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Molecular Mechanism of Type I Collagen Homotrimer Resistance to Mammalian Collagenases*[S]

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Type I collagen cleavage is crucial for tissue remodeling, but its homotrimeric isoform is resistant to all collagenases. The homotrimers occur in fetal tissues, fibrosis, and cancer, where their collagenase resistance may play an important physiological role. To understand the mechanism of this resistance, we studied interactions of $\alpha1(I)_3$ homotrimers and normal $\alpha1(I)_2\alpha2(I)$ heterotrimers with fibroblast collagenase (MMP-1). Similar MMP-1 binding to the two isoforms and similar cleavage efficiency of unwound $\alpha1(I)$ and $\alpha2(I)$ chains suggested increased stability and less efficient unwinding of the homotrimer triple helix at the collagenase cleavage site. The unwinding, necessary for placing individual chains inside the catalytic cleft of the enzyme, was the rate-limiting cleavage step for both collagen isoforms. Comparative analysis of the homo- and heterotrimer cleavage kinetics revealed that MMP-1 binding promotes stochastic helix unwinding, resolving the controversy between different models of collagenase action.

Type I collagen cleavage is crucial for normal tissue homeostasis, tissue repair, cancer invasion, and many other normal and pathological processes. Collagenases of the matrix metalloproteinase (MMP)[2] family are major mammalian proteases involved in the physiological cleavage of the collagen triple helix (1). They include MMP-1, MMP-8, MMP-13, and membrane-bound MMP-14, although MMP-2 is also capable of the triple helix cleavage. Understanding and modulating the activity of these enzymes are important for developing treatments for a variety of disorders (2).

Remarkably, $\alpha1(I)_3$ homotrimers of type I collagen were found to be resistant to all mammalian collagenases (3, 4). Unlike normal MMP-susceptible $\alpha1(I)_2\alpha2(I)$ heterotrimers, the homotrimeric isoform of type I collagen is not produced in healthy non-fetal tissues (we have not confirmed the only report (5) of the homotrimers in normal skin (3)). Instead, the homotrimers were found in fetal tissues (6), genetic disorders associated with the $\alpha2(I)$ chain deficiency (7–9), fibrotic tissues (10–12), carcinomas (3, 13–17), and fetal and cancer cell cultures (3, 18–23). Factors controlling their synthesis are not known, but these MMP-resistant molecules may play an important role in tissues with elevated MMP activity. For instance, they are produced by cancer cells but not by tumor-recruited fibroblasts (3). Their fibers may thus form MMP-protected roadways for cancer invasion.

Why are the homotrimers resistant to all mammalian collagenases? This question is important not only in the context of the homotrimer role in development and pathology but also for better understanding of collagenases. The cleavage of type I collagen involves collagenase binding, local triple helix unwinding, and sequential cutting of individual chains inside the catalytic cleft (24). However, the sequence of these steps and the unwinding mechanism remain controversial (25). The universal resistance of the homotrimers to all collagenolytic MMPs is particularly puzzling because of distinct differences in interactions of MMP-1, MMP-2, and MMP-14 with collagen (26, 27).

In this study, we investigated the homotrimer interactions with MMP-1. Because of preferential $\alpha2(I)$ chain interaction with the enzyme (24), we expected the homotrimer resistance to be associated with reduced MMP-1 binding, but our measurements revealed a different answer. The data clearly pointed to the homotrimer resistance to local unwinding at the MMP cleavage site. To better understand the results, we analyzed different kinetic models of collagen cleavage. This analysis confirmed the qualitative interpretation of the data and provided further evidence for the crucial role of the unwinding step in the cleavage of normal and mutant collagens in various disorders.

EXPERIMENTAL PROCEDURES

Collagen Purification and Fluorescent Labeling—Human type I collagen heterotrimers were purified from culture media of CRL-2127 fibroblasts (American Type Culture Collection). Human type I collagen homotrimers were purified from culture media of fibroblasts from an $\alpha2(I)$-chain-deficient patient (8). The cells were generously provided by Dr. P. H. Byers, University of Washington. Fibroblasts were grown to

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Equations S1–S21, Fig. S1, and additional references.

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[2] The abbreviations used are: MMP, matrix metalloproteinase; APMA, aminophenylmercuric acetate; AF, AlexaFluor.

confluence at 37 °C in Dulbecco's modified Eagle's medium (Invitrogen), 10% fetal bovine serum (Gemini BioProducts), and 2 mM GlutaMAX™ (Invitrogen) in the presence of 5% CO_2. After 24 h of stimulation with 50 μg/ml ascorbate in 0.1% fetal bovine serum, secreted collagen was purified from the media by ammonium sulfate precipitation, pepsin treatment, and NaCl precipitation at acidic pH (28). Type III collagen content was reduced below 1% for the heterotrimers and below 4% for the homotrimers by NaCl precipitation at neutral pH (29). Mouse heterotrimers and homotrimers were purified from tail tendons of wild type and homozygous oim mice, respectively, by pepsin extraction and NaCl precipitation (30). Labeling with AlexaFluor (AF) 488, 546, or 647 (Invitrogen) or Cy5 (GE Healthcare) was performed as described previously (28). The dye concentration was adjusted to 1 dye per 3–5 collagen triple helix labeling efficiencies. The unreacted dye was removed by collagen precipitation with 0.9 M NaCl in 0.5 M acetic acid, dialysis, or size-exclusion chromatography on G-50 spin mini-columns (GE Healthcare). Purified collagen was transferred into 2 mM HCl and characterized by circular dichroism in a J810 spectrometer (Jasco) and electrophoresis on pre-cast 3–8% Tris acetate mini-gels (Invitrogen). The gels were scanned on an FLA5000 fluorescence scanner (Fuji Medical Systems) and analyzed by MultiGauge 3.0 software supplied with the scanner.

Collagen Cleavage—Recombinant human pro-MMP-1, MMP-13, MMP-1(E200A), MMP-1(ΔC), and MMP-3(ΔC) were prepared and purified as described previously (24). Pro-MMP-1 and pro-MMP-1(E200A) were activated with 1 mM aminophenylmercuric acetate and 1/15 molar amount MMP-3ΔC at 37 °C for 1–2 h. The activated enzyme was separated on G-50 spin mini-columns.

Binary mixtures of type I collagen homo- and heterotrimers, in which one component was labeled with AF488 and the other with Cy5, were transferred into 50 mM Tris-HCl (pH 7.5), 0.15 M NaCl, 10 mM $CaCl_2$, 0.05% Brij 35, and 0 or 1 M glycerol (TNC buffer) and incubated with activated MMP-1, MMP-13, MMP-1(E200A), MMP-1(ΔC), or a combination of MMP-1(E200A) and MMP-1(ΔC). To stop the reaction, sample aliquots were mixed with lithium dodecyl sulfate gel-loading buffer (Invitrogen) supplemented with 20 mM EDTA. The aliquots were denatured at 60 °C and analyzed on 3–8% Tris acetate mini gels. Testing revealed no detectable effects of 1 M glycerol (used to prevent collagen fibrillogenesis) or fluorescent labeling on the collagen cleavage rate.

Equilibrium Microdialysis—MMP-1(E200A) binding was measured by equilibrium microdialysis in 50-μl chambers separated by a 100,000 molecular weight cutoff cellulose acetate membrane (Harvard Apparatus). One chamber was filled with a mixture of 0–65 μM MMP-1(E200A) and 0.8–11 μM AF488-labeled mouse tail tendon collagen in TNC buffer with 1 M glycerol. The other chamber was filled by the same buffer without collagen and no or lower MMP-1(E200A) concentration. After a 2–3-day incubation at room temperature, the collagen and MMP-1(E200A) concentrations in each chamber were determined from light absorption at 495 nm (AF488 absorption) and 280 nm (corrected for the contribution of AF488-labeled collagen), respectively. No collagen leakage through the dialysis membrane was observed. Collagen cleavage during the dialysis was negligible, as verified by gel electrophoresis. Bound MMP-1(E200A) was determined based on the difference in total MMP-1(E200A) concentration in the two chambers.

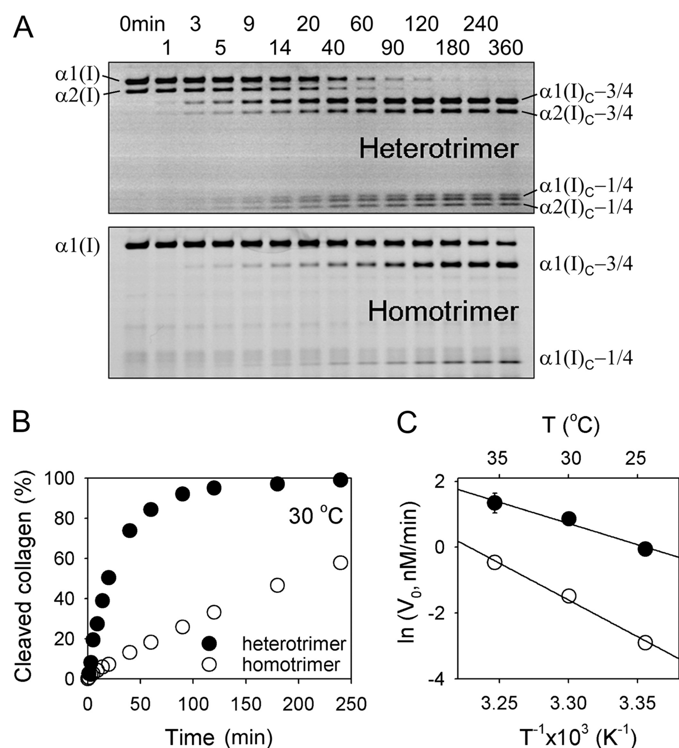


FIGURE 1. **Cleavage of 1:1 mixture (50 μg/ml) of human homotrimers (labeled with Cy5) and heterotrimers (labeled with AF488) by recombinant human MMP-1 (2 nM).** *A*, electrophoresis of sample aliquots at different reaction times; the long (3/4) and short (1/4) cleavage fragments of the α1(I) and α2(I) chains are labeled with subscript *C*. The same gel area is shown in AF488 (*top*) and Cy5 (*bottom*) fluorescence; inverse labeling produced the same results. *B*, cleaved fraction of the α1(I) band. *C*, temperature dependence of V_0 determined from the initial slopes of the cleavage curves. The lines show Arrhenius plots with 44 (homotrimer) and 26 (heterotrimers) kilocalories/mol activation energies.

Confocal Microscopy—Collagen fibers were reconstituted from AF546-labeled homotrimers and AF647-labeled heterotrimers in SecureSeal hybridization chambers (Grace Bio-Labs, Inc.) at 32 °C and imaged in an LSM 510 Inverted Meta microscope (Carl Zeiss) with a controlled environment chamber set at 37 °C (31). After initial imaging, the sample buffer was replaced with MMP-1 solution in TNC, and the sample was quickly remounted in the microscope. The lasers, filters, digital zoom, z-oversampling, and scanning rate were optimized to reduce photo damage and photobleaching. Low laser power, longer laser wavelength (≥543 nm), and short exposure were required to avoid irreversible accumulation of inactivated MMP-1 at fiber surfaces that prevented complete cleavage.

RESULTS

Cleavage Kinetics—Human homo- and heterotrimers were labeled by different fluorescent dyes, mixed together, and co-processed with activated recombinant human MMP-1 in the same sample tube at different temperatures. Comparison of gel electrophoresis patterns at different times after the addition of recombinant human MMP-1 (Fig. 1*A*) revealed the same ¾ and ¼ length fragments after the homo- and heterotrimer cleavage. The initial cleavage rate V_0 (Fig. 1*B*) was slower for the homotri-

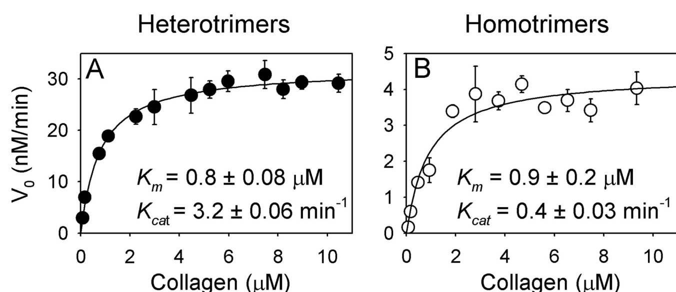


FIGURE 2. **V_0 dependence on the collagen concentration at 25 °C.** *Solid lines* are fits to Equation 1. $k_{cat} = V_{max}$/[MMP-1] was calculated from the enzyme concentration ([MMP-1]) in the solution, although a fraction of MMP-1 could be inactive. Fibrillogenesis was inhibited with 1 M glycerol, which had no effect on the cleavage rate.

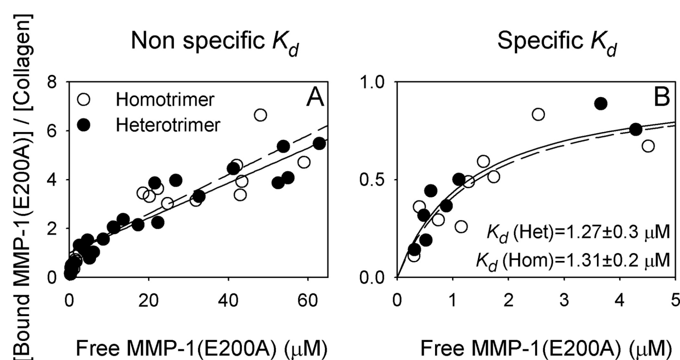


FIGURE 3. **Equilibrium binding of MMP-1(E200A) to homo- and heterotrimeric mouse-tail-tendon type I collagen at room temperature.** *A*, number (*N*) of bound MMP-1(E200A) at 10–65 μM concentration of free molecules was well fitted by a *straight line* with the intercept at *N* = 1. *B*, after subtracting this fitted low affinity binding, the number of MMP-1(E200A) bound below 5 μM was consistent with high affinity binding (K_d = 1.3 ± 0.3 μM) at a single site. *Hom*, homotrimer (*dashed lines* in *A* and *B*); *Het*, heterotrimer (*solid lines* in *A* and *B*).

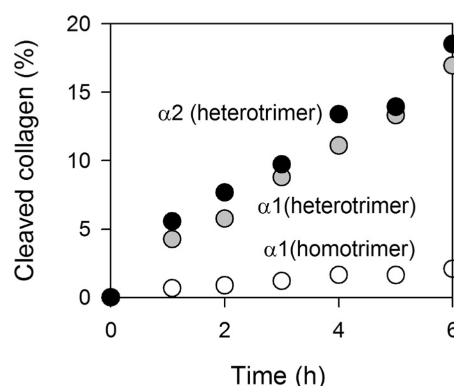


FIGURE 4. **Cleavage of the α1(I) and α2(I) chains in 1:1 mixture of mouse homo- and heterotrimers (0. 2 μM each) by 5.8 μM MMP-1(ΔC) combined with 11 μM MMP-1(E200A) at 25 °C.**

mers, ~17 times at 25 °C and ~ 6 times at 35 °C (Fig. 1*C*). The smaller difference at 35 °C indicated higher activation energy for the homotrimer cleavage. Aside from lower overall enzyme efficiency, the results were essentially the same for the homo- and heterotrimer cleavage by MMP-13 (supplemental Fig. S1).

The dependence of the initial cleavage rate V_0 on collagen concentration [*C*] was consistent with Michaelis-Menten kinetics (Fig. 2) as shown in Equation 1,

$$V_0 = V_{max}[C]/(K_m + [C]) \quad \text{(Eq. 1)}$$

where V_{max} is the maximum cleavage rate and K_m is the Michaelis constant.

For the heterotrimers, K_m = 0.8 ± 0.08 μM was as reported by Welgus *et al.* (32). To our surprise, K_m = 0.9 ± 0.2 μM for the homotrimers was also the same. The slower homotrimer cleavage was entirely attributable to the difference in V_{max}. (A similar K_m value was also reported for homotrimers refolded from denatured α1(I) chains (4), but those data were inconclusive because refolding could result in improper chain alignment altering the cleavage site.)

Enzyme Binding—The same K_m value for the homo- and heterotrimers was inconsistent with stronger MMP-1 binding to the heterotrimers, which we had expected based on reported (24) preferential MMP-1 interactions with the α2(I) chain. To assess preferential binding directly, we measured the equilibrium dissociation constant (K_d) for MMP-1 (E200A), in which Glu-200 was substituted with Ala to inhibit peptide bond hydrolysis (24). For these and subsequent measurements, we used mouse tail tendon collagen. The cleavage of mouse collagen by recombinant human MMP-1 was slower, but the relative rate for homo- *versus* heterotrimers was identical to human collagen.

Equilibrium microdialysis experiments revealed low affinity binding (K_d > 50 μM) of at least five MMP-1(E200A) per collagen triple helix (Fig. 3*A*) and high affinity binding (K_d = 1.3 ± 0.3 μM) of a single MMP-1(E200A) at the cleavage site (Fig. 3*B*). The binding to the homo- and heterotrimers was identical. The high affinity K_d value was consistent with that reported in Ref. 24 for guinea pig heterotrimers and with the K_m value in our kinetic experiments. Thus, the homotrimer resistance to MMP-1 could not be attributed to weaker enzyme binding and had to be related to triple helix unwinding and/or cleavage rate of individual chains.

Triple Helix Unwinding and Chain Cleavage—To distinguish the helix unwinding and cleavage of individual chains, these steps had to be decoupled (MMP-1 is released from collagen only after it sequentially cleaves all three chains (24)). Therefore, we investigated the homo- and heterotrimer cleavage by the catalytic domain of MMP-1 (MMP-1(ΔC)) mixed with catalytically inactive MMP-1(E200A). In the mixture, MMP-1(E200A) binds to the triple helix and promotes local unwinding of the chains, exposing them to independent cleavage by MMP-1(ΔC) (24).

We did not observe noticeable collagen cleavage by either MMP-1(ΔC) alone or MMP-1(E200A) alone within 6–8 h, as described previously (24, 33, 34). When 5.8 μM MMP-1(ΔC) was combined with 11 μM MMP-1(E200A), we observed ~10 times faster chain cleavage in the heterotrimers than homotrimers (Fig. 4). The cleavage efficiency (fraction cleaved) of the α2(I) and α1(I) chains within the heterotrimers was similar, suggesting that the slower α1(I) chain cleavage in the homotrimers was related to the resistance of the latter to triple helix unwinding by MMP-1(E200A).

Fiber Cleavage—Without genetic α2(I) chain deficiency (8, 9), the homotrimers are just a fraction of type I collagen (3, 10–17). They co-assemble with the heterotrimers into mixed fibers (31), which may then be susceptible to collagenases due to heterotrimer cleavage.

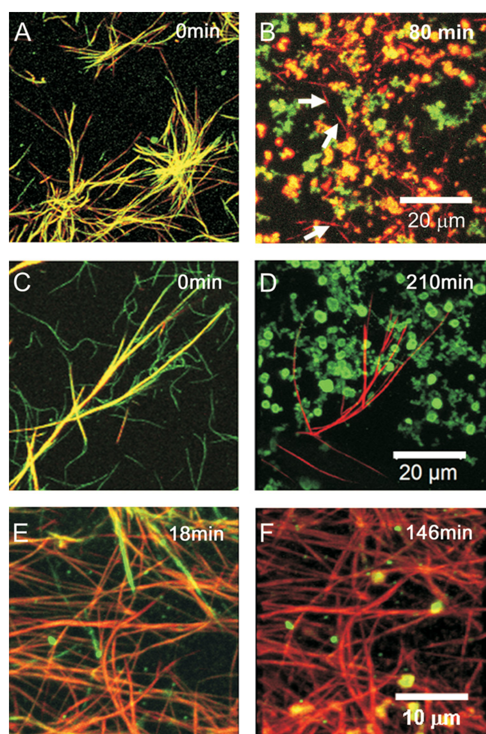


FIGURE 5. **Confocal projections of fibers reconstituted from hetero- (*green*) and homotrimeric (*red*) mouse tail tendon collagen before and after digestion with recombinant human MMP-1.** *A* and *B,* fibers reconstituted from a 1:1 mixture of AF546-labeled homotrimers and AF647-labeled heterotrimers (0.2 mg/ml). Different sample areas were imaged before (*A*) and after (*B*) replacing the buffer with 150 nM MMP-1 in TNC and incubating for 1.5 h at 37 °C. *C* and *D,* fibers reconstituted by adding an ice-cold heterotrimer solution (0.2 mg/ml) to preformed homotrimer fibrils (0.2 mg/ml) and incubating at 32 °C for several days. Different sample areas were imaged before (*C*) and after (*D*) replacing the buffer with 150 nM MMP-1 in TNC and incubating for 3.5 h at 37 °C. *E* and *F,* fibers reconstituted by adding an ice-cold homotrimer solution (0.2 mg/ml) to preformed heterotrimer fibrils (0.2 mg/ml) and incubating at 32 °C for several days. The same area of the sample was imaged after incubating with 150 nM MMP-1 at 37 °C for 18 min (*E*) and 2.5 h (*F*).

To test the cleavage of such mixed fibers, we reconstituted them by *in vitro* fibrillogenesis from fluorescently labeled mouse homo- and heterotrimers and incubated them with MMP-1 at 37 °C. We observed complete disintegration of most fibers after several hours (Fig. 5, *A* and *B*), but we also observed small residual fibers composed almost entirely of the homotrimers (shown by *arrows* in Fig. 5*B*). The residual homotrimer fibers likely originated from segregated regions (31) composed of the homotrimers with few or no heterotrimers.

Next, we tested the cleavage of fibers prepared by addition of heterotrimers to preexisting homotrimer fibers (Fig. 5, *C* and *D*) or by addition of homotrimers to preexisting heterotrimer fibers (Fig. 5, *E* and *F*). MMP-1 rapidly degraded fibers containing few or no homotrimers as well as heterotrimers deposited onto surfaces of homotrimer fibers. At the same time, homotrimer fibers (Fig. 5*D*) and heterotrimer fibers with homotrimers deposited onto their surfaces (Fig. 5*D*) were protected from the degradation. Such fibers are likely to survive multiple tissue remodeling cycles and grow due to further buildup of the collagenase-resistant molecules.

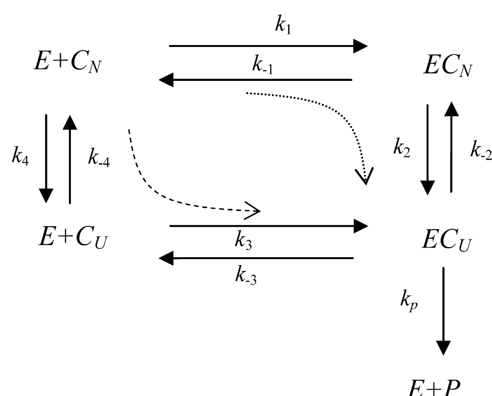
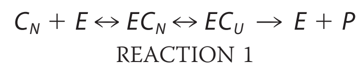


FIGURE 6. **Collagen cleavage pathways.** Each step is characterized by the corresponding rate constant *k*. Sequential cleavage of three collagen chains is represented with a single irreversible step. A model with three irreversible cleavage steps leads to substantively similar predictions.

DISCUSSION

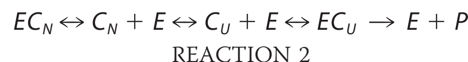
MMP-1 Binding Is Essential for Efficient Triple Helix Unwinding at the Cleavage Site in Type I Collagen—It is widely accepted that enzyme binding, helix unwinding at the cleavage site, and sequential hydrolysis of the chains are the three principal steps in collagen cleavage by MMPs, but two different mechanisms that account for these steps have been proposed. Within one mechanism, the unwinding is promoted by MMP binding, as summarized by Reaction 1 (24),



$$C_N + E \leftrightarrow EC_N \leftrightarrow EC_U \rightarrow E + P$$

REACTION 1

where C_N and C_U indicate collagen in which the MMP cleavage site is in a native (helical) state and in a locally unwound state, respectively; E is the enzyme; EC_N and EC_U are enzyme-collagen complexes; and P denotes triple helix cleavage products. Alternatively, local triple helix unwinding at the cleavage site is postulated to precede enzyme binding, and the EC_N complex is considered to be nonproductive (25) as shown in Reaction 2,



$$EC_N \leftrightarrow C_N + E \leftrightarrow C_U + E \leftrightarrow EC_U \rightarrow E + P$$

REACTION 2

To avoid *a priori* assumptions about the effect of MMP-1 on the local triple helix unwinding, we combined both of these mechanisms into a more general scheme (Fig. 6), within which Reaction 1 corresponds to postulating $k_3 = k_{-3} = k_4 = k_{-4} = 0$ and Reaction 2 corresponds to postulating $k_2 = k_{-2} = 0$. Analysis of previously published data (24) based on this scheme (supplemental material) confirmed much more efficient triple helix unwinding in the presence of bound MMP-1 ($k_2/k_{-2} \gg k_4/k_{-4}$) and dominant role of the upper pathway (Reaction 1).

MMP-1 Binds with the Same Affinity to Homo- and Heterotrimeric Type I Collagen—Potentially, each of the three steps in the cleavage pathway could contribute to the homotrimer resistance to MMP-1. However, the same Michaelis constant (K_m) for the homo- and heterotrimers suggests that the binding step contribution may be excluded (Fig. 2); K_m is determined primarily by MMP-1 binding affinity to collagen (supplemental material). Direct measurements of the binding affinity for MMP-1(E200A) support this conclusion (Fig. 3).

Indeed, the corresponding equilibrium dissociation constant (K_d) is the same for homo- and heterotrimers and close to the K_m value of MMP-1 ($K_d = 1.3 \pm 0.3\ \mu$M and $K_m = 0.9 \pm 0.2\ \mu$M).

Note that nonspecific enzyme binding and migration along fibers might play some role in fiber cleavage (35) but not in cleavage of solubilized collagen. Indeed, the nonspecific binding away from the cleavage site is negligible at relevant ($\ll 1\ \mu$M) MMP-1 concentrations and similar for homo- and heterotrimers (Fig. 3).

Type I Homotrimers Resist Local Triple Helix Unwinding by MMP-1—Not only MMP-1 binds to homo- and heterotrimers with the same affinity, but it also hydrolyzes unwound α1(I) and α2(I) chains with the same efficiency (Fig. 4). We also observed similar hydrolysis efficiency of the α1(I) and α2(I) chains in molecules with the MMP cleavage site intrinsically unwound due to different mutations. In the latter experiments (to be reported elsewhere), the chains were cleaved by MMP-1(ΔC) without MMP-1(E200A).

These findings are consistent with the earlier results of Chung *et al.* (24). Chung *et al.* (24) reported that the α1(I) chain was more readily cleaved by MMP-1(ΔC) than the α2(I) chain in the presence of MMP-1(E200A), which let them propose that MMP-1(E200A) preferentially binds the unwound α2(I) chain. This binding renders the α1(I) chain more susceptible to cleavage by "cutter" enzymes, such as MMP-1(ΔC) and human leukocyte elastase. However, more efficient cleavage of the α1(I) chain by MMP-1(ΔC) was apparent in Ref. 24 only from the ratio of uncleaved α1(I) and α2(I) chains toward the end of the experiment and not from the fractions of cleaved α1(I) and α2(I) chains at the beginning, consistent with the present results (Fig. 4). These observations may be explained by stronger interaction of the unwound α2(I) chain with MMP-1(ΔC) than with MMP-1(E200A), resulting in just a small difference in the susceptibility of the α1(I) and α2(I) chains to cleavage by MMP-1(ΔC). Such a difference may be noticeable only toward the end of the experiment, when most of the chains have been cleaved. A much larger difference in the susceptibility of unwound α1(I) and α2(I) chains to cleavage by human leukocyte elastase (24) may then be explained by weaker interaction of the α2(I) chain with human leukocyte elastase than with either MMP-1(ΔC) or MMP-1(E200A), resulting in a stronger effect of the α2(I) chain binding to MMP-1(E200A) on the cleavage.

A slower cleavage of homotrimers may be explained only by their resistance to the local triple helix unwinding at the cleavage site. This conclusion follows not only from excluding the binding and chain hydrolysis steps but also from thermodynamic analysis of our results within the Fig. 6 cleavage scheme (supplemental material), and from qualitative interpretation of the experiments in Fig. 4. The resistance of α1(I) homotrimers to the local unwinding may also explain their universal resistance to different collagenolytic MMPs (3), because the unwinding is required for the cleavage by all of these enzymes.

MMP-1 Facilitates Stochastic Triple Helix Opening Rather than Actively Unwinding It—Although the unwinding is promoted by MMP-1 binding (24), MMP-1 does not seem to *actively* unwind the helix. Unlike DNA helicases, MMP-1 does not utilize ATP hydrolysis (an energy source is necessary for active unwinding), and models of active unwinding appear to be inconsistent with the observed cleavage kinetics (25). Our data support this conclusion and provide an additional insight into the unwinding mechanism. Indeed, the cleavage kinetics for both homo- and heterotrimers is well described by the model in which MMP-1 binding simply promotes reversible unwinding by shifting the equilibrium between the native helical (EC_N) and locally unwound (EC_U) states of collagen (Fig. 6). Thermodynamic analysis of this model (see supplemental material) suggests that most molecules remain in the native state after MMP-1 binding ($[EC_U]/([EC_U] + [EC_N]) \ll 1$), at least in the case of the homotrimers. In other words, MMP-1 binding facilitates stochastic unwinding of the cleavage site by destabilizing the native helical state (increasing k_2 compared with k_4) and/or stabilizing the unwound state (decreasing $k_{-2} + k_{-3}$ compared with k_{-4}).

Type I Homotrimers Resist Local Unwinding Because of Higher Triple Helix Stability Near the MMP Cleavage Site—The heterotrimers may be less resistant to the local unwinding because the unwound α2(I) chain preferentially binds in the catalytic cleft of MMP-1 (24), stabilizing the enzyme interaction with unwound chains. However, this preferential binding appears to be weak, as suggested by similar cleavage efficiency of the α2(I) and α1(I) chains by MMP-1(ΔC)/MMP-1(E200A) mixtures (in normal collagen) and by MMP-1(ΔC) alone (in mutant collagens with intrinsically unwound cleavage site). Such weak preferential binding is unlikely to be sufficient for 5–10-fold decrease in the homotrimer cleavage rate.

More likely, α1(I) homotrimer resistance to the local unwinding originates from inherently higher stability of the homotrimer triple helix at the cleavage site. This interpretation is not immediately obvious from comparing the amino acid sequences of the α1(I) and α2(I) chains; such analysis is complicated by multiple nontrivial relationships between the chain sequence and local helix stability (36, 37). However, it is consistent with higher overall stability of the homotrimers (30, 38), partial α2(I) chain dissociation from the triple helix at the MMP-1 cleavage site (39, 40), and higher activation energy for the homotrimer cleavage (Fig. 1*C*).

Type I Homotrimers May Alter Tissue Remodeling—Because type I homotrimers were found in a variety of pathological conditions (3, 7–17, 41, 42), their resistance to MMPs may have important implications. For instance, the homotrimers may promote cancer invasion by resisting collagenases massively secreted in tumors and supporting proliferation and migration of cancer cells (3). In fibrosis, they may hinder collagen degradation by MMPs.

In addition, our observations suggest that even a minor fraction of the homotrimers may alter tissue remodeling. Indeed, segregation of the homo- and heterotrimers (31) may result in formation of collagenase-resistant fiber regions composed primarily of the homotrimers (Fig. 5, *A* and *B*). These regions may nucleate homotrimer fibers that will grow instead of being disassembled during multiple tissue remodeling cycles, eventually causing tissue disorganization (Fig. 5, *C* and *D*). This effect may be further amplified by the presence of other collagens (*e.g.* type III and V) in heterotypic fibers, resulting in pathogenic accu-

mulation of collagenase-resistant fibers. We are presently testing this hypothesis.

One crucial unanswered question is why oim mice, in which all type I collagen is homotrimeric, develop glomerular sclerosis (43) but not a generalized fibrosis. Similarly, homozygous Col1a1$^{r/r}$ mice, with an altered α1(I) chain sequence at the primary MMP cleavage site, have some sclerotic collagen deposits (44–46) but no generalized fibrosis. In contrast, MMP-14 knock-out mice have a shortened life span due to severe, generalized collagen turnover deficiency (47). The assumption that MMP-14 is the primary mouse collagenase may explain the collagen turnover deficiency in MMP-14 knockouts (47), but not nearly normal turnover of MMP-14-resistant collagen in oim and Col1a1$^{r/r}$ mice. Apparently, MMP-resistant collagen can be degraded via some alternative mechanisms. Furthermore, given its multiple substrates (48), MMP-14 may be crucial for collagen turnover not just as a collagenase but because it affects fibroblast function and motility, for example. Better understanding of homotrimeric collagen turnover in oim mice may help to solve this puzzle.

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