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Role of calcium in the conformational dynamics of factor XIII activation examined by hydrogen–deuterium exchange coupled with MALDI-TOF MS

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ABSTRACT

Factor XIII catalyzes formation of γ-glutamyl-ε-lysyl crosslinks within fibrin clots. FXIII A_2 can be activated proteolytically with thrombin and low mM Ca^{2+} or nonproteolytically with high monovalent/divalent cations along with low mM Ca^{2+}. Physiologically, FXIII A_2 is poised to respond to transient influxes of Ca^{2+} in a Na^+ containing environment. A successful strategy to monitor FXIII conformational events is hydrogen–deuterium exchange (HDX) coupled with mass spectrometry. FXIII A_2 was examined in the presence of different cations (Ca^{2+}, Mg^{2+}, Ba^{2+}, Cu^{2+}, Na^+, $TMAC^+$, and EDA^{2+}) ranging from 1 to 2 mM, physiological Ca^{2+} concentration, to 50–500 mM for nonproteolytic activation. Increases in FXIII solvent exposure could already be observed at 1 mM Ca^{2+} for the dimer interface, the catalytic site, and glutamine substrate regions. By contrast, solvent protection was observed at the secondary cleavage site. These events occurred even though 1 mM Ca^{2+} is insufficient for FXIII activation. The metals 1 mM Mg^{2+}, 1 mM Ba^{2+}, and 1 mM Cu^{2+} each led to conformational changes, many in the same FXIII regions as Ca^{2+}. FXIII could also be activated nonproteolytically with 500 mM tetramethylammonium chloride ($TMAC^+$) and 500 mM ethylenediamine (EDA^{2+}), both with 2 mM Ca^{2+}. These different HDX studies help reveal the first FXIII segments that respond to physiological Ca^{2+} levels.

Introduction

Blood coagulation involves a cascade of enzyme activations that ultimately concludes in a soluble fibrin network. This network is covalently crosslinked by the transglutaminase factor XIII (FXIII)[1] to form an insoluble clot [1–3]. Due to the critical role of FXIII in coagulation, there is a need to better understand the conditions that influence FXIII activation/activity as well as the structural dynamics of this enzyme system.

In plasma, FXIII is a protransglutaminase with a tetrameric structure A_2B_2, where the B subunits act as a carrier for the catalytically active A subunits [4]. By contrast, cellular FXIII exists in platelets, placenta, and monocytes as an A_2 dimer. Recombinant FXIII A_2 has been successfully crystallized [5], and the resultant structure exhibits well-defined and sequentially folded domains that include the activation peptide (1–37), the β sandwich (38–184), the catalytic core (185–515), β-barrel 1 (516–628), and β-barrel 2 (629–730). See Fig. 1A and B.

When FXIII is activated physiologically, thrombin (IIa) cleaves the N-terminal activation peptide (residues 1–37). The presence of low mM Ca^{2+} assists in the dissociation of the B subunits from plasma FXIII. In addition, Ca^{2+} promotes exposure of the catalytic C314 from both plasma and cellular FXIII resulting in an active A_2 dimer ($FXIIIa^*$) [6–8]. Like other transglutaminases (TGases), the active site of FXIII consists of a thiol-containing catalytic triad (C314, H373, and D396), but FXIII is unique in the fact that it exists as a dimer. In the presence of a suitable acyl-donor glutamine containing substrate, C314 of FXIIIa forms a thioester bond and ammonia is released. The acyl-donor substrate is then covalently linked to a primary amine (lysine) acyl-acceptor forming γ-glutamyl-ε-lysyl cross-links involving the α- and γ-chains of fibrin which stabilizes the growing clot [9].

In addition to IIa proteolytic activation, FXIII A_2 can be nonproteolytically activated when the Ca^{2+} concentration is greater than 50 mM ($FXIIIa^{\circ}$ or $FXIIIa^{Ca}$) [10,11]. FXIII A_2 has also been observed in an activated state in the presence of >150 mM NaCl and low mM Ca^{2+} ($FXIIIa^{Na}$) [12]. A related form of nonproteolytic FXIII activation has been documented in platelets [12–14]. Thrombin-stimulated platelets may exhibit increased Ca^{2+} levels that promote formation of an active intracellular FXIII species which has not been hydrolyzed at the R37–G38 peptide bond. In plasma,

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[1] *Abbreviations used:* FXIII, recombinant human cellular factor XIII; FXIII(a), unactivated or activated factor XIII; $FXIIIa^{Ca}$, calcium activated factor XIII; $FXIIIa^{Na}$, sodium activated factor XIII; $FXIIIa^{IIa}$, thrombin activated factor XIII; IIa, thrombin; TGase, transglutaminse; TG2, transglutaminase 2; IAA, iodoacetamide; MALDI-TOF MS, matrix-assisted laser desorption–ionization time-of-flight mass spectrometry; HDX, hydrogen–deuterium exchange; GDH, glutamate dehydrogenase; GEE, glycine ethyl ester; $TMAC^+$, tetramethylammonium chloride; EDA^{2+}, ethylenediamine.

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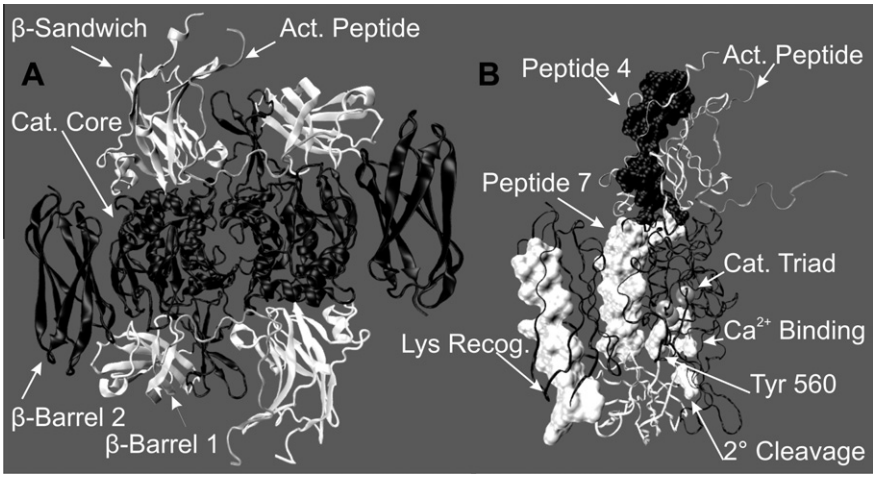

Fig. 1. The FXIII A_2 zymogen 2.1 Å crystal structure 1F13 [39]. (A) A cartoon model to illustrate the intimate contact at the dimer interface with the domains being color coded (gray) activation peptide, (white) β-sandwich, (black) catalytic core, (white) β-barrel 1 and (black) β-barrel 2. (B) Ribbon view of one FXIII-A monomer with the domains represented by the same colors as (A). The regions of interest are labeled: activation peptide (1–37), Gln recognition peptide 4 (72–97), Gln recognition peptide 7 (190–230), catalytic triad (C314, H373 and D396), Tyrosine 560, secondary cleavage site (513–514) and the proposed lysine recognition region (646–658). These figures were created using VMD [52].

these different forms of nonproteolytic activation are minimized by the presence of the B_2 subunits [15]. Regardless of the mode of activation, FXIIIa function can be hindered following hydrolysis at the secondary cleavage site (K513–S514) by thrombin [16,17]. Occupation of a nearby site with Ca^{2+} and other select metals can help protect this secondary hydrolysis from occurring [5,18].

Even though the catalytic C314 is only alkylated by iodoacetamide (IAA) after activation [19–21], the crystal structures for $FXIIIa^{IIa}$ and $FXIIIa^{Ca}$ lack any major structural changes when compared to that of zymogen [5,22,23]. We have used MALDI-TOF mass spectrometry approaches coupled with hydrogen–deuterium exchange or chemical modification to probe FXIII conformation changes occurring in solution. During activation, portions of the FXIII catalytic core and the A_2 dimer interface were found to be more accessible to solvent [24,25]. By contrast, the addition of an inhibitory peptide with a glutamine isostere caused selected FXIII regions to become more protected from solvent [26]. As expected from the secondary cleavage studies, Ca^{2+} binding hindered solvent exposure in a FXIII segment around 513–514. Inhibition at the active site could lead to further long-range decreases in HDX in this region.

A recent paper by Kristiansen and Andersen questions our use of borate buffer supplemented with $CaCl_2$ for mass spectrometry based projects. Our HDX studies have not, however, been carried out under deactivated concentrations (0.3 mM $CaCl_2$) as they presume, but at 1 mM $CaCl_2$ following either proteolytic or nonproteolytic activation. According to their paper, FXIII A_2 that is activated nonproteolytically at 50 mM $CaCl_2$ and then buffer exchanged into 1 mM $CaCl_2$ maintains 90% of its enzyme activity [27]. Moreover, many of the conformational events that we reported as being important for FXIII activation have been confirmed by HDX studies by Andersen and Faber using a buffer system of 200 mM Hepes, 150 mM NaCl, and 50 mM $CaCl_2$ [28].

The focus of our present HDX work was to further investigate the conformational changes that occur to FXIII due to monovalent and divalent cation binding. FXIII A_2 was monitored in the presence of Ca^{2+}, Mg^{2+}, Ba^{2+}, Cu^{2+}, Na^{+}, tetramethylammonium chloride ($TMAC^{+}$) or ethylenediamine (EDA^{2+}) with concentrations ranging from physiological Ca^{2+} concentration, 1–2 mM, up to 50–500 mM. On its own, the low mM Ca^{2+} condition is unable to support FXIII activity. The HDX effects observed in this environment thus provide valuable information on early conformational changes needed in preparation for activation. Often these effects further increased as the Ca^{2+} concentration was raised to the levels required for nonproteolytic activation (50–500 mM). Several of the conformational changes observed with physiological 1 mM Ca^{2+} also occurred with the other divalent metals whereas other effects were distinct for a particular metal. In the current investigation, we also characterized a novel method of nonproteolytic FXIII activation utilizing high concentrations of organic cations $TMAC^{+}$ or EDA^{2+}. Knowledge gained from these different HDX studies will help identify the elusive structural roles of different divalent and monovalent cations. A greater understanding of the conformational dynamics of FXIII will aid in the development of new therapeutic strategies to control excessive bleeding, thrombosis, and/or atherosclerosis.

Materials and methods

Factor XIII preparation and synthetic peptides

Recombinant human cellular factor XIII A_2 (FXIII A_2) was generously provided by Dr. Paul Bishop (ZymoGenetics, Inc., Seattle, WA). After reconstituting the lyophilized FXIII in 18 MΩ deionized water, FXIII was buffer exchanged into 6.67 mM borate at pH 8.3. The concentration of FXIII was determined on a Cary 100 UV/vis spectrophotometer. The absorbance was monitored at 280 nm and concentration calculated with the FXIII extinction coefficient of 1.49 ml/mg cm. Aliquots (36 μl) of 16.7 μM FXIII in 6.67 mM borate were dried in a SpeedVac (Savant) and stored at −70 °C until future use.

The β casein derived FXIII substrate peptide K9 (Ac-LGPGQSK-VIG-OMe) was synthesized by Peptides International (Louisville, KY). K9 was reconstituted in 18 MΩ deionized water and the concentration was confirmed by quantitative amino acid analysis (AAA Service Laboratory, Inc., Boring, OR). Purity was assessed by HPLC and mass spectrometry approaches.

Transglutaminase activity assay

Factor XIII activity was determined using a modified version of the Dade-Behring Berichrom Assay [29,30]. Briefly, this assay utilizes a coupled reaction involving both FXIII and glutamate dehydrogenase (GDH). FXIIIa reacts with the acyl-donor K9 releasing NH_3 and the transglutaminase reaction concludes when the