Previously we have shown that THP-1 cells synthesize matrix metalloproteinase-9 (MMP-9) where a fraction of the enzyme is strongly linked to a proteoglycan (PG) core protein. In the present work we show that these pro-MMP-9-PG heteromers have different biochemical properties compared with the monomeric form of pro-MMP-9. In these heteromers, the fibronectin II-like domain in the catalytic site of the enzyme is hidden, and the fibronectin II-like-mediated binding to gelatin and collagen is prevented. However, a fraction of the pro-MMP-9-PG heteromers interacted with gelatin and collagen. This interaction was not through the chondroitin sulfate (CS) part of the PG molecule but, rather, through a region in the PG core protein, a new site induced by the interaction of pro-MMP-9 and the PG core protein, or a non-CS glycosaminoglycan part of the PG molecule. The interaction between pro-MMP-9-PG heteromers and gelatin was weaker than the interaction between pro-MMP-9 and gelatin. In contrast, collagen I bound to pro-MMP-9-PG heteromers and pro-MMP-9 with approximately the same affinity. Removal of CS chains from the PG part of the heteromers did not affect the binding to gelatin and collagen. Although the identity of the PG core protein is not known, this does not have any impact on the described biochemical properties of the heteromer or its pro-MMP-9 component. It is also shown that a small fraction of the PG, which is not a part of the pro-MMP-9-PG heteromer, can bind gelatin. As for the pro-MMP-9-PG heteromers, this was independent of the CS chains. The structure that mediates the binding of free PG to gelatin is different from the corresponding structure in the pro-MMP-9-PG heteromer, because they were eluted from gelatin-Sepharose columns under totally different conditions. Although only a small amount of pro-MMP-9-PG heteromer is formed, the heteromer may have fundamental physiological importance, because only catalytic amounts of the enzyme are required to digest physiological targets.

A large number of genetically unrelated proteoglycans are known to contain highly negatively charged glycosaminoglycan (GAG) chains. Such core proteins, substituted with GAG chains, constitute an entity of glycoproteins called proteoglycans (PGs). There are several types of GAG chains, where chondroitin sulfate (CS) and heparin/heparan sulfate (HS) are two major types (1). All GAG chains are unbranched, and they contain a variable number of negatively charged sulfate groups that are important for their function (2). Some PGs are associated with cells, whereas others are secreted and are a part of the extracellular matrix (ECM). Almost all mammalian cells synthesize PGs. Monocytes and macrophages synthesize PGs, which are mainly substituted with CS chains (CSPG) and only a minor proportion of HS (3, 4). In resting monocytes most of the CSPG is not released but sorted to the endocytic pathway and degraded (4). However, when monocytes are stimulated and differentiated to macrophages, both the biosynthesis and the secretion of CSPG are increased (4). The human monocyte cell line THP-1 secretes PGs such as versican, perlecan, and serglycin (5, 6). The biological role of the secreted PGs such as serglycin from macrophages is not clear, but it has been shown that it binds to other molecules released from the cells through interaction with the GAG chains (7, 8), suggesting that this and other PGs may act as a kind of carrier molecule. It has also been shown that serglycin is constitutively produced by multiple myeloma plasma cells and can inhibit the bone mineralization process (9).

The family of matrix metalloproteinases (MMPs) consists of more than 20 different secreted and membrane-bound mammalian enzymes that are zinc- and calcium-dependent (10–12). Together, the MMPs are able to degrade most ECM proteins, as well as regulating the activity of serine proteinases, growth factors, cytokines, chemokines, and cell receptors (10, 12–14). Thus, MMPs have complicated biological functions playing a role in normal and pathological conditions (10, 15, 16).

All MMPs are composed of various modules, including a pro- and catalytic domain. In addition, all the secreted MMPs with the exception of the two matrilysins (MMP-7/-26) also contain a C-terminal hemopexin-like domain (10, 12). Typically, the secreted MMPs bind to the ECM proteins and proteoglycans (17). The two gelatinases MMP-9 and MMP-2 contain a unique inserted domain in their catalytic region, i.e. a module containing three fibronectin II-like repeats (FnII) (10, 12). This domain is similar but not identical in the two gelatinases and is involved...
in the binding of denatured collagens, elastin, and native collagen (18–23). The three FnII-like repeats in the catalytic site of MMP-2 and MMP-9 may facilitate the localization of these enzymes to connective tissue matrices. They also appear to be of importance for the degradation of macromolecules such as elastin, gelatin, and collagens IV, V, and XI but do not influence the degradation of chromogenic substrates (23–27).

MMP-9 (92-kDa gelatinase) is produced by a variety of cell lines, including monocytes and macrophages (28). MMP-9 is produced as a monomer as well as various dimer forms (29–34). The homo- and several of the hetero-dimer forms are reduction-sensitive. Hence, the proteins are either covalently linked to each other through disulfide bonds or a very strong reversible interaction where intramolecular disulfide bonds are essential. Recently, we discovered that THP-1 cells produce a new type of reduction-sensitive heteromer, where pro-MMP-9 is linked to the core protein of one or several PGs (34). In the present investigation we have studied the binding properties of this pro-MMP-9-PG heteromers. We have especially focused on the binding to gelatin (denatured collagen) and collagen I.

EXPERIMENTAL PROCEDURES

Materials

Trichloroacetic acid, formaldehyde, Tris, urea, and sodium acetate were from Merck. Ethanolamine and EDTA were from Fluka. Acrylamide, Coomassie Brilliant Blue G-250, and Triton X-100 were from BDH. Safranin O (no. S-2255), cetylpyridinium chloride, phosphor 12-myristate 13-acetate (PMA), DMSO, Hepes, Brij-35, silver nitrate, alkaline phosphatase-conjugated antibodies, chondroitin sulfate C, EDTA, gelatin, calf skin collagen, and alkaline phosphatase-conjugated antibody were purchased from Sigma. Proteinase-free chondroitin ABC lyase (cABC) was from Seikagaku Kogyo Co. Human recombinant TIMP-1, mouse monoclonal antibodies against human MMP-9 and TIMP-1 were from Calbiochem. Gelatin-Sepharose, Q-Sepharose, Superose 6 (HR10/30), Sephadex G-50 (fine), and TIMP-1 were from Calbiochem. Gelatin-Sepharose, (cABC) was from Seikagaku Kogyo Co. Human recombinant chondroitin sulfate C, EDTA, gelatin, gelatin, and collagens IV, V, and XI but do not influence the degradation of chromogenic substrates (23–27).

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**Biosynthesis of PGs**

The human leukemic monocyte cell line THP-1 was a kind gift from Dr. K. Nilsson, Dept. of Pathology, University of Uppsala, Sweden. The cells were cultured in RPMI 1640 medium with 10% fetal bovine serum, 50 μg/ml streptomycin, and 100 units/ml penicillin. To isolate secreted cell-synthesized PGs and pro-MMP-9-PG heteromers, the cells were washed three times in serum-free medium and then cultured for 72 h in serum-free RPMI 1640 medium with 0.1 μM PMA as described earlier (34). Conditioned medium was harvested, and loose cells were pelleted by centrifugation at 1000 rpm for 10 min. Pro-MMP-9 and pro-MMP-9-PG heteromers were thereafter isolated as described below.

**35S Labeling of GAG Chains**

To label the GAG chains with [35S]sulfate, 15 × 10⁶ THP-1 cells were incubated for 72 h in 20 ml of serum-free RPMI 1640 medium containing 50 μg/ml streptomycin, 100 units/ml penicillin, 0.1 μM PMA, and 50 μCi/ml of [35S]sulfate. Conditioned medium was thereafter harvested, loose cells removed through centrifugation at 1000 rpm for 10 min. The cell-free conditioned medium was then applied to a Sephadex G-50 column to separate free [35S]sulfate from PG-labeled [35S]sulfate. The radioactively labeled PG was thereafter isolated as described below.

**Biosynthesis and Isolation of Free GAG Chains**

To obtain biosynthesis of free GAG chains, 0.067–0.67 mM hexyloxyside was added to the cell cultures (35). In some of the cultures, [35S]sulfate was added. To isolate the free GAG chains and the small amount of intact PG formed, the harvested conditioned medium was first subjected to Q-Sepharose chromatography as described for the isolation of PG. Because this method does not separate the free GAG chains from the intact PG, the pooled and desalted fractions from the second Q-Sepharose column containing GAG and PG was applied to a Superose 6 (HR10/30) column. This column was run in 0.05 M sodium acetate, 0.05 M Tris/HCl, and 0.25% Chaps, pH 8.0. The flow rate was 0.4 ml/min, and fractions of 1 min were collected. Aliquots of the collected fractions were analyzed either by the Safranin O method (see below) or by scintillation counting.

Free [35S]-labeled GAG chains were also obtained by treating isolated [35S]PG by NaOH (final concentration, 0.5 M) overnight at room temperature. Prior to neutralization with HCl, 1.0 M Tris-HCl, pH 8.0, was added to the NaOH-treated PG to give a buffer concentration of 0.3 M.

**Detection of Free and PG-bound CS Chains**

Free and PG-bound CS chains were quantified spectrophotometrically by the Safranin O method (36) as described previously (34).

**Isolation of Secreted PG and Pro-MMP-9-PG Heteromers**

Secreted PG and the pro-MMP-9-PG heteromers were isolated by Q-Sepharose anion-exchange chromatography as described previously (34).

**Purification of Pro-MMP-9 from the THP-1 Cells**

The pro-MMP-9 in conditioned medium from the THP-1 cells was partly purified by subjecting the culture medium to a gelatin-Sepharose column (pre-equilibrated with 0.1 M Hepes buffer, pH 7.5), after PGs and pro-MMP-9-PG heteromers had been removed by Q-Sepharose anion exchange chromatography (without Urea present). Both the pro-MMP-9 monomer and homodimer forms bound to the gelatin-Sepharose column. The column was thoroughly washed with 0.1 M Hepes buffer, pH 7.5, containing 1.0 M NaCl and 5 mM EDTA, and then the bound pro-MMP-9 was eluted from the column with 10% of DMSO in 0.1 M Hepes/5 mM EDTA buffer, pH 7.5. The eluted and pooled MMP-9 fractions were passed over a Sephadex G-50 (fine) column, run in 10 mM Hepes, pH 7.5, followed by...
interaction of pro-MMP-9-PG heterodimer with collagen

concentration in a SpeedVac (Savant). SDS-electrophoresis under reducing conditions, followed by either silver or Coomassie Blue staining, showed two bands, a major at 92 kDa and a minor band at 28 kDa. Western blotting revealed that the 92-kDa band was pro-MMP-9, and the 28-kDa band was TIMP-1. The amount of pro-MMP-9 was estimated spectrophotometrically at 280 nm using an $e_{280} = 114.360 \text{ M}^{-1} \text{ cm}^{-1}$ (37), ignoring the contribution of TIMP-1.

**cABC Lyase Treatment**

The PG-bound CS chains were removed by digestion for 2 h at 37 °C with 0.2–1.0 unit of cABC/ml in 0.05 M Tris-HCl, pH 8.0, containing 0.05 M sodium acetate.

**Gelatin Zymography**

SDS-substrate PAGE was done as described previously (34) with gels (7.5 cm × 8.5 cm × 0.75 mm) containing 0.1% (w/v) gelatin in both the stacking and the separating gel, and 4 and 7.5% (w/v) of polyacrylamide, respectively. Gelatinase activity was evident as cleared (unstained) regions, and the area of these regions was quantified by the GelBase/GelBlot™ Pro computer program from Ultra Violet Products.

**3H Labeling of Calf Skin Collagen**

Acid-soluble calf skin collagen was labeled with tritium by reductive methylation of the amino groups as described previously (38). Collagen denatured for 5–10 min at 90 °C resulted in gelatin.

**Gelatin and Collagen I Binding Assays**

_Gelatin-Sepharose Binding Assay—250 μl of [35S]PG (3 × 10^6 to 9 × 10^6 cpm) was applied to a 1.0-ml column of gelatin-Sepharose. As gelatin is known to bind to gelatinases through hydrophobic interactions, and bound enzyme is eluted from gelatin-Sepharose columns with DMSO (21, 37), various hydrophobic compounds such as DMSO (5%), Brij-35 (0.05%), and Triton X-100 (0.5%) in 0.1 M Hepes buffer, pH 7.5, were used to elute the bound radioactive material. Both the material that passed through the column and the eluted material were analyzed for radioactivity._

To determine if the pro-MMP-9-PG and pro-MMP-9-PG core protein complexes bind to gelatin-Sepharose, 150 μg of PG (untreated and cABC-treated) in 75 μl of 0.1 M Hepes buffer, pH 7.5, was applied to a 300 μl column. Both the fractions that passed through the column and the fractions eluted with DMSO (5%), Brij-35 (0.05%) and Triton X-100 (0.5%) in 0.1 M Hepes buffer, pH 7.5, were analyzed on gelatin zymography.

_Gelatin and Collagen I Micro-well Binding Assay—To covalently link collagen type I or gelatin to micro-wells, equal amounts (3.0 μg/ml) of macromolecule in 0.1 M Na2CO3/HCl buffer, pH 9.6, were added to 384-well Nunc Immobilizer Amino plates and incubated for 2 h at room temperature. After wash in the same carbonate buffer, nonspecific binding sites were blocked by incubating the wells with 10 μM ethanolamine in the same carbonate buffer for 1 h. The wells were thereafter washed three times in 0.1 M Hepes buffer, pH 7.5._

Various amounts of pro-MMP-9 in 0.1 M Hepes buffer, pH 7.5, containing 5.0 mM EDTA were added to the wells for 1 h. The wells were then rinsed three times in washing buffer (0.1 M Hepes/5.0 mM EDTA, pH 7.5). The bound pro-MMP-9 was eluted with washing buffer containing 10% DMSO/0.05% Brij-35 and then subjected to gelatin zymography. The amount of eluted enzyme was estimated by using the GelBase/GelBlot™ Pro computer program, and dissociation curves were obtained using a four-parameter non-linear curve-fitting algorithm (SigmaPlot, SPSS Corp., Chicago, IL) to calculate the amount of enzyme required to give a 50% saturation.

Because the exact amount of protein in the pro-MMP-9-PG heteromers was not possible to determine, the affinity of collagen I or gelatin to the pro-MMP-9 and its heteromers was determined by estimating the concentration of DMSO needed to release 50% of the bound enzyme and enzyme-heteromers from the respective ECM-coated wells. In these experiments, a suitable concentration of pro-MMP-9, pro-MMP-9-PG, or pro-MMP-9-PG core protein (in 0.1 M Hepes buffer, pH 7.5, containing 5.0 mM EDTA) was added to the well for 1 h. Then the wells were rinsed three times in washing buffer. Bound enzyme and enzyme-heteromers were eluted with increased concentrations of DMSO in washing buffer containing 0.05% Brij-35. Released enzyme and enzyme-heteromers were detected by gelatin zymography. The percentage of DMSO required to release 50% enzyme/enzyme-heteromers was calculated from dissociation curves using a four-parameter non-linear curve-fitting algorithm (SigmaPlot). All experiments were performed in duplicate and repeated at least four times.

To determine whether gelatin and collagen I bind to the same site on gelatin, competitive binding studies were performed. In these studies, micro-wells with bound gelatin were first incubated for 1 h at room temperature with 12 μl of 70 nM purified pro-MMP-9. The wells were then washed three times with washing buffer and again incubated for 1 h with 12 μl of 0.7 μg/μl pro-MMP-9-PG heteromers (based on GAG determination using the Safranin O method) at room temperature. As controls, the gelatin-coated micro-wells were incubated with pro-MMP-9 or pro-MMP-9-PG heteromer alone. The wells were then rinsed three times with washing buffer. In some experiments the wells were first rinsed three times with a washing buffer containing 1 M NaCl followed by three times rinse in washing buffer. The latter procedure was used to test if the heteromers could bind to the micro-well bound pro-MMP-9 through its GAG chains. The bound pro-MMP-9 and pro-MMP-9-PG heteromers were eluted with 10% DMSO (in washing buffer) and subjected to gelatin zymography.

To determine whether gelatin and collagen I bind to the same site in pro-MMP-9-PG heteromers, another competitive binding study was performed. In this study, micro-wells with bound collagen were incubated for 1 h at room temperature with 12 μl of either 40 nM of purified pro-MMP-9 or 4.75 μg/μl of pro-MMP-9-PG heteromers (based on GAG determination using the Safranin O method) that has been premixed with different amount of gelatin (167–8333 μg/ml). Pro-MMP-9 was here used as a control of the assay, because it has been shown that both collagen and gelatin binds to the FnII repeats in the active
site of the enzyme (19, 22). As controls, the collagen-coated micro-wells were incubated with pro-MMP-9 or pro-MMP-9-PG heteromers alone. The wells were then washed three times with washing buffer. The bound pro-MMP-9 and pro-MMP-9-PG heteromers were eluted with 10% DMSO (in washing buffer) and subjected to gelatin zymography.

**Trichloroacetic Acid Precipitation Assay**—Because trichloroacetic acid does not precipitate PG (39), binding of gelatin to PG and proteins associated with PG was determined with a slightly modified form of the previously described solubel gelatinase assay method (38, 40). Briefly, 100 μl of purified PG in 0.1 M Hepes, pH 7.5, was mixed with 50 μl of the 3H-labeled gelatin solution (2.3 mg/ml or 6.6 × 10^6 cpm/mg). Trichloroacetic acid (60%) was added to give a final concentration of 20%. The assay tubes were centrifuged for 4 min at 20,000 × g using an Eppendorf Minifuge. Then 80 μl of the supernatant was added to 3 ml of Ultima Gold XR, and the radioactivity was quantified in a scintillation counter.

In another type of experiment, 80 μl of the above mentioned supernatant was neutralized with NaOH, and then 20 μl of the neutralized supernatant was subjected to SDS-PAGE. Thereafter, the gel was soaked in Amplify and dried. Undegraded and degraded [3H]gelatin was then detected with autoradiography.

**Statistical Analysis**

All assays were performed in at least triplicate with data presented as mean ± S.D., using the Student t test.

**RESULTS**

*Isolation of PG and Pro-MMP-9-PG Heteromer*—The conditioned serum-free media from PMA-treated THP-1 cells incubated for 72 h in the presence or absence of [35S]sulfate was applied to Q-Sepharose (ion exchange) chromatography as described previously (34, 41). This column separates PG and pro-MMP-9-PG heteromers from free pro-MMP-9 (Fig. 1a). 10–15% of the synthesized pro-MMP-9 was strongly linked to PG, as also shown previously (34). The PG fraction that was eluted from the Q-Sepharose column thus contains a mixture of various PGs and pro-MMP-9-PG heteromers. This mixture was used in the different experiments to study the interaction between the pro-MMP-9-PG heteromers with gelatin and collagen I, as well as PG and gelatin.

*PG Isolated from THP-1 Cells Contains Mostly CS Chains*—The PMA-differentiated cells were incubated in the presence of [35S]sulfate. Analysis of the conditioned medium showed that the [35S]sulfate was almost exclusively incorporated in the synthesized GAG chains (not shown). To determine which types of GAG chains were synthesized by the THP-1 cells, [35S]PG was treated with cABC, which degrades CS but not HS chains. Approximately 98% of the radioactivity was removed from the [35S]PG after cABC treatment, clearly indicating that the PG was almost exclusively substituted with CS chains. Hence, no more than ~2% of the GAG chains can be of the HS type. However, because cABC treatment has been shown to leave small stubs of CS on the PG core protein that may contain a sulfate group (42, 43), it can be assumed that the small amount of radioactivity remaining linked to the purified proteins can at least partly be ascribed to these stubs. If the radioactivity left on the PG after cABC treatment is due to the presence of PG that contains only HS chains, this would be detected in SDS-PAGE as bands that do not change in size after the cABC treatment. As controls, the collagen-coated wells were washed three times with washing buffer. The bound pro-MMP-9 and pro-MMP-9-PG heteromers were eluted with 10% DMSO (in washing buffer) and subjected to gelatin zymography.

**Figure 1.** Summary chart for separation of pro-MMP-9 (monomer and homodimer), PGs, and pro-MMP-9-PG heteromers. The charts show the fate of the PGs and pro-MMP-9-PG heteromers that is referred to in various parts of the text. The PGs were labeled with [35S]sulfate and detected by scintillation counting, whereas the pro-MMP-9, including the heteromers, was detected by gelatin zymography. a, summarizes the amount of the pro-MMP-9, pro-MMP-9-PG, and PGs in the conditioned media from PMA-stimulated THP-1 cells that binds or not to Q-Sepharose. When [3H]-labeled material was analyzed, ~3% of the labeled macromolecules did not bind to the Q-Sepharose column. This unbound material consisted of sulfated glycoproteins and not PGs (data not shown). PGs and pro-MMP-9-PG heteromers bound to the column and were eluted in the same fractions (see "Experimental Procedures"). In contrast, the monomer and homodimer forms of pro-MMP-9 did not bind to the column. Of the total pro-MMP-9 synthesized, between 10 and 15% are linked to a PG core protein (pro-MMP-9-PG heteromer) (34). b, describes experiments where the isolated PGs (including pro-MMP-9-PG heteromers) are tested for the ability to bind to a gelatin-Sepharose column. The results reveal that the PGs and pro-MMP-9-PG heteromers are heterogeneous. The fractions of bound and unbound PGs include the pro-MMP-9-PG heteromers and hence reflect the entire amount of PGs. Shown also is the relative amount of bound PGs eluted with DMSO, Brij-35/ Triton X-100, and NaCl compared with the entire amount of PGs. The quantitative estimation of bound and unbound pro-MMP-9-PG heteromers is based on gelatin zymography and, hence, only reflects relative quantities of the enzyme complexes. Here, the amount of pro-MMP-9-PG eluted with either DMSO, Brij-35, or Triton X-100 is presented relative to the amount of bound complex to the column. Based on the quantitative estimation of bound and unbound PGs as well as bound and unbound pro-MMP-9-PG heteromers to the gelatin-Sepharose column, it was possible to estimate that ~0.1% of the total amount of PGs are pro-MMP-9-PG heteromers.
separating gel (Fig. 2). After cABC treatment, only weak bands could be detected even after 7 days of exposure of the film to the dried gel. These bands occurred both at the position of the untreated control [35S]PG as well as at positions with reduced molecular weight. The majority of the radioactivity appeared at the same position as the tracking marker dye and is most likely due to the cABC-produced radioactive disaccharides. This suggests that only a very small amount of the radioactivity left on the PG after cABC treatment can be ascribed to PGs that contain only HS chains.

**PG Isolated from THP-1 Cells Binds Gelatin**—In contrast to other proteins, trichloroacetic acid does not precipitate PGs due to the presence of GAG chains (39). Thus, if a protein such as gelatin binds to purified PG, it can be expected that the bound protein would remain in solution along with the PG in the presence of trichloroacetic acid, while unbound proteins are precipitated. In the present work, [3H]labeled gelatin was incubated with the PG isolated from THP-1 cells. Trichloroacetic acid was added to give a final concentration of 20%, which precipitates peptides larger than 5 kDa, followed by centrifugation. The amount of radioactivity left in the supernatant increased with increasing concentrations of intact PG (Fig. 3a).

![Graph](image)

**FIGURE 3.** Isolated PGs bind gelatin. *a*, different amounts of the isolated PGs were mixed with 115 µg of [3H]-labeled gelatin (6.6 × 10⁶ cpm/mg). The amount of radioactivity in the supernatant after trichloroacetic acid precipitation was determined and presented as percent relative to the total amount of gelatin used in the assay. Corrections for background counts in the controls that lack PGs have not been done. The controls show that ~8% of the [3H]-labeled gelatin is not precipitated. In this representative experiment each point shows the mean ± S.D. where *n* = 4. The quantification of PGs was done by the Safranin O method. *b*, different amounts of PGs were mixed with gelatin as described in *a*. After the trichloroacetic acid precipitation, the supernatant was neutralized with NaOH, and an aliquot was applied to 7.5% SDS-PAGE. After electrophoresis the gel was treated as described under “Experimental Procedures” and analyzed with autoradiography. Lanes 1–5 contain [3H]-labeled gelatin mixed with different amount of PG as indicated under the figure, and lane 6 is a positive control of [3H]-labeled gelatin that has not been precipitated with trichloroacetic acid. Molecular masses of standard markers in kilodaltons are shown on the left.

As controls, [3H]-labeled gelatin was incubated with cABC-treated PG, buffer alone, or THP-1 culture medium where PGs had been removed by Q-Sepharose chromatography. In isolated PG where the CS chains have been removed by cABC degradation, the amount of radioactivity in the supernatant was the same as in the other two controls (data not shown).
To verify that the radioactivity in the supernatant after trichloroacetic acid precipitation was due to intact gelatin, and not degraded gelatin, 3H-labeled gelatin was added to increasing concentrations of PG. After trichloroacetic acid precipitation, supernatants were neutralized with NaOH, applied to SDS-PAGE electrophoresis, and analyzed with autoradiography. Increasing concentrations of PG resulted in increasing amounts of intact gelatin in the supernatant, whereas no gelatin was detected in the supernatant from the controls without PG (Fig. 3b). Also when 3H-labeled gelatin was mixed with cABC-treated PG, no intact gelatin could be detected in the supernatant (data not shown). Thus the radioactivity left in the supernatant after trichloroacetic acid precipitation was due to intact gelatin bound to the PG and not due to degraded gelatin.

Intact PG but Not Free CS Chains Prevents Soluble Gelatin from Being Precipitated by Trichloroacetic Acid—To gain insight into the mechanism behind the interaction between gelatin and PG, we first investigated whether the binding was to the CS chains or a protein component of the isolated PG. Previously it has been reported that heparin binds to gelatin (44), whereas others did not detect interaction between these two molecules (45). To explore whether the CS chains are involved in the binding of soluble gelatin to PG, we isolated free CS chains from heparin-stabilized T-SPase-treated THP-1 cells. The intact PG and free CS chains were then separated on a Q-Sepharose column. To separate free CS chains from intact PG a Superose-6 column was used. Experiments with [35S]sulfate-labeled material showed that ~80% of the CS chains were free (peak P2) and 20% were bound to PG core protein (peak P1) as shown in Fig. 4a. In experiments with unlabeled material, fractions from the two peaks were pooled. The material in the two pools was mixed with 3H-labeled gelatin, followed by trichloroacetic acid precipitation. The P2 pool containing mainly free CS chains prevented only negligible amount of the 3H-labeled gelatin from being precipitated compared with the effect produced by an equal amount of intact PG, i.e. the P1 pool (Fig. 4b). Thus, it can be concluded that 3H-labeled gelatin did not bind to the CS chains in the PG, but to either the PG core protein or a protein linked to the PG, such as pro-MMP-9. Alternatively, free CS chains cannot prevent bound gelatin from trichloroacetic acid precipitation.

Only a Small Fraction of the PG Secreted from THP-1 Cells Binds to Gelatin—To determine how much of the secreted PG that binds to gelatin, [35S]PG was applied to gelatin-Sepharose chromatography (Fig. 1b). Most of the radioactive material passed through the column, and only 0.5–1.5% was bound (Figs. 1b and 5a). Because gelatin is known to bind to gelatinases through hydrophobic interactions, and bound enzyme is eluted from gelatin-Sepharose columns with DMSO (21, 37), various hydrophobic compounds such as DMSO, Brij-35, and Triton X-100 were used to elute the bound radioactive material. Only a minor fraction (1–2%) of the bound PG was eluted with 5.0% DMSO (Figs. 1b and 5a). Further elution with 0.05% Brij-35 resulted in additional release of 26–51% of the bound PG (Fig. 5a). Most of the remaining PG (29–40%) was eluted with 0.5% Triton X-100 (Fig. 5a). To assure that all the radioactive material bound to the column had been eluted, the entire column material was dissolved in scintillation fluid after Triton X-100 elution. This showed that 19–32% of the total radioactive material was left on the column in the various experiments. This material could be eluted with 1 M NaCl (Fig. 1b). Approximately the same values for the eluted [35S]PG was obtained when the elution started with Brij-35, followed by DMSO, and ended with Triton X-100 (data not shown). When 0.5% Triton X-100 was used as the starting eluant, all of the bound radioactive material was eluted, except for the peak eluted with DMSO (data not shown). Our results show that there is a heterogeneity in the nature of the PG, because most of the PG secreted from THP-1 cells did not bind to gelatin, and the small amount that bound was eluted in different fractions depending on the hydrophobic eluant.

A Large Part of the Pro-MMP-9-PG Heteromer Binds to the Gelatin—Previously we have shown that 10–15% of the pro-MMP-9 secreted by THP-1 cells is strongly linked to a PG core protein forming a pro-MMP-9-PG heteromer (34). This heteromer was separated from the free pro-MMP-9 (monomer and homodimer) during the first Q-Sepharose purification step of PG (Fig. 1a). To investigate what extent the pro-MMP-9-PG heteromer binds to gelatin, the bound fraction eluted from Q-Sepharose chromatography, containing unlabeled PG and MPM-9-PG heteromers, was subjected to gelatin-Sepharose chromatography. The bound material was eluted under the same conditions as in Fig. 5a. To determine in which fractions the pro-MMP-9-PG heteromer was found, each fraction was
Interaction of Pro-MMP-9-PG Heterodimer with Collagen

![Graph](image)

As shown by cABC treatment of $^{35}$S-labeled PG, >98% of the GAG chains were of the CS type. Further, we have previously shown that the pro-MMP-9-PG heteromers mainly contain CS chains (34), as was also confirmed in material used in the present study (Fig. 6a).

Previously we showed that the pro-MMP-9-PG heteromers could be activated by Ca$^{2+}$ ions, but not with the classic organomercurial activator of MMPs, p-aminophenylmercuric acetate (41). As shown in Fig. 6 (b and c), the pro-MMP-9-PG heteromers in both the pass-through and the bound fractions could be activated by Ca$^{2+}$ ions.

The CS Chains Do Not Take Part in the Binding of Pro-MMP-9-PG Heteromers to Gelatin—The CS chains in the PGs were removed by cABC, and the obtained PG core proteins were applied to gelatin-Sepharose chromatography. The eluted fractions were analyzed by gelatin zymography. ~20% of the pro-MMP-9-PG core protein heteromers bound to the gelatin-Sepharose column, whereas the rest passed through the column (data not shown). In repeated experiments the percentage of bound heteromer varied between 15 and 30%. The amount of bound material was approximately the same for both intact and cABC-treated material, clearly indicating that the CS chains are not involved in the binding of the heteromers to gelatin. To verify that the CS chains did not prevent binding of the heteromers to gelatin, the pro-MMP-9-PG fraction that passed through the gelatin-Sepharose column was treated with cABC.

![Diagram](image)
The formed pro-MMP-9-PG core proteins were then applied to a new gelatin-Sepharose column. All pro-MMP-9-PG core protein complexes passed through the column (data not shown), showing that the CS chains did not prevent the heteromers from binding to gelatin.

Pro-MMP-9-PG Heteromers Have Weaker Affinity to Gelatin Compared with Pro-MMP-9 Monomer—To determine the affinity of pro-MMP-9, pro-MMP-9-PG, and pro-MMP-9-PG core protein to gelatin and collagen I, these pro-MMP-9 variants were added to gelatin and collagen I-coated micro-wells. The bound pro-MMP-9 variants were eluted with DMSO, because this compound has been shown to release both pro-MMP-9 (21, 37) and its variants (see Fig. 5) from gelatin-Sepharose. The amount of pro-MMP-9 and its heteromers eluted from the micro-wells was determined by gelatin zymography. Normally, the affinity between various MMPs, recombinant collagen binding domains from MMP-2/-9, and various matrix molecules has been determined by adding increasing amounts of the former compounds to micro-wells coated with a certain matrix molecule (18, 22). However, it was not possible to determine the exact amount of the pro-MMP-9-PG heteromers as well as its molecular size because of the heterogeneity of the material. Therefore it was not possible to compare the affinity based on the concentration of the pro-MMP-9 variants. Instead, the affinity was based on the concentrations of DMSO needed to dissociate 50% of the pro-MMP-9 variants from the gelatin and collagen I matrix.

To establish the method, increasing concentrations of purified pro-MMP-9 were added to gelatin and collagen I-coated micro-wells. After thorough washes, the bound enzyme was dissociated from the ECM molecules using 10% DMSO. Typical zymography gels are shown in Fig. 7 (a and b). Saturation curves occurred in all cases for the binding to gelatin (Fig. 7c). In contrast, binding of pro-MMP-9 to collagen I gave saturation curves only in ~50% of the experiments. A 50% saturation occurred at 24 ± 3 nm (n = 4) pro-MMP-9 for gelatin, whereas 33 ± 7 nm (n = 5) pro-MMP-9 when saturation curves were obtained with collagen I. This shows that pro-MMP-9 has a weaker affinity to collagen I than to gelatin as also shown by others (22). Based on these experiments, we decided to use 20–25 nm pro-MMP-9 in the following affinity experiments. Similar experiments were performed to establish the amount of pro-MMP-9-PG and PG core protein heteromers needed to obtain enough bound material to study the affinity to gelatin and collagen I (data not shown). No pro-MMP-9 or pro-MMP-9-PG heteromers were bound to wells coated with either bovine serum albumin or 0.01 M ethanolamine.

To determine the affinity to gelatin, the three MMP-9 variants were added to gelatin-coated micro-wells. After incubation and rinsing, different concentrations of DMSO were used to elute the bound MMP-9 variants. As shown in Fig. 8, larger concentrations of DMSO were needed to release pro-MMP-9 than pro-MMP-9-PG heteromers from the gelatin, clearly indicating that pro-MMP-9-PG and pro-MMP-9-PG core protein bind weaker to gelatin than pro-MMP-9.

Identical experiments were performed to determine the affinity to collagen I. Fig. 8 shows that the three MMP-9 variants have a similar affinity, in contrast to what was observed for gelatin. All these experiments were repeated several times, and Fig. 8i summarizes the results. As can be seen, a higher concentration of DMSO was needed to elute pro-MMP-9 from gelatin than from collagen I. This confirms the results from the saturation experiments (Fig. 7) as well as what has been reported by other investigators that pro-
FIGURE 8. The strength of the interaction between pro-MMP-9 and its heteromer forms with gelatin and collagen I coated micro-wells. Nunc micro-wells were coated with either 3 µg of gelatin (a, c, and e) or collagen I (b, d, and f) as described under "Experimental Procedures." After thorough rinses with washing buffer, 12 µl of pro-MMP-9 (15.8 nM) (a and b), pro-MMP-9-PG heteromers (280 µg/ml) (c and d), and pro-MMP-9-PG core protein heteromers (280 µg/ml) (e and f) were added to the wells and incubated for 1 h at room temperature. Wells were thereafter rinsed three times with washing buffer to remove unbound enzymes. Bound enzymes were released using 12 µl of elution buffer containing various concentrations of DMSO as indicated, and the eluted material was thereafter mixed with 3 µl of sample buffer. 6 µl of eluted sample mixture was analyzed by zymography, and the amount of bound pro-MMP-9 was quantified using the GelBase/GelBlot™ pro program. The amount of bound pro-MMP-9, pro-MMP-9-PG, and pro-MMP-9-PG core protein is shown in g and h. Curve fitting and hence the amount of DMSO needed to release 50% of the bound enzymes was achieved using a four-parameter non-linear curve-fitting algorithm (SigmaPlot). In the representative experiments with the pro-MMP-9 variants bound to gelatin (g) a 50% release was calculated to occur with 0.93% of DMSO for pro-MMP-9, 0.12% for pro-MMP-9-PG, and 0.19% for pro-MMP-9-PG core protein. The corresponding values for the pro-MMP-9 variants bound to collagen I (h) are 0.33% for pro-MMP-9, 0.15% for pro-MMP-9-PG, and 0.26% for pro-MMP-9-PG core protein. In the curve fit for pro-MMP-9 (g) and pro-MMP-9-PG core protein (h), the samples eluted with 2.25% (a) and 0.20% (f) DMSO, respectively, have been omitted from the curves due to a volume error in the application of the sample to the zymography gel. In i, the strength of the interactions of the various pro-MMP-9 forms with gelatin and collagen I is summarized. The results are presented as mean ± S.D., and the number of independent experiments (N) is shown in the figure. *, p < 0.05 compared with pro-MMP-9 bound to gelatin.
MMP-9 binds stronger to gelatin than to collagen I (22). Further, our results show that the pro-MMP-9-PG and the pro-MMP-9-PG core protein complexes have an almost identical affinity to both ECM molecules (Fig. 8).

Binding of Pro-MMP-9-PG to Gelatin Is Not through the FnII Domain in the Pro-MMP-9 Part of the Complex—In the fraction of the pro-MMP-9-PG heteromers that binds gelatin (Fig. 1b), it is possible that this interaction is mediated by the FnII domain in the proenzyme. To determine whether this is the case or not, competitive binding studies were performed. Pro-MMP-9-PG heteromers bound to gelatin-coated wells that first had been saturated with pro-MMP-9 (Fig. 9a). To investigate whether the binding of the pro-MMP-9-PG heteromers to the gelatin-coated wells saturated with pro-MMP-9 is due to an interaction between the free enzyme and the GAG chains of the PG, wells were rinsed with washing buffer containing 1 M NaCl. This washing procedure had no effect on the binding of the pro-MMP-9-PG heteromers to the pre-coated wells, showing that the heteromer binds to the gelatin and not to the gelatin-bound pro-MMP-9. Further, these results reveal that the heteromer did not bind to gelatin through the FnII domains in the pro-MMP-9 part of heteromers. It also suggests that the two regions on the gelatin molecule that bind pro-MMP-9 and pro-MMP-9-PG heteromers are well physical separated, because the two different molecules can bind simultaneously.

The Same or an Overlapping Region in the Pro-MMP-9-PG Heteromers Binds Gelatin and Collagen I—It is known that gelatin and collagen bind to the same region (the FnII-like domain) in the catalytic site of MMP-9, and that the former molecule has a stronger interaction (22). To determine whether gelatin and collagen bind to the same or an overlapping site in the pro-MMP-9-PG heteromer, competition studies were performed. Micro-wells were coated with collagen I, and then pro-MMP-9 and pro-MMP-9-PG heteromers mixed with increasing concentrations of gelatin were added to the wells. As shown in Fig. 9b, increasing concentrations of gelatin prevented the binding of both pro-MMP-9 and pro-MMP-9-PG heteromer to the collagen-coated wells. The left part of Fig. 9b displays pro-MMP-9, which was used as a positive control for the method. Because gelatin prevents the binding of pro-MMP-9-PG to the wells, it can be concluded that gelatin and collagen bind to the same or an overlapping site in the heteromer.

DISCUSSION

PGs are known to interact with a lot of molecules, including ECM proteins like collagen and gelatin (46). The present work with the monocytic cell line THP-1 shows that pro-MMP-9-PG complexes are heterogeneous with respect to their ability to bind collagen and gelatin. A significant fraction of the heteromers bound gelatin and collagen as summarized in Fig. 1. Only a minor fraction of the total amount of PGs bound to gelatin. Similar to the pro-MMP-9-PG complexes, the PGs that were not linked to pro-MMP-9 were heterogeneous with respect to their ability to bind gelatin. The discussion will focus on the ability of these fractions to interact with the two ECM molecules, as well as on the heterogeneity within these fractions.

The Classic Gelatinase Assay Is Not Always Reliable—The classic quantitative gelatinase activity assay is based on the use of radioactive labeled gelatin (38, 40). Enzyme-generated fragments <5 kDa cannot be precipitated by 15–20% of trichloroacetic acid. Hence, the amount of radioactivity left in the supernatant after trichloroacetic acid precipitation is used as a measure of the enzyme activity. As shown in the present work, this assay cannot be used when gelatin is bound to PGs, because PG prevents trichloroacetic acid precipitation of bound proteins. Therefore, when this assay is used, one should always check that the gelatin left in the supernatant is degraded.

PG Did Not Bind Gelatin through the CS Chains—GAG chains bind a large number of proteins through hydrogen and
Binding of gelatin to PGs secreted from the THP-1 cells as described in the present work is not likely to occur through the CS chains. This is based on the following observations: (i) only detergents could elute the bound PG from gelatin-Sepharose (Figs. 1b and 5a), hence the binding is through hydrophobic interactions. (ii) Only a minor fraction of the secreted PGs binds gelatin (Figs. 1b and 5a). If the binding had been through the CS chains, one would expect that much more PG would have bound to the gelatin-Sepharose column, because CS chains are known to be relatively homogeneous and represent >98% of the GAG chains in the isolated PGs from our PMA-treated THP-1 cells. (iii) The secreted PGs did not prevent gelatin from binding to intact proteoglycan (Fig. 4). These results fit well with a previous study that showed that gelatin cannot bind heparin (45). However, it cannot be totally excluded that gelatin interacts with some other type of GAG chains linked to the PG core protein, although the CS counts form >98% of the GAG chains. 

The Pro-MMP-9–PG Heterodimer Did Not Bind Gelatin through CS Chains—The binding of gelatin to the pro-MMP-9–PG complex (Fig. 1b) did not appear to involve the CS chains, because enzymatic removal of the CS chains by cABC did not prevent binding. In addition, the affinity was approximately the same with or without the CS chains. Because gelatin is not bound to the heteromers through the CS chains, the interaction must be either to the PG core protein, the pro-MMP-9, or another protein associated with the heteromer. As for the free PGs, it cannot be excluded that gelatin interacts with some other type of GAG chains linked to the PG core proteins in the heteromer.

Binding to Gelatin Involves Various Structures—PG bound to gelatin-Sepharose was eluted sequentially with the detergents Brij-35 and Triton X-100 (Fig. 5a). As the two detergents have similar hydrophobic properties and were used in different concentrations, it must be assumed that there are various structures in the PG molecules that bind gelatin. The pro-MMP-9–PG heteromers were only eluted with DMSO and not by the detergents (Figs. 1b and 5b), indicating that gelatin binds to a structure in this heteromer that is different from the corresponding binding structures in the PGs.

The FnII Domain of Pro-MMP-9 in the Pro-MMP-9–PG Heteromer Is Not Involved in the Binding of Gelatin—The pro-MMP-9–PG heteromer that was bound to a gelatin-Sepharose column or gelatin-coated micro-wells could be eluted with DMSO but not with Brij-35 or Triton X-100 (Fig. 1b). It is known that gelatin is bound to pro-MMP-9 through the three FnII repeats in the catalytic site of the enzyme (18, 21, 22). This interaction is due to hydrophobic forces, and the two molecules can be dissociated by DMSO (21, 37). Because the pro-MMP-9–PG heteromers are also dissociated from gelatin by DMSO, although at lower DMSO concentrations than needed for pro-MMP-9 dissociation (Fig. 8), it could be expected that the heteromers are bound to gelatin through its pro-MMP-9 component. However, an excess of pro-MMP-9 could not prevent the binding of pro-MMP-9–PG heteromers to gelatin coated micro-wells (Fig. 9a). This clearly indicates that the heteromer did not interact with gelatin through the FnII domain in the catalytic site of the pro-MMP-9 part. Thus the FnII domain in the catalytic site of pro-MMP-9 is masked both in the pro-MMP-9–PG heteromers that did not bind gelatin, as well as in those heteromers that bound gelatin as indicated in the schematic model of a heterodimer (Fig. 10).

There are several possible models that can explain the interaction between pro-MMP-9–PG heteromers and gelatin. (i) The PG in the heteromer is identical with one of the PGs that are eluted with Brij-35 or Triton X-100 (Fig. 1b). In that case, the strong interaction between the PG and pro-MMP-9 must have hidden the gelatin-binding domain in both of these proteins. In addition, a new binding site in the PG core protein and/or pro-MMP-9 must have been induced. (ii) The PG itself was not able to bind gelatin. In this case, the strong interaction between the PG and pro-MMP-9 must have induced a new binding site in the PG core protein and/or pro-MMP-9. It is known from previous experiments that the interaction between pro-MMP-9 and PG core protein(s) has resulted in hiding as well as inducing new functional sites in pro-MMP-9. This was seen in activation experiments using p-aminophenylmercury acetate and calcium (41). In these experiments p-aminophenylmercury acetate, a known activator of pro-MMP-9, did not induce activation of the enzyme in the heteromer. Further, Ca$^{2+}$, a known stabilizer of MMP-9, acted as an activator of the enzyme in the heteromer (41). (iii) The PG part of the heteromer is different from those PGs that...
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MMP-9 Dimers and Complexes Have Altered Biochemical Characteristics Compared with the MMP-9 Monomer—MMP-9 is known to form various types of dimers, including homo- and heterodimers that involve the C-terminal hemopexin-like domain of the enzyme (29–34). Some of these dimers are detected in SDS-PAGE under non-reducing conditions, but not under reducing conditions. Hence these dimers are reduction-sensitive and assumed to be linked through one or several S-S bridges. Recombinant MMP-9 hemopexin domain (PEX9) also formed a reduction-sensitive homodimer (48). X-ray crystallography revealed that the reduction sensitivity of the dimer was not due to an intermolecular disulfide bridge but an intramolecular disulfide bond between the conserved Cys-516 and Cys-704 connects blades I and IV and is critical for the structural integrity of PEX9. There is a remaining free cysteine residue within the PEX9 domain, Cys-674, which is buried and hence not involved in the dimerization of PEX9. The crystal structure showed that the dimerization was due to non-covalent and mainly hydrophobic interactions of the two PEX9 domains where most of the dimer contacts involved blade IV. In addition, one salt bridge between the C terminus of PEX9(A) and the side chain of Arg-677 of PEX9(B) also contributed to the PEX9 dimer contact.

Formation of different MMP-9 complexes results in altered biochemical properties of the enzyme. In cells that produce both pro-MMP-9 and TIMP-1, these two molecules are bound together through their C-terminal domains, and the presence of TIMP-1 affects the activity of the enzyme (28). When pro-MMP-9 forms a dimer with collagenase, the binding to TIMP-1 is prevented (21). There are conflicting data concerning whether the pro-MMP-9 homodimer is able to form a complex with TIMP-1 (29, 32, 48). There are however characteristics that are somewhat different between the pro-MMP-9 monomer and homodimer, because the monomer is more rapidly activated by MMP-3 than the homodimer (32). In its heterodimer form with neutrophil gelatinase-associated lipocalin, pro-MMP-9 can bind TIMP-1 and form a ternary complex (49). Activation of the enzyme with plasma kallikrein and HgCl₂ is enhanced in the pro-MMP-9-neutrophil gelatinase-associated lipocalin complex (50), and the enzyme is protected from degradation (51). The interaction between the C-terminal domain of pro-MMP-9 and a PG core protein has also been shown to alter the ability of pro-MMP-9 to be converted into an active enzyme (41). The pro-MMP-9 monomer and homodimer are known to be activated by the organomercurial compound p-aminophenylmercury acetate, whereas the pro-MMP-9-PG complex was not activated by this compound (41). In contrast to this, Ca²⁺, which is known to stabilize MMP-9 and other MMPs but not induce activation of the proenzymes, induced an autoactivation of the pro-MMP-9 in the complex. The presence of Ca²⁺ also resulted in activated enzyme forms released from the complex, due to cleavage of both a part of the PG core protein as well as the C-terminal hemopexin domain of the enzyme (41). Both MMP-9 and MMP-2 interact with gelatin as well as collagen through the three FnII-like modules in their catalytic domain (18, 21, 22). This interaction is also important for the ability of these enzymes to degrade these physiological substrates, but has no effect on their degradation of other physiological substrates or chomogenic peptide substrates (18–20, 24, 25, 27). In the present work it is shown that, when pro-MMP-9 is bound to PG core proteins, the enzyme cannot bind gelatin. Hence, the interaction with PG core proteins results in hiding of the gelatin binding sites in the FnII-like modules of the enzyme. Thus, the interaction between pro-MMP-9 and PG core proteins has resulted in changes of several biochemical properties of the enzyme. Based on this it is tempting to assume that an activation of pro-MMP-9-PG, which results in an active MMP-9 that is still attached to the PG core protein, will have altered biochemical properties compared with unbound active MMP-9. Such properties may include substrate specificity, catalytic efficiency, ability to interact with inhibitor molecules, and hence an altered regulation of the enzyme activity.

Putative Physiological Functions of the MMP-9-PG Complex—Because the pro-MMP-9-PG complexes are produced and secreted from cells, and is not an artifact produced during the isolation procedure (34), the complex is likely to play a physiological role. Despite the small amount of pro-MMP-9-PG heteromer formed, the complex may have fundamental physiological importance, because only catalytic amounts of the enzyme are required to digest physiological targets. Also, the heteromers have biochemical properties different from the monomeric enzyme. The PG part of the heteromers may mediate binding to proteins that are not recognized by monomeric...
PGs are known to interact with structural ECM molecules as well as cell surface receptors through either their core proteins or the GAG chains. Among the various structural ECM and cell surface receptor molecules to which PGs can bind are collagens, laminins, fibronectin, fibrin, elastin, fibbrillins, hyaluronic acid, CD44, epidermal growth factor receptor, integrins, and selectins (46). Several types of molecules such as proteinases, proteinase inhibitors, growth factors, cytokines, and chemokines bind to the GAG chains of PGs (1, 7). This binding appears to be of importance for signaling events where the bound molecules have an altered activity. In other cases the bound molecules are protected from degradation. MMPs such as MMP-2 and MMP-7 have been found associated with GAG chains, the former through its C-terminal hemepxin-like domain (17, 52, 53). In contrast to MMP-2, MMP-9 does not bind to GAG chains (54, 55), but instead can be bound strongly to one or more PG core proteins (34). The nature of the PG in the pro-MMP-9-PG heteromer is still not known. We have previously shown that THP-1 cells synthesize serglycin (6), and the PG in the pro-MMP-9-PG heteromer may therefore be serglycin. It is interesting to note that serglycin is a potent inhibitor of bone mineralization in vitro (9). Because both the inorganic (calcium and phosphate) and the organic (collagen I) components of the bones have a high turnover rate, it is tempting to assume that, under conditions where PGs like serglycin accumulate, the presence of a pro-MMP-9-PG heteromer would have resulted in a calcium-induced activation and release of the bound gelatinase, which may participate in the remodeling process. Tumor cells migrate to various tissues, organs, and body cavities. The synthesized PGs and proteolytic enzymes like MMPs seem to be of importance for the migration of cancer cells. The invasive capability of the monocytic leukemia cell line THP-1 increases after PMA stimulation, and this appears to be correlated to an increase in CD147 (EMMPRIN), MMP-2, and MMP-9 (56). It may be assumed also that the MMP-9-PG heteromers can be of importance for cellular migration and invasion.

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