CHAPTER II

LITERATURE REVIEWS

Blood coagulation

The blood coagulation system is consisted of a series of coagulation factors that functionally specific plasma protein. These proteins interact in a highly ordered and predefined sequence with the single object of converting the soluble protein fibrinogen to an insoluble formation of fibrin which consolidates and stabilize the primary haemostatic plug (Pallister, 1994, p. 456).

Mechanism of blood coagulation

The Coagulation is the process of blood forms clots. The stop of blood loss from a damaged vessel. A damaged blood vessel wall is plugged by fibrin to stop bleeding. The mechanism consists of 2 stages of primary and secondary hemostasis.

1. Primary Hemostasis

The first step was the response of platelets from the blood vessels and contraction of blood vessels that is stimulated from reflex stimulation of the nervous system and of chemicals such as serotonin and TXA_2 includes 4 steps.

1.1 The adhesion of platelets to collagen with vWF bound the GPIb / IX receptor on the wall of platelets and collagen.

1.2 Platelet shapes change from the discoid shape into the pseudopod that was ready to release of granule contents and attach themselves.

1.3 Platelet degranulation occurs largely as a result of the fusion of the cytoplasmic granules with the surface-connected tubular system. Degranulation is accompanied by enhanced phosphatidyl inositol and arachidonic acid metabolism, both of which promote platelet aggregation (Pallister, 1994, p. 456).

1.4 Platelet aggregation is the platelet binding to platelets with fibrinogen as a mediator between the receptor on the platelet surface. This receptor is a glycoprotein called GPIIb / IIIa. Results of this process will lead to a platelet plug that plugs the site of injury (นิโลบล เนื่องตัน, 2539, หน้า 73).

2. Secondary Hemostasis

Secondary hemostasis is the formation of fibrin through the coagulation cascade. The clotting sequences were divided into 2 pathways.

2.1 Intrinsic pathway

The intrinsic pathway (also called the contact activation pathway) is much less significant to hemostasis under normal physiological conditions than is the extrinsic pathway. However, abnormal physiology can fore to activation of thrombosis via the intrinsic clotting cascade. The intrinsic pathway was thought to be started by activation of contact factors and involves the subsequent activation of kallikrein, factor XII, factor XI, and factor IX, leading to factor X activation (Schoenmakers, et al., 2005).

When factors contact with a negatively charged surface such as glass or the vascular basement membrane, factor XII becomes factor XIIa. The high molecular weight kininogen (HMWK) helps conversion of FXII to FXIIa. The factor XIIa converts prekallikrein to kallikrein. The kallikrein accelerates the conversion of factor XII to factor XIIa. The factor XIIa proteolytically cleaves factor XI to factor XIa. Then, factor XIa proteolytically cleaves factor IX to factor IXa. The factor IXa factor Xa and thrombin, proteolytically cleave factor VIII to factor VIIIa. The factor VIIIa converts factor X to its active form, factor Xa (พรวรีย์ ลำเจียกเทศ และคณะ, 2540, หน้า 8-9).

2.2 Extrinsic pathway

This pathway is started by the formation of a complex between factor VIIa and a tissue factor on cell surfaces. When a damage to the endothelium permits factor VII to come into contact with the tissue factor, the tissue factor activates factor VII to factor VIIa. The factor VIIa/tissue factor complex work with Ca^{2+} converts factor X to its active form, factor Xa (Riddel, et al., 2007).

2.3 Common Pathway

The common pathway start with the activation of factor X within the intrinsic pathway and/or the extrinsic pathway. The first protease of the common pathway is factor Xa. Factor Xa, and in the presence of factor V, Ca^{2+}, and phospholipids, converts prothrombin to thrombin. Thrombin converts fibrinogen to fibrin to generate the fibrin clot. Thrombin also activates factor XIII to factor XIIIa and mediates the covalent cross-linking of the fibrin polymers to form a stable fibrin

network. Thrombin can catalyze the formation of the cofactors factor Va and factor VIIIa, resulting in effective increase of coagulation (Riddel, et al., 2007).

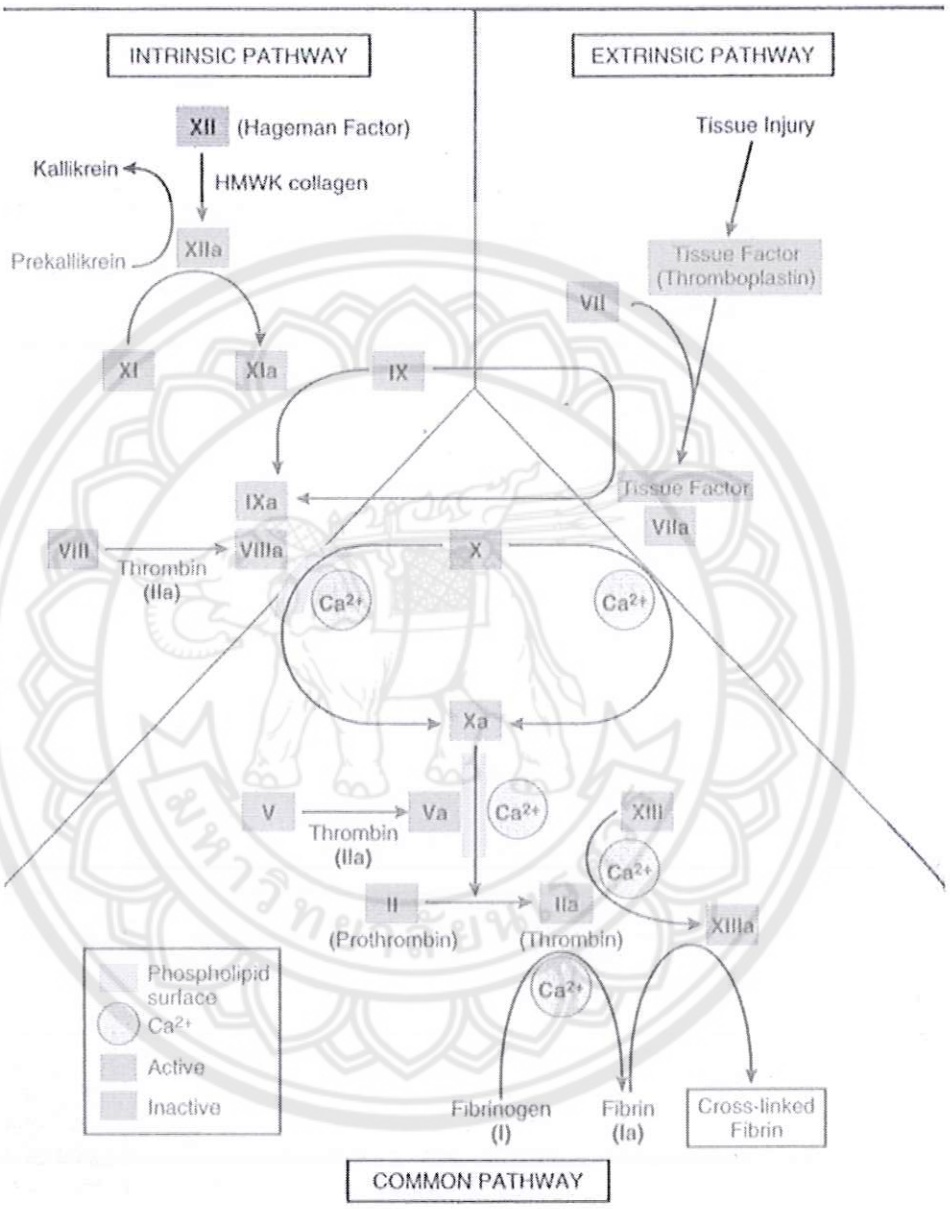

Figure 1 The coagulation cascade model

Source: Riddel, et al., 2007

Component of the fibrinolytic system

Most enzymes of the fibrinolytic system are serine proteases, because their active site consists of a catalytic triad composed of the amino acids serine, aspartic acid and histidine.

Plasminogen

Human Plasminogen in plasma is synthesized in the liver (Raum, et al., 1980). However, other sources have been identified that include adrenal glands, kidney, brain, testis, heart, lung, uterus, spleen, thymus, and gut (Zhang, et al., 2002). Plasminogen is a 92 kDa, single-chain proenzyme glycoprotein consisting of 791 amino acids; it contains 24 disulfide bridges and five homologous kringles (Forsgren, et al., 1987). Tissue or urinary plasminogen activators cleave of a single Arg–Val peptide bond at position 561-562 of plasminogen converting plasminogen to plasmin. The two-chain plasmin molecule is composed of a heavy chain containing the five kringles (N-terminal), and a light chain (C-terminal) containing the catalytic triad, composed of His603, Asp646 and Ser741 (Forsgren, et al., 1987). N-terminal glutamic acid of native plasminogen (Glu-plasminogen) is readily converted by limited plasmic digestion to modified forms with N-terminal lysine, methionine, or valine, commonly designated Lys-plasminogen. Lys-plasminogen is much more readily activated to plasmin than Glu-plasminogen. Glu-plasminogen exists in a closed conformation, whereas Lys-plasminogen presents a more open conformation, which is a preferential substrate for plasminogen activators (Castellino and Ploplis, 2005). Hydrolysis of the Lys77-Lys78 peptide bond gives rise to a conformationally modified form of the zymogen that more readily binds fibrin, and is activated 10–20 times more rapidly than Glu-PLG (Hoylaerts, et al., 1982; Holvoet, et al., 1985). Lys-PLG does not normally circulate in plasma, but has been identified on cell surfaces (Holvoet, et al., 1985).

Tissue plasminogen activator (tPA)

Tissue plasminogen activator (tPA), one of two major endogenous plasminogen activators, is a 72 kDa serine protease, a single polypeptide chain consists of 527 amino acids (Rijken and Collen, 1981). Plasmin converts tPA to a two-chain form by hydrolysis of the Arg–Ile peptide bond at position 275-276. In contrast to the single chain precursor form of most serine proteases, single chain tPA is enzymatically active. The fibrinolytic activities of one chain and two chain

plasminogen activators were compared in two different test systems. The single molecular form tPA shows less activity than the two molecular forms tPA in the fluid phase, however, both forms demonstrate the same activity (lysis time) in fibrin bound (Rijken, et al., 1982). The N-terminal region of tPA is composed of 5 structural domains with homologies to other proteins: a finger domain (fibronectin- like), including residues 4–50; an epidermal growth factor domain consisting of residues 50–87; two kringles including residues 87–176 and 176–262, and structure homologous to plasminogen and a serine protease domain. The region established by residues 276–527 represents the serine protease part with the catalytic triad site, composed of His322, Asp371 and Ser478. The tPA molecule comprises three potential N-glycosylation sites with high mannose carbohydrate on Asn117, Asn184 and complex oligosaccharide on Asn448 and an O-linked α-fucose attachment site at Thr61 (Harris, et al., 1991). The carbohydrate moieties of tPA may modulate its functional activity, regulate its binding to cell surface receptors and specify degradation pathways (Cesarman and Hajjar, 2005). The tPA is synthesized and secreted mainly from endothelial cells. The release of tPA is managed by a variety of stimuli, such as thrombin, histamine, bradykinin, adrenaline, acetylcholine, Arg vasopressin, gonadotropins, exercise, venous occlusion and shear stress. Its circulating half-life is exceptionally short (5-6 min). Although expressed by extravascular cells, the major intravascular activator of plasminogen is tPA (Cesarman and Hajjar, 2005). Kinetic analysis of tPA is a poor plasminogen activator in the absence of fibrin. In the presence of fibrin, however, its activity is two orders of magnitude higher. The kinetic model indicates that both t-PA and plasminogen bind to fibrin in a sequential and ordered way, yielding a cyclic ternary complex in which t-PA has a markedly enhanced affinity for its substrate plasminogen (Hoylaerts, et al., 1982).

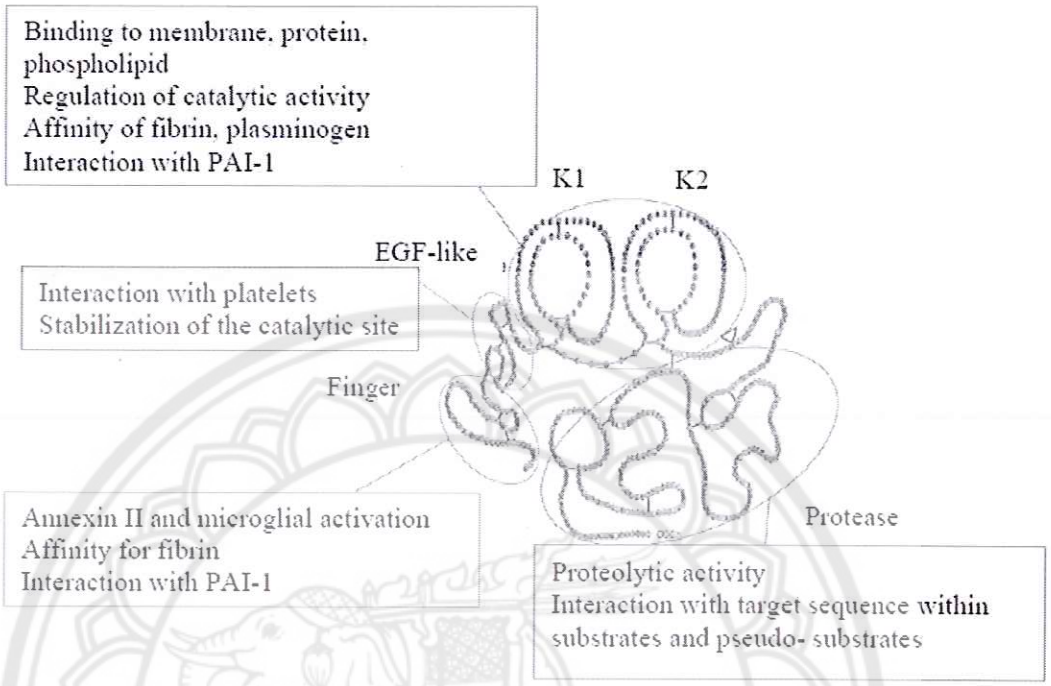

Figure 2 Functional domains of tPA

Source: Manuel, et al., 2008

Table 1 Some properties of the main components of the fibrinolytic system

Component	M_r (Dalton)	Number of amino acids	Catalytic triad of serine proteases	Plasma concentration	
				(lg mL^{-1})	(molarity)
Plasminogen	92,000	791	-	200	2 μM
Plasmin	85,000	715	His603, Asp646, Ser741	-	-
tissue-type plasminogen activator (t-PA)	70,000	530 (527)	His322, Asp371, Ser478	0.005	70 pM
urokinase-type plasminogen activator (u-PA)	54,000	411	His204, Asp255, Ser356	0.002	40 pM
urokinase-type plasminogen activator receptor (u-PAR)	55,000	313	-	-	-
α2-Antiplasmin	70,000	464	-	70	1 μM
plasminogen activator inhibitor-1 (PAI-1)	52,000	379	-	0.02	400 pM
thrombin-activatable fibrinolysis inhibitor (TAFI)	60,000	401		5	75 nM

Source: Rijken and Lijnen, 2009

The mechanism of fibrinolysis system

Fibrinolysis is an analogous series of transformations of zymogens to proteolytic enzymes, which, in the present of cofactor on cell surfaces, convert plasminogen to plasmin, which can hydrolyze the fibrin network, thereby dissolve it (Colman, et al., 2006, p.17). In fibrinolytic system, which refers to fibrin degradation, is activated by blood coagulation. The process of fibrin degradation by plasmin

consists of 2 steps: plasminogen activator activates plasminogen to plasmin, and plasmin degrades fibrin to fibrin fragments called fibrin degradation product or FDP.

The major fibrinolytic protein, plasmin, is derived from plasminogen, an inactive zymogen which circulates in blood. In its native form, plasminogen exists as a single chain polypeptide with a glutamic acid residue at the N-terminal position (glu-plasminogen) (Dudani, 2000). The action of plasmin on Glu-plasminogen results in the cleavage of the NH_2- terminal pre-activation peptide by hydrolysis of one or several of the following bonds: Arg68–Met69, Lys77–Lys78, or Lys78–Val79 and the final product obtained is Lys-plasminogen (lacking the first 76 amino acids and having lysine at the N-terminus), Lys-plasminogen is more easily activated to plasmin than Glu-plasminogen by activators such as tissue plasminogen activator (tPA) and urokinase (uPA) (Castanon, et al., 2007; Holvoet, et al., 1985). The activation of plasminogen by tPA is strongly enhanced in the presence of fibrin, while the activation by u-PA is slightly influenced by fibrin (Nishino, et al., 2000). Both uPA and tPA are secreted as single-chain polypeptides. The single-chain form of uPA is an inactive proenzyme, whereas the two-chain form is the active enzyme (Eaton, et al., 1984; Inge, et al., 2008). The single chain of tPA, almost fully active enzyme, can be further processed into a two-chain fully active form by a cleavage after Arg-310 catalyzed by plasmin, tissue kallikrein or factor Xa (Inge, et al., 2008). Structural motifs known as kringles are required for the interaction of plasminogen and tPA with fibrin, target proteins and cells.

Plasminogen has five kringles whereas tPA has two. These kringles are homologous to one another and are known as lysine-binding sites because of their affinity for omega amino carboxylic acids (Dudani, 2000; Collen and Lijnen, 1991). The tPA is fibrin specific. This is due to the fibrin binding property of tPA and the stimulation of its plasminogen activation activity by fibrin. The finger and the kringle 2 domain are assumed to be involved in the affinity of tPA to fibrin and/or the stimulation of the plasminogen activation by fibrin and fibrin degradation products (Kohnert, et al., 1996).

Tissue type plasminogen activator (tPA) activates fibrinolysis by cleaving plasminogen to plasmin. This activator can also act as an anticoagulant (Gaffney and Edgell, 1996). Plasmin, the enzyme responsible for the degradation of fibrin, is formed

when plasminogen is partly cleaved by tPA on the surface of fibrin. Both plasminogen and tPA bind to fibrin under circumstances in which tPA is protected from inhibition by PAI-1, and assembly of plasminogen and tPA on cell surface-binding sites enhances plasmin generation and protects cell-bound plasmin from inactivation by an α-antiplasmin (Pernod, et al., 1998). This process facilitates both the activation of plasminogen and the degradation of fibrin (Epstein, et al., 2000).

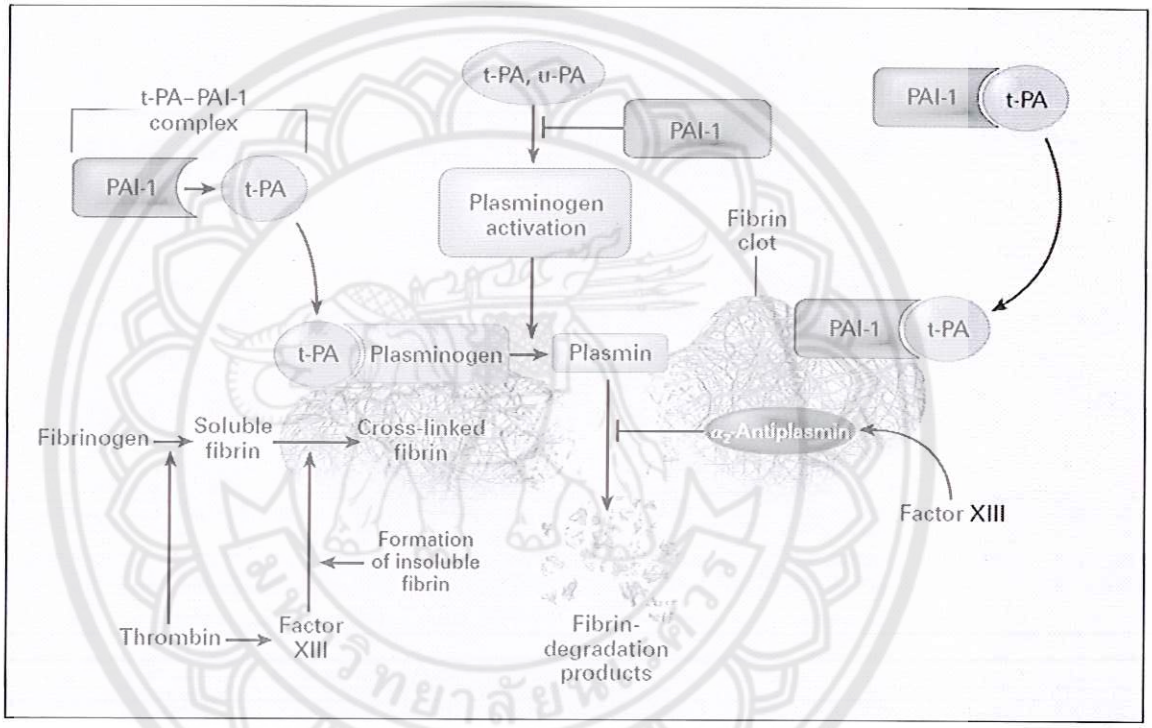

Figure 3 Activation and Inhibition of the Fibrinolytic Pathway

Source: Epstein, et al., 2000

Diseases caused by defective fibrinolysis focus on thrombosis

Optimal haemostasis requires the interaction of numerous components of the blood vessel wall, platelets, coagulation system and the fibrinolytic system. Defects affecting any of these mechanisms can predispose to a hemorrhagic or thrombotic diathesis (Pallister, 1994, p. 481).

Hypofunction of the fibrinolytic system alters the haemostatic balance and can lead to thrombosis by inhibiting clot dissolution while clot formation occurs unimpeded (Antony J. Comerota, 1995, p. 33).

1. Plasminogen Deficiency

The main operation of plasmin is to dissolve fibrin through a series of proteolytic cleavages. Incomplete fibrinolysis has been associated with thrombovascular disease (Indiana Hemophilia & Thrombosis Center, 2012).

2. Decreased Levels of Tissue Plasminogen Activator (tPA)

Tissue plasminogen activator (tPA) is synthesized by endothelial cells. When tPA, a serine protease, is released, it converts plasminogen to plasmin. Practically, decreased release of tPA could lead to a hypercoagulable state due to decreased fibrinolysis (Indiana Hemophilia & Thrombosis Center, 2011-2012).

3. Increased Levels of Plasminogen activator inhibitor 1 (PAI-1)

Plasminogen activator inhibitor 1 (PAI-1) functions as the primary inhibitor of plasminogen activator in plasma. Increased levels of PAI-1 could lead to excessive inhibition of tPA, leading to decreased activation of fibrinolysis and a thrombotic proclivity (Indiana Hemophilia & Thrombosis Center, 2012).

Thrombolytic therapy

Thrombolytic therapy has been established as an important modality in the treatment of various thromboembolic disorders. Thrombolytic therapy as it is practiced today involves the activation of the body own mechanism for dissolving fibrin.

The action of thrombolytic drugs or fibrinolytic drugs is to convert plasminogen to plasmin. Plasmin is a serine protease that cleaves fibrin to fibrin degradation product. The drugs used for pulmonary embolism, deep venous thrombosis and myocardial infarction.

1. Streptokinase (SK)

SK is a protein with a molecular mass of 47 kDa. It is produced by beta-hemolytic streptococci. SK is the oldest of the clinically available thrombolytic drugs. SK does not cleave plasminogen directly, rather first forming and equimolar stoichiometric 1:1 complex of plasminogen or plasmin with SK. This complex of plasmin or plasminogen is true activator, which can convert plasminogen to plasmin.

The plasminogen-SK activator behaves as a serine protease (Antony, J. Comerota, 1995, p. 89; สุรศักดิ์ เสาแก้ว, 2549, หน้า 9). SK has a plasma half-life of 30 min. SK is also associated with bleeding complications due to its non-fibrin specificity (Kumar, 2010).

2. Urokinase (UK)

UK is a protein with a molecular mass of 31.6-54 kDa. It is extracted from human kidney cell lines. UK activates plasminogen directly (Antony J. Comerota, 1995, p. 99-100). The initial half-life of UK was 6-9 minutes upon administration to the patients with acute myocardial infarction (Kumar, 2010).

3. Tissue plasminogen activator (tPA)

tPA is synthesized by endothelial cells with a molecular mass of 70 kDa. tPA converts plasminogen that binds to fibrin, to plasmin faster than free plasma plasminogen. The physiologic role of tPA is to cleave the Arg-Val peptide bond of plasminogen , converting to plasmin, and thus to accelerate the process of fibrinolysis (Antony J. Comerota, 1995, pp. 105). tPA has a plasma half-life of 8 min (สุรศักดิ์ เสาแก้ว, 2549, หน้า 9).

Considering the three types of drugs in this group, SK is the best stimulus plasma plasminogen, followed by UK and tPA consequently. The drug of thrombosis at present is expensive and it has certain side effects, for example, bleeding.

Table 2 Property of thrombolytic drugs

	SK	UK	rt-PA
Half-life (min)	16, 83	15	8
Fibrin enhancement	1+	2+	4+
Plasma proteolytic state	4+	3+	2+
Bleeding complications	4+	4+	4+
Allergy	Yes	No	No
Antigenicity	Yes	No	Not known
Expense	1+	3+	4+

Source: สุรศักดิ์ เสาแก้ว, 2549, หน้า 10

Chromogranin A

The secretory granules of adrenal medullary chromaffin cells synthesize, store and then liberate catecholamines by exocytosis after cell stimulation with acetylcholine. Chromaffin granules contain a great number of molecules, essentially protein, which are co-secreted with the catecholamines as shown in Figure 4. The soluble acidic proteins of chromaffin granules have been collectively called chromogranins (Blaschko, et al., 1967).

Granins consist of single-polypeptide chains of approximately 180 to 700 amino acid residues, bearing an amino-terminal signal peptide that directs the movement of the preproteins from ribosomes to the endoplasmic reticular lumen and, hence, the Golgi complex, where further post-translational modifications occur (Laurent, et al., 2003). The granins are products of distinct genes and are uniquely acidic secretory protein characterized by an excess of acidic amino acid residues resulting in a net negative charge. The granins are ubiquitously distributed in the secretory cells of the nervous, immune and diffuse neuroendrocrine (NE) system of vertebrates and invertebrates (Helle, et al., 2004).

Chromogranin A (CgA) was originally identified as the major soluble protein secreted from the secretory granules of chromaffin cells of adrenal medulla in which it is co-stored and co-released with catecholamines (Huttner, et al., 1991).

CgA is synthesized as a pro-peptide that is directed by N-terminally located signal peptide into the trans-golgi regulated pathway then subjected to cell and tissue specific cleavage by variety of proteases present in the storage granules. CgA can be secreted into the extra cellular space where it is cleaved by circulating proteases, giving rise to peptides of various sizes (Bruno, et al., 2006).

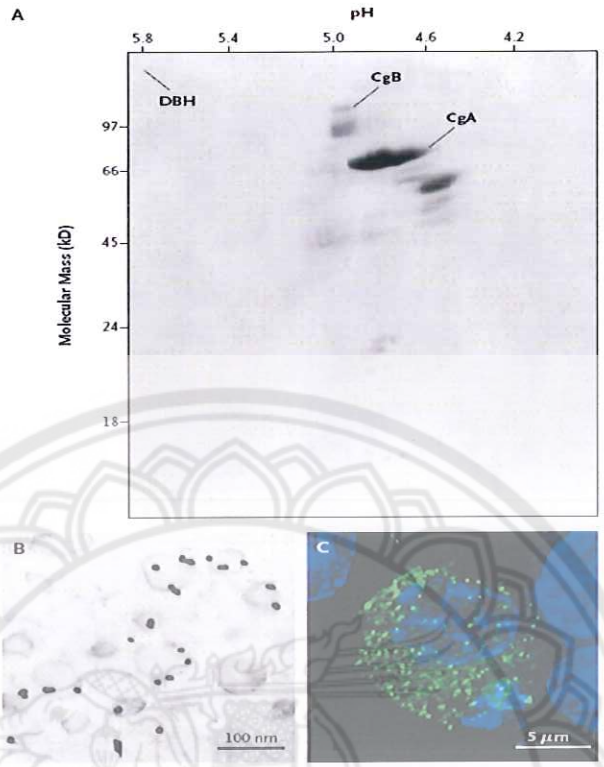

Figure 4 Localization of Chromogranins with Dense-Core Secretory Granules

Source: Taupenot, et al., 2003

Panel A shows soluble-core proteins in bovine chromaffin vesicles after two-dimensional gel electrophoresis, followed by Coomassie blue staining. Panel B shows an electron micrograph of bovine chromaffin cells. Panel C shows the subcellular distribution of a hCgA –enhanced green fluorescent protein (EGFP) chimeric photoprotein, in living sympathoadrenal PC12 cells (Taupenot, et al., 2003).

Structural biology

The gene coding for human CgA has been localized on chromosome 14q32. CgA is coded by a unique gene coding for a protein of 449 amino acid residues; the first 18 represent the signal sequence responsible for the passage of the nascent protein to, and through, the rough endoplasmic reticulum membrane. CgA is remarkably heat stable, hydrophilic and acidic protein with large hydrodynamic volume, mostly in random coil (60–65%) and α helix (25–40%) conformations, with a PI of 4.5-5, due to

high content of glutamate acid (Yoo, et al., 1990). Multiple pairs of basic amino acids along the molecule, more abundantly distributed in the C terminal part, represent potential proteolytic sites for specific proteases, thereby producing defined CgA-derived peptides that can possess biological functions. The N-terminal part possesses a disulphide bridge formed by two cysteine residues at amino acid positions 17 and 38 that appears important for several CgA-related biological activities as shown in Figure 5. The summary of physicochemical properties of CgA shown in Table 3. In the case of mature bovine CgA, the protein is composed of 431 residues with a molecular mass of 45 kDa, while human CgA is a 439 amino acid long protein with a molecular mass of 46 kDa (Bruno, et al., 2006; Marie Helene, et al., 1993; Colombo, et al., 2002).

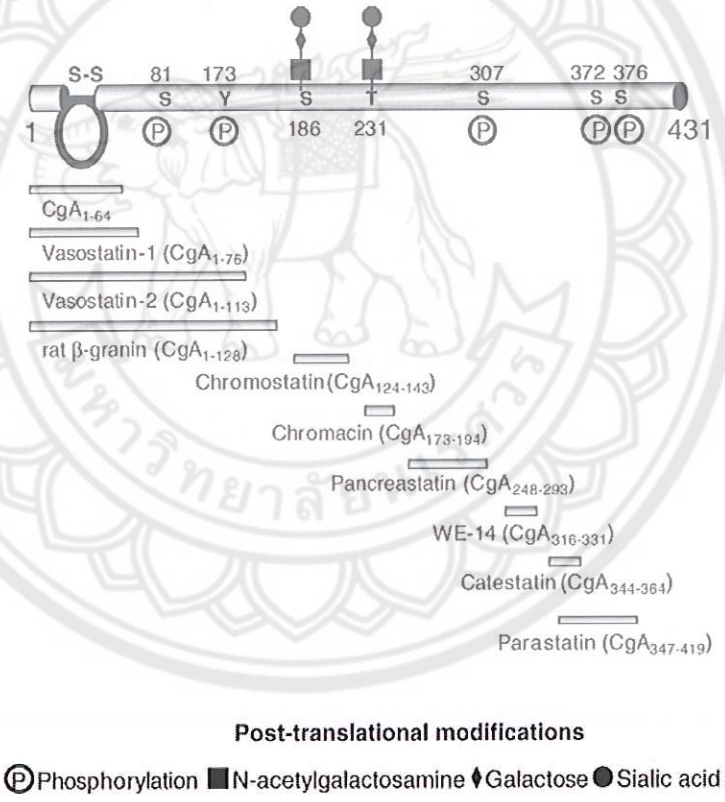

Figure 5 Schematic illustration of Chromogranin A-derived peptides

Source: Strub, et al., 1997

Table 3 Physicochemical properties of CgA

property	CgA
Chromosome localization	14 (human)
Amino acid residues	431-445
Molecular mass (kD)	
- Calculated	49-52
- apparent	74-80
Acidic residues(%)	25
Isoelectric point	4.5-5
Multibasic sites	8-10
Disulfide-bonded loop	yes
Calcium binding	yes
thermostability	yes
sulfation	yes
O-glycosylation	yes
N-glycosylation	no
phosphorylation	yes

Source: Laurent, et al., 2003

Certainly, several cleavage products of human CgA have been identified to date and have been shown to exhibit important biological functions. For example vasostatin (human CHGA1–76); pancreastatin (human CHGA250–301) and catestatin (human CHGA352–372), as shown in Figure 6 (Nitish and Mahapatra, 2008).

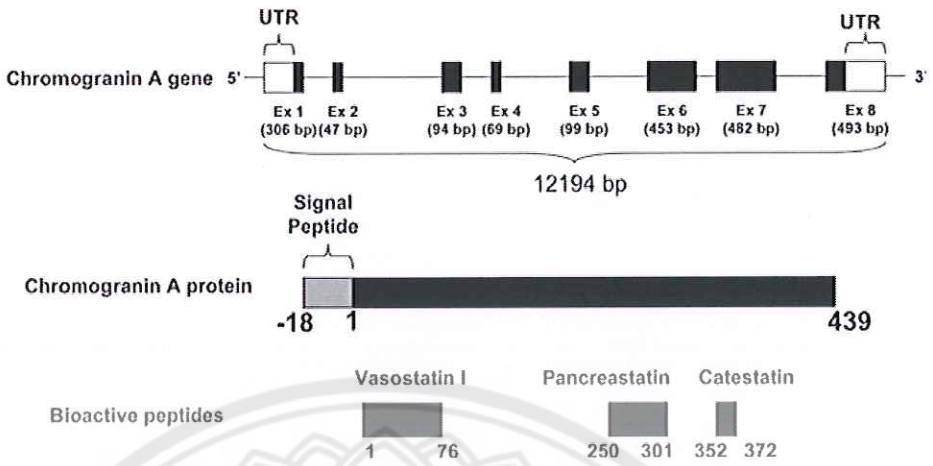

Figure 6 Schematic representation of human chromogranin A gene, protein, and some of its biologically active peptides

Source: Mouland, et al., 1994

The hCgA gene located on chromosome 14q32 and consists of eight exons giving rise to a 2043 nucleotide transcript, of which 1374 nucleotide is processed for translation. hCgA protein consists of 457 amino acid residues, which matures to a 439 amino acid residues protein after removal of the signal peptide. The mature protein undergoes proteolytic cleavages to produce several biologically active peptides (Mouland, et al., 1994).

Biological function

Granins serve as precursor proteins that can be proteolytically processed by prohormone or proprotein convertases at multiple cleavage sites to produce a large number of small bioactive peptides, with a wide range of proposed biological activity. Evidence for biological activity in granin-derived peptides has successively accumulated since pancreastatin was first demonstrated to inhibit insulin secretion in 1986 shown in Figure 7.

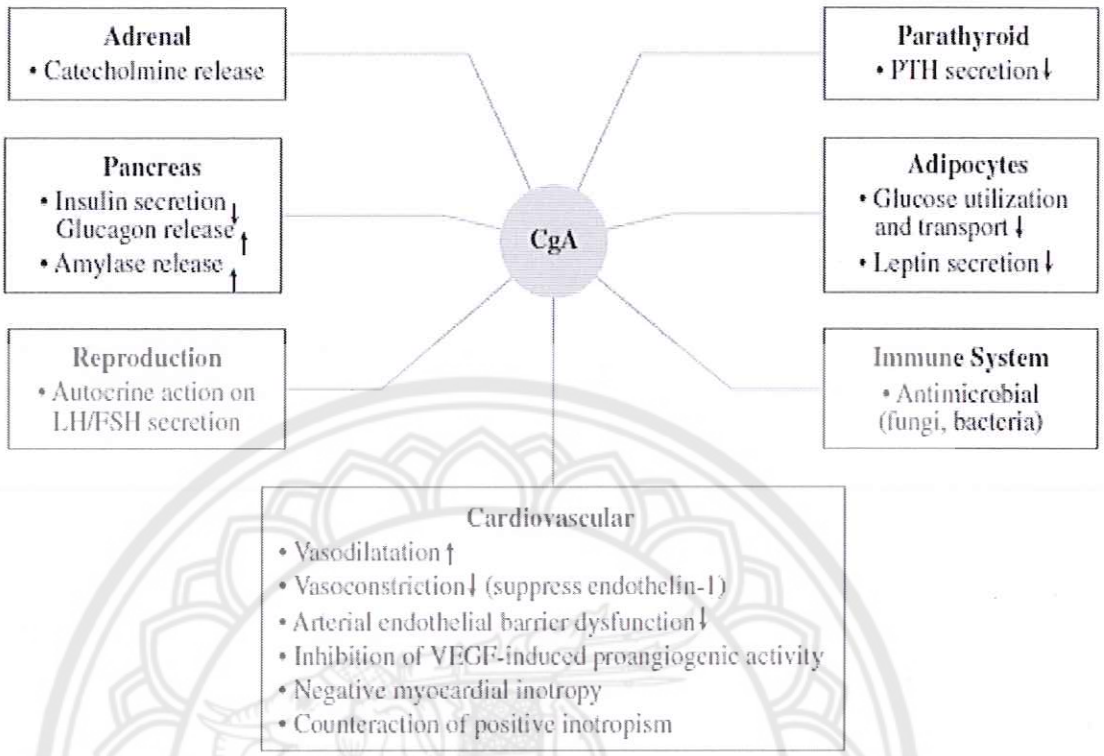

Figure 7 Biological function of CgA

Source: Modlin, et al., 2010

CgA shows diverse physiological interactions. CgA (or its derivatives) is an inhibitor of catecholamine, parathormone, insulin, and leptin secretion but elevates glucagon and amylase release. In addition to its effects on endocrine organs, CgA also regulates reproductive and cardiovascular functions as well as having antimicrobial effects (Modlin, et al., 2010). The normal levels of circulating CgA via its vasostatin-I and catestatin domains may provide a homeostatic buffer function against wear and tear of the cardiovascular elements before onset of disease such as during periods of excessive activation from a wide range of environmental and intrinsic stimuli (Helle, 2010).

In gastrointestinal physiopathology, CgA is over-expressed within endocrine cells from Crohn disease patients (El, et al., 1997). CgA-derived peptides are shown to decrease acetic acid induced effects colonic motility (Ghia, et al., 2005).

CgA has been recognized as a useful tissue and serum marker of neuroendocrine tumors and a prognostic indicator in heart failure (Ceconi, et al., 2002). A growing body of evidence suggests that CgA is not only an important diagnostic and prognostic marker, but that it could also play important functions in tumor biology and cardiovascular physiology that deserve to be investigated (Corti, 2004).

Several hypotheses regarding the biological function of CgA have been proposed. They are summarized in Table 4 (Nobels, et al., 1998).

Table 4 Proposed functions of chromogranin A (CgA)

Intracellular function Modulation of proteolytic processing of peptide hormones and Neuropeptides: Acts as nucleus for aggregation and precipitation of peptide hormones and neuropeptides to form dense core vesicles. Directs peptide hormones and neuropeptides to the regulated pathway of secretion.
Extracellular function Precursor of biologically active peptides with auto-, para-and/or endocrine function.

Source: Nobels, et al., 1998

The presence of numerous paired basic amino acids in granins suggests that they function as prohormones, giving rise to bioactive peptides as a result of post-translational proteolytic processing (Taupenot, et al., 2003). Peptides derived from CgA are shown in Table 5.

Table 5 Proposed Actions of Granin-Derived Peptides

Fragment and Position Chromogranin A	Biologic Activity of the Fragment or Its Orthologue
Bovine CgA 1–40	Increases release of calcitonin and calcitonin gene–related peptide from lung tumor cells, inhibits vasoconstriction, inhibits parathyroid hormone secretion from parathyroid chief cells
Chromofungin (bovine CgA 47–66)	Exerts antifungal activity
Vasostatin I (bovine CgA 1–76)	Inhibits vasoconstriction, promotes fibroblast adhesion, inhibits parathyroid hormone secretion from parathyroid chief cells, triggers microglial-cell–mediated neuronal apoptosis and exerts bacteriolytic and antifungal effects
Vasostatin II (bovine CgA 1–113)	Inhibits vasoconstriction and parathyroid hormone secretion
Prochromacin (bovine CgA 79–431)	Exerts bacteriolytic and antifungal effects
Chromacin I and II (bovine CgA 173–194 and bovine CgA 195–221)	Exerts bacteriolytic and antifungal effects
Pancreastatin (porcine CgA 240–288)	Inhibits insulin release from pancreatic-islet beta cells; promotes hepatic glycogenolysis; decreases insulin-induced glycogen synthesis in skeletal myocytes, hepatocytes and adipocytes; stimulates amylase release from pancreatic acini; decreases gastric acid release from parietal cells; diminishes glucose uptake by skeletal muscles in humans
Catestatin (bovine CgA 344–364)	Inhibits catecholamine release from the adrenal medulla
Parastatin (porcine CgA 347–419)	Inhibits parathyroid hormone secretion from parathyroid chief cells

Source: Taupenot, et al., 2003

The structural determinants of these activities are located in different regions of the N-terminal domain. Peptide 1-40, containing the Cys17-Cys38 disulfide bridge, induces vasodilator effects and inhibits parathormone secretion. CgA and vasostatin-1 can modulate, in an indirect manner, fibroblast- and smooth muscle cell adhesion (Ratti, et al., 2000). Other studies showed that CgA, at nanomolar concentration, may increase deposition of basement membrane components by mammary epithelial cells, and alter ductal morphogenesis in vitro, suggesting a role of CgA in cell adhesion (Corti, 2004).

Proteolytic processing of natural CgA with plasmin decreases its anti-adhesive activity and induces pro-adhesive effects in fibronectin or serum dependent fibroblast adhesion assays (Colombo, 2002). The interplay between CgA and plasminogen/plasmin system could provide a novel mechanism for regulating fibroblast adhesion (Corti, 2004).

Chromaffin cells and plasminogen activation system

In the chromaffin cells of the adrenal medulla, the catecholamines are stored in subcellular particles termed chromaffin granules (Blaschko, et al., 1957) Furthermore, the chromaffin cells express components of the plasminogen/plasmin system including its major activator, tissue plasminogen activator (tPA) and high specific cellular receptor for plasminogen (Jiang, et al., 2002).

Plasminogen comprises five triple disulfide bond kringle structures that modulate the interaction of plasminogen with other proteins (Castellino and McCance, 1997). The kringles of plasminogen expressed lysine binding site mediate the binding of plasminogen to the cell (Miles, et al., 2002). Interaction between plasminogen and receptor of chromaffin cells is specific because unrelated proteins do not affect the interaction of radiolabeled plasminogen with the cells (Parmer, et al., 2000).

Synthesis and expression of tPA is demonstrated in several chromaffin cell sources including rat pheochromocytoma PC12 cells, primary cell culture of bovine adrenal medullary chromaffin cells and human pheochromocytoma (Parmer, et al., 1997). In addition, tPA-release occurs in parallel with catecholamines from catecholamine storage vesicles of chromaffin cells in response to specific secretagogues stimulation (Jiang, et al., 2002).

Plasminogen activation and plasmin function have been shown to be promoted by colocalization of plasminogen and its activator on cell surfaces (Plow, et al., 1991). Moreover, activation of plasminogen may occur in a regulated fashion in response to cellular stimulation with exocytotic release of tPA from its intracellular storage compartment to conversion of plasminogen to the active serine protease plasmin, which is localized and concentrated at the cell surface by its specific binding sites (Jiang, et al., 2002).

At the cell surface, tPA activates plasminogen to plasmin then plasmin processes the major secretory protein found in catecholamine storage vesicles of chromaffin cell in adrenal medulla, CgA, into bioactive peptides that inhibit secretagogue-stimulated catecholamine release. CgA-(360-373) is selectively liberated by plasmin from human CgA at early time points and is stable even after prolonged exposure to plasmin (Jiang, et al., 2001). CgA derived peptides provide a negative feedback mechanism for regulating catecholamine responses during stress (Parmer, et al., 2000).

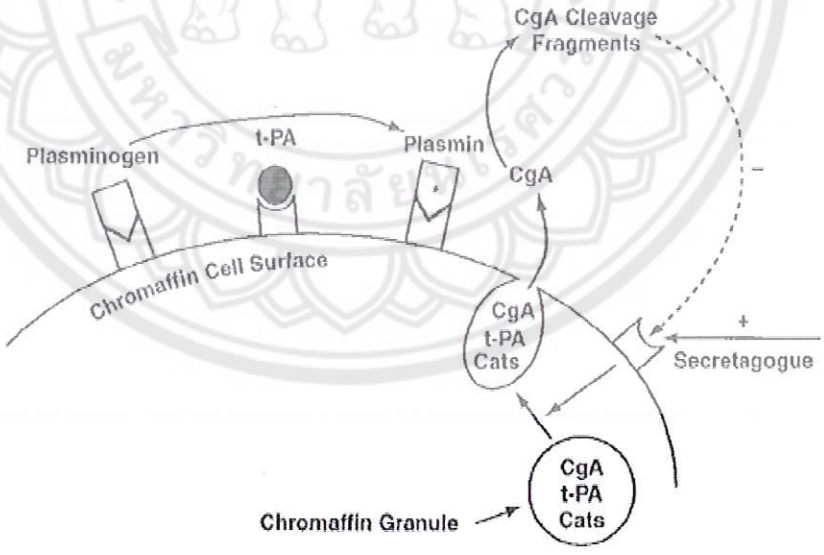

Figure 8 Proposed working model for a local (autocrine/paracrine) chromaffin cell plasminogen/plasmin system and its interactions with CgA

Source: Parmer, et al., 2000

Recent studies suggest a novel role of CgA in the plasminogen activation system for the regulation of catecholamine release in chromaffin cells. Furthermore, CgA stimulates plasminogen activation in dose dependent fashion. These results suggest that CgA play a role in stimulation of plasminogen activation by t-PA, analogous to the well-established stimulating effect of fibrin on plasminogen activation in the circulation and vasculature (Yasothornsrikul and Parmer, 2006). Furthermore CgA fragment (pancreastatin) increased the fibrinolytic activities of urokinase-type plasminogen activator (u-PA) (Nagakawa, et al., 1999).