Published in final edited form as:
*Thromb Res*. 2008 ; 123(2): 352–354. doi:10.1016/j.thromres.2008.03.016.

# Location of the multimerin 1 binding site in coagulation factor V: An update

**Samira B. Jeimy**, **Mary Ann Quinn-Allen**, **Nola Fuller**, **William H. Kane**, and **Catherine P.M. Hayward***

*HSC 2N31, Pathology and Molecular Medicine, McMaster University, 1200 Main St. West, Hamilton, ON, Canada L8N 3Z5. Division of Hematology, Duke University Medical Center, Durham, North Carolina, United States, 27710*

## Abstract

Activated coagulation factor V (FVa) is an important cofactor that accelerates thrombin production. In human blood, 25% of the factor V (FV) is stored in platelets, complexed to the polymeric, FV binding protein multimerin 1 (MMRN1). The light chain of FV is required for MMRN1 binding, and its C2 domain contains a MMRN1 binding site that overlaps phospholipid binding residues essential for FVa procoagulant function. The homologous structures and roles of the FVa light chain C1 and C2 domains led us to investigate if the C1 domain also contains a MMRN1 binding site. The MMRN1 binding properties of FV constructs were tested by modified enzyme-linked immunoassays, before and after thrombin activation. The constructs tested included the combined C1 and C2 domain deleted FV, and B-domain deleted forms of FV containing C1 domain point mutations or combined C1 and C2 domain phospholipid binding site mutations. The MMRN1 binding site in FV/FVa was mapped to a large region that included the C1 domain phospholipid binding residues Y1956 and L1957. The FV construct with combined C1 and C2 domain phospholipid binding site mutations had no MMRN1 binding, highlighting the critical role of the FV C1 and C2 domain phospholipid binding residues in MMRN1 binding. Our data update the information on the structural features of FV and FVa important for MMRN1 binding, and suggest that the extended MMRN1 binding site in the C1 and C2 domains is important for the storage of FV-MMRN1 complexes in platelets.

Activated FV (FVa) is a key coagulation cofactor that accelerates the production of thrombin by the prothrombinase complex [1]. In blood, approximately 25% of the factor V (FV) is retained in platelet α-granules, where FV is stored as a partially activated cofactor complexed to the polymeric protein multimerin 1 (MMRN1) [2,3]. In humans, MMRN1-FV binding is predominantly noncovalent, although 25% of platelet FV is disulfide linked to MMRN1 [3]. Noncovalent FV-MMRN1 binding involves an extended region of the FV light chain C2 domain, that overlaps the FVa membrane binding site [4]. Recently solved FVa crystal structures and mutagenesis studies of FV function have provided evidence that the C domains of FV use structurally similar features to promote FVa binding to phospholipid membranes [5–8]. Nonetheless, the functions of the FV C1 domain are not as well characterized as those of the C2 domain. The possibility that the C1 domain contributes to MMRN1 binding is supported by the absent MMRN1 binding of FV constructs lacking the entire light chain, compared to the impaired MMRN1 binding of FV constructs lacking only the C2 domain [4]. These observations led us to investigate a potential MMRN1 binding site in the FV C1 domain.

*Corresponding author. Room 2N31, McMaster University Medical Centre, 1200 Main St. W, Hamilton, Ontario, Canada L8N 3Z5. Tel.: +1 905 521 2100x73373; fax: +1 905 521 2338. E-mail address: haywrdc@mcmaster.ca (C.P.M. Hayward).

Recombinant MMRN1 was produced and affinity purified as described [9] to test the binding of recombinant FV constructs (tested in serum-free culture media from transfected COS-7 cells), using established methods [4]. Constructs tested included: FV lacking both C domains (dC1C2) [10], a B domain deleted (dB) FV construct with C1 domain point mutations [11], and dBFV mutated at both C domain phospholipid binding sites (Y1956, L1957, W2063, W2064)A [12], which has a dramatically reduced affinity for phosphatidylserine (PS) and an impaired ability to support thrombin generation [12]. The markedly impaired synthesis of A3 domain deleted FV [10] precluded an evaluation of its MMRN1 binding. Control constructs (with previously characterized MMRN1 binding properties) [4] included: wild type FV and constructs lacking the B domain (dB), entire light chain (dLC), or just the C2 domain (dC2) [10]. Enzyme-linked immunosorbent assays (ELISA) described previously [4] were used to quantify the recombinant proteins, and to test FV binding to immobilized MMRN1. Results for MMRN1 binding were expressed as a percentage of the O.D. reading obtained for the corresponding wild-type FV construct. All experiments were performed in the absence of phospholipid vesicles, as MMRN1 and FV were previously shown to independently bind to the negatively charged lipid, PS [4]. Analysis of variance (ANOVA), followed by Fischer's test of least significant difference (LSD), were used to determine which constructs had significantly reduced binding to MMRN1 relative to dB wild-type FV ($p<0.05$), based on triplicate determinations from three separate experiments. Data on MMRN1 binding sites were mapped onto a three dimensional representation of human FVa (coordinate file provided by Dr. B. Villoutreix, Paris, France [13]) using ICM software package (Molsoft, CA, USA).

FV constructs D1892A, (K1954, H1955)A, D1955A, K1958A, K1980A, (F1900, L1901, Y1903)A, Y1917A, Y1956A, (Y1956, L1957)A, R2023A, and (R2023, R2027) A had reduced MMRN1 binding (p values <0.05; range of reductions: 40 – 55% compared to dBWT; Fig. 1). These constructs also had impaired MMRN1 binding after conversion to FVa, suggesting that the same C1 residues contribute to MMRN1 binding, before and after FVactivation. In contrast to the reduced MMRN1 binding of the FV construct mutated at the C1 phospholipid binding site (Y1956, L 1957)A, the FV construct with both C domain phospholipid binding sites mutated [(W2063, W2064, Y1956, L1957)A] had no detectable MMRN1 binding, like dC1C2 FV (p values< 0.001 compared to dBWT; results not different from background; p=0.79). These data indicated that C1 and C2 phospholipid binding residues are essential for MMRN1 binding, and that the homologies between the FV C1 and C2 domains extend to that of MMRN1 binding. Interestingly, a similar essential role for C domain membrane binding sites has been shown for factor VIII binding to von Willebrand factor [14].

Fig. 1 illustrates that the FV/FVa MMRN1 binding site maps to a large area in the C1 and C2 domains, including the homologous membrane binding residues that support FVa PS binding and procoagulant function. Within the MMRN1 binding site, the C2 domain phospholipid binding site is more critical for MMRN1 binding, based on the greater (>90%) reduction in MMRN1 binding when these residues are mutated (indicated in Fig. 1) [4], compared to less striking reductions (< 55%) reductions for mutations at other C2 [4] and C1 domain residues. The more important role of the C2 domain binding site in MMRN1 binding is consistent with a recent report of an autoantibody against the FV C2 domain, associated with a transient platelet FV deficiency, that ablated FVa procoagulant function and FV-MMRN1 binding [15]. The homologous roles of the FV C1 and C2 domains in MMRN1 binding could be important for the normal costorage of FV and MMRN1 in platelets.

## Acknowledgements

Supported by grant 42450 from the Canadian Institutes of Health Research (C.P.M.H.), and grant HL43106 from the National Institutes of Health (W.H.K). C.P.M.H. is the recipient of a Career Investigator Award from the Heart and Stroke Foundation of Ontario and a Canada Research Chair in Molecular Hemostasis. S.B.J. is the recipient of a Doctoral Research Award from the Canadian Institutes of Health Research.

## References


1. Kane, WH.; Factor, V. Hemostasis and Thrombosis: Basic Principles and Clinical Practice. Vol. 5. Colman, RW.; Marder, VJ.; Clowes, AW.; George, JN.; Goldhaber, SZ., editors. Philadelphia: J.B. Lippincott Company; 2006. p. 177-92.
2. Tracy PB, Eide LL, Bowie EJ, Mann KG. Radioimmunoassay of factor V in human plasma and platelets. Blood 1982;60:59–63. [PubMed: 7082847]
3. Hayward CP, Fuller N, Zheng S, Adam F, Jeimy SB, Horsewood I, Quinn-Allen MA, Kane WH. Human platelets contain forms of factor V in disulfide-linkage with multimerin. Thromb Haemost 2004;92:1349–57. [PubMed: 15583744]
4. Jeimy SB, Woram RA, Fuller N, Quinn-Allen MA, Nicolaes GA, Dahlback B, Kane WH, Hayward CP. Identification of the MMRN1 binding region within the C2 domain of human factor V. J Biol Chem 2004;279:51466–71. [PubMed: 15452129]
5. Adams TE, Hockin MF, Mann KG, Everse SJ. The crystal structure of activated protein C-inactivated bovine factor Va: Implications for cofactor function. Proc Natl Acad Sci 2004;101:8918–23. [PubMed: 15184653]
6. Macedo-Ribeiro S, Bode W, Huber R, Quinn-Allen MA, Kim SW, Ortel TL, Bourenkov GP, Bartunik HD, Stubbs MT, Kane WH, Fuentes-Prior P. Crystal structures of the membrane-binding C2 domain of human coagulation factor V. Nature 1999;402:434–9. [PubMed: 10586886]
7. Izumi T, Kim SW, Greist A, Macedo-Ribeiro S, Fuentes-Prior P, Bode W, Kane WH, Ortel TL. Fine mapping of inhibitory anti-factor V antibodies using factor V C2 domain mutants. Identification of two antigenic epitopes involved in phospholipid binding. Thromb Haemost 2001;85:1048–54. [PubMed: 11434683]
8. Kim SW, Quinn-Allen MA, Camp JT, Macedo-Ribeiro S, Fuentes-Prior P, Bode W, Kane WH. Identification of functionally important amino acid residues within the C2-domain of human factor V using alanine-scanning mutagenesis. Biochemistry 2000;39:1951–8. [PubMed: 10684644]
9. Adam F, Zheng S, Joshi N, Kelton DS, Sandhu A, Suehiro Y, Jeimy SB, Santos AV, Massé JM, Kelton JG, Cramer EM, Hayward CP. Analyses of cellular multimerin 1 receptors: in vitro evidence of binding mediated by alphaIIbbeta3 and alphavbeta3. Thromb Haemost 2005;94:1004–11. [PubMed: 16363244]
10. Ortel TL, Devore-Carter D, Quinn-Allen M, Kane WH. Deletion analysis of recombinant human factor V. Evidence for a phosphatidylserine binding site in the second C-type domain. J Biol Chem 1992;267:4189–98. [PubMed: 1740460]
11. Saleh M, Peng W, Quinn-Allen MA, Macedo-Ribeiro S, Fuentes-Prior P, Bode W, Kane WH. The factor V C1 domain is involved in membrane binding: identification of functionally important amino acid residues within the C1 domain of factor V using alanine scanning mutagenesis. Thromb Haemost 2004;91:16–27. [PubMed: 14691564]
12. Peng W, Quinn-Allen MA, Kane WH. Mutation of hydrophobic residues in the factor Va C1 and C2 domains blocks membrane-dependent prothrombin activation. J Thromb Haemost 2005;3:351–4. [PubMed: 15670043]
13. Autin L, Steen M, Dahlback B, Villoutreix BO. Proposed structural models of the prothrombinase (FXa-FVa) complex. Proteins 2006;63:440–50. [PubMed: 16437549]
14. Jacquemin M, Benhida A, Peerlinck K, Desqueper B, Vander EL, Lavend'homme R, d'Oiron R, Schwaab R, Bakkus M, Thielemans K, Gilles JG, Vermylen J, Saint-Remy JM. A human antibody directed to the factor VIII C1 domain inhibits factor VIII cofactor activity and binding to von Willebrand factor. Blood 2000;95:156–63. [PubMed: 10607698]
15. Jeimy SB, Krakow EF, Fuller N, Tasneem S, Hayward CP. An acquired factor V inhibitor associated with defective factor V function, storage and binding to multimerin 1. J Thromb Haemost 2008;6:395–7. [PubMed: 18047547]

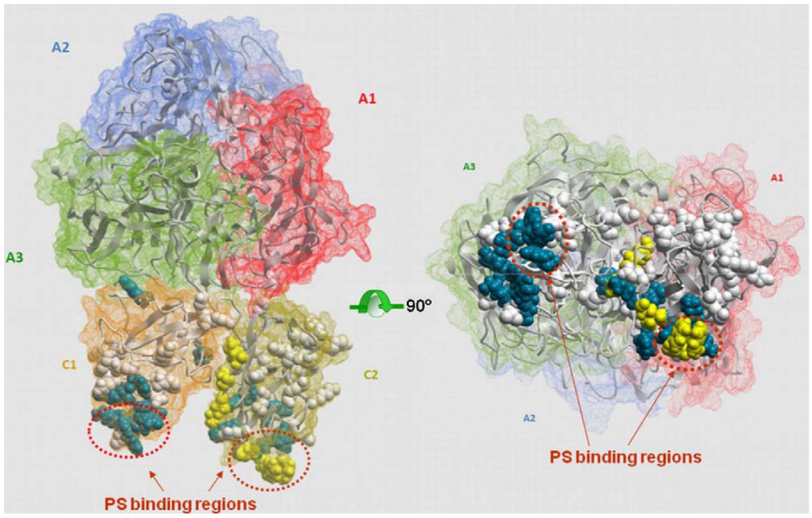


**Figure 1.**

Location of the MMRN1 binding site in the C domains of FV. The molecular model compares the location of C1 domain MMRN1 binding residues to known C2 domain MMRN1 binding residues [4]. Mutation of residues shaded yellow results in >90% loss of MMRN1 binding by ELISA, while mutation of residues shaded blue results in a 40–55% loss of MMRN1 binding. C domain residues not involved in MMRN1 binding are shown in white. The molecule on the right shows the reference orientation, with domains indicated and PS binding residues circled. The molecule on the left is rotated 90° along the X-axis.