von Willebrand Factor Proteolytic Processing and Multimerization Precede the Formation of Weibel-Palade Bodies

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We investigated the intracellular site of pro-von Willebrand factor (pro-vWF) cleavage and multimerization, as well as the fate of the propolypeptide (von Willebrand antigen II) after cleavage. Analysis of subcellular fractions of endothelial cells metabolically labeled with sulfate showed that both cleavage and covalent multimerization occur after sulfation and precede the formation of Weibel-Palade bodies. Because sulfation is a processing step localized to the trans-Golgi network (TGN), our results indicate that multimerization and proteolytic cleavage also occur in this organelle. After cleavage, the propolypeptide remains noncovalently associated with the mature vWF subunit. This association is promoted by a high calcium concentration and an acidic pH (conditions thought to prevail in the TGN) and explains the 1:1 stoichiometry of the propolypeptide and mature vWF found in Weibel-Palade bodies. The propolypeptide remains an integral part of the large multimeric vWF aggregates in the Weibel-Palade body until secretion. When secretion occurs under slightly acidic conditions, such as may be found in poorly perfused wounds, the propolypeptide remains associated with the endothelial surface-bound vWF, and may thus participate in the wound healing process.

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tide and aggregated m-vWF that could explain the cotargeting of these two proteins to the Weibel-Palade bodies.

MATERIALS AND METHODS

Materials. All chemicals were from Sigma (St Louis, MO), unless specified. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) supplies were from Biorad (Richmond, CA). The rabbit antiserum to vWF was from Diagnostica Stago (Asnieres, France). Secondary antibodies were from Cappel (Duham, NC). [35S]Sulfate and L-[35S] cysteine (>1,000 Ci/mmol) were from New England Nuclear (Boston, MA). Octyl glucoside (octyl-β-D-glucopyranoside) was from Calbiochem (La Jolla, CA).

Cell culture and metabolic labeling. Endothelial cells were obtained from human umbilical veins as described and grown in McCoy’s 5A medium supplemented with 20% fetal calf serum (FCS), 50 mg/mL endothelial cell mitogen (Biomedical Technologies, Stoughton, MA) and 100 mg/mL porcine heparin. Cells were used in the first or second passage.

For equilibrium labeling of Weibel-Palade bodies, cells were incubated with 40 μCi/mL [35S]cysteine in the same medium for 2 days. For pulse-chase labeling with radioactive sulfate, preconfluent endothelial cells grown on gelatin-coated dishes were preincubated for 40 minutes in sulfate-free Hank’s Balanced Salt Solution supplemented with 10% dialyzed FCS, RPMI amino acids, and vitamin supplements, and then incubated in the same medium with [35S]sulfate (1 mCi/mL) for the indicated times. After two washes with phosphate-buffered saline (PBS) supplemented with 1 mmol/L MgSO4, the cells were further incubated in McCoy’s medium with 20% FCS.

Cell fractionation. The procedure was performed essentially as described. Cells from three dishes of endothelial cells (150-mm diameter) were homogenized in 0.8 mL homogenization buffer (HB: 250 mmol/L sucrose, 1 mmol/L EDTA, 20 mmol/L Tris/HCl, pH 7.2) with 50 strokes of a Dounce homogenizer (No. 19; Kontes Glass Co, Vineland, NJ). A postnuclear supernatant was obtained by centrifugation at 40,000 g for 10 minutes and layered over a sucrose step gradient made of 35% Percoll, 250 mmol/L sucrose, pH 7.2, in 10-mL Oakridge tubes (Naigco, Rochester, NY). A gradient was generated by centrifugation at 40,000 rpm for 60 minutes in a Sorvall RC-5B centrifuge (SM24 rotor). Twelve 0.8 mL fractions were collected from the top and were supplemented with 5 mmol/L EDTA, 1 mmol/L phenylmethylsulfonyl fluoride (PMSF), and 10 μmol/L pepstatin A and stored at −80°C. Alternatively, the fractions were recovered free of Percoll by floatation through Nycodenz and pelleted by dilution and recentrifugation as described.

vWF content of the fractions was measured by enzyme-linked immunosorbent assay (ELISA). Galactosyl transferase activity was assayed with N-Acetyl glucosamine as an acceptor using the method of Chaney et al.

Immunoprecipitation and gel electrophoresis. Percoll fractions were diluted with 3 vol of lysis buffer (100 mmol/L Tris/HCl, pH 8.0, 5 mmol/L EDTA, 0.5% NP40, 0.5% Triton X-100, 0.1% SDS, 5 mg/mL ovalbumin) supplemented with protease inhibitors (10 μmol/L pepstatin A, 1 mmol/L PMSF, 1 mmol/L iodoacetic acid, and 1 mmol/L N-methyl maleimide). Alternatively, cell monolayers (grown in 10-cm2 dishes) were collected by scraping in 1 mL lysis buffer and preclarified by centrifugation at 14,000g for 5 minutes. The lysates were incubated for 2 hours at room temperature with 20 mg protein A Sepharose, preincubated with 50 μL anti-vWF serum and 15 mg each of two purified monoclonal antibodies to the propolypeptide, BR5 and CM10. The Sepharose beads were then washed four times in lysis buffer and once in 20 mmol/L Tris/HCl, pH 8.0, and the bound proteins were recovered by boiling for 5 minutes in electrophoresis sample buffer, supplemented with 50 mmol/L DTT where indicated. This protocol allows quantitative immunoprecipitation of m-vWF and propolypeptide.

Protein samples were analyzed by SDS-PAGE according to Laemmli. Gels with [35S]sulfate-labeled samples were dried and analyzed with a Molecular Dynamics Phosphorlager. Model 425B. Gels with [35S]-cysteine labeled samples were processed for fluorography. SDS-agarose gel electrophoresis was performed as described.

Aggregation studies. Weibel-Palade bodies were purified from endothelial cells labeled to equilibrium with [35S]-cysteine and were recovered by floatation on Nycodenz. When this preparation was analyzed by SDS-PAGE and fluorography, propolypeptide and m-vWF were the only prominent radiolabeled bands detected. The Weibel-Palade body pellet was resuspended in hypertonic buffers, either acidic (MES 10 mmol/L, pH 6.4) or neutral (Tris/HCl 10 mmol/L, pH 7.4), each supplemented either with 10 mmol/L CaCl2 or 1 mmol/L EGTA. When EGTA was used, the buffer contained also 15 mmol/L KCl to equalize the osmolality. The buffers were also supplemented with nonionic detergents to ensure lysis of the granule membranes. The samples were incubated on ice for 1 hour and layered over a sucrose step gradient made of 1 mL 1.3 mol/L sucrose and 1.5 mL of 0.8 mol/L sucrose (made up with the buffer corresponding to the sample loaded), and were centrifuged for 90 minutes at 200,000 g in a SW55 Ti swing-out rotor in an ultracentrifuge (Model L-8-80M; Beckman). The supernatant and the pellet were recovered and analyzed by SDS-PAGE and fluorography. (No radiolabeled material could be recovered from the 0.8/1.3 mol/L sucrose interface).

Immunofluorescence. Cells grown on gelatin-coated glass coverslips were incubated for 15 minutes in a buffer containing 10 mmol/L PIPES, 135 mmol/L NaCl, 5 mmol/L KCl, 0.5 mmol/L MgSO4, 1 mmol/L CaCl2, and 0.1% glucose, at a pH of 6.4 or 7.4, in the presence of 10 μmol/L A23187 or vehicle (dimethylsulfoxide [dimSO]) 0.1%. The cells were then fixed in 3.7% formaldehyde (diluted in the same buffer as used for the cell incubation), and permeabilized in 0.5% Triton X-100 in PBS. For double-label staining, cells were incubated sequentially with the antipropolypeptide monoclonal antibody BR5 (ascites fluid 1:1,000), rhodamine-conjugated goat antibody to mouse IgG 1:100, rabbit antiserum to human vWF 1:250, and fluorescein-conjugated goat antibody to rabbit IgG 1:500. Each incubation was performed for 30 minutes at 37°C.

Electron microscopy. Pelleted fractions were fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, and stained with uranyl acetate and lead citrate. Ninety-five nanometer sections were cut after embedding in Epon 812, and were examined with a Philips CM10 transmission electron microscope.

RESULTS

Time course of pro-vWF processing in relation to sulfation. vWF is one of several proteins in endothelial cells that is modified posttranslationally by the addition of sulfate to N-linked carbohydrates. Sulfation is thought to occur in the TGN, and is a useful marker for this compartment. Pulse-chase labeling studies with radioactive sulfate therefore allow us to restrict the analysis of pro-vWF processing to the events occurring in the TGN or more distally in the secretory pathways. After 40 minutes of incubation in a sulfate-free medium, endothelial cells were metabolically labeled with [35S]sulfate for 10 minutes, followed by a chase period of 0 to 2 hours. Pro-vWF and its cleavage products were visualized by immunoprecipitation, SDS-PAGE, and autoradiography (Fig 1). At the end of the 10-minute labeling period, pro-vWF was the main immunoprecipitated molecular species. During the chase period, pro-vWF was converted from www.bloodjournal.orgFrom guest on November 16, 2017. For personal use only.
More than half of the pro-vWF was found to be cleaved to m-vWF and propolypeptide, with a rapid time course. Thus, it appears that pro-vWF proteolytic processing occurs rapidly after sulfation. Sulfated vWF was found in the culture medium in increasing amounts over time, and could be detected already after a 10-minute chase period. The ratio of pro-vWF to m-vWF in the medium remained similar over the observed period, indicating that the cleavage is mainly an intracellular process.

Intracellular localization of pro-vWF processing. Endothelial cells were fractionated over Percoll gradients to separate Weibel-Palade bodies from Golgi membranes. vWF was measured in individual fractions by ELISA (Fig 2A). The high-density peak (fractions 8 through 11) had previously been shown to be highly enriched in Weibel-Palade bodies. This was confirmed by electron microscopy, which showed a high content of electron-dense, elongated, membrane-bound structures with a cross-sectional diameter of 0.1 to 0.2 mm (Fig 2B). The low-density peak coincided with the trans-Golgi marker, galactosyl transferase (Fig 2A). The endoplasmic reticulum was also located to this peak (see below).

By electron microscopy, these pelleted fractions contained abundant membranes, some of which retained the shape of stacks typical of the Golgi apparatus (Fig 2C). To investigate the stability of Weibel-Palade bodies in Percoll, and whether part of the vWF found in the low-density fractions was artifically released from the granules during the fractionation procedure, Weibel-Palade bodies (fractions 8 through 11) obtained from endothelial cells labeled to equilibrium with [35S]-cysteine were subjected to a second fractionation. Radiolabeled vWF was located in the same high-density fractions from which it originated, demonstrating the stability of Weibel-Palade bodies in Percoll (data not shown).

To determine whether pro-vWF proteolytic processing occurs in the TGN or in the Weibel-Palade bodies, cells were labeled with [35S]sulfate (1 mCi/mL) for 20 minutes, further incubated in label-free medium, and fractionated. Individual fractions were subjected to immunoprecipitation with anti-vWF and antipropolypeptide monoclonal antibodies, and were analyzed by SDS-PAGE. At the end of the 20-minute labeling period (Fig 3A), labeled vWF could be detected only in light fractions, in a distribution closely paralleling the activity of the trans Golgi marker galactosyl transferease (see Fig 2A). The transit of vWF from the TGN to immature and mature secretory granules would be expected to translate into a progressive shift of the labeled protein to denser fractions. Such a shift could indeed be detected, but with a slower time course than that of cleavage. After a 20-minute chase period, labeled m-vWF became visible in fractions 6 and 7 (data not shown), and then continued to accumulate progressively in the typical Weibel-Palade body fractions (Fig 3B and C). The findings that pro-vWF proteolytic processing occurs rapidly after sulfation but largely, if not entirely, before vWF shifts into denser fractions strongly suggest that pro-vWF cleavage occurs in the TGN.

In control experiments, fractionation and vWF immunoprecipitation were performed on cells metabolically labeled for 20 minutes with [35S]-cysteine. At this short time after synthesis, no proteolytic cleavage would be expected because pro-vWF is still located in the endoplasmic reticulum. Pro-vWF, but not the cleavage products, could be detected (mostly in fractions 3 and 4), indicating the absence of artifactual cleavage during the fractionation or the immunoprecipitation procedures (data not shown).

Intracellular localization of multimer formation. Next, we determined the multimeric pattern of vWF located in the different cellular fractions. To minimize the contamination by vWF from other organelles, Golgi fractions (3 and 4) were prepared from cells after labeling for 20 minutes, whereas Weibel-Palade body fractions (8 through 11) were prepared after a 2-hour labeling period and an 18-hour chase period. vWF was purified by immunoprecipitation and the samples

![Fig 1. Time course of pro-vWF cleavage in relation to sulfation. Endothelial cells were labeled with [35S]-sulfate (1 mCi/mL) for 10 minutes, and lysed after a chase period indicated (in minutes) above each lane. Immunoprecipitation with an antiserum to vWF and two antipropolypeptide monoclonal antibodies was then performed on the cell lysates (C) and on the corresponding culture medium (M). The antigens were electrophoresed on a 5% polyacrylamide gel after reduction, and the gel was analyzed with a Phosphorimager. The two bars on the right indicate the migration of the 200-kD and the 97-kD MW markers.](image)
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were analyzed unreduced on 2% agarose gels (Fig 4). Weibel-Palade bodies contained only high MW multimers, in agreement with the results of Sporn et al.8 The Golgi fractions contained dimers as well as large multimers. The multimers were mostly of the same size as those found in the Weibel-Palade bodies, whereas the conditioned medium was enriched in small multimers. These results indicate that large vWF multimers can be formed in the TGN, before their incorporation into the Weibel-Palade bodies.

Association of propeptides with m-vWF. As can be seen from Fig 3, the ratio of m-vWF to propolypeptide remains constant after cleavage, whether in the Golgi fractions (Fig 3A) or in the Weibel-Palade bodies (Fig 3C). The stoichiomi-

Fig 2. Percoll density gradient fractionation of an endothelial cell postnuclear supernatant. (A) A postnuclear supernatant obtained from human umbilical vein endothelial cells was loaded on a 35% Percoll gradient before centrifugation. Twelve fractions of 0.8 mL each were collected from the top. The values for vWF are given in micrograms per fraction, those for galactosyl transferase (galact.-T) in nanomoles per hour per fraction. (B and C) Pelleted membranes from fractions 3 and 4 (C) and 9 through 11 (B) were examined by electron microscopy. The field in (B) shows numerous Weibel-Palade bodies, together with rare other organelles or fragmented organelles. The field in (C) shows mixed membranes; some structures retain a shape that strongly evokes Golgi stacks. Bar in (B) = 500 nm (same magnification in [b] and [c]).

Fig 3. Subcellular localization of pro-vWF cleavage. Cells were labeled with 1 mCi/mL [35S]-sulfate for 20 minutes (A and B) or for 1 hour (C). Cells were fractionated over a Percoll gradient as in Fig 2A, after a chase period of 0 (A), 4 (B), or 18 (C) hours. Each fraction was immunoprecipitated with an antisera to vWF and two antipropolypeptide monoclonal antibodies, and the antigens were analyzed reduced on a 5% polyacrylamide gel. The fraction numbers are as indicated and the two bars on the right indicate the migration of the 200-kD and the 97-kD MW markers. The arrowheads indicate the position of migration of (from the top) pro-vWF, m-vWF, and propolypeptide.
etry of m-vWF and propolypeptide in Weibel-Palade bodies is 1:1. This suggests that either m-vWF and the propolypeptide are targeted independently, although with identical efficiency, or that they remain noncovalently associated after cleavage. To test this later hypothesis, we examined whether the ionic conditions thought to exist in the TGN favor a noncovalent association between the propolypeptide and m-vWF. Purified, [35S] cysteine-labeled Weibel-Palade body fractions were incubated for 1 hour either at pH 6.4 or 7.4, in the presence of calcium or EGTA. The buffers were supplemented with 0.75% octyl-glucoside, a nonionic detergent. The samples were then layered over a sucrose gradient and subjected to high-speed centrifugation and the supernatant and the pellet were analyzed by SDS-PAGE and fluorography (Fig 5). In all four buffers tested, m-vWF was found in the high-speed pellet. This suggests that m-vWF remains in an aggregated state (resistant to a nonionic detergent), independently of the ionic conditions tested. At pH 6.4 in the presence of calcium, the propolypeptide was also found in the pellet, whereas at pH 7.4 in the presence of EGTA, the propolypeptide was recovered in the supernatant, indicating a noncovalent, calcium- and pH-dependent interaction between the propolypeptide and the aggregated m-vWF. High calcium and acidic pH tested individually supported this interaction only weakly.

Similar results were obtained with other nonionic detergents (2% Lubrol, 1.5% octyl-glucoside, 0.5 mg/mL saponin). In control experiments, m-vWF could not be pelleted after the Weibel-Palade bodies were incubated with 2% SDS. Thus, dissociated vWF multimers are not large enough to be pelleted under the conditions used, indicating that recovery in the pellet reflects higher aggregation of the m-vWF multimers.

pH-dependent association of the propolypeptide and m-vWF after Weibel-Palade release. After exocytosis, part of the released vWF remains trapped under the cells, associated with the extracellular matrix and the plasma membrane, in large patches easily recognizable by immunofluorescence, whereas the propolypeptide is found in the supernatant only. We next examined whether changes in extracellular pH conditions could affect the propolypeptide distribution after secretion. To induce exocytosis, endothelial cells were incubated for 15 minutes with the calcium ionophore A23187 in PIPES buffer either at pH 6.4 or pH 7.4, fixed, permeabilized, and stained by double-label immunofluorescence with antibodies to vWF and propolypeptide. Weibel-Palade bodies were shown with both antibodies. No staining for extracellular vWF was detected in unstimulated control cells (data not shown). After stimulation with A23187 at pH 7.4, large extracellular patches of vWF could be seen; these patches stained only faintly or not at all with antibodies to the propolypeptide (Fig 6a and b). In contrast, at pH 6.4, the patches were stained very brightly with antibodies to both vWF and propolypeptide (Fig 6c and d). This observation suggests that, at pH 6.4, the propolypeptide remains noncovalently associated with m-vWF released from Weibel-Palade bodies and deposited extracellularly.

DISCUSSION

Our results strongly suggest that, in endothelial cells, the proteolytic processing of pro-vWF occurs predominantly if not entirely in the TGN. This conclusion is based on the finding that cleavage rapidly follows sulfation, in a compartment of low density in Percoll gradients that is therefore distinct from the storage granules. This compartment has the same density as the TGN as determined by the marker en-
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Fig 5. pH- and calcium-dependent association of propolypeptide with m-vWF aggregates. Weibel-Palade bodies purified from cells labeled to equilibrium with [35S] cysteine were incubated for 1 hour with 0.75% octyl-glucoside in buffers described above each lane. The preparation was layered over a sucrose step gradient. After high-speed centrifugation (200,000 g, for 90 minutes), the supernatant (S) and the pellet (P) were analyzed on a 7% polyacrylamide gel, the fluorogram of which is shown. The bars on the right indicate the migration of MW markers (from the top: 200, 97, 69, and 30 kDa).

It has been shown that the enzyme furin (also called PACE) can cleave pro-vWF when the two genes are cotransfected into COS cells. This calcium-dependent serine protease is believed to be ubiquitously expressed and located in the TGN. The finding that pro-vWF processing also occurs in the TGN lends further support to the view that furin is the native pro-vWF converting enzyme. It was previously suggested that pro-vWF is processed within the Weibel-Palade bodies, to account for the finding that both the propolypeptide and m-vWF are found in stoichiometric amounts in purified Weibel-Palade bodies, or in cell culture supernatants after stimulated release. The only known endoproteases located to secretory granules are the enzymes PC1 and PC2, recently shown to be converting enzymes for proinsulin. However, the expression of these enzymes appears to be restricted to neuro-endocrine cells. Furthermore, transfection studies such as those performed with furin have shown that pro-vWF is not a substrate for either of these enzymes.

The 1:1 stoichiometry of m-vWF and propolypeptide in the Weibel-Palade bodies can now be explained by our observation of a noncovalent association between these two proteins. This interaction was shown at pH 6.4 but not at pH 7.4, and was also dependent on the presence of calcium. We suggest that, before cleavage, the propolypeptide folds back to interact with the mature portion of pro-vWF, and that this interaction is maintained after cleavage, in the TGN and later in the Weibel-Palade bodies. This is reminiscent of the proposal of Wise et al.15 that the uncleaved propolypeptide masks the factor VIII binding site of pro-vWF, which is located in the N-terminal region of the mature subunit. Although the intragranular pH of Weibel-Palade bodies has not been measured directly, it can be assumed that it is equally or more acidic than in the TGN, as it is the case in other types of secretory granules. Our results also imply that, in the Weibel-Palade bodies, the propolypeptide is not excluded from the vWF aggregates (see Fig 5) and may be part of the granule membrane (see Fig IC and Weibel and Palade).

The pH- and calcium-dependent association of the propolypeptide with the mature subunit before cleavage may also be a key initiating event in the multimerization process. In intact endothelial cells, vWF multimerization and storage in intact endothelial cells, vWF multimerization and storage
are inhibited in the presence of chloroquine or ammonium chloride, which increase the pH of acidic cellular compartments. In vitro, the multimerization of purified pro-vWF dimers seems to be mediated by the intrinsic disulfide isomerase activity of the propolypeptide. This process is triggered by lowering the pH and is also dependent on the presence of calcium (Mayadas and Wagner, unpublished observations). Our results now show that multimerization is already initiated in a distal Golgi subcompartment and thus precedes the formation of Weibel-Palade bodies. Given the similar pH and calcium requirements, the noncovalent interaction between the propolypeptide and the mature subunit could actually initiate the multimerization process by bringing the "enzyme" close to its substrate. Multimerized vWF may be selectively incorporated into nascent Weibel-Palade bodies. However, it should be noted that the formation of covalent multimers is not a prerequisite for storage. When the activity of the prosequence in promoting interchain disulfide bond formation is inhibited by site-specific mutagenesis, the protein still forms elongated aggregates in Weibel-Palade body-like structures upon expression in heterologous cells, indicating that the capacity of the molecules to form noncovalent multimers and to be stored was preserved.

Our data show that storage (i.e., the formation of Weibel-Palade bodies) is a slower process than constitutive release (compare Figs 1 and 3), implying that vWF destined for storage has a longer residence time in the TGN. We suggest that multimerization promotes vWF retention in the TGN, thus favoring storage by prolonging the availability of vWF for incorporation into the nascent Weibel-Palade bodies. Retention in the TGN may also prolong the exposure to the processing enzyme, thus ensuring the completion of pro-vWF cleavage.

The propolypeptide is required for the targeting of vWF to storage granules. However, a mutation that prevents the prosequence cleavage (Arg to Gly at residue 763) abolishes storage, although the pro-vWF mutant undergoes normal multimerization. This suggests that cleavage not only precedes but may actually be a prerequisite for targeting. It has been proposed that the aggregation of secretory proteins is a signal for their targeting to the regulated pathway. One possibility, therefore, is that the propolypeptide, after a conformational change induced by cleavage, promotes the assembly of multimers into aggregates. Our experiments show that, in Weibel-Palade bodies, m-vWF remains in an aggregated form, even after the removal of the propolypeptide at neutral pH, implying that the propolypeptide is not directly involved in the cross-linking of m-vWF multimers. It is possible that the propolypeptide initiates aggregation, e.g., by promoting the proper alignment of the multimers, but is no longer necessary to maintain the aggregates' stability. A more likely hypothesis is that a critical function of the propolypeptide is to prevent the aggregation of pro-vWF at neutral pH in the endoplasmic reticulum and the Golgi appa-
ratus. Once pro-vWF molecules reach the more acidic TGN, cleavage of the propolypeptide may unmask or form sites on m-vWF that are necessary for aggregation, thus initiating the formation of Weibel-Palade bodies. This hypothesis does not rule out an additional role for the propolypeptide in targeting. It has been suggested that regulated secretory proteins interact with a membrane receptor that mediates the targeting to storage granules. The propolypeptide, non-covalently associated with the mature subunit, may bind to such a receptor. Neither mature vWF nor the prosequence are stored when expressed individually (Mayadas and Wagner, Voorberg et al, and Journet and Wagner, unpublished observations).

The regulated release of vWF, as well as of propolypeptide, from endothelial cells can be triggered by thrombin or by inflammatory mediators such as histamine and complement components C5b-9. It has recently been shown that the propolypeptide is a chemotactic factor for macrophages and neutrophils, and that it also induces the expression of tissue factor by endothelial cells. These observations could be all the more relevant if the propolypeptide remains trapped at the site of vascular injury. Trapping in the matrix could be mediated by the collagen binding site in the D2 domain of the propolypeptide. Our results raise the additional possibility that retention of the propolypeptide may also occur as a result of its interaction with vWF either on the surface of stimulated endothelial cells or in the subendothelial matrix. This would occur under acidic conditions such as may be found in poorly perfused areas of tissue injury. The propolypeptide expressed together with vWF and P-selectin at the site of injury may therefore contribute to the wound healing process.

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