PART I

Introduction

CHAPTER 1

Overview of hemostasis

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Introduction

The maintenance of blood fluidity and protection from blood leakage provide major biophysical challenges for the organism. Nature has evolved a highly complex, integrated, and dynamic system which balances the presentations of procoagulant, anticoagulant, and fibrinolytic systems. These systems function collectively to maintain blood within the vasculature in a fluid state while at the same time providing potent leak attenuating activity which can be elicited upon vascular perforation to provide the rapid assembly of a thrombus principally composed of platelets and fibrin to attenuate extravascular blood loss. The dynamic control of this system is such that the coagulation response is under the synergistic control of a variety of blood and vascular inhibitors, resulting in a process that is regionally restricted to the site of vascular damage and does not propagate throughout the vascular system. The rapid coagulation response is also tightly linked to the vascular repair process during which the thrombus is removed by the fibrinolytic system which is also activated regionally to provide clot removal coincident with vascular repair.

A list of important procoagulant, anticoagulant, and fibrinolytic proteins, inhibitors, and receptors can be seen in Table 1.1.

Importance of complex assembly to coagulation

Laboratory data combined with clinical pathology lead to the conclusion that the physiologically relevant hemostatic mechanism is primarily composed of three procoagulant vitamin K-dependent enzyme complexes (which utilize the proteases factor VIIa, factor IXa, and factor Xa) and one anticoagulant vitamin K-dependent complex (which utilizes the proteases thrombin, meizo, and α-thrombin) [1,2] (Figure 1.1). These complexes—extrinsic factor Xase (tissue factor–factor VIIa complex), intrinsic factor Xase (factor VIIIa–factor IXa complex) [3], and the protein Case complex (thrombin–thrombomodulin) [4]—are each composed of a vitamin K-dependent serine protease, a cofactor protein and a phospholipid membrane; the latter provided by an activated or damaged cell. The membrane-binding properties of the vitamin K-dependent proteins are a consequence of the post-translational γ-carboxylation of these macromolecules [5]. The cofactor proteins are either membrane binding (factor Va, factor VIIIa), recruited from plasma, or intrinsic membrane proteins (tissue factor, thrombomodulin). Cofactor–protease assembly on membrane surfaces yields enhancements in the rates of substrate processing ranging from 10^5 to 10^9-fold relative to rates observed when the same reactions are limited to solution-phase biomolecular interactions between the individual proteases (factor VIIa, factor IXa, and factor Xa) and their corresponding substrates [6–8] (Figure 1.2a). Membrane binding, intrinsic to complex assembly, also localizes catalysis to the region of vascular damage. Thus, a system selective for regulated, efficient activity presentation provides for a regionally limited, vigorous arrest of hemorrhage.

Additional complexes associated with the "intrinsic" pathway are involved in the surface contact activation of blood [3]. However, the association of the contact-initiating proteins (factor XII, prekallikrein, high-molecular-weight kininogen) with hemorrhagic disease is uncertain [9].

Of equal importance to the procoagulant processes is regulation of anticoagulation by the stoichiometric and dynamic inhibitory systems. The extant effectiveness of the inhibitory functions is far in excess of the potential procoagulant responses. These inhibitory processes provide activation thresholds, which require presentation of a sufficient concentration of tissue factor prior to significant thrombin generation [10]. Antithrombin and tissue factor pathway inhibitor [11] are the primary stoichiometric inhibitors while the proteolytic thrombin–thrombomodulin–protein C system (protein Case, Figure 1.1) is dynamic in its function.

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Table 1.1 Procoagulant, anticoagulant, and fibrinolytic proteins, inhibitors, and receptors.

Protein	Molecular weight (kDa)	Plasma concentration		Plasma $t_{1/2}$ (days)	Clinical manifestation[a]		Functional classification
		nmol/L	μg/mL		H	T	
Procoagulant proteins and receptors							
Factor XII	80	500	40	2–3	−		Protease zymogen
HMW kininogen	120	670	80		−		Cofactor
LMW kininogen	66	1300	90				Cofactor
Prekallikrein	85/88	486	42				Protease zymogen
Factor XI	160	30	4.8	2.5–3.3	+/−		Protease zymogen
Tissue factor	44			N/A			Cell-associated cofactor
Factor VII	50	10	0.5	0.25	+	+/−	VKD protease zymogen
Factor X	59	170	10	1.5	+		VKD protease zymogen
Factor IX	55	90	5	1	+		VKD protease zymogen
Factor V	330	20	6.6	0.5	+	+	Soluble pro-cofactor
Factor VIII	285	1.1–1.5[b]	0.3–0.4[b]	0.3–0.5	+	−	Soluble pro-cofactor
VWF	255 (monomer)	Varies	10		+		Platelet adhesion carrier for FVIII
Factor II	72	1400	100	2.5	+	−	VKD protease zymogen
Fibrinogen	340	7400	2500	3–5	+	+/−	Structural protein cell adhesion
Factor XIII	320	94	30	9–10	+	+/−	Transglutaminase zymogen
Anticoagulant proteins, inhibitors, and receptors							
Protein C	62	65	4	0.33		+	Proteinase zymogen
Protein S	69	300	20	1.75		+	Inhibitor/cofactor
Protein Z	62	47	2.9	2.5	+/−		Cofactor
Thrombomodulin	100	N/A	N/A	N/A			Cofactor/modulator
Tissue factor pathway inhibitor	40	1–4	0.1	6.4×10^{-4} to1.4×10^{-3}			Proteinase inhibitor
Antithrombin	58	2400	140	2.5–3			Proteinase inhibitor
Heparin cofactor II	66	500–1400	33–90	2.5	+	+/−	Proteinase inhibitor
Fibrinolytic proteins, inhibitors, and receptors							
Plasminogen	88	2000	200	2.2			Proteinase zymogen
t-PA	70	0.07	0.005	0.00167			Proteinase zymogen
u-PA	54	0.04	0.002	0.00347			Proteinase zymogen
TAFI	58	75	4.5	0.00694		+	Carboxypeptidase
PAI-1	52	0.2	0.01	<0.00694			Proteinase inhibitor
PAI-2	47/60	<0.070	<0.005	−			Proteinase inhibitor
α2-Antiplasmin	70	500	70	2.6	+		Proteinase inhibitor
u-PAR	55						Cell membrane receptor

H, hemorrhagic disease/hemophilia; HMW/LMW, high/low molecular weight; N/A, not applicable; PAI-1, plasminogen activator inhibitor–1; T, thrombotic disease/thrombophilia; TAFI, thrombin activatable fibrinolysis inhibitor; t-PA, tissue-type plasminogen activator; u-PA, urokinase-type plasminogen activator; uPAR, urokinase receptor; VKD; vitamin K-dependent proteins; VWF, von Willebrand factor.
+, Presence of phenotype; −, absence of phenotype; ±, some individuals present with the phenotype and others do not.
[a]Clinical phenotype; the expression of either hemorrhagic or thrombotic phenotype in deficient individuals.
[b]Modified from [36,37].

Extrinsic pathway to blood coagulation

The initiating event in the generation of thrombin involves the binding of membrane-bound tissue factor with plasma factor VIIa [12]. The latter is present in blood at ∼0.1 nM [∼1–2% of the factor VII concentration (10 nM)] [13]. Plasma factor VIIa does not express proteolytic activity unless it is bound to tissue factor; thus factor VIIa at normal blood level has no significant activity toward either factor IX or factor X prior to its binding to tissue factor. The inefficient active site of factor VIIa permits its escape from inhibition by the antithrombin present in blood. Vascular damage [14] or cytokine-related presentation of the active tissue factor triggers the process by interaction with activated factor VIIa, which increases the catalytic constant k_{cat} of the enzyme and increases the rate of factor X activation by four orders of magnitude [15]. This increase is the result of the improvement in catalytic efficiency and the membrane binding of factor IX and factor X.

The tissue factor–factor VIIa complex (extrinsic factor Xase) (Figure 1.2) catalyzes the activation of both factor IX and factor

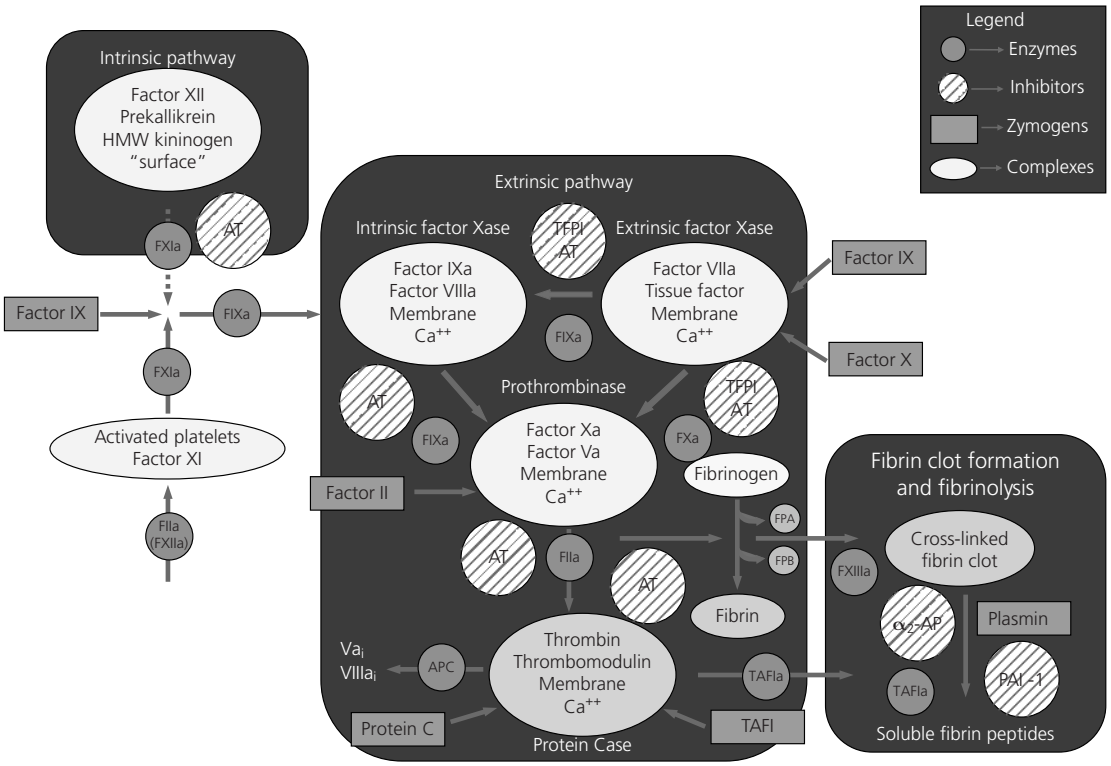

Figure 1.1 Overview of hemostasis. Coagulation is initiated through two pathways: the primary extrinsic pathway (shown on the right) and the intrinsic pathway (historically called the *contact* or *accessory pathway*, shown on the left). The components of these multistep processes are illustrated as follows: enzymes (*circles*), inhibitors (*hatched circles*), zymogens (*boxes*), or complexes (*ovals*). Fibrin formation is also shown as an oval. The intrinsic pathway has no known bleeding etiology associated with it, thus this path is considered accessory to hemostasis. Upon injury to the vessel wall, tissue factor, the cofactor for the extrinsic factor Xase complex, is exposed to circulating factor VIIa and forms the vitamin K-dependent complex, the extrinsic factor Xase. Factor IX and factor X are converted to their serine proteases factor IXa (FIXa) and factor Xa (FXa), which then form the intrinsic factor Xase and the prothrombinase complexes, respectively. The combined actions of the intrinsic and extrinsic factor Xase and the prothrombinase complexes lead to an explosive burst of the enzyme thrombin (IIa). In addition to its multiple procoagulant roles, thrombin also acts in an anticoagulant capacity when combined with the cofactor thrombomodulin in the protein Case complex. The product of the protein Case reaction, activated protein C (APC), inactivates the cofactors Va and VIIIa. The cleaved species, factors Va_i and $VIIIa_i$, no longer support the respective procoagulant activities of the prothrombinase and intrinsic Xase complexes. Once thrombin is generated through procoagulant mechanisms, thrombin cleaves fibrinogen, releasing fibrinopeptide A and B (FPA and FPB) and activate factor XIII to form a cross-linked fibrin clot. Thrombin–thrombomodulin also activates thrombin activatable fibrinolysis inhibitor (TAFIa) that slows down fibrin degradation by plasmin. The procoagulant response is downregulated by the stoichiometric inhibitors tissue factor pathway inhibitor (TFPI) and antithrombin (AT). TFPI serves to attenuate the activity of the extrinsic factor Xase trigger of coagulation. Antithrombin directly inhibits thrombin, factor IXa, and factor Xa. The intrinsic pathway provides an alternative route for the generation of factor IXa. Thrombin has also been shown to activate factor XI. The fibrin clot is eventually degraded by plasmin-yielding soluble fibrin peptides. α-AP, α-antiplasmin; HMW, high molecular weight; PAI-1, plasminogen activator inhibitor-1. Modified from [32].

X, the latter being the more efficient substrate [16]. Thus, the initial product formed is factor Xa. Feedback cleavage of factor IX by membrane-bound factor Xa enhances the rate of generation of factor IXa in a cooperative process with the tissue factor–factor VII complex [17].

The initially formed, membrane-bound factor Xa activates small amounts of prothrombin to thrombin [18]. This initial prothrombin activation provides the thrombin essential to the acceleration of the hemostatic process by serving as the activator for platelets [19], factor V [20], and factor VIII [21] (Figure 1.1). Once factor VIIIa is formed, the factor IXa generated by tissue factor–factor VIIa combines with factor VIIIa on the activated platelet membrane to form the "intrinsic factor Xase" (Figure 1.2a), which becomes the major activator of factor X. The factor VIIIa–factor IXa complex is 10^9-fold more active as a factor X activator and 50 times more efficient than tissue factor–factor VIIa in catalyzing factor X activation [22,23]; thus, the bulk of factor Xa is ultimately produced by the factor VIIIa–factor IXa complex (Figure 1.2).

As the reaction progresses, factor Xa generation by the more active "intrinsic factor Xase" complex exceeds that of the "extrinsic factor Xase" complex [24]. In addition, the "extrinsic factor Xase" complex is subject to inhibition by tissue factor pathway inhibitor (Figure 1.2b) [25]. As a consequence, most (>90%) of factor Xa is ultimately produced by the factor VIIIa–factor IXa complex in the tissue factor-initiated hemostatic

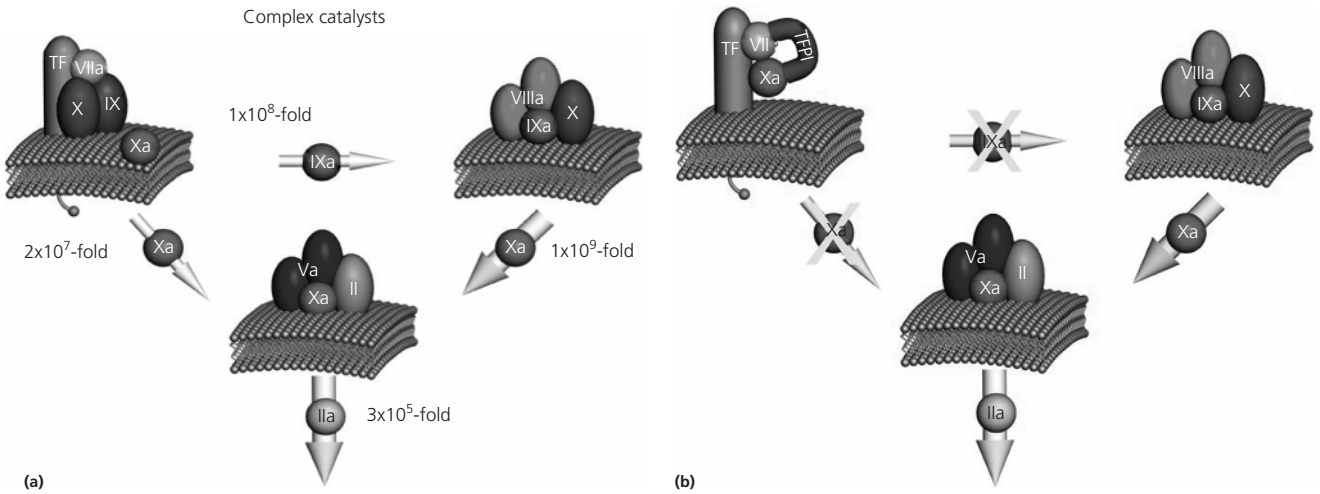

Figure 1.2 Vitamin K-dependent complex assembly. **(a)** The factor Xa generated by the tissue factor–factor VIIa complex activates a small amount of thrombin which activates factor V and factor VIII leading to the presentation of the intrinsic factor Xase (factor VIIIa–factor IXa) and prothrombinase (factor Va–factor Xa) complexes. At this point in the reaction, factor IXa generation is cooperatively catalyzed by membrane-bound factor Xa and by the tissue factor–factor VIIa complex. The thick arrow representing factor Xa generation is cooperatively catalyzed by factor VIIIa-factor IXa and by the tissue factor-factor VIIa complex. **(b)** The tissue factor pathway inhibitor (TFPI) interacts with the tissue factor–factor VIIa–factor Xa product complex to block the tissue factor-initiated activation of both factors IX and factor X. Inhibition of the extrinsic factor Xase complex results in the factor VIIIa–factor IXa complex (intrinsic factor Xase), becoming the only viable catalyst for factor X activation. These slides were modified and used with permission from the "Dynamics of Hemostasis," Haematologic Technologies, K.G. Mann, 2002 [35]. (See also Plate 1.2.)

processes. In hemophilia A and hemophilia B, the "intrinsic factor Xase" complex cannot be assembled, and amplification of factor Xa generation does not occur [26]. Factor Xa combines with factor Va on the activated platelet membrane receptors and this factor Va–factor Xa "prothrombinase" catalyst (Figure 1.2a) converts prothrombin to thrombin. Prothrombinase is 300 000-fold more active than factor Xa alone in catalyzing prothrombin activation [6].

Attenuation of the procoagulant response

The coagulation system is tightly regulated by the inhibitory systems. The tissue factor concentration threshold for reaction initiation is steep and the ultimate amount of thrombin produced is largely regulated by the concentrations of plasma procoagulants and the stoichiometric inhibitors and the constituents of the dynamic inhibition processes [24]. Tissue factor pathway inhibitor blocks the tissue factor–factor VIIa–factor Xa product complex, thus effectively neutralizing the "extrinsic factor Xase" complex (Figure 1.2b) [27]. However, tissue factor pathway inhibitor is present at low abundance (~2.5 nM) in blood and can only delay the hemostatic reaction [28]. Antithrombin, normally present in plasma at twice the concentration (3.2 μM) of any potential coagulation enzyme, neutralizes all the procoagulant serine proteases primarily in their uncomplexed states [11].

The dynamic protein C system is activated by thrombin binding to constitutive vascular thrombomodulin (protein Case). This complex activates protein C to activated protein C (Figure 1.1) [4]. Activated protein C competes in binding with factor Xa and factor IXa and cleaves factor Va and factor VIIIa, eliminating their respective complexes [20]. The protein C system, tissue factor pathway inhibitor, and activated protein C cooperate to produce steep tissue factor concentration thresholds, acting like a digital "switch," allowing or blocking thrombin formation [10].

In humans, the zymogen factor XI which is present in plasma and platelets has been variably associated with hemorrhagic pathology [29]. Factor XI is a substrate for thrombin and has been invoked in a "revised pathway of coagulation" contributing to factor IX activation (Figure 1.1) [30]. *In-vitro* importance of the thrombin activation of factor XI is evident only at low tissue factor concentrations [26].

Factor XII, prekallekrein, and high-molecular-weight kininogen (Figure 1.1) do not appear to be fundamental to the process of hemostasis [31]. The contribution of these contact pathway elements to thrombosis remains an open question and further experimentation is required to resolve this issue [31].

Conclusion

Advances in genetics, protein chemistry, bioinformatics, physical biochemistry, and cell biology provide an array of information with respect to normal and pathologic processes leading to hemorrhagic or thrombotic disease. The challenge for the 21st

century will be to merge mechanism-based, quantitative data with epidemiologic studies and subjective clinical experience associated with the tendency to bleed or thrombose and with the therapeutic management of individuals with thrombotic or hemorrhagic disease. *In-vitro* data and clinical experience with individuals with thrombotic and hemorrhagic disease will ultimately provide algorithms which can combine the art of clinical management with the quantitative science available to define the phenotypes vis-á-vis the outcome of a challenge or the efficacy of an intervention [28–34].

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