Involvement of Fibronectin Type II Repeats in the Efficient Inhibition of Gelatinases A and B by Long-chain Unsaturated Fatty Acids*

The matrix metalloproteinases gelatinase A (MMP-2) and gelatinase B (MMP-9) are implicated in the physiological and pathological breakdown of several extracellular matrix proteins. In the present study, we show that long-chain fatty acids (e.g. oleic acid, elaidic acid, and cis- and trans-parinaric acids) inhibit gelatinase A as well as gelatinase B with Kᵢ values in the micromolar range but had only weak inhibitory effect on collagenase-1 (MMP-1), as assessed using synthetic or natural substrates. The inhibition of gelatinases depended on fatty acid chain length (with C₁₈ > C₁₆, C₁₄, and C₁₀), and the presence of unsaturations increased their inhibitory capacity on both types of gelatinase. Ex vivo experiments on human skin tissue sections have shown that micromolar concentrations of a long-chain unsaturated fatty acid (elaidic acid) protect collagen and elastin fibers against degradation by gelatinases A and B, respectively. In order to understand why gelatinases are more susceptible than collagenase-1 to inhibition by long-chain fatty acids, the possible role of the fibronectin-like domain (a domain unique to gelatinases) in binding inhibitory fatty acids was investigated. Affinity and kinetic studies with a recombinant fibronectin-like domain of gelatinase A and with a recombinant mutant of gelatinase A from which this domain had been deleted pointed to an interaction of long-chain fatty acids with the fibronectin-like domain of the protease. Surface plasmon resonance studies on the interaction of long-chain fatty acids with the three individual type II modules of the fibronectin-like domain of gelatinase A revealed that the first type II module is primarily responsible for binding these compounds.

Matrix metalloproteinases (MMPs)¹ compose a family of at least 23 related zinc-dependent endopeptidases (1) that are collectively able to degrade extracellular matrix proteins such as collagens, laminins, fibronectin, elastin, and proteoglycans. They are consequently implicated in physiological remodeling of connective tissue occurring in embryonic development and repair (2–4). Most of them are secreted as inactive proenzymes and are then extra- or pericellularly activated by other MMPs or serine proteinases (5). Their catalytic activities are strictly controlled by endogenous specific inhibitors designated as tissue inhibitors of metalloproteinases (TIMPs) (6) and also α₂-macroglobulin (7). The balance between activated MMPs and TIMPs determines the overall MMP proteolytic activity and consequently the extent of extracellular matrix degradation. Local disruption of the MMP-TIMP balance can lead to pathological degradative processes including rheumatoid arthritis, atherosclerosis, tumor growth, and metastasis (8–10). MMPs are multidomain enzymes containing propeptide, catalytic and, except matrilysin (MMP-7), MMP-23, and endometastase/matri-lisin-2 (MMP-26), hemopexin-like domains. Gelatinase A (MMP-2) and gelatinase B (MMP-9) contain in addition three tandem copies of a 58-amino acid fibronectin type II (FN-II) module (11, 12) inserted within their catalytic domain (13). The basic fold of the FN-II modules is composed of a pair of β sheets that form a hydrophobic pocket accessible to solvent (13). The FN-II repeats confer high affinity binding of these enzymes to gelatin and insoluble elastin (14–16), a prerequisite for their efficient proteolysis. Long-chain unsaturated fatty acids inhibit the expression and activity of aggrecanases (17). They bind to serine proteinases such as neutrophil elastase (18, 19) and plasmin (20, 21) and modulate their catalytic activities. Studies by Suzuki et al. (22) show that oleic acid, 18-carbon fatty acid with one double carbon bond in the cis position, partially inhibits the formation of lung metastases from subcutaneous implantation of colon carcinoma cells in athymic mice. We recently reported that oleic acid inhibited in a dose-dependent manner the hydrolysis of the fluorogenic substrate Mca-L-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH₂ (23) by gelatinase A (24, 25). We report here an in vitro investigation showing that fatty acids, depending on their chain length and degree of unsaturation, inhibit gelatinases A

¹ The abbreviations used are: MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinases; FN-II, fibronectin type II; pro-ΔVal₁⁵⁹–Gln₁⁶⁰ or pro ΔFN-II gelatinase A, progelatinase A mutant with amino acids Val¹⁵⁹–Gln₁⁶⁰ deleted; Mca, [7-methoxycoumarin-4-yl] acetyl; Dpa, [3-‘2’,4‘-dinitrophenyl]-1,2,3-diaminopropionyl]; APMA, p-amino phenylmercuric acetate; Me₂SO, dimethyl sulfoxide; SPR, surface plasmon resonance.
TABLE I

Inhibition of gelatinases A and B activities by fatty acids

<table>
<thead>
<tr>
<th>Long-chain fatty acids</th>
<th>Trivial names</th>
<th>Gelatinase A</th>
<th>Gelatinase B</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.0</td>
<td>Capric</td>
<td>NI a</td>
<td>NI</td>
</tr>
<tr>
<td>14:0</td>
<td>Myristic</td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td>c9–14:1</td>
<td>Myristoleic</td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td>16:0</td>
<td>Palmitic</td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td>c9–16:1</td>
<td>Palmitoleic</td>
<td>29.3 ± 5.5</td>
<td>34.3 ± 4.9</td>
</tr>
<tr>
<td>18:0</td>
<td>Stearic</td>
<td>35.2 ± 5.6</td>
<td>47.4 ± 7.7</td>
</tr>
<tr>
<td>c9–18:1</td>
<td>Oleic</td>
<td>4.3 ± 0.4</td>
<td>6.4 ± 0.4</td>
</tr>
<tr>
<td>t9–18:1</td>
<td>Elaidic</td>
<td>4.4 ± 0.3</td>
<td>5.8 ± 0.3</td>
</tr>
<tr>
<td>c9, t11, t13, c15–18:4</td>
<td>cis-Parinaric</td>
<td>8.1 ± 1.2</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td>All t9, 11, 13, 15–18:4</td>
<td>trans-Parinaric</td>
<td>5.7 ± 0.4</td>
<td>1.9 ± 0.6</td>
</tr>
</tbody>
</table>

a NI, no or less than 10% of inhibition, at [I]i = 40 μM.

and B with similar efficiency but only have low inhibitory capacity toward collagenase-1.

We conclude that interaction between FN-II domain and unsaturated fatty acids leads to gelatinase inhibition because of the following: (i) oleic acid displayed markedly weaker inhibitory capacity toward gelatinase A deleted in FN-II modules; (ii) fatty acids bound avidly to the first FN-II module; and (iii) this module totally prevented the oleic acid-mediated inhibition of full-length gelatinase A. The physiological relevance of our findings was substantiated by ex vivo experiments on human skin tissue sections demonstrating that C18-unsaturated fatty acid efficiently impeded the collagenolytic and elastolytic activities of gelatinases A and B, respectively.

INCLUSION MECHANISM OF GELATINASES BY LONG-CHAIN FATTY ACIDS—The inhibitory effect of fatty acids (from C10 to C18, either saturated or cis- or trans-unsaturated) against gelatinase A (full-length or FN-II-deleted forms), gelatinase B, or collagenase-1 was analyzed using the fluorescent quenched substrate Mca-PLGL-(Dpa)-AR-NH2. Two hundred picomolar of each MMP species were preincubated for 15 min at 22 °C with 0–40 μM fatty acid in a 50 mM HEPES buffer, pH 7.5, containing 150 mM NaCl and 5 mM CaCl2. The assays were initiated by adding 2 μM Mca-PLGL-(Dpa)-AR-NH2. The final concentration of dimethyl sulfoxide (Me2SO) used to dissolve fatty acid and fluorogenic substrate never exceeded 1% (v/v). The reaction was allowed to proceed at 22 °C for 20 min (gelatinase A), 60 min (gelatinase B), or 180 min (collagenase 1) and then was stopped by adding 10 mM EDTA. Under these experimental conditions, MMPs generated a similar intensity of fluorescence, allowing the comparison of the inhibition results. The effect of substitution of the carboxylic end by alcohol or hydroxamate group on the inhibitory potency of oleic acid against gelatinase A was similarly evaluated. The rate of substrate cleavage was measured in quadruplicate for each fatty acid or derivative concentration examined, using a Perkin Elmer LS 50B spectrophotofluorimeter with excitation and emission wavelengths of 325 and 387 nm, respectively. Less than 5% of the substrate was hydrolyzed during the rate measurements. Addition of fatty acid after the digestion of fluorogenic substrate had no effect on the fluorescent signal. Nonlinear regression analysis with the Grafit computer software (R. J. Leatherbarrow, Erithacus Software) allowed us to calculate the best estimates of the equilibrium dissociation constant of the enzyme-inhibitor complex or inhibition constant Ki, using the integrated Equation 1 (29),

\[
\frac{v}{v_0} = 1 - \frac{[I] + K_i}{2[E]} - \frac{[E] + [I] + K_i^2 - 4[E][I]^{1/2}}{2[E]} 
\]

where \(v\) is the rate of substrate hydrolysis in the presence of inhibitor; \(v_0\) is the rate in its absence; and [E] and [I] are the initial concentrations of enzyme and inhibitor, respectively. The inhibitory effect of oleic, elaidic, or stearic acids (0–40 μM) was further evaluated against gelatinase A, using a natural substrate, i.e. heat-denatured [1H]collagen type I. Briefly, 1.6 nm gelatinase A was first mixed with 0–40 μM fatty acid in a 50 mM Tris-HCl buffer, pH 7.4, containing 150 mM NaCl and 10 mM CaCl2 for 15 min at 22 °C before incubation with radiolabeled natural substrate for 20 h at 37 °C (30). The 50% inhibitory concentrations (IC50) of fatty acids for gelatinase A were determined using the Grafit computer software.

KINETIC ANALYSIS OF OLEIC ACID INHIBITION OF FULL-LENGTH AND ΔFN-II GELATINASES A—For each enzyme, the mode of inhibition of oleic acid was analyzed with the Grafit computer software, using the graphical methods of either Dixon or Cornish-Bowden (see Ref. 31).
sensor chip was washed for 5 min with 40 mM α-ethyl-β-D-glucopyranoside in water. Fatty acid (20 μl of an 8 mM solution in Me₂SO) was then injected. The process of spontaneous fatty acid adsorption on the sensor chip was monitored. When the sensogram reading began to level out, the flow rate was briefly increased to 100 μl/min to suppress the multiple lipid layers formed on the sensor chip surface. An additional injection of 10 mM NaOH (10 μl) was performed to regenerate the sensor chip and to reach a stable baseline. To assess the extent of coverage of the sensor chip surface by both fatty acids, we injected 10 μl of 0.1 g/liter bovine serum albumin in 4.4 mM Na₂HPO₄ buffer, pH 7.4, 130 mM NaCl, 3 mM KCl. The amount of bovine serum albumin bound to the sensor chip surface corresponded to 43 resonance units, a value much lower than that typically obtained with a surface fully coated with dimyristoyl phosphatidylcholine or palmitoyloleoyl phosphatidylcholine. Binding experiments with col 123 were performed in the same buffer. After each cycle, the sensor chip was regenerated by injecting 10 μl of 10 mM NaOH. These regeneration conditions allowed to restore the base-line level observed before each injection. The col 123 binding kinetics were measured at six different concentrations of analyte, i.e., 3.125, 6.25, 12.5, 25, 50, and 100 μM. We similarly studied oleic acid interactions with the isolated FN-II modules of gelatinase A, i.e., col 1, col 2, or col 3. The binding curves were analyzed using the nonlinear data fitting software BIAevaluation to obtain the rate constants of association (kₐ) and dissociation (kₐ) and the equilibrium constants of dissociation (Kₑ).

Role of FN-II Modules in the Inhibition of Gelatinase A by Oleic Acid—Oleic acid (5 μM) and gelatinase A (200 μM) were preincubated for 15 min at 22 °C with molar fold excess (1–100-fold in comparison with gelatinase A) of the trimodular protein col 123 or the single module proteins col 1, col 2, or col 3, and residual enzymatic activity was measured with Mca-PLGL-(Dpa)-AR-NH₂ (2 μM), as described above. Whatever the concentration used, col 123, col 1, col 2, or col 3 did not exhibit inhibitory effect against gelatinase A.

Computerized Morphometric Analysis of Elaidic Acid Inhibition of Skin Tissue Degradation by Gelatinases A and B—A set of three skin tissue sections was laid on a coated polylysine microscopic slide (Biorad) and overlaid with 10 μl of 50 mM Tris-HCl buffer, pH 7.4, containing 5 mM NaCl and 5 mM CaCl₂ (assay buffer) or the same buffer containing 50 mM APMA-activated gelatinase A or gelatinase B. The preparations were then incubated for 4 h at 37 °C in a moist chamber. After incubation, the tissue sections were rinsed and stained with polyphenolic catechin-fuschin and red sirus for staining of elastic and collagen fibers, respectively (32). Elastic fibers appeared in deep blue-black, and the background was poorly stained and collagen fibers were revealed in red-orange. This allowed a further quantitative estimation of the area (A₀) and volume (V₀) fractions occupied by these fibers. For eladic acid inhibition assays, gelatinase A or gelatinase B were first incubated with 0, 1, or 10 μM fatty acid for 15 min at 22 °C; alternatively, tissue sections were preincubated with 1 or 10 μM eladic acid or Me₂SO-containing assay buffer as negative control for 30 min at 37 °C before adding gelatinase A or gelatinase B. The microscopic slides were then observed under a Zeiss standard 14 microscope equipped with CF 126 PHR video camera, and computerized analyses of the elastic and collagenic fibers were carried out as described previously (32). Briefly, three skin serial sections were used for each assay, and 10 fields of dimensions 0.7 × 0.7 mm were analyzed for each skin tissue section. Black and white images generated by the video camera were converted into 256 different gray levels using a Sophotec NUM 600 image memory, transferred to a BFM 186 microcomputer, and finally analyzed using a software for mathematical morphology. The area fraction (A₀) occupied by the elastic and collagen fibers was automatically calculated. The area fraction represents the surface of fibers as a function of the tissue area analyzed. For the elastic fibers, the volume fraction (V₀) was also determined and corresponds to \(A₀ × k\), where \(k < 1\) represents the Weibel correction factor \(k = \frac{d}{d + t}\), with \(d\) and \(t\), elastic fiber diameter, and section thickness, respectively, in μm). The average diameter of the elastic fibers was obtained semi-automatically using skin tissue sections treated or not and a calibrated slide.

RESULTS

Relationship between Fatty Acid Structure and Inhibition of MMP Activity—The effect of fatty acids varying in chain length and degree of unsaturation was first evaluated on gelatinases A and B using the fluorogenic Mca-PLGL-(Dpa)-AR-NH₂ substrate. Table I shows that both enzymes were inhibited with a similar efficiency by fatty acids. Inhibition depended on their alkyl chain length (with C₁₈ > C₁₆, C₁₄, and C₁₀). The presence of unsaturations in fatty acids increased the inhibition of both gelatinases. Furthermore, gelatinases A and B inhibition did not depend on the cis-trans configuration of double bond(s) since oleic and eladic acid, its trans-counterpart, displayed similar Ki values; also, no difference was observed between cis- and trans-parinaric acids. Such polyunsaturated fatty acids were, however, more efficient as inhibitors toward gelatinase B versus gelatinase A (Table I).

When they were tested against collagenase-1, only unsaturated fatty acids with 18 carbon atoms exhibited a low inhibitory effect, with a Ki value equal to 59.6 ± 5.7 μM for oleic acid.
Contrary to data obtained with gelatinases, a trans-configuration of the unsaturation slightly improved collagenase-1 inhibition; 40 mM of oleic or elaidic acids inhibited by 30 and 40%, respectively, the degradation of fluorogenic substrate by collagenase-1.

Effect of Derivatization of the COOH-terminal End Group of Oleic Acid on Its Gelatinase A Inhibitory Potential—Changing carboxylic end group of oleic acid to hydroxamic group, a more potent bidentate ligand for catalytic zinc, did not dramatically improve gelatinase A inhibition, with the $K_i$ value only decreasing from 4.3 $\pm$ 0.4 to 1.8 $\pm$ 0.2 mM (Fig. 1). Also, replacement of carboxylic group by an alcohol group, not considered as a zinc ligand, did not strikingly impair the inhibitory activity of oleic acid, with the $K_i$ value only rising from 4.3 $\pm$ 0.4 to 6.3 $\pm$ 0.3 mM (Fig. 1).

Involvement of FN-II Repeats in the Inhibition of Gelatinases A and B by Fatty Acids—Our data suggested that FN-II repeats, present in gelatinases A and B but absent in collagenase-1, could represent selective targets for fatty acids. We therefore evaluated the inhibitory capacity of oleic, elaidic, and stearic acids against gelatinase A deleted in FN-II repeats. Fig. 2 illustrated the weak inhibition of oleic and elaidic acids toward truncated gelatinase A; as shown above for collagenase-1, oleic acid was less efficient than its trans-counterpart, elaidic acid, with $K_i$ values of 32.5 $\pm$ 3.0 and 12.3 $\pm$ 1.6 mM, respectively. Again, stearic acid did not inhibit truncated gelatinase A (Fig. 2).

Kinetic Analysis of Full-length and ΔFN-II Gelatinases A Inhibition by Oleic Acid—The mode of inhibition of each enzyme by oleic acid was evaluated. In both cases, Dixon plots (data not shown) were consistent with oleic acid acting as either competitive or mixed inhibitor, with $K_i$ values graphically estimated to 1.6 and 26.3 mM for full-length and truncated gelatinases A, respectively. Cornish-Bowden plot allowed us to distinguish between these two possibilities (Fig. 3). The inter-
enzymatic activity was measured with Mca-PLGL-(Dpa)-AR-NH₂ (2 A by oleic acid. Oleic acid (5

A concentration. All experiments were done in duplicate.

section of lines above the abscissa in the Cornish-Bowden plot was consistent with a mixed mode of inhibition of oleic acid against the full-length gelatinase A (Fig. 3A), whereas it appeared to act as a weak competitive inhibitor toward truncated gelatinase A (Fig. 3B).

Binding of Fatty Acids to the FN-II Modules of Gelatinase A—Potential interactions between fatty acids and the gelatinase A FN-II repeats were studied by SPR analyses using a recombinant peptide corresponding to the three FN-II modules (coll 123) of gelatinase A and oleic or stearic acids. Fatty acids were linked to the surface of a sensor cell as described under “Experimental Procedures.” Solutions of coll 123 were allowed to bind to the immobilized oleic acid or stearic acids, and data were analyzed as a function of time. The results of typical binding assays are shown in Fig. 4, A and B. The association rate constant (kₐ), dissociation rate constant (k_d), and dissociation equilibrium constant (K_D) of coll 123 for oleic acid or stearic acid were calculated from SPR analyses, using the nonlinear data fitting software BLAevaluation (Table II). Oleic and stearic acids bound coll 123, with K_D of 41 ± 1 and 36.1 ± 2 μM, respectively. Furthermore, SPR analysis demonstrated that oleic acid interacted with the first FN-II module, coll 1, with a K_D value of 62 ± 4 μM, close to that obtained with the entire FN-II domain, coll 123; on contrary, the second and third FN-II modules weakly bound oleic acid with dissociation constants of 210 ± 90 and 3900 ± 1400 μM, respectively.

We next investigated whether the ability of oleic acid to bind FN-II modules was related to its gelatinase inhibitory capacity. Gelatinase A activity was measured in the presence of increasing concentrations of trimodular protein coll 123 and an oleic acid concentration producing 50% inhibition, i.e., 5 μM. Increasing concentrations of coll 123 relieved the inhibition of gelatinase A, and the inhibitory effect of oleic acid was totally abolished by a 100-fold molar excess of coll 123 (Fig. 5A). In the same way, we analyzed the effect of isolated FN-II modules, i.e. coll 1, coll 2, and coll 3, on gelatinase A inhibition by oleic acid. A 50-fold molar excess of coll 1 restored up to 95% gelatinase A activity in the presence of 5 μM oleic acid (Fig. 5B). In contrast, only a slight or no restoration of gelatinase A activity was observed in the presence of coll 2 or coll 3.

**Effect of Fatty Acids on the Degradation of Gelatin by Gelatinase A—** Denatured collagen (gelatin) represents physiological substrate for gelatinase A. We also evaluated the effect of oleic, elaidic, and stearic acids on gelatinolytic activity of gelatinase A (Fig. 6). The IC₅₀ values of oleic and elaidic acids, determined using heat-denatured type I collagen, were equal to 1.6 ± 0.3 and 1.3 ± 0.3 μM, respectively, versus 9.6 ± 0.9 μM obtained for stearic acid, their saturated counterpart (Fig. 6). By using the synthetic fluorogenic substrate, at such concentrations, oleic and elaidic acids inhibited −25% of the activity of gelatinase A, whereas 10 μM stearic acid caused any detectable inhibition.

**Effect of Elaidic Acid on the Degradation of Human Skin Collagen and Elastin by Gelatinases A and B**—We previously showed, by quantitative morphometric analysis, that gelatinase A preferentially degraded collagen fibers, whereas gelatinase B displayed elastolytic activity (32). By using a similar approach, the inhibitory effect of elaidic acid on the ex vivo collagenolysis (Fig. 7, A–C) and elastolysis (Fig. 7, D–F) by gelatinases A and B, respectively, has been investigated on human skin tissue sections. In control sections (Fig. 7A), skin collagen fibers appeared as thick homogenous bundles. When skin sections were incubated with 50 ng gelatinase A, the number of collagen fibers decreased (Fig. 7B); they often ap-

![Fig. 5. Effect of FN-II modules on the inhibition of gelatinase A by oleic acid.](image-url)
Inhibition Mechanism of Gelatinases by Fatty Acids

FIG. 7. Ex vivo effect of elaidic acid on ex vivo collagenolysis and elastolysis. Human skin tissue sections were overlaid with buffer alone as control (A and D), or containing 50 nM gelatinases A or B (B and E), or 50 nM gelatinases A or B preincubated with 10 nM elaidic acid (C and F) and then incubated 4 h at 37 °C before staining by red Sirius to visualize collagen fibers (A–C) or polyphenolic catechin-fuschin for elastin fibers (D–F). e, epidermis; d, dermis; v, vessel; arrowhead, collagen bundles; arrow, mature elastic fibers.

peared as scattered bundles, with most of them exhibiting teased extremities. When gelatinase A was preincubated with 1 or 10 µM elaidic acid, a dose-dependent inhibition of collagen fiber hydrolysis was observed (Fig. 7C and Table III). Pretreatment of skin tissue sections by elaidic acid concentration as low as 1 µM impeded degradation of collagen fibers by gelatinase A (Table III).

As shown in Fig. 7D, control skin sections stained with polyphenolic catechin dye revealed an intact elastic fiber system around the vessel. When skin sections were incubated with 50 nM gelatinase B, an important alteration of the elastic fiber network could be evidenced (Fig. 7E). Elastolytic activity was markedly diminished when gelatinase B was preincubated with 1 or 10 µM elaidic acid (Fig. 7F), as assessed by morphometric analyses (Table III). Also, preincubation of skin tissue with 1 µM elaidic acid partially protected tissue against elastolytic activity of gelatinase B (Table III).

DISCUSSION

We recently reported that oleic acid inhibited in a dose-dependent manner gelatinase A activity (24, 25). In this study, we first compared the effect of various fatty acids toward gelatinases A and B and collagenase-1. By using a synthetic fluoro-

genic substrate, the extent of MMP inhibition by fatty acids was related to both fatty acid chain length and to the presence of at least one double carbon bond, as previously evidenced for neutrophil elastase (18). Similar gelatinase inhibition was obtained with cis-unsaturated fatty acids and their trans isomers, in contrast with the stringent conformational requirement observed for neutrophil elastase inhibition (18).

The fatty acid carboxylate end group, a potential zinc-coordinating group, was initially suspected to be the main driving force in fatty acid-mediated gelatinase inhibition (24). However, the hydroxamate derivative of oleic acid did not significantly improve its inhibitory capacity (24), whereas 200- and 500-fold enhancements in inhibitory capacity from carboxylate to hydroxamate inhibitors were previously reported for matrix-lysin and collagenase-1, respectively (33, 34); in addition, the alcohol analogue of oleic acid, a compound unable to chelate the catalytic zinc, was also found nearly as effective as oleic acid in inhibiting gelatinase A, suggesting that, at least for gelatinases, zinc chelation was not the main determinant in enzyme inhibition by fatty acids.

We further examined the possibility that, as for other hydrophobic inhibitors, fatty acids could occupy the catalytic site of MMPs. The catalytic domains of all MMPs display structural homology (35). However, at the vicinity of the catalytic zinc, S1 invagination was found to differ in size and shape among the various MMPs. Based on x-ray crystallographic and homology modeling studies (36), MMPs may be classified into two broad structural subgroups depending on the depth of S1'. Gelatinases A and B possess a deep S1' pocket with leucine at position 214; in collagenase-1, leucine residue is replaced by arginine that partly occludes that enzyme subsite (37). Differences observed in fatty acid inhibition efficiency between gelatinases and collagenase-1 could therefore result from the difference in the depth of the S1' pocket. However, the marked difference in extent of inhibition between full-length gelatinase A and ΔFN-II gelatinase A suggests that inhibition must involve additional mechanism(s). For ΔFN-II gelatinase A, the weak oleic acid inhibition was characterized as fully competitive by different analyses, indicating that unsaturated fatty acid could lie on to the enzyme-extended active site. In contrast, oleic acid behaved as a more potent partially competitive inhibitor toward full-length gelatinase A, pointing to other binding modes possibly implicating the FN-II domain of the enzyme.

The FN-II domain of gelatinases consists of three FN-II homology units (11, 12). Comparison of secondary structures between FN-II modules of gelatinase A and kringle domains in plasmin revealed a remarkable concordance in the position of β sheets, suggesting an overall structural homology between those structures (38). The kringles mediate the binding of multidomain proteins to other proteins. The presence of FN-II modules allows gelatinases A and B to bind and degrade insoluble elastin, a highly hydrophobic extracellular matrix macromolecule (16, 39). Binding of long-chain fatty acids to kringles was found to modulate plasmin activity toward both synthetic substrate and fibrinogen (20, 21). The involvement of the FN-II modules of gelatinase A in its inhibition by fatty acids was therefore investigated. Binding of oleic acid to plasma fibronectin has been documented previously (40). SPR experiments showed that col 123, a recombinant protein containing the three FN-II repeats of gelatinase A (14, 27), equally bound oleic and stearic acids, suggesting that such an interaction did not involve the double carbon bond of the fatty acid but somehow contributed to the higher inhibition efficiency of unsaturated fatty acids versus their saturated forms. The FN-II domain could act as a “helping hand” in terms of additional binding or
Inhibition Mechanism of Gelatinases by Fatty Acids

### Table III

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Collagen fibers&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Elastin fibers&lt;sup&gt;b&lt;/sup&gt;</th>
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<tr>
<td></td>
<td>Area fraction&lt;sup&gt;(Aₐₐ)&lt;/sup&gt;</td>
<td>Hydrolysis</td>
</tr>
<tr>
<td>Control</td>
<td>65.0 ± 4.7</td>
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<tr>
<td>GelA</td>
<td>38.3 ± 3.2</td>
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<tr>
<td>GelA + 1 μM EA</td>
<td>45.3 ± 1.4</td>
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</tr>
<tr>
<td>GelA + 10 μM EA</td>
<td>55.0 ± 3.0</td>
<td>15</td>
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<tr>
<td>1 μM EA + GelA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.5 ± 1.9</td>
<td>27</td>
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<tr>
<td>Control</td>
<td>6.50 ± 4.7</td>
<td>41</td>
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<tr>
<td>GelB</td>
<td>2.5 ± 0.2</td>
<td>38</td>
</tr>
<tr>
<td>GelB + 1 μM EA</td>
<td>3.2 ± 0.4</td>
<td>20</td>
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<tr>
<td>GelB + 10 μM EA</td>
<td>3.8 ± 0.3</td>
<td>5</td>
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</tbody>
</table>

<sup>a</sup> The percentage of collagen fibers solubilized by gelatinase A (GelA) was calculated from $A_{AA}$ (control) and $A_{AA}$(assay) to correspond to values obtained in the absence or presence of enzyme, respectively.

<sup>b</sup> The percentage of elastin fibers solubilized by gelatinase B (GelB) was calculated from $V_{VV}$ (control) and $V_{VV}$(assay) to correspond to values obtained in the absence or presence of enzyme, respectively.

For the protection assays, skin tissue sections were first incubated with 1 μM elaidic acid, rinsed carefully, and overlaid with the gelatinase solution.

orienting the fatty acid. According to the x-ray structure of progelatinase A (13), the three FN-II modules turn outward to form a “three-pronged fishhook.” The basic structure of each module is composed of a pair of β sheets connected with a short α helix. The β sheets form a hydrophobic pocket allowing sub-strate binding. Interestingly, although the three FN-II modules were shown to act cooperatively to bind gelatin (14, 27, 41), oleic acid specifically bound the first FN-II module of gelatinase A. In addition, at a concentration as low as 15 mM, the first FN-II module, but not the second or third FN-II modules, completely abolished gelatinase A inhibition by 5 μM oleic acid. Preliminary data (not shown) demonstrated that the first FN-II module, but not the second and third modules, served as a nucleating site to form oleic acid micromicles. Altogether, these results suggest that more than 1 mole of oleic acid could bind the first FN-II module of gelatinase A.

Fatty acids were more potent in inhibiting gelatinase activity on protein substrate than on a synthetic substrate. A direct involvement of the FN-II domain in binding of gelatinases A and B to gelatin and native collagen has been extensively reported (14, 15, 42–45). The stearic acid efficiency in inhibiting denatured type I collagen degradation by gelatinase A was thus in agreement with the ability of the FN-II domain to bind to the saturated acid. Computerized morphometric analysis of skin tissue sections has shown that gelatinase B degraded elastic fibers and fibrillin, whereas gelatinase A preferentially degraded type III collagen fibers (32). By using a similar experimental model, elaidic acid efficiently impaired degradation of collagen and elastic fibers by gelatinase A and gelatinase B, respectively, by both inhibiting gelatinase activity and protecting fibers against hydrolysis. These data strongly suggest that besides their direct inhibitory effect toward MMPs, fatty acids could also bind and protect extracellular matrix macromolecules, as demonstrated previously (40) for oleic acid protection of fibronectin against neutrophil elastase or cathepsin G.

Hydrolysis of elastic fibers in the arterial walls is a critical step in the development of atherosclerosis (46, 47). By using an ex vivo model of aortic explants cultured with or without monocytes/macrophage–like cells, Katayama et al. (48) reported a fragmenta-tion of elastic fibers of the aortic explants by gelatinase B. Targeted gene disruption of gelatinase B suppressed development of abdominal aortic aneurysms in mice (49), emphasizing the role of this MMP in such a degenerative condition associated with aging and atherosclerosis. The gelatinase inhibition by fatty acids as we have disclosed in an in vitro study and then confirmed in an ex vivo model on human skin tissue (32) could thus explain, at least partly, the relationship between a Mediterranean-style diet, in which olive oil is the main source of fat, and protection from cardiovascular disease (50); it could also be involved in the reduction of lung metastasis formation by colon carcinoma cells in athymic mice after treatment by oleic acid (22).

In conclusion, our data provide in vitro and ex vivo evidence that fatty acids could contribute to regulate the extracellular matrix breakdown by inhibiting gelatinases A and B. This inhibition was related to fatty acid chain length and to the presence of double carbon bond(s) and was driven mainly by interaction of fatty acid with the first FN-II module of gelatinase A.

Acknowledgments—We are indebted to Dr. Jacques Cohen (Faculty of Medicine, Reims, France) for assistance with the BLAcore X system. We thank Dr. Patrick Henriet (Institute of Cellular Pathology, Brussels, Belgium) for critical reading of this manuscript. Dr. Marie-Paule Mingeot (Faculty of Pharmacy, Brussels, Belgium) provided valuable advice about fatty acid chemistry.

REFERENCES

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doi: 10.1074/jbc.M011664200 originally published online February 21, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M011664200

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