

Sticky and promiscuous plasma proteins maintain the equilibrium between bleeding and thrombosis

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Summary

A vascular fissure requires a patch that must be provided by constituents of the cellular and fluid phases of flowing blood. The principal components involved in primary haemostasis are platelets, collagen and von Willebrand factor (vWF). Platelets, the cellular elements of the patch, are inert until they encounter conditions that trigger their activation. Platelet adhesion and aggregation at the site of vascular injury lead to the formation of a platelet plug and to a local activation of the coagulation cascade. The resulting final product of blood coagulation is a fibrin network that stabilises the primary platelet plug. Most coagulation factors are zymogens of serine proteases. They are converted from an inactive form to an active enzyme by limited proteolytic cleavage of one or a few peptide bonds. The coagulation reactions must become extinguished as soon as the patch in the injured blood vessel has been established. Several inhibitors, present in excess in plasma, neutralise the surplus of remaining proteases, and the fibrinolytic system dissolves the plug after the surrounding tissue has been repaired.

In fulfilling their function to control the fluidity and integrity of the vascular system, the plasmatic and cellular haemostatic players undergo

multiple interactions of two kinds: they recognize and bind, often irreversibly, to several partners which are present in their immediate environment. On the other hand, some haemostatic factors, such as fibrinogen and von Willebrand factor, enhance their stickiness by polymerisation of identical subunits carrying multiple adhesive sites. Several haemostatic plasma proteins and their cellular surface receptors are involved in or may be affected by other homeostatic systems, such as immune response, complement activation, cytokine release, cell proliferation, growth and differentiation. These diverse functions are only possible because of the modular structure of participating proteins. In the process of evolution a series of structural modules have been incorporated into protein molecules as their integral domains by exon duplication and shuffling. Owing to variable conformations of the resulting multi-domain proteins, the same modules may perform different tasks and be recognized only by specific ligands, thus controlling the delicately balanced system of haemostasis.

Key words: thrombin; fibrinogen; von Willebrand factor

Introduction

The vascular system provides oxygen and nutrients to tissues and removes waste products. It also has the ability to repair damage to blood vessel walls and prevent blood loss from the wound. Blood vessels occasionally become severely hurt, but even in health, the capillaries are permanently exposed to slight injuries due to mechanical or chemical lesions. Maintenance of the integrity of the vascular system is absolutely vital for survival.

Fortunately, the haemostatic system both recognizes the vascular damage and recruits the appropriate combination of cells and enzymes to produce an insoluble plug that will stop blood loss.

Homeostatic systems often involve proteolytic enzymes, not only those engaged in blood coagulation but also other proteases active in inflammation, blood pressure regulation, and complement activation. They may be found as participants of cascades in which a protease produced in one step generates another protease in the next step. The function of proteases must be carefully regulated. The key property of proteases is that their formation from inactive precursors is an irreversible reaction. Following plugging of the injured site, the activated enzymes must disappear to insure that the blood coagulation ceases. Clots that grow too

large or break away from their site of origin cause blockage of blood vessels, or thrombosis. The circulating protease inhibitors support this sensitive balance between coagulation and anticoagulation.

Particularly important in haemostasis are positive and negative feedbacks in which an enzyme generated later in a cascade acts to enhance or impair its own production. In pathologic conditions, congenital or acquired conditions may lead to

bleeding, or the disordered thromboregulation may result in arterial or venous thrombosis. The response of the coagulation process is generally limited to the site of injury. The system is normally quiescent but becomes active within seconds after injury. Activation of platelets by thrombin initiates the assembly of the prothrombinase complex leading to conversion of fibrinogen to fibrin which deposits in the platelet plug.

Thrombin

Thrombin plays a central role in haemostasis and thrombosis. It is a serine protease possessing both procoagulant and anticoagulant properties. Thrombin catalyses cleavage of fibrinopeptides from the soluble plasma protein fibrinogen. The resulting fibrin monomers spontaneously polymerise to fibrin fibers, a major constituent of the blood clot. Fibrin network becomes covalently crosslinked by factor XIIIa that has, in a parallel activation reaction, been produced by the thrombin-induced cleavage of the inactive zymogen factor XIII. Thrombin is also a powerful agonist for a variety of cellular responses to vascular injury (table 1). It is a very potent activator of platelet aggregation and release. Thrombin activates platelets by binding to and cleaving the transmembrane protease-activated receptors (PAR)-1, and thus initiates activation of glycoprotein (GP) receptors IIb/IIIa on the platelet surface [1], resulting in crosslinking of activated platelets by fibrin(ogen) and other plasma proteins with distinct amino acid sequences (Arg-Gly-Asp = RGD) [2]. Human platelets also respond to thrombin

through PAR-4 and GP Ib [3]. By a positive feedback mechanism an amplification of prothrombin activation is achieved through cleavage of coagulation factors XI, VIII and V.

Upon binding to endothelial cell surface protein thrombomodulin, the specificity of thrombin switches so that it can activate protein C which in turn inactivates factors VIII and V and thus shuts down the coagulation cascade [4]. Thrombin also inhibits fibrinolysis by activating thrombin activable fibrinolysis inhibitor (TAFI) [5, 6]. After the formation of a stable clot has been completed, the activity of thrombin has to be readily abolished. This is accomplished by incorporation of active thrombin in the fibrin clot and by its binding to inhibitors, such as antithrombin III, heparin cofactor II, α_2 -macroglobulin, or protein C inhibitor [7–9].

In addition to the above haemostatic functions, thrombin induces or stimulates expression of a number of cellular receptors. By binding to various cells thrombin induces synthesis or release of several proteins involved in haemostatic, inflam-

Table 1
Thrombin functions in response to vascular injury.

Cleavage of fibrinopeptides: conversion of fibrinogen to fibrin monomer
Activation of factors V, VII, VIII, XI: positive feedback of coagulation cascade
Activation of factor XIII: stabilisation of the fibrin network by factor XIIIa
Activation of platelets: platelet aggregation and secretion
Release of von Willebrand factor from endothelial cells and platelets
Stimulation of tissue factor synthesis and release from endothelial cells
Activation of protein C following binding to thrombomodulin on endothelial cells: main anticoagulation process (negative feedback)
Release of tissue factor pathway inhibitor, annexin V and nexins (PN-1 and PN-2) from endothelial cells: anticoagulant negative feedback
Release of tissue plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA) from endothelial cells
Release of plasminogen activator inhibitor (PAI-1) from endothelial cells and platelets
Activation of thrombin-activable fibrinolysis inhibitor (TAFI).
Translocation of endothelial cell membrane E-selectin (also called CD62E or ELAM-1), mediating interactions with leukocytes
Translocation of P-selectin (also denoted as GMP-140 or PADGEM) in platelets and endothelial cells
Induced expression and release of prostacyclin (inhibitor of platelet aggregation) and platelet aggregating factor (PAF) from endothelial cells
Release of platelet-derived growth factor (PDGF)
Stimulation of endothelial cell proliferation
Neurite retraction in nerve cells
Chemotactic activity for monocytes and neutrophils
Mitogenic activity towards fibroblasts, macrophages, lymphocytes and vascular smooth muscle cells

matory, proliferative, chemotactic, and reparative processes. Thrombin is a player with many faces and playing many roles. To fulfill all its functions the molecule of thrombin contains a number of structural configurations that enable it to associate with very specific and different interacting partners, with whom it can undergo different and sometimes contrary tasks (table 2). Thrombin is one of the most promiscuous proteins, having at least 20 known associates. Traditionally, interactions among proteins have been studied sequentially one by one. Improvements in analytical technology over the past couple of years [10, 11] may allow us to identify the numerous members of interacting proteins.

Not only thrombin but also other proteins involved in the control of haemostasis are very social; almost all are known today to associate with a variety of partners. They turn up in several different complexes or pathways. Even in patients lacking some components of the normal coagulation set, the haemostatic equilibrium is not completely overthrown, suggesting that redundant mechanisms are present which may compensate for the missing participants. Four proteins involved in haemostasis, in addition to thrombin, and their ligands are listed in table 2: fibrinogen, thrombospondin, vitronectin, and von Willebrand factor (vWF).

Table 2
Multiple ligands of some representative proteins involved in haemostasis.

Thrombin	fibrin(ogen)	thrombospondin	vitronectin	von Willebrand factor
fibrinogen	thrombin	integrin $\alpha_5\beta_1$	integrin $\alpha_V\beta_3$	platelet glycoprotein Ib
fibrin	factor XIIIa	integrin $\alpha_V\beta_3$	integrin $\alpha_V\beta_5$	integrin $\alpha_V\beta_3$
factor V	integrin $\alpha_V\beta_1$	integrin $\alpha_{IIb}\beta_3$	integrin $\alpha_V\beta_1$	integrin $\alpha_{IIb}\beta_3$
factor VIII	integrin $\alpha_M\beta_2$	platelet glycoprotein IV (CD36)	integrin $\alpha_{IIb}\beta_3$	factor VIII
factor XI	integrin $\alpha_V\beta_5$	fibrinogen	urokinase receptor	fibrin
factor XIII	integrin $\alpha_{IIb}\beta_3$	von Willebrand factor	plasminogen	glycosaminoglycans
protein C	integrin $\alpha_4\beta_2$	thrombin	PAI-1*	sulfatides
TAFI*	plasminogen	factor IXa	thrombin-antithrombin	collagen types I-VI
thrombomodulin	tissue plasminogen activator	factor Xa	glycosaminoglycans	botrocetin**
glycosaminoglycans	PAI-2*	plasminogen	collagen	ristocetin**
antithrombin III	α_2 -antiplasmin	histidine-rich glycoprotein	osteonectin	
heparin cofactor II	factor Xa	neutrophil elastase	tenascin	
protein C inhibitor	fibronectin	cathepsin G	complement gC1q receptor	
nexin I	fibulin-1	fibronectin	complement C5b-C9	
hirudin**	entactin	collagen types I, V	streptococci	
platelet glycoprotein Ib α	histidine-rich glycoprotein	laminin	staphylococci	
PAR1* on platelets	lipoprotein(a)	glycosaminoglycans		
PAR1 on endothelial cells	protamine**	glycolipids		
PAR2 on endothelial cells	heparin	calcium		
PAR4 on platelets	hyaluronic acid			
calcium	ICAM*			
	collagen			
	calcium			
	various cells***			
	streptococci			
	staphylococci			

* TAFI: thrombin activable fibrinolysis inhibitor; PAR: protease-activated receptor; PAI: plasminogen activator inhibitor; ICAM: intercellular cell adhesion molecule
 ** non-physiologic ligands
 *** endothelial cells, macrophages, fibroblasts, monocytes, hepatocytes, erythrocytes

Fibrinogen

Transformation of soluble fibrinogen to the insoluble fibrin clot in the ruptured blood vessel is brought about by a sequence of events that have been perfected during the evolution of the fibrinogen molecule during more than 500 million years. Following cleavage of fibrinogen by thrombin, a binding site for the terminal D domains of fibrinogen is exposed in the central E domain leading to associations between monomeric fibrin molecules (figure 1). The dimeric nature of fibrinogen molecules allows the propagation of each polymer in two directions, and the initial protofibrils undergo in turn lateral interactions producing bundles of fibrin strands [12, 13]. The initial non-covalent bonds become stabilised by the introduction of crosslinks between adjacent fibrin monomers of a protofibril and between protofibril strands, catalyzed by factor XIIIa.

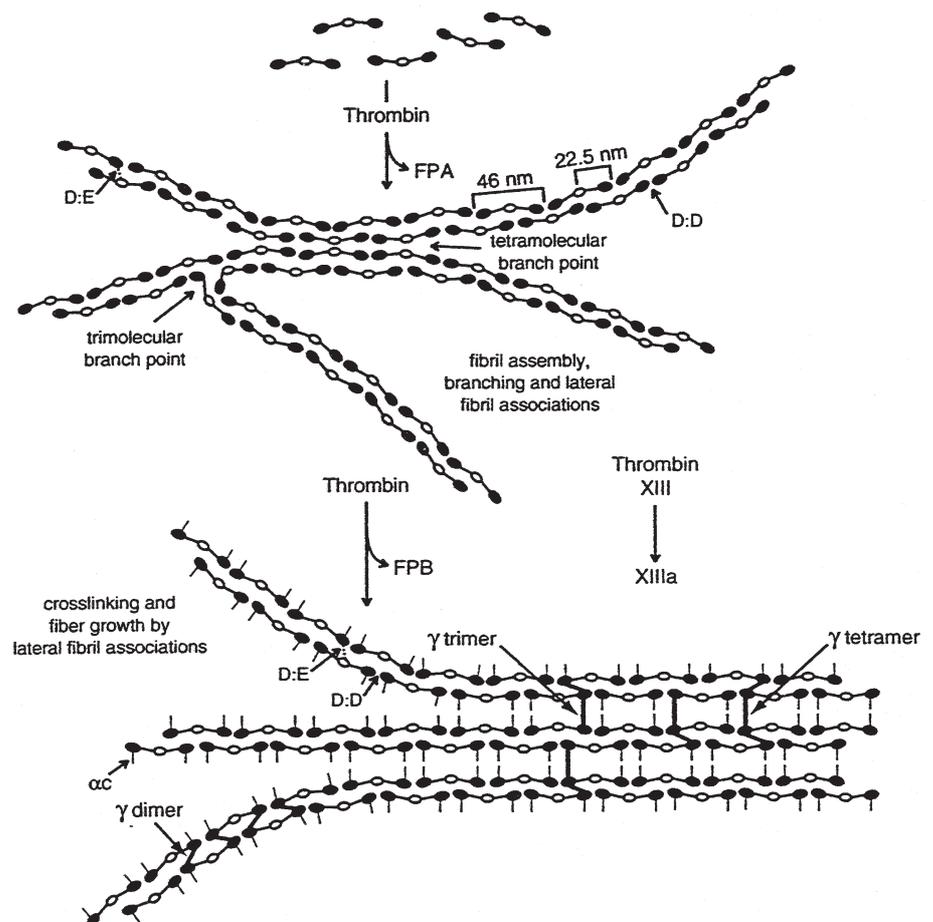
Calcium is required for normal fibrin polymerisation. The same calcium ion, which binds to a high-affinity binding site on the fibrinogen mol-

ecule, also affects the digestion of fibrin molecules in the course of clot dissolution by fibrinolytic enzymes. Fibrin clots are not designed to remain permanently in the damaged blood vessel, but must be dissolved by the fibrinolytic system composed of plasmin(ogen) and plasminogen activator(s) [13, 14]. Fibrinogen not only contains binding sites for these enzymes responsible for clot dissolution, but also binds the inhibitor α_2 -antiplasmin that takes care of the active plasmin after its task has been accomplished.

Fibrinogen is essential for the aggregation of platelets following their activation by thrombin or by other agonists. Fibrinogen contains binding sites for glycoprotein receptors IIb/IIIa on the platelet membranes [15]. As a dimeric molecule, it serves as an ideal adhesive between activated platelets. Finally, fibrinogen recognizes and binds to a number of other proteins and cells (table 2), as well as to various strains of bacteria.

Figure 1

Schematic diagram of fibrin polymerisation and crosslinking. After release of fibrinopeptide A (FPA) by thrombin the assembly of fibrin begins with non-covalent interactions between E and D domains to form end-to-middle staggered overlapping double-stranded protofibrils. Subsequent to cleavage of fibrinopeptides B (FPB) carboxy terminal domains of the α -chains (α C) interact with other α C domains, thus promoting lateral fibril associations. Factor XIIIa introduces the first covalent crosslinks between carboxy terminal sites in the γ chains, and at a later stage also between α chains. Reproduced with permission from [12].



Thrombospondin

Thrombospondin is a large trimeric glycoprotein that is a major constituent of platelet α -granules [16]. Upon release thrombospondin binds to several glycoprotein receptors on the platelet surface. A number of protein ligands have been attributed to thrombospondin (table 2). The role of thrombospondin in platelet adhesion is controversial: both adhesive and antiadhesive properties of thrombospondin have been reported [17]. Throm-

bospondin is also synthesised and secreted by endothelial cells, fibroblasts, monocytes and macrophages. In addition to its involvement in haemostasis [18], thrombospondin has been reported to serve diverse other functions, to stimulate and inhibit cell growth and migration, and to be involved in cell apoptosis, angiogenesis and tumor progression [19].

Vitronectin

Vitronectin is an adhesive plasma glycoprotein that participates not only in platelet aggregation [20] and blood coagulation but also in the regulation of complement assembly, cell differentiation, proliferation, and morphogenesis [21]. An important role is ascribed to the binding of vitronectin to plasminogen activator inhibitor-1 (PAI-1), since the complex formation with vitronectin increases

the half-life of PAI-1 and enables the inhibitor to become stabilised in the platelet plug at the site of the vessel injury. Due to its interactions with integrins and with urokinase receptors, vitronectin may serve a regulatory function in urokinase receptor-related invasiveness, and differentiation of cells.

von Willebrand factor

von Willebrand factor (vWF) is the main protein component of plasma involved in primary haemostasis. It acts as a bridge between the injured vessel wall and specific receptors (GP Ib) on the platelet surface. It has been postulated that conformational changes occurring following interaction of vWF with subendothelial components (microfibrillar structures, possibly collagen type VI), result in exposure of its binding sites for the GP Ib receptors and thus initiate the deposition of platelets at the site of vascular injury [22]. High fluid shear forces may additionally act on the conformation of vWF and initiate platelet adhesion and thrombus formation. It is conceivable that proteoglycans also contribute to binding of vWF to exposed subendothelium, since glycosaminoglycans are abundantly present in the extracellular matrix and represent structures onto which vWF may attach.

Platelet glycoprotein complex IIb/IIIa expressed on the surface of an activated platelet is an adhesive receptor ($\alpha_{IIb}\beta_3$) that, like all other integrins, shows high binding affinity for proteins containing amino acid sequence RGD. Several plasma proteins, such as fibrinogen, fibronectin, thrombospondin, vitronectin, and vWF, but also a series of other cellular, extracellular matrix and plasma proteins share this sequence. Different integrins

with RGD recognition specificity can apparently discriminate among RGD-containing ligands [23]. Because the concentration of fibrinogen in human plasma is two orders of magnitude higher than that of vWF, most of the available GP IIb/IIIa receptors on activated platelets will become occupied and crosslinked by fibrin(ogen). However, vWF can support platelet aggregation even in the absence of fibrinogen, albeit less efficiently, as shown in patients with afibrinogenemia [24]. At high fluid shear rates, such as those prevalent in arterioles, fibrinogen alone cannot by itself support formation of stable platelet aggregates, whereas vWF can [25]. The initial rate of platelet aggregation is even faster in the absence of fibrinogen, suggesting that vWF and fibrinogen compete for binding to activated GP IIb/IIIa.

The biologic half-life of factor VIII is strongly reduced in patients with vWF deficiency. As long as factor VIII is complexed to vWF it appears to be protected from the action of activated protein C. Upon thrombin-catalysed activation of factor VIII the complex between vWF and factor VIII dissociates, and factor VIIIa becomes fully available to perform its cofactor role in the generation of factor Xa [26], but also exposes its own vulnerable Achilles' heel to activated protein C.

Multiple partners of other haemostatic and fibrinolytic factors

All plasma proteins involved in blood coagulation have affinities for multiple ligands. Thus, activation of the central participants (vitamin K-dependent coagulation factors II, VII, IX and X) of the coagulation cascade occurs following their binding to specific cofactors on the negatively charged phospholipids derived from platelet membranes during activation [27]. The volume fraction of the phospholipid vesicles is several orders of magnitude smaller than the bulk plasma volume, and the resulting concentrations of vitamin K-dependent factors, as well as their reaction rates, are several orders of magnitude higher (10^5 – 10^6 -fold) than in the plasma. The assembly of clotting complexes on phospholipid membranes is schematically depicted in figure 2. Each of these complexes requires calcium ions and consists of a serine protease and a cofactor protein. They are attached to the negatively charged membranes of the activated platelets. Factor X can become converted to factor Xa by two activating complexes, by the tissue factor (TF)-factor VIIa complex (extrinsic tenase), and by the factor VIIIa (composed of heavy chain VIII_H and light chain VIII_L)-factor IXa complex (intrinsic tenase) [27]. The TF-VIIa complex is capable of activating both factor X and factor IX, but factor X appears to be the preferred substrate. In the next step factor Xa activates prothrombin (II) in the presence of the activated cofactor Va (composed of heavy chain Va_H and light chain Va_L). The complex of factors Xa and Va, attached via calcium ions to the negatively charged membrane phospholipids, is denoted as prothrombinase. A similar complex (protein C-ase), consisting of thrombin (IIa) and thrombomodulin (TM), located on the endothelial cell membrane, is responsible for proteolytic cleavage of protein C

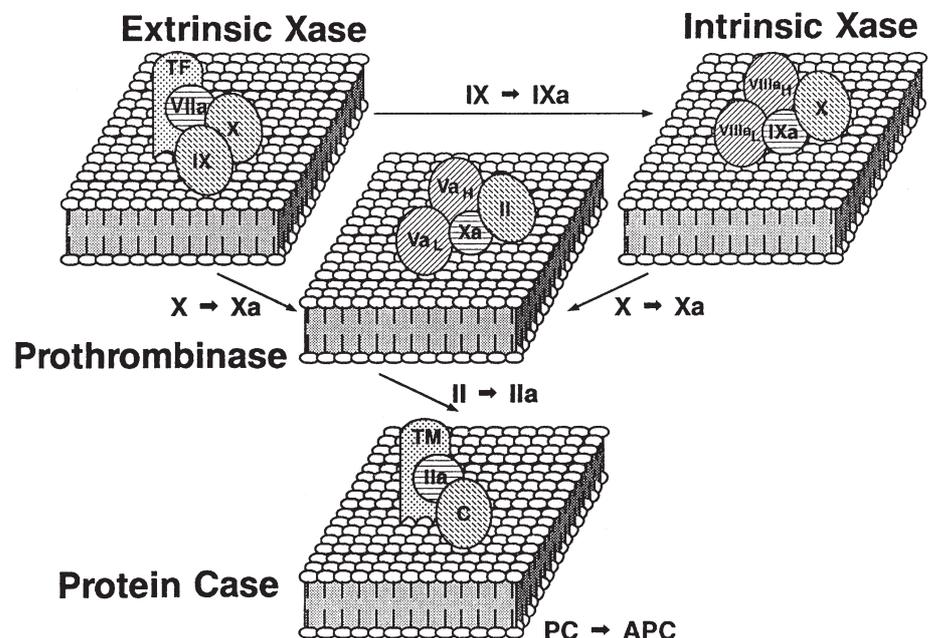
to activated protein C (APC), the major anticoagulant enzyme of the blood coagulation system.

In a similar manner, the components involved in the contact phase of blood coagulation (factors XII and XI, prekallikrein and high molecular weight kininogen) have affinity for each other and for negatively charged surfaces, such as glass, kaolin, dextran sulfate, or ellagic acid. Again, the contact phase is not only responsible for *in vitro* activation of the coagulation cascade but also participates in diverse systems of host defense, e.g. kinin generation, complement activation, activation of renin-angiotensin system, and fibrinolysis. In fact, the physiological significance of the contact system in haemostasis is still elusive, since individuals with a deficiency of factor XII, prekallikrein, or high molecular weight kininogen do not bleed excessively and do not require replacement therapy following haemostatic challenge from trauma or surgery.

Multiple interactions have also been observed between the components of the fibrinolytic system. Thus, tissue-type plasminogen activator, plasminogen, and α_2 -antiplasmin bind to fibrin monomers and fibrin degradation products, whereas urokinase-type plasminogen activator binds to a specific cellular receptor (u-PAR). The latter binding seems to be crucial for its activity. Inhibition of fibrinolysis may occur either at the level of plasminogen activator by plasminogen activator inhibitors (PAI), or at the level of plasmin, mainly by α_2 -antiplasmin. Several molecular interactions have been observed between the fibrinolytic system and matrix metalloproteinases (MMP) [28]. Both systems may cooperate in generating the proteolytic activity. Plasmin was shown to mediate activation of proMMP-3 and

Figure 2

Schematic representation of the vitamin K-dependent enzyme complexes responsible for activation of factors X and IX (Xase), II (prothrombinase) and protein C (protein C-ase). Each serine protease is associated with the specific cofactor on a phospholipid surface. TF, tissue factor; TM, thrombomodulin; APC, activated protein C. Reproduced with permission from [27].



proMMP-9, but on the other hand MMP-3 cleaves off the fibrin binding site from plasminogen, as well as the u-PAR binding site from urokinase, and thus impairs the activation of plasminogen.

By use of amino acid sequencing and X-ray crystallography of the coagulation proteins, as well as by analysis of the coding regions of their genes, similar structural units have been discovered in proteins associated with formation and dissolution of blood clots. In the process of evolution nature has moved discrete structural modules, such as gla module, kringle, epidermal growth factor module, calcium binding domain and others, from one protein to another, thus creating new types of proteins

with different functions and competent for very specific tasks. For example, the gla module, indispensable for binding to phospholipid membranes, is shared by all four vitamin K-dependent coagulation factors (II, VII, IX and X), and by proteins C, S and Z. They also share the epidermal growth factor module with factor XII, tissue plasminogen activator, and urokinase. Kringles were found in plasminogen, tissue plasminogen activator, urokinase, and factors II and XII [29]. It is assumed that the function of modules is to bind to appropriate ligands as well as to affect post-translational modifications, since the primitive ancestral proteins were less specialised than its descendants.

Effect of multimerisation on adhesion properties of proteins

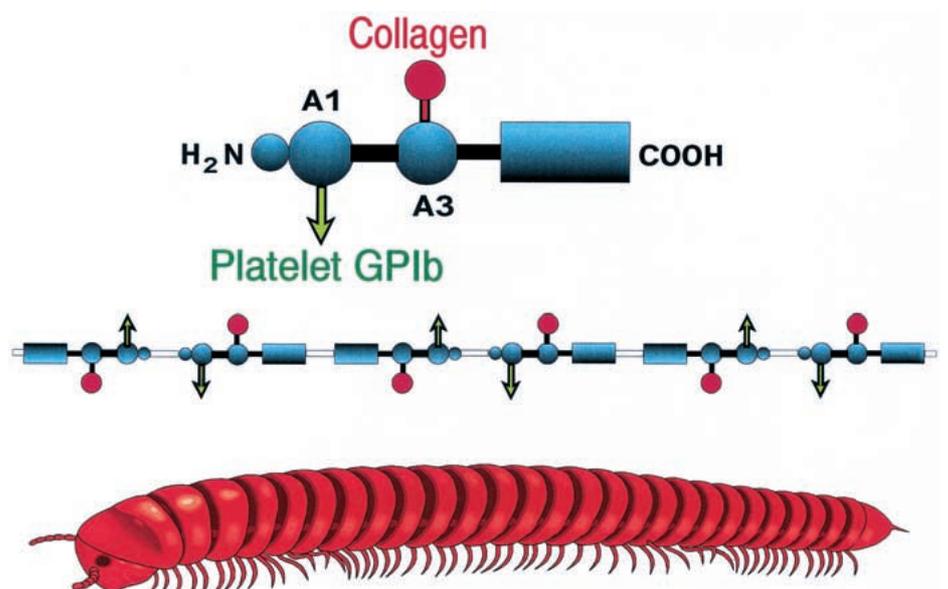
The fibrinogen molecule has a symmetric dimeric structure with sticky domains at both ends. This dimeric structure not only supports aggregation of activated platelets via binding of fibrinogen to glycoproteins IIb/IIIa on two adjacent platelets, but also facilitates linear multimerisation of fibrin monomers due to exposure of binding sites in the central region of the symmetric molecule following removal of fibrinopeptides A by thrombin. After initiation of polymerisation, fibrinopeptides B are also cleaved, exposing further polymerisation sites that interact laterally to associate the individual protofibrils. The resulting fibers form a three-dimensional network (figure 1). Platelet crosslinking and aggregation by dimeric fibrinogen molecules and the resulting fibrin network stabilise the platelet plug and arrest the bleeding.

Polymerisation of a protein not only determines its solubility but also strongly enhances its binding affinity for cellular receptors. vWF circulates in plasma as a mixture of multimers containing a variable number of subunits, linked together by covalent disulfide bonds. The largest multimers

may be as large as 20,000 kD. They represent the largest known proteins in human plasma. Initial platelet adhesion is mediated by sequences within the A1 and A3 domains: the A1 domain binds to platelet glycoprotein Ib whereas the A3 domain contains the principal collagen-binding site (figure 3). vWF normally does not interact with circulating platelets. However, after binding to collagen, a conformational change occurs in vWF that allows interaction between the A1 domain and platelet receptor Ib. The presence of repeating protomeric subunits, each endowed with a set of binding sites for collagen and platelet glycoproteins Ib and IIb/IIIa, leads to unusually strong interactions between vWF, the denuded subendothelium, and receptors GP Ib and IIb/IIIa on platelet membranes. Decreased polymeric size of vWF, observed in patients with von Willebrand disease type 2A, results in an impaired platelet adhesion to subendothelium under high fluid shear stress, suggesting that binding affinities of smaller vWF multimers for collagen and platelet glycoprotein Ib are not sufficiently strong to resist high

Figure 3

Schematic model of vWF structure. Binding sites for platelet glycoprotein Ib and collagen, involved in the initial event of primary hemostasis, are situated on the A1 and A3 domains, respectively. Multiple interactions of repeating binding sites with collagen and platelet receptors are dramatically increased in highly polymeric forms of vWF synthesized in endothelial cells and megakaryocytes. Only the large vWF multimers are capable of supporting platelet adhesion to the denuded subendothelium of an injured blood vessel under high shear forces encountered in the circulation. The millipede whose multiple legs help to resist its gravity under unfriendly weather conditions invented a similar solution of the adhesion problem.



shear forces of flowing blood, acting upon adhering platelets. Electron microscopic studies have shown that about 90% of the vWF in a resting solution is present in the form of linear polymers coiled upon themselves in a 'ball of yarn' form [30]. It is conceivable that the vWF undergoes a shear stress-induced conformational transition from a globular state to an extended chain conformation, thus exposing the binding sites for collagen and platelet receptors. Multimerisation confers colossal stickiness to vWF, as is required for platelet adhesion in the process of primary haemostasis. The polymeric vWF can be compared to a millipede (figure 3) that can stick to the bottom side of a leaf and resist the winds and showers of rain and cling to the twigs of an oak tree under these unfriendly conditions.

Multimeric forms of vWF found in normal blood apparently do not bind to resting platelets. However, under high shear forces, binding of vWF to glycoprotein Ib occurs even in the absence of platelet activation and release, suggesting that it was vWF from plasma rather than from α -granules that had been bound to platelet receptors [31]. Shear stress-induced binding of vWF was significantly increased in the blood of patients with thrombotic thrombocytopenic purpura (TTP), a dramatic platelet clumping disorder that is characterized by the presence in plasma of unusually large vWF multimers, similar to those secreted by activated endothelial cells [32]. This observation suggested that the deficiency of a vWF depolymerase may be responsible for the dramatic platelet

clumping episodes in TTP [33]. It is likely that the vWF multimers in the circulating blood are slowly, but continuously, cleaved to smaller, less adhesive forms by a specific vWF-cleaving protease. A complete deficiency of vWF-cleaving protease in the plasma of patients with chronic relapsing episodes of TTP has recently been demonstrated [34]. This observation suggests that not only too small but also too large vWF multimers in the circulation may become detrimental.

In conclusion, varied adhesive structures of proteins enable them to stick to specific substrates and receptors, but also to identical protein molecules or even to parts of themselves. Similar interactions and associations among proteins and cellular surfaces also play an important role in other systems, such as immune response, complement activation, release of cytokines, cell proliferation, growth and differentiation. A congenital or an acquired disturbance in the behaviour of these adhesive interactions is often responsible for deranged homeostatic equilibrium and for resulting pathologic conditions. The mapping of the eukaryotic proteome as a network of protein interactions may provide a rational approach to our understanding of these disorders and to their treatment.

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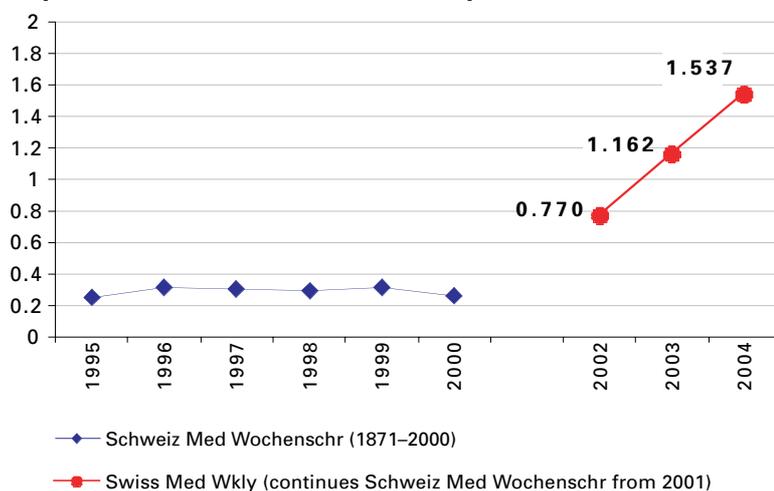
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