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Review Article

Functional impact of oxidative posttranslational modifications on fibrinogen and fibrin clots

Marissa Martinez [a], John W. Weisel [b], Harry Ischiropoulos [a,*]

[a] Department of Pediatrics and Department of Pharmacology, Children's Hospital of Philadelphia, University of Pennsylvania, Philadelphia, PA 19104, USA
[b] Department of Cell and Developmental Biology, Raymond and Ruth Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

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ABSTRACT

Fibrinogen is a circulating multifunctional plasma protein vital for hemostasis. Activation of the coagulation cascade converts soluble fibrinogen to insoluble polymerized fibrin, which, along with platelets, forms the hemostatic clot. However, inappropriate formation of fibrin clots may result in arterial and venous thrombotic disorders that may progress to life-threatening adverse events. Often thrombotic disorders are associated with inflammation and the production of oxidants. Fibrinogen represents a potential target for oxidants, and several oxidative posttranslational modifications that influence fibrinogen structure and function have been associated with disease pathogenesis. Here, we review various oxidative modifications of fibrinogen and the consequences of these modifications on protein structure and the ability to form fibrin and how the resulting alterations affect fibrin architecture and viscoelastic and biochemical properties that may contribute to disease.

Contents

Introduction to fibrinogen and fibrin structure and biochemistry

Fibrinogen is synthesized in hepatocytes and secreted into the blood [1] where it circulates with a half-life of about 3 days [2]. Fibrinogen is a 340-kDa hexamer, composed of two pairs of three nonidentical chains termed Aα, Bβ, and γ. Specific sites in each of these chains are subject to oxidative modification (Fig. 1) as elaborated below. Together the chains comprise a symmetrical molecule composed of one globular E region flanked on each side by globular D regions [3–6] that are connected by three-stranded α-helical coiled-coils [4–6]. The E region, which is composed of all three chains, contains fibrinopeptides A (FpA) and B (FpB) [4–6]. Cleavage of these peptides by thrombin exposes knobs "A" and "B", resulting in the formation of fibrin monomers [7–11]. Positively charged A and B knobs have complementary negatively charged binding sites within the γ and β nodules in the D regions of adjoining monomers termed holes "a" and "b" [12,13]. Knob–hole associations each result in the formation of half-staggered, double-stranded protofibrils [14,15], which associate laterally to form fibrin fibers [14,16] and ultimately the branching network structure of the hemostatic or thrombotic clot [17]. The formation of fibrin clots using either isolated fibrinogen or platelet-poor plasma (PPP) after the addition of thrombin and

Abbreviations: FpA/FpB, fibrinopeptide A/B; PPP, platelet-poor plasma; PTM, posttranslational modification; tPA, tissue plasminogen activator; VTE, venous thromboembolism; MPO, myeloperoxidase; GSH, glutathione; GSNO, *S*-nitrosoglutathione

* Corresponding author. Fax: +1215 590 4267.
E-mail address: ischirop@mail.med.upenn.edu (H. Ischiropoulos).

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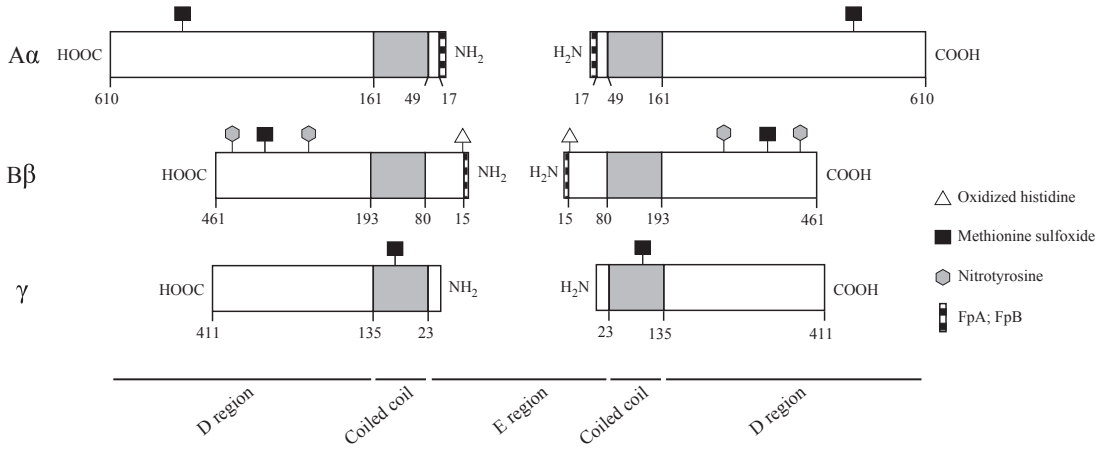

Fig. 1. Sites of oxidative PTMs on fibrinogen. Schematic representations of the Aα, Bβ, and γ chains of fibrinogen drawn to scale in length. Residue numbering follows the removal of the signal peptide. FpA and FpB (stripes) are localized toward the N-termini of the Aα and Bβ chains, respectively. Coiled-coil domains are represented in gray. His^{16} is located within the "B" knob. Met^{476}, Met^{367}, and Met^{78} are localized to the αC region, the carboxy termini of the Bβ chains, and the coiled-coils of the γ chain, respectively. Both Tyr^{292} and Tyr^{422} are localized within the carboxy termini of the Bβ chains; however, Tyr^{292} is located near the "b" holes.

Ca^{2+} can be monitored spectrophotometrically by following turbidity changes over time (Fig. 2A). This assay yields some generalized structural and kinetic information about fibrin clot formation, but does not provide specifics on properties such as rate of FpA and FpB release, which requires HPLC methodology [18], or fibrin clot structure, which can be obtained by scanning electron microscopy (Fig. 2B) [19] or confocal microscopy [20].

Fibrin is covalently crosslinked by the transglutaminase Factor XIII (FXIII), which provides stability and elasticity to the clot [20–23]. FXIII is activated (FXIIIa) by thrombin through cleavage of the N-terminal activation peptide [24]. FXIIIa crosslinks fibrin via the formation of ε-(γ-glutamyl) lysine bonds within and between α and γ chains, creating γ dimers, α polymers, and α–γ heteropolymers [25,26]. The rate and extent of FXIIIa crosslinking of fibrin, made by addition of thrombin plus FXIII and Ca^{2+} to either isolated fibrinogen or PPP, can be determined by quenching the reaction and running the products on an SDS–PAGE gel with crosslinked fibrin products identified and quantified by Western blot analysis and densitometry (Fig. 2C).

Physiological lysis of the fibrin clot proceeds through the enzyme plasmin, which is converted from its inactive zymogen precursor, plasminogen, by the enzyme tissue plasminogen activator (tPA) [27]. This reaction requires fibrin as a cofactor [28] and the kinetics of lysis can be followed by changes in turbidity (Fig. 2A). Plasmin binds and cleaves fibrin at several high-affinity lysine residues, one of which is located in the flexible region of the carboxy termini of the Aα chains termed αC regions [29,30], subsequently exposing additional lysine residues that plasmin further cleaves into smaller fragments [29]. Endogenous inhibitors of fibrinolysis target both tPA and plasmin and include plasminogen activator inhibitor-1, α_2-antiplasmin, and thrombin-activatable fibrinolysis inhibitor.

Fibrin is a highly extensible, viscoelastic polymer [22], allowing it to deform appropriately in response to shear stress. Changes in clot structure due to posttranslational modifications (PTMs), elevated fibrinogen concentration, or interactions with other proteins have been shown to alter the viscoelastic properties of fibrin clots. Specifically, increased clot stiffness was significantly associated with coronary artery disease [31], as well as those at risk for thrombotic events such as smokers [32] and diabetics [33]. Traditionally, rheometry has been used to measure the elastic (stiffness) and inelastic (viscosity) properties of fibrin clots in vitro, although some studies have also utilized thromboelastography. Defined by the storage modulus (G') and loss modulus (G''), respectively, these properties dictate the ability of the clot to store or dissipate energy from the application of applied shear stress. The ratio of the loss modulus to the storage modulus, tan δ, is also used as a measure of the relative proportion of inelastic to elastic components. The relationships between viscoelastic properties, fibrin clot structure, and pathology remain unclear. Most studies have observed that increased fiber density or decreased porosity, coupled with decreased individual fiber diameter, results in stiffer clots with delayed lysis, fostering a prothrombotic state [20,31,33]. In other cases, clot structural heterogeneities, including fiber clusters or bundles, have been observed as a consequence of PTMs and have varying effects on viscoelastic properties [32].

In addition to forming the fibrous network structure of a thrombus, fibrinogen also plays an important role in platelet aggregation. Individual platelets bind residues (400–411) located within the carboxy termini of the γ chains of fibrin(ogen) via their integrin receptor, αIIbβ3 [34]. These linkages tether platelets together resulting in platelet aggregation.

Fibrinogen is a critical protein in the formation of the clot, both in the fibrin network and in platelet aggregation, which are ultimately required for the generation of the hemostatic thrombus. Perturbations in these functions may influence the formation and properties of the fibrin network and promote pathological states, including thrombosis and thromboembolism. Indeed, epidemiological studies have indicated that increased levels of circulating fibrinogen are an independent predictor of coronary heart disease and, in some cases, of premature death from cardiovascular disease [35–38]. Despite these sound correlations, a causative association between high levels of fibrinogen and cardiovascular disease has not been firmly established. Evidence suggests that oxidative PTMs may contribute to alterations in fibrinogen function and affect pathology. Here we review the impact of oxidative modifications on the structure and function of fibrinogen and discuss their relationship with various disease states.

Oxidative modifications of fibrinogen

Oxidative stress has been widely implicated in physiological processes such as aging, in disease pathogenesis ranging from carcinogenesis to atherogenesis, and in the etiology of arterial and venous thrombosis. Proteins are major targets for oxidants, and fibrinogen, which comprises a large percentage of plasma proteins (~4%), is a likely target for oxidative PTMs.