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Research Article

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Properties of Human Asialo-Factor VIII

A RISTOCETIN-INDEPENDENT PLATELET-AGGREGATING AGENT

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ABSTRACT Human Factor VIII desialylated by treatment with *Vibrio cholerae* neuraminidase (ASVIII) aggregates human platelets in the absence of ristocetin in platelet-rich plasma and, to a lesser extent, in washed platelet suspensions. Aggregation is accompanied by thromboxane formation and is completely inhibited by EDTA. Aspirin blocks the second phase of aggregation and abolishes thromboxane production. Subaggregating doses of ASVIII and of either ADP, epinephrine, or collagen produce prompt and complete platelet aggregation. Bernard-Soulier syndrome platelets either did not aggregate with ASVIII (two cases) or showed markedly decreased aggregation (one case). Factor VIII complex was prepared from the plasma of two patients with variant von Willebrand's disease (sialic acid content 142 and 75 nmol/mg, respectively); neither protein generated platelet-aggregating activity upon desialylation. [^{3}H]ASVIII binds rapidly to platelets at 37°C, while tritiated, fully sialylated factor VIII binds to a negligible extent. As little as 1–2 µg ASVIII bound/10^9 platelets is capable of inducing platelet aggregation. ASVIII may be a useful tool for investigating platelet-Factor VIII interactions in the absence of ristocetin. Furthermore, desialylated Factor VIII might play a physiologic role in Factor VIII-mediated platelet reactions in vivo.

INTRODUCTION

In addition to its importance in the coagulation mechanism the Factor VIII complex (FVIII)[1] is essential to

[1] *Abbreviations used in this paper*: ASVIII, desialylated Factor VIII complex; FVIII, Factor VIII complex; [^{3}H]ASVIII, tritiated ASVIII; [^{3}H]FVIII, tritiated FVIII; PRP, platelet-rich plasma; VIII:Ag, FVIII-related antigen; VIII:C, FVIII coagulant activity; VIII:Rcf, FVIII-related ristocetin cofactor activity: vWD, von Willebrand's disease; WP, fresh water platelets.

the interaction of platelets with the vessel wall (1). The latter role was initially suggested by the observation of a prolonged bleeding time in patients with severe von Willebrand's disease (vWD), and by the correction of the bleeding time in such patients after infusion of some FVIII-rich plasma fractions (2). Furthermore, histological studies of biopsies of bleeding time sites in vWD patients showed an absence of platelet adhesion to vessel walls, which could be normalized by prior infusion of plasma (3, 4). Finally, adhesion of platelets to de-endothelialized rabbit (5) or human (6) arterial tissue is dependent on the presence of FVIII in the tissues or in the circulating plasma.

A great deal of investigation has been directed toward an understanding of the mechanism of platelet aggregation induced by the antibiotic ristocetin (1, 2, 7, 8), which depends upon the presence of FVIII-related ristocetin cofactor activity (VIII:Rcf). In some patients with vWD, however, there is a lack of correlation between the bleeding time and VIII:Rcf levels (9, 10). Furthermore, infusion of some VIII:Rcf-rich concentrates does not correct the prolonged bleeding time in patients with vWD despite normalization of plasma VIII:Rcf levels (11). These observations suggest that the mechanism of Factor VIII-dependent ristocetin-induced platelet aggregation may differ from the mechanism of Factor VIII-dependent platelet-vessel wall interaction.

Little attention has been paid to the observation that desialylated human FVIII is capable of aggregating platelets directly (12–14). Because desialylation of Factor VIII represents a possible mechanism for Factor VIII-mediated platelet-vessel wall interactions independent of substances such as ristocetin, we have studied some of the properties of desialylated Factor VIII as a platelet aggregating agent.

METHODS

Purification of proteins. Human cryoprecipitate was either prepared as described by Pool et al. (15), or was obtained from the American National Red Cross, Bethesda, Md. (we thank Dr. Doris Menaché-Aronson for making this ma-

terial available). Batches of 18–35 cryoprecipitates were re-cryoprecipitated and resuspended at 20°C in 200–300 ml of a buffer consisting of 0.02 M Tris, 0.02 M sodium citrate, 0.02 M ε-aminocaproic acid (EACA), pH 7.0, and further purified essentially by the method of Newman et al. (16), as modified by Switzer and McKee (17). Gel-filtration was performed on a 5 × 80-cm column of Sepharose 6B (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) equilibrated with a buffer consisting of 0.02 M imidazole, 0.15 M NaCl, 0.01 M sodium citrate, 0.02 M EACA, pH 6.5. The void volume peak containing FVIII was concentrated by ultracentrifugation for 3.5 h at 220,000 g, as originally suggested by Thelin and Wagner (18), and stored at 4°C at concentrations of 0.8–2.2 mg/ml. FVIII from two patients with variant vWD was purified by cryoprecipitation in the presence of ethanol (16), which was followed by the steps previously outlined, with the following modifications: the bentonite absorption step was omitted, and a narrower (2.5 × 70 cm) Sepharose 6B column was used. For experiments utilizing this material, normal FVIII was prepared in an identical fashion.

Fibrinogen was purified from citrated human plasma by the glycine precipitation method of Kazal et al. (19). Galactose oxidase (Worthington Biochemical Corp., Freehold, N. J.) was freed of proteolytic activity by chromatography on DEAE cellulose (DE 52, Whatman Ltd., Springfield Mill, Kent, England) according to Morell et al. (20), or by gel filtration on Sepharose 6B according to Hatton and Regoeczi (21). No proteolysis of FVIII or fibrinogen could be detected after 3 h exposure to the repurified enzyme at 37°C, when measured by loss of FVIII coagulant activity (VIII:C) or by a change in reduced SDS gel electrophoretic patterns.

Desialylation of Factor VIII. The purified FVIII solution was dialyzed overnight against 0.15 M NaCl, made 0.008 M in CaCl₂ by addition of a 0.1 M solution, and adjusted, if necessary, to pH 5.9 with 0.005 M HCl. Desialylation was carried out with protease-free *Vibrio cholerae* neuraminidase (Cal-biochem-Behring Corp., American Hoechst, San Diego, Calif.) using 30 U/mg FVIII. Mixtures were incubated 3 h at 37°C, at which time desialylation was complete. Controls were run omitting only the neuraminidase. Both the sample and control were passed through an antineuraminidase antibody affinity column (kindly supplied by Dr. Jose Martinez, Cardeza Foundation, Jefferson Medical College) and then dialyzed overnight at 4°C against several changes of a buffer containing 0.02 M Tris, 0.15 M NaCl, pH 7.0. Aliquots were assayed for VIII:C and VIII:Rcf and stored at 4°C. Under these conditions, VIII:C and VIII:Rcf were stable for at least 3 d. Desialylated human fibrinogen and prothrombin were prepared in an analogous manner. SDS polyacrylamide gel electrophoresis was performed on 5% gels according to the method of Weber and Osborn (22). Sialic acid content was measured, either after acid hydrolysis (pH 1.1–1.2) for 1 h at 80°C, or after neuraminidase treatment by the thiobarbituric acid method of Warren (23). Protein measurement was done by the method of Lowry et al. (24).

Platelet aggregation, thromboxane measurement, and coagulation assays. Blood was collected into 1/9 vol of 3.2% sodium citrate and centrifuged at 230 g for 10 min at room temperature to obtain platelet-rich plasma (PRP). Fresh washed platelets (WP) were prepared as described by Walsh (25) and resuspended in Ca⁺⁺-free Tyrode's buffer. Formalin-treated platelets were prepared as described by Allain et al. (26), and resuspended in Tyrode's buffer free of both Mg⁺⁺ and Ca⁺⁺. Platelet aggregation studies were carried out at a final platelet concentration of 3–4 × 10⁸ platelets/ml in a Payton dual channel aggregometer (Payton Associates, Inc., Buffalo, N. Y.) with 0.4–0.5 ml PRP or WP. ADP was obtained from the Sigma Chemical Co. (St. Louis, Mo.), epineph-

rine from Elkins-Sinn, Inc. (Cherry Hill, N. J.), collagen from Hormon-Chemie (Munich, Germany), and ristocetin from Lenau (Copenhagen, Denmark). When fibrinogen was added to WP, the final concentration was 0.3–0.7 mg/ml. To measure thromboxane B₂, samples of PRP were removed from the aggregrometer cuvette 6 min after addition of the aggregating agent and were centrifuged at 15,000 g for 2 min. Thromboxane B₂ was measured in the supernate as described by Lewy et al. (27). VIII:C, FVIII-related antigen (VIII:Ag), and VIII:Rcf were assayed by standard methods (26, 28–31).

Radiolabeling of Factor VIII. Purified FVIII was tritium labeled at galactose residues, as described by Morell et al. (32), omitting peroxidase. FVIII (1–1.5 mg/ml) was incubated with an approximately equal volume of galactose oxidase (80 U/mg FVIII) for 3 h at 37°C, after which 2–3 mCi [³H]-sodium borohydride (high specific activity, Amersham Corp., Arlington Heights, Ill.) was added per milliliter of reaction mixture and the mixture stirred for 30 min. The sample was passed through a 1.5 × 26-cm Sepharose 6B column equilibrated with 0.02 M Tris, 0.15 M NaCl, pH 7.3. The FVIII was concentrated to ~1 mg/ml either against 20% polyethylene glycol 20,000 (Carbowax, Fisher Scientific Co., Fairlawn, N. J.) in the same buffer or by immersion in dry Sephadex G-50 or G-25. [³H]FVIII was desialylated as described above for intact FVIII.

Tritiated desialylated FVIII ([³H]ASVIII) was treated with *S. pneumoniae* β-galactosidase (a protease-free, semi-purified preparation supplied by Dr. Gilbert Ashwell, National Institutes of Health), as described (33, 34). A control with no enzyme was incubated in parallel. TCA-soluble radioactivity was measured at 24, 48, and 72 h. An aliquot of the 72-h enzyme-desialylated FVIII (ASVIII) incubation mixture was gel-filtered on Sephadex G-200.

For binding studies, up to 50 μl of either tritiated FVIII ([³H]FVIII) or [³H]ASVIII was added to 0.5 ml of PRP containing 4 × 10⁸ platelets/ml, while stirring in the aggregometer. 10 μl [¹⁴C]sucrose (New England Nuclear, Boston, Mass.) was included with the PRP when initially prepared in order to correct for plasma trapped in the platelet pellet. The time-course of [³H]ASVIII binding to platelets was studied at a final [³H]ASVIII concentration of 50 μg/ml. Binding as a function of [³H]ASVIII concentration was measured in the range of 5–88 μg/ml. For these studies [³H]ASVIII was added to PRP and stirring was continued for 6 min, after which the samples were centrifuged in an Eppendorf microfuge at 15,000 g for 2 min, and the supernates rapidly removed. The tubes were inverted and allowed to drain, and the pellet was removed with a cotton swab. The pellets and 100 μl of the supernate were oxidized in a Packard Tricarb 306 oxidizer (Packard Instrument Co., Inc., Downers Grove, Ill.) and [³H]-H₂O and [¹⁴C]O₂ were determined by liquid scintillation counting (Packard Tricarb scintillation spectrometer). Platelet-bound ASVIII, expressed as microgram per 10⁹ platelets, was derived from the percentage of tritium bound, and corrected for the interstitial medium. To check for specificity of binding to platelets, [³H]FVIII and [³H]ASVIII, at a final concentration of 21.5 μg/ml, were incubated for 0.5 h at 37°C with PRP and with erythrocytes or lymphocytes resuspended in platelet-poor plasma. Stirring was omitted, so that platelet aggregation would not be a variable. Erythrocytes were washed three times in a buffer containing 0.02 M Tris, 0.15 M NaCl, pH 7.3, and resuspended at a concentration of 2 × 10⁸ cells/ml in platelet-poor plasma. Lymphocytes were separated by Ficoll-Hypaque centrifugation and plastic adherence (35), and resuspended at a concentration of 6 × 10⁶ cells/ml in platelet-poor plasma.

Patients. PRP was obtained from three patients with the Bernard-Soulier syndrome (36). In all three patients platelet

aggregation to ristocetin was absent and was not corrected by the addition of cryoprecipitate. Two patients have a severe bleeding disorder, while the third bleeds primarily after trauma or surgical procedures. Clinical and laboratory descriptions of these patients have been published (37, 38).

FVIII was prepared from the plasmas of two patients with a variant form of vWD, desialylated as described above, and tested for aggregating activity in normal PRP. Both patients have spontaneous epistaxes and skin bruising; one patient (G.W.) bled after tooth extraction while the second patient (W.K.) has not had any surgery or extractions.

RESULTS

Purification and desialylation of FVIII. FVIII, before reduction, did not enter 5% SDS-polyacrylamide gels. After reduction, a single band was seen at a protein load of 20–40 μg/gel. FVIII did not react on immuno-diffusion in 1% agarose against antisera to fibrinogen, fibronectin, α_2-macroglobulin, or β-lipoprotein, but showed a single line against antiserum to human FVIII. Mean specific activities of VIII:C, VIII:Ag, and VIII:Rcf in these preparations were 36, 107, and 123 U/mg protein, respectively, and the sialic acid content was 131 ± 16 nmol/mg protein (Table I). In Table I the activity of control FVIII, exposed to the desialylating conditions but not to neuraminidase, has been defined as 100%. Although small losses (5–20%) of VIII:C occasionally occurred in the control FVIII preparation, presumably related to the acid pH at which the reaction is carried out, desialylation per se produced little or no loss of VIII:C. No loss of VIII:Rcf was detected in fresh preparations of either control or ASVIII, whether the assay was done with formalinized platelets in a test tube or in an aggregometer, or with freshly washed platelets in an aggregometer.

General aggregating properties of ASVIII. ASVIII produces aggregation of platelets in citrated PRP (Fig. 1a). Asialofibrinogen and asialoprothrombin (data not shown) neither aggregate PRP nor interfere with ASVIII-induced aggregation of PRP. ASVIII-induced platelet aggregation in PRP is accompanied by thromboxane formation (46 pmol/ml). In the presence of aspirin, only primary aggregation is seen and thromboxane formation is inhibited (0.72 pmol/ml). WP do

TABLE I
Effect of Neuraminidase Treatment on Human FVIII

	Normal FVIII*	ASVIII
	%	%
VIII:C (n = 6)	100	93.5 ± 14.8
VIII:Rcf (n = 6)	100	117 ± 12
Sialic acid (n = 8)	100‡	6.9 ± 7.4

* Normal FVIII was exposed to desialylating conditions in the absence of neuraminidase.

‡ Normal FVIII sialic acid content 131 ± 16 nmol/mg protein.
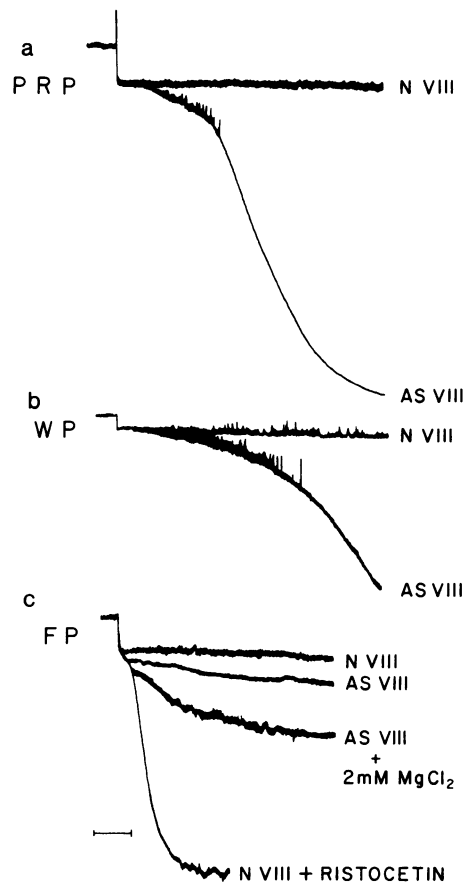

FIGURE 1 Effect of ASVIII on platelet aggregation. ASVIII was used at final concentrations of 50–60 μg/ml. Platelet aggregation was performed at 37°C on PRP; WP and formalinized platelets were prepared as described in Methods. The horizontal bar denotes 1 min elapsed. N VIII, normal, fully sialylated FVIII.

not respond to ASVIII as promptly or completely as do platelets in PRP (Fig. 1a vs. b); occasional preparations of WP aggregate poorly, if at all. Aggregation of WP is not enhanced by the addition of fibrinogen, but is restored toward normal when WP are resuspended in platelet-poor plasma. Aggregation of PRP and WP is inhibited by sodium EDTA at concentrations >2 mM. ASVIII produces little or no macroscopic agglutination of formalinized platelets (Fig. 1c), although small aggregates can be seen under the microscope. ASVIII agglutination of formalinized platelets is enhanced, but only slightly, by addition to the system of 2 mM MgCl$_2$ (Fig. 1c) or 2 mM CaCl$_2$ (not shown).

In different preparations, a minimum final concentration of 5–15 μg/ml ASVIII is necessary to produce platelet aggregation in PRP (Fig. 2a). The rapid phase of aggregation is preceded by a lag phase, which is shorter with increasing concentrations of ASVIII; at a concentration of ASVIII >15 μg/ml aggregation is es-
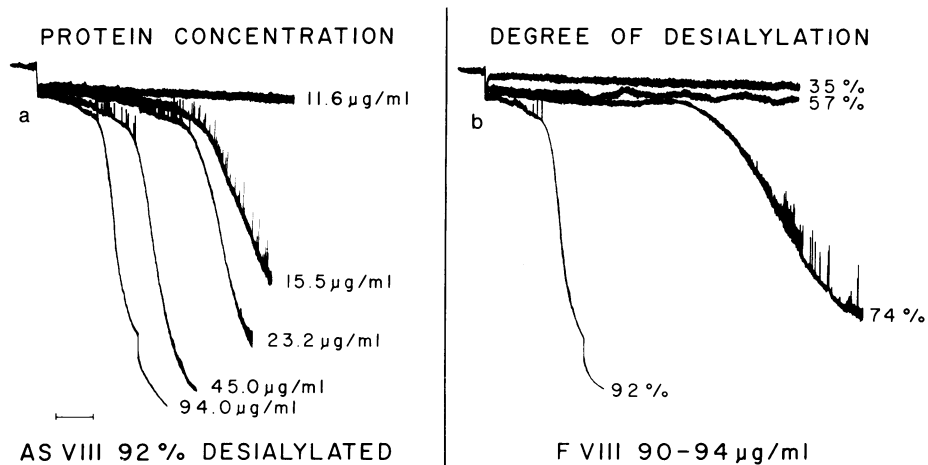
FIGURE 2   Effect of protein concentration of desialylated FVIII (a) and degree of desialylation of FVIII (b) on platelet aggregation. Aggregation was performed in stirred PRP at 37°C, as described in Methods. Final concentrations of ASVIII in the aggregometer cuvette are shown in *a* and percentage of sialic acid removed in *b*. The horizontal bar denotes 1 min elapsed.

sentially complete, regardless of the duration of the lag phase. A minimum of 60–70% desialylation must be achieved to produce a potent aggregating agent (Fig. 2b). Further desialylation causes progressive shortening of the lag phase.

When nonaggregating doses of ASVIII are combined with subaggregating doses of ADP, collagen, or epinephrine, prompt and complete platelet aggregation accurs. No such effect is produced when the same concentration of normal FVIII is added to subaggregating doses of ADP.

Deficient response of Bernard-Soulier syndrome platelets to ASVIII. ASVIII aggregation of PRP from each of three patients with the Bernard-Soulier syndrome was defective or absent (Fig. 3). Platelets from patients I and II, who have severe bleeding tendencies, showed no response to ASVIII. Platelets from patient III, who has only a moderate bleeding disorder, showed a very delayed response to ASVIII, although complete aggregation eventually occurred.

Effect of desialylation of variant vWD Factor VIII. The plasma FVIII activities of the two patients with variant vWD are shown in Table II. In the final Sepharose 6B gel-filtration step of the preparation of patient FVIII void volume, VIII:Ag accounted for only 30% of the total, whereas 85% of the normal control VIII:Ag appeared in the void volume. Void volume fractions from the patients were pooled and concentrated and analyzed for sialic acid content. The FVIII in one patient had a normal sialic acid content, whereas in the second patient the sialic acid content was half normal (Table II). Desialylated patient FVIII did not produce platelet aggregation even at final concentrations of 50–75 µg/ml.

Binding of [³H]ASVIII to platelets. [³H]FVIII contained 0.6–1.5 µCi/mg protein. Addition of tritiated borohydride to normal FVIII not pretreated with galactose oxidase resulted in incorporation of slightly <15% of the counts incorporated into the enzyme-treated material. β-Galactosidase treatment of [³H]ASVIII cleaved 75% of the total radioactivity from the protein. Labeling did not alter VIII:C, VIII:Rcf, or gel electrophoretic characteristics of the protein. The peak of radioactivity in reduced SDS polyacrylamide gels corresponded exactly to the position of the stained FVIII subunit band. [³H]ASVIII had the same characteristics as nonlabeled ASVIII with respect to VIII:C, VIII: Rcf, and gel electrophoretic characteristics. [³H]-ASVIII and cold ASVIII competed on an equimolar
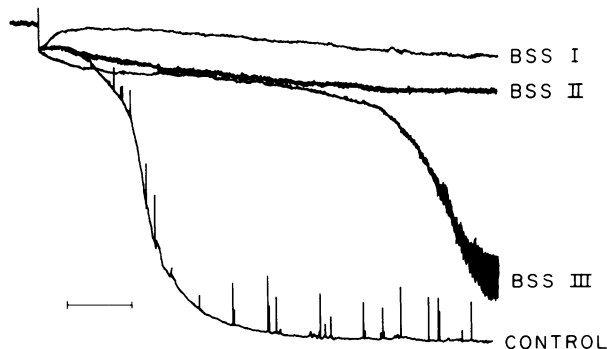
FIGURE 3   Effect of ASVIII on Bernard-Soulier syndrome (BSS) PRP. ASVIII was added to PRP at a final concentration of 50–60 µg/ml, and platelet aggregation was followed as described in Methods. The horizontal bar denotes 1 min elapsed.

basis in binding to platelets (Fig. 4). The specific activity of the [³H]ASVIII was the same as that of [³H]FVIII exposed to the desialylating conditions in the absence of neuraminidase, which indicates that none of the tritium had been incorporated into sialic acid residues.

Binding of [³H]ASVIII to platelets in stirred PRP was complete within 3 min (Fig. 5a). When binding was studied as a function of [³H]ASVIII concentration (Fig. 5b), a rapid increase in amount bound was seen, up to [³H]ASVIII concentrations of 20–25 μg/ml, followed by a slower accumulation of bound [³H]ASVIII, over the range studied. In this series of experiments, all concentrations of [³H]ASVIII produced platelet aggregation, although the lag phase increased with decreasing [³H]ASVIII concentration. Thus, with this preparation, as little as 2 μg [³H]ASVIII bound/10⁹ platelets was capable of inducing aggregation, although with a long (5-min) lag phase. There was little or no binding of [³H]FVIII to platelets in stirred PRP, even when ADP was added to produce platelet aggregation. When lymphocytes or erythrocytes resuspended in platelet-poor plasma were incubated with 22.5 μg/ml [³H]FVIII or [³H]ASVIII, without stirring, <1.5% of added counts bound to the cells. Under the same conditions, <1.5% [³H]FVIII, but 9.0% [³H]ASVIII, bound to platelets. Asialofibrinogen, which does not inhibit ASVIII-induced platelet aggregation, did not block [³H]ASVIII binding to platelets.

DISCUSSION

Vermylen et al. (12–14) observed that desialylated human FVIII aggregated human platelets in PRP, but did not aggregate washed platelets unless fibrinogen was added; aggregation was inhibited by EDTA. The potential significance of ASVIII-platelet interactions has not been explored further. The data reported here demonstrate that ASVIII is a specific and potent platelet-aggregating agent, which apparently requires an intact platelet for full expression of its aggregating potency. The inability of ASVIII to aggregate Bernard-Soulier syndrome platelets, which are deficient in platelet membrane glycoprotein I (36), suggests that
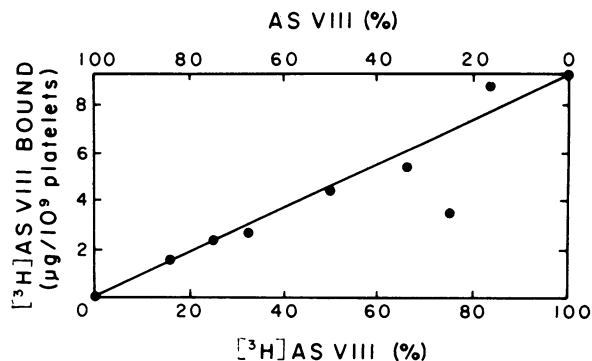

FIGURE 4 Binding of ³H-ASVIII to platelets in stirred PRP. The relative proportion of [³H]ASVIII and unlabeled ASVIII was varied in each of two sets of experiments which have been combined in this figure. Final total concentrations of ASVIII in the two experiments were 25 μg/ml and 50 μg/ml, respectively.

ASVIII, like normal FVIII in the presence of ristocetin (39), interacts with the platelet via the glycoprotein I complex.

The role of the carbohydrate side chains of FVIII in its interaction with platelets has been the subject of intensive investigation (12–14, 34, 40–42). In our hands, desialylation of normal FVIII does not reduce its VIII:Rcf, although further treatment with galactose oxidase substantially diminishes both this activity and direct ASVIII aggregating activity.[2] The reason other investigators have not observed the direct platelet aggregating activity of ASVIII is probably related to their use of WP or formalinized platelets, since in these systems, particularly in the absence of divalent cations, ASVIII does not produce significant aggregation.

The interaction of ASVIII with platelets shows both cellular and protein specificity. ASVIII binds to platelets, but not to lymphocytes or erythrocytes, whereas normal FVIII binds to platelets in only negligible amounts unless ristocetin is added, as has previously been shown by many investigators (8, 42–46). ASVIII prepared from two vWD variants has no platelet aggregating activity, which indicates that removal of negative charge from the FVIII complex is insufficient to explain this activity. In addition, asialofibrinogen, asialoprothrombin, and other asialoproteins, as shown by us and others (14), do not induce platelet aggregation. Finally, asialofibrinogen does not inhibit either ASVIII-induced platelet aggregation or ASVIII binding to platelets.

The precise mechanism whereby ASVIII binds to platelets is not clear. Ashwell and co-workers (20, 33, 47) have described a liver-membrane-associated pro-

TABLE II
FVIII-related Parameters of vWD Variant Patients

	Normal	G.W.	W.K.
Template bleeding time, *min*	2–7	>12	>15
VIII:C, *μ/ml*	0.48–1.5	0.42	0.38
VIII:Ag, *μ/ml*	0.49–2.0	0.70	0.65
VIII:Rcf, *μ/ml*	0.46–2.1	<0.03	<0.03
Sialic acid, *nmol/mg*	131±16	142	75

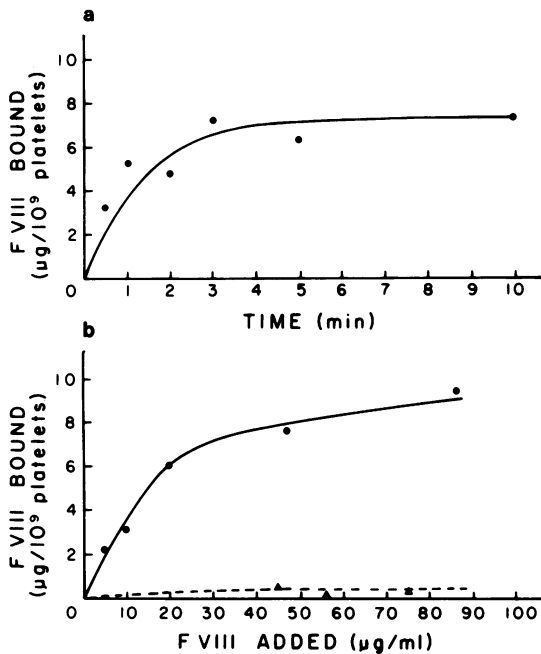[2] De Marco, L., and S. S. Shapiro. Unpublished observations.

FIGURE 5 Binding of [³H]ASVIII to platelets in stirred PRP. See Methods for details. (a) Time dependence of binding at 50 μg/ml final concentration of [³H]ASVIII. (b) Effect of [³H]-ASVIII concentration on amount of ASVIII bound. The abscissa shows final concentrations of [³H]ASVIII. ●, [³H]-ASVIII; ▲, normal [³H]FVIII.

tein capable of binding a number of different desialylated serum glycoproteins; this binding is inhibited by EDTA. Preliminary data indicate that EDTA does not block the binding of ASVIII to platelets, suggesting a mechanism different from that involved in the liver-membrane-associated receptor.

Evidence that abnormalities in the carbohydrate moiety of FVIII are causal in the pathogenesis of vWD is conflicting. Gralnick et al. (48) have reported decreased periodic acid-Schiff staining of FVIII preparations from three patients with variant vWD and substantial amounts of circulating VIII:Ag. Howard et al. (49) have described decreased concanavalin A binding to purified FVIII from an additional patient. Gralnick et al. (50) found the sialic acid content of semipurified FVIII preparations from two patients to be 17.5 and 14.5 nmol/mg protein, whereas the sialic acid content of normal and hemophilic controls was ~40 nmol/mg protein. Zimmerman et al. (51), on the other hand, studied 13 patients with variant vWD, including 1 previously reported by Gralnick and co-workers, using semiquantitative polyacrylamide gel periodic acid-Schiff staining of FVIII immunoprecipitates; only Gralnick's patient had decreased periodic acid-Schiff staining. In our hands, the sialic acid content of normal FVIII is 131 ± 16 nmol/mg protein, a figure in

general agreement with those more recently reported by others for purified preparations (34, 40, 41). We measured the sialic acid content of purified FVIII from two patients with type IIa (10) vWD: One patient's FVIII had an entirely normal sialic acid content (142 nmol/mg), whereas the other was only 50% of normal (75 nmol/mg). Thus, heterogeneity exists even within the type IIa classification. The relationship between abnormalities in multimeric forms of FVIII, observed in many patients with variant vWD (52, 53), and defects in carbohydrate structure is presently unclear.

The mechanism whereby FVIII exerts its hemostatic effects is still unknown. Although most in vivo studies of FVIII-platelet interactions use platelet aggregation as an end point, there is little or no evidence that FVIII is required for this phenomenon in vivo (3–4). To the contrary, there is persuasive evidence that FVIII, in some form, is essential for the interaction of platelets and the vascular subendothelium (5–6). Several recent articles have suggested an enhancing role for FVIII in platelet adhesion to collagen and in collagen-induced platelet aggregation (54–57). Our data demonstrate that ASVIII induces thromboxane synthesis and that even small amounts of ASVIII act synergistically with ADP and epinephrine, as well as with collagen, to promote platelet aggregation under conditions in which fully sialylated FVIII has no effect. The presence of even small amounts of ASVIII in the blood or in the subendothelium might enhance platelet adhesion to subendothelium and promote subsequent platelet aggregation. Since sialidases and sialyltransferases have been identified in platelets (58, 59) and other cells (60, 61), as well as in circulating blood, the potential exists for the presence of ASVIII in vivo. Thus, in addition to its utility as a tool for the study of FVIII-platelet interactions in the absence of ristocetin, ASVIII may be a physiologically important mediator of the hemostatic effects of Factor VIII.

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