The role of the D1 domain of the von Willebrand factor propeptide in multimerization of VWF


While studying patient plasma containing an unusual pattern of von Willebrand factor (VWF) multimers, we discovered a previously unreported phenomenon: heavy predominance of dimeric VWF. Genomic analysis revealed a new congenital mutation (Tyr87Ser) that altered the final stages of VWF biosynthesis. This mutation in the propeptide (VWFpp) resulted in synthesis of dimeric VWF with an almost complete loss of N-terminal multimerization. The multimer pattern in patient plasma appears to result from separate alleles synthesizing wild-type or mutant (dimeric) VWF, with homodimers composing the predominant protomeric species. We have expressed VWF protein containing the Tyr87Ser mutation and analyzed the intracellular processing and resulting VWF biological functions. The expressed dimeric VWF displayed a loss of several specific functions: collagen binding, factor VIII binding, and ristocetin-induced platelet binding. However, granular storage of dimeric VWF was normal, demonstrating that the lack of multimerization does not preclude granular storage. Although the tertiary structure of the VWFpp remains unknown, the mutant amino acid is located in a region that is highly conserved across several species and may play a major role in the multimerization of VWF. Our data suggest that one function of the highly cysteine-rich VWFpp is to align the adjacent subunits of VWF into the correct configuration, serving as an intramolecular chaperone. The integrity of the VWFpp is essential to maintain the proper spacing and alignment of the multiple cysteines in the VWFpp and N-terminus of the mature VWF. (Blood. 2002;100:1699-1706)

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relatively normal distribution of the remaining VWF multimers. In this report, we describe how a congenital defect located in the VWFpp results in a complete loss of VWF multimerization but maintenance of normal vesicular storage of VWF. The natural intramolecular chaperone function of the VWFpp has been altered by this defect. Heterozygosity in this patient presented a unique opportunity to study a VWF dimer mutant in vitro and determine the extent to which the VWFpp functions intracellularly during VWF multimer assembly in the secretory pathway.

Materials and methods

 Isoelectric focusing

 Total cellular RNA and genomic DNA were prepared from platelets and leukocytes, respectively, isolated from 50 mL peripheral blood as previously described.14,15

 Reverse transcription—PCR and cDNA sequencing

 Reverse transcription of purified platelet RNA was performed as previously described.14,15 with the use the VWF antisense primer a1144-1130 (CA TT -Nco1-1130 (CA TACGAGGAACTCAGATCCTGAGAAGAGG) and antisense primer a1115-1081: Nsi1 (CAAAATGGACATGTGTTGACAGTCTGGAGAAGGAGG). Lower-case letters indicate nucleotides altered for the purpose of introducing restriction sites. Second-round polymerase chain reactions (PCRs) were performed under the same conditions, except that the antisense primer was changed to a1059-1026: Nsi1 (GAAGATGCAGCAGAGCAGTAACCTGGTAGTCCTCCACG).

 Tyr87Ser mutant plasmid construction

 The VWF expression vector pW198 (a gift of Dennis Lynch, Dana Farber Cancer Center, Boston, MA) was digested with restriction enzymes XhoI and KpnI (Promega), and the resulting 525-bp fragment was cloned into pGEM7-Zf’ for use as mutagenic PCR template (Promega). Two separate first-round PCRs were performed with the use of the M13 forward universal primer (M13f) with aVWF271-249−mutant nucleotide (aVWF271-249-m) (ATCTCCCAAGAAGACACGGAGAGG) producing a 590-bp product, and sVWF249-271-m (CCTCTCGGTGTCCTTTGGGGAAT) with M13 reverse universal primer (M13r) producing a 174-bp product. A second round of PCR was performed on 2 mL of the first-round products and amplified with M13f and M13r. The resulting PCR fragment was then cloned into the XhoI/KpnI site of pG3vW-1 (an intermediate cloning vector constructed by cloning the 3302-bp SalI-KpnI fragment of pVW198 into the SalI-KpnI site of pGEM3-Zf’ (Promega)). Finally, the vector expressing the Tyr87Ser-VWF defect (pW198-Br) was constructed by a 3-fragment ligation with the use of the mutant 3302-bp SalI/KpnI fragment of pG3vW-1, and the 2415-bp KpnI/BamHI and the 7083-bp BamHI/SalI fragments of pVW198. Introduction of the patient’s mutation into the previously described8 VWFpp expression vector.

Cell culture

 Established cell culture lines were used in the course of this study: monkey kidney cells (COS-7; ATCC, Rockville, MD); mouse pituitary tumor cells (AtT-20/D16v-F2; ATCC); and human embryonic kidney cells (HEK 293T; provided by D. Ginsburg, University of Michigan, Ann Arbor, MI). AtT-20 cells are an adrenocorticotropic hormone (ACTH)–synthesizing model cell line that does not synthesize VWF, but will store VWF when transfected with VWF cDNA.10,16,17 COS-7 and HEK-293T cells are nonstorage cell lines used for high expression of VWF without VWF storage. All cell lines were cultured at 37°C in a 5% CO2 humidified incubator in either complete AtT-20 or HEK293T medium as previously described, or complete COS medium (RPMI 1640 [Life Technologies, Rockville, MD]; 10% fetal bovine serum [FBS]; and 2 mM L-glutamine).

Transient and stable expression of VWF

 VWF expression vectors were transiently transfected into COS-7 cells and HEK-293T cells with the use of lipofectamine (Life Technologies). Transfected conditions were previously determined.3,17 Briefly, 24 hours prior to transfections, cultured cells were plated at 5 × 105 cells (COS-7) in 60-mm culture dishes or at 4 × 106 cells (AtT-20) in 100-mm dishes. Cells were incubated in OPTI-MEM I-reduced serum medium (Life Technologies) diluted with either 15 µg DNA plus 40 µg lipofectamine or 8 µg DNA plus 192 µg lipofectamine for 5 hours at 37°C for the COS-7 and AtT-20 cell transfections, respectively. The DNA/lipid complexes were removed and replaced with complete media (with 10% FBS), and the plasmids were transiently expressed for 72 hours. Conditioned media were harvested from the cells, centrifuged to remove cellular debris, and analyzed by solid-phase capture enzyme-linked immunosorbent assays (ELISAs)15 and functional fluid-phase assays.18,19 The transfected cells were then lysed or fixed for immunofluorescent staining (see “Immunofluorescence staining”). AtT-20 cells were used to establish stable transfections of wild-type VWF (WT-VWF) (pW198.1) or Tyr87Ser-VWF mutant (pW198-Br) with the use of the selection agent G418 (Sigma, St Louis, MO) at 0.3 mg/mL. After several weeks of selection, positive colonies were selected by analysis of the secreted VWF in the conditioned media by VWF antigen-capture ELISA.

Antibodies used

 Antibodies used in this study include AP-1, AWV-1, AWV-3, AWV-5, AWV-17, MBC 33.5, and polyclonal anti-VWF antibodies. The Hybridoma Core Laboratories at the Blood Center of Southeastern Wisconsin (Milwaukee) produced all above antibodies. AP-1 is a monoclonal antibody (mAb) against GPIbα that inhibits the platelet GPIbα binding of VWF. The monoclonal antibodies AWV-1, AWV-3, AWV-5, and AWV-17 recognize distinct epitopes in mature VWF. MBC 33.5 is an anti-VWFpp mAb.

 Multimer analysis

 Expressed VWF was immunoprecipitated from conditioned media with the use of mAb AWV-1 (anti-VWF) or MBC 33.5 (anti-VWFpp) covalently coupled to sepharose 4B agarose beads (Pharmacia Biotech, Piscataway, NJ), and eluted as previously described with the use of a buffer containing 8 M urea/65% sodium dodecyl sulfate (SDS)/13 mM Tris (tris(hydroxymethyl)-aminomethane)/1 mM EDTA (ethylenediaminetetraacetic acid)/0.05% bromophenol blue (pH, 8.8).16 The eluted samples were electrophoresed through 0.65% or 1.5% HGT(P) agarose (FMC Bioproducts, Rockland, ME) gels containing 1% SDS for 16 hours at 40 V . The protein was transferred to nitrocellulose for 30 minutes at 35 V followed by 4 hours at 50 V. Membranes were blocked with 5% nonfat dry milk, incubated overnight with mAbs AWV-5 and AWV-17, and then incubated for 2 hours with horseradish peroxidase (HRP)–conjugated goat antiamouse immunoglobulin G (IgG) (Pierce, Rockford, IL). Membranes were treated with electrogenerated chemiluminescent (ECL) substrate (Amersham Life Science, Arlington Heights, IL), and bands were detected by exposure to autoradiography film.

Platelet-binding assays

 Binding of expressed VWF to platelets was determined by means of a modification of the platelet-binding assay previously described by our laboratory.19 In these assays, conditioned media samples from transiently transfected cells were diluted to equal amounts of VWF (determined by ELISA) and mixed with125I-labeled AWV-1 (anti-VWF mAb). After 60 minutes, formalin-fixed human platelets were added to this mixture in the presence or absence of either ristocetin (1.2 mg/mL) or botrocetin.

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(1.0 μg/mL) under nonstirring conditions. After 30 minutes, reactions were centrifuged at 10,000 g for 10 minutes. Both platelet pellets and supernatants were quantitated to determine the amount of VWF bound to the platelets as determined by measuring the 125I-AVW-1. All data were averaged from 6 independent assays of separate transient transfections and normalized to WT-VWF, which has been established as 100% binding. Controls included media from nontransfected cells and diluted normal pool plasma.

Collagen binding
Binding of expressed VWF to collagen was determined in a microtiter plate assay. Immunol 1 96-well plates (Dynatech, Chantilly, VA) were coated with 300 ng per well of collagen Type III from human placenta (Sigma). Fixed amounts of expressed recombinant VWF (rVWF) (100 ng/mL) were added to the collagen plate and incubated for 60 minutes. Samples were incubated with polyclonal anti-VWF antiserum for 30 minutes and then incubated with AP-conjugated goat antirabbit IgG (Pierce) for 30 minutes. Color was developed with Immunoblot Substrate (Life Technologies) and read at 492 nm in the ThermoMax microplate reader (Molecular Devices). The relative amount of VWF bound (VWF-CB) was calculated by means of SOFTmax, version 2.34 (Molecular Devices), on the basis of a standard curve generated by plating serially diluted pooled normal human plasma.

FVIII binding
Binding of FVIII to VWF was measured in a microtiter plate assay essentially as described by Nishino et al.20 and modified by Kroner et al.17,18 Samples were diluted to a final VWF concentration of 10 μM/mL. Equal volumes were added to an AVW-1-coated microtiter plate (Immulon 1) and incubated for 2 hours. Plates were washed and incubated for 1 hour at 37°C with 50 μL recombinant human FVIII (Baxter Healthcare, Glendale, CA) ranging in concentration from 0 to 4 U/mL. Plates were washed, and the VWF-bound FVIII was determined by chromogenic assay by means of the Chromogenix Coatest FVIII/C4 kit (diaPharma, West Chester, OH). Plates were incubated at 37°C for the enzymatic reactions, and the resulting chromogenic substrate conversion was read at 405 nm in the ThermoMax microplate reader. The amount of FVIII bound was calculated by means of SOFTmax software, on the basis of a standard curve generated by the addition of serially diluted r-hFVIII to microtiter plates wells prior to the chromogenic FVIII assay.

Immunofluorescence staining
Cultured cells transfected with VWF plasmids were analyzed for the subcellular localization of VWF with immunofluorescent antibody labeling of the expressed antigens and confocal laser scanning microscopy, as previously described.21 Transfected AtT-20 cells were grown on 35-mm culture plates (Fisher Scientific, Itasca, IL) in Dulbecco modified Eagle medium/G418 media for 72 hours. Negative controls (nontransfected and mock-transfected cells) were processed in parallel with each immunofluorescence labeling experiment. The cells were processed and immunofluorescently labeled by the sequential antibody staining method.10,22 Purified anti-VWF monoclonal and polyclonal antibodies, and anti-VWFp monoclonal antibodies, were used as primary antibodies. Fluorescein isothiocyanate (FITC)– and Texas Red (TXR)–conjugated donkey IgG (H + L) (Fab’2 fragments) (Jackson ImmunoResearch, West Grove, PA) were the secondary antibodies (diluted at 1:200 and 1:1000, respectively).

Submarine gel analysis
To examine the formation of homodimers and heterodimers of VWF, 2 types of VWF were expressed. Wild-type VWF was coexpressed with hereditary persistence of pro-VWF (HPP-VWF) that contains a disrupted furin-cleavage site. The HPP-VWF mutation was identified in a patient and described by Kroner et al.17,18 Transfected AtT-20 cells were grown on 35-mm culture plates (Fisher Scientific, Itasca, IL) in Dulbecco modified Eagle medium/G418 media for 72 hours. Negative controls (nontransfected and mock-transfected cells) were processed in parallel with each immunofluorescence labeling experiment. The cells were processed and immunofluorescently labeled by the sequential antibody staining method.10,22 Purified anti-VWF monoclonal and polyclonal antibodies, and anti-VWFp monoclonal antibodies, were used as primary antibodies. Fluorescein isothiocyanate (FITC)– and Texas Red (TXR)–conjugated donkey IgG (H + L) (Fab’2 fragments) (Jackson ImmunoResearch, West Grove, PA) were the secondary antibodies (diluted at 1:200 and 1:1000, respectively).

Results
Identification and expression of mutant VWF
In the process of studying a family with clinical symptoms of type 1 VWD, we discovered that the mother and the daughter had a previously unreported multimer pattern. A heavy predominance of VWF dimers was observed in both plasmas (Figure 1, lanes 1, 2), equaling approximately 50% of the total VWF antigen, while the remainder of the VWF appeared to multimerize normally. A single missense mutation (260A>C) was identified in exon 4 (D1 domain), converting Tyr87 to a serine (Tyr87Ser) (Figure 2). When a VWF expression vector containing this mutation was transfected into COS-7 cells, the expressed VWF consisted predominantly of dimers, with a nearly complete loss of multimerization beyond the dimer band (Figure 3, lanes 2, 3).
The binding of VWF to platelet GPIb. The expressed WT-VWF bound of VWF, which contains the GPIb-binding domain, and induces the 20% to 25% of rFVIII as compared with wild-type VWF. Binding of FVIII results with rVWF samples from transient transfections of WT, Arg854Gln, Tyr87Ser, and mock plasmids. Binding to collagen was measured as described previously (Figure 2). Dimeric VWF species were identified by the presence of a 100-kDa band in SDS-PAGE gel electrophoresis.

Effect of dimeric VWF on platelet binding

To determine whether the VWFpp mutation had an effect on the platelet-binding function of VWF, we analyzed the binding capability of expressed VWF in platelet-binding assays performed with ristocetin and botrocetin as agonists (Table 1). The Antibody rosetting assay was used to induce the interaction between VWF and the platelet GPlbo receptor. Biotinylated VWF was added to platelet-rich plasma, and the platelet rosetting was measured by flow cytometry. Patient plasma exhibited what appeared to be dimers from the abnormal allele and normal multimers from the normal allele. The ratio of FVIII bound to VWF captured was between 53% and 62% (normal plasma controls were 91%). In this assay, a heterozygote 2N VWD control displayed a binding ratio of 53%, indicating that the patient’s plasma possesses a degree of FVIII binding similar to that of the control 2N VWF.

Intracellular storage of Tyr87Ser-VWF

Since multimerization occurs prior to VWF regulated storage, we next addressed whether this VWFpp mutation would affect VWF intracellular storage. When expressed in AT-20 cells, the Tyr87Ser dimeric VWF and WT-VWF were secreted at similar levels (303 ± 18 versus 264 ± 27 ng VWF per 1 × 10^6 cells, respectively). To determine whether a dimeric VWF species could be stored in secretory granules, AT-20 cells expressing mutant and wild-type VWF were stained with anti-VWF mAbs to assess intracellular localization of VWF. The punctate granular pattern detected by indirect immunofluorescent microscopy of cells expressing Tyr87Ser-VWF (Figure 4B) appears as small and medium-sized granules similar to those observed in WT-VWF expressing AT-20 cells (Figure 4A). Additional experiments focused on the capability of the mutant propeptide to direct noncontiguous VWF protein to storage. When the Tyr87Ser-VWFpp and mature VWF (Δpro) plasmids were cotransfected into AT-20 cells as 2 separate gene products (in trans), both the mutant propeptide (Figure 5, top panel) and VWF (Figure 5, center panel) were stored colocalized in granules as shown in the lower panel of Figure 5.

Further functional characterization of the dimeric VWF species

We questioned whether the other functions of VWF might be similarly affected. We explored the potential effects on the critical VWF functions of collagen and FVIII-binding by the dimeric VWF species. Since VWF interactions with subendothelial matrix proteins such as collagen are necessary to promote platelet adhesion and wound repair, we analyzed the ability of the recombinant VWF dimers to bind collagen. Compared with the VWF expressed by WT-VWF-transfected cells, the rVWF dimers expressed by Tyr87Ser-VWF–transfected cells displayed only 23% of the collagen-binding potential (Table 1).

In a solid-phase assay for VWF-to-FVIIIIB activity, mAb-captured expressed rVWF was incubated with rFVIII in varying amounts (see “Materials and methods”). Table 1 compares the binding of FVIII results with rVWF samples from transient transfections of WT, Arg854Gln, Tyr87Ser, and mock plasmids. The Arg854Gln is a type 2N mutant construct shown to bind only 20% to 25% of rFVIII as compared with wild-type VWF.

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Table 1. Functional assays of expressed recombinant VWF proteins

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<th>Expressed VWF type</th>
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<td>Ristocetin</td>
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<td>Arg854Gln-VWF (2N)</td>
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All of the transfected VWF samples listed in the first column were analyzed simultaneously. While Tyr87Ser-VWF displayed normal botrocetin-induced platelet binding (97% of WT-VWF), this mutant Tyr87Ser-VWF had reduced ristocetin-induced platelet binding (17% of WT-VWF). VWF binding to collagen type III was also markedly reduced by mutant (dimeric) VWF. The FVIII binding of expressed mutant Tyr87Ser was compared with WT-VWF, Arg854Gln-VWF (type 2N VWD), and mock-conditioned media and found to be reduced.

Discussion

Our laboratory has been investigating the role of the VWFpp in the processing of the mature VWF molecule. A key congenital mutation was discovered in the VWFpp resulting in an unusual multimerization pattern in which there is a marked increase of the high-molecular weight VWF multimers when studied with low-percentage agarose electrophoresis. The abnormality (Tyr87Ser) in this study is one that affects the later stages of VWF biosynthesis: the generation of VWF multimers. When this mutation (Tyr87Ser) was introduced into a VWF expression vector, the secreted VWF was nearly identical from message from the same allele. In addition to wild-type VWF, a second construct (HPP-VWF) expressing pro-VWF with a disrupted furin-cleavage site was used to create a larger protein that could be distinguished from wild type by SDS-agarose gel electrophoresis. This HPP-VWF protein has been shown to multimerize normally. The 2 VWF constructs were expressed independently and coexpressed in HEK293T cells. The resulting conditioned media were analyzed on submature Metaphor-agarose gels that better define dimeric VWF molecules (Figure 6). As shown in Figure 6, our results demonstrate that there was not significant formation of heterodimers (less than 10%, lane 1, middle band). Instead, the predominant species appears to be mainly homodimers (39% HPP-VWF; lane 1, upper band, and 52% WT-VWF; lane 1, lower band). These experiments were repeated with the use of a VWF deletion mutant (A3 domain deleted) with the HPP-VWF. Similar results were obtained: 8% heterodimer, 46% HPP-VWF homodimer, and 46% ΔA3-VWF homodimer (data not shown).

Discussion

Our laboratory has been investigating the role of the VWFpp in the processing of the mature VWF molecule. A key congenital mutation was discovered in the VWFpp resulting in an unusual multimerization pattern in which there is a marked increase of the high-molecular weight VWF multimers when studied with low-percentage agarose electrophoresis. The abnormality (Tyr87Ser) in this study is one that affects the later stages of VWF biosynthesis: the generation of VWF multimers. When this mutation (Tyr87Ser) was introduced into a VWF expression vector, the secreted VWF was nearly exclusively the dimeric subunit (Figure 3). Previous experiments indicated that multimerization is a process that is separate from dimerization. Our studies confirm these findings; the Tyr87Ser VWFpp defect directly affects the multimerization process of VWF. Furthermore, lack of multimerization did not preclude storage in AtT-20 cells (Figures 4 and 5), confirming that multimerization and granular storage are 2 independent processes.

We theorize that the multimer pattern presented in Figure 1 is the result of multimers derived from separate alleles synthesizing wild-type (full-length multimers) and mutant VWF (dimer molecules) with little evidence for production of heterodimers. Recently, Bodo et al.24 reported that a Cys1141Arg mutation in VWF had a dominant negative effect, causing endoplasmic reticulum retention of the normal subunit as a result of heterodimer formation with the mutant subunit. In this report, we did not find a random formation of heterodimer. In our study, we observed that less than 10% of the dimer population consisted of heterodimers (Figure 6). This result was obtained with the use of essentially wild-type VWF proteins or A3 domain–deleted VWF. We have found the propensity for dimer formation to be primarily homodimeric, indicating that assembly of dimers (C-terminal) may occur cotranslationally. The drive for homodimer formation could be the result of C-terminal interaction with the nearest neighboring VWF monomer, which most likely would be a monomer being produced by the same polysome and therefore the same mRNA molecule.

Although the mutation affects the propeptide, the inhibitory effect of this defect manifests itself in a dramatic loss of several critical functions for the processed VWF protein. While the ability of the dimeric rVWF to participate in botrocetin-induced platelet binding was unaffected, the dimeric rVWF displayed diminished ristocetin-induced platelet binding, collagen binding, and FVIII binding (Table 1). The decrease in platelet binding to these dimers in the presence of ristocetin is not surprising, since it is known that high-molecular weight multimers have a greater predilection for platelets in the presence of ristocetin. In contrast, botrocetin is not affected by the multimeric size of VWF. Even though the binding sites for collagen in the A1 and A3 domains are not mutated, there
was a marked decrease in rVWF dimer binding to type III collagen (Table 1). This could be attributed to the loss of HMW multimers since it has been shown that the highest MW multimers bind collagen with the greatest avidity.\(^1,6\) The dimeric rVWF also binds FVIII, but at reduced levels, similar to type 2N VWD controls. This suggests a dependency of FVIII binding on VWF multimer size, and rVWF binding of FVIII may depend on the degree of cooperation between neighboring FVIII-binding sites in more fully multimerized VWF molecules.

Defects and deletions in the propeptide of VWF have demonstrated the obligatory nature of VWFpp in the multimerization process. Experiments involving deletion of parts of VWFpp (ΔD1 and ΔD2) or the entire VWFpp (Δpro-VWF) demonstrated that deletion of these regions resulted in the expression of only the dimeric form of VWF and prevented the synthesis of VWF multimers.\(^9,27\) When transfected in trans, the separate and noncontiguous VWFpp retains the ability to correctly orientate the mature-only subunits of Δpro-VWF to create multimers in vivo\(^27\) and in vitro.\(^28\) In patients with a heterozygous abnormality, the functional wild-type VWFpp is involved in multimerization of the wild-type VWF molecules and is unavailable to function in trans. Naturally occurring mutations in the VWFpp (Tyr87Ser [this study], Arg273Trp,\(^29\) Asn528Ser,\(^30\) Gly550Arg,\(^31\) Cys623Trp,\(^25\) and 625insGly\(^25\) ) all demonstrate altered multimerization, presumably resulting from their VWFpp defects, yet none are located in close proximity to any functional areas. An intact VWFpp appears to be prerequisite for the N-terminal multimerization of VWF in the trans Golgi network (TGN).

Propeptides of peptide hormones have been reported to act upon their mature subunits as intramolecular chaperones, including

**Figure 5. Mutant Tyr87Ser-VWFpp facilitation of intracellular storage of VWF.** The mutant Tyr87Ser-VWFpp functions in trans to facilitate intracellular storage of VWF. The Tyr87Ser-VWFpp was coexpressed in trans with Δpro (mature VWF) as 2 separate plasmids. The fixed and permeabilized cells were immunolabeled as described in “Materials and methods.” Transfected cells were labeled with anti-VWF polyclonal antibodies (detected by FITC) and anti-VWFpp monoclonal antibodies (detected by TXR) to visualize the intracellular localization of VWF and VWFpp. Panel A shows anti-VWFpp staining (red); panel C shows anti-VWF staining (green); and panel B represents the merge of VWFpp and VWF (colocalization is shown in yellow). Tyr87Ser-VWFpp directs mature VWF into storage granules.

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**Figure 6. Facilitation of multimerization.** The propeptide functions in cis and trans to facilitate multimerization. The question of homodimer versus heterodimer formation in VWF biosynthesis was addressed by the coexpression of HPP-VWF (wild-type VWF containing a disrupted furin-cleavage site) with wild-type VWF. When these 2 VWF proteins were coexpressed in HEK293T cells, the resulting protein expression showed nearly exclusive formation of homodimers (lane 1). As a size reference, each single plasmid transfection was included in the gel analysis (WT-VWF, lane 2, and HPP-VWF, lane 3). The immunoblot shown was scanned and the band densities were calculated. Each peak is listed with the scanned area and percentage of total area per gel lane. Only 8.6% of the total VWF detected in lane 1 is heterodimeric. Dimer formation appears to be primarily homodimeric.

**Figure 7. Proposed intramolecular chaperone function of the VWFpp.** The propeptide of VWF functions as an intramolecular chaperone. The contiguous molecule folds VWF into the correct orientation necessary for proper intermolecular bridging by the cysteines in the adjacent dimers (panel A). The VWFpp role may be to tether the molecules together, allowing the cysteines to interact. The Tyr87Ser VWF defect and possibly other type IIC VWD defects cause the region to assume an incorrect configuration (panel B), and thus prevent the proper contact between adjacent dimers that results in a loss of N-terminal multimerization.
Figure 7. The propeptide serves as a molecular chaperone during maturation process as an intramolecular chaperone. In our model, it is our hypothesis that the propeptide of VWF functions during the multimerization process of von Willebrand factor (vWF) in the intracellular trafficking of factor VIII to von Willebrand factor storage granules. J Clin Invest. 1998;101:613-624.

Figure 8. High degree of homology in the D1 domain across species. The VWFpp of human, porcine, bovine, canine, and murine VWF have been aligned. Shown in this Figure is a 60-aa span of the D1 domain for 5 species and the Tyr87Ser mutation in human sequence. Murine VWF has been reported for only a small portion of the D1 domain, shown here from residues 74 through 120. Only differences are shown, with - depicting a perfect homology match. Between the 5 species’ VWFpps, there is a very strong homology throughout this region in the D1 domain of perfectly conserved and well-conserved amino acids (86% to 95%), indicating that the Tyr87Ser mutation may alter the configuration of the region.

References


The role of the D1 domain of the von Willebrand factor propeptide in multimerization of VWF