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REVIEW ARTICLE

Protein S: a Central Regulator of Blood Coagulation

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SUMMARY

Background: **Protein S is a central regulator of coagulation as it critically participates in down-regulation of both extrinsic and intrinsic pathways of the coagulation cascade. In this review, we aim to provide an update on protein S and its anticoagulant functions as a central hemostatic regulator.**
Methods: **Electronic databases including, Google, Google Scholar, PMC, PubMed, Science Direct, and Scopus were rigorously searched using the terms protein S, hemostasis, natural anticoagulants, regulators of coagulation, and coagulation inhibitors for the completion of this descriptive review.**
Results: **Literature review shows that protein S is a potent cofactor for activated protein C (APC) in the regulation of the intrinsic pathway and a cofactor for tissue factor pathway inhibitor (TFPI) in the regulation of the extrinsic pathway. The strong association between protein S deficiency either hereditary or acquired and increased risk for venous thrombosis indicates the important and central role of protein S in controlling the initiation and propagation phase of coagulation cascade and that protein S is an important determinant for optimal activity of both APC and TFPI in coagulation regulation.**
Conclusions: **Available evidence suggests that the role of protein S in the down-regulation of blood coagulation is mainly mediated through its high affinity binding to negatively charged phospholipid surfaces. This high affinity binding to negatively charged phospholipids helps bring the anticoagulant proteins to the membranes, resulting in efficient and targeted regulation of coagulation. In the shade of current COVID-19 pandemic, protein S deficiency has been found to be a leading cause of thrombotic complications associated with COVID-19.**
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INTRODUCTION

Blood coagulation is an automated biological mechanism used by all mammalian species to stop bleeding after vascular injury. Vascular damage results in the activation of platelets, coagulation factors, and vascular endothelium itself that results in the formation of blood clots at the site of injury. After trauma or an injury, tissue factor (TF) is released by endothelial cells and initiates the extrinsic pathway of coagulation by forming a complex with circulating factor VII (FVII) [1]. TF-FVIIa activates the zymogen factor X (FX) to its procoagulant active form FXa that converts prothrombin

into thrombin [2]. The generated thrombin via the extrinsic pathway in trace amounts is not sufficient to form a stable clot, but can efficiently propagate the signal and initiates the activation of the intrinsic pathway by activating factor XI (FXI) to FXIa which activates factor IX (FIX) to FIXa. In the presence of platelet phospholipids (PL) and calcium ions, FIXa with its cofactor factor VIIIa (FVIIIa) forms the tenase complex that generates more FXa. The extrinsically-generated thrombin also activates FV to FVa [3]. FVa is a cofactor of FXa in the prothrombinase complex assembled on negatively charged phospholipids in the presence of calcium ions. The prothrombinase complex amplifies the production of thrombin which converts fibrinogen into fibrin; the final product of blood coagulation (Figure 1). Activated blood coagulation factors are kept restricted at the site of injury by soluble proteins known as natural anticoagulants.

Natural anticoagulants

Although blood coagulation is a complex process, it is tightly regulated and governed by anticoagulant pathways to avoid systemic clotting and to restrict clot formation at the site of injury.

Antithrombin

Antithrombin is a member of the serpin family of serine protease inhibitors and a major inhibitor of the coagulation serine proteases. Thrombin is the main target of antithrombin. Other coagulation factors such as FIXa, FXa, FXIa, and FVIIa are also inhibited by thrombin with various efficiencies [4].

Protein C and activated protein C (APC)

Protein C is a vitamin K-dependent zymogen, which is activated by the thrombin-thrombomodulin complex on endothelial cells to an active serine protease i.e. activated protein C (APC). APC is a major anticoagulant protein, which inhibits the coagulation cascade by proteolytic inactivation of FVa and FVIIIa (the important cofactors of the prothrombinase and tenase complexes) in a reaction that is stimulated by protein S [5,6]. The mechanism of FVa inactivation by APC is described as sequential cleavage at residues Arg^{306}, Arg^{506}, and Arg^{679}. Cleavage at Arg^{506}, which is kinetically favored and more rapid than cleavage at Arg^{306}, results in the formation of a partially active intermediate, which is fully inactivated through cleavage at Arg^{306} [7,8]. Cleavage at Arg^{679} seems to be less significant for the regulation of FVa activity by APC.

Tissue factor pathway inhibitor (TFPI)

TFPI is a main regulator of the extrinsic pathway of blood coagulation, which inhibits the procoagulant activity of both FVIIa and FXa. TFPI first forms a complex with FXa via its Kunitz-2 domain called as slow-tight binding mechanism. After which the TFPI-FXa complex binds to TF-FVIIa via the Kunitz-1 domain of TFPI forming an inactive quaternary complex [9].

Protein S

Molecular structure of protein S

Protein S is vitamin K-dependent plasma glycoprotein with a molecular weight of about 75 KDa formed of 635 amino acid residues. Structurally, the amino terminus of protein S starts with the Gla domain which comprises 11 gama-carboxy glutamic acid residues (residues 1 to 46). This domain is important in calcium-dependent binding to negatively charged phospholipid surfaces [10]. The Gla domain is followed by a disulfide-bridged loop called thrombin sensitive region (TSR) (residues 47 to 75) [11] and by four epidermal growth factor (EGF)-like domains (residues 76 to 242). This domain plays a structural and stabilizing role [12,13]. The C-terminus of protein S unlike other vitamin K-dependent coagulation factors does not possess a serine protease region. Instead it contains a huge domain called the sex hormone binding globulin (SHBG)-like domain consisting of two laminin G-type domains (residues 243 to 635) [14,15] (Figure 2).

Synthesis and biological forms of protein S

Hepatocytes and endothelial cells are the main sites of protein S synthesis [16,17]. Other cells including megakaryocytes, osteoblasts, platelets, T-cells, vascular smooth muscle cells, and tumor cells also synthesize protein S [18,19]. Protein S circulates in plasma at a concentration of 350 nM where 60% exists in tight complex with C4BP (Figure 3) while the remaining 40% circulates in free form and represents the molar excess of protein S above 200 nM concentration of C4BP [20,21]. Protein S also exists in plasma in cleaved form. The thrombin sensitive domain of protein S (TSR) is susceptible to the proteolytic cleavage by elastase and by proteases released from activated platelets. This region is also sensitive to cleavage by proteases such as thrombin and factor Xa. Thrombin cleaves at Arg^{49} and Arg^{70} only in the absence of calcium ions, whereas FXa cleaves at Arg^{60} in the presence of calcium ions and phospholipids (Figure 4) [22-25]. Therefore, 10 - 20% of protein S present in plasma circulates in cleaved form. Accordingly, the free and C4BP-bound protein S in plasma exists in either an intact or cleaved form.

Expression and activation

The coding gene of protein S is called PROS1 gene, which is located near to the centromere on chromosome 3q11.2. PROS1 gene spans about 80 kb of genomic DNA and has 15 exons which code for a precursor protein of 676 amino acid residues. Mature protein S consists of 635 amino acids residues resulting from the post-translational modification of the precursor protein [26,27]. Similar to other vitamin K-dependent proteins, protein S requires carboxylation of its glutamic acid residues to become biologically active. This carboxylation involves a γ-glutamyl carboxylase and a reduced form of vitamin K resulting in the addition of CO_2 molecules to the γ-carbon of glutamic acid residue.

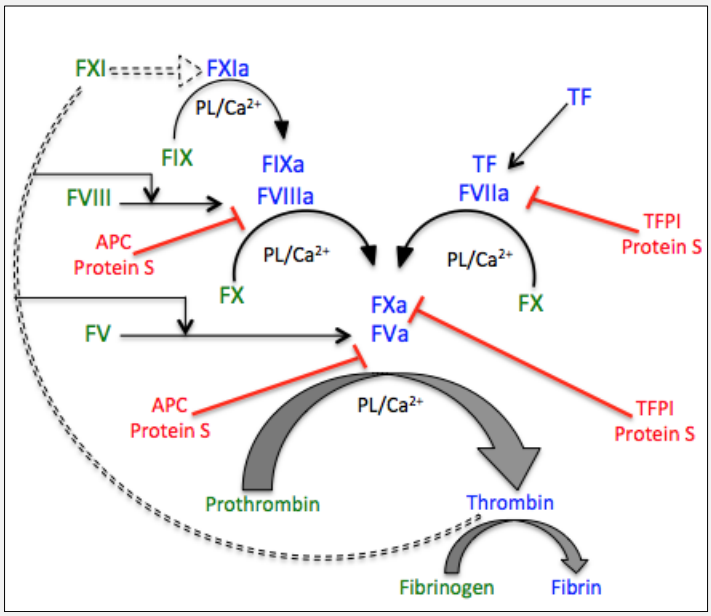

Figure 1. The blood coagulation pathway and its down-regulation by TFPI, APC, and protein S.

The scheme describes the intrinsic and extrinsic pathways of coagulation. Inactive coagulation factors (green), activated coagulation factors (blue). Anticoagulant proteins (red), phospholipids (PL), and calcium ions (Ca^{2+}) (black).

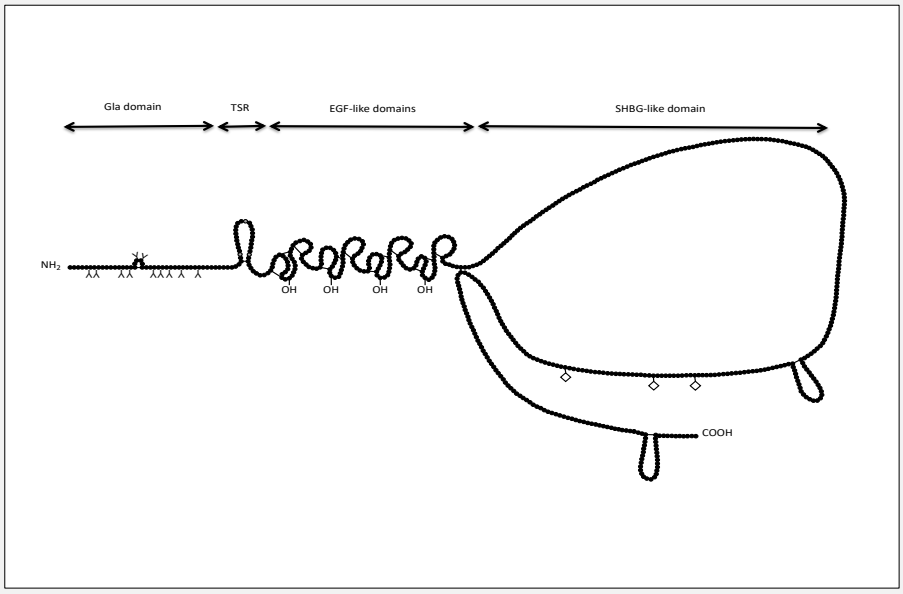

Figure 2. Molecular structure of protein S. Mature protein S is composed of 635 amino acids arranged into multiple domains.

At the amino terminus, protein S consists of a γ-carboxyglutamic acid (Gla) domain with 11 Gla residues indicated with ϒ, a thrombin sensitive region followed by four epidermal growth factor (EGF)-like domains in which each one has three disulfide bridges and one β-hydroxylated amino acid residue, and a sex hormone binding globulin (SHBG)-like domain which contains two disulfide bridges and three glycosylation sites indicated by diamond shapes.

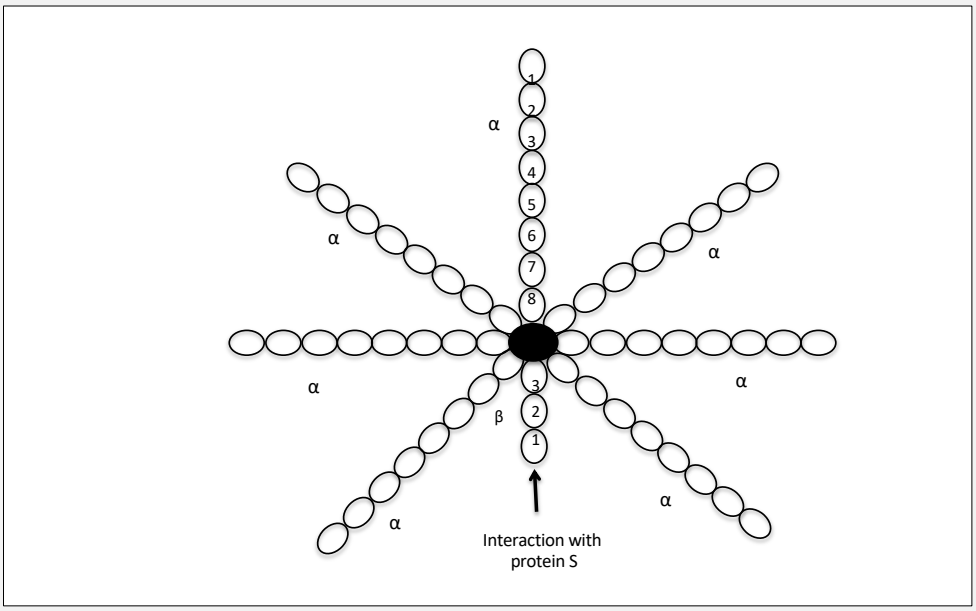

Figure 3. Schematic illustration of the structure of C4b-binding protein (C4BP).

C4BP has 7 identical α chains, each chain is composed of seven or eight complement control protein (CCP) domains and one β-chain consists of three CCP. The α and β-chains are covalently connected to a central core via disulfide bonds. The binding site of protein S is located on the CCP-1 domain of the β-chain.

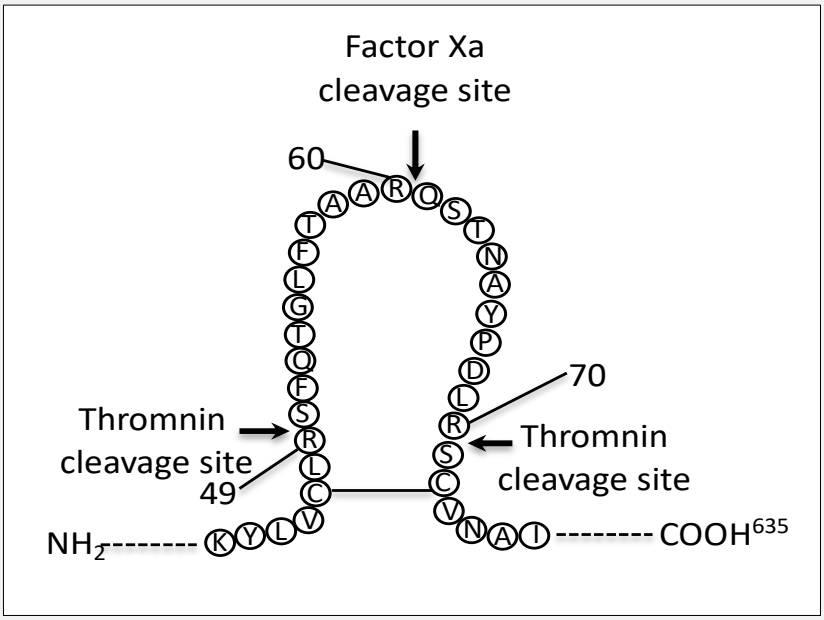

Figure 4. Cleavage sites of thrombin and factor Xa in protein S.

The thrombin sensitive region of protein S is shown with amino acid single letter codes. Thrombin and factor Xa cleavage sites are indicated by arrows.

Functions of protein S

The functions of protein S can be categorized into two main groups including non-anticoagulant and anticoagulant.

Non-anticoagulant functions of protein S

Protein S is known as a multifunctional protein because of its involvement in many biological processes such as inflammation, angiogenesis, apoptosis, and cancer [28]. Protein S is involved in inflammation through its binding to the CCP1 of the beta-chain of C4-binding protein (C4BP) which supports the ability of C4BP to bind with apoptotic and necrotic cells and to prevent inflammation caused by activation of complement by these cells. Also, the binding of protein S to negatively charged phospholipids mediates appropriate clearance of apoptotic cells by phagocytes, since protein S acts as a bridging molecule between the apoptotic cells and phagocytes [29,30]. Additionally, protein S has been reported to be a ligand for Tyro3, Axl, and Mer (TAM) receptors which is a unique family of receptor tyrosine kinases (RTKs). Through this interaction, protein S plays a role in signaling pathways involved in angiogenesis and cancer development [28].

Anticoagulant functions of protein S

APC-dependent activity of protein S

The first-described and intensively studied anticoagulant function of protein S was its APC cofactor activity [31]. APC regulates the activity of the procoagulant FVa and FVIIIa (two important cofactors of the prothrombinase and tenase complexes) [5,6]. Protein S significantly enhances the proteolysis of FVa by APC at Arg^{306} about 20-fold [32]. Additionally, Protein S and intact FV are cooperative cofactors of APC in FVIIIa inactivation by cleavage mechanism at Arg^{336} and Arg^{562} residues [33,34]. For a long time this anticoagulant role of protein S as a potent cofactor of APC was assigned only for the intact free form of protein S, since different studies reported that the APC cofactor function of protein S is inhibited by binding to C4BP and proteolysis of the thrombin sensitive region [35,36]. Later, it was reported that binding of protein S to C4BP inhibits its APC cofactor activity in FVa inactivation only, and protein S-C4BP is a cofactor of APC in FVIIIa inactivation. A study has also shown that protein S-C4BP complex is a cofactor of APC in FVa inactivation. Protein S dependent APC cleavage of Arg^{306} is enhanced in the presence of protein S-C4BP about 10-fold while protein S-independent APC cleavage of Arg^{506} is inhibited 3 to 4-fold [37]. These findings indicate that C4BP-bound protein S may have a role in coagulation regulation as APC cofactor even though this role seems to be less efficient than the role of free protein S.

TFPI-dependent activity of protein S

TFPI plays a critical role in controlling blood coagulation. It is an important regulator of the extrinsic pathway of coagulation, which inhibits the procoagulant activity of both FVIIa and FXa [9]. Protein S acts as non-enzymatic cofactor of TFPI [38]. It acts as a cofactor for TFPI by enhancing the formation of FXa-TFPI complex and the subsequent inhibition of the TF-FVIIa complex.

Direct and independent anticoagulant activity of protein S

Since its discovery protein S was only known as anticoagulant by its APC and TFPI dependent cofactor activities. However, it has been shown that protein S has direct inhibitory effects on coagulation by inhibiting FIXa directly, and disruption of the interaction between protein S and FIXa causes an increased rate of thrombus formation in mice [39]. This new-found function of protein S implies an unexploited target for antithrombotic therapeutics.

Clinical implications

Impairment of the anticoagulant system increases the risk of thrombosis-related diseases. Protein S deficiency is a risk factor for thrombotic diseases which, however, is more clearly observed in thrombophilic families [40, 41] than in the general population [42,43]. Hereditary as well as acquired conditions can cause protein S deficiency. Based on plasma levels of total and free protein S antigen and on the functional activity of protein S, three types of hereditary protein S deficiency have been defined. Type I is a quantitative disorder in which total and free protein S antigen levels and activity are low. Type II is a qualitative disorder in which total and free protein S levels are normal but activity is reduced. Type III is characterized by a normal total protein S level but reduced free form and activity.

Hereditary protein S deficiency

Many mutations in the PROS1 gene encoding protein S have been identified [44]. Homozygous PROS1 mutations result in severe thrombotic complications and purpura fulminans, while heterozygous mutations are compatible with life and are associated with increased risks of venous and arterial [45] thrombotic complications [46]. In heterozygous mutations, protein S antigen levels are reduced to the range between 35 - 60%. About 2% of venous thrombosis cases are diagnosed with heterozygous protein S mutations [46]. Presence of other genetic mutations such as FV-Leiden and prothrombin G20210A mutations increase thrombosis risk in protein S deficient individuals [47,48].

Acquired protein S deficiency

Development of acquired protein S deficiency is mainly related to increased protein S consumption, redistribution of bound form of protein S and impairment of its biological synthesis. Many studies reported a marked reduction in the plasma level of protein S in patients with acute liver failure and cirrhosis, which is consistent with the fact that the liver and endothelial cells are the main sites of protein S synthesis [49,50]. Also, hormonal changes such as occur during pregnancy and oral contraceptive use in women are well known leading causes for the development of acquired protein S deficiency even though the exact underlying mechanism is still not fully understood [51,52]. As protein S is a vitamin K-dependent protein, its plasma level is also reduced during treatment with vitamin K antagonists [53]. In the shade of current COVID-19 pandemic a positive

association was found between decreased activity of protein S and thromboembolic events in COVID-19 patients [54].

Diagnosis of protein S deficiency

Two types of assays are used for the diagnosis of protein S deficiency. Immunoassay measures the antigenic levels of free and total protein S in plasma. This assay enables diagnosis of type I and III protein S deficiency. Functional assays (clotting assays) are also used for the diagnosis of protein S deficiency and they represent the only way to detect qualitative (type II) protein S defects. Generally, functional assays are based on measuring the ability of protein S to increase clotting time of plasma in the presence of APC [55]. The major commercially available PS functional assays are based on measurement of the prothrombin time (PT-based assay), FXa-induced clotting (Factor Xa-based assay) or the activated partial thromboplastin time (aPTT-based assay). Unfortunately, available functional assays sometimes give unreliable results that could lead to misdiagnosis of protein S deficiency with high false results due to existence of confounding factors such as presence of lupus anticoagulant and use of a therapeutic anticoagulant rivaroxaban. On the other hand, presence of FV Leiden mutation and high levels of prothrombin, FVIIIa, and FVIIa lead to false low results or underestimation of actual activity of protein S [56,57]. Presence of factor V Leiden mutation, one of the most common inherited disorder, effects the reliability of results of functional protein S assays [58]. Furthermore, it is also a fact that available functional assays of protein S only quantifies the APC cofactor activity of protein S.

New functional assays that could enable quantification of both APC and TFPI cofactor activity of protein S are highly required. In addition to that, more analytical methods should be adopted to detect protein S disorders without being influenced by the presence of factor V Leiden mutation [59]. Besides the antigenic levels and functional protein S assays, molecular analysis could be used to confirm diagnosis of inherited protein S deficiency in patients who are diagnosed with low protein S antigen levels and/or low protein S activity. Although gene sequencing is an important test to detect variations in protein S gene and to confirm hereditary deficiency, the use of this test is mainly limited to specialized laboratories and research centers. Furthermore, it can be challenging because there are many different conditions that can temporarily lower the levels of protein S in the blood (acquired protein S deficiency) [60].

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None.

Declaration of Interest:
The authors declare no conflict of interest.

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