The binding of factor VIII to von Willebrand factor (vWF) is essential for the protection of factor VIII against proteolytic degradation in plasma. We have characterized the binding kinetics of human factor VIII with vWF using a centrifugation binding assay. Purified or plasma vWF was immobilized with a monoclonal antibody (MoAb RU1) covalently linked to Sepharose (Pharmacia LKB Biotechnology, Uppsala, Sweden). Factor VIII was incubated with vWF-RU1-Sepharose and unbound factor VIII was separated from bound factor VIII by centrifugation. The amount of bound factor VIII was determined from the decrease of factor VIII activity in the supernatant. Factor VIII binding to vWF-RU1-Sepharose conformed to the Langmuir model for independent binding sites with a $K_d$ of 0.46 ± 0.12 nmol/L, and a stoichiometry of 1.3 factor VIII molecules per vWF monomer at saturation, suggesting that each vWF subunit contains a binding site for factor VIII. Competition experiments were performed with a recombinant vWF (ΔA2-rvWF), lacking residues 730 to 910 which contain the epitope for MoAb RU1. ΔA2-rvWF effectively displaced bound factor VIII, confirming that factor VIII binding to vWF-RU1-Sepharose was reversible. To determine the association rate constant ($k_{on}$) and the dissociation rate constant ($K_d$), factor VIII was incubated with vWF-RU1-Sepharose for various time intervals. The observed association kinetics conformed to a simple bimolecular association reaction with $k_{on} = 5.9 \pm 1.9 \times 10^6 \text{M}^{-1} \text{s}^{-1}$ and $K_d = 1.6 \pm 1.2 \times 10^{-3} \text{s}^{-1}$ (mean ± SD). Similar values were obtained from the dissociation kinetics measured after dilution of preformed factor VIII-vWF-RU1-Sepharose complexes. Identical rate constants were obtained for factor VIII binding to vWF from normal pooled plasma and to vWF from plasma of patients with hemophilia A. The kinetic parameters in this report allow estimation of the time needed for complex formation in vivo in healthy individuals and in patients with hemophilia A, in which mononoclonally purified or recombinant factor VIII associates with endogenous vWF. Using the plasma concentration of vWF (50 nmol/L in monomers) and the obtained values for $k_{on}$ and $K_d$, the time needed to bind 50% of factor VIII is $\approx 2$ seconds.

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by centrifugation. Using this assay, we characterized the kinetics of factor VIII binding to vWF.

MATERIALS AND METHODS

All experiments were performed at 22°C in 150 mmol/L NaCl, 50 mmol/L TRIS·HCl, pH 7.4, containing 0.1% Tween-20 (E. Merck AG, Darmstadt, Germany), 1% bovine serum albumin fraction V (Sigma Chemical Co, St Louis, MO), and 2 mmol/L CaCl₂ (TBS), unless indicated otherwise. Centrifugation steps were performed in an Eppendorf microfuge at 10,000g for 10 seconds.

Proteins. Recombinant factor VIII was a generous gift from Dr R.J. Kaufman (Genetics Institute, Cambridge, MA). This preparation was essentially free of vWF as measured with an enzyme-linked immunosorbent assay (ELISA). Human vWF devoid of factor VIII was purified from human cryoprecipitates (kindly provided by A. Knevelman and H.J.C. de Wit from the Red Cross Blood Bank Friesland, Leeuwarden, The Netherlands) using gel-filtration chromatography as described previously. Fractions were collected and assayed for vWF:Ag, vWF:Ag multimers and factor VIII activity. The first fractions contained high multimers, followed by intermediate and low multimeric vWF. Fractions were pooled and stored at −70°C until use, except for the experiments with high and low multimeric vWF, where fractions were not pooled and stored separately.

Production of a deletion variant vWF (ΔA2-rvWF). A detailed description of the construction and expression of a recombinant vWF, which lacks the A2 repeat (residues 730 to 910) in baby hamster kidney cells will be published elsewhere (Lankhoh, H. et al., submitted). Briefly, site-directed mutagenesis was used to delete the coding region for the A2 domain, and the deletion construct was cloned in the pNUT-expression vector. After transfection, stable cell lines were selected with methotrexate. AA2-rvWF was purified in our laboratory.

Human vWF was prepared in our laboratory (Dakopatts, Glostrup, Denmark), with normal pooled plasma as a standard. The polyclonal rabbit antibodies against human vWF (Dakopatts, Glostrup, Denmark), 1% bovine serum albumin fraction V (Sigma Chemical Co, St Louis, MO), and ristocetin-induced platelet aggregation, equivalent to normal factor VIII, FVIIIfc, was far below the detection limit of the assay, Cmin = 0.2 pg/mL, as measured using an ELISA using unconjugated and peroxidase-conjugated polyclonal rabbit antibodies against human vWF (Dakopatts, Glostrup, Denmark), with normal pooled plasma as a standard. The multimeric structure was assessed by using the Pharmacia Phast Gel System (Pharmacia LKB Biotechnology, Uppsala, Sweden) as described. Factor VIII concentrations were determined by using a chromogenic substrate assay (Factor VIII Coatest; Chromogenix, Mölndal, Sweden). The change in absorbance at 405 nm was measured using a V-max reader (Molecular Devices Corp, Menlo Park, CA). Normal pooled plasma was used as a reference. The concentrations of factor VIII and vWF in normal pooled plasma were taken from literature as 0.2 µg/mL and 10 µg/mL, respectively. The molecular weights used to determine concentrations were as follows: factor VIII, 540,000; purified and plasma vWF monomer, 270,000; and ΔA2-rvWF monomer, 250,000.

Preparation of RU1-Sepharose. MoAb RU1 was coupled to cyanogen bromide-activated Sepharose CL-4B (Pharmacia LKB Biotechnology, Uppsala, Sweden) to a final concentration of 1 mg MoAb RUI/mL of Sepharose, according to the manufacturer’s instructions. After coupling and blocking of residual active sites, RU1-Sepharose was incubated for 30 minutes with TBS containing 4% bovine serum albumin to prevent nonspecific binding. vWF was added to a 50% suspension of RU1-Sepharose in a final volume of 1 mL and allowed to bind. During incubation the sample was mixed and after 1 hour the vWF-RU1-Sepharose was washed five times by resuspension and repeated centrifugation in TBS.

Association experiments. Factor VIII (150 µL) was incubated with vWF-RU1-Sepharose (50 µL; 50% suspension in TBS). During incubation the tubes were mixed by inversion. After 10 to 600 seconds, the factor VIII bound to vWF-RU1-Sepharose and unbound factor VIII were separated by centrifugation and the factor VIII concentration in the supernatant was measured. Initial experiments performed at 22°C and 37°C yielded similar rate constants, thus subsequent experiments were performed at 22°C.

Competition experiments. Because ΔA2-rvWF lacks the epitope for MoAb RU1, it cannot compete with vWF for binding to RU1-Sepharose. vWF-RU1-Sepharose (100 µL; 50% suspension in TBS) was incubated with factor VIII (100 µL) for 30 minutes, and then ΔA2-rvWF (200 µL; final concentration 0 to 35 nmol/L) was added. After 2 hours, the samples were centrifuged and the supernatants were tested for factor VIII activity.

Dissociation experiments. The dissociation rate constant (koff) for the factor VIII-vWF complex was determined in dilution experiments. Factor VIII (15 µL) was added to vWF-RU1-Sepharose (250 µL, 50% suspension in TBS) and mixed. After 30 minutes, the mixture was diluted 50-fold by adding TBS. At different time intervals, 300-µL aliquots containing buffer and Sepharose beads were removed, centrifuged, and the supernatants were assayed for factor VIII activity.

Data analysis. Equilibrium binding data of factor VIII to vWF-RU1-Sepharose were analyzed by the Langmuir model for independent binding sites:

\[ C_{eq} = \frac{C_{max} \cdot FVIIIfc}{K_d + FVIIIfc} \]

(1)

with \( C_{eq} \) the amount of factor VIII bound to vWF-RU1-Sepharose, \( C_{max} \), the maximal binding capacity of the vWF-RU1-Sepharose, \( K_d \), the dissociation constant, and \( FVIIIfc \) the concentration of unbound factor VIII. It should be noted that this model does not take into account the possibility that the adjacent binding sites on the multimeric vWF interact. Application of this model to binding data obtained for various factor VIII concentrations and a fixed vWF concentration allowed establishment of the binding stoichiometry and of the dissociation constant.

In competition experiments the nonsementable fraction of factor VIII (FVIIIfc) of total added factor VIII (FVIIIfc) was measured as function of the ΔA2-rvWF concentration. In this situation the binding of factor VIII to vWF-RU1-Sepharose is again described by Equation 1, whereas the binding to ΔA2-rvWF is described by a similar equation:

\[ C_{eq,ΔA2} = \frac{C_{max,ΔA2} \cdot FVIIIfc}{K_{d,ΔA2} + FVIIIfc} \]

(2)

where \( C_{eq,ΔA2} \) is the maximal binding capacity of ΔA2-rvWF and \( K_{d,ΔA2} \) is the dissociation constant for factor VIII binding to ΔA2-rvWF. These experiments were performed in the presence of high concentrations of vWF. As a result the concentration of unbound factor VIII, FVIIIfc, was far below the \( K_d \) and Equations (1) and (2) can be simplified to:


Using the relations, $C_{eq}$, independent factor VIII binding site per vWF subunit. Therefore, association reaction:

$$
FVIII_{eq} = \frac{FVIII_0}{1 + \frac{K_d}{K_d + C_{eq,0} + C_{max,0}}}
$$

Equilibrium binding results were compatible (see Fig 1), with one independent factor VIII binding site per vWF subunit. Therefore, binding kinetics were modeled as a simple reversible bimolecular association reaction:

$$
factor VIII \cdot vWF \rightleftharpoons k_c \rightarrow k_{off}
$$

where $C$ represents the factor VIII-vWF complex and $k_c$ and $k_{off}$ are the association and dissociation rate constants for the reaction. These constants are related to the dissociation constant $K_d = k_{off}/k_c$. The rate of change of the concentration of the factor VIII-vWF complex with time is given by the differential equation:

$$
\frac{dC}{dt} = k_c(FVIII_0 - C(vWF_0 - C) - k_{off}C)
$$

with $FVIII_0$ and $vWF_0$ the total added factor VIII and vWF monomer concentrations. The concentration of factor VIII-vWF complexes at equilibrium, $C_{eq}$, expressed in terms of $FVIII_0$ and $vWF_0$, is given by:

$$
C_{eq} = p = \frac{1}{2}[FVIII_0 + vWF_0 + K_d] - \sqrt{(FVIII_0 + vWF_0 + K_d)^2 - 4FVIII_0vWF_0]}
$$

The solution of Equation 4 is given by:

$$
C(t) = \frac{1 - \frac{q}{P} - \frac{C_0}{C_0 - \frac{p}{q}}}{}
$$

where $C_0$ represents the initial concentration of FVIII-vWF complexes, and

$$
p = C_{eq}
$$

and

$$
q = \frac{FVIII_0vWF_0}{p}
$$

In the association experiments, $C_0 = 0$, and in the dissociation experiments, $C_0$ is calculated from Equation 5, using the dilution factor and the concentrations of factor VIII and vWF during the incubation. Binding constants and reaction rates were estimated by an ordinary least-squares fit of Equation 1, 3, or 6 to the experimental data (Fig. P. Biosoft, Cambridge, UK) and are presented as estimate ± standard error of the estimate (SEE), unless indicated otherwise.

**RESULTS**

**Binding of vWF and factor VIII to RU1-Sepharose.** The concentration of vWF bound to RU1-Sepharose was determined by measuring the vWF concentrations in the supernatant after incubation with RU1-Sepharose, and by subtracting these values from the amount initially present in the incubation mixture. Typically, 70% to 80% of added vWF bound to RU1-Sepharose under these conditions, and no vWF release could be measured after subsequent washing steps. Identical binding was found for purified and plasma vWF.

Aspecific binding of factor VIII could result in an overestimation of factor VIII binding. However, in control experiments where factor VIII was added to RU1-Sepharose we observed more than 98% recovery of factor VIII, which indicated that aspecific binding was less than 2% and was therefore considered to be negligible. In preliminary experiments, factor VIII (0.5 nmol/L) was added to an excess of vWF-RU1-Sepharose. After 30 minutes, samples were centrifuged and supernatants were assayed for factor VIII activity. The amount of factor VIII remaining in the supernatant was $8.8\% \pm 2.6\%$ (mean ± SD, $n = 4$) of the initial amount. This factor VIII also did not bind to newly added vWF-Sepharose, and therefore most likely represents activated factor VIII that does not bind to vWF but is normally detected in the chromogenic assay. We corrected for this fraction of nonbinding factor VIII in the data analysis.

**Equilibrium binding isotherm.** To determine whether the affinity of vWF for factor VIII was affected by binding of vWF to RU1-Sepharose, increasing amounts of factor VIII were incubated with a constant amount of vWF-RU1-Sepharose (2.1 nmol/L) for 30 minutes and the time course of binding was followed until equilibrium was reached. The concentration of bound factor VIII was obtained by subtracting the equilibrium value of unbound factor VIII from the total added factor VIII concentration. Figure 1 shows that factor VIII binds to vWF-RU1-Sepharose in a dose-dependent saturable manner. An adequate fit to these data was obtained using the model for (identical) independent binding sites given in Equation 1. The dissociation constant ($K_d$) and the number of binding sites ($C_{max}$) were estimated by a least-squares fit of Equation 1 to the data: $K_d = 0.46 \pm 0.12$ nmol/L, and $C_{max} = 2.8 \pm 0.3$ nmol/L. The obtained value of $C_{max}$ corresponds to a binding stoichiometry of 1.3 molecules factor VIII per vWF monomer, indicating that every vWF monomer is accessible for factor VIII binding.

**Studies with ΔAA2-rvWF.** To compare factor VIII binding to solution-phase vWF and to vWF immobilized to RU1-Sepharose, competition experiments were performed in which a vWF mutant was incubated with preformed factor VIII-vWF-RU1-Sepharose complexes. The mutant (ΔAA2-rvWF) was chosen because it lacks the A2 domain (residues 730 to 910) that contains the epitope for MoAb RU1, and therefore does not bind to MoAb RU1. First, factor VIII (0.16 nmol/L) was incubated with vWF-RU1-Sepharose (vWF concentration 8.0 nmol/L) for 30 minutes. Then ΔAA2-rvWF (0 to 35 nmol/L) was added, and after 2 hours of incubation, the samples were centrifuged and the factor VIII
Fig 1. Binding isotherm for the interaction between factor VIII and vWF-RU1-Sepharose. Factor VIII (0.5 to 5 nmol/L) was incubated with vWF-RU1-Sepharose (vWF concentration 2.1 nmol/L) for 10 to 600 seconds. Unbound and bound factor VIII were separated by centrifugation at 10,000 g for 10 seconds. Supernatants were tested for the concentration of unbound factor VIII. Each point represents the concentration of bound factor VIII at equilibrium, calculated according to Equation 6. The solid line represents the least squares fit of the binding isotherm of Equation 1 to these data. Estimated values of the dissociation constant and maximal binding were: $K_d = 0.46 \pm 0.12$ nmol/L and $C_{max} = 2.8 \pm 0.3$ nmol/L, respectively. Buffer: 150 mmol/L NaCl, 50 mmol/L TRIS-HCl, pH 7.4, 0.1% Tween-20, 1% bovine serum albumin, 2 mmol/L CaCl$_2$, $T = 22^\circ$C.

activity in the supernatant was measured. This activity represents the sum of unbound factor VIII and factor VIII bound to $\Delta$A2-rvWF. Control experiments indicated that the presence of $\Delta$A2-rvWF did not interfere with cofactor activity of factor VIII in the chromogenic assay used (data not shown). Figure 2 shows that addition of $\Delta$A2-rvWF resulted in a dose-dependent increase of the factor VIII concentration in the supernatant. By a least-squares fit of Equation 3 to the data, using the binding parameters for vWF-RU1-Sepharose determined in Fig 1, a dissociation constant of $0.32 \pm 0.04$ nmol/L was found. This result provides further evidence that factor VIII binding to vWF-RU1-Sepharose is reversible, and that immobilizing vWF via an MoAb to Sepharose does not alter the binding affinity for factor VIII.

Association experiments. The rate of factor VIII-vWF complex formation was determined by incubating factor VIII with vWF-RU1-Sepharose for various time intervals, and by assaying the concentration of unbound factor VIII in the supernatant. Initial concentrations of factor VIII and vWF were chosen so that complex formation was slow enough to be followed in time, ie, with concentrations below the plasma concentrations for both factor VIII and vWF. Figure 3 shows the kinetics of binding of factor VIII (0.49 nmol/L) to vWF-RU1-Sepharose (vWF concentration 2.1 nmol/L). Adequate fit was obtained by using the simple bimolecular association reaction of Scheme 1 (Equation 6). The values for the association and dissociation rate constants were: $k_{on} = 5.9 \pm 1.9 \times 10^6$ M$^{-1}$ s$^{-1}$ and $k_{off} = 2.5 \pm 0.4 \times 10^{-3}$ s$^{-1}$, respectively ($\Delta$A2-rvWF concentration 0.16 nmol/L). The dissociation constant at equilibrium ($K_d$) was calculated as $k_{off}/k_{on}$, was $0.27 \pm 0.22$ nmol/L.

Association experiments were also performed in which varying amounts of vWF (1.5 or 8 nmol/L) were coupled to
a constant amount of RU1-Sepharose and incubated with a low (0.02 nmol/L) or high (0.34 nmol/L) factor VIII concentration. As expected, the rate of complex formation increased with increasing factor VIII and vWF concentrations, whereas the estimated rate constants were similar ($k_{on} = 5.2$ and $4.3 \times 10^{-4} \text{ M}^{-1} \text{s}^{-1}$ and $k_{off} = 3.9$ and $2.2 \times 10^{-9} \text{ s}^{-1}$, respectively). This indicates that the model of bimolecular association (Scheme 1) provides adequate description and that complex formation is not influenced by a transport rate limitation.

The purified vWF used in the preceding experiments contained a mixture of high and low vWF multimers, as present in plasma. To investigate the influence of multimeric size of vWF on the rate of complex formation, vWF subfractions containing only high or low multimers were used to immobilize vWF to RU1-Sepharose, and association experiments were performed. Binding of high and low multimeric vWF to RU1-Sepharose was similar (data not shown). Table 1 shows that similar values were found for the rate constants of association and dissociation, indicating that factor VIII-vWF interaction is independent of the degree of vWF multimerization.

Because the coupling of vWF to RU1-Sepharose is followed by a washing procedure, it also permits selective binding of vWF from plasma. We performed binding experiments with vWF from normal pooled plasma and from plasma from patients with severe hemophilia A. As shown in Table 2, vWF from normal pooled plasma and from plasma of patients with severe hemophilia A exhibits kinetic rate constants comparable to purified vWF.

**DISCUSSION**

In the present study we developed a method that allowed the measurement of the association and dissociation kinetics of the factor VIII-vWF complex with a time resolution of 15 seconds. vWF was immobilized by binding to an MoAb (MoAb RU1) coupled to Sepharose beads. Free factor VIII and factor VIII associated with vWF bound to the Sepharose could be separated by centrifugation. The data presented here show that factor VIII-vWF complex formation is a rapid
process with an association rate constant of $5.9 \pm 1.9 \times 10^6 \text{M}^{-1} \text{s}^{-1}$ and a dissociation rate constant of $1.6 \pm 1.2 \times 10^{-4} \text{s}^{-1}$. In addition, we found identical rate constants for vWF from normal pooled plasma and from plasma of patients with hemophilia A.

The dissociation constant, $K_d = 0.46 \pm 0.12 \text{nmol/L}$, found in equilibrium experiments, was in good agreement with the $K_d$ values for the factor VIII-vWF interaction previously reported by us and others. The stoichiometry found in this study is consistent with the value of $1:1$ (molecules of factor VIII per subunit of vWF) reported by Lollar et al., who analyzed the interaction between the corresponding porcine proteins. It is also consistent with the value that we found previously in experiments with vWF conjugated to 15-nm colloidal gold. In experiments with vWF immobilized directly or indirectly via an antibody on the wells of a microtiter plate, a different stoichiometry of $1:50$ to $1:100$ was found. We previously observed that in solution, vWF binding to MoAbs with epitopes distinct from the factor VIII binding region per se does not interfere with the $1:1$ stoichiometry of factor VIII binding. Therefore, it appears that binding of vWF directly to a plastic surface or indirectly via an MoAb causes steric restrictions, which then results in a loss of factor VIII binding capacity of the majority (98% to 99%) of the vWF subunits. However, in this study all vWF subunits retained their binding capacity when bound to Sepharose with an antibody, and the value of $K_d$ found in competition experiments (see Fig 2) indicates that binding of vWF to RU1-Sepharose does not significantly affect the affinity of vWF for factor VIII binding.

The observed association and dissociation kinetics were adequately described by assuming one single class of binding sites for factor VIII and vWF. The value $k_{on} = 5.9 \pm 1.9 \times 10^6 \text{M}^{-1} \text{s}^{-1}$ found for the association rate constant of the factor VIII-vWF complex is well below the diffusion limit of $10^9 \text{M}^{-1} \text{s}^{-1}$, and typical for protein-protein reactions in solution. The value $k_{off} = 1.6 \pm 1.2 \times 10^{-4} \text{s}^{-1}$ for the first-order rate constant of dissociation and the half-life of dissociation of 430 seconds found in this study agrees well with the results from Leyte et al., who reported a first-order dissociation rate constant of $0.9 \times 10^{-3} \text{s}^{-1}$ and a half-life of dissociation of 780 seconds. The dissociation of bound factor VIII from vWF-RU1-Sepharose in exchange experiments with ΔA2-rvWF supports the reversibility of the factor VIII-vWF interaction. It also indicates that factor VIII can dissociate from vWF in the presence of an excess of vWF, and in the absence of thrombin. This is consistent with our previous observation in vivo, in which 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 assay used in this study showed no difference in factor VIII-vWF binding kinetics between low and high multimeric vWF. Likewise, the calculated values for the dissociation constants were similar. This extends our earlier observation that both forms of vWF have the same affinity toward factor VIII (Koppelman S, et al, *Blood*, in press). It is important to note that vWF was bound to RU1-Sepharose beads with a diameter of 100 μm, which could introduce a rate limit for the mass transfer of factor VIII from solution to the Sepharose surface and thereby influence the rate of association. To evaluate this possibility, we performed association experiments with different densities of vWF bound to RU1-Sepharose. Identical values were found for the rate constant of association, indicating that the observed rate constants reflect intrinsic kinetics and are unaffected by transport limitations.

The porous structure of the Sepharose beads, with a 20 × 10^6 Dalton exclusion size of the pores, also could present a point of concern with respect to the accessibility for factor VIII binding. Our equilibrium binding data show that all vWF monomers are accessible for factor VIII binding. In addition, no apparent deviations from the simple bimolecular association model were found in measurements of the association and dissociation kinetics. More convincingly, restricted accessibility could only be anticipated for low molecular vWF multimers, with molecular dimensions similar to factor VIII, whereas our data in Table 1 show that the kinetics observed for low and high multimers are not significantly different.

The site of complex formation between factor VIII and vWF in the human body is not precisely known. The liver is the major site of factor VIII synthesis, as shown by immunohistochemical localization studies, whereas vWF is synthesized by vascular endothelial cells and megakaryocytes. Interestingly, hepatic sinusoidal endothelial cells do not contain vWF:Ag or Weibel-Palade bodies, which suggests that these cells are different from their vascular counterparts in that they do not synthesize vWF. Directly after synthesis and secretion, factor VIII enters the space of Disse, the narrow space between the hepatocyte and the endothelium lining the sinusoid. The endothelium of the hepatic sinusoids contains fenestrations with openings up to 1 μm that permit plasma vWF to gain access to the surface of the hepatic cells. Thus, complex formation most likely occurs in the liver in the space of Disse, where factor VIII and vWF first meet. The rate of complex formation, expressed as the half-time for complex formation, can be calculated as $t_{1/2} = \ln2/(k_{on} \times \text{vWF concentration} + k_{off})$. Based on the plasma concentration of vWF (50 nmol/L in monomers), together with the estimates for $k_{on}$ and $k_{off}$ obtained in this study, the time needed to bind 50% of factor VIII is approximately 2 seconds.

The results obtained in the present work also provide a theoretical basis for the successful treatment of patients with hemophilia A with factor VIII concentrates presently available. Since the 1970s, therapy in patients with severe hemophilia A depended on cryoprecipitates, a product that retains the normal amount and distribution of vWF multimers observed in plasma. However, during the period from 1980 to 1990, monoclonally purified and recombinant factor VIII concentrates became available, and those products have become the standard in the treatment of patients with hemophilia A. However, it should be noted that these concentrates contain little or no vWF, and therefore factor VIII is dependent on endogenous vWF with which it has to form a
complex to be protected against proteolytic degradation in plasma. Although clinical studies have proven that the plasma half-life of monoklonally purified and recombinant factor VIII is not substantially different from that of less pure concentrates, the time needed for complex formation between infused factor VIII and endogenous vWF was hertofore unknown. Our results show that vWF from patients with hemophilia A resembles normal vWF with respect to factor VIII binding kinetics. Moreover, infused factor VIII is bound rapidly to the plasma vWF, with more than 95% binding in 12 seconds, which is extremely rapid in comparison to the half-life of 1 hour observed for factor VIII in the absence of vWF in plasma.

Rapid complex formation may also serve to stabilize assembly of factor VIII subunits at the site of secretion, or to prevent factor VIII from interacting with components that bind with lower affinity, such as factor IXa (Kd = 15 nmol/L) and phospholipids (Kd = 2 to 4 nmol/L).

Previous studies have established that factor VIII can be activated by factor Xa during the initial phase of factor X activation. Because factor VIII bound to vWF is protected from activation by activated factor X, rapid complex formation between factor VIII and vWF may be important in regulating factor VIII activity by inhibiting this feedback activation of factor VIII.

In summary, the kinetic experiments in this study show that factor VIII forms a complex with vWF in plasma, with a half-life of complex formation of 2 seconds. This rapid complex formation between vWF and factor VIII, either in the liver or after infusion of factor VIII in patients with hemophilia A, may be important in the stabilization and regulation of factor VIII activity in plasma.

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