SUBUNIT STRUCTURE AND ASSEMBLY OF VON WILLEBRAND FACTOR POLYMER

Complementary Analysis by Electron Microscopy and Quasielastic Light Scattering

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Von Willebrand factor (vWF) is a polymeric plasma glycoprotein (1, 2) that promotes platelet adhesion at sites of vascular injury (3). In plasma, vWF polymers of varying length are assembled from 230,000-d monomers and are maintained in the polymeric state by disulfide bonds (4). Biologic activity, defined in vitro as the ability of vWF to agglutinate platelets in the presence of the cationic polypeptide antibiotic, ristocetin, is directly related to multimer size and to the presence of disulfide bonds (5). We assessed the size and shape of vWF multimers by electron microscopy (EM) and by quasielastic light scattering (QLS) analysis of vWF in solution. In addition, we used QLS techniques to demonstrate the noncovalent assembly of vWF protomers, and to derive the dissociation constant for this process. Biologic activity was then correlated with the size and shape parameters defined from this analysis.

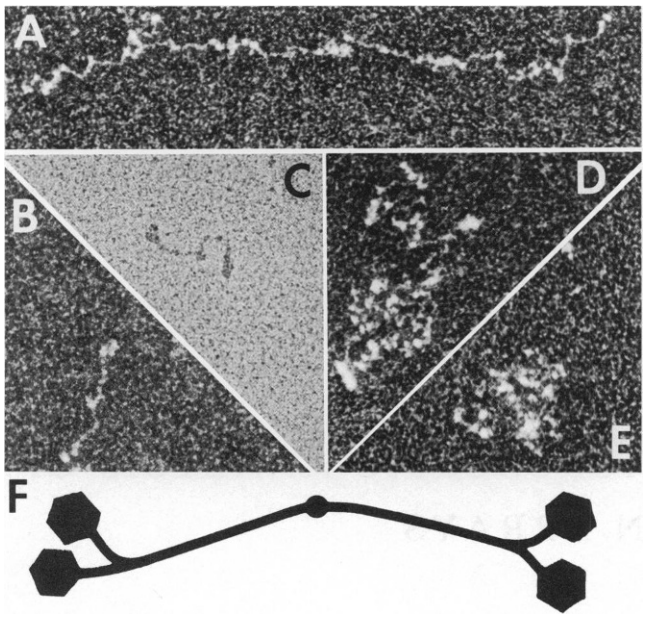

FIGURE 1 Electron micrographs of vWF. An extended multimer of native vWF is demonstrated in (*a*), protomers are shown in (*b*) and (*c*); coiled multimers are depicted in (*d*) and (*e*). Specimens were metal coated with 8×10^{-7} gm/cm^2 of tungsten and photographed either in brightfield (*c*) with a magnification of 169,600 × or in darkfield (*a, b, d, e*) with a magnification of 128,000 ×. A schematic model of the protomer is shown in (*f*).

RESULTS AND DISCUSSION

By high-resolution light and darkfield EM (Fig. 1), vWF was found to be a flexible, linear polymer ranging in contour length from 100 to 1,300 nm. In any given EM field, ~85% of the polymers were coiled upon themselves with maximal diameters ranging from 60 to 200 nm and a calculated mean radius of gyration, $\overline{R}_g$, of 99 ± 56 nm. Repeating protomers were discernible in the uncoiled polymers which, themselves, were filamentous and measured 100 nm × 1.5–2.0 nm. These protomers contained a coiled central globular region and two somewhat larger globular heads on each end of the filamentous backbone.

Analysis of the same vWF sample by quasielastic light scattering (QLS) revealed a similar $\overline{R}_g$, 83 ± 37 nm, and a mean Stoke's radius, $\overline{R}_h$, of 59 ± 25 nm (Table I). These measurements and the ratio, $\overline{R}_g/\overline{R}_h = 1.4$, indicate that in solution, as well, the average vWF polymer is not fully extended but, rather, assumes a shape intermediate between that of a rigid rod and an oblate ellipsoid of revolution (Table II).

At physiologic concentrations of vWF (~20 μg/ml), disulfide bond reduction with 2-mercaptoethanol reduced average polymer size ($\overline{R}_g$ and $\overline{R}_h$), while at higher concentrations (~100 μg/ml), no change in size was detectable by QLS. Changes in temperature and vWF concentration produced reversible changes in $\overline{R}_h$, thereby permitting calculation of the dissociation constant for protomer-polymer assembly. The data fit best a model that assumes the basic protomer to be a dimer of 230,000-d monomers associating end-to-end in a noncooperative manner (6). With these assumptions, the K_D for protomer-polymer

TABLE I
VON WILLEBRAND FACTOR SIZE AND SHAPE PARAMETERS

Method	$\overline{R}_g$ (nm)	$\overline{R}_h$ (nm)	$\overline{R}_g/\overline{R}_h$
QLS*	83 ± 37	59 ± 37	1.4
EM	99 ± 56		

*Measurements were performed at 28° in 10 mM Tris, pH 7.8, 0.15 M NaCl using an assumed solvent viscosity (equivalent to water) of 0.8327 centipoise.

TABLE II
DEPENDENCE OF R_g/R_h ON SHAPE

Shape	R_g/R_h
Sphere	0.77
Oblate Ellipsoid	0.94
Rod	1.87

assembly was 0.77 μg/ml at 5°C, 2.4 μg/ml at 25°C, and 7.7 μg/ml at 37°C.

Biologic activity, as measured by ristocetin-dependent platelet agglutination using nephelometry (7), was found to depend directly on polymer size (Fig. 2), with both $\overline{R}_g$ and $\overline{R}_h$ varying similarly with activity. The ratio of the slopes of these lines is one, suggesting that the overall solution shape of vWF does not vary with changes in multimer length.

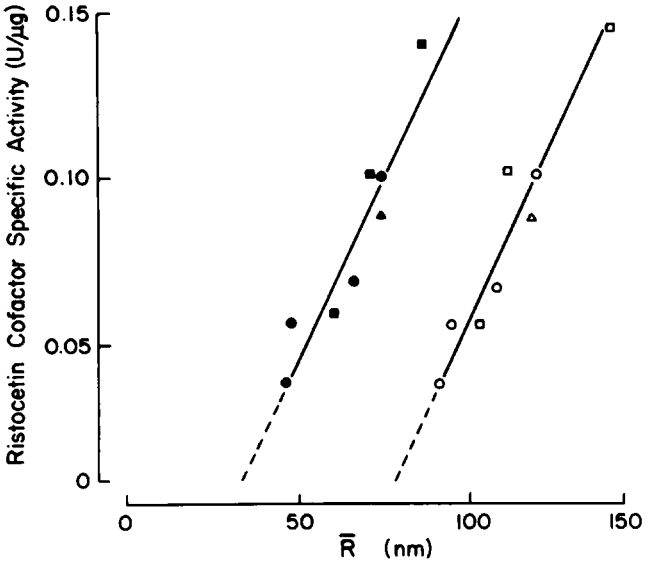

FIGURE 2 Ristocetin-dependent platelet-agglutinating activity of vWF as a function of solution radii. Fractions of three different preparations (closed symbols) of vWF obtained from Sephacryl S-1000 column chromatography (Sephadex), used to purify vWF from a cryo-precipitate of plasma and to fractionate its multimer distribution (8), were tested for biologic activity (i.e., platelet agglutination); the same fractions were analyzed by QLS. Activity (one unit defined as that found in 1 ml of normal human plasma) is plotted as a function of $\overline{R}_g$ (open symbols) and of $\overline{R}_h$ (closed symbols).

These data show that: (*a*) vWF is a flexible, linear polymer composed of repeating protomers; (*b*) the flexible, coiled configuration of the polymer noted by EM exists in solution as well; (*c*) disulfide bonds maintain polymer size at physiologic concentrations, but noncovalent forces support protomer association at higher concentrations and are possibly important for intracellular assembly; and (*d*) size and shape are directly correlated with biologic activity.

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QUANTITATIVE ELECTRON MICROSCOPIC ANALYSIS OF MICROCRYSTALLINE PROTEIN ARRAYS

A Few Precautionary Notes

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Electron microscopic studies of macromolecular microcrystals (1) have shown that, despite the 7 Å-resolution limit imposed by radiation damage for unstained materials, hope remains to use the 3 Å data (for example) found in electron diffraction patterns. While initial image studies utilize negatively stained preparations (~20 Å resolution)

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