent with the vulnerable site hypothesis, as fluctuations in the triple helix were not observed until after MMP-1 binding (59). As indicated earlier in the studies of MMP movement on fibrils, local unfolding was not sufficient for hydrolysis (36). Support for the vulnerable site hypothesis comes from the action of the CAT domains of MMP-1 and MMP-8 against type I collagen (58). However, high concentrations of CAT domains were utilized to obtain hydrolysis, and a prior study demonstrated that the MMP-8 CAT domain had a different pattern of type I collagen hydrolysis compared with full-length MMP-8 (74). A highly temperature-dependent collagenolytic activity was observed for the MMP-13 CAT domain but not for full-length MMP-13, indicating that the activity of the CAT domain was based on partial denaturation of the substrate (54). Although the MMP-12 CAT domain hydrolyzes interstitial collagens (11), it appears to possess unique properties that allow it to destabilize the triple helix (75, 76).

It has been noted that although MMP-1 and MMP-8 have similar collagenolytic mechanisms, MMP-2 and MT1-MMP have mechanisms distinct from MMP-1 and MMP-8 (50, 56, 77, 78). In the case of MMP-2 (and MMP-9 as well), interaction with collagen is primarily via the fibronectin type II-like modules within the CAT domain, not the HPX domain (3). All three fibronectin type II-like modules contribute to collagen binding, with the greatest effects observed for modules 2 and 3 (56, 79). Individual residues involved in collagen binding are primarily Arg (positions 252, 296, and 368) and aromatics (Phe^{297}, Tyr^{302}, Tyr^{323}, Tyr^{329}, Trp^{374}, and Tyr^{381}) (79). It has been proposed that MMP-2 preferentially binds the α1(I) chain and grossly distorts the triple helix, followed by initial hydrolysis of the α2(I) chain (77).

A mechanism has been proposed for *C. histolyticum* collagenolysis based on x-ray crystallographic analysis of the collagenase module (activator + CAT domains), the polycystic kidney disease-like domain, and one or both of the collagen-binding domains (CBDs) and mutagenesis analysis of substrate binding and/or hydrolysis of *C. histolyticum* class I collagenase (ColG) (80–82). The CBDs of ColG promote interaction with fibrils, not individual triple helices, along the fibril axis. Mutagenesis analysis of ColG CBD binding to THP Gly-(Pro-Hyp-Gly)$_8$ revealed Thr^{957}, Tyr^{970}, Leu^{992}, Tyr^{994}, and Tyr^{996} as participating in binding, with all of these residues centrally located on one face of the CBD (80). The ColG CBD binds unidirectionally to the undertwisted C terminus of the triple helix but does not facilitate unwinding (83). The polycystic kidney disease-like domain swells the collagen but does not unwind it (18, 82). The ColG collagenase module forms a saddle-shaped two-domain architecture that "squeezes" the fibril, facilitating enzyme (CAT domain) accessibility to monomeric triple helices (81). Initial collagen contact is made with the CAT domain, followed by a closing of the saddle and contact by the activator domain. Only the open state was observed in x-ray crystallographic analysis, whereby the opening between the CAT and activator domains matched that of collagen microfibrils (40 Å) (81). Removal of the activator domain or the Gly-rich hinge region between the activator and CAT domains greatly decreased collagenolytic activity. The mechanical energy for substrate unwinding comes from the release of stored ordered water upon hydrolysis (81). This is consistent with force measurement studies that concluded that *C. histolyticum* collagenase processes collagen independent of an unwinding transition (84). Supporting this notion is the much lower activation energy for *C. histolyticum* collagenase hydrolysis of fibrillar type I collagen compared with MMP-1 (33).

The entrance to the cathepsin K active site is 5 Å wide (85), and thus, manipulation of the triple helix in a similar fashion as in MMPs is anticipated. Collagenolytic activity of cathepsin K is lost by the T67L/L205A double mutation, as this mutation ren-

ders the S_2 subsite unable to accommodate Pro (86). Efficient collagenolytic activity requires a complex between cathepsin K and chondroitin sulfate (87).

Facilitation of Collagen Catabolism

Cell surface collagenolysis may be facilitated by collagen-binding integrins providing strain on the collagen, protease-binding partners, and/or protease dimerization. Binding of a THP by the $\alpha2\beta1$ integrin results in disruption of interactions between Arg and Glu side chains in the ligand and significant changes in main chain conformation, reflected in the bending of the triple helix (88). Strain could be induced by integrin-collagen interactions and/or cellular traction forces (57).

The reported effects of strain on collagenolysis have been contradictory. Molecular dynamics simulations indicated that force stabilizes the MMP cleavage site in heterotrimeric type I collagen, slowing proteolysis (89). However, homotrimeric type I collagen possesses a more stable cleavage site, so force enhances proteolysis by destabilizing the cleavage site (89). MMP-1 hydrolysis of a homotrimeric model of type I collagen was increased by 81-fold by a mechanical load (57), whereas similar force enhanced MMP-1 catalysis of heterotrimeric type I collagen by 8-fold (84). These data suggested that heterotrimeric type I collagen was more unwound than homotrimeric type I collagen, and hence, the effect of strain on further unwinding the triple helix was less pronounced in the former case (84). Conversely, strain of reconstituted type I collagen fibrils increased degradation time by MMP-8 (90). The discrepancy between the single-molecule study (84) and the fibrillar collagen study (90) may be due to effects on diffusive transport of the MMP in fibrils (84).

In one study, applied force had little effect on *C. histolyticum* collagenase processing of heterotrimeric type I collagen (84), whereas in another, the application of force significantly reduced *C. histolyticum* collagenase activity (91). The different results could be due to the mixtures of collagenases used. *C. histolyticum* collagenase activity was decreased by increasing strain in corneal tissue (92). As the enzyme processed the tissue, the same applied load strained the remaining tissue to a greater degree, limiting diffusion and slowing collagenolysis (92).

Related to strain, fibronectin binds to type I collagen at Gly^{788}–Gly^{799}, near the classic collagenase cleavage site (93). Fibronectin binding destabilizes the collagen triple helix, potentially facilitating MMP collagenolysis (93).

Many "soluble" collagenolytic MMPs have cell surface binding partners, including the $\alpha2\beta1$ integrin (MMP-1) and CD44 (MMP-9) (94). MT1-MMP has numerous cell surface binding partners, including tetraspanins, the $\alpha2\beta1$ and $\alpha v\beta3$ integrins, and CD44 (94–96). The HPX domain of MT1-MMP binds to tetraspanins CD63 and CD151 (96). MT1-MMP association with CD151 modulates collagenolysis in that knockdown of CD151 decreases collagenolysis (96).

Highly efficient collagenolysis requires homodimerization of MT1-MMP, where association includes interactions of the HPX domain (3). Homodimerization is symmetrical, involving Asp^{385}, Lys^{386}, Thr^{412}, and Tyr^{436} in blades II and III of the HPX domain (97). MT6-MMP also forms homodimers through a disulfide bond in the stem region (98). MMP-1 and MMP-9 can form an active heterodimeric complex capable of fibrillar type I collagen catabolism (63).

Cell surface-bound MT1-MMP has only a partially decreased collagenolytic activity upon deletion of the HPX domain (21). This suggests that other factors contribute to enzyme activity on the cell surface, possibly by straining the collagen.

Conclusion

Interstitial collagenolytic activity is a convergent evolutionary process. The initial steps of MMP-1 collagenolysis have been experimentally derived, and individual residues involved in this process have been identified. In addition, the roles of specific collagen residues in MMP substrate specificity have been quantified. Binding partners that modulate the activity of collagenolytic enzymes have begun to be identified. Collagenolytic MMPs utilize subtly different mechanisms for processing triple helices, and these differences may be exploited to develop selective inhibitors. As further information on interstitial collagenolytic processes is obtained, inhibition can be fined-tuned to be disease- or pathogen-specific.

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