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Protein S: A multifunctional anticoagulant vitamin K-dependent protein at the crossroads of coagulation, inflammation, angiogenesis, and cancer

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Abstract

Since its discovery in 1970, protein S (PS) has emerged as a key vitamin K-dependent natural anticoagulant protein at the crossroads of multiple biological processes, including coagulation, apoptosis, atherosclerosis, angiogenesis/vasculogenesis, and cancer progression. Following the binding to a unique family of protein tyrosine kinase receptors referred to as Tyro-3, Axl and Mer (TAM) receptors, PS can lead to regulation of coagulation, phagocytosis of apoptotic cells, cell survival, activation of innate immunity, vessel integrity and angiogenesis, and local invasion and metastasis. Because of these dynamics and multiple functions of PS, which are largely lost following invalidation of the mouse PROS1 gene, this molecule is currently intensively studied in biomedical research. The purpose of this review is to provide a brief chronicle of the discovery and current understanding of the mechanisms of PS signaling, and how PS and their signaling partners regulate various cellular functions, with a particular focus on TAM receptors.

Keywords: Protein S; Protein tyrosine kinase receptors; Signal transduction; Coagulation; Inflammation; Apoptosis; Angiogenesis; Metastasis

1. Introduction

Protein S (PS) was randomly discovered in the 1970s as a new vitamin K-dependent plasma glycoprotein in Seattle and therefore, named after the city of its discovery [1]. PS

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is a fascinating natural anticoagulant with potentially multiple biologic functions. In humans, PS is encoded by the *PROS1* gene that is located near the centromere on chromosome 3q11.2 [2]. The *PROS1* gene spans ∼80 kb of genomic DNA and has 15 exons that code for a protein of 635 amino acid residues with a predicted molecular mass of ∼69-kDa [3].

PS, which lacks enzymatic activity, functions primarily as an anticoagulant cofactor to the activated protein C (APC) in the inactivation of activated factors V (FVa) and VIII (FVIIIa), which are respectively the cofactors of the prothrombinase and tenase complexes in the coagulation cascade [4,5]. After binding to the phospholipid's surfaces, it helps localizing APC in proximity to FVa and FVIIIa. This relocation enhances APC-induced cleavage of FVa and FVIIIa by 20- and 3-fold, respectively, and leads to regulation of thrombin generation [6,7]. More recently, it was reported that PS has also an APC-independent activity in vitro by directly inhibiting both prothrombinase and tenase activity. This APC-independent anticoagulant activity is mediated by PS, which acts as a cofactor of tissue factor pathway inhibitor (TFPI) and specifically stimulates the inhibition of factor Xa [8,9]. Perhaps, a more direct evidence for the physiological importance of PS as a guardian in controlling thrombin generation and fibrinolysis was the discovery of some disorders of PS referred to as (PS) deficiencies (PSD) [10]. To date, over 220 mutations of the *PROS1* gene resulting in either protein truncation or single amino acid substitutions were reported [11]. Hereditary PSD manifests as an autosomal dominant trait and the heterozygous are at enhanced risk of venous thromboembolism (VTE) during adulthood while the carriers of a double mutated gene suffer from complete PSD and from purpura fulminans at an early age that requires fresh frozen plasma administration [12].

PS circulates in the plasma at a 350 nM concentration (25 mg/L), of which 60% forms a non-covalent 1:1 stoichiometric complex with the β-chain containing C4b binding protein (C4BP), a protein involved in the complement system comprising seven α-chains and one or no β-chain [13]. The remaining 40% is free and corresponds to the molar excess of PS over C4BP [14]. In principle, FVa inactivation by APC proceeds via a biphasic reaction that consists of a rapid and a slow phases, which are associated with cleavages at Arg506 and Arg306 of the heavy chain of factor Va, respectively [15]. Earlier studies have led to the concept that optimal proteolysis of factor Va by APC requires the presence of the cofactor PS and that the binding of PS to C4BPβ+ inhibits the function of free PS as a cofactor for APC in factor Va degradation [16]. However, this concept is still inconsistent and only partially understood as recent evidence indicates that the complexed form still constitutes a cofactor for APC that enhances APC-catalyzed proteolysis at R^{306} 10-fold whereas cleavage at R^{506} is inhibited by approximately 4-fold [17].

PS in plasma is mainly synthesized and secreted by hepatocytes where it has an anti-coagulant function, but other cells like megakaryocytes, endothelial cells, Leydig and Sertoli cells, osteoblasts, dendritic cells and T cells, vascular smooth muscle cells, and tumor cells also synthesize and secrete PS [18]. Intriguingly, in these cells PS has no anticoagulant function effects but rather functions to activate a unique family of receptors' protein–tyrosine kinase (PTK) made up of the three members Tyro3, Axl, and Mer also referred to as TAM receptors (TAMRs). As a TAMR ligand, PS participates in a variety of physiological processes distinct from hemostasis, including cell proliferation/survival, apoptosis, regulation of inflammatory cytokine release, atherosclerosis, vasculogenesis, and cancer development [18–21].

In this review, we will not consider APC-dependent and -independent anticoagulant activities of PS, which have been amply described elsewhere [4,22–24]. We will focus on recent developments of how PS interaction with distinct TAMRs can affect many different aspects of cell behavior and discuss the emerging pathophysiological roles of this exciting ligand receptor system in various diseases.

2. PS structure

Structurally, human PS in its mature form is a single-chain (75-kDa) glycoprotein of 635 amino acids resulting from post-translational modifications of a 676 amino acid precursor. Like other vitamin K-dependent proteins, PS requires carboxylation of its glutamic acid (Glu) residues to become biologically active. This process 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 acids forming γ-carboxyglutamic acid (Gla) residues on the N-terminal Gla domain [25]. Human PS is encoded by the PROS1 gene composed of 15 exons that encode for the different domains of the protein precursor [3] (Fig. 1A and B). Exon 1 codes for the signal peptide of 24 amino acids (aa 1–24), and exon 2 for the propeptide (aa 25–41) which are both removed by proteolytic cleavage prior to protein secretion, yielding a mature protein of 635 residues. The secreted mature protein contains the N-terminal Gla domain (aa 42–86) encoded by the 3′ end of exon 2 and exon 3, which is required for the binding to negatively charged phospholipids. This binding also requires conformational changes of the Gla domain triggered by the binding of Ca^{2+} resulting in the exposure of three hydrophobic residues that are essential for tight and stable phospholipid binding [26]. Following the Gla domain, exon 4 codes for a disulphide-bridged thumb loop (aa 87–113), one of the regions that has been shown to be susceptible to thrombin cleavage by both thrombin and free FXa, and its removal inhibits PS anticoagulant activity [27–29]. Experimental evidence suggests that the thumb loop shared by several other plasma proteins, has strong stabilizing properties on the structure of Gla module [30]. An interesting feature of PS is the existence of four epidermal growth factors (EGF)-like domains in tandem, an evolutionary conserved domain found in a number of plasma proteins as well as extracellular portions of plasma membrane protein receptors, that are encoded