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## The Crystal Structure of Activated Protein C-inactivated Bovine Factor Va: Implications for Cofactor Function

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*Within the blood coagulation cascade, the process by which blood clots, factor Va serves as an essential protein cofactor for the efficient generation of thrombin, the primary blood-clotting enzyme. Upon the formation of the prothrombinase complex with factor Xa on a membrane surface, factor Va increases the catalytic efficiency of factor Xa 300,000-fold. The crystal structure of activated protein C (APC)-inactivated factor Va (Va<sub>i</sub>) is the first view of this multi-domain cofactor and begins to address questions regarding its mechanism, including membrane binding, chain association, and complex assembly.*

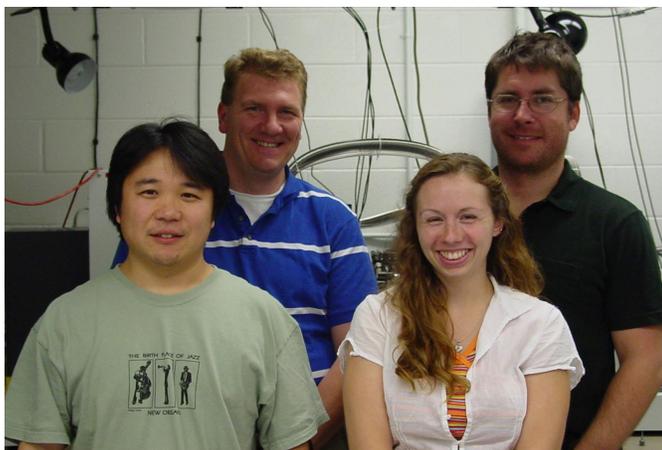
The majority of deaths throughout the world can be either directly or indirectly attributed to an imbalance in hemostasis, which leads to thrombosis – a blood clot. Under normal circumstances hemostasis is defined as the process that stops bleeding by activating the blood coagulation cascade. An action central to this cascade is the catalytic acceleration of each step through the assembly of the vitamin K-dependent enzyme complexes. This culminates in the conversion of prothrombin, an inactive zymogen in blood plasma, to its enzyme counterpart, thrombin, by the *prothrombinase* complex.

bin, to the membrane surface of activated platelets. Condensation of this enzyme-substrate pair on a surface contributes to the explosive generation of thrombin observed *in vivo*. The reaction is regulated through the inactivation of factor Va by APC, which removes the A2 domain, forming factor Va<sub>i</sub> and shutting down thrombin production. A common polymorphism in which a single APC cleavage site is removed, factor V<sup>Leiden</sup>, significantly increases the risk of thrombosis by sustaining factor Va activity.

We recently solved the crystal structure of bovine factor Va<sub>i</sub>. This

structure reveals a novel domain architecture that suggests a new mechanism for cofactor function (**Figure 1**). Our structure includes two A domains (A1 & A3), each encompassing two plastocyanin-like folds, and two C domains (C1 & C2) with weak homology to the discoidin family of lipid-binding proteins. In fact, historical data concerning the membrane binding ability of factor V has been localized to the C domains, primarily the C2 domain, and previous hypothetical models have proposed that membrane interaction is mediated strictly by the C2 domain. However, we found that the C domains in factor Va<sub>i</sub> are oriented in such a way that both domains may potentially interact with the membrane.

Factor V circulates in the plasma as a pro-cofactor comprised of six domains (A1-A2-B-A3-C1-C2). Thrombin activates factor V by excising the B domain, forming the heterodimer factor Va, composed of heavy chain (A1-A2) and a light chain (A3-C1-C2) associated in a calcium-dependent manner. During the initial stages of coagulation, factor Va actively recruits the enzyme, factor Xa, and the substrate, prothrom-



Authors: (Back row, from left) Stephen J. Everse and Ty E. Adams. (Front row) Kihachiro Umezaki and Sara K. Briggs

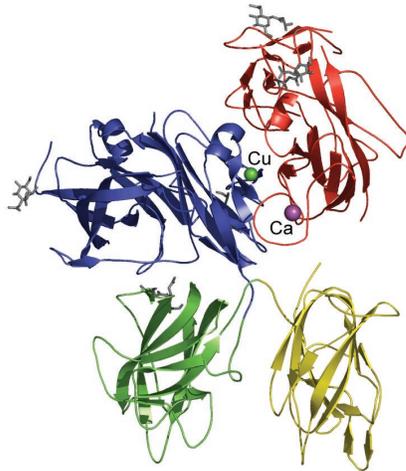
At the base of each C domain folds several loops adopt an extended conformation and expose several hydrophobic residues at the apex of these loops (**Figure 2**). In the literature, these loops are referred to as "spikes" and have been postulated to be critical phospholipid-binding sites. Indeed, mutations of these loops within the

C2 domain have been shown to reduce or knockout cofactor activity. Recent information has also shown that mutation of key hydrophobic residues within the spikes of the C1 domain decreases the membrane binding potential and activity of the cofactor. Along with our structure,

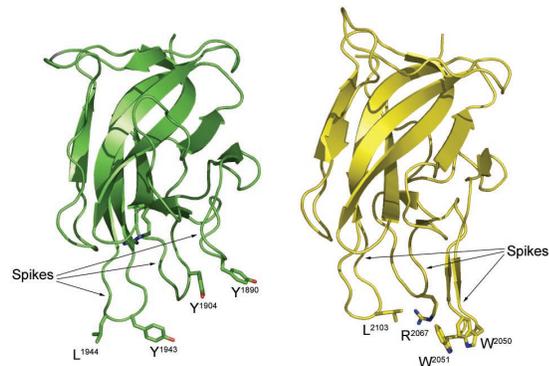
this suggests that the C1 domain is important for membrane interaction and cofactor function.

Our structure provided the first glimpse into how this cofactor, as well as the homologous cofactor factor VIII, functions in coagula-

tion. We can now use this information to develop improved methods of controlling coagulation *in vivo* for the treatment of thrombosis in such cases as factor V<sup>Leiden</sup> and explain hemophilia in various factor VIII mutations.



**Figure 1.** Ribbon diagram of the structure of bovine factor Va<sub>1</sub> (PDB ID code 1SDD). The domains are colored as follows: A1 (red), A3 (blue), C1 (green), and C2 (yellow). The two metal ions, calcium and copper, are shown as magenta and green spheres, respectively. The five carbohydrates are shown in grey as ball-and-stick.



**Figure 2.** Membrane binding domains. The three loops that compose the "spikes" in each domain are marked with arrows. Residues important for membrane interaction are shown as ball-and-stick.