Comparison of Factor VIII-Related von Willebrand Factor Proteins Prepared From Human Cryoprecipitate and Factor VIII Concentrate

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Both commercial factor VIII concentrate and cryoprecipitate have factor VIII coagulant activity and factor VIII antigen (VIII:Cg), but the concentrate lacks the von Willebrand activity (VIII:WF) found in the cryoprecipitate. In an effort to find a possible cause for this anomaly we compared the structures of VIII:WF proteins responsible for the VIII:Cg and VIII:WF activities obtained from the two sources. Samples of cryoprecipitate and concentrate were chromatographed on agarose gel, and the resultant VIII:WF protein-rich void volume fractions were analyzed by first- and second-dimension sodium dodecyl sulfate gel electrophoresis. Proteins were first separated on a 1.5 % polyacrylamide–0.5 % agarose gel and then reduced and electrophoresed into a 3 % polyacrylamide–0.5 % agarose gel. VIII:WF protein from both cryoprecipitate and concentrate was multimeric, with polymers composed of $M_r = 2.4 \times 10^8$ subunits. However, the multimers of the VIII:WF proteins from the two sources differed in average molecular weight and structure. Cryoprecipitate VIII:WF multimers were predominantly in the molecular weight range above $5 \times 10^9$ and were uniformly separated from each other by increments of $M_r = 6.5 \times 10^9$. In contrast, concentrate VIII:WF multimers were relatively smaller, predominantly in the molecular weight range from $1.4 \times 10^8$ to $5 \times 10^9$. The polymeric bands were separated from each other by spaces equivalent to approximately $M_r = 5 \times 10^8$ alternating with distances of $M_r = 2.5 \times 10^9$. These differences in molecular weight and structure are possible causes for the functional property demonstrated here that VIII:WF protein from cryoprecipitate had 2.6 times more VIII:WF activity per $M_r = 2.4 \times 10^8$ subunit than VIII:WF protein from concentrate.

Factor VIII von Willebrand-related (VIII:WF) protein is involved with optimal platelet adhesion to the subendothelium as well as transport and stabilization of factor VIII coagulant activity* (VIII:C).1,2 The antigenic properties (VIII:Cg) of VIII:WF protein and the ability of VIII:WF protein to aggregate platelets in the presence of the antibiotic ristocetin have been used to quantitate the protein.3,5 Reduced levels or abnormal forms of VIII:WF protein are associated with von Willebrand's disease.6,7 However, the relationship between

*Abbreviations for factor VIII activities include the following: VIII:C, factor VIII procoagulant activity; VIII:WF protein, the major glycoprotein obtained when factor VIII is purified from plasma, having a subunit mass of $2.4 \times 10^8$; VIII:Cg, antigenic determinants associated with VIII:WF protein; VIII:WF activity, the activity of factor VIII that supports ristocetin-induced platelet aggregation. Other abbreviations include the following: Clg, cold insoluble globulin; SDS, sodium dodecyl sulfate; PEG, polyethylene glycol; PMSF, phenylmethyl sulfonyl fluoride; KIU, kallikrein inhibitory unit; AHF, antihemophilic factor.
VIIIIR:WF structure and function is not well understood, largely because of difficulties in purifying and examining the structure of this high-molecular-weight trace constituent of plasma.

It is generally agreed that VIIIIR:WF protein from cryoprecipitate has a $M_r > 10^6$ and is composed of identical disulfide-linked subunits of $M_r \approx 2 \times 10^5$. Studies with porcine$^{10}$ and human$^{11}$ VIIIIR:WF protein strongly suggest that the protein is a multimeric polymer. For example, Fass et al.$^{10}$, working with porcine VIIIIR:WF protein, observed polymeric aggregates with an estimated $M_r$ as large as $20 \times 10^6$, and they hypothesized that these forms were composed of a basic repeating unit consisting of six to eight $M_r = 2 \times 10^5$ subunits. The functional properties of these aggregates, at least in vitro, are not uniform: the larger multimers have a greater tendency to bind to platelets in the presence of ristocetin than the smaller ones.$^{12}$ It is also apparent that the functional properties of VIIIIR:WF protein in vivo are strongly dependent on purification procedures. Several investigators$^{13,14}$ have reported that blood-bank-prepared cryoprecipitate corrects the prolonged bleeding time in patients with von Willebrand's disease, whereas commercially produced factor VIII AHF concentrate does not. It has been suggested$^{15}$ that this lack of activity is the result of proteolytic degradation of the larger VIIIIR:WF protein aggregates during the purification process.

In the present study we examined the structure and molecular weight distribution of VIIIIR:WF multimers obtained from cryoprecipitate and concentrate to further elucidate possible causes for their different functional properties. A first- and second-dimension SDS gel electrophoresis method was developed that proved useful in assessing the purity and molecular weight distribution of VIIIIR:WF multimers prepared from the two sources. This procedure demonstrated that not only do VIIIIR:WF multimers from factor VIII concentrate lack the higher-molecular-weight forms observed in VIIIIR:WF protein from cryoprecipitate but also they have a different polymeric structure that yields a heterogeneous rather than homogeneous banding pattern on SDS gels.

**MATERIALS AND METHODS**

The following chemicals were purchased from the suppliers indicated: Al(OH)$_3$ gel (Amphojel without flavor) from Wyeth Laboratories; fatty-acid-free bovine albumin type F, Aprotinin, bentonite, and dithiothreitol from Sigma Chemical Co., St. Louis, Mo.; kaolin (acid-washed) from Fisher Scientific, Medford, Mass.; $N,N'$-methylenebisacrylamide (ultrapure, electrophoresis grade), acrylamide, and $N,N',N''$-tetramethylenediamine (TEMED) from Polysciences, Warrington, Pa.; Sea Kem (ME) agarose, used for electrophoresis, from Marine Colloids, Rockland, Me.; dimethyl sulfoxide from Pierce Chemical Co., Rockford, Ill.; SDS from British Drug House, Poole, England. All other chemicals were reagent grade. Column chromatographic medium included Agarose A-15m from Bio-Rad, Richmond, Calif. Lyophilized antihemophilic concentrates were obtained as gifts from Abbott Laboratories, South Pasadena, Calif., Armour Pharmaceutical Co., Phoenix, Ariz., Cutter Laboratories, Berkeley, Calif., Merieux Institute, Miami Fla., and Hyland Division Travenol Laboratories, Costa Mesa, Calif. Cryoprecipitate was obtained from the American Red Cross. Rabbit anti-VIIIIR:Ag antisera was purchased from Behring Diagnostics, Sommerville, N.J. Human Clg was prepared by the method of Engvall and Ruoslahti.$^{16}$ Myosin, used for an $M$, marker, was generously contributed by Dr. Renee Lu, Boston Bio Med Research Institute, Boston, Mass. Ristocetin was acquired from Bio/Data Corp., Horsham, Pa. Centrolex P, purchased from Centr Soya, Chicago, Ill., was used as a platelet substitute in the VIIIIR:C assay. Cylindrical SDS gels were formed and electrophoresed in glass tubes (0.5 cm ID X 12 cm) held in a Bio-Rad model 150 A gel apparatus. Slab gels were formed in a Bio-Rad model 220 slab gel device and run on the horizontal slab gel bed model 92-1 made by MRA.
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Corp., Boston, Mass. A sheet of porous polyethylene 1/4 inch thick was obtained from Bell Art, Pequannock, N.J.

Quantitative Determination of Factor VIII Activities

The assay for VIII:C was carried out by a previously described method modified by adding the sample to be tested to the kaolin-phospholipid-hemophiliac plasma mixture just prior to the addition of CaCl₂.

The quantity of VIIIR:WF activity was determined by aggregation of washed formaldehyde-fixed platelets in the presence of nistocetin. Each assay was performed at 37°C by adding 0.1 ml of sample to 0.375 ml of washed platelets (3 × 10^9 platelets/ml 0.025-M TRIS-HCl-0.125-M NaCl-0.02% NaN₃, pH 7.3), incubating the mixture for 3 min, and adding 25 μl of solution containing 25 g of ristocetin per liter of TRIS-saline buffer. A blank containing 0.2 ml of platelets in 0.3 ml of TRIS buffer was used in the aggregometer. A standard reference curve was constructed on log-log paper by plotting the slope of the platelet aggregation curve, obtained from serial dilutions of normal pooled plasma in TRIS buffer, versus the percentage of plasma in the dilution.

Quantitative electroimmunoassays and crossed immunoelectrophoresis studies were performed by the methods used by Over et al.

The relative amounts of VIIIR:WF protein in cryoprecipitate and concentrate were also assessed by two-stage SDS gel electrophoresis.

Comparison of VIII R: WF Protein Obtained From Cryoprecipitate and Concentrate

Samples of cryoprecipitate were prepared by mixing a solution of 6 ml of veronal buffered saline, pH 7.35, containing 0.1% albumin, 2-mM PMSF, 20 KIU Aprotinin/ml, and 0.02% NaN₃ (veronal-inhibitor buffer) with 36 ml of cryoprecipitate. Additional Aprotinin and diisopropyl fluorophosphate were added to raise the final concentrations of these reagents in the 40 ml of solution to 5 KIU/ml and 2 mM, respectively. The solution was absorbed with 1.2 ml of Al(OH)₃ for 10 min and centrifuged for 10 min at 6000 g. The supernatant was then incubated for 10 min with 0.4 g of bentonite, centrifuged at 6000 g for 10 min to remove the bentonite, and applied to a 37.5-cm column of agarose A-15m equilibrated with the veronal-inhibitor buffer. The column was eluted at a flow rate of 2.5 ml/min.

Lyophilized concentrate samples from the various manufacturers containing 250-375 VIII:C units were dissolved in 40 ml of the veronal-inhibitor buffer. The solutions were treated with Aprotinin, diisopropyl fluorophosphate, Al(OH)₃, and bentonite and were chromatographed on the A-15m column in the same manner as the cryoprecipitate samples.

In both cases the void volume peaks containing factor VIII activities were pooled, precipitated by the addition of PEG 4000 to a final concentration of 15%, and incubated overnight at 4°C. The samples were then centrifuged at 7000 g for 30 min at 4°C, and the precipitates were dissolved in a buffer of 0.02-M sodium citrate, 0.3-M NaCl, 2.5-mM PMSF, and 20 KIU of Aprotinin/ml, pH 7.0. Sample aliquots were stored at −80°C. All of the concentrate samples from the various manufacturers were chromatographed and examined by first-dimension SDS gel electrophoresis (vide infra). The Hyland AHF concentrate was used in further experiments involving crossed immunoelectrophoresis, second-dimension SDS gel electrophoresis, and platelet aggregation in the presence of ristocetin.

C₁g was removed from some sample aliquots by gelatin-Sepharose absorption. Samples containing as much as 10 μg of C₁g in a 0.5-ml aliquot were incubated for 1 hr at 22°C with 0.3 ml of gelatin-Sepharose. The Sepharose beads were removed by centrifugation at 6000 g for 10 min. C₁g could not be detected in the supernatant by a crossed immunologic assay sensitive to 5 μg of C₁g per milliliter of solution.

Human IgM and fibrinogen purified from pooled normal plasma and cross-linked with dimethyl suberimidate served as M₁ markers on SDS gels.

First-Dimension SDS Gel Electrophoresis

Cylindrical SDS gels (0.5 × 9.5 cm) composed of 1.5% polyacrylamide-0.5% agarose were made by the same procedure used in preparing 3% polyacrylamide-0.5% agarose gels, but the concentration of acrylamide was halved. The 1.5% polyacrylamide-0.5% agarose gels did not adhere to the walls of the glass tubes; tissue paper held to the bottoms of the tubes with sections of rubber tubing kept the gels
from slipping out during electrophoresis. Possible gel loss through flotation was prevented by placing a
5-mm-diameter porous polyethylene disk edgewise into the top of each gel tube after sample application
but before buffer overlay. The gel sample buffer was also the same,17 containing 2% SDS, 8-M urea,
2-mM acid EDTA, and 0.05-M TRIS-HCl, pH 8.0.

Samples of protein to be used for SDS gel electrophoresis were prepared either by mixing the sample
directly with an equal volume of denaturing gel sample buffer or by precipitating the protein first with
15% PEG 4000 and then dissolving the precipitate in gel sample buffer. The latter procedure was used
with low protein concentrations. The SDS-treated samples were heated at 65°C for 15 min, and 30 µl
aliquots containing 25–50 µg of protein were applied to each gel. Electrophoresis was performed at 75 V
for 0.5 hr and then at 120 V for 1.5 hr. Staining was carried out by incubating the gels overnight in a
solution of 35 mg of Coomassie blue R-250/liter, 10% isopropanol, and 10% acetic acid. The gels were
destained in a solution of 7.5% acetic acid and 5% methanol.

Second-Dimension SDS Gel Electrophoresis

Slab gels (3% polyacrylamide-0.5% agarose) 3 mm thick were made as described previously,17 but
were formed as 11 X 14.5-cm sections to fit on the horizontal MRA slab gel bed. When in place on the
MRA apparatus, the top glass plate covering the gel was removed, and a sample well 0.5 X 9.5 cm was
cut into the slab 2 cm from the cathode wick to hold the cylindrical gel for the second-dimension
electrophoresis separation. A small quantity of 0.5% agarose was used to seal the bottom of the well to
prevent leakage of reducing sample buffer. Additional sample wells 1 X 3 mm were cut on either side of
the large well to hold protein used for M4 markers.

Protein electrophoresed on the 1.5% polyacrylamide-0.5% agarose gel was reduced for second-
dimension analysis by incubating the gel in 6.5 ml of sample buffer (completely surrounding the gel),
containing 1% dithiothreitol, at 37°C for 20 min. The sample buffer was removed, and the tube
containing the gel was flushed with nitrogen, sealed with a stopper, and incubated overnight at 22°C.
Second-dimension gel electrophoresis was carried out at constant voltage: 75 V for the first 0.5 hr and
140 V for the remaining 3.5 hr. Reduced Clg, myosin, and fibrinogen Aα, Bβ, and γ chains were used as
M4 markers of M4 = 2.2 X 105,22 2 X 105,23 6.8 X 104, 5.6 X 104, and 4.8 X 104,24 respectively. Staining
with Coomassie blue and destaining were performed as with the first-dimension gels.

RESULTS

Agarose Gel Chromatography of Cryoprecipitate and Concentrate

VIII:C and VIIIIR:WF activities were isolated in the void volume peak of the
A-15m agarose gel column to remove most of the non-VIII contaminants (Fig. 1). The ratio of VIII:C to VIIIIR:WF was greater in all of the concentrate samples than
in the cryoprecipitate samples. The optical absorbance of the cryoprecipitate void
volume peak was much higher than that from samples of concentrate, reflecting the
presence of non-VIII-related proteins and chylomicrons. The same regions of
column eluate for both chromatographed cryoprecipitate and concentrate were
concentrated and used for further experiments. It should be noted that in order to
avoid procedures that would alter the polymeric structure or molecular weight
distribution of VIIIIR:WF multimers, the samples were treated initially and
chromatographed in the presence of proteolytic inhibitors. Methods such as
extraction of cryoprecipitate with low-ionic-strength buffer and PEG fractiona-
tion25 were not used, since they could lead to enrichment of lower-molecular-weight
multimers.18

Crossed Immunoelectrophoresis

VIIIIR:Ag in cryoprecipitate was compared with that in concentrate by crossed
immunoelectrophoresis (Fig. 2). The VIIIIR:Ag immunoprecipitin curve obtained
from concentrate was asymmetric, with its peak shifted slightly toward the anode, as compared with the symmetric curve of VIIIIR:Ag from cryoprecipitate. Assuming, in this case, that migration distance is primarily a function of size rather than charge, one can infer from this shift that there is a greater percentage of smaller, faster-migrating VIIIIR:WF multimers in concentrate than in cryoprecipitate.

Fig. 2. Crossed immunoelectrophoresis of VIIIIR:Ag contained in the void volume peak of chromatographed AHF concentrate and cryoprecipitate. The arrows and broken line indicate the more anodal peak position and asymmetry of the concentrate VIIIIR:Ag precipitin line, as compared with that from cryoprecipitate.
First-Dimension SDS Gel Electrophoresis

Samples of the void volume fraction from chromatographed cryoprecipitate and concentrate were electrophoresed on 1.5% polyacrylamide–0.5% agarose SDS gels (Fig. 3). Samples from both sources contained, in addition to VIIIIR:WF protein, IgM, Clg, fibrinogen monomer and dimer, and unidentified protein(s) with a monomer form having approximately the same mobility as fibrinogen dimer. Clg had an apparent $M_r = 8.5 \times 10^5$ on these gels, twice the molecular weight determined by sedimentation equilibrium. The cause of this anomaly is not known. The relative concentrations of void volume proteins differed among individual preparations, but samples of cryoprecipitate generally contained higher levels of material unrelated to VIIIIR:WF protein than did samples of concentrate.

In all samples two major characteristics distinguished cryoprecipitate VIIIIR:WF multimers from those found in concentrate. Cryoprecipitate VIIIIR:WF protein appeared to be a multimer with polymer molecular weight increasing in a uniform or homologous manner (Fig. 3A). Using cross-linked fibrinogen and cross-linked IgM as molecular-weight markers, and assuming that the VIIIIR:WF multimers migrate similarly, we estimated the molecular-weight difference between multimers to be $6.5 \times 10^5 \pm 0.7 \times 10^5$ (SD), or two to three $2.4 \times 10^5$ subunits per increment. The molecular weight of VIIIIR:WF multimers from concentrate increased in a nonuniform or heterogeneous manner. Migration distances between polymers equivalent to $M_r = 2.5 \times 10^5 \pm 0.5 \times 10^5$ alternated with spaces analogous to $M_r = 5 \times 10^5 \pm 0.5 \times 10^5$. The concentrate VIIIIR:WF multimer thus appeared to be composed of a repeating “doublet.” On heavily loaded gels an additional band could be discerned between doublets. Adsorption of Clg with gelatin-Sepharose from samples of both cryoprecipitate and concentrate did not change the VIIIIR:WF multimer pattern.

The second major difference between VIIIIR:WF multimers from the two sources was in their range of molecular weights. Approximately 85% of the multimers from all samples of concentrate from the various manufacturers were between
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M_r = 1.4 \times 10^6 and 5 \times 10^6, whereas 75\% of the cryoprecipitate multimers were above M_r = 5 \times 10^6 (Fig. 3B).

Second-Dimension SDS Gel Electrophoresis

Void volume protein obtained from chromatographed AHF concentrate and cryoprecipitate was examined further by second-dimension SDS gel electrophoresis. This procedure involved incubating the first-dimension gel in dithiothreitol to reduce proteins to their constituent monomeric subunits (Figs. 4 and 5).

All VIIIIR:WF multimers from cryoprecipitate (Fig. 4) were reduced to monomeric subunits of M_r = 2.4 \times 10^5. The subunits of other proteins detected by this procedure included the heavy chain of IgM, the subunits of Clg and \(\alpha_2\)-macroglobulin, and the \(\alpha\alpha\), B\(\beta\), and \(\gamma\) chains of fibrinogen. The unidentified protein(s) appeared to be a multimer with a major protomer of M_r = 5 \times 10^6.

The protein contained in the void volume of chromatographed concentrate was similarly examined by second-dimension SDS gel electrophoresis (Fig. 5). Trace components with M_r = 1.4 \times 10^5 were observed in the second-dimension gel under the 2.4 \times 10^5 subunits of VIIIIR:WF. This material is probably a degraded form of the M_r = 2.4 \times 10^5 subunit, since it is expected that a noncovalently bound contaminant of this molecular weight would be separated from the M_r > 10^6 multimers on the first-dimension gel. Other than this trace component, however, there was no difference between the reduced VIIIIR:WF multimers from cryoprecipitate and concentrate, despite the great differences in average molecular weights and polymer migrations of the unreduced proteins.

**Fig. 4.** Second-dimension SDS gel electrophoresis of void volume proteins obtained by chromatography of cryoprecipitate on agarose A-15m before (first-dimension) and after (second-dimension) reduction with dithiothreitol. The arrows indicate (1) the M_r = 2.4 \times 10^5 subunit of VIIIIR:WF protein, (2) an unidentified protein and its multimers, (3) the heavy chain of IgM, (4) a subunit of Clg, (5) an \(\alpha_2\)-macroglobulin subunit, and (6) the polypeptides of fibrinogen.
FIRST DIMENSION (non-reduced)

SECOND DIMENSION (reduced)

Fig. 5. Second-dimension SDS gel electrophoresis of void volume proteins obtained by chromatography of AHF concentrate on A-15m agarose. The arrows indicate (1) the M_2.4 \times 10^6 subunit of VIIIIR:WF protein, (2) trace components (not clearly seen in this photograph) of M = 1.4 \times 10^4, (3) a CIg subunit, (4) an unidentified protein, (5) the heavy chain of IgM, and (6) and (7) the polypeptides of fibrinogen dimer and monomer, respectively.

Comparison of VIIIIR:WF Activities Obtained From Cryoprecipitate and Concentrate

The ratio of VIIIIR:WF activity to VIIIIR:WF protein was also considered (Table 1). The samples of cryoprecipitate and concentrate compared here had 22 VIIIIR:Ag U/ml and 86 VIIIIR:Ag U/ml, respectively. Two-stage SDS gel electrophoresis was also performed to determine the relative amounts of M_2.4 \times 10^6 VIIIIR:WF subunit present in each sample. Conceivably, for an equal amount of subunit, the smaller, faster-moving VIIIIR:WF multimers of concentrate could give disproportionately higher antigen levels than VIIIIR:WF from cryoprecipitate. However, the relative concentration of subunit was in proportion to the amount expected by the immunologic technique. The ratios of VIIIIR:WF activity to VIIIIR:Ag were 0.28 for concentrate and 0.72 for cryoprecipitate. Thus, for the particular samples examined, VIIIIR:WF protein from cryoprecipitate was 2.6

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<th>VIIIIR:WF (U/ml)</th>
<th>VIIIIR:Ag (U/ml)</th>
<th>VIII Subunit (arbitrary units)*</th>
<th>VIIIIR:WF/VIIIIR:Ag</th>
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<tr>
<td>Concentrate</td>
<td>24 ± 2 (SD)(n = 5)</td>
<td>86 ± 7 (SD)(n = 7)</td>
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<tr>
<td>Cryoprecipitate</td>
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<td>22 ± 2 (SD)(n = 7)</td>
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<td>0.72</td>
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*Serial dilutions of cryoprecipitate and concentrate samples were used to obtain the linear relationship between subunit peak area and sample dilution. The slope of the line derived from concentrate dilutions was four times greater than that obtained from cryoprecipitate.
times more effective as a platelet aggregating agent in the presence of ristocetin than VIIIR:WF protein from concentrate.

DISCUSSION

The polymeric nature of VIIIR:WF protein reported here is consistent with the observations of other investigators who have used techniques such as crossed immunoelectrophoresis, agarose gel electrophoresis, and SDS gel electrophoresis to detect structural heterogeneity. It is also in agreement with the electron microscopy studies of Marder et al., who observed purified VIIIR:WF protein to consist of various sizes. However, this Mr heterogeneity was not confirmed by the ultracentrifugation Mr analysis of Legaz et al. These investigators employed 6-M guanidine HCl as a disaggregating agent and determined that VIIIR:WF was a single protein of Mr = 1.2 X 10^6. It is possible that only one polymeric form of VIIIR:WF protein was isolated by the techniques used by these investigators or that the protein aggregates are not held together by covalent bonds and disaggregate in the presence of guanidine, but not with 1% SDS–8-M urea. Even if the latter is tenable, given the results presented here and elsewhere, VIIIR:WF protein most likely exists as a multimer under normal ionic strength conditions.

VIIIR:WF multimers from cryoprecipitate have, on the average, high molecular weights and are composed of a uniform repeating unit, in contrast to the lower-molecular-weight doublets of VIIIR:WF protein from concentrate. The regular spacings between the multimer bands of cryoprecipitate VIIIR:WF protein are equivalent in this SDS gel system to Mr = 6.5 X 10^5 ± 0.7 X 10^5. Our estimate of molecular weight is based on the migration of the unreduced multimer in 1.5% polyacrylamide–0.5% agarose gels compared with the migration of cross-linked fibrinogen and IgM. The value of two subunits per increment would be in accord with the evidence reported by Counts et al., who employed a 2% polyacrylamide–0.5% agarose gel in their studies. By comparing the migration distance of partially reduced multimers to that of multimers formed from synthetically cross-linked, fully reduced and alkylated subunits, these investigators determined that the subunit dimer was the basic repeating unit. Only even-numbered multimers were observed in the former series, whereas both even and odd were present in the latter series.

The smaller heterogeneous multimers of VIIIR:WF from concentrate are in part the product of selective enrichment of the lower-molecular-weight forms during the commercial purification process. This enrichment may take place at the stage of extracting cryoprecipitate with a low-ionic-strength buffer, which is consistent with the higher solubility of lower-molecular-weight forms of VIIIR:WF protein. The doublet pattern is presumably the result of proteolysis, as suggested by the trace component with Mr = 1.4 X 10^5. However, nondegradative processes may also be responsible. For example, intact disulfide bonds could preserve a linear, globular, or mixed assembly of subunits each type having a different migration rate for equivalent Mr. The round, rod-shaped, and hook-shaped structures reported by Tan and Andersen in their electron microscopic study of VIIIR:WF protein may be the physical causes for these heterogeneities.

Whatever the cause of differences between VIIIR:WF protein from cryoprecipitate and concentrate, the difficulty in detecting these differences emphasizes the
caution that must be exercised when comparing the physical properties attributed to VIIIIR:WF protein obtained by different laboratories. The purity of VIIIIR:WF protein has frequently been assessed by examining the protein in its intact and reduced states on 4% or 5% polyacrylamide gels. Polymeric heterogeneity of the intact protein could not be detected on such highly cross-linked gels, and on reduction VIIIIR:WF protein from both sources would appear to have identical $M_r = 2.4 \times 10^5$ subunits. Similarly, conventional crossed immunoelectrophoresis does not have the resolving power to distinguish between heterogeneous and homogeneous polymers.

The high-molecular-weight population of VIIIIR:WF protein in cryoprecipitate and the greater ability of cryoprecipitate to aggregate platelets in the presence of ristocetin, as compared with VIIIIR:WF protein in concentrate, are in agreement with the observations of several other groups. Koutts and Zimmerman have reported that only the larger forms of human VIIIIR:Ag bind to human platelet membrane preparations. It is most likely that electrophoretically fast migrating VIIIIR:Ag found by crossed immunoelectrophoresis in some variants of von Willebrand’s disease is the result of the smaller size of the VIIIIR:WF multimer population, analogous to the immunoelectrophoresis results reported here.

Similarly, the ability of cryoprecipitate, in contrast to concentrate, to stop the prolonged bleeding of von Willebrand’s patients may be related to the size differential of VIIIIR:WF protein from the two sources. VIIIIR:WF activity as measured by aggregation of platelets in the presence of ristocetin appeared to be separable from the activity responsible for correcting prolonged bleeding. Possibly a slightly lower molecular-weight range of multimers or a lower concentration of higher-molecular-weight multimers is sufficient for the ristocetin reaction to occur but not for the events yielding in vivo cessation of bleeding. However, our work and that of others suggest that the closer to unity the ratio of VIIIIR:Ag to in vitro VIIIIR:WF activity in a given factor VIII preparation, the better the in vivo activity of the preparation.

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REFERENCES

7. Sultan Y, Simeon J, Caen JP: Electrophoretic heterogeneity of normal factor VIII/von Willebrand protein, and abnormal electrophoretic...
Comparison of factor VIII-related von Willebrand factor proteins prepared from human cryoprecipitate and factor VIII concentrate

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