2. PLASMA COAGULATION FACTORS

Pamela Sakkinen and Russell P. Tracy

Overview
The role of thrombosis in precipitating acute cardiovascular disease (CVD) events has been well known since the early 1970s [1]. It is now becoming increasingly apparent that thrombosis may also be involved with the chronic development of CVD [2-4]. Clot formation or thrombosis can be conceptualized as a balance between procoagulant and anticoagulant and fibrinolytic forces [5] (Figure 2-1). Although some factors, such as thrombin, may have more than one role, this “pseudoequilibrium” provides a schema for assessing the relative coagulant balance.

Traditionally the hemostatic system has been divided into intrinsic and extrinsic pathways, which come together at the prothrombinase complex in a common pathway. The components of the intrinsic system are contained entirely within the vasculature, whereas the extrinsic system involves components from both the blood and the vasculature itself. There are interactions between components of the intrinsic and extrinsic pathways, however, and this division does not exist strictly in vivo. Tissue factor (TF)—factor Vila complex, the major trigger to coagulation, activates small amounts of both factor IX of the intrinsic pathway and factor X of the common pathway [6] (Figure 2-2A). Activation of factor X to factor Xa and of prothrombin to thrombin results in autoamplification through activation of the obligate cofactors, factors V and VIII, of factor VII by thrombin, and of factor Xa. Thrombin further activates platelets to provide cellular binding sites for the assembly of vitamin K–dependent protein (VKDP) complexes and catalyzes activation of factor XIII. Measurements of prothrombin fragment 1–2 (F1–2), thrombin–antithrombin complexes (TAT), and fibrinopeptide A (FPA) provide indices of thrombin generation, neutralization, and action, respectively. Activation peptides, released when a zymogen is activated to an enzyme, can also be quantitated as measures of enzymatic activity [7].

While the classic model of the clotting cascade has provided a logical understanding of hemostasis, it is evident that it does not account for various clinical coagulation pathologies, for example, the bleeding diatheses experienced by hemophiliacs. A study attempting to simulate in vivo conditions has proposed that factor Xa produced on the phospholipid surface has a different role than factor Xa produced by the TF–factor Vila complex [8]. The inactivation of factor Xa by tissue factor pathway inhibitor (TFPI) has also been postulated to interfere with the extrinsic pathway in vivo [9]. Alternatively, Mann and colleagues have proposed a model of factor X activation based on kinetic experiments in systems containing elements of both pathways, which provides a plausible explanation for the bleeding that occurs in the factor VIII and IX deficiencies (Figure 2-2B) [10]. The key to this model is the activation of factor IX through an inactive intermediate, factor IXa. Factor IXa is initially produced by the small amount of factor Xa formed by the TF–factor Vila complex. Once factor IXa is formed, it becomes the preferred substrate of TF–factor Vila. Action of TF–factor Vila on the intermediate factor IX produces factor IXa (factor IX(a)). From this point on, the factor IXa–factor Vila complex exceeds the TF–factor Vila complex in the generation of factor Xa. Therefore, deficiencies of factors IX or VIII under normal physiological conditions would result in inadequate hemostasis.

A common theme in major reactions of the coagulation cascade is the role of the phospholipid surface [11]. The Xase complex (factor IXa–factor VIIIa), as well as the prothrombinase and protein case reactions, are localized to a phospholipid surface. The components of the reaction include a vitamin K–dependent (VKD) enzyme, a cofactor protein that binds the enzyme, lipid surface, and ionized calcium (Figure 2-3). Although the components of each reaction are substrate specific, they assemble in similar patterns and provide the same advantage — amplified kinetic rates of reaction through a localized reaction complex protected from plasma dilution and plasma inhibition factors [12,13] (Figure 2-4). It is thought that physiologically the Xase and prothrombinase reactions occur on the same phospholipid surface, with the
Part A: Scientific Principles

Procogulation
Examples
Proenzymes (e.g., Factor X)
Enzyme cofactors (e.g., Tissue Factor)
Structural proteins (e.g., fibrinogen)
Appropriate surface (e.g., that of an activated platelet, monocyte)

Anticoagulation
Examples
Proenzymes (e.g., Protein C)
Enzyme inhibitors (e.g., Antithrombin III)
Cofactors (e.g., Thrombomodulin, Tissue Factor Pathway Inhibitor)

Prothrombinase
Examples
Plasminogen activators
Plasmin
Cellular plasminogen receptors
Appropriate surface (e.g., fibrin)

Fibrinolytic
Examples
Plasminogen activator inhibitors (e.g., PAI-1)
Plasmin inhibitors (e.g., alpha-2-antiplasmin)
Lp(a), may compete with plasminogen for plasminogen receptor

FIGURE 2-2. A: The traditional view has the intrinsic and extrinsic pathways coming together at the activation of factor X. Newer information suggests that TF-factor VIIa activates factor IX to factor IXa as well. The model of Mann and colleagues (B) proposes that an intermediate form of factor IX (factor IX α) is preferred by TF-factor VIIa over factor X. The action of factor IXα results in the formation of factor IXa. Factor IXa is then responsible for the majority of factor Xa formed. Obligate cofactors are shown in parentheses.

FIGURE 2-1. Thrombosis as a pseudoequilibrium between pro- and anti-coagulant and fibrinolytic factors. Increases in procoagulant or antifibrinolytic factors favor thrombosis, whereas anticoagulant and fibrinolytic factors prevent clot formation and enhance clot dissolution, respectively.

product of the first reaction becoming the enzyme of the second, but this has not been demonstrated in vivo [13].

The major role of thrombin as a procoagulant is to enhance the hydrolysis of fibrinogen to form fibrin. Thrombin acts sequentially at the amino terminus of fibrinogen, initially cleaving the two A-α chains of fibrinogen, followed by excision of terminal peptides of the two B-β chains, releasing FPA and FPB peptides, respectively. Although other enzymes, such as tissue plasminogen activator (t-PA), can catalyze the site-specific hydrolysis of fibrinogen, resulting in formation of fibrin monomer I, they do so relatively slowly, and the measurement of FPA is felt to be specific for thrombin activity. Fibrin monomer I can be crosslinked by factor XIIIa to form stable fibrin strands [14].

Fibrin polymerization occurs in sequential steps. First, exposure of the new N termini results from cleavage of the A-α and B-β chains of fibrinogen in the central region of the molecule. These new N termini bind weakly to the terminal D domains of the fibrin II monomers. Factor XIIIa catalyzes bond formation between terminal D domains. The final step of fibrin polymerization is covalent crosslinking of the α chains.

There are four major proteins involved in the regulation of coagulation: TFPI, protein C, its cofactor protein S, and antithrombin III (AT III; Figure 2-5). In vitro studies have demonstrated that TFPI inhibits factor Xa, and TF-factor VIIa in a factor Xa-dependent reaction [15], and animal studies have shown that decreased levels of TFPI predispose to coagulation [16,17]. Measurement of levels in human populations in various states of health and disease, however, have been less straightforward in defining an in vivo anticoagulant role for TFPI. Whereas some studies have shown an elevation of TFPI in procoagulant states [18–20], suggesting TFPI increases to compensate for increases in procoagulant activity, others have shown decreased or normal levels of TFPI in thrombotic disease [20–22]. Recent data from cross-sectional analysis are consistent with TFPI