How is Cardiovascular Health Influenced by Peptides of the Blood Coagulation Pathway?

The blood coagulation pathway serves as an important matrix for generating proteins and peptides that work to regulate hemostasis, which when impaired could result in the development of coagulation related disorders such as thrombosis. Peptides that are involved in the blood coagulation pathway bind to and interact with specific components of the coagulation system in order to promote cellular effects that influence cardiovascular health. Their roles in vascular disease may also stem from their constitutive expression in vascular cells mediated via unique activation mechanisms that help to dictate and regulate smooth muscle contraction, vessel tone permeability etc.

This background white paper reflects on the nature and actions of peptides involved in the blood coagulation pathway, such as those derived from or acting upon Thrombin, Fibrinogen, Plasmin & Thromboplasmin and how they influence cardiovascular health. In addition, this white paper will serve as an excellent guide to choose peptides not only for specific applications such as neuro-hormonal modulation, vasoactive or anti-inflammation but also as indicators of cardiac health. Peptides within the realm of cardiovascular physiology function also as agonists and antagonists, the latter being well known for receptor antagonizing effects, which serve as important tools to study the molecular mechanisms of receptor-mediated signal transduction as with the thrombin activated receptors.
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INTRODUCTION

Peptides are one of the mechanistic elements that play a major role in cardiovascular diseases (CVD)*, which comprise of a group of disorders of the heart and/or blood vessels:

- Diseases of the heart muscle and valves include congenital heart disease, deep vein thrombosis and pulmonary embolism (WHO, 2014).
- Disorders of blood vessels that supply to specific organs such as the heart can result in coronary heart disease. In the vessels leading to the brain, cerebrovascular disease can result, and in the blood vessels that supply the arms and legs, peripheral arterial disease can occur.

Over the past few decades much focus on cardiovascular disease research has centered towards understanding the disease mechanism from a clinical, pathophysiological, genetic and molecular standpoint, including exploring novel agents such as dual-acting neuro-hormonal modulators, vasoactive and anti-inflammatory peptides as potential therapeutic agents (von Lueder TG & Krum H, 2015). With peptides presenting as promising research and development targets, their diverse and important roles in cardiovascular physiology impart a central prominence to cardiovascular research.

This background white paper delves into the role of cardiac-related peptides, specifically those involved in one of the major pathways of cardiovascular disease, the blood coagulation pathway.

The blood coagulation pathway is responsible for maintaining blood hemostasis by regulating and balancing thrombogenic (clotting) and anti-thrombogenic (subsequent clot dissolution) mechanisms.

Peptides involved in the Blood Coagulation are derived from or act upon the proteins, Thrombin, Fibrinogen, Plasmin, and/or Thrombospondin (Fig 1). They play important roles in maintaining hemostasis balance, which when impaired could result in the development of coagulation related disorders such as thrombosis, myocardial infarction, disseminated intravascular coagulation etc.

Peptides Involved in Blood Coagulation Pathway

Peptides that are involved in the Blood Coagulation Pathway play important roles in maintaining hemostasis balance which has implications for coagulation related disorders. These peptides or peptide fragments are derived from or act upon blood coagulation related proteins such as Thrombin, Fibrinogen, Plasmin, and/or Thrombospondin whose cellular effects are governed by specific proteases. It has been long observed that the presence of non-catalytic domains among these proteases serve to mediate interactions which in turn help in the regulation of blood coagulation and fibrinolysis.

This white paper will focus on thrombin and its receptors, fibrinopeptides, plasmin and thromboplasmin as important players that affect diverse functions related to coagulation.

THROMBIN, PARs AND RECEPTOR SIGNALING

Thrombin is a serine protease of the chymotrypsin family and is produced by the enzymatic cleavage of two sites on prothrombin (its inactive precursor) by activated Factor Xa. Thrombin mediates the conversion of monomeric fibrinogen into polymeric fibrin (Fig 2).

Acting as a platelet agonist, thrombin activates platelets allowing them to interact with fibrin resulting in thrombus formation (blood clot) and also plays key roles in the coagulation pathway (Fig 3). Both thrombin and platelets are important in acute arterial thrombosis and in maintaining hemostasis. Blockade of platelet activation by thrombin has been considered as a useful antithrombotic strategy given that thrombosis is a major cause of morbidity and mortality.

* CVD is the leading global cause of death. It accounts for 17.3 million annual deaths and is projected to grow to over 23.6 million in 2030 (Mozaffarian D et al 2015). The cause of CVD events typically involve the presence of a combination of risk factors, such as tobacco use, unhealthy diet and obesity, physical inactivity and harmful use of alcohol, hypertension, diabetes and hyperlipidemia (von Lueder TG & Krum H, 2015).
How is Cardiovascular Health Influenced by Peptides of the Blood Coagulation Pathway?

Opposing Actions of Thrombin in Hemostasis Regulation (Pro and Anti-coagulant)

Thrombin has opposing roles in hemostasis in that it acts as both a pro-coagulant as well as an anti-coagulant (Table 1). Following vascular injury, loss of blood is controlled via an intricate mechanism of hemostasis involving thrombin. Thrombin plays an important role not only in clot formation and inhibition but also in cell signaling and other processes that influence fibrinolysis and inflammation.

As a pro-coagulant, thrombin converts fibrinogen into insoluble fibrin which anchors platelets to the wound/lesion site to initiate the wound healing process (Coughlin SR, 2000). This process is supported by tissue factors (Factor XIII or transglutaminase) to stabilize the clot and inhibit fibrinolysis (Fig 4). Thrombin also triggers expression of pro-coagulant activity on the platelet surface, which in turn supports additional thrombin generation (Hughes PE & Pfaff M, 1998).

Thrombin’s chief pro-coagulant roles support fibrin formation and wound healing via platelet activation.

Once the fibrin/hemostatic plug has filled the lesion site to repair the wound, via thrombin’s pro-coagulant role, a mechanism to shut down this process is essential to prevent further migration of the fibrin plug to the healthy endothelium adjacent to the site. This is when, thrombin’s anti-coagulant roles come into play.

As an anti-coagulant, thrombin functions to down-regulate the coagulation process as it (fibrin-bound from its pro-coagulant property) complexes with thrombomodulin present on the endothelial cell surface and activates protein C, an anti-coagulant proteinase (Fig 5). Since thrombomodulin has high-affinity for thrombin, it binds to thrombin and effectively removes thrombin away from the hemostatic plug site and makes thrombin less susceptible to cleave fibrinogen to form fibrin.

Thrombin’s chief anti-coagulant roles support activation of protein C, thrombin inhibition, and down-regulation of coagulation.

Table 1. Functions of Thrombin in Coagulation Pathway

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<td>Binding to Thrombomodulin</td>
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<td>Platelet activation</td>
<td>Activation of Protein C</td>
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<td>Support pro-inflammatory molecular signaling and expression of chemokines.</td>
<td>Decreased fibrinogen cleavage</td>
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Other pro-coagulant roles of thrombin include, shape changes in platelets, release of the platelet activators ADP, serotonin and thromboxane A2, as well as chemokines and growth factors (Coughlin SR, 1998), mobilizing the adhesion molecule P-selectin and the CD40 ligand to the platelet surface and activating the integrin αIIb/β3 towards mediating platelet aggregation. Thrombin has the ability to exert atherogenic actions such as inflammation, enhanced oxidative stress, and leukocyte recruitment into the atherosclerotic plaque, migration and proliferation of smooth muscle cells, apoptosis and angiogenesis. Thrombin shows a pronounced pro-inflammatory property, which may influence the onset and progression of atherosclerosis.

Protease-activated receptors (PARs)

PARs or Protease Activated Receptors, function as receptors for Thrombin. These G-protein coupled receptors (GPCR) are seen as contributors to different pathways including those of the circulatory and cardiovascular system, nervous system, gastrointestinal system, airways and also the skin. They can regulate many biological processes that are critical in disease, including coagulation, proliferation and survival, inflammation, neurotransmission and pain (Ossovskaya VS and Bunnett NW, 2004). While endothelial PARs help in the regulation of vessel tone and permeability, those in smooth muscles mediate contraction, proliferation and hypertrophy.

Four PARs are known in mice and humans with specific activation modes. Human PAR1, PAR3, and PAR4 can be activated by Thrombin. PAR1 and PAR4 are direct thrombin receptors. Mouse PAR3 does not by itself mediate transmembrane signaling but instead functions as a cofactor for the cleavage and activation of mouse PAR4 by thrombin. PAR2 is activated by trypsin and tryptase as well as by coagulation factors VIIa and Xa, but not by thrombin (Coughlin SR, 2000).
PARs (Thrombin Receptor) Mediated Signaling

PARs are irreversibly activated through a distinct proteolytic cleavage mechanism mediated by ligand interactions with residues residing in the second extracellular loop as opposed to interactions formed within a transmembrane helix pocket as observed in a typical GPCR type signal transducing mechanism.

The signaling process begins with a series of events involving the PAR-1 sequence, LDPRSFLLRNPNDKYEFP consisting of key domains (see inset- Key domains in PAR-1 sequence) involved in its activation. Firstly, thrombin recognizes the N-terminal extracellular domain (exodomain) "LDPR". This sequence docks in the active site of thrombin. The PAR-1 'hirudin-like' sequence, DKYEFP binds to Thrombin’s fibrinogen-binding exosite. Thrombin then cleaves the peptide bond between receptor residues Arg 41 and Ser 42 unmasking a new N-terminus, beginning with the sequence SFLLRN that functions as a ‘tethered ligand’. The tethered ligand docks intramolecularly with PAR-1 thereby activating the PAR-1 to effect transmembrane signaling. Thus PAR1 is, in essence, a peptide receptor that carries its own ligand, the latter being active only after receptor cleavage (Coughlin SR, 2000). Figure 6 represents the proteolytic activation site of human PAR1 by the unmasking of its tethered ligand.

SFLLRN is also referred to as thrombin receptor activator for peptide 6 or TRAP-6. Synthetic SFLLRN peptide corresponds to the amino terminal peptide sequence, which mimics the tethered ligand sequence, functioning as an agonist independently of receptor cleavage. It mimics thrombin-signaled cell responses in platelets.

With structure-function studies, it has been established that the serine at the N-terminus position is important for full receptor activation, while leucine at the 3rd position behaves as a connector unit to form a bioactive conformation and leucine at the 4th position interacts directly with the receptor. The guanidine group of Arg-5 is also important for the electrostatic interaction with the acidic groups of the receptor. Another group essential for receptor activation was found to be the hydrogen atoms of the phenylalanine residue at position 2. (Fujita T, et al 1999).

KEY DOMAINS IN PAR-1 SEQUENCE

LDPR: N-terminal exodomain
SFLLRN: New N-terminal ‘tethered ligand’ following thrombin cleavage
DKYEFP: Hirudin-like sequence

Tethered Ligand: The term "tethered ligand" refers to the new N-terminal sequence formed following the cleavage of N-terminal portion of a PAR family member by serine protease (Offermans S & Rosenthal W, 2004).

Once activated, PARs undergo conformational changes within transmembrane helices that facilitate interaction with heterotrimeric G proteins. Activated PAR-1 couples to multiple heterotrimeric G protein subtypes of the G-protein α subunit including G12/13, Gq and Gi, and initiates a variety of signaling molecules important for inducing cell shape changes, cellular growth and motility, adhesion molecule expression, secretion of vasoactive factors etc.

Typical GPCR main signaling events are outlined in figure 7. It includes ligand binding to the receptor coupled with heterotrimeric G proteins with an inactive GDP bound on the α subunit of G protein. Upon ligand binding, GPCR functions as a guanine nucleotide exchange factor and converts GDP to GTP with the release of the α subunit and beta-gamma dimer. These two further activate downstream signaling pathways such as adenylate cyclase producing important second messengers such as cAMP for nuclear translocation and expression.

Implications of PAR Activation

Important cellular functions of PARs include platelet activation. PAR1 and PAR4 are essential for thrombin-induced human platelet activation. The signaling events contributing to platelet activation can be several and different routes are being explored to understand specific activation mechanisms. For example, an MMP-2-integrin binding mediated activation of platelets has been proposed for PAR-1 wherein an undescribed tethered ligand was observed to cause G-protein activation and subsequent signaling (Sebastiano M, et al 2017). In another recent study, a sphingolipid-mediated thrombin stimulation of NF-kB signaling in platelets was studied to cause platelet activation via PAR-4 and not PAR-1(Chen WF, et al 2013).

In addition to coupling to heterotrimeric G proteins for effecting cell signals, activated PARs can also interact with various adaptor proteins to facilitate signal transduction independent of heterotrimeric G protein coupling (Soh UJH, et al 2010). For instance, signaling effects can be achieved via interaction with β-arrestins and transforming growth factor β-activated kinase-binding protein-1 (TAB1), which functions as scaffolds to activate yet another signaling pathway, the mitogen-activated protein kinase (MAPK) (Stalheim L, et al 2005).
Thus, even though PAR-mediated receptor activation occurs through the distinct ‘tethered ligand’ mechanism of proteolytic cleavage, the routes adapted following receptor activation for downstream cellular signaling and effects are governed by functional selectivity and may involve several pathways.

PAR Agonists

Agonist peptides function very similar to the parent peptide. Thrombin receptor agonist peptides include SFLLRN, the heptapeptide corresponding to the PAR-1 tethered ligand and another agonist peptide corresponding to amino acids 42 to 55 of the human thrombin receptor (SFLLRNPNDKYEFP). The latter is known to fully reproduce the action of thrombin on MMPs in vascular endothelial cells. This peptide, also referred to as TRP-14 may signal a variety of thrombin’s responses and has been shown to cause platelet aggregation that can evoke a wide range of G-protein dependent responses (Duhamel-Clérin E et al, 1997).

Examples of Agonist PAR peptides include TFFLRN and its amidated version as a PAR-1 agonist; SLIGKV-NH2 is a PAR-2 agonist, TFRGAP-NH2 is a PAR-3 agonist and, GYPGQV is a PAR-4 agonist peptide. Table 2 summarizes the four types of PARs depicting their structural and functional sequence motifs and agonist sequences.

PAR-1 agonists including TFFLRN and TFLLRNPNDK are known to mediate cellular effects of thrombin much like the PAR-1 peptide, SFLLRN. The TFLLRNPNDK-amide agonist peptide binds to PAR-1 thus mimicking the ‘tethered ligand’ formation that thrombin generates via proteolytic cleavage of the substrate. Another peptide (SFLL) derived from SFLLRNPNDK sequence functions as a peptide agonist corresponding to the N-terminal region of the human platelet thrombin receptor exposed after cleavage by thrombin. In some instances, the arginine at the 5th position of SFLLRN is replaced with a citrulline rendering the short peptide to have improved biological activity. Other PAR-1 agonists include TFRIFD-NH2 and GFIYF-NH2 with modifications done to evaluate biological activity. Antagonists of PAR-1 that function specific to the thrombin receptor are considered promising therapeutic candidates for thrombosis. They include YFLLRNP and SFALRNP (S-para Fluoro-Aad-LRNP) with modifications done for studying their antagonistic actions (Macfarlane SR, et al 2001).

PAR-2 agonists include amidated SLIGRL, SLIGKV, and further modified LIGRLQ, RKPNDK each with trans-cinnamoyl and 3-marcaptopropionyl/cyclohexylalanine modifications respectively. PAR-2 agonists activate PAR-2 and of those, the SLIGRL and SLIGKV peptides correspond to PAR-2 tethered ligand. Two PAR-2 antagonists, FSLLRY and LSIGRL have been shown to block trypsin activation of PAR-2 in kidney cells (Macfarlane SR, et al 2001).

PAR-3 agonists include TFRGAP and SFNGGP of which, the former peptide allosterically regulates PAR 1 signaling and that targeting PAR-3 may counter the effects of PAR-1 activation seen in endothelial responses such as vascular inflammation (Macfarlane SR, et al 2001).

PAR-4 agonists include AYPGKF, AYPGQV and GYPGQV. PAR-4 peptides typically can be acted upon by both trypsin and thrombin. The PAR-4 agonists are PAR-4 activating peptides with various roles. For example, AYPGKF stimulates thromboxane production by platelets, GYPGQV stimulates TNF-alpha secretion from human leukemic mast cells, and GYPGKF cause contraction in muscles. A PAR-4 antagonist, YPGKF with modification to its N-terminal end has been shown to inhibit thrombin and platelet aggregation following PAR-4 agonist induction (Macfarlane SR, et al 2001).

Other notable thrombin receptor-activating peptides (TRAP) include a thrombin-related peptide, also called hTRAP. TRAP antagonists have been known to inhibit thrombin- or TRAP-stimulated platelet aggregation in vitro (Ahn H-S et al, 1997).

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<th>Activation Molecule(s)</th>
<th>Mechanism of Signaling</th>
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<td>PAR-1*</td>
<td>N-SFLLRNPNDKYEFP-C</td>
<td>TFFLRN TFLLRNPNDK</td>
<td>Thrombin (human)</td>
<td>cAMP modulated; Ligated ligand mediated</td>
</tr>
<tr>
<td>PAR-2</td>
<td>N-SLIGKV-NH2</td>
<td>SLIGRL SLIGKV</td>
<td>Trypsin Tryptase Factor Vila, Xa</td>
<td>cAMP modulated; Ligated ligand mediated</td>
</tr>
<tr>
<td>PAR-3*</td>
<td>N-TFRGAP-NFNGGP-C</td>
<td>TFRGAP SFNGGP</td>
<td>Thrombin (human)</td>
<td>Allosteric PAR-1 regulation</td>
</tr>
<tr>
<td>PAR-4*</td>
<td>N-AYPGKF-AYPGQV-GYPGQV-C</td>
<td>AYPGKF AYPGQV GYPGQV</td>
<td>Trypsin &amp; Thrombin</td>
<td>IP3/DAG modulation</td>
</tr>
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Table 2. Summary of PARs and Agonists with domain patterns/alignments involved in Thrombin Receptor Signaling.

* Direct Thrombin Receptors; a tethered ligand cleavage motif; b)ion binding histidine-like site; XXXX - sequences of the extracellular domain II (tethered ligand binding domain) with conserved residues underlined; N (N terminal); C (Cterminal).

Thrombin Receptor Antagonists

In general, receptor antagonists are useful tools to study the molecular mechanism of receptor-mediated signal transduction. Based on the structure of thrombin receptor-tethered ligand, SFLLRNPNDK, peptides have been designed to harbor a potential antagonizing group which may be chemical groups such as beta-mercaptopropionyl or cyclohexylalanine. Such modifications can prevent the receptor confirmation from changing for activation. Clinical development of PAR-1 antagonists that can have effects such as attenuating arterial thrombosis are underway and would help to understand the role of receptor antagonists in disease better, such as that of PAR-1 in atherosclerosis.

FIBRINOPEPTIDES

Fibrinogen is a complex 340-kDa plasma glycoprotein formed with two sets of chains linked together by disulfide bonds. It is the principal protein involved in the formation of fibrin (clot) and thus acts as a precursor in fibrin formation following its cleavage by thrombin and subsequent release of polypeptide fragments.

Fibrinogen and its components play important roles in the hemostatic balance.
**Fibrinopeptides** are cleaved products of fibrinogen, which is composed of 3 non-identical polypeptide chains, $\alpha$, $\beta$, and $\gamma$. These chains are linked together by 29 disulfide bonds and form several structural regions, 2 distal D, one central E, and 2 $\alpha C$ regions (Medved et al, 2009) (Fig 8a). The conversion of monomeric fibrinogen into polymeric fibrin is mediated by thrombin, which binds to the central region of fibrinogen and catalyzes cleavage of the 2 short peptides, the 16-residue fibrinopeptide A (FPA) followed by the 14-residue fibrinopeptide B (FPB), located at the N-termini of the $\alpha$ and $\beta$ chains, respectively (Blomback, 1996). Liberation of FPA and FPB exposes the E domain of fibrinogen resulting in the formation of fibrin monomers, which associate and cross-link to form a semi-solid network, while the fibrinopeptides remain soluble in plasma. The residual protein, fibrin monomer, polymerizes to form fibrin clot (Fig 8b).

Elevated FPA levels have been reported in patients with coronary heart disease (Neri Serneri et al, 1981) and appear to be a marker during the course of coronary thrombosis (Eisenberg PR et al, 1985). Further, fibrin formation associated with active thrombosis leads to significantly higher plasma levels of FPB and thus measurement of plasma FPB levels is considered a more sensitive and specific serologic marker for acute thrombosis (Mikami and Takao, 2007).

Other related peptide(s) include a fibrinogen binding inhibitor peptide whose sequence is derived from the 600-411 residues at the C-terminal end of the gamma chain of fibrinogen. This peptide is known for its importance in platelet aggregation and is considered as one of the common ligands for glycoprotein recognition and binding (Farrell DH et al, 1992). Another fibrinogen-derived inhibitory peptide, fibrinogen, gamma chain (377-395) attenuates microglia activation and suppresses relapsing paralysis in multiple sclerosis. It has been observed that targeting this gamma-fibrinogen epitope could represent a potential therapeutic strategy for multiple sclerosis and other neuroinflammatory diseases associated with blood-brain barrier disruption and microglia activation.

**PLASMIN AND ITS INHIBITORS**

Plasmin is a serine protease that plays a role in breaking down fibrin (fibrinolysis) (figure 9) and also elicits roles in wound healing and maintenance of liver homeostasis. It is released as a zymogen (plasminogen) from the liver and in humans, presents as two forms - type I plasminogen (containing two glycosylation moieties) that is readily recruited to the blood clots, and type II plasminogen (containing a single $O$-linked sugar) that is recruited to the cell surface.

Both plasmin and fibrinogen activator inhibitor peptides are involved in the fibrinolysis pathway and play important roles in the regulation of fibrin formation and lysis. The generation of plasmin is aided by tPA and plasminogen. As plasmin remains bound to fibrin it is relatively protected from the fibrinolysis or antiplasmin. Antiplasmin thus serves to inactivate free plasmin as it is cross-linked to fibrin.

Inhibitors of fibrinolysis include Plasminogen Activator Inhibitor (PAI-1), also called Serpin E1 (a serine protease inhibitor), thrombin activated fibrinolysis inhibitor (TAPI) and $\alpha_2$-antiplasmin, also called serpin peptidase inhibitor. PAI-1 serves as the primary inhibitor of tissue plasminogen activator (tPA), while $\alpha_2$-antiplasmin also inhibits tPA and urokinase. The anti-plasmin peptide, $\alpha_2$-antiplasmin also serves as a substrate for factor XIIIa by cross-linking to it and helps Factor XIIIa to stabilize the clot against fibrinolysis.
Elevated PAI-1 is a risk factor for thrombosis and atherosclerosis and plasminogen activator inhibitors serve as indicators of cardiac disease. It is also believed that Angiotensin II, a potent vasoconstrictor, increases synthesis of PAI-1 thereby exacerbating the development of atherosclerosis (Vaughan, 2005). It is the most potent and rapidly acting of the plasmin inhibitors and is thought to be important in the regulation of fibrinolysis in vivo. In assay development, its role in potentiating fibrin to promote nerve regeneration by enzymatically incorporating exogenous neurite-promoting heparin-binding peptides has been explored (Sakiyama S et al, 1999).

**THROMBOSPONDIN**

Thrombospondins (TSPs) are a family of five extracellular calcium binding matrix glycoproteins that mediate cell to cell interactions owing to their ability to bind a variety of ligands through their multiple domains. Upon secretion, they are either incorporated into the extracellular matrix or can undergo extracellular proteolysis by thrombin or plasmin during fibrinolysis. As potent inhibitors of angiogenesis TSPs play important roles in cell proliferation, apoptosis, inflammation and atherosclerotic response.

TSP peptides interacts and bind to components of the fibrinolytic system comprising of plasminogen, urokinase etc, and promotes cellular effects in coronary heart disease. Their roles in vascular disease stem from their constitutive expression in various cell types and are also thought to be mediated by thrombin-induced platelet activation (Gutierrez LS, 2008).

The family of TSP is separated into two groups since TSP-1 and TSP-2 are trimers (Group A) and TSP-3, TSP-4 and TSP-5/COMP (cartilage oligomeric matrix protein) are pentamers (Group B) (Krishna et al, 2013). TSP-1 is a 450 kDa homotrimeric glycosylated protein and each TSP-1 subunit consists of N-terminal and C-terminal globular (G) domains which are connected by a thin strand. The N-domain is cleaved by several proteases (such as thrombin, plasmin, cathepsins, elastases, trypsin and chymotrypsin) (Bonneyo & Hoylaerts, 2008).

Thrombospondin-1 (TSP-1) is an important extracellular matrix component that influences the function of vascular smooth muscle cells, endothelial cells, fibroblasts and inflammatory cells with important implications for cardiovascular disease. There are multiple domains in the TSP-1 sequence that can interact with a number of cellular receptors and hence effecting varied cellular functions (figure 10). For example, the heparin binding domain present in the N-terminal of TSP-1 binds to heparin sulfate proteoglycans, whereas the peptide sequence CSVTCG present in the TSR1 of TSP-1 interacts with CD36 (cluster of differentiation 36), a member of the scavenger receptor class B. The RGD sequence of the TSR3 interacts with a variety of integrin β subclass receptors and the C-terminal cell-binding domain binds to CD47 (cluster of differentiation 47) (Lawler et al, 1998). TSP-1 also interacts with fibronectin and fibrinogen and binds to components of the fibrinolytic system such as plasminogen, urokinase and its inhibitor PAI-1 and to cathepsin G and elastase. TSP-1 promotes the formation of platelet and monocyte rosettes in patients with severe coronary artery stenosis and atherothrombotic cerebral ischaemia (Jurk et al, 2010).

TSP-1 is considered a biomarker for cardiovascular disease since it is constitutively expressed in aortic valves and higher levels of TSP-1 have been reported in large blood vessels with atherosclerotic lesions and also in the presence of diabetes. TSP-1 is expressed at higher plasma concentrations in peripheral arterial disease. With its involvement in vascular diseases, it is thought that TSP-1 expression could originate from thrombin-induced platelet activation due to its release from α-granules and in addition, many vascular cell types also constitutively secrete and express TSP-1(Krishna et al, 2013). TSP-1 peptide analogues have been evaluated for therapeutic roles in cardiovascular disease owing to their anti-angiogenic, anti-proliferative and pro-apoptotic effects of various TSP-1 domains. A study using a peptide antagonist of TSP-1, LSKL which selectively blocks glucose and angiotensin II-stimulated TGF-β signaling suggests the use of TSP-1 antagonists as a therapeutic strategy to modify fibrotic complications in diabetes. It has also been suggested that blockade of TSP-1-dependent TGF-β activation may be a novel therapeutic strategy to prevent or reverse cardiac fibrosis (Belmadani et al, 2007).

**CONCLUSION**

Several proteins, proteases and peptides play significant roles in regulating blood coagulation and related signaling events functioning as a whole proteomic system affecting cardiac physiology and health. In doing so, they not only serve as important biomarkers but also present as viable pipeline drug candidates for cardiovascular disease. Given particularly the unique activation mechanisms that some of the cardiovascular proteins/peptides exhibit, targets such as PARs are considered attractive not only for the development of therapeutics in cardiovascular disease but also as emerging candidates for cancer, respiratory and central nervous systems, and gastrointestinal system disorders as well. While potential therapeutic application of peptides in cardiovascular disease has gained momentum with the development of novel targets based on peptidomimetic modulation, the advancement in synthesis technologies and high-throughput platforms continue to drive cardiovascular peptides as promising drug candidates.
REFERENCES