International Journal of Mass Spectrometry 302 (2011) 139–148

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Structural characterization of both the non-proteolytic and proteolytic activation pathways of coagulation Factor XIII studied by hydrogen–deuterium exchange mass spectrometry

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ARTICLE INFO

Article history:
Received 28 May 2010
Received in revised form 31 August 2010
Accepted 9 September 2010
Available online 17 September 2010

Keywords:
Factor XIII
Hydrogen–deuterium exchange
Enzyme activation
Structure–activity relationship
Transglutaminases
rFXIII

ABSTRACT

Activated Factor XIII is a 83 kDa transglutaminase crucial in the final steps of blood coagulation. FXIII can be activated both via a proteolytic pathway and a non-proteolytic pathway. Activation in plasma takes place by a thrombin catalyzed cleavage after residue Arg37 (FXIIIa′) and a Ca^{2+} dependent conformational change (FXIIIa*). The non-proteolytic activation is the result of a conformation change only in the absence of thrombin or other proteolytic cleavage (FXIIIa◦). Hydrogen/deuterium exchange (HX) detected by mass spectrometry (MS) has proven a powerful technique for analyzing the conformational properties of proteins in solution. In this study, we apply HX-MS analyses on the entire span of conformational states of recombinant FXIII, i.e., rFXIII, rFXIIIa′, rFXIIIa* and rFXIIIa◦. 79 peptic peptides were used for analysis providing 90% coverage of the 83 kDa non-redundant sequence of FXIII. The HX-MS data show increased deuterium exchange along the dimer interface suggesting weakened dimer interaction in all rFXIIIa′, rFXIIIa* and rFXIIIa◦. Apart from this, rFXIIIa′ resembles zymogen rFXIII and no conformational changes seem to have taken place. In contrast, extensive changes occur upon full activation to either rFXIIIa* or rFXIIIa◦. All domains of rFXIII are involved, but the major changes occur in the catalytic core and β-barrel 1 domains. Furthermore, these experiments show highly similar HX-MS data for rFXIIIa* and rFXIIIa◦ clearly demonstrating that both the thrombin dependent and the non-proteolytic activation pathways of rFXIII produce similar activated conformations.

1. Introduction

Activated Factor XIII (FXIII; EC 2.3.2.13) is a transglutaminase catalyzing intermolecular ε-(γ-glutamyl)lysine isopeptide bonds [1]. FXIII activity is crucial in the final steps of blood coagulation by cross-linking fibrin monomers into a tight fibrin network. Furthermore, a number of anti-fibrinolytic, pro-haemostatic and adhesive proteins are cross-linked to the clot thereby providing a mechanically strong fibrin structure with increased resistance to fibrinolysis by plasmin or other proteolytic enzymes. FXIII is also found intracellular where the cross-linking enzyme activities are involved in tissue remodeling and also in cytoskeleton remodeling that occurs, for instance, during platelet activation [2–4]. (See [5–9] for reviews on FXIII structure and function.)

The catalytic activity is located in the 83 kDa FXIII – A subunit which forms a stable non-covalent dimer structure in solution. The dimer structure of rFXIII has been characterized various times by X-ray crystallography (Fig. 1A) [10–12]. Each FXIII–A subunit consists of an N-terminal activation peptide (residues 1–37), a β-sandwich domain, the large catalytic core domain holding the active site residues Cys314, His373 and Asp396 and finally two β-barrel domains (Fig. 1A and D). The dimer structure of rFXIII shows the activation peptide of one rFXIII subunit extending towards the catalytic core domain of the opposite rFXIII subunit and thus close to the opposite active site (Fig. 1A). Plasma FXIII furthermore contains two 80 kDa non-catalytic carrier FXIII-B subunits and thus circulates as a hetero-tetramer. Intracellular FXIII consists of the FXIII-A subunits only.

In plasma, thrombin hydrolyzes the activation peptide of FXIII between residues Arg37-Gly38 resulting in FXIIIa′ (Fig. 2B). This event does not by itself create functional enzyme activity, e.g., the active site Cys314 is not solvent exposed in FXIIIa′ [13,14] and thrombin cleaved FXIIIa′ retains a zymogen conformation [15]. In the presence of physiological plasma concentrations of Ca^{2+} and

Abbreviations: ESI, electrospray ionization; MALDI, matrix assisted laser desorption/ionization; MS, mass spectrometry; HX, hydrogen/deuterium exchange; HEPES, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; CID, collision induced dissociation; UPLC, ultra performance liquid chromatography; FXIII, Factor XIII; rFXIII, recombinant FXIII (consisting of the A_2 subunits); FXIIIa*, thrombin activated FXIII; FXIIIa′, thrombin cleaved but not active; FXIIIa◦, FXIII activated without proteolysis; TG, transglutaminase.

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doi:10.1016/j.ijms.2010.09.010

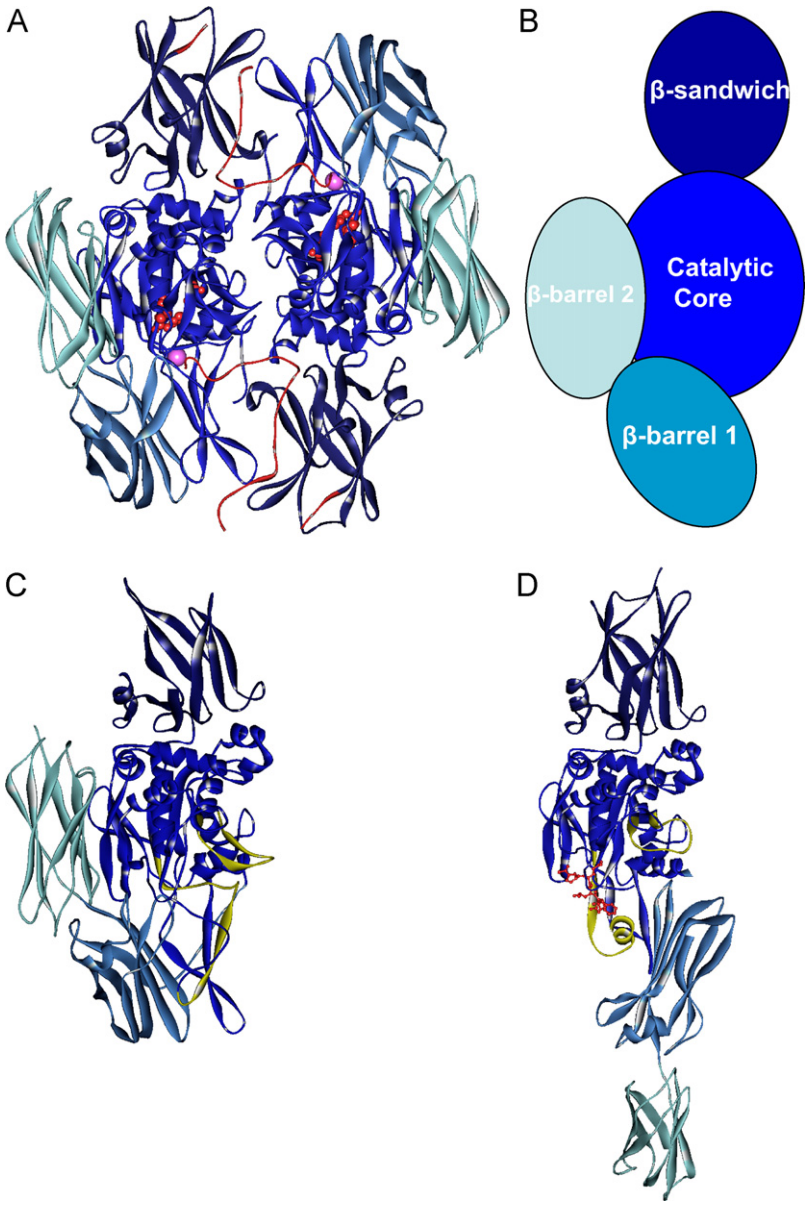

Fig. 1. rFXIII and recombinant TG2 structures and schematic domain overview. (A) The structure of rFXIII dimer. The domains are, from N- to C-terminus; activation peptide (red), β-sandwich (dark blue), catalytic core domain (blue), β-barrel 1 (light blue) and β-barrel 2 (light turquoise). The catalytic core domain contains the active site residues (Cys314, His373 and Asp396, red ball and stick) and the primary Ca^{2+} binding site (violet). (B) Schematic overview of the domain organization in rFXIII and non-activated TG2. Domain coloring is similar to panel A. (C) Non-activated TG2 and (D) activated TG2 showing the structural rearrangements in TG2 upon activation. Domain coloring is similar to panel A. Notice the reorientation of β-barrel 1 upon activation. The peptide active site inhibitor (red ball and stick) and residues 300–320 (α-helical in panel D) and 357–372 are highlighted (yellow). TG2 is monomeric and does not contain an activation peptide. The structures are from pdb-entries 1GGU, 1KV3 and 2Q3Z, respectively [10,23,26].

fibrin, FXIIIa′ undergoes a conformational change resulting in the active enzyme FXIIIa* [16–18] (Fig. 2C). During the conformational rearrangement the activation peptide and FXIII-B subunits are released. This thrombin dependent activation pathway is dominant in plasma where thrombin and fibrin is generated under coagulation conditions FXIIIa* can also be created from FXIIIa′ *in vitro* in the absence of fibrin by raising the Ca^{2+} concentration to 15 mM or higher [19]. Alternatively, zymogen FXIII is also able to activate non-proteolytic in the absence of thrombin or other proteases. Effective non-proteolytic activation of FXIII-A_2 occurs only at 50 mM Ca^{2+} and above *in vitro* [13,16]. However, even low amounts of Ca^{2+} in combination with, e.g., 1 M NaCl, chaotropic ions or other stimuli is sufficient for activation to occur [19,20]. This activation is solely an effect of a conformation change, without the release of the activation peptide, and results in rFXIIIa° (Fig. 2D). The latter activation pathway is dominant for intracellular FXIII where no thrombin is present [21,22].

FXIII belongs to a larger class of transglutaminase enzymes. The known structures of zymogen transglutaminases (FXIII, TG2 and TG3) are very similar as exemplified in Fig. 1A and C showing the structures of zymogen rFXIII and recombinant non-activated TG2, respectively [23]. Human transglutaminases are activated and regulated in numerous ways. Some transglutaminases are dependent on a proteolytic cleavage whereas others rely on conformational activation only. However, all TGs depend on Ca^{2+} for activity and activation [24]. TG2 is activated by Ca^{2+} but also inhibited by the presence of GTP/GDP nucleotide [23,25]. TG2 has been structurally characterized by X-ray crystallography in the non-active form, in the absence of nucleotides, as well as with a peptide inhibitor bound in the active site, respectively [26]. This structure of active TG2