CONCISE REPORT

von Willebrand Factor Released From Weibel-Palade Bodies Binds More Avidly to Extracellular Matrix Than That Secreted Constitutively

By Lee Ann Sporn, Victor J. Marder, and Denisa D. Wagner

Large multimers of von Willebrand factor (vWf) are released from the Weibel-Palade bodies of cultured endothelial cells following treatment with a secretagogue (Sporn et al, *Cell* 46:185, 1986). These multimers were shown by immunofluorescent staining to bind more extensively to the extracellular matrix of human foreskin fibroblasts than constitutively secreted vWf, which is composed predominantly of dimeric molecules. Increased binding of A23187-released vWf was not due to another component present in the releasate, since releasate from which vWf was adsorbed, when added together with constitutively secreted vWf, did not promote binding. When iodinated plasma vWf was overlaid onto the fibroblasts, the large forms bound preferentially to the matrix. These results indicated that the enhanced binding of the vWf released from the Weibel-Palade bodies was likely due to its large multimeric size. It appears that multivalency is an important component of vWf interaction with the extracellular matrix, just as has been shown for vWf interaction with platelets. The pool of vWf contained within the Weibel-Palade bodies, therefore, is not only especially suited for platelet binding, but also for interaction with the extracellular matrix.

VON WILLEBRAND FACTOR (vWf) mediates platelet adhesion to the vessel wall by forming a bridge between glycoprotein Ib on the platelet surface and an as yet unidentified component of the vascular subendothelium.[1] Endothelial cells synthesize vWf[2] as a series of disulfide-bonded multimers that range in size from 0.5 to 20 million daltons.[1] Predominantly small multimers of vWf are secreted constitutively, and the large multimeric forms are stored in the Weibel-Palade bodies.[3] The contents of the Weibel-Palade bodies can be released from cultured endothelial cells upon exposure to nonphysiologic secretagogues such as the calcium ionophore A23187 and phorbol myristate acetate,[4,5] or physiologic stimuli such as thrombin[4,6] and fibrin.[7] For several reasons, the large multimers of vWf are considered to be functionally important: First, only the largest vWf multimers are specifically missing from the plasma of patients with type IIa von Willebrand disease,[8] and second, the largest multimers of vWf have a higher affinity for glycoprotein Ib than do the low molecular weight forms.[9-11] In addition, we now show that the large multimers of vWf found within the Weibel-Palade bodies bind the most avidly to the extracellular matrix, the in vitro counterpart of the basement membrane.

MATERIALS AND METHODS

Cell culture and labeling. Endothelial cells were obtained from human umbilical veins as previously described[12,13] and grown in McCoy's 5a medium (Flow Laboratories, McLean, VA) containing 20% undialyzed fetal bovine serum. Human foreskin fibroblasts (HFF) in fourth passage were a gift from Sue Schmidt (Infectious Disease Unit, University of Rochester), and were grown under the same conditions. For continuous metabolic labeling, cell cultures were grown in the same medium in the presence of [^{35}S]-methionine (25 μCi/mL, 1132 Ci/mmol) (New England Nuclear, Boston, MA). Cells were treated with the calcium ionophore A23187 (Sigma, St Louis) using a solution of 10 μmol/L A23187 in McCoy's 5a medium with 20% fetal bovine serum. Iodinated plasma vWf was a generous gift from Dr Philip Fay (Hematology Unit, University of Rochester) and was prepared as described previously.[14]

Antisera. The preparation and characterization of monospecific antisera against human vWf used for immunofluorescence were previously described.[11,12] For vWf purification, antiserum was purchased from Calbiochem-Behring (La Jolla, CA). Immunofluorescence staining using anti-vWf antiserum was carried out as previously described.[12]

Purification of vWf and gel electrophoresis. Cells were lysed and vWf was immunopurified as described previously.[15] Two-percent agarose gels were prepared with 0.1% sodium dodecyl sulfate (SDS) in 0.05 mol/L phosphate, pH 7.0; running buffer was 0.1 mol/L phosphate buffer, pH 7.0, with 0.1% SDS.

Adsorption of vWf from A23187 releasate. Protein A-sepharose (Sigma) (100 mg/mL of sample) was preincubated with anti-vWf antiserum (100 μL/mL of sample) then washed three times with McCoy's 5a medium. Releasate was shaken with this mixture for 1.5 hours at room temperature, Sepharose beads were centrifuged and the supernatant used for the HFF overlay experiment described in the text.

RESULTS AND DISCUSSION

This study compares binding of vWf secreted constitutively and that released from Weibel-Palade bodies to the extracellular matrix of HFF. Prior to conducting binding studies, we have tested the stability of the various multimeric forms of vWf while in contact with HFF for an extended incubation period. Endothelial cells were metabolically labeled with [^{35}S]-methionine for three days and culture

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Submitted January 9, 1987; accepted January 28, 1987.

Supported in part by Grant No. HL 30616 from the National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD, and by Training Grant No. ES-07026 from the National Institutes of Health. The research was done during the tenure of an Established Investigatorship from the American Heart Association (D.D.W.) and with funds contributed in part by the New York State Affiliate, Inc.

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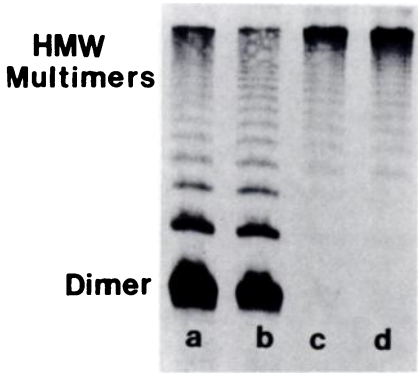

Fig 1. Multimeric composition of vWf used in matrix-binding experiments. vWf secreted constitutively (a, b) or released by A23187 (c, d) from metabolically-labeled endothelial cells both after (b, d) or before (a, c) a 24-hour incubation with HFF was immunopurified and electrophoresed on a 2% agarose gel. Constitutively secreted vWf contains a broad range of multimers, but predominantly dimer molecules, while that released from cells by A23187 treatment is highly enriched in the high molecular weight (HMW) vWf multimers. There was no significant change in multimeric composition of vWf after incubation over HFF for 24 hours.

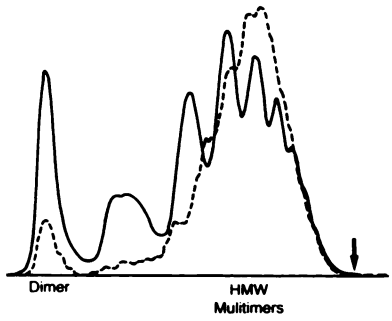

Fig 3. The multimeric pattern of vWf bound to HFF. Iodinated plasma vWf (starting material) was overlaid onto a confluent flask of HFF for 48 hours, at which time the HFF were washed extensively and lysed. Samples of both starting material and cell lysate were electrophoresed, nonreduced on the same 2% agarose gel and submitted to autoradiography. The densitometric scan of starting material (——) is from a short exposure of the gel, and the scan of bound vWf is from a longer exposure (---). The figure is provided as a comparison of multimeric profiles and is not representative of relative quantities of vWf in the two samples. Arrow points to the origin of the gel.

medium containing constitutively secreted vWf was removed. Following a six-hour chase period in unlabeled culture medium, cells were subjected to A23187 treatment for ten minutes. Half of each sample was then placed on a confluent flask of HFF for 24 hours and the remaining half incubated under the same conditions but in the absence of cells. vWf was immunopurified from all samples. Figure 1 compares the multimeric pattern of vWf secreted constitutively (A, B) and released by A23187 (C, D) with and without incubation with HFF. This experiment indicated that the presence of the HFF did not alter the multimeric pattern or cause significant degradation of vWf.

The comparative study of binding of constitutively secreted vWf and A23187-released vWf to the extracellular matrix of HFF was then conducted as follows. Human umbilical vein endothelial cells metabolically labeled for three days were subjected to A23187 treatment for ten minutes. The released material was aged for 24 hours in a tissue culture flask at 37°C so that the multimers were of comparable age with those secreted constitutively by cultures labeled continuously for 24 hours. vWf was immunopurified from both samples. The 24-hour medium sample and the A23187 releasate contained approximately the same number of counts per minute in vWf per volume; therefore, the two samples contained a similar number of vWf subunits but in different multimeric forms (Fig 1). Equal volumes of these media from unlabeled identical flasks prepared in parallel were then overlaid onto cultures of HFF grown on glass coverslips. After 72 hours incubation on the HFF, the coverslips were washed extensively, and stained by immunofluorescence using anti-vWf antiserum. Figure 2A represents control cells cultured in the absence of vWf, treated in the same manner and showing no matrix staining. The medium sample containing the constitutively secreted vWf produced only a trace of matrix decoration (Fig 2B). In contrast, the vWf released by A23187 produced a strong fluorescent signal (Fig 2C). The strength of the fluorescent signal produced by purified plasma vWf has been shown to be dependent on the vWf concentration (weight/volume) in the culture medium.[16] Since equal concentrations of vWf were placed on HFF, it appeared that the A23187-released vWf bound more avidly to the extracellular matrix.

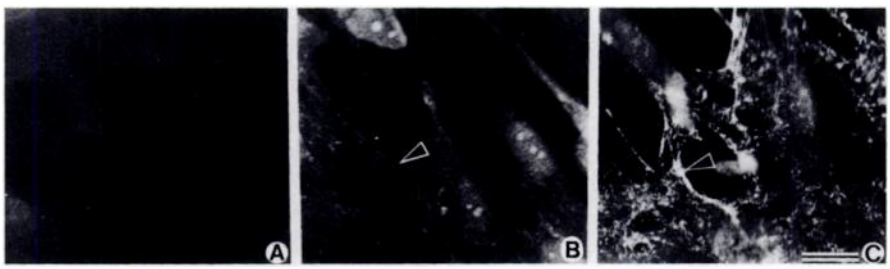

Fig 2. Comparison of binding to extracellular matrix of vWf secreted constitutively and released by A23187. vWf secreted without stimulation over a 24-hour period (B) and an equivalent amount of A23187-released vWf (C) were placed on HFF grown on glass coverslips. (A) represents control fibroblast cultures to which no vWf was added. After 72 hours, the coverslips were fixed, permeabilized, and stained by fluorescence with anti-vWf antiserum. Arrowheads point to extracellular matrix filaments decorated with vWf. A23187-released vWf produced a much stronger fluorescence signal indicating that it bound more extensively. Bar = 20 μm.

To address the possibility that a component other than vWf in the A23187 releasate enhanced binding of the protein to the extracellular matrix, a mixing experiment was performed (not shown). Culture medium containing constitutively secreted vWf was mixed with A23187 releasate, from which vWf was removed by adsorption (described in Materials and Methods) and then incubated with the HFF. The presence of the vWf-depleted A23187 releasate did not cause an increase in fluorescent signal in comparison to controls that were mixed with fresh medium only. This experiment does not, however, rule out the possibility that a yet unknown modification exists in the vWf stored within the Weibel-Palade bodies that could cause enhanced binding to the matrix. Most likely the difference in affinity for the extracellular matrix of constitutively secreted vWf and A23187-released vWf is due to differences in multimeric size (ref 3, Fig 1). This idea is supported by the study of small multimeric forms of vWf synthesized by endothelial cells in the presence of monensin or ammonium chloride. These small multimers do not incorporate into the extracellular matrix of endothelial cells, nor do they bind to the matrix of HFF as seen by immunofluorescent staining with anti-vWf antiserum.[17,18]

For more direct evidence that the large multimeric sizes of vWf bind better to the extracellular matrix, vWf purified from plasma and containing all multimeric forms was iodinated and placed on HFF culture. After 48 hours, media was removed and cells were washed extensively and lysed. To check the efficiency of our lysing procedure, an equivalent amount of cold vWf was placed on HFF grown on glass coverslips; the cells were lysed, and the coverslip was stained by fluorescence with anti-vWf antiserum. As no staining was observed, this revealed that the lysing procedure removed all cellular material and extracellular matrix. Without further processing of the samples, a small amount of both iodinated starting material used for the HFF overlay and cell lysate were electrophoresed, nonreduced on a 2% agarose gel. A comparison of the densitometric scans of vWf in starting material and of bound vWf revealed that the bound vWf was highly enriched in the high molecular weight (HMW) multimers (Fig 3). When the starting material and bound vWf were compared reduced by analysis on polyacrylamide gels, no apparent change in the subunit size was observed indicating that it was not significantly degraded or covalently crosslinked by a transglutaminase to another extracellular matrix component (not shown).

Since the multimers of vWf are composed of many identical subunits arranged in a linear array,[19] enhanced binding of the large multimers could be due to multiple interactions along the length of the vWf molecule with available binding sites in the extracellular matrix. The notion that the polyvalency of the multimers of vWf is important to its function is not new. Low–molecular weight forms of vWf exhibit enhanced ristocetin cofactor activity following their adsorption to colloidal gold.[20] This indicates that dimers and other small multimers possess the potential for interaction with platelets, but this potential is manifested only following formation of larger multimers. The importance of polyvalency has been reported for other adhesive systems as well. For example, the species-specific reaggregation of cells in the marine sponge *Microciona prolifera* is mediated by a proteoglycanlike aggregation factor. High-affinity interaction between cells could be reconstituted by chemical crosslinking of fragments of the aggregation factor containing low-affinity cell binding sites.[21]

In our previous studies, we have shown that the vWf released from the Weibel-Palade bodies is highly enriched in the largest multimeric forms of vWf synthesized by the cells.[3] The present observations reveal that this same pool of molecules is not only best suited for interaction with platelets, but also for interaction with the extracellular matrix, the in vitro model of the basement membrane.

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