The crystal structure of activated protein C-inactivated bovine factor Va: Implications for cofactor function

Ty E. Adams, Matthew F. Hockin*, Kenneth G. Mann, and Stephen J. Everse†

Department of Biochemistry, College of Medicine, University of Vermont, 89 Beaumont Avenue, Burlington, VT 05405

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In vertebrate hemostasis, factor Va serves as the cofactor in the prothrombinase complex that results in a 300,000-fold increase in the rate of thrombin generation compared with factor Xa alone. Structurally, little is known about the mechanism by which factor Va alters catalysis within this complex. Here, we report a crystal structure of protein C inactivated factor Va (A1-A3-C1-C2) that depicts a previously uncharacterized domain arrangement. This orientation has implications for binding to membranes essential for function. A high-affinity calcium-binding site and a copper-binding site have both been identified. Surprisingly, neither shows a direct involvement in chain association. This structure represents the largest physiologically relevant fragment of factor Va solved to date and provides a new scaffold for the future generation of models of coagulation cofactors.

In developed countries, the majority of deaths can be directly or indirectly attributed to an imbalance in hemostasis, leading to thrombosis. These thrombi are a natural result of the coagulation cascade, a process characterized by the localized, but “explosive,” generation of α -thrombin and the subsequent formation of a platelet-fibrin clot at the site of vascular injury (reviewed in ref. 1). Central to this cascade is the catalytic acceleration of each step through the assembly of the vitamin K-dependent enzyme complexes. The best studied complex, prothrombinase, is composed of the serine protease factor Xa, the cofactor protein factor Va, and calcium ions on a phospholipid membrane. The formation of this complex accelerates the conversion of prothrombin to α -thrombin by a factor of 3×10^5 relative to factor Xa alone (2). This rate enhancement is partly a consequence of factor Xa and prothrombin interactions with the membrane, but more importantly the increase is due to interactions with factor Va that alter both the K_M and k_{cat} of the reaction process. Factor Va binds tightly to the platelet membrane ($K_d \approx 10^{-9}$ M) and serves as the “glue” by increasing the affinity of factor Xa for the membrane by a factor of 10^2 to 10^5 (3) and influencing the catalytic efficiency of prothrombin activation (k_{cat} increases $\approx 3 \times 10^3$) (2).

Produced in hepatocytes, factor V is secreted into the plasma as a single chain, composed of six domains (A1-A2-B-A3-C1-C2), that is devoid of coagulant activity (4, 5). Studies of bovine factor V reveal cleavage sites for α -thrombin at Arg-1536, Arg-1006, and Arg-713, forming the activated molecule factor Va, a heterodimer composed of a single heavy (A1-A2, residues 1–713) and light (A3-C1-C2, residues 1537–2183) chain associated in a calcium-dependent manner (6, 7). Activation results in the removal of the B domain and exposure of the factor Xa-binding site on factor Va, which leads to assembly of the prothrombinase complex and the subsequent rapid generation of thrombin (8, 9). It remains unclear whether the factor Xa-binding site is simply masked by the B domain or is formed by conformational changes resulting from its removal.

One of the key reactions in down-regulating coagulation is the inactivation of factor Va by the anticoagulant activated protein C (APC) (10). APC cleaves at Arg-505 and Arg-306, leading to the spontaneous release of the A2 domain and a complete loss

of cofactor activity (11). The remaining fragment, factor Va_i, is composed of the A1 domain noncovalently associated with the light chain (Fig. 1A). Individuals carrying mutations in factor V at any of the APC cleavage sites, such as factor V^{Leiden}, have an increased risk of thrombosis due to incomplete inactivation of factor Va (12).

Factor V shares strong functional and sequence homology with factor VIII (anti-hemophilic factor). Both have an identical domain organization with the B domains that act as large activation peptides (comprising nearly half of each procofactor), with no detectable homology either to each other or to any other known protein. The A domains (≈ 330 aa) of factors V and VIII share $\approx 40\%$ sequence identity with each other and roughly 30% with the A domains of ceruloplasmin (13). The C domains (≈ 150 aa) of factors V and VIII are $\approx 43\%$ identical and have no strong homology to any other known proteins. There is a weak homology with the discoidin-like proteins, a family of proteins involved in cell adhesion (14). Recent structures of recombinant C2 domains from both factor V and factor VIII are consistent with those observed in other discoidin domain-containing proteins (15, 16).

Membrane binding of factor Va is mediated through interactions involving the light chain. Specifically, these interactions have been localized to the C2 domain (17). Antibodies to the C2 domain of both factors V and VIII have been shown to interfere with membrane binding and inhibit cofactor function (18, 19). Deletion of the entire C2 domain results in a complete loss of phosphatidylserine-specific membrane binding (20). Alanine-scanning mutagenesis within the C2 identified several key polar and hydrophobic amino acids as necessary for achieving maximal cofactor function (21, 22).

Overall, the biophysical properties of the prothrombinase complex have been described in exquisite detail, yet the structural basis of its interactions remains elusive. An understanding of how factor Va influences the catalytic activity of factor Xa is crucial for deciphering the function of this complex and may provide targets for the treatment of hemostatic disorders. Here, we present the 2.8-Å crystal structure of factor Va_i, which reveals a domain arrangement that predicts a more extensive membrane binding. Identification of the high-affinity calcium-binding site, as well as the location of a copper ion, suggests a possible mechanism for heavy- and light-chain association. Using this information, we can begin to develop new paradigms for the function of these cofactors *in vivo*.

Materials and Methods

Bovine factor Va was purified by using a modified procedure from Nesheim *et al.* (23). Bovine APC was a generous gift from Haematologic Technologies (Essex Junction, VT).

Abbreviations: APC, activated protein C; rmsd, rms deviation; EM, electron microscopy.

Data deposition: The atomic coordinates and structure factors have been deposited in the Protein Data Bank, www.pdb.org (PDB ID code 1SDD).

*Present address: Department of Human Genetics, Howard Hughes Medical Institute, University of Utah, 15N 2030E Suite 5440, Salt Lake City, UT 84112-5331.

†To whom correspondence should be addressed. E-mail: stephen.everse@uvm.edu.

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Fig. 1. The structure of bovine factor Va. (A) Schematic drawing of the structure of bovine factor Va. The extent and names of the five domains, metal-binding sites, and phosphorylation sites are indicated. Dashed lines and outlined fonts depict the A2 domain that is removed in factor Va_i. (B) Ribbon diagram of bovine factor Va_i indicating the positions of the carbohydrates (orange) and the metals (Ca²⁺, gray; Cu²⁺, pink). A van der Waals surface representation is shown in the background. Domains are color coded throughout all figures as follows: A1, red; A3, blue; C1, green; and C2, yellow. All structural figures were prepared by using PYMOL (46).

Inactivation of Bovine Factor Va by Bovine APC. Bovine factor Va (40 μ M) was extensively dialyzed against 20 mM Hepes, 150 mM NaCl, and 2 mM CaCl₂ (pH 7.4) (HBS-Ca). Factor Va was incubated with 100 μ M phospholipid vesicles (75% phosphatidylcholine:25% phosphatidylserine) at 37°C for 1 hr. Bovine APC was added (250 nM), and the sample was incubated at 37°C for 3 hr. Factor V activity was monitored by single-stage clotting assays. The sample was loaded onto a Poros HQ20 (4.6 $\times$ 100 mm) equilibrated in 20 mM Hepes and 2 mM CaCl₂ and eluted with a gradient elution of 0–500 mM NaCl in equilibration buffer over 10 min. Fractions identified by SDS/PAGE as containing A2-domainless factor Va_i were pooled and analyzed for residual factor Va activity. Purified protein was stored in HBS-Ca at –20°C.

Crystallization and Data Collection. Purified bovine factor Va_i in 20 mM Hepes, 150 mM NaCl, and 2 mM CaCl₂ (pH 7.4) was crystallized at $\approx$ 6.5 mg/ml by the vapor diffusion sitting-drop method at 12°C against 200 mM MgCl₂ and 16% polyethylene glycol (PEG) 3350 (pH 5.0). After 5–21 days, diffraction quality crystals appeared (Table 1). Three isomorphous heavy atom derivative crystals were identified from native crystals soaked in mother liquor containing either 10 mM tetrakismercuroxymethane (TAMM), 10 mM ethylmercury (EtHg), or 2.5 mM lead acetate (PbAc) (Table 2, which is published as supporting information on the PNAS web site).

Data Processing and Structure Refinement. All diffraction data sets were processed by using DENZO, and individual data sets were scaled and merged by using SCALEPACK (24). All data were

Table 1. Data collection and refinement statistics

	Native*
Resolution limits, Å	30 to 2.8
Space group	$P2_12_12_1$
Cell dimensions, Å	$a = 63.37$ $b = 86.56$ $c = 229.20$
Reflections	30,822
Completeness, [†] %	97.4 (94.9)
Redundancy	3.6
$I/\sigma^†$	16.9 (4.1)
$R_{\text{sym}}, ^\dagger$ %	6.9 (29.4)
Model details	
No. of protein atoms	7,012
No. of solvent molecules	390
Additional ligands	5 NAG, 1 Cu^{2+} , 1 Ca^{2+}
Average B-factor, Å ²	
Protein main chain	46.2
Protein side chain	47.3
Solvent molecules	50.9
$R_{\text{factor}} (R_{\text{free}})$, %	23.3 (29.2)
rmsd from ideal geometry	
Bond lengths, Å	0.008
Bond angles, °	1.412
Residues in allowed Ramachandran regions, %	98.5

*Collected at the Cornell High Energy Synchrotron Source A-1 beamline ($\lambda = 0.935$ Å) using an Area Detector Systems Co. Quantum-210 charge-coupled device detector.

[†]Data in parentheses represent the highest resolution shell (2.90 to 2.80 Å).

subsequently scaled to the native data by using SCALEIT (25), and heavy-atom sites were determined by SOLVE (26). Heavy-atom refinement and phasing were carried out by using the maximum likelihood program MLPHARE in the CCP4 program suite (25). A single round of density modification in SOLOMON (27) was followed by additional heavy-atom refinement, and phasing yielded phase estimations at 3.7 Å with a final figure of merit of 0.83. The resulting map was not immediately interpretable. The partial phase information was used in a molecular replacement search by using the 6D phased rotation/translation program BRUTEPTF[‡] with the previously solved factor V C2 domain (PDB 1CZT) and factor Va A1 domain model (PDB 1FV4) as search models. The search results yielded two unique A domain solutions with correlation coefficients of 0.198 and 0.186, as well as two unique C domain solutions with correlation coefficients of 0.249 and 0.217. Model phases combined with experimental phases produced interpretable density and allowed for manual model fitting and rebuilding of the molecular replacement solution. The structure was refined with alternating rounds of refinement, including simulated annealing by using CNS (28) and model rebuilding in o (29) (Table 1).

Results and Discussion

Domain Structure and Organization. The bovine Va_i structure is composed of two of the three A domains from factor V (A1 & A3) and both C domains (C1 & C2) (Fig. 1A). Each A domain is comprised of two linked cupredoxin-like β -barrels and shares high structural conservation with each other and the three A domains of ceruloplasmin [rms deviation (rmsd) between 0.98 and 1.37 Å for 268 C α atoms] (30). A single metal ion is observed within each A domain, and the site is distinct from the metal-binding sites found in ceruloplasmin. The factor Va_i C domains

Fig. 2. Comparison of homologous cofactor models. Domain orientation of the model of factor Va (PDB ID code 1FV4) (A) (45), the cryoEM structure of factor VIIIa (B) (47), and the crystal structure of bovine Va_i (C). The sizes and orientation of the ovals were scaled to match the cryoEM C2 domain.

can be described as a distorted jelly-roll β -barrel with a high degree of structural similarity between the C1 and C2 (rmsd 0.96 Å for 157 C α atoms). The structure of these is very similar to the recombinant C2 structures of human factors V and VIII (rmsd 0.61–0.87 Å for 159 C α atoms) (15, 16).

One of the most exciting aspects of the structure is the unique domain arrangement (Fig. 1B). Consistent with earlier models, the A1 and A3 domains are arranged around a pseudo-threefold axis similar to that observed in ceruloplasmin. Several disordered loops are not visible in our structure, including residues flanking the additional bovine APC cleavage site found within the A3 domain. Within the A1 domain, the disordered loops are localized along one edge of the domain and may be due to partial destabilization of the domain caused by the removal of the A2 domain. Looking down the threefold axis within the A domains, the C domains are aligned “edge-to-edge,” forming a platform upon which the A domains rest. This model is completely different from models in which the C1 was predicted to be stacked above the C2 domain (Fig. 2). In our factor Va_i structure, the C domains are side-by-side, suggesting that both domains may be important in membrane binding.

Domain Interfaces. The interface between the C1 and C2 domains buries <700 Å² of surface area and contains neither a substantial electrostatic or hydrophobic character. In fact, only three hydrogen bonds exist between the two domains, two of which occur within the four amino acid linker between the disulfide bonds in the C1 (Cys-1866–Cys-2020) and C2 (Cys-2025–Cys-2180) domains. These interactions, in conjunction with a hydrogen bond between Asp-1863 in the A3 domain and Ser-2026 in the C2 domain, may restrain the linker between the C1 and C2 domains, thereby restricting the orientation of the C2 domain with respect to the rest of the molecule.

The interface between the C1 and A3 domains contains both hydrophobic and electrostatic interactions that bury 1,758 Å² of surface area. One end of the interface is anchored by hydrophobic interactions between residues from the A3 domain (Leu-1860 and Val-1862) and the C1 domain (Leu-1931, Val-1996, and Val-2022). The other end of the interface predominantly involves hydrogen bonds and salt bridges between a loop (Phe-1966–Val-1974) that interrupts a β -strand in the C1 domain (Asn-1962–Asn-1980) and charged residues within the A3 domain.

In contrast, the A1 domain does not substantially interact with the C2 domain. Whether this lack of interaction is physiologically relevant or the result of relaxation of the domain due to the excision of the A2 domain is unclear and must await a factor Va structure. This hypothesis may also explain why the A1 domain has the highest average B-factors among the four domains. The lack of interactions between the A1 domain and light chain is entirely mediated by means of interactions with the A3 domain.

[‡]Strokopytov, B. & Almo, S., American Crystallographic Association Annual Meeting, July 21–26, 2001, Los Angeles, CA, P218 (abstr.).

Fig. 3. Stereo images of the metal-binding sites in factor Va_i. (A) The copper-binding site in the A3 domain (blue) with anomalous density for the copper is shown at 3 σ . The trigonal planar coordination geometry is shown with dashed lines. Nearby residues from the A1 domain (backbone shaded red) are shown, and the distance to the closest residue is shown in red. (B) The octahedral coordination geometry (dashed lines) of the calcium-binding site in the A1 domain (red).

Within this reciprocally contoured surface, we observe a network of hydrogen bonds dispersed throughout the entire 2,662 Å² of buried surface area.

Metal-Binding Sites. In our structure, we clearly see the anomalous signal for a copper ion within the buried surface between the A1 and A3 domains (Fig. 3A). Experimental evidence has demonstrated that both factor V and VIII bind a single copper atom (31, 32). A functional role for copper in factor V or Va has not yet been ascertained, but, in factor VIII, a type II copper leads to $\approx$ 100-fold affinity between the factor VIII subunits (33). In our structure, ligands to the Cu²⁺ include His-1802, His-1804 (both predicted), and Asp-1844 in a trigonal planar coordination geometry. Although homology modeling predicted that a Cu²⁺ in factor Va would bridge the heavy and light chains (34), the metal in our structure is >5 Å from any potential ligand in the A1 domain. Therefore, this copper ion may have a structural role in providing additional stabilization of the A1·A3 interface rather than directly linking the two domains.

Chain association is required for factor Va function and has been shown to be dependent on a divalent cation (7). Factors V and Va contain a single high-affinity Ca²⁺ site as well as several low-affinity sites (35). The occupancy of the high-affinity site is essential for the interaction of the heavy and light chains and the subsequent activity of factor Va (7, 36). Historically, this Ca²⁺ was believed to bridge the heavy and light chains; however, our factor Va_i structure clearly reveals that the Ca²⁺ is entirely coordinated by ligands in the A1 domain (Fig. 3B). These ligands include the side chains of both Asp-111 and Asp-112, along with the main chain carbonyl oxygens of Lys-93 and Glu-108. Recent mutational data support a role for Ca²⁺ binding in both factors Va and VIIIa at this site (37, 38).

Because chain association cannot be directly attributed to the coordination of Ca²⁺, we anticipate that the loop comprising Lys-93–Asp-112 adopts a conformation that results in several essential interactions between the A1 and the A3 domains. For example, the carboxylate side chain of Glu-96 forms a hydrogen bond with His-1804 in the A3 domain, and the terminal amino group of Lys-93 forms a hydrogen bond to the backbone carbonyl of Trp-1840. These interactions, along with a hydrophobic stacking of Tyr-100 and Leu-1842, suggest that disruption of the Ca²⁺ binding loop may interfere with the packing of the A3

Fig. 4. Potential C domain membrane interactions. (A) The membrane-binding spikes of the C1 (Left) and C2 (Right) domains. The domains are displayed in similar orientations with respect to the overall β -barrel fold. Residues potentially involved in membrane binding are shown. (B) Packing interactions of the tryptophans from spike C2-1 (2050 and 2051) with a hydrophobic pocket in the A3 domain (white, hydrophobic; blue, polar) from a neighboring molecule.

domain against the A1 domain, which may be sufficient to force the dissociation of the heavy and light chains of factor Va.

Membrane Interactions. Protruding from the bottom of the β -sandwich in each C domain are three β -hairpin loops, referred to as “spikes,” that form a pocket lined with both hydrophobic and polar amino acids (Fig. 4A) (15). Factor Va_i spike C2-1 (Ser-2045–Trp-2055) contains two tryptophans (Trp-2050 and Trp-2051) at its apex extending away from the pocket. Macedo-Ribeiro *et al.* (15) identified two crystal forms of the recombinant factor V C2 domain in which this spike moved by 7 Å. They hypothesized that this movement resulted in the exposure of the phospholipid-binding pocket and allowed membrane binding. In our factor Va_i structure, these tryptophans are constrained by crystal-packing interactions with an A3 domain from a neighboring molecule (Fig. 4B), burying them into a hydrophobic cleft on the A3 domain. In factor Va, this cleft may be masked by interactions with the A2 domain, yet these tryptophans clearly have a high propensity for inserting into a hydrophobic environment. In agreement with other studies, these tryptophans are the most likely point of lipid bilayer insertion during membrane binding of the C2 domain. However, conclusions regarding the physiological role of the movement of this loop with respect to membrane interaction must await a structure with lipid bound.

Given the position of the C1 domain relative to the C2 domain, it also has the potential to interact with the membrane. Like the C2 domain, the C1 domain contains three spikes although one spike (C1-1, Glu-1886–Trp-1891) contains a five-residue deletion eliminating the two putative membrane-inserting tryptophans. Nevertheless, at the apex of spike C1-3 (Gly-1939–Tyr-1948), Leu-1944 is solvent exposed and in position to insert into the membrane. The C1 spikes also contain several tyrosine residues (Tyr-1890, C1-1; Tyr-1904, C1-2; Tyr-1943, C1-3) located at or near the apex of each loop. Unlike the tryptophans on the C2 spikes, the tyrosines would not insert into, but rather

Fig. 5. Model of factor Va. Overlaid structure of ceruloplasmin (PDB 1KCW, yellow) on the bovine Va_i structure (black). For clarity, the ceruloplasmin A domain representing the A2 domain is depicted in red as a surface representation. Measurements do not include extended loops. (Right) Image rotated 90° about a vertical axis.

could interact favorably with, phospholipid membranes (39, 40). A very recent report using alanine-scanning mutagenesis identified these leucine and tyrosine residues on the C1-3 spike as important in prothrombinase activity (41). Additionally, two arginine residues in human factor Va (Lys-2010 and Arg-2014 in the bovine molecule) were shown to have a significant impact on function. In our structure, these particular residues are solvent exposed, lie on opposite sides of the domain, and could potentially interact with negatively charged phospholipid head groups on the membrane surface.

Structure Validation. Although a structural rearrangement due to APC inactivation cannot be completely ruled out, several pieces of evidence argue against this possibility. First, reconstructions of factor Va using electron microscopy (EM) depict a molecule extending ≈ 100 Å from the cell membrane (42), and these dimensions correlate well with the more recent 15-Å EM projection structure of factor VIIIa (43). In homology models of factors Va and VIIIa based on these EM data, a variety of domain orientations have been proposed (Fig. 2). Most notably, the C1 domain was predicted to stack upon the C2 domain vertically outward from the membrane, thereby lifting the A domains to a height appropriate for interaction with its specific enzyme partner, factors Xa and IXa, respectively. Our structure (Fig. 2C) has dimensions similar to the EM-derived values, with the differences attributed to the missing A2 domain. Second, overlaying ceruloplasmin on the A1 and A3 domains (rmsd 1.3 Å for 544 C α atoms) places the missing A domain exactly between them, without overlap (Fig. 5). The addition of this A domain (representing the A2 domain of factor Va) increases the height of the structure to 112 Å, well within the experimental error of the EM measurements. Third, fluorescence resonance energy transfer (FRET) data predict that the APC active site is 94 Å above the surface of the membrane (44). Inspection of the APC cleavage site (Arg-505) in the potential A2 domain reveals

that it lies ≈ 90 Å above the putative membrane surface whereas, when the C domains are stacked on top of one another, this site is only 75 Å above the membrane surface (45).

Conclusions. The structure of factor Va_i answers several important questions regarding factor Va function, including metal disposition, chain association, and membrane binding. We demonstrate that the Ca²⁺ is coordinated completely within the A1 domain and neither Ca²⁺ or Cu²⁺ plays a direct role in chain association. We believe that Ca²⁺ orders a critical loop within the A1 domain to allow for constructive interactions between the A1 and A3 domains. This hypothesis is supported by mutational studies of residues within this loop, as exemplified by the E96A mutation in factor Va, where the two chains remain associated in the presence of Ca²⁺ yet show a reduced cofactor activity (37). In our structure, Glu-96 does not participate in Ca²⁺ binding but instead interacts with the A3 domain. As well, removal of the copper ion results in no loss of factor Va cofactor function within the prothrombinase complex (unpublished data). Because we cannot attribute any particular function to copper binding, it may simply be a remnant of the cupredoxin-like protein fold.

The placement of the C domains adjacent to one another provides a platform that lifts the A domains to a height above the membrane surface appropriate for interaction with their physiologic partners (factor Xa, prothrombin, and APC). Our data in combination with recent mutational studies allow us to propose that both C domains contribute to the factor Va binding to the membrane surface. We suggest that membrane binding may be initiated by the C2 domain. The structural flexibility between this domain and the rest of the molecule would then allow the C1 domain to locate its cognate lipid within the membrane, thereby strengthening the overall affinity of factor Va for the platelet surface.

Due to its high degree of functional and structural homology to factor Va, the structure of factor Va_i provides a basis for

construction of a model of factor VIIIa. Because factor VIII deficiency is the causative agent of hemophilia A, modeling studies will be enhanced by the rich database of clinically relevant factor VIII mutations and will provide a more coherent approach to the design of pharmaceuticals for the treatment of hemophilia as well as other thrombotic disorders.

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- Mann, K. G., Butenas, S. & Brummel, K. (2003) *Arterioscler. Thromb. Vasc. Biol.* **23**, 17–25.
- Nesheim, M. E., Taswell, J. B. & Mann, K. G. (1979) *J. Biol. Chem.* **254**, 10952–10962.
- Krishnaswamy, S. & Mann, K. G. (1988) *J. Biol. Chem.* **263**, 5714–5723.
- Nesheim, M. E., Myrmet, K. H., Hibbard, L. S. & Mann, K. G. (1979) *J. Biol. Chem.* **254**, 508–517.
- Mann, K. G. & Kalafatis, M. (2003) *Blood* **101**, 20–30.
- Esmon, C. T. (1979) *J. Biol. Chem.* **254**, 964–973.
- Krishnaswamy, S., Russell, G. D. & Mann, K. G. (1989) *J. Biol. Chem.* **264**, 3160–3168.
- Nesheim, M. E. & Mann, K. G. (1979) *J. Biol. Chem.* **254**, 1326–1334.
- Guinto, E. R. & Esmon, C. T. (1984) *J. Biol. Chem.* **259**, 13986–13992.
- Walker, F. J., Sexton, P. W. & Esmon, C. T. (1979) *Biochim. Biophys. Acta* **571**, 333–342.
- Kalafatis, M. & Mann, K. G. (1993) *J. Biol. Chem.* **268**, 27246–27257.
- Dahlback, B., Carlsson, M. & Svensson, P. J. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 1004–1008.
- Gitschier, J., Wood, W. I., Goralka, T. M., Wion, K. L., Chen, E. Y., Eaton, D. H., Vehar, G. A., Capon, D. J. & Lawn, R. M. (1984) *Nature* **312**, 326–330.
- Fuentes-Prior, P., Fujikawa, K. & Pratt, K. P. (2002) *Curr. Protein Pept. Sci.* **3**, 313–339.
- Macedo-Ribeiro, S., Bode, W., Huber, R., Quinn-Allen, M. A., Kim, S. W., Ortel, T. L., Bourenkov, G. P., Bartunik, H. D., Stubbs, M. T., Kane, W. H. & Fuentes-Prior, P. (1999) *Nature* **402**, 434–439.
- Pratt, K. P., Shen, B. W., Takeshima, K., Davie, E. W., Fujikawa, K. & Stoddard, B. L. (1999) *Nature* **402**, 439–442.
- Ortel, T. L., Quinn-Allen, M. A., Keller, F. G., Peterson, J. A., Larocca, D. & Kane, W. H. (1994) *J. Biol. Chem.* **269**, 15989–15905.
- Ortel, T. L., Moore, K. D., Quinn-Allen, M. A., Okamura, T., Sinclair, A. J., Lazarchick, J., Govindan, R., Carmagnol, F. & Kane, W. H. (1998) *Blood* **91**, 4188–4196.
- Arai, M., Inaba, H., Higuchi, M., Antonarakis, S. E., Kazazian, H. H., Fujimaki, M. & Hoyer, L. W. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 4277–4281.
- Ortel, T. L., Devore-Carter, D., Quinn-Allen, M. A. & Kane, W. H. (1992) *J. Biol. Chem.* **267**, 4189–4198.
- Kim, S. W., Quinn-Allen, M. A., Camp, J. T., Macedo-Ribeiro, S., Fuentes-Prior, P., Bode, W. & Kane, W. H. (2000) *Biochemistry* **39**, 1951–1958.
- Nicolaes, G. A., Villoutreix, B. O. & Dahlback, B. (2000) *Blood Coagul. Fibrinolysis* **11**, 89–100.
- Nesheim, M. E., Katzmman, J. A., Tracy, P. B. & Mann, K. G. (1981) *Methods Enzymol.* **80**, 249–285.
- Otwinowski, Z. & Minor, W. (1997) *Methods Enzymol.* **276**, 307–326.
- Collaborative Computational Project (1994) *Acta Crystallogr. D* **50**, 760–763.
- Terwilliger, T. C. & Berendzen, J. (1999) *Acta Crystallogr. D* **55**, 849–861.
- Abrahams, J. P. & Leslie, A. G. W. (1996) *Acta Crystallogr. D* **52**, 30–42.
- Brunger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszewski, J., Nilges, M., Pannu, N. S., et al. (1998) *Acta Crystallogr. D* **54**, 905–921.
- Jones, T. A., Zou, J. Y., Cowan, S. W. & Kjeldgaard, M. (1991) *Acta Crystallogr. A* **47**, 110–119.
- Zaitseva, I., Zaitsev, V., Card, G., Moshkov, K., Bax, B., Ralph, A. & Lindley, P. (1996) *J. Biol. Inorg. Chem.* **1**, 15–23.
- Mann, K. G., Lawler, C. M., Vehar, G. A. & Church, W. R. (1984) *J. Biol. Chem.* **259**, 12949–12951.
- Bihoreau, N., Pin, S., de Kersabiec, A.-M., Vidot, F. & Fontaine-Aupart, M.-P. (1994) *Eur. J. Biochem.* **222**, 41–48.
- Wakabayashi, H., Koszelak, M. E., Mastri, M. & Fay, P. J. (2001) *Biochemistry* **40**, 10293–10300.
- Villoutreix, B. O. & Dahlback, B. (1998) *Protein Sci.* **7**, 1317–1325.
- Hibbard, L. S. & Mann, K. G. (1980) *J. Biol. Chem.* **255**, 638–645.
- Laue, T. M., Lu, R., Krieg, U. C., Esmon, C. T. & Johnson, A. E. (1989) **28**, 4762–4771.
- Zeibdawi, A. R., Grundy, J. E., Lasia, B. & Prydzial, E. L. (2004) *Biochem. J.* **377**, 141–148.
- Wakabayashi, H., Freas, J., Zhou, Q. & Fay, P. J. (2004) *J. Biol. Chem.* **279**, 12677–12684.
- Rinia, H. A., Boots, J. W., Rijkers, D. T., Kik, R. A., Snel, M. M., Demel, R. A., Killian, J. A., van der Eerden, J. P. & de Kruijff, B. (2002) *Biochemistry* **41**, 2814–2824.
- Roderick, S. L., Chan, W. W., Agate, D. S., Olsen, L. R., Vetting, M. W., Rajashankar, K. R. & Cohen, D. E. (2002) *Nat. Struct. Biol.* **9**, 507–511.
- Saleh, M., Peng, W., Quinn-Allen, M. A., Macedo-Ribeiro, S., Fuentes-Prior, P., Bode, W. & Kane, W. H. (2004) *Thromb. Haemostasis* **91**, 16–27.
- Stoylova, S., Mann, K. G. & Brisson, A. (1994) *FEBS Lett.* **351**, 330–334.
- Stoylova, S. S., Lenting, P. J., Kembell-Cook, G. & Holzenburg, A. (1999) *J. Biol. Chem.* **274**, 36573–36578.
- Yegneswaran, S., Wood, G. M., Esmon, C. T. & Johnson, A. E. (1997) *J. Biol. Chem.* **272**, 25013–25021.
- Pellequer, J. L., Gale, A. J., Getzoff, E. D. & Griffin, J. H. (2000) *Thromb. Haemostasis* **84**, 849–857.
- DeLano, W. L. (2002) *The PyMOL Molecular Graphics System* (DeLano Scientific, San Carlos, CA).
- Stoilova-McPhie, S., Villoutreix, B. O., Mertens, K., Kembell-Cook, G. & Holzenburg, A. (2002) *Blood* **99**, 1215–1223.