The Affinity and Stoichiometry of Binding of Human Factor VIII to von Willebrand Factor

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To study the interaction between factor VIII and von Willebrand factor (vWF), binding experiments were performed using immobilized plasma vWF. Plasma was obtained from healthy donors and from patients with severe hemophilia A. For normal and hemophilic vWF, the dissociation constants (kd) for binding of factor VIII to vWF were 0.21 ± 0.04 and 0.22 ± 0.05 nmol/L, respectively. At saturation, the stoichiometry was one factor VIII molecule per 50 vWF monomers. In gel-filtration experiments, vWF was saturated by 23 times more factor VIII. However, when this FVIII-vWF complex was immunobilized on microtiter plates, the ratio of factor VIII/vWF decreased to the same ratio as in the solid-phase binding assay. To exclude any effect of antibody binding, colloidal gold particles with a diameter of 15 nm were coupled to purified vWF. This vWF-gold complex remained immunoreactive toward polyclonal and monoclonal antibodies, and was able to bind factor VIII, specifically, saturably, and reversibly. After incubation of vWF-gold with factor VIII, unbound and bound factor VIII were separated by centrifugation. Binding isotherms of these fluid-phase binding experiments indicated a kd of 0.32 ± 0.09 nmol/L and a stoichiometry of ∼0.5 factor VIII molecule per vWF monomer. We conclude that vWF-binding to a surface, with or without an antibody, may induce a conformational change causing a dissociation of bound factor VIII from vWF.

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Factor VIII is an X-linked gene product that accelerates the activation of factor X by factor IXa in the coagulation process. A deficiency of factor VIII results in hemophilia A, a bleeding disorder affecting 1 in 10,000 males. In plasma, factor VIII is bound to von Willebrand factor (vWF), a glycoprotein that plays an essential role in hemostasis by promoting the attachment of platelets to the injured vessel wall. vWF circulates in plasma as a series of disulfide-bonded multimers with molecular-weight values up to 20 × 10^6 daltons. In vivo, the survival of factor VIII is strongly dependent on the formation of a noncovalent complex with vWF. This is also shown by von Willebrand disease type Normandy (recently classified as type 2Ny), in which several amino acid substitutions cause an abnormal factor VIII-vWF interaction. In vitro, complex formation was shown to protect factor VIII against inactivation by activated protein C and activation and inactivation by factor Xa.

The binding site for vWF on factor VIII is located on the amino-terminal region of the light chain. Studies with monoclonal antibodies (MoAbs) that inhibit the interaction have further localized this domain on factor VIII to amino acids 1670-1684 and 1673-1689. Recently, the presence of an additional binding site on the carboxy-terminal region of the factor VIII light chain has been suggested by a binding study using a fusion protein consisting of glutathione S-transferase and the C2 domain of factor VIII. On vWF, a major factor VIII binding site resides within the 34- to 36-amino-terminal tryptic fragment and, more specifically, within the first 272 amino acid residues of the mature vWF molecule, with a crucial role for residues 78 through 96 and a sequence around residue 53.

An intriguing issue remains the stoichiometry of the factor VIII-vWF complex in plasma. By convention, the amount present in 1 mL of normal human plasma is one unit for both proteins. For factor VIII, the reported specific activities range from 2,300 to 8,000 U/mg. This corresponds to 0.1 to 0.4 μg/mL and ~1 nmol/L (assuming a molecular mass of 240 kD). vWF circulates in plasma at a concentration of ~10 μg/mL. Because each monomer (with a molecular mass of 270 kD) can circulate as a dimer or a multimer with varying length, vWF is not commonly expressed in molar concentrations. Nonetheless, according to weight ratios, only one factor VIII molecule is bound per 50 vWF monomers in plasma. This ratio remains unchanged in deficiency states such as von Willebrand disease, and concomitant increases of factor VIII and vWF concentrations have been observed in disorders associated with acute-phase reactions.

Factor VIII binding to vWF has been investigated using various techniques such as coating vWF directly on plastic, binding vWF with a MoAb, gel filtration, and ultracentrifugation. Using the first two techniques, binding affinities (kd) of 0.1 to 0.2 nmol/L were found, whereas the stoichiometry was one factor VIII molecule per 50 to 100 vWF monomers—the same as in plasma. In contrast, the last two techniques showed a stoichiometry close to the theoretically possible: one factor VIII molecule bound to one vWF monomer. However, it is important to note that in the ultracentrifugation experiments described by Lollar and Parker, porcine factors were used. The plasma concentration of porcine factor VIII and vWF are 2 to 3 μg/mL and 4 to 8 μg/mL, respectively, indicating that porcine vWF is already more than 50% saturated with factor VIII in vivo.

In this report, we compared the binding of factor VIII to vWF from normal human volunteers and to vWF from patients with severe hemophilia A using a solid-phase binding assay. To investigate the effect of surface adsorption of vWF on the complex formation with factor VIII, we used gel-filtration chromatography of the factor VIII-vWF complex for comparison. In addition, we used a third technique in which purified vWF was coupled to 15 nm colloidal gold particles...
MATERIALS AND METHODS

Reagents. Benzamidine-HCl and bovine serum albumin (BSA) fraction V were from Sigma Chemical Co (St Louis, MO). Triton X-100 and Tween 20 were from E. Merck AG (Darmstadt, Germany). Sephacryl 300 HR was from Pharmacia (Uppsala, Sweden). The factor VIII Coatest assay was purchased from Chromogenix (Molndal, Sweden). Cephalin and kaolin were from Boehringer Mannheim GmbH (Mannheim, Germany). Factor VIII-deficient plasma was obtained from a patient with severe hemophilia A or purchased from Organon Teknika Co (Turnhout, Belgium).

Patients. Ten adult patients with severe, cross-reactive material-negative hemophilia A gave informed consent for blood collection for the described experiments. None of the patients had antibodies against factor VIII, or were suffering from bleeding or infection. All had previously been treated with factor VIII concentrates, but they did not receive factor VIII concentrates for at least 5 days before venepuncture. Blood was collected in a 10-ml collection volume 3.8% trisodium-citrate and centrifuged at 4°C at 1,200 g for 15 minutes and plasma was stored at −70°C until use.

Recombinant factor VIII. Recombinant factor VIII was generously provided by Dr R.J. Kaaman (Genetics Institute, Cambridge, MA). The specific activity was 2,900 U/mg protein. The amount of vWF present, tested with an enzyme-linked immunosorbent assay (ELISA), was 0.0001 U/mg protein.

Human von Willebrand factor. Von Willebrand factor was purified from human cryoprecipitates (kindly provided by Dr. J.A. van Mourik, Central Laboratory, Amsterdam, The Netherlands) as described previously. Non–heat-treated vials were used, because heat treatment induces cross-linking of vWF with fibrinogen and fibrin.

Preparation of colloidal gold. Colloidal gold solution was prepared according to a protocol by Dr. H. Meyer, INSERM U.143, Paris, France) directed against the binding site for factor VIII on vWF was used to block the factor VIII–vWF interaction.

Solid-phase binding assay. Solid-phase binding experiments were performed, as described. In short, 96-well plates (Costar, Cambridge, MA) were coated with 100 μL of a solution of 2 μg/mL MoAb RU-1 in 50 mM/L NaHCO3 (pH 9.6) overnight at 4°C. After washing with 150 mM/L NaCl, 50 mM/L TRIS-HCl, pH 7.4, 0.1% Tween-20 (wash buffer), the wells were blocked with 3% BSA in wash buffer for 1 hour at 37°C. Normal plasma was diluted (1:200) in 150 mM/L NaCl, 50 mM/L TRIS-HCl, pH 7.4, 0.1% Tween-20, 0.5% BSA (diluting buffer). The patient plasma was first tested for vWF-antigen using an ELISA and also diluted to a final vWF concentration of 5 mU/mL. Subsequently, vWF-containing samples were added to the antibody-coated wells and incubated for 16 hours at 4°C. The precise amount of immobilized vWF in the solid-phase binding assay was determined by measuring the concentrations of vWF in the sample before and after binding to the antibody. By subtracting both concentrations, and taking into account the volume of the wells, the amount of vWF bound to the antibody was calculated. The antibody was not saturated in this assay, because higher signals could be obtained by using less diluted plasma. Endogenous factor VIII was removed by incubating the wells for 10 minutes with 150 mM/L NaCl, 50 mM/L TRIS-HCl, pH 7.4, 0.1% Tween-20, and 0.25 mM/L CaCl2. The plates were then washed and factor VIII was added in diluting buffer. After washing twice, bound factor VIII was determined with the factor VIII Coatest, essentially according to the manufacturer’s instructions. The CaCl2 needed to assemble the tenase complex in this assay was supplied to the incubation mixture by starting the reaction with 25 mM/L CaCl2. Hydrolysis of the substrate was measured as the absorbance rise at 405 nm, using a V-max microplate reader (Molecular Devices Corp, Menlo Park, CA). Factor VIII concentration was determined by reference to a standard curve prepared with a normal plasma pool of 40 donors. Factor VIII bound to BSA-coated wells (usually less than 10% of total binding) was subtracted from total binding to obtain specific binding.

Preparation of colloidal gold. A Sephacryl 300 HR column (100 × 1.6 cm) was equilibrated with Michaelis buffer (28.5 mM/L Na-barbital; 28.5 mM/L Na-acetate, pH 7.4; 116 mM/L NaCl; 0.5% BSA; 0.005% Triton-X-100; 1 mM/L benzamidine; 0.02% NaN3). All gel-filtration experiments were performed at 4°C. Factor VIII (100 μL, 25 mM/L) was either directly applied to the column, or after incubation (30 minutes) with 30 μL of normal or hemophilic plasma. Fractions were collected at a flow rate of 20 mM/L and assayed for factor VIII coagulant activity and vWF antigen using a one-stage coagulation assay (KC-10 Coagulometer, Amelung GmbH, Lemgo, Germany) and a vWF-ELISA, respectively. After incubation with vWF, factor VIII eluted in two peaks with a ratio of 23 in units per unit (0.5 factor VIII molecule/vWF monomer) in the first peak (see Results). This saturated vWF was added to microtitre plates coated with MoAb RU-1, and incubated for 2 hours at 37°C. Thus, the amount of vWF bound was determined as described above. After binding, the amount of factor VIII bound and in the supernatant was measured with the factor VIII Coatest.

Preparation of colloidal gold. Colloidal gold solutions were prepared essentially as described. Briefly, a 0.0125% solution of HSAuCl3, and a reducing mixture consisting of 0.2% tri-sodium citrate-dihydrate and 0.001% tannic acid in distilled water were prepared. The amount of tannic acid added to the reducing mixture determines the diameter of the gold particles. We used 0.001% tannic acid to produce a homogeneous gold sol of 15 nm particles. Both solutions were warmed to 60°C, rapidly mixed, and stirred for 30 to 45 minutes at 60°C until the color changed to red. After boiling for 5 minutes, the gold sol was cooled and stored at 4°C until use.

Coupling of purified vWF to colloidal gold. The binding of a protein to gold is pH-dependent, and in general, stable complexes can be obtained at a pH equal to or slightly higher than the isoelectric point of the protein involved. Because the reported isoelectric point of human vWF is between 5.7 and 5.9, the pH of the colloidal solution was adjusted to 6.1. Because the purified vWF was kept in 150 mM/L NaCl, 50 mM/L TRIS-HCl, pH 7.4, it was dialyzed against 10 mM/L TRIS-HCl, pH 6.1, to remove the salt. The minimal concentration of vWF required to stabilize the colloidal gold particles was determined with a concentration-variable adsorption isotherm as described. After conjugation, 1% BSA (final concentration) was added for maximal stabilization. Subsequently, the vWF-gold solution was centrifuged and resuspended three times to remove unbound vWF. All centrifugation steps were performed at 10,000g.
for 15 minutes. The vWF-gold conjugate was kept in 150 mmol/L NaCl, 50 mmol/L TRIS-HCl, pH 7.4, 0.1% Tween-20 and 1% BSA during purification steps and in binding experiments. In an attempt to verify the morphology of vWF after conjugation with colloidal gold, the samples of the conjugate were adsorbed on EM-grids and examined in a JEOL-1200-CX electron microscope, as described.  

**Factor VIII binding to vWF-gold.** Samples (80 to 100 µL) of vWF-gold at fixed concentrations were incubated with factor VIII at increasing concentrations for 30 minutes at 37°C. After centrifugation, aliquots of the supernatant (10 to 25 µL) were removed and tested with a one-stage coagulation assay to determine the concentration of unbound factor VIII. Similar samples which were not centrifuged yielded the total amount of factor VIII. Bound factor VIII was calculated by subtracting the unbound factor VIII from the total amount of factor VIII. Binding isotherms were obtained by varying the concentration of factor VIII (0 to 6 nmol/L) with a fixed concentration of vWF-gold (see Table 1). The time course of factor VIII binding was studied in a similar manner, except that the factor VIII concentration was constant (0.3 nmol/L) and exposure times ranged from 5 to 40 minutes. Control studies were performed in which clotting times were recorded with fixed amounts of factor VIII in either the absence or presence of vWF-gold. Colloidal gold coated with 1% BSA was used to provide for nonspecific binding (usually <20% of total binding).

**Calculations.** In the solid-phase binding assays and in the experiments with vWF-gold, data were fitted employing a non-linear regression program (EnzFitter Software, Elsevier, Amsterdam, The Netherlands).

**RESULTS**

**Solid-phase binding assay.** The interaction between factor VIII and vWF from healthy individuals and from patients with severe hemophilia A was studied by immobilizing plasma vWF with MoAb RU-1. In this assay, the presence of vWF does not interfere with factor VIII determination. Previous reports indicated that factor VIII binding to immobilized vWF was maximal after 30-60 minutes of incubation and could be reversed by the addition of 0.25 mmol/L CaCl₂ or an excess of unbound vWF. Normal and hemophilic vWF bound similarly to this antibody (~0.4 mU/well) as measured with a vWF-ELISA. Factor VIII bound to this immobilized vWF in a dose-dependent manner. Scatchard analysis of binding data demonstrated the presence of a single class of binding sites with k_d = 0.21 ± 0.04 mmol/L (n = 4) for normal pooled plasma (data not shown). For the 10 patients with hemophilia A, a mean k_d of 0.22 ± 0.05 mmol/L was found. For both normal and patient plasma, the number of factor VIII binding sites was equivalent to ~0.4 mU factor VIII per well. Thus, the stoichiometry at saturation was ~1 in units (one factor VIII molecule/50 vWF monomers). A typical experiment with vWF from a single patient is shown in Fig 1.

Several control experiments were performed. First, purified vWF was directly coated to microtiter plates and factor VIII binding was determined. Second, MoAb CLB-RAg 35 was used to immobilize vWF instead of MoAb RU-1. Third, factor VIII was added to vWF prior to adding the formed factor VIII-vWF-complexes to wells coated with MoAb RU-1. Fourth, rabbit immunoglobulins against mouse immunoglobulins were used (as a spacer for MoAb RU-1). In all these experiments, essentially the same binding characteristics were obtained (data not shown). To exclude a possible denaturing effect from washing with 0.25 mol/L CaCl₂, this step was omitted in a binding experiment with hemophilic vWF. Again, no differences in binding were observed. Finally, the amount of immobilized vWF was tested before and after binding experiments, with a peroxidase-conjugated anti-vWF antibody. This amount did not change during the binding experiments.

**Gel-filtration chromatography.** Previous reports indicated that factor VIII remains associated with vWF during gel-filtration chromatography on Sepharose CL-4B (fractionation range for globular proteins 6 × 10⁴ - 2 × 10⁵ daltons), and that factor VIII bound to vWF elutes in the void volume with the free factor VIII eluting at larger elution volumes. With this technique, twelve times the amount of factor VIII normally present in plasma was shown to bind to vWF. In order to determine the effect of binding of a monoclonal antibody against vWF on preformed factor VIII-vWF complexes, vWF was “saturated” with factor VIII and subjected to gel-filtration chromatography. After elution, fractions were added to antibody-coated wells. When 30 µL of normal citrate plasma (vWF antigen 1 U/mL) was applied to the column, vWF eluted in the void volume (~80 mL). Subsequently, recombinant factor VIII (100 µL, 25 nmol/L) was applied, which eluted in the included volume (~110 mL) with a recovery of 25% (Fig 2). When the same amount of factor VIII was preincubated with 30 µL citrate plasma for 30 minutes and then applied to the column, factor VIII eluted in two peaks, with a total recovery of ~90%. In the first peak, the ratio of factor VIII/vWF was 23 ± 3 (n = 3) in units/units (0.5 factor VIII molecule/vWF monomer), whereas in the second peak only free factor VIII was found. This value was constant in the first 4-5 fractions of the peak. However, conclusions concerning the binding of factor VIII according to the multimeric distribution of vWF were not possible because of the elution of free factor VIII in the later
FACTOR VIII-VON WILLEBRAND FACTOR INTERACTION

Fig 2. Sephacryl 300 HR chromatography of factor VIII, with or without vWF. When factor VIII (100 μL, 25 nmol/L) was applied to the column, all of the factor VIII activity was found in the included volume (~110 mL) (C). After incubation with 30 μL of normal pooled plasma, factor VIII eluted in two peaks (α, representing factor VIII bound to vWF (δ) in the void volume (~80 mL) and unbound factor VIII in the included volume.

fractions of this peak. The same results were obtained when hemophilic plasma was used (data not shown). In a similar experiment, MoAb 418 (400 nmol/L, final concentration) directed against vWF was added to a sample of citrate plasma and incubated for 15 minutes, and then factor VIII was added. In the presence of MoAb 418, which blocks the factor VIII-vWF interaction, factor VIII eluted in a single peak in the included volume, as in the absence of vWF (not shown).

Thus, the factor VIII eluting in the void volume represents factor VIII specifically bound to vWF. In contrast, preincubation of purified vWF with MoAb RU-1 or CLB-RAg 35 had no effect on factor VIII binding.

Aliquots (100 μL) from the first peak (Fig 2) were added to microtiter plates coated with MoAb RU-1 and incubated for 2 hours at 37°C. A double vWF-ELISA (see above) revealed that approximately 30% of the vWF binds during this incubation. After binding, the supernatant was removed and assayed for factor VIII activity with a chromogenic assay. After washing, the bound factor VIII was determined in the same way. The factor VIII activity in the supernatant was 65 ± 6% (n = 3) of the initial amount, whereas bound factor VIII was decreased to a factor VIII/vWF ratio of 1.3 ± 0.6 (n = 3) in units/units (1 factor VIII molecule/40 vWF monomers). These results suggest that factor VIII dissociates from vWF upon binding of vWF to an antibody on a surface.

Labeling of vWF with colloidal gold. To investigate the binding of factor VIII to human vWF without the interference of antibodies or surfaces, purified vWF was coupled to colloidal gold particles with a diameter of 15 nm. In a concentration-dependent adsorption isotherm, the curve showed optimal stabilization at a vWF concentration of 20 μg/mL. Therefore, purified vWF (25 U/mL) in 10 mmol/L TRIS-HCl, pH 6.1, was diluted 10 times in a solution of 15 nm colloidal gold (pH 6.1), and mixed by gentle inversion.

After 15 minutes at room temperature, BSA was added to a final concentration of 1%. After washing by centrifuging three times, the amount of un conjugated vWF was less than 1% the amount of conjugated vWF. When analyzed by electron microscopy, vWF conjugated with colloidal gold showed characteristics that resembled that of un conjugated native vWF, ie, some vWF multimers were depicted as elongated strands, whereas most were in a ball-of-yarn configuration.

Factor VIII binding to vWF-gold. First, the time needed for binding of factor VIII to vWF was determined. Factor VIII (0.3 nmol/L) was incubated with vWF-gold (0.1 U/mL) at various time intervals (5 to 40 minutes), followed by centrifugation. Complex formation was already maximal after 5 minutes (data not shown). Although the binding possibly proceeds during the centrifugation step, this suggests that factor VIII binding to vWF is a rapid process.

To compare the binding affinity of vWF-gold and nonlabeled vWF, samples with different mixtures of both (total vWF concentration, 0.125 U/mL) were incubated with factor VIII (0.125 nmol/L). After 30 minutes, the samples were centrifuged and the residual amount of factor VIII-C in the supernatant was determined. Figure 3 shows that factor VIII binding corresponded to the percentage vWF-gold, demonstrating that free vWF and vWF-gold had the same affinity for factor VIII.

To investigate whether binding of factor VIII to vWF-gold was reversible, factor VIII (1.0 nmol/L) was incubated with vWF-gold (0.1 U/mL). After 30 minutes, increasing amounts of nonconjugated vWF (up to a 100-fold excess)
were added and incubated for 3 hours. We hypothesized that when factor VIII dissociates from vWF-gold it either remains free or reassociates with nonconjugated vWF. Either way, factor VIII will remain in the supernatant during centrifugation. After centrifugation, the remaining factor VIII in the supernatant was measured with an ELISA and compared with initial values for each sample. Here, we preferred an ELISA for the detection of factor VIII potential disturbance by the excess of nonlabeled vWF. As concentration used in this ELISA (1 mol/L NaCl) dissociates essentially reversibly under these conditions.

To assess the binding affinity and stoichiometry of factor VIII binding to vWF-gold, increasing amounts of factor VIII were added to vWF-gold and incubated for 30 minutes at 37°C. For each concentration, two samples were prepared, one of which was not centrifuged to provide for the total amount of factor VIII. Control experiments showed that the one-stage coagulation assay was not disturbed by exposure to colloidal gold particles (data not shown). After centrifugation, the residual factor VIII in the supernatant was quantified, and the amount of factor VIII bound was calculated. As shown in Fig 4, nonlabeled vWF effectively displaced previously bound factor VIII. After 3 hours and with a 100-fold excess of nonlabeled vWF, ~80% displacement had occurred. Thus, factor VIII binding to vWF-gold appears essentially reversible under these conditions.

Table 1. Binding of Factor VIII to vWF Coupled to 15 nm Colloidal Gold Particles

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Kd (nmol/L)</th>
<th>Capacity Factor VIII (nmol/L)</th>
<th>vWF Gold (nmol/L)</th>
<th>Stoichiometry Factor VIII/vWF</th>
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Increasing amounts of factor VIII (0 to 6 nmol/L) were incubated with vWF-gold for 30 minutes at 37°C. After centrifugation (10,000g, 15 minutes) the remaining amount of factor VIII in the supernatant was determined. Scatchard transformation of data was performed using a nonlinear regression program. Stoichiometry data were converted from U/U to molecule/monomer with the following molecular-weight values and plasma concentrations: factor VIII, 240 kD and 0.2 µg/mL; vWF, 270 kD and 10 µg/mL.
sults. Because no information was available concerning the binding of factor VIII to vWF from patients with hemophilia A, we investigated this interaction with vWF from 10 patients with this bleeding disorder. Both the dissociation constant and stoichiometry were comparable with normal vWF. The high-affinity association between factor VIII and vWF from patients with hemophilia A found in this study may be important in achieving clinically effective factor VIII levels in those patients. With the present prophy-
axis consisting of monoclonaIy-purified and recombinant factor VIII, little or no vWF is infused into the patient. As a consequence, factor VIII is dependent on endogenous vWF, with which it has to form a complex in order to be protected against proteolytic degradation.

To assess whether binding of vWF to an antibody or a surface influences the binding of factor VIII, we used filtration techniques. In the presence of vWF, factor VIII eluted in two peaks; in the first peak, 23 U factor VIII eluted with 1 U vWF (0.5 factor VIII molecule/vWF monomer). Zucker et al. performed similar studies with BaSO4-treated plasma as a source of vWF; in agreement with our findings, they also found that vWF can be loaded with much more factor VIII than present in plasma. When we incubated this FVIII-vWF complex with an antibody directed to vWF coated on microriter plates, considerably less factor VIII was bound (~1 factor VIII molecule/40 vWF monomers). In addition, the same stoichiometry was found when factor VIII was incubated with purified vWF directly coated on microriter plates. Thus, a clear distinction in factor VIII binding to vWF in solution and vWF immobilized to a surface is evident from these experiments.

As a third technique we used colloidal gold to label purified vWF. Binding of proteins to colloidal gold is a complex phenomenon, mediated by hydrophobic and ionic interactions. This method has been used to label factor X, fibrinogen, and various other proteins. We used 15-nm colloidal gold to label purified human vWF, given the dimensions of monomeric vWF of ~60 nm. The structure of vWF may be an advantage, because multimeric proteins have a larger number of possible contacts with a metal surface. Factor VIII binding was not influenced by labeling with colloidal gold (Fig 3). Also, factor VIII bound to vWF-gold was shown to dissociate in the presence of an excess of nonlabeled vWF (Fig 4). Thus, nonlabeled vWF can compete with vWF-gold not only in the initial formation of the factor VIII-vWF-gold complex, but also after the FVIII-vWF complex has been formed. This is consistent with our previous observation in vivo, that radiolabeled factor VIII in complex with lower multimeric vWF was redistributed over all multimeric forms of vWF, after infusion into patients with hemophilia A.

The discrepancy in binding of factor VIII to vWF in the solid-phase binding assay and vWF in solution is remarkable. In this study, two different methods were used to estimate factor VIII activity: the factor VIII Coatest assay and the one-stage coagulation assay. However, control experiments showed that both assays gave the same results when recombinant factor VIII is used (data not shown). Kaufman et al. used both assays to detect recombinant factor VIII in culture
activated protein C. Thus, processes at the site of synthesis and complex formation, as well as proteolytic breakdown in the plasma, may both regulate the factor VIII/vWF ratio in vivo.

In conclusion, our data show that the binding affinity of vWF from patients with hemophilia A for factor VIII is similar to normal vWF. The solid-phase binding assay gives the same value for the stoichiometry of the factor VIII/vWF complex as observed in plasma. In contrast, 50% of the vWF monomers can bind a factor VIII molecule when gel-filtration techniques are used or when vWF is labeled with colloidal gold. The dissociation constant for factor VIII and vWF in solution is 0.32 ± 0.09 nmol/L. Labeling with colloidal gold may prove valuable in studying the interaction of vWF with its other ligands, such as heparin, collagen, and platelet glycoproteins.

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The affinity and stoichiometry of binding of human factor VIII to von Willebrand factor

AJ Vlot, SJ Koppelman, MH van den Berg, BN Bouma and JJ Sixma