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Inhibitory mechanisms of activated matrix metalloproteinase-9 on platelet activation

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Abstract

The intracellular mechanisms underlying the signaling pathways of activated matrix metalloproteinase-9 (MMP-9) in platelets are not yet completely understood. Therefore, the aim of this study was to further examine the effects of activated MMP-9 in preventing platelet aggregation. In this study, activated MMP-9 time-dependently (3–60 min) inhibited platelet aggregation in washed human platelet suspensions stimulated by agonists. However, activated MMP-9 had no significant effect on the binding of FITC-triflavin to the platelet glycoprotein IIb/IIIa complex. Triflavin is a specific antagonist of the glycoprotein IIb/IIIa complex purified from snake venom. Moreover, activated MMP-9 (21 and 90 ng/ml) markedly decreased the fluorescence intensity of platelet membranes tagged with diphenylhexatriene. The thrombin-evoked increase in pHi was inhibited in the presence of activated MMP-9 (21 and 90 ng/ml). In addition, activated MMP-9 (21 and 90 ng/ml) markedly reduced the electron spin resonance (ESR) signal intensity of hydroxyl radicals in collagen (1 μg/ml)-activated platelets. These results indicate that the antiplatelet activity of activated MMP-9 may involve the following pathways: (1) activated MMP-9 may initially induce conformational changes in platelet membranes and hydroxyl radical formation, leading to inhibition of platelet aggregation; and (2) activated MMP-9 also inhibits the Na^+/H^+ exchanger, leading to reduced intracellular Ca^{2+} mobilization, and ultimately to inhibition of platelet aggregation. This study further provides new insights concerning the effects of activated MMP-9 on platelet aggregation.
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Keywords: Matrix metalloproteinase-9; Platelet aggregation; Membrane fluidity; Na^+/H^+ exchanger; Hydroxyl radical

1. Introduction

Matrix metalloproteinases (MMPs) are a family of zinc-containing endoproteinases that share the same structural domains but differ in substrate specificity, cellular sources, and inducibility. MMPs are important in the resorption of extracellular matrices in both normal physiological processes and pathological states (Dollery et al., 1995). These enzymes are responsible for the degradation of extracellular matrices such as collagen, laminin, and proteoglycans (Birkedal-Hansen, 1995). Therefore, MMPs have been implicated in the tissue remodeling which accompanies inflammation, bone resorption, wound healing, thrombosis, atherosclerosis, and tumor invasion (Ray and Stetler-Stevenson, 1994). Most MMPs are synthesized and secreted as inactive proenzymes (Lijnen, 2001). MMP expressions appear to be strictly regulated by tissue inhibitors of metalloproteinases (TIMPs) (Ray and Stetler-Stevenson, 1994; Birkedal-Hansen, 1995). Under some pathological conditions such as tumor cell metastasis, inflammation, thrombosis, and atherosclerosis, MMP expressions may be significantly increased over those of TIMPs.

MMP-9, also known as gelatinase B, has a broad range of substrate specificities for different native collagens (types IV, V, VII, and X) as well as denatured collagens (gelatin) and elastin (Ray and Stetler-Stevenson, 1994; Birkedal-Hansen, 1995). MMP-9 is secreted as a 92-kDa proenzyme and can be changed into an 86-kDa active form (Birkedal-Hansen, 1995). Vascular smooth muscle and endothelial cells are known to synthesize and release MMPs including MMP-9 (Ray and Stetler-

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Stevenson, 1994; Sawicki et al., 1997). Furthermore, Fernandez-Patron et al. (1999) reported that non-aggregated platelets release MMP-9 basally. Platelet aggregation induced by subthreshold (approximately 30% maximal) concentrations of collagen or thrombin increased MMP-9 release to its maximum, suggesting that this protein may be involved in the process of hemostasis and/or thrombosis.

We previously demonstrated that human platelets can release MMP-9, and that it significantly inhibited platelet aggregation stimulated by agonists (Sheu et al., 2004). We therefore suggest that activated MMP-9 may play an important role in regulating platelet aggregation. In that study, activated MMP-9 concentration-dependently (15–90 ng/ml) inhibited platelet aggregation stimulated by agonists in human platelet suspensions. We suggest that the inhibitory effect of activated MMP-9 may inhibit the activation of phospholipase C, followed by inhibition of phosphoinositide breakdown, protein kinase C activation, P47 protein phosphorylation, and thromboxane A_2 formation, thereby leading to inhibition of intracellular Ca^{2+} mobilization (Sheu et al., 2004). On the other hand, activated MMP-9 also activates the formation of nitric oxide/cyclic GMP, resulting in inhibition of platelet aggregation (Sheu et al., 2004). In that study, we partially resolved the mechanisms of activated MMP-9 in platelet aggregation; however, the detailed intracellular mechanisms underlying activated MMP-9–platelet interactions have still not yet been completely resolved. We therefore further examined the effect of activated MMP-9 in human platelets, and utilized the findings to characterize the mechanisms involved in activated MMP-9–platelet interactions.

2. Materials and methods

2.1. Materials

Pro-MMP-9 was purified from human neutrophil granulocytes (Calbiochem, San Diego, CA). Collagen (type I, bovine Achilles tendon), U46619, diphenylhexatriene (DPH), ADP, prostaglandin E_1 (PGE_1), 5,-5-dimethyl-1 pyrroline *N*-oxide (DMPO), arachidonic acid, heparin, apyrase, osmium tetroxide, *p*-aminophenylmercuric acetate (APMA), bovine serum albumin (BSA), and thrombin were purchased from Sigma Chemical (St. Louis, MO). BCECF-AM and fluorescein isothiocyanate (FITC) were purchased from Molecular Probe (Eugene, OR). *Trimeresurus flavoviridis* venom was purchased from Latoxan (Rosans, France).

2.2. Platelet aggregation

Human platelet suspensions were prepared as previously described (Sheu et al., 1999a). This study was approved by the Institutional Review Board of Taipei Medical University (IRB, TMU), and human volunteers gave informed consent also. In brief, blood was collected from healthy human volunteers who had taken no medicine during the preceding 2 weeks, and was mixed with acid/citrate/glucose (9:1, vol/vol). After centrifugation at 120×*g* for 10 min at room temperature, the supernatant (platelet-rich plasma; PRP) was supplemented with prostaglandin E_1 (PGE_1) (0.5 μM) and heparin (6.4 IU/ml), then incubated for 10 min at 37 °C and centrifuged at 500×*g* for 10 min. Washed platelets were finally suspended in Tyrode's solution containing BSA (3.5 mg/ml) and adjusted to about 4.5×10^8 platelets/ml. The final concentration of Ca^{2+} in Tyrode's solution was 1 mM.

A turbidimetric method was applied to measure platelet aggregation using a Lumi-Aggregometer (Payton, Canada). Pro-MMP-9 was activated by AMPA (10 μM) as described by Marcy et al. (1991). Platelet suspensions (4.5×10^8 platelets/ml, 0.4 ml) were pre-warmed to 37 °C for 2 min, then activated MMP-9 (21 ng/ml) and a solvent control (10 μM AMPA) were added 3 min before the addition of agonists. The reaction was allowed to proceed for the indicated time (0, 3, 30, and 60 min), and the extent of aggregation was expressed in light-transmission units.

2.3. Analysis of the platelet surface glycoprotein IIb/IIIa complex by flow cytometry

Triflavin, a specific fibrinogen receptor (glycoprotein IIb/IIIa complex; $\alpha_{IIb}\beta_3$ integrin) antagonist, was prepared as previously described (Sheu et al., 1996). Fluorescence-conjugated triflavin was also prepared as previously described (Sheu et al., 1996). The final concentration of FITC-conjugated triflavin was adjusted to 1 mg/ml. Human platelet suspensions were prepared as described above. Aliquots of platelet suspensions (4.5×10^8/ml) were preincubated with activated MMP-9 (21 and 90 ng/ml) for 3 min, followed by the addition of 2 μl FITC-triflavin. The suspensions were then incubated for another 5 min, and the volume was adjusted to 1 ml/tube with Tyrode's solution. The suspensions were then assayed for fluorescein-labeled platelets using a flow cytometer (Becton Dickinson, FACScan System, San Jose, CA). Data were collected from 50 000 platelets per experimental group. All experiments were repeated at least 4 times to ensure reproducibility.

2.4. Measurement of membrane fluidity using a fluorescent probe

The intensity of fluorescence in human platelets was measured as described previously (Kitagawa et al., 1984). Briefly, platelets (4.5×10^8/ml) were preincubated with various concentrations of activated MMP-9 (21 and 90 ng/ml) for 3 min followed by the addition of a diphenylhexatriene (DPH) (0.5 μM) solution for another 6 min. The relative fluorescence intensity of platelets was measured in a fluorescence spectrophotometer (Hitachi F4500, Tokyo, Japan) at 37 °C.

2.5. Determination of lactate dehydrogenase

Lactate dehydrogenase (LDH) was measured according to previously described methods (Wang et al., 1998). Platelets (4.5×10^8/ml) were preincubated with various concentrations of activated MMP-9 (90 ng/ml) for 30 min, followed by centrifugation at 15 000×*g* for 5 min. An aliquot of supernatant was incubated with phosphate buffer containing 0.2 mg β-

NADH for 20 min. Thereafter, 100 μl of pyruvate solution were added, and the absorbance was read at a wavelength of 334 nm using a UV–visible recording spectrophotometer (UV-160; Shimazu, Kyoto, Japan). A maximal value of LDH was constructed from sonicated platelets.

2.6. Platelet pHi measurement

Platelet pHi was measured with the fluorescent probe, 2′,7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester (BCECF-AM), according to a previously described method (Touyz and Schiffrin, 1993). Washed platelets were incubated with 5 μM BCECF-AM at 37 °C for 30 min in a Hepes-buffered solution (HBS, pH 7.4) and then centrifuged at 450×*g* for 8 min. Washed pellets were finally suspended in buffer and adjusted to 4.5×10^8/ml. Leukocyte contamination was less than 0.01%. Aliquots of this platelet suspension (50 μl) were transferred to a cuvette containing 2 ml HBS in a dual-excitation wavelength spectrofluorometer (CAF 110, Jasco, Japan). Fluorescence signals for BCECF-AM were recorded at 430- and 490-nm excitation wavelengths with an emission wavelength of 530 nm (5-nm slit). The background fluorescence of the platelets was subtracted from each reading. Calibration was carried out after diluting the BCECF-loaded platelets in a high-K^+ buffer in the presence of nigericin (0.2 mg/ml) (Touyz and Schiffrin, 1993). In all experiments, platelets were stimulated by thrombin (0.1 U/ml) to trigger the Na^+/H^+ exchanger.

2.7. Measurement of free radicals in platelet suspensions by electron spin resonance (ESR) spectrometry

The ESR method used a Bruker EMX ESR spectrometer as described previously (Iuliano et al., 1994). In brief, platelet suspensions (0.4 ml) were pre-warmed to 37 °C for 2 min, and then activated MMP-9 (21 and 90 ng/ml) or an isovolumetric vehicle solution (10 μM AMPA) was added for 3 min before the addition of collagen (1 μg/ml). The reaction was allowed to proceed for 5 min, followed by the addition of 100 mM DMPO for the ESR study. The rate of free radical-scavenging activity is defined by the following equation: inhibition rate = 1 − [signal height (activated MMP-9) / signal height (control)] (Yamaguchi et al., 1999).

ESR spectra were recorded at room temperature on a Bruker EMX ESR spectrometer using a quartz flat cell designed for aqueous solutions. Conditions of ESR spectrometry were as follows: 20 mW of power at 9.78 GHz, 1-G modulation, and 100-G scanning in 42 s, with 10 scans accumulated.

2.8. Statistical analysis

The experimental results are expressed as the means ± S.E.M. and are accompanied by the number of observations. Data were assessed using analysis of variance (ANOVA). If this analysis indicated significant differences among the group means, then each group was compared using the Newman–Keuls method. A P value of <0.05 was considered statistically significant.

3. Results

3.1. Time-inhibition effect of activated MMP-9 on platelet aggregation in human platelets

Zymographic analysis confirmed that pro-MMP-9 (92 kDa) was activated by AMPA (10 μM) to form an 86-kDa activated MMP-9, as shown by gelatinolytic activities (data not shown). As previously described (Sheu et al., 2004), activated MMP-9 (15–90 ng/ml) concentration-dependently inhibited platelet aggregation stimulated by collagen (1 μg/ml), arachidonic acid (60 μM), and U46619 (1 μM), a prostaglandin endoperoxide analogue in washed human platelets and platelet-rich plasma. It similarly inhibited ADP (20 μM)-induced platelet aggregation in the presence of fibrinogen (200 μg/ml) (Sheu et al., 2004). In contrast to activated MMP-9, neither pro-MMP-9 (10 μg/ml) nor AMPA (10 μM) significantly inhibited collagen (1 μg/ml)-induced platelet aggregation (Sheu et al., 2004). In the present study, we found that activated MMP-9 (21 ng/ml) also time-dependently (3–60 min) inhibited agonist-induced platelet aggregation in washed human platelets which reached a maximal inhibitory effect at about 60 min (Fig. 1). This result indicates that activated MMP-9 not only concentration-dependently but also time-dependently inhibited agonist-induced platelet aggregation.

3.2. Effect of activated MMP-9 on triflavin binding to the collagen-activated glycoprotein IIb/IIIa complex in human platelets

Triflavin is a specific glycoprotein IIb/IIIa ($\alpha_{IIb}\beta_3$ integrin) antagonist purified from *T. flavoviridis* snake venom (Sheu et al., 1996, 1999b).

In this study, the relative intensity of the fluorescence of FITC-triflavin (2 μg/ml) bound directly to collagen (1 μg/ml)-activated platelets was 186.8 ± 18.1 (Fig. 2A), and it was

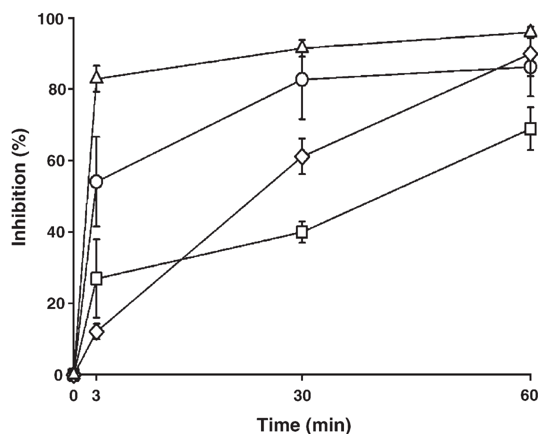

Fig. 1. Time-inhibition curve of activated MMP-9 on collagen (1 μg/ml, ○)-, arachidonic acid (60 μM, △)-, U46619 (1 μM, □)-, and ADP (20 μM, ◊)-induced platelet aggregation in human platelet suspensions. Platelets were preincubated with activated MMP-9 (21 ng/ml) and then incubated for 3–60 min at 37 °C, respectively. Aggregation agonists were then added to trigger platelet aggregation. Data are presented as a percentage of the control (means ± S.E.M., $n=4$).

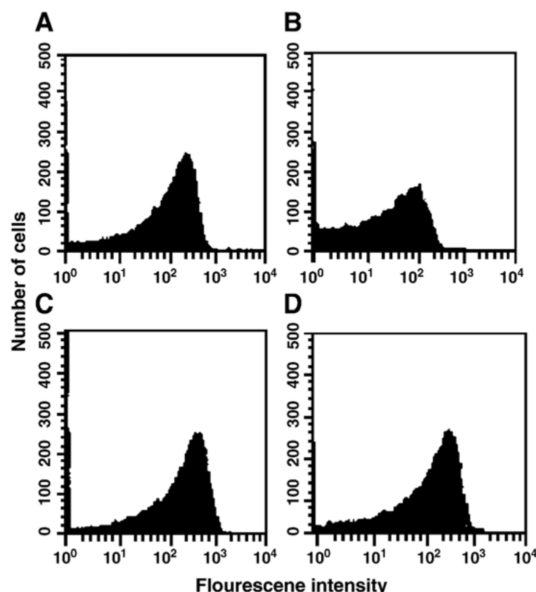

Fig. 2. Flow cytometric analysis of FITC-triflavin binding to collagen-activated platelets in the absence or presence of activated MMP-9. (A) The solid line represents the fluorescence profiles of FITC-triflavin (2 μg/ml) in the presence of the vehicle solution (10 μM AMPA) as a positive control; (B) in the presence of EDTA (5 mM) as the negative control; or in the presence of activated MMP-9 (C, 21 ng/ml and D, 90 ng/ml). Profiles are representative examples of five similar experiments.

markedly reduced in the presence of 5 mM EDTA (negative control, 58.4±2.3) (Fig. 2B). At concentrations of 21 and 90 ng/ml, activated MMP-9 had little effect on FITC-triflavin binding to the glycoprotein IIb/IIIa complex in platelet suspensions (218.9±36.1 at 21 ng/ml; 195.5±12.8 at 90 ng/ml) (Fig. 2C, D), indicating that the mechanism of activated MMP-9's inhibitory effect on platelet aggregation does not involve binding to the glycoprotein IIb/IIIa complex.

3.3. Effect of activated MMP-9 on platelet membrane fluidity

Platelet membrane fluidity was measured in DPH-labeled human platelets. The addition of DPH (1 μM) to the platelet suspensions markedly increased the fluorescence intensity (52.4±6.7 vs. 2887.2±173.5, $n=4$, $P<0.001$) (Fig. 3A, B). The addition of activated MMP-9 (21 and 90 ng/ml) to platelet preparations resulted in a concentration-dependent decrease in the DPH-related fluorescence intensity (1050.8±80.9 at 21 ng/ml vs. 794.2±18.5 at 90 ng/ml, $n=4$, $P<0.05$) (Fig. 3C, D). Neither pro-MMP-9 (10 μg/ml) nor AMPA (10 μM) significantly effected DPH-labeled human platelets (data not shown). This result implies that the inhibitory effect of activated MMP-9 on platelet aggregation may be due, at least partly, to the influence of platelet membrane fluidity.

3.4. Effect of activated MMP-9 on LDH released from platelet cytosol

In this study, activated MMP-9 (90 ng/ml) treatment of human platelets for 10 min did not significantly increase LDH activity compared with resting platelets (resting platelets, 21.7±1.9 units vs. activated MMP-9-treated platelets, 34.0±6.5 units, $n=4$), even when the incubation time of activated MMP-9 with platelets was prolonged to 60 min (data not shown). This result indicates that although activated MMP-9 (21 and 90 ng/ml) significantly changed the fluidity of platelet membranes, it did not, by itself, affect platelet permeability or induce platelet cytolysis under this range of concentrations, clearly disproving the cytotoxic effect of activated MMP-9 on platelets in this study.

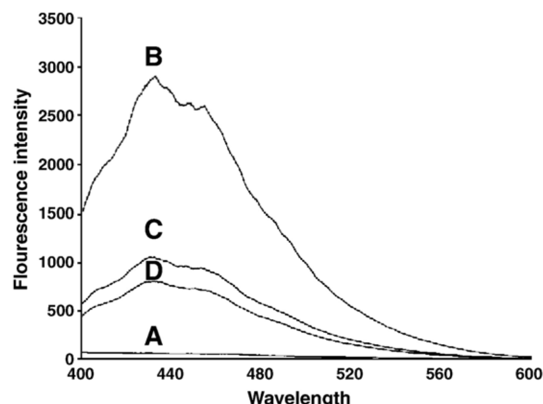

Fig. 3. Fluorescence emission spectra of platelet membranes in the (A) absence or (B) presence of DPH (1 μM). Curves C and D are the emission spectra of membranes labeled with DPH in the presence of activated MMP-9 (C, 21 ng/ml and D, 90 ng/ml). Profiles are representative examples of four similar experiments.

3.5. Effect of activated MMP-9 on thrombin-evoked pHi changes in platelets

Thrombin can trigger more-pronounced pHi changes than collagen in human platelets. Thus, we chose to use thrombin instead of collagen in this study. Fig. 4 shows that the addition of thrombin (0.1 U/ml) resulted in an increase in BCECF fluorescence equivalent to an increase in pHi values of about 0.151±0.008 (Fig. 4). This thrombin-evoked increase in pHi values was markedly inhibited in the presence of activated

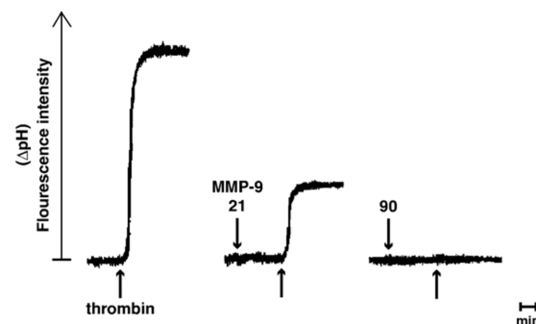

Fig. 4. Effect of activated MMP-9 on the thrombin-triggered intracellular pH increase in BCECF-AM-loaded platelets. Platelet suspensions (4.5×10^8/ml) were preincubated with BCECF-AM (5 μM), followed by the addition of thrombin (0.1 U/ml) to trigger intracellular alkalinization in the presence or absence of activated MMP-9 (21 and 90 ng/ml). Profiles are representative examples of four similar experiments.

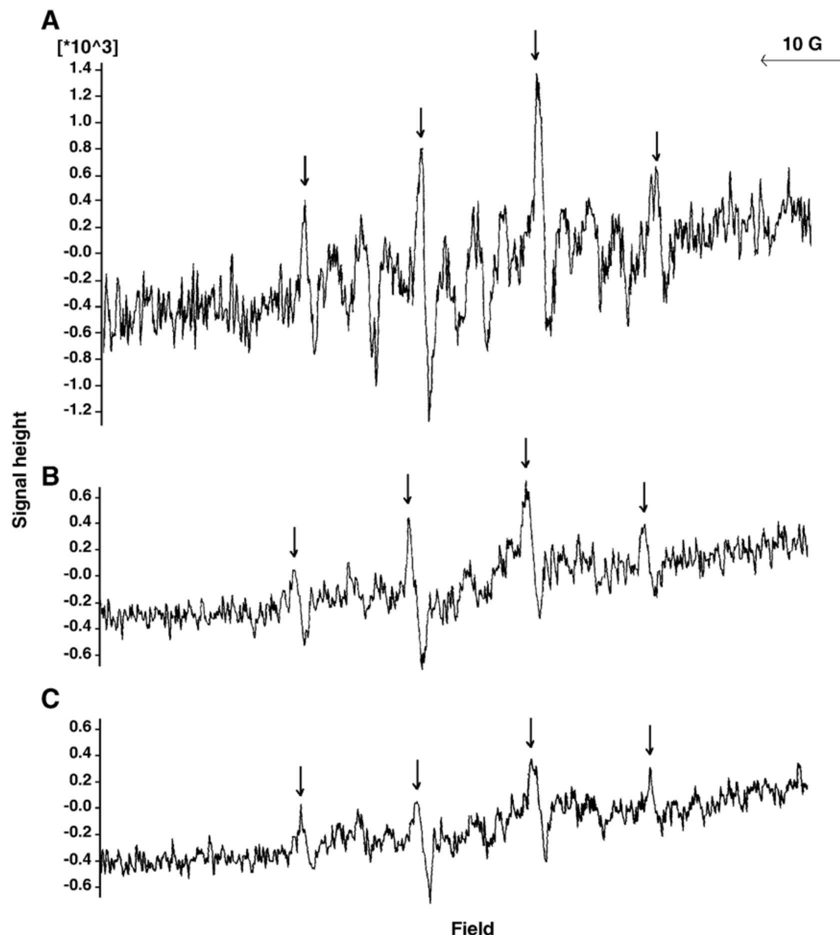

Fig. 5. Electron spin resonance (ESR) spectra of activated MMP-9 in the inhibition of hydroxyl radical formation in collagen-activated platelets. Platelet suspensions (4.5×10^8/ml) were preincubated with (A) the vehicle solution (10 µM AMPA) or activated MMP-9 at (B) 21 ng/ml and (C) 90 ng/ml for 3 min, and then collagen (1 µg/ml) was added to trigger platelet aggregation as described in Materials and methods. The reaction was allowed to proceed for 5 min, followed by the addition of DMPO (100 mM) for the ESR experiments. The spectrum is a representative example of five similar experiments.

MMP-9 (0.084 ± 0.006 at 21 ng/ml; 0.034 ± 0.010 at 90 ng/ml) (Fig. 4). Neither pro-MMP-9 (10 µg/ml) nor AMPA (10 µM) significantly inhibited the thrombin-evoked pH increase in human platelets (data not shown). In addition, cariporide (0.5 µM), an Na^+/H^+ exchange inhibitor (Baumgarth et al., 1998), significantly inhibited the thrombin-evoked pHi increase by about 90% in this study (data not shown).

3.6. Free radical-scavenging activity of activated MMP-9 in collagen-activated platelets

In this study, a typical ESR signal of the hydroxyl radical was observed, as shown in Fig. 5. Activated MMP-9 (21 and 90 µg/ml) markedly suppressed hydroxyl radical formation by about 47% and 63% ($n = 5$), respectively. This observation may provide in vitro evidence of the usefulness of the free radical-scavenging activity of activated MMP-9 in activated platelets.

4. Discussion

The principal objective of this study was to further describe the detailed inhibitory mechanisms involved in the inhibition of agonist-induced human platelet aggregation by activated MMP-9. The inhibition was directly proportional to the amount of activated MMP-9 used and the incubation time. Activated MMP-9-induced inhibition of platelet aggregation was rapid in onset reaching a maximum within 30–60 min of incubation (Fig. 1). Although the action mechanisms of various platelet aggregation agonists, such as collagen, ADP, U46619, and arachidonic acid differ, activated MMP-9 significantly inhibits platelet aggregation stimulated by all of them (Sheu et al., 2004). This implies that activated MMP-9 may block a common step shared by these inducers. These results also indicate that the site of action of activated MMP-9 is not at the receptor level of individual agonists. Triflavin acts by binding to the glycoprotein IIb/IIIa complex on the platelet surface membrane, resulting in interference with the interaction of fibrinogen with its specific receptor (Sheu et al., 1996, 1999b). There is now a multitude of evidence suggesting that the binding of fibrinogen to the glycoprotein IIb/IIIa complex is the final common pathway for agonist-induced platelet aggregation. Therefore, we decided to further evaluate whether or not activated MMP-9 binds directly to the platelet glycoprotein IIb/IIIa complex, leading to inhibition of platelet aggregation induced by

agonists. In this study, we found that activated MMP-9 had no significant effect on FITC-triflavin binding to the glycoprotein IIb/IIIa complex, indicating that the antiplatelet activity of activated MMP-9 is possibly not directly due to interference with the binding of fibrinogen to its specific receptor on the platelet membrane. Conformational changes in the plasma membrane and/or changes in membrane fluidity represent a generally accepted mechanism for the antiplatelet effect of numerous drugs, including local anesthetics, chlorpromazine, and beta-blockers (Kobayashi et al., 1986). Therefore, we wondered whether activated MMP-9 might also inhibit platelet aggregation by influencing membrane fluidity. To test this hypothesis the fluorescent probe, DPH, was used to label biological membranes. Measurements using the fluorescent probe technique demonstrated that activated MMP-9 is capable of direct interaction with platelet membranes. In this study, activated MMP-9 (21 and 90 ng/ml) concentration-dependently decreased the DPH-relative fluorescence intensity of platelet membranes (Fig. 3). Activation of platelets is accompanied by a decrease in membrane fluidity as reflected by an increase in fluorescence intensity of DPH-labeled platelets (Pribluda and Rotman, 1982). Reorganization of cytoplasmic contractile and structural proteins is essential for platelet functions (Pribluda and Rotman, 1982). Therefore, a decrease in membrane fluidity (producing relatively rigid membranes) in activated platelets may affect the cytoskeletal network. This finding suggests that changes in platelet membrane fluidity may be the primary mechanism responsible for the antiplatelet effect of activated MMP-9.

Activation of platelets by a variety of agonists (i.e., thrombin and ADP) is associated with stimulation of the Na^+/H^+ exchanger (Kimura et al., 1992). This mode of activation of the Na^+/H^+ exchanger usually induces a rise in cytosolic Ca^{2+}, granule secretion, stimulation of shape change, and aggregation (Kimura et al., 1992). Basal pHi is normally maintained within a narrow range, and even small changes in pHi may have significant effects on platelet activity. In many cell types (i.e., smooth muscle cells), Na^+/H^+ exchange activity is regulated by $[Ca^{2+}]i$ (Nieuwland et al., 1994). Furthermore, Kimura et al. (1992) reported that cyclic nucleotides modulate Na^+/H^+ exchange in human platelets. Inhibition of Na^+/H^+ exchange by cyclic GMP has also been demonstrated in other cells, such as epithelia of the urinary system (Cha et al., 2005). Activated MMP-9 (21 and 90 ng/ml) increased the formation of cyclic GMP in human platelets as described previously (Sheu et al., 2004). Therefore, relationships among cyclic nucleotides (i.e., cyclic GMP) and the Na^+/H^+ exchanger may play an important role in mediating the antiplatelet activity of activated MMP-9.

Reactive oxygen species (i.e., hydrogen peroxide and hydroxyl radicals) derived from platelet activation may affect cells with which they come into intimate contact, such as the endothelium, and this could result in amplification of platelet reactivity during thrombus formation. Furthermore, reactive oxygen species act as second messengers during the initial phase of platelet activation processes (Iuliano et al., 1994). Mirabelli et al. (1989) showed an increase in cytosolic Ca^{2+} concentration upon platelet exposure to oxidative stress. Platelets primed by exposure to subthreshold concentrations of AA or collagen are known to be activated by nanomolar levels of hydrogen peroxide, and this effect is mediated by hydroxyl radicals formed in an extracellular Fenton-like reaction (Pietraforte et al., 2002). It is also evident that some of the hydrogen peroxide produced by platelets is converted into hydroxyl radicals, as platelet aggregation can be inhibited by hydroxyl radical scavengers (Leo et al., 1995). In this study, we found that activated MMP-9 inhibited platelet aggregation possibly, at least partially, through inhibition of free radical formation in activated platelets.

In conclusion, results of this study further suggest that the inhibitory effect of activated MMP-9 may possibly involve the following mechanisms. Activation of MMP-9 may be due to induction of conformational changes in membrane fluidity, with a resulting influence on phospholipase C activity followed by inhibition of PKC activation; intracellular Ca^{2+} mobilization subsequently inhibits free radicals (such as hydroxyl radicals) released from activated platelets. In addition, activated MMP-9 also triggers the formation of cyclic GMP which subsequently inhibits the Na^+/H^+ exchanger. This leads to reduced intracellular alkalinization and intracellular Ca^{2+} mobilization, and ultimately inhibition of platelet aggregation. The most important finding in this study provides further new insights concerning the effects of activated MMP-9 on platelet aggregation. However, no data in the literature are available concerning MMP-9 receptor(s) on platelets or other cells; this thus needs to be further identified. MMP-9 appears to represent a novel platelet-regulatory system based on its anti-aggregatory effect. Therefore, it is possible that an imbalance in the generation and/or release of MMP-9 contributes to pathological vascular diseases associated with platelet aggregation such as stroke, myocardial infarction, and thrombosis. We therefore believe that our present results indicate a physiologically relevant function for MMP-9 as a negative feedback regulator during platelet activation. When MMP-9 accumulates in the microenvironment of a generating thrombus, the recruitment and activation of platelets is thus prevented. However, the physiological relevance of a direct anti-aggregatory effect of MMP-9 still remains to be further studied.

Acknowledgments

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