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Review article

Role of heparin and non heparin binding serpins in coagulation and angiogenesis: A complex interplay

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ABSTRACT

Pro-coagulant, anti-coagulant and fibrinolytic pathways are responsible for maintaining hemostatic balance under physiological conditions. Any deviation from these pathways would result in hypercoagulability leading to life threatening diseases like myocardial infarction, stroke, portal vein thrombosis, deep vein thrombosis (DVT) and pulmonary embolism (PE). Angiogenesis is the process of sprouting of new blood vessels from pre-existing ones and plays a critical role in vascular repair, diabetic retinopathy, chronic inflammation and cancer progression. Serpins; a superfamily of protease inhibitors, play a key role in regulating both angiogenesis and coagulation. They are characterized by the presence of highly conserved secondary structure comprising of 3 β-sheets and 7–9 α-helices. Inhibitory role of serpins is modulated by binding to cofactors, specially heparin and heparan sulfate proteoglycans (HSPGs) present on cell surfaces and extracellular matrix. Heparin and HSPGs are the mainstay of anti-coagulant therapy and also have therapeutic potential as anti-angiogenic inhibitors. Many of the heparin binding serpins that regulate coagulation cascade are also potent inhibitors of angiogenesis. Understanding the molecular mechanism of the switch between their specific anti-coagulant and anti-angiogenic role during inflammation, stress and regular hemostasis is important. In this review, we have tried to integrate the role of different serpins, their interaction with cofactors and their interplay in regulating coagulation and angiogenesis.

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1. Introduction

Serpins (serine protease inhibitors) are a complex and broadly distributed superfamily of proteins present in plasma and/or tissues. They are composed of 350–500 amino acids and share a highly conserved structure comprising of 3 β-sheets (A-C) and 7–9 α-helices (A-I). Most serpins have molecular weight (MW) in the range of 40–60 kDa. So far, maximum MW of 105 kDa has been observed in C1 inhibitor where ~50% of the weight is attributed to post translational glycosylation [1]. Presently, there are well over 3000 serpin and serpin-like members identified in the genome of organisms representing all forms of life, like metazoan, plantae, viruses and 36 confirmed human serpins [2]. Phylogenetically serpins are divided into 16 clades (designated A through P) and 10 highly diverged "unclassified orphans". Most of the serpins inhibit serine proteases but there are others that inhibit caspases and papain-like cysteine proteases [3,4]. In addition to their inhibitory function several serpins such as ovalbumin (storage), angiotensinogen (blood pressure regulation) and thyroid binding globulin (hormone transport) perform non-inhibitory functions.

Serpins are ubiquitous in nature and perform diverse functions at all levels of evolution. They regulate host defense functions in various organisms by inhibiting serine peptidases which are activated in response to fungal and bacterial infections and activates Toll-like receptors that results in expression of antimicrobial peptides [5]. In *Drosophila melanogaster* serpins spn27A and spn1, regulate dorsal-ventral axis formation and maintain immunoregulation by regulating Toll-like receptors respectively [6,7]. Serpins AFXa (AaSRPN25) and alboserpin are the known inhibitors of FXa and contribute to malaria parasite lysis and activates Toll pathway [8]. Most of the plant serpins need to be characterized but it has been suggested that they are involved in regulating immune responses by inhibiting proteases of plant pathogens [9]. Serpins such as serp-1 and serp-2 have evolved in viruses to evade host immune response where serp-1 inhibits tissue and urokinase type plasminogen activators (tPA and uPA), plasmin, FXa and serp-2 inhibits granzyme B and caspases 1 and 8 [10]. SW-AT-1 is a serpin-type trypsin and chymotrypsin inhibitor in silkworm (*Bombyx mori*) playing role in defence responses [11].

Owing to their molecular flexibility and anti-protease activity, serpins serve novel biochemical and biological functions in the human body (Fig. 1) and control proteolytic pathways related to human health and diseases. They are involved in vital physiological processes like blood coagulation, fibrinolysis, inflammation, complement activation and apoptosis. Serpins such as SERPINA1 (antitrypsin), SERPINA3 (antichymotrypsin), SERPINA4 (kallistatin), SERPINA5 (protein C inhibitor), SERPINA10 (protein Z-dependent protease inhibitor), SERPINB1 (monocytes neutrophil elastase inhibitor), SERPINB2 (plasminogen activator inhibitor-2), SERPINB3 (squamous cell carcinoma antigen-1), SERPINB4 (squamous cell carcinoma antigen-2), SERPINB6 (protease inhibitor-6), SERPINB7 (megsin), SERPINB8 (cytoplasmic antiprotease 8), SERPINB9 (cytoplasmic antiprotease 9), SERPIN B10 (bomapin), SERPINB11 (epipin), SERPINB12 (yukopin), SERPINB13 (headpin), SERPINC1 (antithrombin), SERPIND1 (heparin cofactor II), SERPINE1 (plasminogen activator inhibitor I), SERPINE2 (protease nexin I), SERPINF2 (alpha-2-antiplasmin), SERPING1 (C1 inhibitor), SERPINII (neuroserpin) and SERPINI2 (myoepithelium-derived serine protease inhibitor) have been well characterized and are inhibitory in nature [2]. Non-inhibitory serpins in humans include SERPINA6 (corticosteroid-binding globulin), SERPINA7 (thyroxine-binding globulin), SERPINA8 (angiotensinogen), SERPINB5 (maspin), SERPINF1 (pigment epithelium derived factor) and SERPINHI (47 kDa heat-shock protein).

1.1. Serpins bind to cofactors

A major advantage of the serpin fold over small protease inhibitors such as bovine trypsin pancreatic inhibitor is that the inhibitory activity of serpins can be exquisitely controlled by specific cofactors. For example antithrombin (AT), kallistatin, heparin cofactor II (HCII), protease nexin-1 (PN-1) and protein C inhibitor (PCI) binds to heparin. HCII binds to dermatan sulfate and dermatan sulfate proteoglycans (DSPGs), plasminogen activator inhibitor-1 (PAI-1) binds to vitronectin and pigment epithelium-derived factor (PEDF) binds to collagen, hyaluronan and chondroitin sulphate (Table 1). In 1973, Rosenberg and Damus suggested that heparin binds the protease inhibitor AT causing a conformational change within AT, accelerating its reaction with the protease thrombin and formation of an active complex between protease and inhibitor. Proteases, growth factors, chemokines, lipid binding proteins and pathogen proteins are among numerous proteins that bind heparin. Heparin binding residues in some serpins are shown in Fig. 2.

1.2. Mechanism of action of inhibitory serpins

Acidic groups of heparin interact with positive residues on serpins. A large amount of available biochemical and biophysical data reveals that serpins typically use a *suicide substrate-like inhibitory mechanism* for inhibiting the target proteases [12]. In serpins, the region responsible for interaction with target proteases is the reactive centre loop (RCL) which forms an extended, exposed conformation above the body of the serpin scaffold. In the *inhibitory pathway*, the protease initially forms a non-covalent Michaelis-like complex through interactions with residues flanking the scissile bond (P1-P1′). An attack of the active site serine on scissile bond leads to formation of covalent ester linkage between serine-195 of the protease and backbone carbonyl of P1 residue leading to cleavage of peptide bond. At this stage, with the removal of restraint, the RCL starts to insert into β-sheet A and transport covalently bound protease with it. Upon complete loop insertion the protease is translocated by over 70 Å, and its active site is distorted. The alignment of the active site catalytic triad is altered by as much as 3 Å and P1 side chain is removed from S1 pocket [13]. Due to the inherent dependence of serpin mechanism on conformational mobility and a metastable native fold, serpins are highly susceptible to mutations that perturb their functions [14]. These conformational diseases or 'serpinopathies' have been identified in humans