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Structural investigation of the A domains of human blood coagulation factor V by molecular modeling

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Abstract

Factor V (FV) is a large (2,196 amino acids) nonenzymatic cofactor in the coagulation cascade with a domain organization (A1–A2–B-A3–C1–C2) similar to the one of factor VIII (FVIII). FV is activated to factor Va (FVa) by thrombin, which cleaves away the B domain leaving a heterodimeric structure composed of a heavy chain (A1–A2) and a light chain (A3–C1–C2). Activated protein C (APC), together with its cofactor protein S (PS), inhibits the coagulation cascade via limited proteolysis of FVa and FVIIIa (APC cleaves FVa at residues R306, R506, and R679). The A domains of FV and FVIII share important sequence identity with the plasma copper-binding protein ceruloplasmin (CP). The X-ray structure of CP and theoretical models for FVIII have been recently reported. This information allowed us to build a theoretical model (994 residues) for the A domains of human FV/FVa (residues 1–656 and 1546–1883). Structural analysis of the FV model indicates that: (a) the three A domains are arranged in a triangular fashion as in the case of CP and the organization of these domains should remain essentially the same before and after activation; (b) a Type II copper ion is located at the A1–A3 interface; (c) residues R306 and R506 (cleavage sites for APC) are both solvent exposed; (d) residues 1667–1765 within the A3 domain, expected to interact with the membrane, are essentially buried; (e) APC does not bind to FVa residues 1865–1874. Several other features of factor V/Va, like the R506Q and A221V mutations; factor Xa (FXa) and human neutrophil elastase (HNE) cleavages; protein S, prothrombin and FXa binding, are also investigated.

Keywords: APC-resistance; blood coagulation; factor V; protein modeling; thrombosis

The coagulation cascade involves sequential enzymatic activation of serine protease zymogens, with the nonenzymatic cofactors, FV and FVIII, as critical elements. Thrombin cleaves fibrinogen to generate fibrin, the end product of the cascade, which stabilizes the hemostatic clot. FVa, the activated form of FV, serves as the cofactor within the prothrombinase (PT) complex, a multi-molecular machinery which enhances significantly the generation of thrombin (reviewed by Jenny et al., 1994; Dahlbäck & Stenflo, 1994; Rosing & Tans, 1997). FV is homologous to FVIII and both, FV and FVIII deficiencies, can lead to severe bleeding disorder. FV circulates in blood as a single chain 333,000 molecular weight glycoprotein with little or no procoagulant activity (Nesheim et al., 1979). The primary structure of human FV has been reported (Kane & Davie, 1986; Jenny et al., 1987; Kane et al., 1987), and starting from the N-terminus, FV consists of domains A1, A2, B, A3, C1, and C2. The A domains have each about 310 residues, the B domain has about 840 residues and each C domain has about 150 residues. FV is cleaved by thrombin within the B domain at positions R709, R1018, and R1545 (Jenny et al., 1987) to give rise to the active cofactor (FVa), which is composed of a heavy chain (domains A1–A2 and a short segment from the B domain) and a light chain (domains A3–C1–C2). The heavy and light chain are noncovalently bound in the presence of divalent metal ions (Guinto & Esmon, 1982).

The PT complex consists of the serine protease FXa, the nonenzymatic cofactor Va, calcium ions, and an appropriate phospholipid surface. Factor Va, within the PT complex serves as a membrane receptor for FXa, as an effector which enhances FXa catalytic activity for the conversion of prothrombin to thrombin and as promoting the interaction between prothrombin and the prothrombinase complex (Nesheim et al., 1979; van Rijn et al., 1984). The PT complex is about 300,000 to 1,000,000-fold more efficient in the activation of prothrombin than FXa alone (Nesheim et al., 1979; Rosing et al., 1980). FXa weakly binds to intact FV (Suzuki et al., 1982) but seem to interact with relatively high affinity with the heavy and light chains of FVa (Tucker et al., 1983; Annamalai et al., 1987; Kalafatis et al., 1994c) while prothrombin binds only to the FVa heavy chain (Guinto & Esmon, 1984; van de Waart et al., 1984; Luckow et al., 1989). It is known that APC cleavage at FVa R506 reduces the affinity of FVa for FXa (Nicolaes et al.,

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1995) suggesting that the R506 area has contact with FXa. Removal of FVa residues 683–709 results in an impaired FXa-FVa interaction and greatly reduced FVa cofactor activity (Bakker et al., 1994). Both FV and FVa bind with high affinity ($K_d < 10^{-9}$ M) in a calcium independent fashion to membranes that contain negatively charged phospholipids (Bloom et al., 1979; Pusey et al., 1982; van de Waart et al., 1983). It has been proposed that the C2 (Ortel et al., 1992, 1994; Kalafatis et al., 1994a) and A3 (Kalafatis et al., 1990, 1994a) domains of FVa interact through ionic and hydrophobic forces, respectively, with the membrane surface.

Proteolytic inactivation of FVa by APC (Kisiel et al., 1977) is one of the key reactions in the regulation of thrombin formation. APC catalytic activity is stimulated by a nonenzymatic cofactor, protein S (PS), and negatively charged phospholipids (Bakker et al., 1992). APC cleaves human FVa within the heavy chain at R306, R506, and R679 (Kalafatis et al., 1994b). Deficiencies in protein C (PC) and PS have been associated with increased risk for thrombosis. Recently, Dahlbäck et al. (1993) have reported a novel genetic risk factor for thrombosis which involves a single point mutation in the FV gene leading to the R506Q replacement (Bertina et al., 1994; Dahlbäck & Hildebrand, 1994; Greengard et al., 1994; Voorberg et al., 1994). This FV abnormality (FV-R506Q) is referred to as APC resistance (reviewed by Dahlbäck, 1997) and has been further characterized by several groups (Kalafatis et al., 1995; Nicolaes et al., 1995; Aparicio & Dahlbäck, 1996; Egan et al., 1997). APC was proposed to interact with the light chain of FVa (Krishnaswamy et al., 1986) with potential contribution of FVa residues 1865–1874 (Walker et al., 1990) while FVa residues 493–506 could be involved in the binding of PS and FXa (Heeb et al., 1996). APC cleavage at R506 is selectively inhibited by the presence of FXa (Rosing et al., 1995) while PS is thought to counteract the ability of FXa to protect FVa from APC cleavage (Solymoss et al., 1988). PS seems to enhance FVa cleavage at R306 by APC (Rosing et al., 1995; Egan et al., 1997). PS has also an APC-independent activity since it directly inhibits prothrombin activation through interactions with FVa (Heeb et al., 1993) and Xa (Heeb et al., 1994).

Recently, an anticoagulant function for FV has been reported. Intact FV has been proposed to be a synergistic cofactor to the PS-APC system in the degradation of FVIIIa (Shen & Dahlbäck, 1994; Lu et al., 1996; Varadi et al., 1996). It has been suggested that a region from FV B domain could stimulate FVIIIa inactivation by APC (Shen & Dahlbäck, 1994; Lu et al., 1996). Varadi et al. (1996) have shown that FV-R506Q has reduced cofactor activity in the APC degradation of FVIIIa when compared to wild type FV.

The three A domains of FV and FVIII are homologous to the three A domains of CP, the major transport protein for copper in plasma (Takahashi et al., 1984). Interestingly, FV and FVIII also bind copper ion (Mann et al., 1984; Bihoreau et al., 1994). Human CP is a single chain glycoprotein of 1,046 amino acid residues (Takahashi et al., 1984). It belongs to a family of blue copper oxidases, with structural subunits based on the cupredoxin domain (Murphy et al., 1997). The cupredoxin fold is an eight-stranded Greek key β-barrel that was observed first in plastocyanin and azurin (Adman et al., 1978; Colman et al., 1978). FV instability and heterogeneous glycosylation have so far hindered its structural study by X-ray crystallography. Recent advances in computational chemistry combined with analysis of previously reported experimental/clinical data can provide structure-function insights. For instance, theoretical models for factor VIII (Pan et al., 1995; Pemberton et al., 1997) were recently reported using the well established comparative model building method (Greer, 1990). In the present investigation, a model for the A domains of FV was developed using the X-ray structure of CP as template (Zaitseva et al., 1996). This model allows us to propose molecular mechanisms for the rather complex FV/FVa set of interactions and inactivation by APC.

Results and discussion

The structure of human CP has been solved and it was found that its three A domains are arranged in a triangular fashion with each domain made up of two cupredoxin-type folds. Electron microscopy (EM) investigations have been reported for FV/FVa and membrane bound FV/FVa but a 3D structure with atomic resolution was needed to understand further FV/FVa essential cofactor functions and inactivation.

Validation of the model

The overall structure of the FV model shows the basic features of the cupredoxin-type fold. Thus, each A domain is made essentially of two β-barrels. The present model (domains A1, A2, A3) fits into an approximate box of 65 × 75 × 85 Å (1 Å = 0.1 nm). When looking down the pseudo-threefold axis, the three A domains could fit in a circle having a diameter of about 80–90 Å. The three A modules were screened interactively and their 3D structure as well as the distribution of the hydrophobic, polar and charged residues were found in accordance with the X-ray template and known characteristics of protein structure. For instance, the residues expected to be charged at neutral pH are solvent exposed or, if buried, involved in ionic interaction and/or hydrogen bonding. No severe steric clashes were noticed during the modeling process of the individual A domains nor at the interfaces, supporting the quality of the structure.

In the FV model all insertions/deletions as compared to ceruloplasmin are in solvent exposed areas and loop structures (Fig. 1). It is well known that loop structures and insertions are more difficult to predict. Long loops also tend to be flexible, and high resolution structures are seldom obtained experimentally for these regions of a protein. In the present FV model, the areas more difficult to model involved about 40 residues out of 994 and encompass regions of residues 302–317, 442–446, 1659–1673, and 1727–1736. The 302–317 insertion can be built as an extended loop (data not shown) or could have, as presented here, several residues in helical conformation as predicted using the Profile fed neural network systems from Heidelberg (Rost & Sander, 1993) (Fig. 2). This segment contains eight positively charged residues and one glutamic acid and should, thus, be essentially solvent exposed. All these charged residues in this region are solvent exposed, suggesting that the selected conformation is of reasonable accuracy. Such helical structure could indeed be of functional importance (see below). With the conformation selected, the regions of residues 442–446 and 1659–1673 display favorable contact with the remaining part of the protein or are appropriately solvent exposed. In CP, the segment corresponding to FV residues 1727–1736 was missing from the coordinate file but was relatively easy to build.

The disulfide bridges have been reported for the heavy chain of bovine FV (Xue et al., 1994). They are conserved in the CP structure and correspond to the ones proposed in Figure 1 (C139–C165,

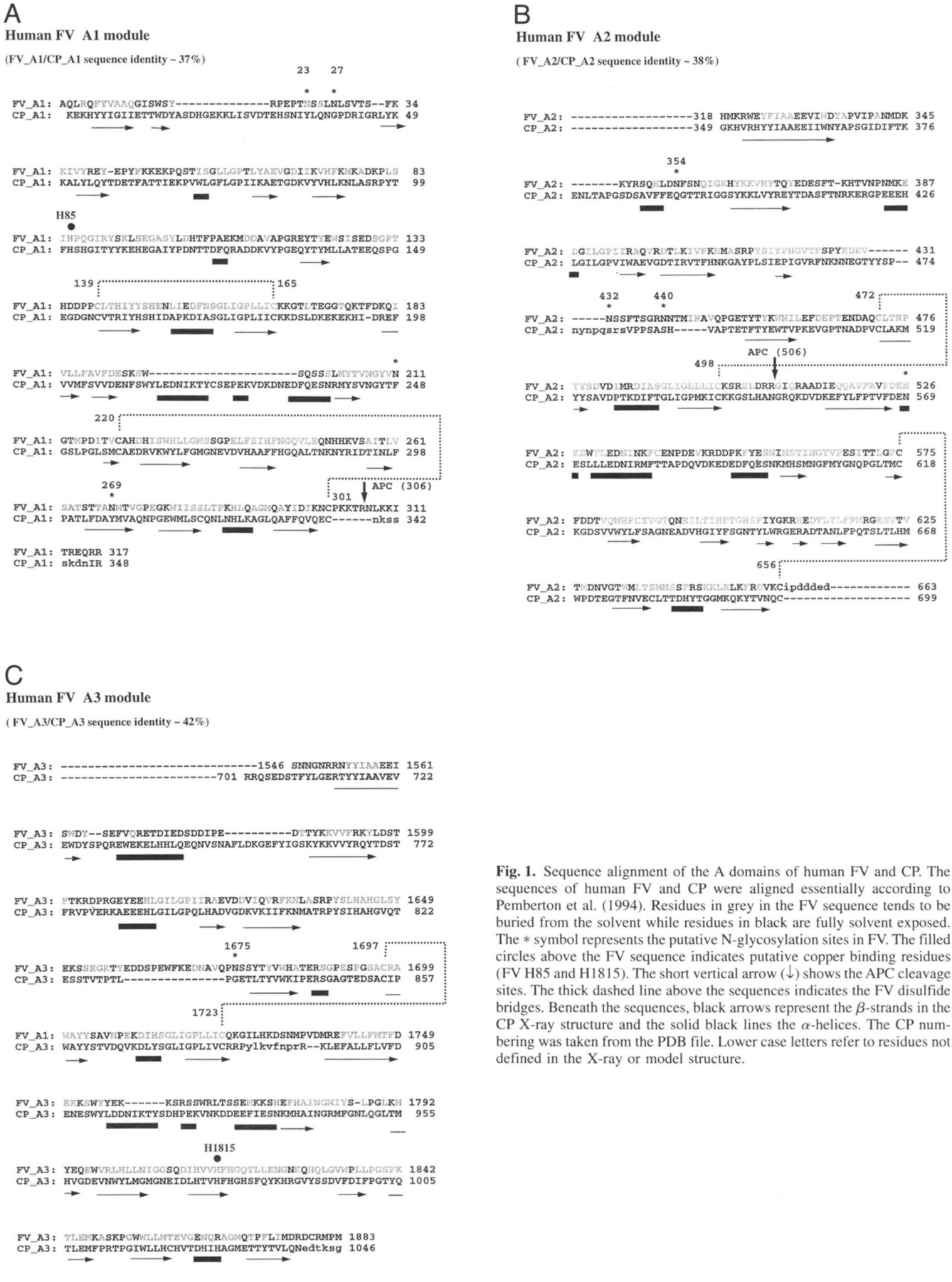

Fig. 1. Sequence alignment of the A domains of human FV and CP. The sequences of human FV and CP were aligned essentially according to Pemberton et al. (1994). Residues in grey in the FV sequence tends to be buried from the solvent while residues in black are fully solvent exposed. The * symbol represents the putative N-glycosylation sites in FV. The filled circles above the FV sequence indicates putative copper binding residues (FV H85 and H1815). The short vertical arrow (↓) shows the APC cleavage sites. The thick dashed line above the sequences indicates the FV disulfide bridges. Beneath the sequences, black arrows represent the β-strands in the CP X-ray structure and the solid black lines the α-helices. The CP numbering was taken from the PDB file. Lower case letters refer to residues not defined in the X-ray or model structure.

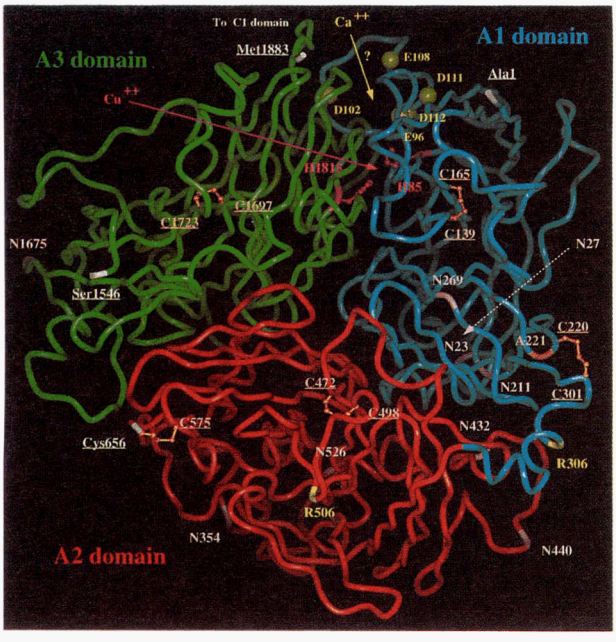

Fig. 2. Ribbon diagram of the three A domains of FV. The model is viewed down the pseudo-threefold axis. The disulfide bridges (ball and stick model) are shown in orange; the asparagines part of a consensus sequence for N-glycosylation are colored light magenta (N27 is on the opposite face of the molecule), alanine 221 possibly involved in FV deficiency, is painted in orange; R306 and R506 (cleavage sites for APC) are in yellow; the N-term of the A1 domain and C-term of the A2 domain are in white, the N-term and C-term of the A3 domain are also shown in white; the two histidines (H85 and H1815), most likely involved in copper binding, are in magenta (ball and stick model); potential residues (E96, D102, E108, D111, D112) involved in calcium binding are shown as CPK sphere (yellow).

C220–C301, C472–C498, C575–656, C1697–C1723) as found after analysis of the model (Fig. 2). Taken together, the above observations strongly suggest that the FV model is correct.

Copper binding site

Metal ions of blue oxidases (e.g., ceruloplasmin) are divided into three spectroscopically distinguishable types, which are referred to as Type I ("blue" copper, with intense optical absorbance around 600 nm), Type II (with no visible absorbance), and Type III (absorbing at 330 nm) (see Adman, 1991). Six copper atoms have been identified in the CP crystal structure (Zaitseva et al., 1996). Three copper ions occupy mononuclear centers while the remaining three ions form a trinuclear cluster. In CP, there are three Type I copper binding sites, two Type III and one Type II. It is known that FVIII contains one copper ion per molecule (Bihoreau et al., 1994). Tagliavacca et al. (1997) have found after site directed mutagenesis experiments that copper ion most likely binds to a Type I site (present in CP) involving at least FVIII C310 (C319 in CP, S282 in FV) in the A1 domain. These authors have also shown that copper binding at this site is of importance for the domain folding, for the proper association of the subunits and for FVIII/FVIIIa activity. Based upon these data we have investigated the FVIII model (Pemberton et al., 1997) and easily found the experimentally proposed copper binding site. This supports the accuracy of the FVIII structure and the fact that ceruloplasmin can be used to model accurately the A domains of both FVIII and FV.

Bovine FV and FVa have been shown to bind a non-type I and non-type III copper ion by atomic absorption and emission spectroscopy (Mann et al., 1984). Moreover, it has been suggested that copper ion in FVIII is liganded to different residues than the copper ion in FV (Tagliavacca et al., 1997). Indeed, when comparing the human FV model and the bovine FV sequence (Guinto et al., 1992) with the CP X-ray structure, only one copper binding site is conserved and corresponds to the Type II copper of CP, which involves H101 and H978 (Table 1). The homologous residues in human FV are H85 and H1815, while CP Y107 and S102, indirectly involved in copper binding, are respectively human FV Y91 and P86. The putative copper binding site in the FV model is consistent with the study reported by Mann et al. (1984). A copper ion, in both FV and FVIII, could contribute to the stabilization of the domain structure and of the A1/A3 interface.

Calcium binding site

It is known that calcium binds to bovine and human FV and that neither the heavy chain nor the light chain alone exhibits significant affinity for this metal ion (Hibbard & Mann, 1980; Guinto & Esmon, 1982). In the FV model, a calcium binding pocket could involve all or some of the following residues at the A1–A3 interface (nearby the copper binding site): E96, D102, E108, D111, D112 (Fig. 2). These negatively charged residues are essentially conserved in the bovine FV, CP, and FVIII sequences. However, other regions cannot be ruled out with the presently available information.

Biostructural pathology

The occurrence of parahemophilia (FV deficiency) is low, by contrast with hemophilia A (FVIII deficiency). Two point mutations within the A modules of FV have been reported so far. One involved a A221V substitution in the A1 domain (Murray et al., 1995) and the second, the R506Q replacement within the A2 domain (Bertina et al., 1994; Greengard et al., 1994; Voorberg et al., 1994; Zöller & Dahlbäck, 1994).

Plasma from the patient presenting with the A221V substitution had reduced FV antigen level and reduced activity. Alanine 221 is located in a loop and is solvent exposed (Figs. 1, 2), thus its replacement by a valine could be tolerated. In the multiple sequence alignment presented by Pemberton et al. (1997), it can be seen that this alanine is conserved in CP and FV but is replaced by a histidine in FVIII. The potential structural problems due to the A to V substitution could be disturbance of a partially buried salt bridge between K304 and E275 and/or direct steric clashes with E275 and/or loss of the C220–C301 disulfide. However, based upon structural analysis of the model, this naturally occurring mutation may not be the reason for the phenotype observed. It would be of interest to explore further the role of this mutation in vitro.

Arginine 506 is solvent accessible and is located at the tip of a loop (Fig. 2). It may have ionic interaction with D504. The R506Q mutation could induce some slight conformational changes but will essentially be unfavorable for the interaction with APC D189 (chymotrypsinogen numbering, see Mather et al., 1996), located at the bottom of the specificity pocket. Intact FV has been shown to be a cofactor, together with PS, in the APC-mediated FVIIIa inactivation (Shen & Dahlbäck, 1994; Lu et al., 1996; Varadi et al., 1996) while the FV-R506Q protein seems to be much less effective as APC cofactor during this process (Varadi et al., 1996). Four

Table 1. *Cerulosplasmin and FV copper binding sites*

Copper binding residues in CP	Equivalent residues in human FV	Equivalent residues in bovine FV	Comments
H637	L594	L598	Type I copper in CP, not conserved in FV
C680	S637	T641	
H685	P642	P646	
M690	L647	L651	
H276	F239	F239	Type I copper in CP, not conserved in FV
C319	S282	S282	
H324	H287	H287	
H975	H1812	H1799	Type I copper in CP, not conserved in FV
C1021	T1858	T1845	
H1026	N1863	I1850	
M1031	M1868	M1855	
H163	H147	Y147	Type III copper in CP, not conserved in bovine FV but partially conserved in human FV
H980	H1817	H1804	
H1020	N1857	D1844	
H101	H85	H85	Type II copper in CP, conserved in FV
H978	H1815	H1802	
H103	Q87	Q87	Type III copper in CP, not conserved in FV
H161	Y145	Y145	
H1022	E1859	E1846	

hypotheses can be made here, (1) the R506 area binds APC in a region located outside the enzyme active site, (2) APC cleavage at R506 is necessary for FV anticoagulant activity, (3) the R506 area interacts with protein S, and (4) the R506 area interacts with FVIIIa.

Membrane binding

A recombinant FV lacking the C2 domain lost its ability to bind to the membrane (Ortel et al., 1994). FVa could also interact with the phospholipid surface via the A3 domain (Krishnaswamy & Mann, 1988; Kalafatis et al., 1990). Bovine FVa residues 1654–1752 (1667–1765 in human), in the A3 domain, could be important for interaction with neutral phospholipids (Kalafatis et al., 1994a). After analysis of the FV model, it can be seen that most of these A3 residues are buried from the solvent and belong to the hydrophobic core of the protein (Fig. 3). Such a hydrophobic peptide could interact with neutral phospholipids but in a nonphysiological way. The amino acids solvent exposed in the FV model belonging to the peptide segment 1667-1765 involve approximately K1667–Y1678, Q1724–E1740, and E1757–R1765 (Figs. 1, 3). The region 1724–1740 is close to M1883 and possibly close to the C2 domain. Thus, if the A3 domain of FV binds to the membrane, the most likely region involves only residues 1724–1740 while the region around residues R1765 and T1767 should not be involved in this process since these residues are accessible to FXa and human neutrophil elastase cleavage, respectively, in the presence of phospholipids (Samis et al., 1997; Thorelli et al., 1997). The region of residues 1667–1678 contains mainly charged amino acids, and this is not consistent with the fact that hydrophobic forces have been

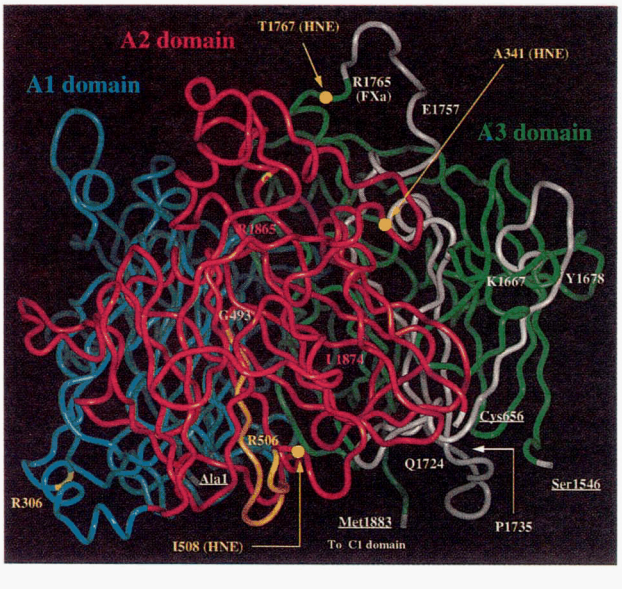

Fig. 3. Potential binding site for PS, APC, and membrane. Ribbon diagram of the three A domains of FV viewed perpendicular to the pseudo threefold axis. FVa residues 493–506, proposed as potential binding site for FXa and PS (Heeb et al., 1996), are shown in orange. In the model structure only residues K499 to R506 are solvent exposed. APC was proposed to bind to FVa residues 1865–1874 (Walker et al., 1990). These residues are shown in magenta in the model but are buried from the solvent. Residues 1667–1765 (A3 domain) could be involved in membrane binding (Kalafatis et al., 1994a). These residues are colored in white and are mainly buried from the solvent but for regions K1667–Y1678, Q1724–E1740, and E1757–R1765 (see text). FXa and HNE cleavage sites are also mentioned.

shown to be essential for the peptide-membrane interaction. Based upon structural analysis of the FV model, we suggest that only the C2 (and maybe the C1) domain interacts with the membrane. The two C domains would serve, in part, as a spacer, positioning the A domains at the appropriate distance from the membrane plane for optimal functional activity. Such hypothesis is consistent with the schematic representation of membrane bound FVa, as shown by EM study (Stoylova et al., 1994) or with the EM investigation of Lampe et al. (1984), which points out that the light chain component (A3–C1–C2) of FVa bound to the membrane surface appears to be largely external to the phospholipids.

It has been reported that the heavy chain of FVa can have direct contact with neutral phospholipids and that this interaction is not ionic in nature (Koppaka et al., 1997). After structural analysis of the A domains, we suggest that departure of the A3 domain from its triangular organization within the A1–A2–A3 structure leads to the exposure of hydrophobic side chains that are buried at the A1–A3 and A2–A3 interfaces. After inactivation of FV/FVa by APC, the interaction of the A domain(s) with the membrane, however, could be of physiological importance.

Factor Xa Protein S binding sites

It has been reported that PS and FXa could interact with FVa residues 493–506 (Heeb et al., 1996). From this peptide, residues K499, S500, R501, D504, R505, and R506 are solvent exposed in the model (Figs. 1, 3). Interestingly, removal of FVa residues 683–709 or APC cleavage at R506 results in an impaired FXa-FVa interaction (Bakker et al., 1994; Nicolaes et al., 1995). These data are consistent because the location of these two regions could be relatively close in space (possibly around 10–20 Å). FVa residues 499–506 could interact with FXa, and this result would explain the observed protective effect of FXa from APC cleavage at R506 (Rosing et al., 1995). Interestingly, residues 558–565 and 1811–1818 from FVIIIa have been proposed to interact with factor IXa (Fay et al., 1994; Lenting et al., 1996). The equivalent segments in FV involve residues 502–509 and 1677–1682, respectively, and most of them are solvent exposed.

It has also been suggested that FVa residues 507–520 could be involved in FXa binding (Heeb et al., 1996). FVa residues I508, R510, A511, A512, D513, I514, and E515 are solvent exposed and could thus have contact with FXa. The fact that FXa hardly binds to intact FV (Suzuki et al., 1982) and the above data suggest that the B domain could have direct or indirect contact with regions surrounding R506.

Two monoclonal antibodies directed either against the A1 or A3 domain of FV inhibited the FVa–FXa interaction (Kalafatis et al., 1994c). Since it has been proposed that the membrane bound FVa light chain alone does not interact with FXa (Pusey & Nelsestuen, 1984), additional information is required in order to determine if the antibodies recognized the FXa binding site at the surface of FVa or if they inhibited the FXa–FVa interaction due to steric hindrance. Since after APC inactivation of FVa, the A2 domain dissociates (Mann et al., 1997) and because in this situation the remaining FV intermediate does not bind FXa, it is likely that key contact areas between FVa and FXa involve the A2 domain. Such hypothesis could be investigated in a near future since a model structure for FXa has just been reported (Sabharwal et al., 1997).

It is known that PS stimulates APC cleavage at FVa R306 (Rosing et al., 1995; Egan et al., 1997) and that PS induces relocation of the APC active site 10 Å closer to the membrane surface (Yegneswaran et al., 1997). The distance between R306 and R506 in the model structure is about 35 Å. It is, however, still very difficult to envision the exact mechanism of action of PS from these data. On the one hand, PS could bind to residues 499-506 and could thus counteract the protective effect of FXa for APC cleavage at R506 and orient APC for optimal cleavage at R306. On the other hand, if PS binds to FVa residues 499–506, it should protect or affect significantly the cleavage at R506 while it seems that PS does not play a role at this site (Egan et al., 1997).

Prothrombin binding sites

Prothrombin forms a 1:1 complex with FVa heavy chain. This interaction is of moderate affinity and calcium independent in the absence of a membrane surface (Guinto & Esmon, 1982; Luckow et al., 1989). However, the Gla domain of prothrombin plays a role in the prothrombin-FVa interaction when phospholipids are present (Dahlbäck & Stenflo, 1980). After inactivation of FVa by APC the A2 domain dissociates and prothrombin binding is lost (Mann et al., 1997). Because within the prothrombinase complex FVa is partially protected from APC cleavages but for the R306 site and since FXa protect APC cleavage at R506 (Rosing et al., 1995), we suggest that prothrombin has direct interaction with the A2 domain of FVa, in areas surrounding R506 but different from the FXa binding site. Possibly, as in the case of FXa binding, FVa residues 683–709 could play a role in the interaction with prothrombin (Bakker et al., 1994).

Factor Xa and neutrophil elastase cleavages

The only FXa cleavage that can be investigated with the present model structure involves human FV residue R1765 (Thorelli et al., 1997). This residue is solvent exposed (Figs. 1, 3) and because the experiments were carried out in the presence of phospholipids, this region should be at about 80 Å from the membrane surface. Human neutrophil elastase cleaves FVa at A341, I508, and T1767 (Samis et al., 1997). These three cleavage sites are not influenced by the presence of phospholipids, indicating that these regions are located outside the membrane binding area. These three residues are found on solvent exposed loops in the FV model (Figs. 1, 3). These data support also the accuracy of the FV model structure.

Activated protein C binding sites and FV/FVa inactivation

APC seems to bind to FVa light chain via a site encompassing FV residues 1865–1874 (Walker et al., 1990). These residues, however, are mostly buried inside the protein core (Figs. 1, 3). This peptide, found to inhibit the inactivation of FVa by APC, should thus act in a nonphysiological way.

FVa is cleaved by APC at residues R306, R506, and R679 (Kalafatis et al., 1994b). These authors have suggested that cleavage by APC of the membrane bound FVa at R506 promotes cleavage at R306 and R679 but Nicolaes et al. (1995) have shown that prior cleavage at R506 is not required for cleavage at R306 after studies of the FV-R506Q protein. The physiological role of APC cleavage at residues R679 is not well defined by contrast with cleavages at R306 and R506. For instance, Egan et al. (1997) have reported that APC cleavage at R679 does not contribute to the inactivation of FVa. The rate of APC cleavage at R506 is about 20-fold higher than at R306 (Nicolaes et al., 1995). In addition, as described above, FXa seems to protect FVa cleavage at R506 while PS has

been suggested to enhance APC cleavage at R306 (Rosing et al., 1995; Egan et al., 1997). It has also been reported that APC cleavages of FVa leads to dissociation of the A2 domain, which then results in a factor Va intermediate with no activity (Mann et al., 1997). This last reaction would resemble the spontaneous dissociation of the A2 domain of FVIIIa that follows thrombin activation (Lollar & Parker, 1990). Finally, intact membrane bound FV seems to be cleaved slowly and sequentially by APC at residues R306, R506, R679, and K994 (Kalafatis et al., 1994b). Such information would suggest that at least R506 and R679 are directly or indirectly protected by the B domain.

In the present model structure, only FV R306 and R506 can be investigated. R306 is solvent exposed and could be located within a distorted helical segment between the A1 and A2 domains (Fig. 2) or on an extended and most likely flexible loop structure (data not shown). Indeed, this segment may undergo conformational changes and adopt both conformations, like in the case of the serpin reactive loop (Wei et al., 1994). In all situations, important structural rearrangements would be required for appropriate docking into the APC active site cleft. As mentioned above, secondary structure prediction indicated that the R306 segment could be in helical conformation. Such a strained structure would be consistent with the fact that after APC cleavages, the A2 domain dissociates. Cleavage of the R306 peptide bond could thus release energy and promote the dissociation of the A2 domain. However, R306 should be at all time accessible to APC and the cleavage at R506 is not required to gain access to the 306–307 peptide bond while, however, some conformational changes after cleavage at R506 may facilitate the cleavage at R306. Arginine 306 has in its direct vicinity several positively charged residues (FV residues K299, K303, K309, K310, R313) not directly counterbalanced by negative ones that could play a role during APC cleavage and binding.

R506 is solvent exposed (Figs. 1–3) and can "plug" easily into the APC specificity pocket but some local conformational rearrangements would be needed, from about the P5–P3 and P2′–P5′ residues (Schechter & Berger, 1967), in order to match the canonical structure necessary for optimal serine protease-substrate/inhibitor interaction (Bode & Huber, 1991). The P2P1P1′ residues adopt already the canonical conformation in the present model, and thus, by contrast with the R306 segment, the R506 peptide within the native FV/FVa structure seems better suited for interaction with the APC active site cleft. Several uncompensated positively charged residues are found in the direct vicinity of R506. These involve R316, R320, R400, R501, R505, and R510. Residue 506 is located between two β-barrels within the A2 domain, thus, after the R506 cleavage, conformational changes are likely to occur and could explain in part the loss of FXa binding.

Interestingly, FVIIIa is cleaved by APC at R336 and R562 (Fay et al., 1991), and these residues are homologous to FVa R306 and R506 respectively. Further work could involve comparison of the FV and FVIII model structures together with docking of the APC X-ray structure (Mather et al., 1996) at its respective cleavage sites.

From factor V to factor Va

EM investigations have been reported for FV, FVa, and membrane bound FVa (Lampe et al., 1984; Dahlbäck, 1986; Fowler et al., 1990; Mosesson et al., 1990; Stoylova et al., 1994). FVa was seen as composed of two domains, each having a diameter of about 80 Å (Dahlbäck, 1986). The overall diameter of the present FV model, when looking down the pseudo-threefold axis, is about 80–90 Å. It is possible that the three A domains represent one of the two spheres seen in this EM study. The two C domains spacing out the A domains of about 80 Å with respect to a membrane surface would be consistent with the schematic diagram reported by Stoylova et al. (1994) and observations made by Lampe et al. (1984). This is also in line with the expected 70–90 Å distance between the membrane plane and the active site cleft of proteases as evaluated by fluorescence energy transfer (Mutucumarana et al., 1992; Yegneswaran et al., 1997). Mosesson et al. (1990) proposed that intact FV has an irregular oblong shape of about 100–200 Å that displays no major structural rearrangement after release of the B domain. Absence of conformational rearrangement would be consistent with the present model structure. Fowler et al. (1990) and Mosesson et al. (1990) have suggested that the heavy and light chains of FVa form a globular structure, while the B domain has an extended rod-like structure projecting out from the globular structure in intact FV. These data together with the fact that the B domain has 25 potential sites for N-glycosylation and that several regions are sensitive to proteases suggest that this domain should have extensive contact with the solvent. It has been reported (Fay & Smudzin, 1989) after fluorescence energy transfer measurements for intact FVIII that the distance between residues C528 (A2 domain) and C1858 (A3 domain) is around 20 Å (25–30 Å in the FVIII model structure). This information also suggests that the A domains are in close contact before activation.

These data, together with the fact that FV and FVa bind copper ion equally well, and the present modeling study, suggest that, after activation by thrombin, the B domain of FV is removed while the three A domains remain in close contact. Because (1) intact FV has reduced affinity for FXa, (2) a FXa binding site at the surface of FVa should be located around R506, (3) APC catalyzes very slowly intact FV, (4) FV and FVa have essentially the same affinity for the appropriate phospholipid surface, (5) the C2 domain binds to the membrane, (6) at least intact FV cleavage at R306 by APC is phospholipid-dependent, (7) the area of R1765 and T1767 are not in contact with the membrane plane, (8) R306, R506, and R1765 have to be at about 80 Å from the membrane, and (9) possibly the entire A3 domain has no direct interaction with the phospholipids, we suggest that only the C2 domain could contact the membrane while the B domain could partially cover the region of R506 but let the region of R306 more exposed.

Conclusion

The quality of the present FV/FVa model structure is strongly supported since the theoretical work is in good agreement with the experimental data (e.g., disulfide bridges; accessibility of residues cleaved by APC, HNE, and FXa; absence of severe steric clashes during the modeling process). Numerous structural features and functions of FV/FVa have been analyzed, and new experiments can be designed based upon the model structure.

Materials and methods

A Silicon Graphics Indigo2 (SGI) R10000 workstation was used for the construction of the models together with the molecular modeling softwares InsightII, Biopolymer, Homology and Discover (Biosym-MSI). Figures were prepared within InsightII and edited with the SGI routine showcase.

The sequences of ceruloplasmin and human FV were aligned according to Figure 1. This alignment follows essentially the one proposed by Pemberton et al. (1997). The sequence identity between the FV and CP A domains is around 40%. The X-ray structure of CP (Zaitseva et al., 1996) (entry 1KCW) was obtained from the Protein Data Bank (PDB) (Bernstein et al., 1977) and used as a template to build the three A domains of factor Va. The insertion regions (Fig. 1) were built from a search (Jones & Thirup, 1986) among some of the high-resolution protein structures present at the PDB (Hobohm & Sander, 1994). The fragments that best accommodate the structure were introduced in the FVa model. Deletions in FV when compared to CP were effected by computationally removing the appropriate residues. Side-chain orientations were then optimized interactively, if needed, using a rotamer library (Ponder & Richards, 1987) while monitoring nonbond energy values. The minor steric clashes, deletion, and bond strains due to the introduction of the new side chains and loop building were regularized by a short energy minimization within Discover. The entire model was then briefly energy minimized. The normalized static solvent accessibility was calculated according to Lee and Richards (1971).

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