Available online at www.sciencedirect.com
SCIENCE DIRECT
Archives of Biochemistry and Biophysics 445 (2006) 36–45
ABB
www.elsevier.com/locate/yabbi

Probing interactions between the coagulants thrombin, Factor XIII, and fibrin(ogen) ☆

Muriel C. Maurer [a,*], Toni A. Trumbo [b], Giulia Isetti [a], Brian T. Turner Jr. [a]

[a] Department of Chemistry, University of Louisville, 2320 South Brook Street, Louisville, KY 40292, USA
[b] Department of Chemistry, Bloomsburg University of Pennsylvania, Bloomsburg, PA 17815, USA

Received 14 September 2005, and in revised form 12 November 2005
Available online 1 December 2005

Abstract

Thrombin cleaves fibrinopeptides A and B from fibrinogen leading to the formation of a fibrin network that is later covalently crosslinked by Factor XIII (FXIII). Thrombin helps activate FXIII by catalyzing hydrolysis of the FXIII activation peptides (AP). In the current work, the role of exosites in the ternary thrombin–FXIII–fibrin(ogen) complex was further explored. Hydrolysis studies indicate that thrombin predominantly utilizes its active site region to bind extended Factor XIII AP (FXIII AP 33–64 and 28–56) leaving the anion-binding exosites for fibrin(ogen) binding. The presence of fibrin-I leads to improvements in the K_m for hydrolysis of FXIII AP (28–41), whereas peptides based on the cardioprotective FXIII V34L sequence exhibit less reliance on this cofactor. Surface plasmon resonance measurements reveal that D-Phe-Pro-Arg-chloromethylketone–thrombin binds to fibrinogen faster than to FXIII a_2 and dissociates from fibrinogen more slowly than from FXIII a_2. This system of thrombin exosite interactions with differing affinities promotes efficient clot formation.

Keywords: Thrombin; Factor XIII; V34L polymorphism; Fibrinogen; Anion-binding exosite-I; Fibrin; Kinetics; Surface plasmon resonance

Thrombin, fibrinogen, and Factor XIII (FXIII) are key players in the latter stages of the blood coagulation cascade. Fibrinogen is a dimer of three chains (Aα, Bβ, and γ) held together by disulfides [1]. This homodimer $(A\alpha B\beta\gamma)_2$ is rod-like and composed of two globular D-regions joined to a centrally located E-region by coiled coils of Aα, Bβ, and γ chains. The N-termini of the fibrinogen chains are grouped together within the E-region. The peptide chains then propagate in the direction of the D-regions. The serine protease thrombin cleaves fibrinopeptides A and B (FpA[1] and FpB, respectively) from the N-termini of the Aα and Bβ chains, thus converting fibrinogen into fibrin [2,3]. Loss of FpA results in fibrin-I; the additional loss of FpB yields fibrin-II. The resultant A and B knobs within the E-region of a fibrin monomer can align with complementary a and b holes in the D-regions of other fibrin monomers to produce a soft clot. In addition to targeting fibrinogen, thrombin also helps to activate the transglutaminase FXIII (FXIII) [4,5]. Thrombin participates in this process by cleaving the R37–G38 peptide bond within the FXIII activation peptide (AP) segment. Activated FXIII later catalyzes the formation of γ-glutamyl-ε-lysyl covalent crosslinks in the fibrin network and in fibrin-enzyme complexes leading

☆ Funding for this project supported by NIH Grant R01 HL68440 and an AHA Grant-in-Aid Award (Ohio Valley Affiliate).
* Corresponding author. Fax: +1 502 852 8149.
E-mail address: muriel.maurer@louisville.edu (M.C. Maurer).

[1] *Abbreviations used:* FpA, fibrinopeptide A; FpB, fibrinopeptide B; AES-I, anion-binding exosite-I; AES-II, anion-binding exosite-II; FXIII, blood clotting Factor XIII; AP, activation peptide; Fbg Aα, peptide segment from the fibrinogen Aα chain; PAR1, protease activated receptor 1; PAR4, protease activated receptor 4; PPACK, D-Phe-Pro-Arg-chloromethylketone; RP-HPLC, reversed-phase high-performance liquid chromatography; MALDI-TOF, matrix assisted laser desorption ionization time of flight; K_m, Michaelis–Menten kinetic constant; k_{cat}, catalytic constant or turnover number; NHS, *N*-hydroxysuccinimide; EDC, *N*-ethyl-*N*′-(dimethyl-aminopropyl)-carbodiimide); k_a, association rate constant; k_d, dissociation rate constant; K_D, equilibrium binding constant; RU, response units.

0003-9861/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.abb.2005.11.009

to formation of a mechanically stable and degradation resistant hard clot.

Factor XIII exists as a heterotetramer in plasma composed of two catalytic a subunits and two regulatory b subunits. Calcium is an important cofactor in activating plasma FXIII and also in promoting dissociation of the b subunits [6,7]. Much research has focused on exploring the interplay between plasma FXIII a_2b_2, thrombin, and fibrin(ogen) [8–11]. Thrombin hydrolysis of plasma FXIII is accelerated in the presence of fibrin-I and involves interactions with anion-binding exosite-I (AES-I) [10]. Comparatively less is known about interactions of the a_2 form that is prevalent in platelets and placental tissue. Once the b subunits are removed from plasma FXIII, it is the activated a_2 form that targets the reactant fibrin(ogen). Activated FXIII a_2 has been demonstrated to catalyze the formation of covalent crosslinks in fibrin and, to a lesser extent, in fibrinogen [12]. Fluorescence studies indicate that a_2 binds to fibrin but with a weaker affinity than a_2b_2. The affinity, however, is increased upon a_2 activation and suggests involvement of a putative FXIII binding site [11].

Kinetic, solution NMR, and X-ray crystallography studies have focused on examining the FXIII AP residues that bind within the thrombin active site [13–18]. Results suggest that the P_4–P_1 positions[2] within the FXIII AP serve as the major anchors to the thrombin surface [14]. Unlike the fibrinogen Aα chain, the P_4 position of a FXIII AP plays a larger role in binding and hydrolysis at the thrombin active site than the P_9 position [14,17]. A common polymorphism in human FXIII is replacement of V34 at the P_4 position with L34. Studies with synthetic peptides and whole enzyme both reveal that this variant of FXIII is more readily activated than the wild type form [13,19,20]. Furthermore, FXIII V34L has the ability to alter fibrin clot structure. Interestingly, the V34L polymorphism has been correlated with cardioprotective benefits [21].

In the current work, the role of exosite interaction in the ternary complex of thrombin, FXIII a_2, and fibrin(ogen) was explored. Synthetic FXIII activation peptides that have the potential to stretch from the thrombin active site to AES-I were studied. As a result, the ability of this exosite to directly promote cleavage of the FXIII AP could be assessed. As the common polymorphism V34L generates a FXIII that is more easily activated, there was interest in knowing whether thrombin hydrolysis of the FXIII AP is influenced differently by the presence of fibrin. New approaches to probe binding interactions among thrombin, FXIII, and fibrin(ogen) were also desired. Surface plasmon resonance (SPR) studies make it possible to readily monitor their association and dissociation rates during the course of the experiment and use this information to obtain equilibrium binding constants. Studies were carried out with an active site blocked D-Phe-Pro-Arg-chloromethylketone (PPACK)–thrombin, thus leaving the anion-binding exosites available for binding fibrinogen and FXIII.

The results obtained suggest that AES-I is reserved for fibrin binding and promotes interactions of the FXIII AP (28–41) V34 activation peptide sequence with thrombin. Extended FXIII activation peptides cannot contribute additional beneficial interactions with thrombin. Binary components of the thrombin–FXIII–fibrin complex exhibit fast association and dissociation rate properties that likely promote efficient clot formation.

Materials and methods

Synthetic peptides

The peptides FXIII AP (28–41) V34, FXIII AP (28–41) V34L, FXIII AP (33–64) V34, and FXIII AP (28–56) V34 were synthesized by SynPep (Dublin, CA, USA). Purity of all peptides was evaluated by analytical RP-HPLC. Molecular masses were verified by MALDI-TOF mass spectrometry. Concentrations for stock solutions in deionized water were determined by quantitative amino acid analysis (Molecular Analysis Facility, University of Iowa, Iowa). See Table 1 for sequences used in this project and related studies.

Thrombin preparation for FXIII AP studies

Bovine plasma citrate or sulfate eluate (Sigma) was dissolved in activation buffer consisting of 50 mM Tris-base, 150 mM NaCl, 0.1% PEG, pH 7.4. The solution was desalted on PD-10 size exclusion columns (Amersham Pharmacia Biotech). Eluent containing thrombin was activated by addition of calcium chloride and *Eschis carinatus* snake venom, incubated at 37 °C, and monitored for fibrinogen clotting ability [13]. As described previously, the acti-

Table 1
Sequences from substrates that include Fibrinogen, Factor XIII, and the Thrombin Receptors PAR1 and PAR4. The sequences displayed may be mapped from the thrombin active site region toward anion binding exosite-I (AES-I)

$P_4P_3P_2P_1P'_1$	AES-1 Region
FXIII AP (28–41)	^{28}TVELQGVVPRGVNL^{41}
FXIII AP (28–41, V34L)	^{28}TVELQGLVPRGVNL^{41}
FXIII AP (33–41)	^{33}GVVPRGVNL^{41}
FXIII AP (33–64)	^{33}GVVPRGVNLQEFLNVTSVHLFKERWDTNKVDH^{64}
FXIII AP (28–56)	^{28}TVELQGVVPRGVNLQEFLNVTSVHLFKER^{56}
Fbg Aα (7–20)	^{7}DFLAEGGGVRGPRV^{20}
Fbg Aα (7–38)	^{7}DFLAEGGGVRGPRVVERHQSACKDSDWPFCSD^{38}
PAR1 (29–45)	^{29}PESKATNATLDPRSFLL^{45}
PAR1 (29–56)	^{29}PESKATNATLDPRSFLLRNPNDKYEPF^{56}
PAR4 (38–51)	^{38}STPSILPAPRGYPG^{51}
PAR4 (38–62)	^{38}STPSILPAPRGYPGQVCANDSDTLE^{62}

Human sequences were taken from the following sources: Factor XIII [22] and Fibrinogen Aα chain [23], PAR1 [24], and PAR4 [25,26].

[2] The P nomenclature system (…P_3, P_2, P_1, P'_1, P'_2, P'_3…) is used to assign the individual amino acid positions on the substrate peptides. The P_1–P'_1 peptide bond becomes hydrolyzed by the enzyme. The peptide amino acids to the left of the cleavage site are labeled P_2, P_3, P_4, etc., whereas as those to the right of the cleavage site are labeled P'_2, P'_3, P'_4, etc.