Identification of the MMRN1 Binding Region within the C2 Domain of Human Factor V*

Samira B. Jeimy‡‡, Rachael A. Woram‡, Nola Fuller‡, Mary Ann Quinn-Allen‡, Gerry A. F. Nicolae**, Bjorn Dahlbäck††, William H. Kane‡, and Catherine P. M. Hayward‡ ‡‡

From the ‡Health Sciences Centre 2N31, Pathology and Molecular Medicine, McMaster University, 1200 Main Street West, Hamilton, Ontario L8N 3Z5, Canada, †Pathology, Duke University Medical Center, Durham, North Carolina 27710, ††Cardiovascular Research Institute Maastricht Department of Biochemistry, Maastricht University, P.O. Box 616, 6200 MD, Maastricht, The Netherlands, and ‡‡Clinical Chemistry, University of Lund, Lund, Malmö, S-20502 Malmo, Sweden

In platelets, coagulation cofactor V is stored in complex with multimerin 1 in α-granules for activation-induced release during clot formation. The molecular nature of the V-MMRN1 binding has not been determined, although multimerin 1 is known to interact with the factor V light chain. We investigated the region in factor V important for multimerin 1 binding using modified enzyme-linked immunosassays and recombinant factor V constructs. Factor V constructs lacking the C2 region or entire light chain had impaired and absent multimerin 1 binding, respectively, whereas the B domain deleted construct had modestly reduced binding. Analyses of point mutated constructs indicated that the multimerin 1 binding site in the C2 domain of factor V partially overlaps the phosphatidylinosine binding site and that the factor V B domain enhances multimerin 1 binding. Multimerin 1 did not inhibit factor V phosphatidylinosine binding, and it bound to phosphatidylinosine independently of factor V. There was a reduction in factor V in complex with multimerin 1 after activation, and thrombin cleavage significantly reduced factor V binding to multimerin 1. In molar excess, multimerin 1 modestly reduced factor V procoagulant activity in prothrombinase assays and only if it was added before factor V activation. The dissociation of factor V-multimerin 1 complexes following factor V activation suggests a role for multimerin 1 in delivering and localizing factor V onto platelets prior to prothrombinase assembly.

Activated coagulation factor V is a key non-enzymatic cofactor that is an essential component of the prothrombinase complex (1, 2). The active form of factor V, factor Va, is generated through consecutive cleavages by thrombin or factor Xa at residues Arg-709, Arg-1018, and Arg-1545, which produce factor Va heterodimers containing a heavy chain (A1 and A2 domains) and light chain (A3, C1, and C2 domains). The B domain of factor V, which facilitates thrombin cleavage and enhances factor V anticoagulant activity, is released on activation (3–7). The incorporation of factor Va into the prothrombinase complex provides binding sites for factor Xa, resulting in a 300,000-fold increase in the $V_{\text{max}}$ of prothrombin activation (8, 9).

In blood, much of the procoagulant factor V is stored in platelets as a complex with the α-granule protein multimerin 1 (MMRN1) for activation-induced release during clot formation (10, 11). Several differences have been noted in the functional properties of platelet and plasma factor V (reviewed in Ref. 12), which are encoded by the same gene (1), but the contributions of MMRN1 1 to these differences are unknown. MMRN1 is recognized to bind factor V, factor Va, and the isolated light chain of factor Va (11). Studies of activated platelets show that factor V-MMRN1 complexes dissociate after exposure to thrombin (11), similar to the way factor VIII-von Willebrand factor (VWF)³ complexes dissociate upon exposure to thrombin to liberate factor VIIIa for tenase complex assembly during coagulation (13). This is notable in light of the ∼40% amino acid sequence similarity shared by factor V and factor VIII in their light chain domains, which contain homologous regions for binding to phosphatidylinosine (PS), and in factor VIII, the binding site for VWF (13, 14). However, despite similarities between factors V and VIII, no significant homology has been found between MMRN1 and VWF, the multimeric proteins that bind these cofactors (15), and unlike VWF MMRN1 does not circulate in normal plasma (11, 13).

Recent studies have demonstrated that structural components within the light chain are important for factor Va phospholipid binding and procoagulant activity (16, 17). A β-sheet protruding loop in the factor Va C2 domain containing two tryptophan residues (Trp-2063 and Trp-2064) was identified to be a key region for these functions (17–19). The intent of our present study was to identify the region of factor V important for MMRN1 binding and its possible overlap with the factor Va phospholipid binding site in the C2 domain. We report that the factor V light chain and its C2 region are important for MMRN1 binding and that the binding of factor V to MMRN1 is

* This work was supported by Grant 42450 from the Canadian Institutes of Health Research (to C. P. M. H.), Grants HL43106 and HL54939 from the National Institutes of Health (to W. H. K), Grant 016.046.330 from the Dutch Organization for Scientific Research (to G. A. F. N.), and Grant 07143 from the Swedish Research Council (to B. D.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†† Recipient of a Career Investigator Award from the Heart and Stroke Foundation of Ontario.

§ Recipient of a Master’s Studentship Award from the Heart and Stroke Foundation of Canada.

* The abbreviations used are: VWF, von Willebrand factor; PS, phosphatidylinosine; dB, lacking B domain; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline; BSA, bovine serum albumin; FV, factor V; WT, wild-type; IIa, thrombin.
MMRN1 Binding Region within the C2 Domain of Human Factor V

Experimental Procedures

Human Factor V Constructs—Recombinant Factor V constructs that were tested for MMRN1 binding included full-length wild-type factor V and constructs lacking the B domain (dB), the entire light chain (dLC), C2 region of the light chain (dC2), both C2 and B domains (dBC2) (16), and a dB chimera construct that contained the C2 region of factor VIII (chimera) (20). Also tested were 30 dB constructs with charge to alanine point mutations in the C2 domain (18), three full-length constructs containing altered residues in the N-linked glycosylation consensus sequence (21), and four full-length constructs with mutated residues in the phospholipid-binding loop of the C2 domain (17).

Enzyme-linked Immunosorbent Assays (ELISAs) and Binding Assays—Recombinant MMRN1 free of human factor V was expressed, affinity purified, and quantitated as described previously (22) for use in binding assays. The recombinant MMRN1 contained 18.5 μg of MMRN1 per unit of antigen (amount contained in 10^2 pooled normal platelets) (10). Because of variability in MMRN1 polymer size, molar concentrations of recombinant MMRN1 were calculated based on the amounts of MMRN1 subunits (average subunit size was 186 kDa).

For ELISA, microtitre plates (Nunc MaxiSorp Immunoplate number 442404, Corning Life Sciences, Concord, MA) were blocked (2 h at room temperature) with phosphate-buffered saline (PBS) containing 2% bovine serum albumin (BSA). Samples were diluted in the same buffer with 0.05% Tween (PBST-BSA), and 3 mM CaCl_2 was added for thrombin cleavage experiments. Wells were washed three times with PBST between incubations (1 h at room temperature).

Factor V constructs used for binding assays were quantitated by a modification of a previously described ELISA (23) in which the human factor V heavy chain antibody HC-5146 (1 μg/ml, Haemtech Technologies, Inc., Essex Junction, VT) was substituted for factor V detection. To test MMRN1 binding to factor V, the assay was further modified as follows: factor V (1 μg/ml) was immobilized by monoclonal antibody FV-1 (0.2 μg/ml) on a polyclonal capture (1 μg/ml sheep anti-human factor V, Affinity Biologicals, Hamilton, ON), before serial incubations with MMRN1 (0.5 μg/ml, monoclonal antibody JS-1 (0.2 μg/ml)) (22), peroxidase-conjugated goat anti-mouse IgG (0.04 μg/ml, Jackson ImmunoResearch, West Grove, PA), and the peroxidase substrate Colorburst® Blue (100 μW/ml, Alerchek, Inc., Portland, ME). Additional assays were conducted to test possible overlap between the MMRN1 binding site and sites for site-directed epitopes (17, 18) recognized by monoclonal antibodies HV-1 (Sigma-Aldrich) which blocks factor Va PS-binding and procoagulant activity, and 6A5, which inhibits factor Va procoagulant activity (20). For these assays, captured factor V was preincubated with 10 μg/ml antibody or control, normal mouse IgG (Zymed Laboratories, Inc., San Francisco, CA). Factor V (1 μg/ml) preincubated with MMRN1 (1,000 dilution, (22)) followed by peroxidase-conjugated donkey anti-rabbit IgG (0.04 μg/ml, Jackson ImmunoResearch). Any measured background binding of MMRN1 to wells without added factor V was subtracted from test results. Wells without added factor V or MMRN1 confirmed the specificity of binding assays for factor V-MMRN1 complexes, as they typically bound <1% of the added binding protein.

Factor V binding to immobilized MMRN1 was tested using non-treated polystyrene microtitre plates (Corning Life Sciences, number 3631), which gave no detectable background factor V binding. For this assay, wells were precoated with affinity-purified recombinant MMRN1 (1 μg/ml) before adding factor V (5 ng/ml). Bound factor V was quantitated using HC-5146, as in the modified factor V ELISA. Analyses of wells without added MMRN1 or factor V confirmed the specificity of this assay for factor V-MMRN1 complexes. For some experiments, recombinant factor V was pretreated with or without 1 unit/ml thrombin (37 °C for 20 min in PBST-BSA containing 3 mM CaCl_2, Enzyme Research Laboratories, South Bend, IN) and the binding of factor V, Va, and the B domain to MMRN1 was evaluated using monoclonal antibodies against the B domain (0.1 μg/ml MMX-30, Ref. 24), heavy chain (1 μg/ml), light chain (1 μg/ml), or light chain (1 μg/ml, samples analyzed with heavy and light chain antibodies after separation on reduced 6% sodium dodecyl sulfate-polyacrylamide gels) confirmed full cleavage of factor V in the thrombin-treated samples. Results for all binding assays were expressed as a percentage of value obtained for the corresponding wild-type factor V (full-length or dB) or untreated full-length wild-type factor V for the thrombin cleavage experiments.

The effect of thrombin on preformed factor V-MMRN1 complexes was evaluated using a previously described ELISA (23). Briefly, factor V-MMRN1 complexes were generated in vitro by preincubating 1 μg/ml MMRN1 with 0–100 ng/ml of full-length or dB wild-type factor V (30 min for 37°C) before adding thrombin (0 or 1 units/ml final concentration in 25 mM Tris-HCl with 0.15 mM NaCl, 3 mM CaCl_2, 2 mM KCl, 10 mg/ml BSA, pH 7.4, 37°C for 20 min) and assaying for residual complexes.

Phospholipid Binding Assay—MMRN1 and factor V binding to phospholipids was studied using microtiter wells precoated with 3 μg/ml bovine brain 1-α-phosphatidylinositol (Sigma) as described previously (16) with minor modifications. MMRN1 bound to PS was detected with JS-1 and peroxidase-conjugated goat anti-mouse IgG. Factor V (wild-type and dB) bound to PS was detected using sheep anti-human factor V (1 μg/ml) followed by a peroxidase-conjugated donkey anti-sheep IgG (0.04 μg/ml, Jackson ImmunoResearch). For some determinations, factor V (100 ng/ml wild-type or dB) was tested after a 2-h preincubation with affinity-purified recombinant MMRN1 (0–22 μg/ml). Analysis of wells without PS confirmed that these assays measured protein binding to PS.

Prothrombinase Assays—Phospholipid vesicles used for prothrombinase assays were prepared using 1.2-dioleoyl-sn-glycero-3-phospho-L-serine and 1,2-dioleoyl-sn-glycero-3-phospho-L-cholesterol (Avanti Polar Lipids, Inc., Alabaster, AL), and phospholipid concentrations were determined as described (25). Phospholipids (3:1 ratio of 1,2-dioleoyl-sn-glycero-3-phosphocholine/1,2-dioleoyl-sn-glycero-3-phospho-L-serine) were suspended in chloroform, dried under a stream of nitrogen gas, and resuspended in 25 mM Tris-HCl buffer with 0.15 mM NaCl, 3 mM CaCl_2, 2 mM KCl, and 20 μg/ml BSA, pH 7.4. Lipid suspensions were passed several times through a membrane extruder (Liposofat, Avestin, Inc., Ottawa, Ontario) equipped with polycarbonate filters to produce vesicles with an average diameter of 100 nm.

Factor V procoagulant activity was examined by prothrombinase assays described previously (18) with minor modifications, using recombinant coagulation factor Va (a gift from Dr. Karl O. M. Wallberg). In the absence of phosphatidylinositol/phosphatidylethanolamine, 1.4 μM prothrombin (Haemtech Technologies, Inc.), and 5 μg factor Xa (Haemtech Laboratories, Inc.), the purified MMRN1 for these assays was stored in 10 mg/ml BSA/PBS to minimize losses. Factor V (0–400 μg to provide 0–40 μM final concentration in prothrombinase reactions) was tested after preincubation (30 min at 37°C) without or with a molar excess of purified MMRN1 (up to 4 μg/ml final concentration after sample dilution in prothrombinase reactions, which was ~400 times the molar amount of factor V and more than the 20-fold molar excess of MMRN1: factor V in platelets (10)). After the preincubation, factor V was activated with thrombin (5 μM final concentration, 37°C) before adding 1 μM of the irreversible thrombin inhibitor Pefabloc® SC (Roche Applied Science, Laval, Quebec) and diluting samples 10-fold to measure factor Va procoagulant activity. For other determinations, MMRN1 (same final concentrations) was added after factor V activation by thrombin. Results for prothrombinase assays were expressed as the percentage of factor Va activity for samples without added MMRN1.

Statistical Analysis—Analyses of variance followed by Fischer’s test of least significant difference were used to determine which mutant factor V constructs had significantly reduced MMRN1 binding relative to the corresponding full-length or dB wild-type factor V and the MMRN1 concentrations that reduced factor V binding to PS. Paired, one-tailed Student’s t tests were used to determine whether factor V C2 domain antibodies inhibited MMRN1 binding, whether thrombin treatment reduced factor V-MMRN1 binding and dissociated preformed factor V-MMRN1 complexes, and whether MMRN1 inhibited factor Va activity in prothrombinase assays.

Figure 1. Binding of MMRN1 to domain deleted factor V (FV) constructs. Results are expressed as a percentage of the binding to wild-type (WT) FV. Bars show means ± S.E. of three independent experiments. Constructs with significantly reduced MMRN1 binding are indicated (*, p < 0.05).
RESULTS

Binding of MMRN1 to Factor V Constructs—Analyses of MMRN1-factor V binding using the assay with immobilized factor V indicated that some domain deleted forms of factor V had impaired MMRN1 binding. Although the dB construct had mildly reduced MMRN1 binding, the dC2 construct had only 10% residual binding, and the dLC, dBC2 constructs, and the chimera construct had no detectable MMRN1 binding (Fig. 1, p < 0.05).

Analyses of the point-mutated, full-length constructs confirmed that the factor V C2 region contained residues important for MMRN1 binding. Four site-directed, full-length C2 mutant constructs had reduced MMRN1 binding (Fig. 2A, p < 0.05). Of these, constructs with mutations at residues W2063 and W2064, which are important for high-affinity binding of factor V to PS (17, 18), had about 20% less MMRN1 binding (Fig. 2A, p < 0.05). A similar decrease in MMRN1 binding was evident for a construct with an S → T substitution at residue 2183 (Fig. 2A, p < 0.05). This substitution has been shown previously to increase the efficiency of N-linked glycosylation at position 2181, which impaired factor V-PS binding (21, 26).

The data for the charge to alanine mutated dB constructs further suggested overlap between the MMRN1 and PS binding sites in factor V (Fig. 2B). 19 of the 30 constructs had reduced MMRN1 binding relative to dB factor V (Fig. 2B, p < 0.05), and of these the 12 with the lowest MMRN1 binding (0–8.8% of wild-type) were mutants W2063A, W2064A, (W2063, W2064)A, R2074A, (R2072, R2074)A, (K2101, K2103, K2104)A, L2116A, (K2157, H2159, K2161)A, R2171A, R2174A, E2189A, and (R2187, E2189)A. Seven C2 mutant constructs with reduced MMRN1 binding (indicated in Fig. 2B) were reported previously to be incorrectly folded (18), suggesting that their abnormal MMRN1 binding could be an artifact. Further evaluation of the MMRN1 binding properties of full-length and dB point mutated factor V constructs using the binding assay with immobilized MMRN1 (data not shown) confirmed results
MMRN1 Binding Region within the C2 Domain of Human Factor V

FIG. 3. Binding of MMRN1 and wild-type FV to immobilized PS. In A, MMRN1 binding (without added FV) to wells precoated with (+) or without (−) PS is indicated by OD450. In B, FV binding to PS was measured after preincubation without or with MMRN1 (up to 400-fold molar excess of MMRN1) and expressed as the percentage bound without MMRN1 (means ± S.E. of three independent experiments). MMRN1 binding to PS occurred without FV (A) and MMRN1 did not significantly inhibit FV binding to PS (B).

FIG. 5. Location of MMRN1 binding sites on the crystal structure of the C2 domain of FV. (The three-dimensional structure data for the C2 domain of factor V can be accessed through the Research Collaboratory for Structural Bioinformatics Protein Data Bank under accession code 1CZT.) Highlighted in the upper panel are residues involved in binding MMRN1 (blue, excludes residues associated with misfolded mutations), 6A5 (green), and other residues not involved in MMRN1 binding (yellow). The lower panel indicates 6A5 (green), HV-1 (purple), and PS (purple) binding residues. Models on the left have been rotated 180° relative to those on the right. Regions in FV important in MMRN1 binding overlapped but were larger than the areas important for PS and HV-1 binding. Analysis of the molecular model was performed using the YASARA package (35).

DISCUSSION

Presently, the molecular nature of MMRN1-factor V binding has not been determined, although MMRN1 is known to interact with the light chain of factor V and Va (11). Using modified enzyme-linked immunoassays and recombinant factor V constructs, we identified important regions for MMRN1 binding (summarized in Fig. 9). We found that discontinuous regions in the C2 domain of factor V, which mapped to an extended linear region on the C2 domain crystal structure (Fig. 5), were important for binding MMRN1. This region overlapped but was larger than sites in the C2 domain implicated in PS binding and procoagulant activity, and it was distinct from the 6A5 binding site, which contributes to factor V procoagulant functions. In addition to Trp-2063 and Trp-2064, Leu-2116, which putatively interact with Trp-2063 and Trp-2064 to modulate factor V-PS binding (18), was also important in MMRN1 binding. Moreover, the S2183T substitution, which led to a higher degree of N-linked glycosylation at Asn-2181 and impaired factor V-PS binding (21, 26), also reduced factor V-MMRN1 binding. Although our data implicated the C2 domain of factor V as the most important region for MMRN1 binding, there may be contributions of other regions of the light chain based on the more impaired MMRN1 binding of constructs lacking the en-

obtained by assays of MMRN1 binding to immobilized factor V. Together, the data implied that the MMRN1 binding site in the C2 domain of factor V involved four discontinuous segments, including residue 2187 and regions between residues 2060–2080, 2114–2116, and 2157–2161 (Fig. 2B). Only part of the region implicated in MMRN1 binding overlapped PS and HV-1 binding sites (Fig. 2B).

In PS binding assays, MMRN1 was identified to bind to PS independently of factor V (Fig. 3A). In addition, MMRN1 did not significantly reduce the binding of full-length (Fig. 3B, p = 0.3) and dB (data not shown) factor V to PS, even at high concentrations (Fig. 3B, 400-fold molar excess of MMRN1 to factor V). Nonetheless, antibody HV-1, which blocks factor V binding to PS, significantly reduced MMRN1 binding to factor V (p < 0.01), whereas antibody 6A5 and normal mouse IgG did not (Fig. 4). When factor V residues important in binding HV-1 and 6A5, MMRN1, and PS were highlighted in a three-dimensional model of the factor V C2 domain (Fig. 5), the MMRN1 binding site was identified to be distinct from the 6A5 binding site, and it spanned a region that overlapped but was larger than the site important for PS binding and procoagulant function.

Effect of Thrombin on MMRN1-Factor V Binding—Further studies were conducted to determine whether the binding of factor V to MMRN1 was altered by thrombin cleavage of factor V. Following thrombin activation, wild-type factor V (Fig. 6) and point mutated constructs (not shown) showed significantly reduced MMRN1 binding (p < 0.01) in assays that used antibodies against the heavy chain and light chain to detect bound factor V and Va. The assay that used the factor V B domain antibody to detect components bound to MMRN1 showed more striking reductions after thrombin cleavage (Fig. 6, p < 0.001), further suggesting that the light chain was more important than the B domain for MMRN1 binding. The exposure of preformed MMRN1-factor V complexes (generated using a range of molar ratios, including the 20:1 molar ratio of MMRN1/factor V in platelets (10)) to thrombin significantly reduced the amount of factor V/Va bound to MMRN1 (Fig. 7 and data not shown, p < 0.01).

Effect of MMRN1 on Factor V/Va Procoagulant Activity—In thrombinase assays, MMRN1 had no detectable effect on factor Va activity when it was added after factor V activation, even in assays done with very low concentrations of phospholipids (Fig. 8). However, when MMRN1 was incubated with factor V before thrombin activation, it had significant but fairly minimal inhibitory effects on factor Va activity (Fig. 8, p < 0.05).
MMRN1 Binding Region within the C2 Domain of Human Factor V

The overlap in the PS, HV-1, and MMRN1 binding sites in the C2 domain suggested that MMRN1 might significantly inhibit factor V-PS binding, particularly because VWF binds to a similar region of the factor VIII C2 domain and blocks factor VIII binding to PS (31). However, we did not detect any inhibitory effects of MMRN1 on factor V-PS binding, and our data offered several potential explanations. Firstly, the MMRN1 binding site in factor V spanned an area larger than the PS binding site, suggesting that factor V might be able to bind PS and MMRN1 simultaneously. Secondly, we found that MMRN1 bound to PS independent of factor V, raising the possibility that factor V binding to PS was preserved because MMRN1 provided an alternative, indirect mechanism for factor V binding to PS.

We identified some interesting parallels between thrombin-mediated dissociation of factor V-MMRN1 complexes with thrombin-mediated dissociation of factor VIII-VWF complexes. Specifically, the ability of factor V to bind MMRN1 was reduced after it was activated to factor Va, and thrombin cleavage promoted significant dissociation of preformed factor V-MMRN1 complexes. The differences in binding of factor V and Va to MMRN1 were consistent with the observation that MMRN1...
binding region in the C2 domain of factor V, an area previously involving cysteine 1085 in the factor V B domain (10). Although recently, we identified that human platelets contain forms of factor V to Va, there was a considerable (84%) reduction in MMRN1 binding when reduced MMRN1 binding. Second, point mutations in the C2 domain had more dramatic effects on MMRN1 binding when compared with the wild type. MMRN1 in platelets suggests that the B domain directly contributes to the MMRN1 binding site of factor V.

We found several lines of evidence that the B domain also modulates binding to other proteins, including factor Xa (33). Factor V thrombin activation (3–7), the B domain of factor V makes contributions to the MMRN1 binding site of factor V. MMRN1 in platelets suggests that the B domain directly contributes to the MMRN1 binding site of factor V.

Reduced factor Va activity in prothrombinase assays when it was added before (but not after) factor V activation. Nonetheless, the inhibitory effects of MMRN1 on factor V procoagulant activity were quite minimal, even though prothrombinase assays were done with a significant molar excess of MMRN1 and activity were quite minimal, even though prothrombinase activity was added before (but not after) factor V activation. Nonethe-

References

1. Kane, W. H. (2001) in Hemostasis and Thrombosis: Basic Principles and Clinical Practice (Colman, R. W., Hirsh, J., Marder, V. J., Clowes, A. W., and George, J. N., eds) pp.157–169, J. B. Lippincott Company, Philadelphia.

2. Nicolaas, G. A., and Dahlback, B. (2002) Arterioscler. Thromb. Vasc. Biol. 22, 530–538.

3. Pfitzner, D. D., Marquetter, K. A., and Kaufman, R. J. (1994) Blood 84, 4214–4225.

4. Thorelli, E., Kaufman, R. J., and Dahlback, B. (1998) J. Biol. Chem. 273, 16140–16145.

5. Thorelli, E., Kaufman, R. J., and Dahlback, B. (1997) Eur. J. Biochem. 247, 12–20.

6. T. R., and Camire, R. M. (2004) J. Biol. Chem. 279, 21643–21650.

7. Pfitzner, D. D., Tomkinson, K. N., Michnick, D., Steligosku, U., and Kaufman, R. J. (1994) Biochemistry 33, 6952–6959.

8. Nesheim, M. E., Taswell, J. B., and Mann, K. G. (1979) J. Biol. Chem. 254, 10953–10962.

9. Rosing, J., Tans, G., Govers-Riemslag, J. W., Zwaal, R. F., and Henker, H. C. (1980) J. Biol. Chem. 255, 274–283.

10. Hayward, C. P., Fuller, N., Zhang, S., Adam, F., Jeimy, S., Horsewood, J., Quinn-Alleen, M. A., and Kane, W. H. (2004) Thromb. Haemostasis 92, 1349–1357.

11. Hayward, C. P., Furmaniak-Kazmierczak, E., Cieutat, A. M., Moore, J. C., Rainbolt, D. F., Nesheim, M. E., Kelton, J. G., and Cote, G. (1995) J. Biol. Chem. 270, 19217–19224.

12. Gould, W. R., Silveira, J. R., and Tracy, P. B. (2004) J. Biol. Chem. 279, 2383–2393.

13. Kane, W. H., and Davie, E. W. (1988) Blood 71, 539–555.

14. Kalafatis, M., Beck, D. O., and Mann, K. G. (2003) J. Biol. Chem. 278, 21550–21561.

15. Hayward, C. P., Hassell, J. A., Denomme, G. A., Rachubinski, R. A., Brown, C., and Kelton, J. G. (1995) J. Biol. Chem. 270, 18246–18251.

16. Ortel, T. L., Devore-Carter, D., Quinn-Alleen, M., and Kane, W. H. (1992) J. Biol. Chem. 267, 4189–4198.

17. Nicolaas, G. A., Vilkootreix, B. O., and Dahlback, B. (2000) Blood Coagul. Fibibrinolysis 11, 89–109.

18. Kim, S. W., Quinn-Alleen, M. A., Camp, J. T., Maceo-Ribeiro, S., Fuentes-Providor, P., Bode, W., and Kane, W. H. (2000) Biochemistry 39, 1951–1958.

19. Peng, W., Quinn-Alleen, M. A., Kim, S. W., Alexander, K. A., and Kane, W. H. (2004) Biochemistry 43, 4385–4393.

20. Ortel, T. L., Quinn-Alleen, M. A., Keller, E. G., Peterson, J. A., Lorcoba, D., and Kane, W. H. (1994) J. Biol. Chem. 269, 15988–15905.

21. Nicolaas, G. A., Vilkootreix, B. O., and Dahlback, B. (1999) Biochemistry 38, 13584–13591.

22. Hayward, C. P., Smith, J. W., Horsewood, P., Warkentin, T. E., and Kelton, J. G. (1995) J. Clin. Investig. 91, 1614–1620.

23. Hayward, C. P., Weiss, H. J., Lages, B., Finlay, M., Hengst, A. C., Zheng, S., Cowie, A., Masse, J. M., Harrison, P., and Cramer, E. M. (2001) Br. J. Haematol. 113, 871–877.

24. Dahlback, B. (1984) Semin. Thromb. Hemostasis 10, 139–145.

25. Krishnaswamy, S., and Mann, K. G. (1988) J. Biol. Chem. 263, 5714–5723.

26. Kim, S. W., Ortel, T. L., Quinn-Alleen, M. A., Yoo, L., Worfolk, L., Zhai, X., Lenta, B. R., and Kane, W. H. (1999) Biochemistry 38, 11448–11454.

27. Guasch, J. F., Cannegieter, S., Reitsma, P. H., Veer-Korthof, E. T., and Bernt, R. (1996) Br. J. Haematol. 91, 32–38.

28. Miletich, J. P., Majerus, D. W., and Majerus, P. W. (1978) J. Clin. Investig. 62, 824–831.

29. Cui, J., O'Shea, K. S., Purkayastha, A., Saunders, T. L., and Ginsburg, D. (1996) Nature 384, 66–68.

30. Maceo-Ribeiro, S., Bode, W., Huber, R., Quinn-Alleen, M. A., Kim, S. W., Ortel, T. L., Boureniotis, G. P., Bartunik, H. D., Stubbs, M. T., Kane, W. H., and Fuente-Providor, P. (1999) Nature 402, 454–459.

31. Andersson, L. O., and Brown, J. E. (1981) Biochem. J. 200, 161–167.

32. Barks, A. R. (2006) Semin. Thromb. Hemostasis 28, 11–22.

33. Steen, M. (2002) Scand. J. Clin. Lab. Invest. Suppl. 237, 5–11.

34. Hayward, C. P., Baiden, D. F., Smith, J. W., Horsewood, P., Stead, R. H., Podar, T. J., Warkentin, T. E., and Kelton, J. G. (1993) J. Clin. Investig. 91, 2630–2639.

35. Krieger, E., Koraimann, G., and Vriend, G. (2002) Proteins 47, 393–402.

36. Yang, T. L., Cui, J., Rehumtulla, A., Yang, A., Moussalli, M., Kaufman, R. J., and Ginsburg, D. (1996) Blood 91, 4583–4599.

37. Guinato, E. R., Emon, C. T., Mann, K. G., and MacGillivray, R. T. (1992) J. Biol. Chem. 267, 2971–2978.

38. Kane, W. H., and Davie, E. W. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 6890–6894.