Zinc: An important cofactor in haemostasis and thrombosis

Trang T. Vu1,2; James C. Fredenburgh1,3; Jeffrey I. Weitz1,2,3,4

1Thrombosis and Atherosclerosis Research Institute, Hamilton, Ontario, Canada; 2Department of Medical Sciences, McMaster University, Hamilton, Ontario, Canada; 3Department of Medicine, McMaster University, Hamilton, Ontario, Canada; 4Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada

Summary
There is mounting evidence that zinc, the second most abundant transition metal in blood, is an important mediator of haemostasis and thrombosis. Prompted by the observation that zinc deficiency is associated with bleeding and clotting abnormalities, there now is evidence that zinc serves as an effector of coagulation, anticoagulation and fibrinolysis. Zinc binds numerous plasma proteins and modulates their structure and function. Because activated platelets secrete zinc into the local microenvironment, the concentration of zinc increases in the vicinity of a thrombus. Consequently, the role of zinc varies depending on the microenvironment; a feature that endows zinc with the capacity to spatially and temporally regulate haemostasis and thrombosis. This paper reviews the mechanisms by which zinc regulates coagulation, platelet aggregation, anticoagulation and fibrinolysis and outlines how zinc serves as a ubiquitous modulator of haemostasis and thrombosis.

Keywords
Zinc, coagulation, contact system, fibrinolysis, platelets

Introduction
Next to iron, zinc is the most abundant transition metal in the body and is critical for maintaining normal physiology (1). Zinc’s biological importance results from its ability to bind proteins and modulate protein-protein interactions and enzymatic activity (2, 3). Accordingly, the levels of zinc are tightly maintained (4, 5). The plasma concentration of zinc ranges from 10 to 20 µM, and virtually all of the zinc is bound to proteins. Although it can bind to numerous haemostatic proteins to modulate their activity, the majority of zinc forms a low affinity interaction with albumin, which serves as a zinc repository (2, 6, 7). Because most of the zinc is protein-bound, the concentration of unbound or free ion in plasma is only 0.5–1 µM (2, 5, 6). However, the plasma concentration of zinc is not static, and can change under certain conditions. For example, because of competition with zinc for albumin binding, zinc levels rise when the plasma concentration of free fatty acids increases (7). A second mechanism for modulating zinc levels involves platelets. Platelets accumulate zinc in their cytoplasm and α-granules, such that the concentration of zinc in platelets is 30- to 60-fold higher than that in plasma (8). In the milieu of activated platelets, the free zinc concentrations can reach 7-10 µM (9). Furthermore, erythrocytes, lymphocytes and neutrophils can also release zinc into the blood (10). Third, the affinity of zinc for plasma proteins is altered when the pH falls because of local ischaemia and subsequent tissue hypoxaemia. A low pH alters the capacity of proteins to bind zinc, thereby modifying its effect on protein structure and function (11-15). The potential for modulation of the local zinc concentration and activity supports the contention that zinc is a dynamic regulator of haemostasis and thrombosis.

The importance of zinc in haemostasis first came to attention in 1982 when bleeding and clotting abnormalities were described in men on a low zinc diet (16). Zinc deficiency is associated with impaired platelet aggregation, and cutaneous bleeding and platelet dysfunction are noted in some zinc-deficient cancer patients; problems that are reversed with zinc supplementation (16-19). Subsequent studies have shown that zinc regulates the coagulation, anticoagulation and fibrinolytic pathways. The interaction of zinc with factor (F)XII, the initiator of the contact pathway and the kalikrein/kinin system, was the first described role for this ion in coagulation (20). Zinc also modulates platelet aggregation and fibrin formation. Release of zinc from activated platelets results in a local surge in the zinc concentration that facilitates propagation of coagulation. Zinc also regulates ancillary steps in anticoagulation and fibrinolytic pathways. Therefore, zinc plays an essential part in regulating multiple reactions in haemostasis and thrombosis. An overview of these roles is summarised in Figure 1.
If zinc is such an important cofactor in these processes, why has it not received greater recognition? Zinc has long been overlooked for several reasons. First, calcium, which was identified as coagulation factor IV, is perceived as the predominant divalent cation because it is more abundant in plasma than zinc and has an obligate role in numerous haemostatic reactions (21). Second, citrate is commonly used as an anticoagulant when preparing plasma for coagulation or platelet function testing or for studies of other haemostatic reactions. Because the affinity of citrate for zinc is higher than that for calcium, it is likely that many zinc-dependent reactions in plasma have been overlooked. Recognising the importance of zinc in haemostasis and thrombosis, this review describes the mechanisms by which zinc modulates coagulation, platelet aggregation, anticoagulation and fibrinolysis.

Role of zinc in the regulation of coagulation

Contact system

Coagulation is initiated by tissue factor (TF) or via the contact system and intrinsic pathway. Although the TF pathway triggers thrombus formation at sites of vascular injury, current thinking is that the intrinsic pathway is important in the propagation phase (22). Thus, emerging evidence indicates that the contact system plays an important role in thrombosis, even though it is not essential for haemostasis (23, 24). This section outlines the role of zinc as a cofactor for initiating contact-mediated activation of coagulation on polyanionic surfaces and for the assembly of contact system proteins on endothelial cells (ECs) and platelets.

FXIIa generation on polyanionic surfaces

The contact system is initiated when FXII comes into contact with a polyanionic surface. One way in which FXIIa is generated is through FXII autoactivation, whereby FXIIa cleaves its own zymogen to amplify activation (21). Because the affinity of citrate for zinc is higher than that for calcium, it is likely that many zinc-dependent reactions in plasma have been overlooked. Recognising the importance of zinc in haemostasis and thrombosis, this review describes the mechanisms by which zinc modulates coagulation, platelet aggregation, anticoagulation and fibrinolysis.
Vu et al. Zinc regulation of haemostasis and thrombosis

induces a conformational change that potentiates surface-independent and surface-dependent autoactivation by at least 10-fold (11, 12, 25, 26). During the process of FXII autoactivation, the zymogen undergoes two distinct conformational changes, which prime it for optimal proteolysis (25, 27). The first conformational change occurs when FXII binds to polyanionic surfaces, whereas the second coincides with zinc binding to histidine residues on FXII (11, 28, 29). Studies of FXIIa generation in prekallikrein (PK)-deficient plasma reveal that polyanionic surface alone is insufficient to induce FXII autoactivation. Instead, optimal FXII autoactivation requires both the surface and zinc (27). These findings suggest that zinc is an essential cofactor for activation of the contact system on artificial surfaces. However, the extent to which zinc is required for contact-mediated activation of coagulation on physiological surfaces, such as nucleic acids and polyphosphates, is as yet unstudied (30, 31).

There is ample evidence that zinc binds FXII and induces conformational changes. These changes could augment the interaction of FXII with surface or with its activators, or enhance FXII cleavage (25-27, 32). There are four zinc binding sites on FXII (26, 33, 34). One binding site is lost when FXII is converted to α-FXIIa, and further conversion to β-FXIIa results in the complete loss of zinc binding; findings that suggest that zinc interacts predominately with the heavy chain of FXII (26). Zinc promotes the binding of FXII and α-FXIIa, but not β-FXIIa, to dextran sulfate. These observations suggest that by binding to the heavy chain, zinc enhances the capacity of FXII to bind to surfaces (29). There also is evidence that zinc induces a conformational change in FXII that renders it a better substrate for its activators, FXIIa and kallikrein (12). Although zinc does not bind directly to the catalytic domain of FXIIa, zinc inhibits FXIIa-mediated activation of PK in a dose-dependent manner, thereby providing further evidence of a zinc-induced conformational change (12, 25). The capacity of zinc to serve as both a cofactor for FXII activation and an inhibitor of FXIIa may provide a regulatory switch for control of the contact system.

In addition to its effect on FXII auto-activation, zinc also potentiates kallikrein-mediated activation of FXII, which provides a second positive feedback loop that further amplifies FXII activation. This pathway is dependent on high-molecular-weight kininogen (HK), a cofactor that bridges PK and kallikrein onto negatively charged surfaces and cells (9, 20, 35, 36). Although PK and kallikrein binds to surfaces independently of HK, activation of FXII by kallikrein is less efficient in the absence of HK. The mechanism by which zinc augments kallikrein-mediated generation of FXIIa may reflect the capacity of zinc to bind HK and to promote its interaction with anionic surfaces (37). Alternatively, the zinc-mediated conformation change in FXII may render it more susceptible to kallikrein cleavage (11). Therefore, there are multiple avenues by which zinc potentiates FXIIa generation on anionic surfaces.

Contact system on endothelial cells

Zinc also facilitates the assembly of the contact system on quiescent ECs (Figure 3). In the presence of zinc, both HK and FXII form high-affinity interactions (Kₐ values of 7-54 nM) with EC binding sites, such as urokinase-type plasminogen activator receptor (uPAR) and complement component C1q receptor (gC1qR). HK localises PK and FXI onto the endothelium. Subsequent activation of PK by prolylcarboxypeptidase (PRCP) generates kallikrein (K), which then activates FXII to generate FXIIa and cleaves HK to release bradykinin (BK). FXIIa cleaves FXI to generate FXIa.
tor (uPAR), complement component C1q receptor (gC1qR), and cytokeratin 1 (CK1) (9, 38-42). The majority of circulating PK and FXI is bound to HK, and HK can bridge PK and FXI to ECs. Because zinc facilitates HK binding to ECs, zinc serves to localise these zymogens onto the endothelial surface (38, 43). The amount of zinc required to facilitate FXII binding is 33- to 50-fold higher than that needed for HK binding (0.3 μM), suggesting that HK is the preferred ligand for the EC binding sites (9, 44, 45). Nonetheless, the local increase in zinc levels that occurs upon platelet activation raises the zinc concentration to levels sufficient for FXII to compete with HK for binding to ECs, which may provide a regulatory switch that modulates the activity of these proteins (9, 46). On the EC surface, prolylcarboxypeptidase converts PK to kallikrein more efficiently than FXIIa; a finding that suggests that prolylcarboxypeptidase is the physiological activator of PK on ECs (47, 48). Once generated, kallikrein cleaves HK to release bradykinin, a potent vasodilator (49). Unlike kallikrein, generation of FXIIa on ECs is dependent on FXIIa and this process is regulated by zinc because the ion localises both factors onto the endothelium (48, 50). Therefore, by mediating the interaction between the proteins in the contact system and the endothelium, zinc regulates thrombosis. Assembly of the contact system on the endothelium is also important for regulating vascular tone, inflammation and angiogenesis; the effects of zinc on these processes are discussed in detail elsewhere (49-53).

Role of zinc in platelet aggregation and fibrin formation

Activation of the coagulation system results in thrombin generation. Thrombin is a potent platelet agonist and induces release of zinc from the platelet cytoplasm and α-granules. The surge in zinc concentrations in the local microenvironment of an expanding thrombus promotes fibrin formation and triggers key signalling events that lead to platelet aggregation. Therefore, zinc release from platelets can be viewed as an essential transition phase that promotes the propagation of coagulation. This section outlines the role of zinc in platelet aggregation and fibrin formation; two processes that are critical for thrombus formation.

Platelet aggregation

Evidence from animal models and humans suggests that zinc is an important effector of platelet aggregation. Tail bleeding times are prolonged in rats with acute zinc deficiency and their platelets exhibit impaired aggregation in response to agonists such as adenosine diphosphate (ADP) and thrombin (16-18). Zinc enhances collagen- and ADP-induced aggregation of washed platelets (59, 60) and, even in the absence of agonists, zinc augments platelet aggregation by promoting fibrinogen binding to αIIbβ3, its cognate receptor on the platelet surface (59, 61). Thus, zinc not only augments agonist-induced platelet aggregation, but also promotes the binding of adhesive proteins to platelets.

The intracellular calcium concentration, which is an important determinant of platelet activation, depends on the dynamic balance between calcium uptake and release (62, 63). Zinc regulates the capacity of platelets to take up extracellular calcium, but does not affect the flux of calcium from intracellular pools (59, 64). Therefore, by enhancing calcium influx, zinc modulates processes important in platelet aggregation. This phenomenon may explain why platelets from zinc deficient animals and humans exhibit impaired aggregation, and why addition of zinc promotes agonist-induced platelet aggregation (16-18).

It is unclear how zinc modulates extracellular calcium uptake. Calcium channels are dependent on sulphydryl (SH) groups for normal function (65). When rats are fed a low zinc diet, the concentration of SH groups on the membrane proteins of their platelets is reduced by about 15% and this is associated with impaired uptake of extracellular calcium and reduced ADP-induced platelet aggregation (66). Therefore, current thinking is that zinc regulates the redox state of SH moieties in the calcium channels. Consequently, in the absence of zinc, SH groups are oxidised and form a disulphide bond, which inactivates the channel (64). Rearrangement of disulphide bonds on the platelet membrane, which is crucial for platelet aggregation, secretion and post-aggregation events, is primarily mediated by protein disulphide isomerase (PDI) (67, 68). Zinc induces oligomerisation of PDI, thereby attenuating its activity (69); an observation that raises the possibility that zinc may modulate the redox state of calcium channels in an indirect fashion. Furthermore, the activation of protein kinase C, a family of calcium-dependent kinases that modulate platelet signalling

Propagation of coagulation occurs on the platelet surface and the first step is mediated by FXIa (reviewed in [54]). There are two mechanisms through which FXI binding to platelets can be modulated. The first involves calcium and prothrombin, while the second requires zinc and HK (55, 56). Zinc facilitates the binding of FXI to glyocalcin, a glycoprotein present on the external portion of glycoprotein Ib (GPIb). However, it is unclear whether zinc acts alone or in concert with HK to localise FXI onto activated platelets (55, 56). On the platelet surface, FXIIa, FXIa or thrombin activates FXI to FXIIa, which in turn activates FIX to FIXa. The intrinsic tenase complex, which consists of FXa and its activated cofactor, FVIIIa, assembles on the activated platelet surface and propagates the initial procoagulant response (55, 56). Because zinc contributes to the regulation of FXIa binding to platelets, it is an important mediator of coagulation.

Protease nexin-2 (PN-2), which is secreted by activated platelets, inhibits FXIa. Zinc reduces the Kᵢ for FXIIa inhibition by PN-2 by at least 3-fold (57). Because platelet-bound FXIa is protected from PN-2 inhibition, whereas free FXIa is not, the release of zinc from platelets may localise FXIa activity to the platelet surface (57, 58).

With zinc concentrations in the platelet cytoplasm reduced, the regulatory switch of FXIIa activity facilitates FXII to FXIIa activation on the platelet surface (59, 61). Thus, zinc not only augments platelet aggregation, but also promotes the binding of adhesive proteins to platelets.
and aggregation, is also regulated via a zinc redox mechanism (70, 71). Taken together, these studies demonstrate the importance of zinc in modulating key signalling events that result in platelet aggregation.

Fibrin formation

Once generated, thrombin converts fibrinogen to fibrin, which then stabilises the platelet plug that forms at sites of injury. Zinc has multiple effects on the fibrin clotting process. Zinc inhibits the amidolytic activity of thrombin in a dose-dependent manner (72, 73). This finding is consistent with the observation that zinc attenuates thrombin-mediated fibrinopeptide A release (74). However, the same group also reported that zinc accelerates the rate of thrombin-induced fibrin clot formation (72, 74). In contrast, neither magnesium nor manganese had an effect on thrombin clot times, suggesting that modulation of thrombin-induced conversion of fibrinogen to fibrin is unique to zinc (72).

It is unclear how zinc attenuates the activity of thrombin, yet promotes fibrin clot formation. One hypothesis is that zinc interacts with fibrinogen/fibrin and potentiates the assembly of fibrin into a three-dimensional network. In support of this concept, zinc binds to fibrinogen and fibrin with $K_d$ values that ranges from 8 to 18 $\mu$M and with a stoichiometry of six zinc atoms per molecule of fibrinogen (75). The zinc binding sites on fibrinogen are likely localised to the D and/or the $\alpha$C-domains because zinc binds to fibrinogen fragments D and X, but not to fragment E (76). Additionally, analysis of fibrin clot structure reveals that zinc, but not manganese, increases the fiber thickness of thrombin-generated fibrin clots. Zinc-containing fibrin fibrils are thicker and have fiber diameters that are ~10-fold greater than those formed in its absence (75, 77). Furthermore, the zinc binding sites on fibrinogen are distinct from those of calcium, a known effector of fibrin polymerisation (75, 77-79). Although zinc enhances the lateral association of protofibrils to a greater extent than calcium, fibrin clots formed in the presence of zinc have reduced visco-elastic strength compared with those formed in the presence of calcium alone. Therefore, zinc modulates fibrin formation in a manner distinct from that of calcium (75, 77, 80). Together, these observations suggest that the ability of zinc to promote fibrin formation is not necessarily the result of altered thrombin proteolysis of fibrinogen, but more likely reflects modulation of fibrin polymerisation (74, 79).

Role of zinc in the regulation of anticoagulant pathways

The increase in the local zinc concentration that occurs upon platelet activation provides a positive feedback mechanism that ensures propagation of coagulation. However, zinc is also involved in several ancillary reactions that counteract coagulation. First, by regulating the assembly of the contact pathway proteins on platelets, zinc attenuates platelet activation. Second, zinc binds to FVIIa and protein S (PS) and attenuates FXa generation, thereby limiting the propagation of the extrinsic pathway. In addition, zinc also modulates the activity of the protein C (PC) anticoagulant pathway and heparin-mediated inhibition of coagulation. This section provides an overview of the mechanisms by which zinc attenuates coagulation and highlights the contention that zinc serves as a dynamic, regulatory switch in haemostasis and thrombosis.

Contact system on platelets

In addition to its effects on platelet aggregation, zinc also modulates platelet activation. Zinc promotes the binding of HK and FXII to GPlb on the platelet surface (81-83). The light chain of HK binds to anionic surfaces, whereas the heavy chain binds to GPlb (81, 84, 85). HK binds to both resting and activated platelets with a $K_d$ value of 1-20 nM. This interaction is dependent on zinc, but not calcium, suggesting that zinc modulates the binding of HK to platelets (20, 36). Current thinking is that cleavage of protease activated receptors 1 and 4 by thrombin triggers signalling events that lead to platelet activation. However, thrombin may also induce a platelet procoagulant response via activation of GPlb (86). Because HK and FXIIa can compete with thrombin for GPlb binding, thrombin-mediated platelet activation is attenuated (81-83, 87). The capacity of zinc to serve as both an activator and an inhibitor of platelet activation may provide a regulatory switch for control of haemostasis.

Inhibition of FVIIa

Extrinsic tenase, the complex of FVIIa with its cofactor TF, activates FX and FIX. The activity of FVIIa is allosterically regulated by TF and divalent cations. In addition to calcium, the crystal structure of $\beta$-aminobenzamidine-inhibited FVIIa in complex with soluble TF reveals two zinc atoms in the protease domain (88). Functional studies indicate that zinc attenuates the amidolytic activity of FVIIa regardless of whether it is in complex with TF, although calcium negates this effect (89, 90). It is likely that zinc targets the protease domain of FVIIa because the amidolytic activity of mutant FVIIa lacking gamma-carboxyglutamic acid (Gla) residues is attenuated by zinc to the same extent as the native protein (89, 90). However, zinc does not significantly alter the affinity of FVIIa for TF (89). Because zinc has the capacity to dampen FXa generation, the ion may attenuate thrombosis in the vicinity of a platelet rich thrombus, where the concentration of zinc is elevated.

Modulation of activated protein C generation and activity

The PC pathway plays a key role in anticoagulation. PC is converted into activated PC (APC) by the thrombin-thrombomodulin (TM) complex on ECs in a calcium-dependent manner (91, 92). By positioning PC in proximity to the thrombin-TM complex, the endothelial cell protein C receptor (EPCR) augments APC generation by 20-fold compared with thrombin-TM alone (reviewed in [93]). Zinc binds to PC and induces a conformational change that...
increases the affinity of PC for EPCR by 5- to 10-fold compared with calcium alone (92). In addition to facilitating phospholipid interactions, the Gla-domains of PC and APC also mediate their interaction with EPCR (94). PC and APC contain 8-10 zinc binding sites, 6-8 of which are localised to the Gla-domain. Therefore, zinc binding to the Gla-domain regulates PC and APC binding to EPCR. The ability of zinc to potentiate APC generation is only observed in the presence of calcium, suggesting that the two ions work in concert to promote APC generation (92). Collectively, these studies suggest that zinc augments APC generation by enhancing the interaction of PC with EPCR.

APC serves as an anticoagulant by inactivating the activated cofactors, FVa and FVIIa (95). In addition to binding to the Gla domain of APC, zinc also binds to its protease domain and induces a conformational change. In the absence of calcium, zinc inhibits the amidolytic activity of APC by ~7-fold, but the impact of this phenomenon on the anticoagulant activity of APC is unclear (92, 96). Zinc attenuates the capacity of APC to inactivate FV in the presence of phospholipid vesicles (96), but not on the EC surface (92); differences that suggest that APC activity varies depending on the membrane surface (97). Together, these studies suggest that zinc acts as a regulatory switch for APC by augmenting its generation and attenuating its activity.

Protein S anticoagulant activity

PS is a cofactor that enhances the anticoagulant activity of APC (98). However, PS also has PC-independent anticoagulant functions that are regulated by zinc. PS binds one zinc atom; an interaction that is lost with some PS purification methods (99, 100). In the absence of zinc, PS augments the capacity of tissue factor pathway inhibitor (TFPI) to inhibit FXa generation by extrinsic tenase (101, 102). However, in the presence of zinc, PS dampens the generation of FXa by extrinsic tenase in a TFPI-independent manner. Thus, in the presence of zinc, PS binds to FX, FXa and TF and attenuates assembly of the extrinsic tenase complex (103). An antibody directed against PS residues 621-635 only recognises zinc-containing PS, suggesting that zinc modulates PS conformation and activity (99). Moreover, zinc increases the affinity of PS for FXa by 16-fold, thereby enhancing PS-mediated inhibition of FXa activity (99, 100). Collectively, these observations suggest that zinc binds PS and modulates its anticoagulant function.

Anticoagulant activity of heparin

Heparin and its derivatives are commonly administered anticoagulants. Heparin exerts its anticoagulant activity by binding to antithrombin (AT) and enhancing its activity (104). There are multiple mechanisms by which zinc alters the anticoagulant activity of heparin. Zinc increases the affinity of heparin for fibrinogen by ~4-fold (105). By promoting heparin-fibrinogen interactions, zinc renders less heparin available to bind to AT, thereby attenuating its anticoagulant activity (105). A consequence of the interaction of heparin with fibrin is the formation of the ternary heparin-thrombin-fibrin complex, which protects fibrin-bound thrombin from inhibition by heparin-catalysed serpins, such as AT and heparin cofactor II (106).

Approximately 10-15% of circulating fibrinogen molecules are heterodimers containing a variant γ-chain (γA/γγ) and their abundance in plasma is associated with cardiovascular disease (107, 108). Because thrombin forms a higher affinity interaction with γA/γγ-fibrin than with γA/γA-fibrin, thrombin bound to γA/γγ-fibrin is more protected from inhibition by AT and heparin cofactor II (106). However, in the presence of heparin, zinc augments the affinity of thrombin for γA/γA-fibrin by 4-fold (105). Consequently, thrombin bound to γA/γA-fibrin in the presence of zinc and heparin is protected from AT to a similar extent as thrombin bound to γA/γγ-fibrin (105).

Pro- and anticoagulant functions of histidine-rich glycoprotein

The capacity of zinc to serve as a pro- and anticoagulant effector is highlighted by its interaction with the abundant plasma protein, histidine-rich glycoprotein (HRG) (15, 109). HRG is composed of multiple domains, the most notable being a histidine-rich region. Zinc binds to this region and induces a conformational change in the molecule, which facilitates HRG binding to various haemostatic factors, including heparin, fibrinogen and FXIIa (15, 110-113).

Binding of HRG to heparin abrogates its anticoagulant activity in both purified and plasma systems (114, 115). This effect is zinc-specific because neither copper nor magnesium influences the reaction (115). These studies suggest that zinc serves as a cofactor for heparin neutralisation by HRG, which dampens the anticoagulant activity of AT, thereby promoting thrombosis.

Conversely, HRG also exhibits zinc-dependent anticoagulant activity. Zinc potentiates the binding of HRG to FXIIa by 1,000-fold (112). By binding FXIIa, HRG dampens FXII autoactivation and FXIIa-mediated activation of FXI; and zinc is a cofactor in these processes (112). Moreover, in the presence of zinc, HRG binds to the carboxy-terminus of the γ-chain of γA/γ′-fibrinogen with a Kd of ~0.8 nM. Because the carboxy-terminus of the γ-chain also serves as a high affinity thrombin binding site, in the presence of zinc, HRG competes with thrombin for binding to this region (113). Fibrin-bound thrombin remains active and is protected from inhibition by fluid phase inhibitors (106, 116). By displacing thrombin from fibrin, zinc-containing HRG may, therefore, have antithrombotic properties.

Role of zinc in modulation of the fibrinolytic pathway

Compared with coagulation and anticoagulation, little is known about the role of zinc in fibrinolysis. Fibrinolysis is initiated when tissue-type plasminogen activator (tPA) or urokinase-type plasminogen activator (uPA) converts plasminogen (Pg) to plasmin (Pn). Pn then degrades fibrin to generate soluble fibrin degradation products. However, the role of zinc in fibrinolysis is poorly understood. Zinc appears to modulate the activity of plasminogen activators, potentially influencing the rate and extent of fibrinolysis. Further studies are needed to elucidate the precise mechanisms by which zinc regulates fibrinolysis.
products (117). In vitro studies suggest that zinc influences the activity of key components of the fibrinolytic pathway.

**Plasmin generation and fibrin degradation**

Zinc may be an effector of fibrinolysis because it modulates the activity of tPA, uPA and Pn. Zinc inhibits the catalytic activity of tPA and Pn in a dose-dependent manner and as a result, Pn generation by tPA is also dampened by zinc (118, 119). Although zinc binds to tPA, it is currently unknown whether it also binds to Pg and/or Pn (118, 119). The effects of zinc on fibrinolysis are unclear because none of the studies performed to-date has included fibrin. It is well-established that tPA binds to fibrin and uPA does not (117, 120). However, zinc, but not calcium or magnesium, promotes binding of single-chain (sc-) uPA to fibrin in a reversible and saturable manner with a Kd value of 300 nM and a stoichiometry of three sc-uPA molecules per fibrin monomer. The binding of sc-uPA to fibrin is specific because neither two-chain (tc-) uPA nor its degradation product, low-molecular-weight tc-uPA, binds fibrin in the presence of zinc (120). Pn and kallikrein convert sc-uPA to tc-uPA, and there is evidence that zinc accelerates this process on the fibrin surface (117). These results are interesting and posit a novel role for zinc in regulating the activity of fibrinolytic proteins.

Despite substantial evidence that zinc regulates the activity of proteins important in fibrinolysis, how this affects fibrin degradation remains unclear. There is some evidence that zinc potentiates lysis by altering fibrin clot structure. On scanning electron images, zinc-containing clots are coarser and composed of thicker fibrils than clots formed in the absence of zinc (75, 77-79). This may explain how zinc affects fibrinolysis because thicker fibers undergo more rapid lysis than thinner fibers (121, 122). Therefore, zinc has the capacity to impact fibrinolysis by modulating processes important in fibrin clot formation, Pg activation and lysis.

**Contact system and fibrinolysis**

Components of the contact system are also involved in fibrinolysis and these processes are regulated by zinc. Zinc mediates the localisation of HK and kallikrein onto the EC surface (40-42) and, in turn, kallikrein cleaves HK to generate bradykinin, a potent inductor of tPA release from ECs (123). Moreover, zinc potentiates FXIa-mediated Pg activation in the presence of kaolin (124). In addition, in the presence of zinc and sulfatides, FXIa is as potent

**Table 1: Overview of the zinc-binding proteins and the effects of zinc on haemostasis and thrombosis.**

<table>
<thead>
<tr>
<th>Target</th>
<th>Binding region</th>
<th>Consequence</th>
<th>Effect on haemostasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor XI/XIIa</td>
<td>Heavy chain</td>
<td>↑ factor XI autoactivation</td>
<td>Coagulation</td>
</tr>
<tr>
<td>High-molecular-weight kininogen (HK)</td>
<td>Light chain</td>
<td>↑ HK binding to polyanionic surfaces</td>
<td>Coagulation</td>
</tr>
<tr>
<td>Platelet calcium channels</td>
<td>Sulfhydryl-groups</td>
<td>↑ platelet aggregation</td>
<td>Coagulation</td>
</tr>
<tr>
<td>Fibrin(ogen)</td>
<td>D and/or αC-domains</td>
<td>↑ lateral association of fibrin protofibrils</td>
<td>Coagulation</td>
</tr>
<tr>
<td>Factor VIIa</td>
<td>Protease domain</td>
<td>↓ amidolytic activity</td>
<td>Anticoagulation</td>
</tr>
<tr>
<td>Protein C/activated protein C (APC)</td>
<td>Gla and protease domains</td>
<td>↓ APC generation on endothelial cell protein C receptor</td>
<td>Anticoagulation</td>
</tr>
<tr>
<td>Protein S</td>
<td>Unknown</td>
<td>↑ factor Xa generation by extrinsic tenase</td>
<td>Anticoagulation</td>
</tr>
<tr>
<td>Histidine-rich glycoprotein</td>
<td>Histidine-rich region</td>
<td>↑ interaction with heparin</td>
<td>Coagulation</td>
</tr>
<tr>
<td>Plasmin(ogen)</td>
<td>Unknown</td>
<td>↓ plasminogen activation by tPA</td>
<td>Antifibrinolytic</td>
</tr>
<tr>
<td>Tissue plasminogen activator (tPA)</td>
<td>Unknown</td>
<td>↓ proteolytic activity</td>
<td>Antifibrinolytic</td>
</tr>
<tr>
<td>Contact system proteins</td>
<td>Numerous</td>
<td>↑ release of tPA from endothelial cells</td>
<td>Profibrinolytic</td>
</tr>
<tr>
<td>Single-chain urokinase plasminogen activator (uPA)</td>
<td>Unknown</td>
<td>↑ interaction with fibrin</td>
<td>Profibrinolytic</td>
</tr>
<tr>
<td>Fibrin</td>
<td>D and/or αC-domains</td>
<td>↑ fibrin fiber thickness</td>
<td>Profibrinolytic</td>
</tr>
</tbody>
</table>
an activator of Pg as uPA (125). Therefore, these studies suggest that the contact pathway is also important in fibrinolysis and that zinc modulates these processes.

Conclusion

Zinc is important in platelet aggregation, coagulation, anticoagulation and fibrinolysis (►Table 1). Zinc promotes initiation of the contact system in both soluble and cellular compartments. Once activated, platelets secrete zinc, which provides a positive feedback mechanism that is essential for the propagation of coagulation. Zinc further stimulates coagulation by potentiating platelet aggregation and fibrin clot formation. Furthermore, zinc has secondary effects on anticoagulant pathways and fibrinolysis, which dampen the procoagulant response. Consequently, zinc not only serves as a regulatory switch that ensures entry into the propagation phase of coagulation, it also limits these processes by activating compensatory inhibitory pathways. The seemingly paradoxical role played by zinc is, in fact, a trait shared with calcium, which also simultaneously participates in numerous pathways in haemostasis.

Zinc binds to proteins and alters their enzymatic activity or promotes protein interactions (2, 113). Several mechanisms exist to regulate local concentrations of zinc and its activity; processes that enable zinc to serve as a dynamic regulator of haemostatic reactions. The concentration of free zinc increases in the vicinity of activated platelets, and its capacity to bind to proteins is modulated by the pH in the local microenvironment. Consequently, the ability of zinc to act as an effector molecule is attenuated under ischaemic, hypoxic or acidic conditions (11, 13-15). Since the processes that maintain haemostasis and thrombosis are spatially and temporally regulated, the effects of zinc on these reactions may also vary depending on the location and sequence of events. Such regulation endows zinc with the capacity to act as a dynamic regulator of haemostasis and thrombosis.

The procoagulant activity of zinc is particularly relevant to contact-initiated coagulation. Because there is increasing evidence that the contact system contributes to thrombosis (23, 24), zinc may be an important modulator of thrombosis. Collectively, these observations suggest that a better understanding of the mechanisms of zinc regulation in haemostasis and thrombosis may lead to novel strategies to mitigate and treat thrombotic disorders.

Conflicts of interest

None declared.

References


32. Liocego; A, Randriahazana HN, Negro R. Influence of Zn(2+) on the kinetic events that contribute to the 500-kDa dextran-sulfate-dependent activation of factor XII (Hageman factor). Eur J Biochem 1997; 246: 204-210.


70. Scully MF, Kakkar VV. Structural features of fibrinogen associated with binding to cleated zinc. Biochim Biophys Acta 1982; 700: 130-135.


Vu et al. Zinc regulation of haemostasis and thrombosis


117. Schoubose I. Factor XII activation of plasminogen is enhanced by contact activating surfaces and Zn(2+). Blood Coagul Fibrinolysis 1997; 8: 97-104.