Thrombin

Ma Hongbao *, Jenny Young **, Cherng Shen ***

* Bioengineering Department, Zhengzhou University, Zhengzhou, Henan 450001, China, mahongbao2007@gmail.com, 718-404-5362
** New Start Company, Brooklyn, New York 11212, USA, youngjenny2008@yahoo.com
*** Department of Electrical Engineering, Chengshiu University, Niaosong, Taiwan 833, China, cherngs@csu.edu.tw; 011886-7731-0606 ext 3423

Abstract: Thrombin is most widely recognized for its role in blood coagulation but the diversity of this protein's functions in the body are far more widespread. There are thousands of articles related to thrombin published each year. This review article describes the major functions of thrombin in the body, considering the different processes separately. [Nature and Science. 2008;6(2):90-93]. ISSN: 1545-0740.

Keywords: coagulation; fibrinogen; thrombin

1. Introduction

Thrombin is most widely recognized for its role in blood coagulation but the diversity of this protein's functions in the body are far more widespread. Indeed, it has been noted by one group of workers that almost every cell type tested (except erythrocytes) was responsive to thrombin (Fenton, et al, 1998). This review article describes the major functions of thrombin in the body, considering the different processes separately.

2. Thrombin in Coagulation

The blood coagulation system comprises a cascade of proteases that cleave precursor enzymes to form active enzymes, forming an extremely effective amplification system that culminates in the formation of thrombin. Thrombin, once formed, then catalyses the conversion of fibrinogen to fibrin to form a clot and, importantly, regulates the system by providing stimulatory and inhibitory feedback. The centrality of thrombin to this system makes it an extremely powerful enzyme in coagulation and makes it the most suitable enzyme to be a target for anticoagulant drug therapy (Fenton, 1998), as to control the action of thrombin would be to control the entire coagulation system.

3. Fibrin formation

The main role of thrombin in coagulation is the cleavage of soluble fibrinogen to form insoluble fibrin, the basis of the haemostatic clot. Fibrinogen cleavage is an orderly process comprising of two distinct steps, both catalysed by thrombin (Mosesson, 1998). The fibrinogen molecule is made up of a central "E" domain, attached to two "D" domains, one on either side. In the first step of fibrinogen cleavage, thrombin cleaves fibrinogen at the "E" domain, releasing fibrinopeptide A (FPA) and exposing a polymerisation site, so-called EA. The exposure of this site allows relatively weak association between this site and a D domain of another fibrin molecule; the molecules are then arranged in an end-to-middle, cross-linking fashion and fibrin polymerisation thus begins to occur. In the second step, thrombin cleaves the E domain again, releasing fibrinopeptide B (FPB) and exposing another fibrin polymerisation site, EB. EB associates with another site on D domains of other molecules, and the cross-linking is strengthened (Mosesson, 1998).

Thrombin's role in fibrin formation does not quite end there, however. The fibrin matrix is strengthened by more cross-linking by factor XIII, a transglutaminase generated by thrombin formed after the formation of the fibrin clot, which cross-links the fibrils, thus stabilizing the clot (Dahlbäck, 2000).

4. Positive feedback

Once the coagulation cascade is initiated and thrombin is generated, the initial stimulus is often "turned off", and the cascade is maintained by the feedback effects of thrombin. This stimulatory feedback comes in the form of activation of factors V, VIII and XI (Narayanan, 1999). Factor V, when activated, associates with activated factor X and cleaves prothrombin to thrombin. Factor VIII, when activated, associates with activated factor IX and activates factor X. Factor XI, when activated, activates factor IX. In this way, the cascade is up-regulated, and a very large amount of product (i.e. fibrin) can be formed from a relatively

small initial stimulus. This role of thrombin makes it an extremely powerful procoagulant and causes it to be the target of many anticoagulant drug therapies.

To complement its role in fibrin clot formation, thrombin also plays a part in inhibition of lysis of that clot (Broze, 1996). This action is carried out by a plasma carboxypeptidase enzyme, which circulates in the blood as an inactive proenzyme and is activated by thrombin. This enzyme, termed "thrombin activatable fibrinolysis inhibitor" (TFPI) inhibits fibrinolysis by cleavage of carboxy-terminal lysine residues on the fibrin polymers (Tilburg, Rosendaal & Bertina, 2000). These residues are important in assembling components of the fibrinolytic system, so their removal inhibits fibrinolysis.

5. Inhibitory feedback

Thrombin regulates its own production by being part of an inhibitory system. This is achieved via binding to a vascular endothelial cell protein called thrombomodulin. This leads to activation of protein C (causing inactivation of coagulation factors V and VIII and thus down-regulation of thrombin generation), and inhibition of thrombin's ability to form fibrin and activate factor XIII, platelets and coagulation feedback stimulatory proteins (Esmon, 2000).

Inhibitory feedback Thrombin regulates its own production by being part of an inhibitory system. This is achieved via binding to a vascular endothelial cell protein called thrombomodulin. This leads to activation of protein C (causing inactivation of coagulation factors V and VIII and thus down-regulation of thrombin generation), and inhibition of thrombin's ability to form fibrin and activate factor XIII, platelets and coagulation feedback stimulatory proteins (Esmon, 2000).

The protein C system of thrombin inhibition is extremely powerful under normal circumstances, and so must be confined to the site of injury. This confinement is achieved by the necessity of binding to thrombomodulin, which is expressed on damaged endothelial cell walls.

As well as inhibiting fibrinolysis, as seen earlier, thrombin also has indirect effects that are stimulatory for this phenomenon. Thrombin is a chemoattractant for neutrophils, which play a role in degradation of the fibrin clot (Sonne, 1988) and thrombin releases plasminogen activators from endothelial cells which generate plasmin. Plasmin then initiates fibrinolysis and inactivates prothrombin (Fenton, et al, 1998). This apparent anomaly, that thrombin plays a role in both enhancement and inhibition of fibrinolysis, may seem confusing but is an example of the high degree of regulation which is integral to maintaining the fine balance of haemostasis. This regulation must be extremely rigorous in order to prevent the catastrophic consequences of both excessive haemorrhage and excessive coagulation. Thrombin is central to this regulation.

6. Thrombin and Platelets

Supplementary to its role in coagulation, thrombin also plays a vital role in primary haemostasis, as an extremely potent activator of platelets. This thrombin-induced platelet activation is critical for adequate haemostasis (Hung, et al, 1992). Platelet activation by thrombin, as by any other platelet agonist, results in intra-platelet events such as activation of phospholipase C, inhibition of adenylate cyclase, and mobilisation of calcium, culminating in platelet aggregation (Hayes, et al, 1994). This activation is initiated by interaction of the thrombin molecule with a receptor on the surface of the platelet, with subsequent activation of various secondary messenger systems, of which the inositol triphosphate/calcium system is probably the most important (Harrison, 2000). The interaction of thrombin with the platelet receptor has been extensively studied. The receptor, a member of the seven-transmembrane domain family, has been cloned (Hung, et al, 1992). The interaction of these two molecules is interesting because the kinetics of the reaction suggest that what actually occurs is not simple ligand-receptor interaction, but something more akin to an enzyme-substrate interaction where thrombin enzymatically cleaves the receptor/substrate (Hayes, et al, 1994). The amino terminal extracellular domain of the receptor contains a cleavage site for thrombin, which is structurally similar to the anticoagulant hirudin and is therefore able to bind thrombin (Liu, et al, 1994). Thrombin cleaves this site between Arg 41 and Ser 42 exposing a new amino terminal domain, which acts as a ligand for the receptor itself - termed a "tethered ligand" (Liu, et al, 1994).

The role of thrombin in platelet activation is not an isolated one, but is closely associated with thrombin's other roles in coagulation, particularly fibrin formation. As platelet aggregation and coagulation go hand-in-hand during bleeding, it is fitting that the molecule that is central to and regulates one system also regulates the other. Whenever fibrin formation is required to achieve haemostasis, platelet aggregation will also be required, and vice versa.

7. Thrombin and Inflammation

Thrombin has various actions in inflammation, a few of which will be discussed. Firstly, thrombin is a chemoattractant for neutrophils (Esmon, 2000) and monocytes (Becker, et al, 1998), that is it induces the cells to move down a chemical gradient to where the thrombin is most concentrated, i.e. the site of injury. This allows the neutrophils and monocytes to carry out their phagocytic role if there is invading bacteria present.

Thrombin stimulates the production from Weibel-Paladi bodies in endothelial cells, of the cell-anchoring protein P-selectin, which is then expressed on their membrane (Esmon, 2000). This molecule is important in the process of leucocyte "rolling", in which leucocytes are loosely bound to the vessel wall and therefore begin to slow down their flow rate, and roll along the endothelium, eventually stopping where they are required. Thrombin also stimulates endothelial cells to produce platelet activating factor (PAF). Although not implied in its name, PAF is a potent activator of neutrophils especially those bound to P-selectin.

In addition to P-selectin, thrombin induces the production of other pro-inflammatory and pro-coagulant substances from endothelial cells including von Willebrand factor, growth factors and cytokines, and induces changes in the endothelial cell itself including shape change and increased permeability (Coughlin 1999).

Thrombin also acts on monocytes to induce production of proinflammatory cytokines such as interleukin 6 (IL6) and IL8, and on endothelial cells to produce other inflammatory cytokines (Cate, 2000). These cytokines, as well as initiating an inflammation response, have been shown to induce thrombin production by mononuclear cells, and thus induce a coagulation response also (Cate, 2000).

In this way, the processes of coagulation and inflammation are linked, with thrombin at the centre of the interaction between the two.

8. Other Roles of Thrombin

As mentioned previously, it has been suggested that thrombin affects almost all cell types in some way, so it is not difficult to imagine that there are more roles of thrombin in the body than we are currently aware of. It has been suggested recently that thrombin plays a part in nervous development, and as such in the pathophysiology of Alzheimer's disease (Turgeon & Houenou, 1997). Alzheimer's disease pathology centres around vascular and cerebral plaques which are composed largely of a protein called amyloid beta protein. Thrombin has been shown to cause the secretion from endothelial cells of the precursor to this protein - amyloid precursor protein (APP) (Ciallella, et al, 1999) and so has been implicated in Alzheimer's disease pathology.

Thrombin causes cell proliferation in a number of cell types including smooth muscle cells and macrophages. This function of thrombin causes it to be implicated in the disease process of atherosclerosis and the build-up of the atherosclerotic plaque which contains many macrophages and smooth muscle cells (Becker, et al, 1998).

9. Conclusion

The biological roles of thrombin are diverse. It is evident that thrombin is a molecule with an extremely wide range of biological roles, and an extremely high amount of regulation associated with it due to its potency as a pro-coagulant and pro-inflammatory mediator. This essay described the main roles of thrombin, and in particular those which interest the haematologist. New functions of thrombin are being discovered at a rapid rate and research is continuing into how we can use and control those functions to our advantage, for example with the development of thrombin-specific anticoagulants such as hirudin.

Correspondence to:
Ma Hongbao, Ph.D.

Bioengineering Department
Zhengzhou University
Zhengzhou, Henan 450001, China
mahongbao2007@gmail.com
718-404-5362

February 25, 2008

References

1. Becker RC, Bovill EG, Seghatchian MJ, Samama MM. Pathobiology of thrombin in acute coronary syndromes American Heart Journal. 1998;136:S19-31.
2. Broze GJ. Thrombin-dependent inhibition of fibrinolysis. Current Opinions in Haematology. 1996;3(5):390-394.
3. Cate H. Pathophysiology of disseminated intravascular coagulation in sepsis. Critical Care Medicine 2000;28(9):S9-11.
4. Ciallella JR, Figeuiredo H, Smith-Swintosky V, McGillis JP. Thrombin induces surface and intracellular secretion of amyloid precursor protein from human endothelial cells. Thrombosis and Haemostasis. 1999;81(4):630-637.
5. Coughlin SR. How the protease thrombin talks to cells. Proc Natl Acad Sci 1999;96(20):11023-11027.
6. Dahlbäck B. Blood coagulation. Lancet. 2000;355(9215):1627-1632.
7. Esmon C. The protein C pathway. Critical Care Medicine. 2000;28(9):S44-48.
8. Fenton JW, Ofosu FA, Brezniak DV, Hassouna HI. Thrombin and antithrombotics Seminars in Thrombosis and Hemostasis. 1998;24(2):87-91.
9. Harrison P MSc. Haematology Lecture Notes. 2000.
10. Hayes KL, Leong L, Henriksen RA, Bouchard BA, Ouellette L, Church WR, Tracy PB. a-thrombin - induced human platelet activation results solely from formation of a specific enzyme - substrate complex. Journal of Biological Chemistry. 1994;269(46):28606-28612.
11. Hung DT, Vu TH, Wheaton VI, Ishii K, Coughlin SR Cloned platelet thrombin receptor Is necessary for thrombin - induced platelet activation. Journal of Clinical Investigation. 199;89(4):1350-1353.
12. Liu L, Freedman J, Hornstein A, Fenton JW & Ofosu FA. Thrombin binding to platelets and their activation in plasma. British Journal of Haematology. 1994;88(3):592-600.