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Biochemical and functional characterization of recombinant von Willebrand factor produced on a large scale

B. E. Fischer*, U. Schlokat, M. Reiter, W. Mundt and F. Dorner

IMMUNO AG, Biomedical Research Center, Uferstrasse 15, A-2304 Orth an der Donau (Austria)

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Abstract. Recombinant von Willebrand factor (r-vWF) was produced in serum-free medium on a large scale in recombinant Chinese hamster ovary cells and was purified from fermentation supernatant by a combination of anion exchange chromatography and herapin affinity chromatography. Heparin affinity chromatography yielded r-vWF polymers of different degrees of multimerization. r-vWF was analysed by qualitative and quantitative functional analysis. We could show that while binding of r-vWF to platelets did not depend on multimerization of the molecule, ristocetin-induced platelet aggregation, binding to collagen and binding to heparin correlated directly with the extent of multimerization. Binding of recombinant coagulation factor VIII (r-FVIII) to r-vWF was studied by real-time biospecific interaction analysis and surface plasmon technology. The data indicated that binding of r-FVIII did not depend on r-vWF multimerization. Real-time biospecific interaction analysis suggested a potential stoichiometry of 2 to 2.5 r-vWF subunits per r-FVIII molecule. Kinetic analysis of the r-vWF-r-FVIII interaction gave a binding rate constant of 3×10^6 M^{-1} s^{-1} and an association constant of 2.5×10^9 M^{-1}. Reaction of r-vWF with carbohydrate-specific lectins demonstrated that r-vWF contained a high proportion of N-glycans composed of mannose, galactose, glucose, *N*-acetylglucosamine and terminal sialic acid. Carbohydrate moities were covalently bound to the protein structure and were quantitatively removed from r-vWF only after protein denaturation. The results demonstrated that r-vWF produced on large scale under serum-free culture conditions exhibited qualitative and quantitative functional properties comparable to human plasma-derived vWF.

Key words. von Willebrand factor; recombinant protein; collagen binding; platelet aggregation; platelet binding; glycosylation; coagulation factor VIII.

The adhesive protein von Willebrand factor (vWF) exists in human plasma as a series of heterogeneous homo-multimers ranging in size from about 450 kDA to more than 20,000 kDa [1–4]. In endothelial cells the precursor polypeptide is produced as a pre-pro-vWF consisting of a 22-residue signal peptide, a 741-residue pro-peptide and a 2050-residue polypeptide found in mature plasma vWF. After removal of the signal peptide, the pro-vwF subunits are engaged in a complex biosynthetic process which is thought to begin with the formation of a primary dimer, consisting of two pro-vWF subunits, linked through disulphide bonds. The dimers associate further through interchain disulphide bonds to form a series of multimers containing a variable number of subunits. vWF pro-peptide is cleaved from multimeric vWF before it is released from intracellular storage sites into circulation [1, 3, 5, 6]. Released, vWF serves a dual purpose in haemostasis. First, it acts as an adhesive protein that serves as a bridge between platelet glycoproteins and the vascular subendothelium.

* Corresponding author.

Second, vWF binds and stabilizes coagulation factor IVIII (FVIII) and circulates with it as a noncovalently linked complex. In this manner, vWF plays a key role in haemostasis by initiating platelet adhesion at sites of vascular injury and by localizing FVIII to sites where it can participate in the generation of thrombin and the fibrin clot [7].

Human plasma concentrates containing vWF are used for therapy in patients with von Willebrand disease. However, preparation of recombinant vWF offers an alternative to the preparation of vWF from human plasma.

vWF was cloned in 1986 [8] and expressed in different heterologous cells [9–24]. r-vWF and r-vWF fragments have been produced on a laboratory scale to study the molecular structure, to detect receptor binding sites and modified regions, as well as to interpret post-translational processing and multimerization [8, 9, 11, 12, 14, 18, 20–23, 25–27].

We have taken up the challenge of preparing r-vWF as an alternative source to plasma-derived human vWF [28–31]. Usually, recombinant cells are cultivated in growth medium supplemented with fetal calf serum to express r-vWF [9, 14, 16–19, 21–27]. However, this method is not suitable for therapeutic r-vWF preparation. Thus, we adapted Chinese hamster ovary (CHO) cells to serum-free culture conditions and optimized the composition of the culture medium for continuous production of r-vWF on a large scale [29–31]. Multimer structure and subunit composition of r-vWF produced under serum-free conditions have been analysed in detail [29]. Preclinical studies and characterization of r-vWF in animal models have been reported [32–35]. In this report we present the biochemical and functional characterization of r-vWF.

Materials and Methods

Materials. Real-time biospecific interaction analysis using surface plasmon resonance technology (SPR) was performed using a BIAcore 2000 from Pharmacia Biosensor (Uppsala, Sweden). Sensor chip CM5 and an amine coupling kit containing *N*-hydroxysuccinimide (NHS), *N*-ethyl-*N'*-(3-diethylaminopropyl)-carbodiimide (EDS) and ethanolamine hydrochloride were obtained from Pharmacia Biosensor. Recombinant von Willebrand factor (r-vWF) produced by large-scale culture of recombinant CHO cells [28, 29], human plasma, human plasma cryoprecipitate and purified monoclonal anti-von Willebrand factor antibody, MAb AvW8/2, were from IMMUNO (Vienna, Austria). Recombinant coagulation factor VIII (Kogenate, r-FVIII) was obtained from Bayer (Leverkusen, Germany). Quantitative determination of vWF antigen (vWF:Ag) was done by enzyme-linked immunosorbent assay (ELISA) (Asserachrom vWF, Boehringer Mannheim). vwF ristocetin cofactor activity (vwF:RistCof) was analysed using stabilized platelets (von Willebrand Reagent, Behringwerke, Marburg, Germany). Fractogel EMD-TMAE and Fractogel EMD-Heparin were from Merck (Darmstadt, Germany). Other reagents used were rabbit anti-human vWF serum (Dakopatts, Glostrup, Denmark), alkaline phosphatase-conjugated, affinity-purified, goat anti-rabbit immunoglobulin G (IgG) (Bio-Rad, Hercules, USA). *N*-glycosidase F, *O*-glycosidase and neuraminidase were from Boehringer Mannheim.

Purification of vWF. Purification of r-vWF from cell culture supernatant of recombinant CHO cells was performed in 20 mM tris/HCl buffer, pH 7.4 (tris buffer), by combination of anion-exchange chromatography and heparin affinity chromatography. Recombinant CHO cell supernatant was applied onto a Fractogel EMD-TMAE column equilibrated with tris buffer. The column was washed with 180 mM NaCl in tris buffer; vWF was then eluted with 280 mM NaCl in tris buffer. Fractions containing vWF were pooled, diluted to 90 mM NaCl with tris buffer and applied onto a Fractogel EMD-heparin column. Weakly bound material was eluted with 100 mM NaCl in tris buffer. vWF was eluted from the Fractogel EMD-heparin column at NaCl concentrations of 160 mM, 230 mM and 280 mM in tris buffer.

Qualitative and quantitative characterization of vWF. Binding activity of vWF to collagen (collagen-binding activity, vWF:CBA) was determined using the method described previously [36]. Briefly, soluble human collagen type III was coated onto microtitre plates. vWF dilutions were incubated with the immobilized collagen. Collagen-bound vWF was quantified using rabbit anti-human vWF IgG-peroxidase conjugate. Multimer analysis of vWF was performed by SDS-1% agarose gel electrophoresis, whereby individual vWF multimers were separated by electrophoresis and then blotted onto nitrocellulose membrane as described previously [28, 29]. vWF multimers were detected by immunoenzymatic staining using a rabbit anti-human vWF serum as primary antibody; the second antibody was alkaline phosphatase-conjugated, affinity-purified, goat anti-rabbit IgG. Staining was performed with the nitroblue tetrazolium choride/bromochloroindolyl phosphate substrate system. Activity of vWF is expressed in units (U). One U of vWF:Ag, vWF:CBA or vWF:RistCof is defined as the amount present in 1 ml of normal pooled plasma. Specific activity in the ratio of functional activity (vWF:RistCof, vWF:CBA) to vWF:Ag.

Real-time biospecific interaction analysis. Monoclonal anti-human von Willebrand antibody AvW8/2 was covalently bound to a sensor chip CM5 surface by reaction of AvW8/2 amines with an *N*-hydroxysuccinimide-ester-activated sensor chip surface [37–39]. Measurement of response units after AvW8/2 immobilization

gave baseline response units (RU_{BL}). r-vWF was dissolved in 10 mM Hepes, pH 7.4, 150 mM NaCl, 1 mM $CaCl_2$, 0.05% surfactant P20 (HBS buffer) to a vWF:Ag concentration of 20 μg/ml. For each experiment a 50-μl sample of vWF dilution was injected over the sensor chip surface at a constant flow rate of 5 μl/min and allowed to interact with the immobilized AvW8/2. For each experiment a constant level of 0.55 ng vWF as bound per mm^2 sensor chip surface. Nonbound vWF was washed from the chip surface by injecting 20 μl of HBS buffer. Measurement of response units after binding of vWF to immobilized AvW8/2 resulted in vWF response units (RU_{vWF}). 60 μl of r-FVIII (2.5 μg/ml in HBS buffer) were injected over the sensor chip surface at a constant flow rate of 5 μl/ml to react with vWF bound to AvW8/2 at the chip surface. Binding of r-FVIII to vWF was recorded continuously. Measurement of response units at the end of the association phase gave FVIII response units (RU_{FVIII}). The flow was continued at the same rate with HBS buffer, and dissociation of r-FVIII was followed continuously. Apparent stoichiometry of the $vWF_{subunit}$-FVIII complex was calculated as follows: $vWF_{subunit}:FVIII = (RU_{vWF} - RU_{BL})/(RU_{FVIII} - RU_{vWF}) \times 330{,}000/220{,}000$. Dissociation rate constants (k_d) and association rate constant (k_a) were calculated as described in detail previously [31, 32] using microcomputer software provided by Pharmacia Biosensor. The equilibrium binding constant (K_a) was obtained as $K_a = k_d/k_a$.

Binding of r-vWF to platelets. Purified r-vWF was diluted to concentrations of vWF:Ag between 1 and 10 μg/ml in 20 mM tris buffer, 150 mM NaCl, pH 7.4. A 200-μl aliquot of the diluted r-vWF solution was mixed with 250 μl of paraformaldehyde-fixed human platelets (200,000/μl) and 50 μl ristocetin (15 mg/ml) in an Eppendorf tube. After an incubation for 15 min at 23 °C, platelets were sedimented by centrifugation (10 min, 15,000 rpm). The supernatant was carefully aspirated, and the platelets were reconstituted in 500 μl of buffer. To determine the concentration of platelet-bound r-vWF, vWF:Ag was determined in the supernatant before and after the incubation. SDS-1% agarose gel electrophoresis was used to analyse r-vWF multimer composition.

Deglycosylation of r-vWF and h-vWF. Deglycosylation of r-vWF was carried out with *N*glycosidase F, *O*-glycosidase and neuraminidase using standard protocols suggested by the manufacturer. Briefly, r-vWF at a protein concentration of 1 mg/ml was incubated at 90 °C for 2 min in 1% SDS solution for protein denaturation and was then diluted to 0.1 mg/ml with 20 mM phosphate buffer, pH 7.2, containing 50 mM EDTA, 10 mM sodium azide and 0.5% Nonidet P-40. Per 10 μg of the glycoprotein, either 2.5 mU *O*-glycosidase or a mixture of 2.5 mM *O*-glycosidase, 2 mU neuraminidase and 0.4 U *N*-glycosidase F were added. The proteins were incubated at 37 °C for 20 h. Samples, taken prior to deglycosylation and after deglycosylation, were analysed by SDS-agarose electrophoresis and for ability to bind (before deglycosylation) or not to bind (after deglycosylation) to O- and N-glycan specific lectins.

Figure 1. Purification of r-vWF. SDS-PAGE analysis using reducing conditions. Samples: (*a*) purified human vWF, (*b*) r-vWF prior to purification, (*c*) r-vWF isolated by anion-exchange chromatography, (*d*) r-vWF/HMW isolated by heparin affinity chromatography.

Detection of glycan structures with digoxigenin-labelled lectins. After SDS-PAGE and electrotransfer of proteins onto nitrocellulose membranes, glycoproteins were incubated with digoxigenin-labelled lectins followed by detection of glycoprotein-bound lectins with alkaline phosphatase conjugated anti-digoxigenin immunglobulin as described previously [40]. Fetuin and asialofetuin at concentrations of 0.5 μg per lane were used as control glycoproteins to certify reactivity of lectins.

Results

Transformed CHO cells were adapted to cell culture medium completely free of bovine serum and any animal-derived protein, and r-vWF was produced by culture of CHO cells at these serum-free conditions. r-vWF

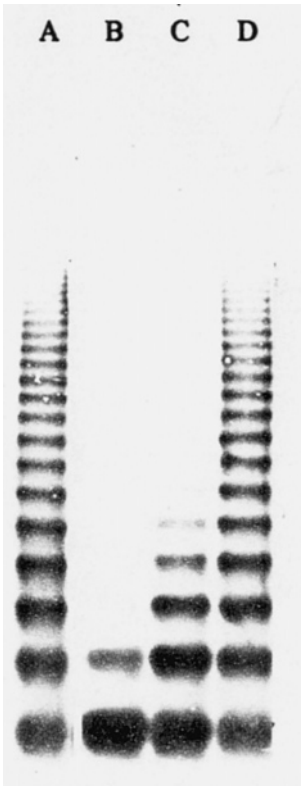

Figure 2. Heparin affinity chromatography of r-vWF. Multimer analysis by SDS-1% agarose gel electrophoresis. Samples: (*A*) r-vWF before purification; (*B*) 160 mM NaCl eluate from heparin affinity chromatography (r-vWF/LMW); (*C*) 230 mM NaCl eluate from heparin affinity chromatography (r-vWF/MMW); (*D*) 280 mM NaCl eluate from heparin affinity chromatography (r-vWF/HMW)

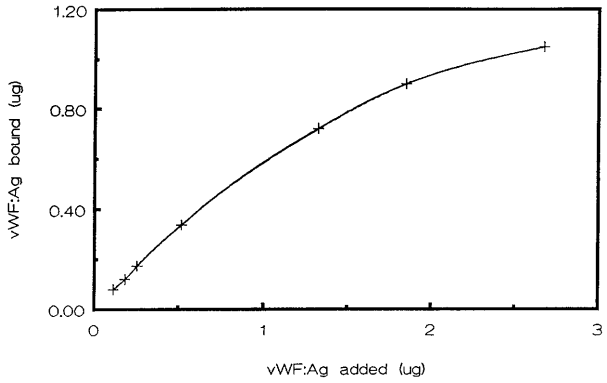

Figure 3. Binding of r-vWF to platelets in the presence of ristocetin as a function of the total amount of r-vWF added.

was purified from culture supernatant of recombinant CHO cells by combination of anion-exchange chromatography and heparin affinity chromatography (figs. 1 and 2). No addition of albumin was necessary prior to, during or after purification to stabilize r-vWF. Purified r-vWF in buffer was stable both at 4 °C (1 week) and −20 °C (6 months) and after lyophilization without adding any stabilizer. Crude r-vWF exhibited a specific activity of 0.15 IU vWF:RistCof/IU vWF:Ag. Anion-exchange chromatography elution using 280 mM NaCl yielded vWF preparations containing the entire multimer pattern, ranging from the first bands representing the first dimer up to the largest vWF-polymers. In contrast, elution of r-vWF from the heparin affinity column with different concentrations of NaCl resulted in r-vWF containing various degrees of multimerization (fig. 2). Treatment of the heparin affinity column with 160 mM NaCl eluted r-vWF composed mainly of the dimer and traces of the tetramer (low molecular weight multimers, r-vWF/LMW). Elution with 230 mM NaCl resulted in r-vWF composed of dimer, tetramer, hexamer and traces of decamer (medium molecular weight multimers, r-vWF/MMW). The complete spectrum of r-vWF-polymers, ranging from the dimer up to the largest polymers, was obtained with 280 mM NaCl (high molecular weight multimers, r-vWF/HMW). This separation of r-vWF-multimers was achieved in the absence of calcium ions. In contrast, when low concentrations of calcium ions were added to the buffer, separation of r-vWF polymers could not be achieved.

r-vWF preparations isolated by heparin affinity chromatography were analysed for vwF:RistCof, vWF:CBA and vWF:Ag. The results are summarized in table 1. A comparison between the multimer composition of individual r-vWF fractions and the functional properties shows that both specific activities, vWF:RistCof/vWF:Ag and vWF:CBA/vWF:Ag, increase with increasing multimerization of r-vWF.

Figure 3 shows the binding of r-vWF/HMW to platelets. It appears that binding of r-vWF to platelets reaches saturation. Binding of r-vWF to platelets did not depend on multimerization (fig. 4).

Interaction of r-vWF and coagulation factor VIII was analysed by real-time biospecific interaction analysis and surface plasmon resonance technology [39]. The sensor chip surface was activated, and the monoclonal anti-human vWF antibody AvW8/2 was covalently immobilized at the chip surface via active-ester coupling to amine. Measurement of response units after coupling yielded the baseline value (RU_{BL}). For each binding analysis 0.55 ng of vWF:Ag as bound per mm^2 of sensor chip surface. This resulted in an increase in the protein mass bound to the sensor chip surface and in an increase in the response units (RU_{vWF}). The difference between RU_{BL} and RU_{vWF} represents a measure of r-vWF bound to the sensor chip surface. Binding of r-FVIII to bound r-vWF resulted in a further increase

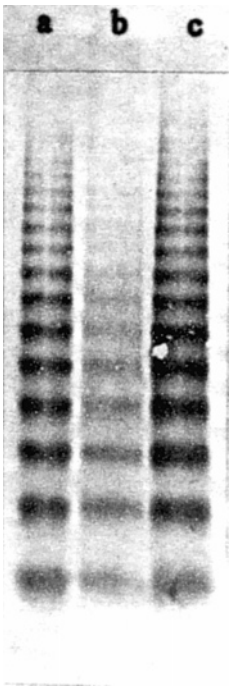

Figure 4. Binding of r-vWF to platelets in the presence of ristocetin. Multimer analysis by SDS-1% agarose gel electrophoresis. Samples: (*a*) r-vWF prior to incubation; (*b*) r-vWF not bound to platelets; (*c*) platelet-bound r-vWF.

Table 1. Functional characterization of r-vWF with various degrees of multimerization. r-vWF was separated into r-vWF/LMW, r-vWF/MMW and r-vWF/HMW by heparin affinity chromatography.

Sample	vWF:RistoCof/vWF:Ag (U/U)	vWF:CBA/vWF:Ag (U/U)
r-vWF/LMW	0.004	0.001
r-vWF/MMW	0.07	0.04
r-vWF/HMW	0.25	0.35

Table 2. Biospecific interaction analysis of vWF and FVIII. Determination of rate constants and equilibrium binding constants.

Sample	Stoichiometry vWF-subunit: FVIII	k_a (M^{-1} s^{-1}) $\times 10^6$	k_d (s^{-1}) $\times 10^{-3}$	K_a (M^{-1}) $\times 10^9$
r-vWF/LMW	2.5:1	3.9	1.8	2.2
r-WF/MMW	2.0:1	3.2	1.4	2.3
r-vWF/HMW	2.0:1	3.0	1.2	2.5

Table 3. Reaction of r-vWF with carbohydrate specific lectins.

Lectin/carbohydrate specificity	r-vWF
SNA/SA 2-6 Gal	−
MAA/SA 2-3 Gal	+
DAS/Gal 1-4 GlnNAc	+
PNA/Gal 1-3 GalNAc	−
GNA/Man 1-2/3/6 Man	+

+, binding of lectin; −, no binding of lectin.

in response units. Flow of r-FVIII was continued until saturation of r-vWF was reached. Response unit measurement at this time yielded the RU_{FVIII} value. The difference between RU_{FVIII} and RU_{vWF} corresponds to the amount of r-FVIII bound to r-vWF. Preparations of r-vWF varying in multimer compositions were used to study the efficiency of r-FVIII binding. Apparent stoichiometry of the complex of $vWF_{subunit}$ to FVIII molecule, apparent association rate constants (k_a), apparent dissociation rate constants (k_d) and equilibrium binding constants (K_a) were determined and summarized in table 2.

To characterize its glycosylation, r-vWF was analysed by SDS-1% agrose gel electrophoresis, blotted onto nitrocellulose membrane and incubated with carbohydrate-specific lectins. Lectins from *Datura stramonium* (DSA), *Galanthus nivalis* (GNA), *Maackia amurensis* (MAA), *Sambucus nigra* (SNA) and peanut (PNA) were used to detect glactose-β(1-4)-*N*-acetylglucosamine units (Gal 1-4 GlnNAc), mannose-α(1-3)/(1-6)/(1-2)-mannose structures (Man 1-2/2/6 Man), sialic acid-α(2-3)-galactose units (SA 2-3 Gal), sialic acid-α(2-6)-galactose structures (SA 2-6 Gal) and galactose-β(1-3)-*N*-acetylgalactomsamine (Gal 1-3 GalNac), respectively. Results are summarized in Table 3. Apparently r-vWF exhibited glycosylation corresponding to the presence of mannose, galactose, glucose, *N*-acetylglucosamine, capped by sialic acid 2-3 galactose bond.

Selective and complete removal of carbohydrates by (i) *N*-glycosidase F and by (ii) a mixture of *N*-glycosidase F, sialidase and *O*-glycosidase from denatured and native r-vWF was followed by SDS-1% agarose gel electrophoresis (fig. 5). Treatment of denatured r-vWF with *N*-glycosidase F resulted in a significant reduction in molecular weight of all multimer bands demonstrating a high proportion of N-glycans in the molecule. Treatment with a combination of *N*-glycosidase, sialidase and *O*-glycosidase resulted in multimers with molecular weights slightly lower than multimers after removal of only the N-glycans. Apparently, r-vWF contained only low levels of *O*-glycoslation. Quantitative removal of glycans from r-vWF was only achieved after denaturation of the molecule, demonstrating that the bonds between N- and O-linked carbohydrate chains and the protein backbone are protected in the three-dimensional protein structure.

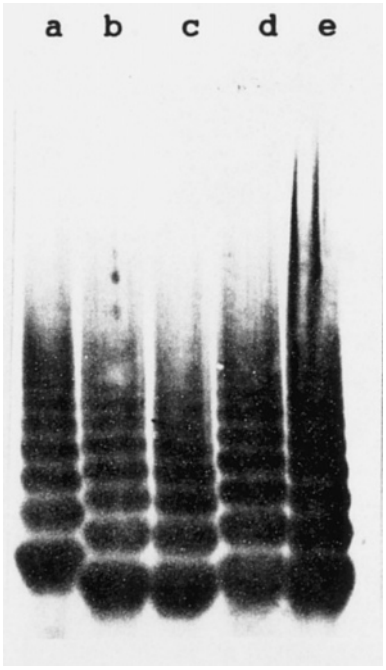

Figure 5. Multimer structure of r-vWF analysed by SDS-1% agarose gel electrophoresis prior to and after deglycosylation. Samples: (*a*) multimer structure of r-vWF prior to deglycosylation; (*b*) multimer structure of r-vWF after denaturation and incubation with *N*-glycosidase F; (*c*) multimer structure of r-vWF after denaturation and incubation with a combination of *N*-glycosidase F, sialidase and *O*-glycosidase; (*d*) multimer structure of r-vWF after incubation with *N*-glycosidase without denaturation; (*e*) multimer structure of r-vWF after incubation with a combination of *N*-glycosidase F, sialidase and *O*-glycosidase without denaturation.

Discussion

Recently we reported the expression of r-vWF under serum-free conditions by large scale, continuous culture of transformed CHO cells and characterized the multimer structure and subunit composition of the recombinant protein [28–31]. Preclinical studies and characterization of r-vWF in animal models have been reported [32–35]. Effects of r-vWF on haemostasis was investigated in depleted mice, as well in dogs and pigs suffering from hereditary von Willebrand disease. Increase in plasma factor VIII level and reduction of blood loss was observed upon injection, demonstrating functionality of r-vWF in vivo [32–35].

In this study we present biochemical and functional qualitative and quantitative characterization of r-vWF. r-vWF was isolated from culture supernatant and purified by anion-exhange chromatography. Heparin affinity chromatography was used to isolate r-vWF fractions with different degrees of polymerization.

Binding analysis demonstrated that all the different multimeric species of r-vWF exhibit platelet-binding activity comparable to the results obtained for human plasma-derived v-WF [41]. Qualitative functional analysis of r-vWF demonstrated that both vWF:CBA/vWF:Ag and vWF:RistCof/vWF:Ag increase with increasing size of the molecule. Low molecular weight r-vWF multimers (vWF dimer and vWF tetramer, r-vWF/LMW) exhibited very low vWF:CBA and vwF:RistCof, while the highest activities were found for high molecular weight r-vWF polymers (r-vWF/HMW). Heparin affinity chromatography demonstrated that heparin affinity of r-vWF increases with an increasing degree of multimerization.

Functional analysis of human plasma-derived vWF has shown previously that the largest molecular weight vWF multimers are most haemostatically effective and that vWF:CBA, vWF:RistCof and heparin binding increase with multimerization of the protein [42, 43]. Thus r-vWF produced under serum-free conditions on a large scale is as functionally active as vWF derived from human plasma.

Interaction of r-vWF with r-FVIII was studied by real-time biospecific interaction analysis. Binding rate constants, association constants and stoichiometry of r-vWF-monomer/FVIII complex at saturation were calculated. At saturation, a stoichiometry of 2 to 2.5 r-vWF subunits per r-FVIII molecule was obtained, independent of the degree of r-vWF polymerization. This result corresponds well with gel filtration experiments by Vlot et al. [44], who found 0.5 molecules of human factor ViII bound per monomer of human plasma-derived vWF. It appears that both the association rate constants and the dissociation rate constants are less affected by the degree of multimerization of r-vWF. The dissociation rate constant of 1.16×10^{-3} s^{-1} determined for r-vWF/HMW and r-FVIII agrees with the dissociation rate constant of 0.9×10^{-3} s^{-1} of human plasma-derived vWF and human FVIII determined by Leyte et al. [45]. Equilibrium binding constants for r-vWF and r-FVIII did not depend on the degree of multimerization of the recombinant protein. Therefore, binding of coagulation factor VIII is similar for r-vWF and human plasma-derived vWF.

Carbohydrate analysis of r-vWF demonstrated that the recombinant molecule is *N*-glycosylated. Apparently, carbohydrate chains are incorporated into the protein structure. Carbohydrate moities such as galactose, glucose, *N*-acetylglucosamine, mannose and terminal sialic acid were detected, demonstrating effective glycosylation of the protein during its recombinant production in CHO cells under serum-free culture conditions. N-glycans containing classic carbohydrate linkage patterns were detected including only 2,3-linked sialic acid residues, but no 2,6-linked sialic acid was detected as usually found in human plasma proteins. r-vWF exhibited only 1,4-linked galactose residues; no terminal 1, 3-linked galactose moieties were detected. Deglycosyla-

tion demonstrated a lower level of O-glycans in the molecule. However, differences in glycosylation apparently did not affect r-vWF function, because important functional properties were similar to human vWF.

Therapeutic plasma concentrates containing human vWF often lack the largest multimers and exhibit various degree of degradation of vWF [42]. Structural damage to plasma-derived vWF is caused by soluble and platelet/leucocyte-associated proteases which may act at the early stages of the manufacturing process of plasma concentrates [42]. An alternative way to prepare von Willebrand factor without degradation of vWF by plasma proteases is by recombinant technology. Our previous structural analyses have demonstrated that r-vWF is not degraded by proteases during the fermentation and purification process. r-vWF is compoased of mature subunits [29–31]. The present results show that r-vWF produced on a large scale under serum-free culture conditions exhibits all qualitative and quantitative functional properties which allow it to mediate platelet aggregation, promote collagen binding and binding of coagulation factor VIII with activity efficiency comparable to human plasma-derived vWF.

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