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TOPICAL REVIEW

Phosphatidylserine Regulation of Coagulation Proteins Factor IXa and Factor VIIIa

Rinku Majumder[1]

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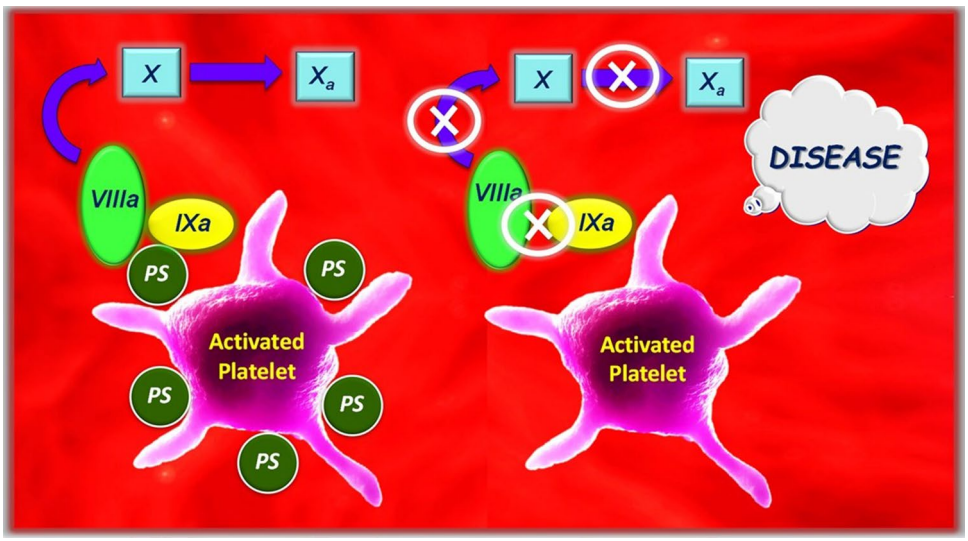
Abstract

Blood coagulation is an intricate process, and it requires precise control of the activities of pro- and anticoagulant factors and sensitive signaling systems to monitor and respond to blood vessel insults. These requirements are fulfilled by phosphatidylserine, a relatively miniscule-sized lipid molecule amid the myriad of large coagulation proteins. This review limelight the role of platelet membrane phosphatidylserine (PS) in regulating a key enzymatic reaction of blood coagulation; conversion of factor X to factor Xa by the enzyme factor IXa and its cofactor factor VIIIa. PS is normally located on the inner leaflet of the resting platelet membrane but appears on the outer leaflet surface of the membrane surface after an injury happens. Human platelet activation leads to exposure of buried PS molecules on the surface of the platelet-derived membranes and the exposed PS binds to discrete and specific sites on factors IXa and VIIIa. PS binding to these sites allosterically regulates both factors IXa and VIIIa. The exposure of PS and its binding to factors IXa/VIIIa is a vital step during clotting. Insufficient exposure or a defective binding of PS to these clotting proteins is responsible for various hematologic diseases which are discussed in this review.

Graphical Abstract



Keywords Phosphatidylserine · Factor IXa · Protein S · Coagulation · Factor Xa

✉ Rinku Majumder
rmajum@lsuhsc.edu

[1] Department of Biochemistry, Louisiana State University Health Sciences Center, MEB-7114, New Orleans, LA 70112, USA

Hemostasis at sites of blood vessel injury and its pathologic counterpart, thrombosis, involve multiple players in the blood coagulation system. Lipids, particularly anionic phospholipids, have long been recognized as key agents

that promote blood coagulation. Specifically, the anionic phospholipid phosphatidylserine (PS) is a crucial regulator molecule that controls production of thrombin from prothrombin (Majumder et al. 2005, 2002). Activation of human platelets exposes PS molecules on the platelet membrane surface, and the exposed PS binds to discrete and specific sites on prothrombin, factor X_a, and factor V_a (Majumder et al. 2005, 2002). Phosphatidylserine binding to these sites allosterically regulates factors X_a and V_a (Majumder et al. 2002; Zhai et al. 2002).

This review is focused on regulation of a key reaction of blood coagulation, i.e., the formation of factor X_a by the intrinsic Xase complex. The intrinsic Xase complex is composed of factor IXa and factor VIIIa and its activity is regulated by phosphatidylserine. The Xase is an excellent example of a proteolytic enzyme in blood coagulation, and the goal is to understand Xase regulation by PS in the Xase-mediated conversion of factor X. Particularly, we examine the allosteric nature of PS-mediated regulation of procoagulant proteins in blood coagulation.

Phosphatidylserine

Phosphatidylserine molecule, not the platelet membrane surface, is the key regulator of prothrombin activation by factor X_a (Majumder et al. 2002; Srivastava et al. 2002). This fact represented a paradigm shift in understanding regulation of blood coagulation. Negatively charged phospholipids, especially phosphatidylserine (PS), have key functions that control activation of factor X by the factor $VIII_a$-factor IX_a (Xase) complex (Gilbert and Arena 1996; Mathur et al. 1997). Phosphatidylserine-containing membranes increase the k_{cat} of the factors $VIII_a$–IX_a complex by more than 1000-fold (Gilbert and Arena 1996). Factor $VIII_a$ and factor IX_a bind specifically and with high affinity to PS-containing membranes (Gilbert and Drinkwater 1993; Mann et al. 1990; Mathur et al. 1997; Mertens et al. 1984). Similarly, the k_{cat} and K_M of factor IX_a-catalyzed activation of factor X in the presence of soluble phosphatidylserine molecule (C6PS) were 0.00038/min and 33 nM, respectively, and the k_{cat}/K_M was 1.1×10^4 M^{-1} min^{-1}, similar to the rate constant in the presence of PS/PC membranes (1.0×10^4 M^{-1} min^{-1}) (Rawala-Sheikh et al. 1990). Thus, as for factor Xa, it is PS (either in solution or in membrane) and not a membrane surface that regulates factor IXa activity (Majumder et al. 2014).

Factor IX_a

Factor IX is a vitamin K-dependent plasma protein that has a crucial function in blood coagulation (Mertens et al. 1984). After a series of posttranslational modifications, the mature protein (M_r 57,000) is a zymogen of serine protease factor IX_a (Mertens et al. 1984). Factor IX is activated by a complex of factor VII_a-tissue factor-Ca^{2+} or by factor XI_a–Ca^{2+} (Mertens et al. 1984). In the intrinsic pathway of blood coagulation, factor IX_a activates factor X by catalyzing the hydrolysis of a single-peptide bond at Arg^{194}-Ile^{195} in factor X (Mathur et al. 1997). In the absence of cofactors phospholipids and factor $VIII_a$, the catalytic efficiency of factor IX_a toward factor X is low ($k_{cat}/K_m = 10^2$ M^{-1} s^{-1}) (Gilbert and Arena 1995; Gilbert and Drinkwater 1993). Additionally, factor IX_a is poorly reactive toward other substrates and inhibitors that are usually highly reactive toward other proteases in this family. However, at optimum concentrations of calcium ions, addition of phospholipid and factor $VIII_a$ increases the catalytic efficiency of factor IXa by 10^6-fold (Gilbert and Arena 1996; Mathur et al. 1997).

Factor $VIII_a$

Factor VIII is synthesized as a protein of M_r 280,000 with significant internal sequence homology to factor V that defines a domain A1–A2–B–C1–C2 (Lollar and Parker 1989). Factor VIII requires proteolytic activation by thrombin or factor X_a to participate optimally in factor X activation (Gilbert et al. 1990). The characterization of the Xase complex consisting of factor IX_a and factor $VIII_a$ on a phospholipid membrane surface has been limited by difficulties in isolating factor $VIII_a$. The thrombin-mediated activation of factor VIII at its plasma concentration ($\cong$ 1 nM) at pH 7.4 is followed by nonproteolytic inactivation (Lollar and Fass 1984), which is accompanied by dissociation of the factor VIII A2 subunit (Lollar et al. 1984; Wakabayashi et al. 2014). The inactivation rate is reduced, but not prevented, by factor IX_a and phospholipids (Lollar et al. 1984). Different research groups reported a wide range of factor $VIII_a$ spontaneous inactivation rates. For example, Lollar et al. (Lollar et al. 1984, 1992) observed 90% loss in activity in 10 min, Griffith et al. (Griffith et al. 1982) reported 20% loss in activity in 10 min, and Fay et al. (Fay et al. 1991) observed 5% loss in activity in 80 min. However, porcine $VIII_a$ was stable at a concentration > 0.2 μM at pH 6.0 (Lollar et al. 1984).

Intrinsic Xase Complex

Blood coagulation occurs by a cascade of enzymatic reactions that represent two independent pathways, intrinsic and extrinsic, that converge to a common pathway with thrombin generation as the endpoint of the reactions (Monroe and Hoffman 2002). Following initiation of the intrinsic coagulation pathway, generated factor XI_a activates factor IX to factor IX_a. Conversely, in the extrinsic coagulation pathway,

factor VII_a forms a complex with tissue factor on a phospholipid membrane surface and activates factor IX (Davie et al. 1991; Lawson and Mann 1991; Mann et al. 1992). Factor IX, activated by these two different pathways, forms "intrinsic Xase complex" together with factor $VIII_a$ and Ca^{2+} on the phospholipid membrane surface (Davie et al. 1991; Monroe and Hoffman 2002). Finally, in the intrinsic pathway of the blood coagulation, the zymogen factor X is converted to factor X_a by the Xase complex that forms on the phospholipid membrane surface.

Function of Phosphatidylserine

The mechanisms are known by which factor X_a assembles with its cofactor factor V_a to form the prothrombinase complex that activates prothrombin to thrombin (Nesheim et al. 1979; Rosing et al. 1980). Assembly of this complex requires negatively charged membranes. The negatively charged phospholipid phosphatidylserine (PS) is a critical component of these thrombogenic membranes (Jones et al. 1985). A breakthrough in the study was the demonstration by the Lentz laboratory that a soluble form of PS, 1,2-dicaproyl-sn-glycero-3-phospho-l-serine (C6PS), binds to discrete sites on factors X_a (Banerjee et al. 2002; Majumder et al. 2003, 2002) and V_a (Majumder et al. 2002). This interaction alters the solution conformations of factors X_a and V_a, promotes factor X_a dimerization (Majumder et al. 2003), and enhances both the catalytic activity of factor X_a and the cofactor activity of factor V_a (Koppaka et al. 1996; Majumder et al. 2002). These studies showed for the first time that a fully active prothrombinase could be assembled with C6PS (Majumder et al. 2002). In addition, these studies not only provided a powerful tool (C6PS) for assembling in solution a normally membrane-associated enzymatic complex, but also the studies showed that the key to forming an active prothrombinase complex is binding of a limited number of PS molecules to specific sites on X_a and V_a and not X_a and V_a binding to a membrane surface. Phosphatidylserine accelerates the enzymatic activity of the prothrombinase complex by as much as 1500-fold (Majumder et al. 2003, 2005, 2002). By contrast, the complex of factor VII_a/tissue factor appears to be stimulated less than tenfold by PS. Moreover, factor IX_a is regulated by molecular PS (Majumder et al. 2014). The intrinsic fluorescence, amidolytic activity, and proteolytic activity of factor IX_a are regulated by PS, and calcium is needed for PS-mediated activation of factor IX_a (Majumder et al. 2014). Direct measurement by equilibrium dialysis confirmed that factor IX_a bound two molecules of C6PS (Majumder et al. 2014) with kds of 1.3 μM and 130 μM, respectively. Circular dichroism showed that factor IX_a undergoes conformational changes in the presence of C6PS and 3 mM calcium (Majumder et al. 2014). To ensure that a molecular form of C6PS and not micelles existed in the experiments, the C6PS critical micelle concentration was measured under each experimental condition, with pyrene as a fluorescent probe (Haque et al. 1995, 1999).

The organization of the components of the factor X activation complex strongly resembles the structure of the prothrombinase complex. Factor VIII is homologous to the procoagulant protein factor V, in amino acid sequence (Griffith et al. 1982; Lollar et al. 1992) and in function as a membrane-bound cofactor (Gilbert et al. 1990; Kane and Davie 1988; Mann et al. 1990). Factor IX has the same modular structure as Factor X: a γ-carboxyglutamate (Gla) domain, two epidermal growth factor-like (EGF) domains (EGF1 and EGF2), and the serine protease domain that occupies the C-terminal half of each molecule (Furie et al. 1999; Stenflo 1977). Because the proteins of the Xase complex are homologous to the proteins of the prothrombinase complex, and PS regulates all the proteins in the prothrombinase complex and factor IX_a in the Xase complex, it is reasonable to hypothesize that PS also has a significant activity in the regulation of the Xase complex.

On the basis of studies with PS/PC membrane and C6PS, we conclude that PS regulates factor X activation by factor IX_a in the presence and absence of factor $VIII_a$ to form the intrinsic Xase complex (Majumder et al. 2014).

Diseases Caused by Improper PS Regulation of Factor IXa/Factor VIIIa

When PS fails to be exposed or is exposed incorrectly, such that PS cannot bind to factor IXa/factor VIIIa, immunological and hematological diseases may occur, such as the Scott syndrome (Wielders et al. 2009), Systemic lupus erythematosus (Kawano and Nagata 2018), Hemophilia A (Croteau et al. 2021) and coagulation abnormalities, like thrombosis. Normally, when a vascular injury occurs, platelets are activated, and PS in the inner leaflet of the platelet membrane is transported to the outer leaflet of the membrane, where it provides a binding site for plasma protein complexes, such as factors $VIII_a$-IX_a (intrinsic Xase).

In Scott syndrome, PS translocation to the platelet membrane is defective. PS is one of the primary apoptotic cell ligands that provides eat-me signals to phagocytes. Upon recruitment of PS to the outer layer of the platelet membrane, phagocytes recognize PS directly or indirectly by cell–cell interactions mediated by specific bridging or adapter molecules on the surfaces of dying cells. Macrophages recognize additional abnormal cell characteristics such as elevated lateral mobility of PS. These interactions initiate signaling when factor Xa formation is impaired and, ultimately, when thrombin formation is impaired (Wielders et al. 2009).

Hemophilia A is an inherited bleeding disorder, caused by a deficiency of factor VIII (Bhatnagar and Hall 2018) that results in insufficient thrombin generation and fibrin formation (Brummel-Ziedins et al. 2009). Persons with Hemophilia A are categorized as having severe (< 1% of normal factor VIII activity), moderate (1–5%), or mild (5–40%) Hemophilia. Circulating microparticles (MP) are procoagulant because their surface contains PS. The level of MPs in plasma is greater in untreated Hemophilia A persons compared with healthy individuals (Brummel-Ziedins et al. 2009). A clinical study of plasma from severe Hemophilia A patients showed that the level of MPs decreased after factor VIII treatment and was inversely correlated with thrombin generation and fibrin formation (Jardim et al. 2017). These findings suggest that MPs may participate in the formation of hemostatic clots in severe Hemophilia A individuals. In an in vivo factor VIII-knockout Hemophilia A mouse model, a threefold increase in total MP level induced by soluble P-selectin infusion normalized the tail vein bleeding time (Hrachovinova et al. 2003). Thus, it is essential to state that PS has a crucial function in regulating factors IXa/VIIIa and in maintaining normal hemostasis.

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Data Availability The proteins and mutants which have been mentioned in the review will be available to all the researchers.

Declarations

Conflict of Interest There is nothing to disclose.

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