Insights into the Structure of Human Blood Coagulation Factor X

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Abstract The solution structure and function of human coagulation factor X (FX) were studied to understand the key structural elements of the enzyme form during activation. FX is a vitamin K-dependent plasma glycoprotein, composed of light and heavy chains, and plays a central role in the coagulation cascade. The calcium-rich γ -carboxyglutamic acid domain is responsible for binding to the membrane, an important step in the coagulation process. After activation, the serine protease domain undergoes a significant relocation, forming a compact multidomain. The S1-specific pocket largely determines the functional activities that activate FX. The enzyme-producing form of FX is more extensive than the active form. The enzyme-producing and activated forms of FX have different conformations in the serine protease domain.

Keywords factor X, factor Xa, serine protease domain, γ -carboxyglutamic acid domain, differences

Introduction

Coagulation factor X and its active form FXa are of great significance for hemostasis and thrombosis. The formation of FXa led to the activation of thrombin to thrombin, followed by the conversion of fibrinogen to fibrin.^[1] Congenital FX deficiency is rare. Recessive hereditary bleeding disorders account for 10% of all rare bleeding disorders, affecting 1 in 1 million people, and may cause nosebleeds, schistosomiasis and gastrointestinal bleeding. In addition to congenital defects, low FX levels may occasionally appear in some disease states.^[2] One FXa molecule produces more than 1,000 thrombin molecules. Evidence suggests that early inhibition of the coagulation cascade at FXa levels may have greater antithrombotic potential, and FXa is an attractive potential specific target for new anticoagulants. The most commonly used clinically used anticoagulants warfarin and heparin series anticoagulants and fondaparinux sodium have different degrees of inhibition on Fxa.^[3] Therefore, an in-depth study of the structure and function of FX and FXa not only helps us understand the important position of FX and FXa in the coagulation process, but also provides new ideas for scientific research and clinical treatment.

Structure

Light chain

The light chain of FX consists of GLA, EGF1 and EGF2 domains. The γ -carboxyglutamic acid (GLA)-rich domain contains 11 GLA residues (Ala1-Gla39 represents the GLA domain), followed by a short hydrophobic stack (residues Phe40-Lys45) and two epidermal growth factor (EGF)-like structures Domain: EGF1 (Asp46-Phe84) and EGF2 (Thr85-Gly128). The calcium-rich GLA domain is responsible for binding to the membrane, an essential step in the coagulation process. The negative charge elicited from the GLA residue string facilitates binding to Ca^{2+} and produces the conformation required for binding to an anionic phospholipid membrane. This surface in the body is provided by activated platelets or other blood cells to deal with vascular damage by exposing the inner surface of its cell membrane. *In vitro*

systems that attempt to simulate blood coagulation primarily use natural or synthetic phosphatidylserine and phosphatidylcholine preparations.

The binding of calcium ion to human FXa, especially to GLA-EGF1, has been widely studied by various experimental methods. NMR studies of the GLA-EGF1 fragment of FXa indicate that calcium binding in the GLA domain plays a key role in reversible membrane binding.^[4,5] In the calcium-bound form, GLA residues bind to Ca^{2+} ions at the core of the GLA domain, forcing the side chains of the hydrophobic residues Phe4, Leu5, and Val8 into the solvent. In the calcium-free GLA domain, GLA residues are exposed to solvents, and Phe4, Leu5, and Val8 residues form hydrophobic clusters inside the protein.^[5] Similarly, calcium ions bind to the GLA-EGF1 hinge of several VKD proteins that contain the EGF domain. The relative orientation of the GLA-EGF1 domain is more ordered in the presence of calcium ions.^[7] Recent time-resolved fluorescence studies of the FXa calcium-binding GLA-EGF1 domain have also shown that the relative orientation of the GLA and EGF1 domains changes when calcium ions bind to the GLA-EGF1 interface,^[8] in the absence of calcium next, the GLA domain of FXa does not bind to the phospholipid surface.^[9]

EGF domains are typically present in extracellular Mosaic proteins and are characterized by the presence of three disulfide bond bridges that are connected in a characteristic manner.^[10] The EGF1 and EGF2 domains in FX, along with other VKD proteins, act as flexible spacers between lipid-bound GLA domains and SP domains. However, their function in protein-protein interactions is not fully understood. The effect of calcium on the relative orientation of gla-egf1 domains has been extensively studied (as described above). The isolated EGF1 domain binds to moderately strong Ca^{2+} , and Kd is 103m.^[11] However, the gla-egf1 binding region binds Ca^{2+} ions and increases the Ca^{2+} ion affinity by a factor of 10.^[4,12] This indicates that Ca^{2+} ions coordinate residues in GLA and EGF1 domains. The existing X-ray crystal structures in this region lack the key GLA domain. In the absence of EGF1, the EGF1 domain may be somewhat disordered in crystal structure. Figure 1 shows the gla-egf1 interdomain region of the calcium ion coordination network with FX. The ligands for calcium ions

are the two backbone carbonyl atoms of Gly47 and Gly64 and the side chains of bha-63, Asp46, and Gln49. Two oxygen atoms from the bha-63 carboxylic acid side chain participate in coordination (although not shown, the Ca^{2+} ion-binding network is similar in activation form). In addition to the six oxygen atoms from the gla-egf1 domain, two water molecules are balanced in the coordination sphere. In the FX and FXa simulation structures, a total of 8 ligands bind to Ca^{2+} ions. During the whole simulation process, it was observed that the calcium coordination network was stable and two water molecules maintained coordination with calcium ions. Yeast contains a large number of EGF2/AP hydrogen bonds that are lost when the activated form is formed without AP in the activated form. Residues Thr136, Lys134, and Arg139 all have different partners in active and zymogen form. The interactions between the EGF2 and SP domains are also very different. Only Asn93-Trp308 and His101-Asp307 interactions are relatively identical in zymogen and activated form. The total number of interactions between the activated EGF2 and SP domains and the zymogen EGF2/(SP+AP) was almost the same.

Figure 1 The calcium-ion coordination network at the GLA-EGF1 domain interface of FX.

Serine protease domain

FXa is produced when a zymogen is cleaved in a heavy chain containing a catalytic serine protease domain. The SP domain of FX consists of 254 residues. A key difference between the active form of FX and the zymogen form is that after the AP at the Arg194-Ile195 (Arg15-Ile16) peptide bond is cleaved, the *N*-terminus of the SP domain of FX A is significantly redirected. After activation, the *N*-terminus of Ile195 (Ile16) was redirected to the interior of the SP domain and stabilized by forming a strong salt bridge (ion pair) with Asp378 (Asp194). This event is essential for triggering events that promote the catalytic activity of FXa.

To study the key differences between the activated and enzyme-producing forms of the SP domain, the electrostatic potential (ESP) near the active site was studied. The ESP profiles of zymogen and activated SP domains are shown in Figures 2a and 2b, respectively. After the backbone atoms of the active site residues Asp282, His286 and Ser379 are aligned, both SP domains are in the same orientation. Various residues and catalytic triads in the active site-binding capsule are labeled in Figure 2a. The cleavage site Arg194-Ile195 in the zymogen is also indicated by a circle (labeled 12) in the figure. Positive potentials (blue areas in the circle) are present at sites involved in peptidyl cleavage of incoming proteolytic enzymes. Although the ESP chart derived from GRAP can only give a qualitative image of the difference between the two structural forms, the changes around the active part are obvious.^[13] The active site pocket in the zymogen is wider than the activated form and more solvent is exposed. The static electricity of the side chain of residue Gln376 (labeled 8 in the figure) helps to determine the specific pocket (S1 site) in activation of FX, which is

significantly different from the zymogen structure. Although the orientation of this side chain limited the accessibility of the active site residue Ser379 (labeled 1 in Figure 2), the same residues in the fermentor structure were replaced. In the simulated FXa, the CA-CA distance between Ser379 and Gln376 (S1 position) (calculated by superimposing the skeleton atoms of the catalytic triad residues of FX and FXa) is 5.28 Å (the corresponding distance in the x-ray crystal structure) (6.61 Å) and 8.21 Å in the simulated fermentor. This deviation of 3.0 Å may be significant because the S1-specificity pocket largely determines the functional activity of activated FX.^[14] Recall also that the ion pair between the *N*-terminus end of Ile195 and Arg378 is located near the S1-specificity pocket. Other specificity pockets around the active site appear to have little change of the backbone $\text{Ca}-\text{Ca}'$ distance. For instance, the $\text{Ca}-\text{Ca}'$ distance between Ser379 and Tyr279 (S4 specificity site) is 7.1 Å in both FXa and FX. The same is true for the distance between Phe356 and Tyr279 (14 Å). The difference in the S1-specificity pocket in FX and FXa is also shown by the differences in the solvent-accessible surface area (SASA) near the active-site region. The SASA around the catalytic residue Ser379 (Ser195) within a 7 Å sphere is evaluated using a probe radius of 1.4 Å. The SASA values were calculated by the ACCESS program in the WHATIF package for nonhydrogen atoms.^[15] This area covers all the catalytic triad residues and several specificity pockets around the active site. A total of 30 residues were found in FXa and 25 residues in FX around Ser379 within the 7 Å radius. The SASA values for this region are 393 Å² and 471 Å², respectively, for FXa and FX.

Figure 2 Electrostatic potential maps of serine protease domains of zymogen (top left) and activated (bottom left) structures of FX generated by the GRASP program.^[13] Both structures represent similar orientation with the backbone alignment of the three catalytic triad (His236, Asp282, and Ser379) residues. Various residues around the active-site pocket are marked. The residues corresponding to the numbering are as follows: 1, Ser379; 2, His236; 3, Asp282; 4, Lys276; 5, Tyr279; 6, Trp399; 7, Ser398; 8, Gln376; 9, Gln240; 10, Na-binding region; 11, Ca²⁺-binding region. The same numbering is used for both activated and zymogen structures. In addition, the cleavage site of zymogen at Arg194-Ile195 (12) is circled in the structure. The ESP of full zymogen structure derived from the last snapshot (6 ns) is also shown in the figure (right). Different domains of FX are marked in the figure. Regions of EGF2 and AP domains that might be involved in negative electrostatic repulsion are marked in circles.

The distance between the active site residues in the SP domain and the surface of the hypothetical phospholipid was investigated. Although earlier fluorescence studies showed an approximate distance of 61 between the surface and the active site, later using the same technique, studies of different fluorescent probes have shown it to be 83 ± 3 for FXa. Similar

measurements have not been performed on fermentogens. The results show that the distance between the plane of calcium ions (the surface of our model) and the Ser379 (Ser195) residues in the active site crack changes with time, and Ser379 residues play an important catalytic role in the active site crack. Figures 3a and 3b show the change in distance between FXa (Figure 3a) and FX (Figure 3b) during the simulation time. The distance in FXa is basically stable within the last 500 ps of the simulation time and remains stable at $\sim 83 \pm 2$ Å. Encouragingly, this is similar to the reported experimental values.^[17] For enzyme-producing types, the distance is calculated to be $\sim 95 \pm 3$ Å. Therefore, the enzyme-producing form of FX is more extensive than the active form, indicating that there are differences in conformation and orientation between active FX and enzyme-producing FX. The reason for the discrepancy can be seen in the ESP chart for foreign exchange. Esps of the complete proenzyme structure obtained from the last snapshot of the MD trajectory (6.2 ns) (Figure 3c), and esps of the egf2-sp domain of FX and FXa (Figures 3a and 3b). There is an electrostatic repulsion between some EGF2 residues of FX and AP (as shown in the circle), which may cause the AP and EGF2 domains of FX to shift. In addition, the recombinant EGF2 and SP interactions (FX and FX a) may result in a wider conformation. When the activating peptide in the coagulation pathway was removed, the rejection of AP residues was removed. Therefore, the surface of the SP domain buried under AP in FX is exposed to FXa. New exposure areas of FXa are primarily hydrophobic or neutral. Therefore, FXa can respond by shrinking to some extent. In Figure 4, we show a snapshot of FX a and FX with GLA and EGF1 domains (ala1-glu82; RMSD 2.4a). As can be seen from the figure, the enzymology and activation forms of FX have significantly different conformations in the SP structural domain.^[17]

Figure 3 Changes in the distance between the plane of GLA calcium ions (three calcium ions in the core of the GLA domain) and Ser379 (backbone atom CA) of FX (a) and FX (b).

Figure 4 The conformational difference between the active and zymogenic forms of FX when the backbone atoms of the GLA and EGF1 domains of the two structures were aligned. Superimposition of GLA/EGF1 domains used the residues 1—82 of both structures. The zymogen structure is shown in thick coils while the activated form is in thin coil. Activation peptide of FX is represented in solid coil form. The SP-bound calcium ions are also shown as spheres.

Calcium-binding site in SP

The consensus calcium-binding loop in the SP domain has an important functional role in protecting the SP domain from proteolysis^[18] and enhancing the amidolytic activity of factor Xa.^[19] Also, the calcium-binding site may participate in the prothrombinase complex formation.^[20] This site is well characterized in trypsin and other serine proteinases.^[21] It has been reported that Ca^{2+} ion potentiates the S-2222 hydrolytic activity of factor Xa by 1.6-fold.^[18]

Conclusion

FX's activated and enzyme-producing structures are complete. These two structures share similar domain motifs in the light chain. However, after activation, FX was redirected such that the enzymatic structure was on average 10 times longer than the long-axis activated structure. Compared with the active structure, the enzyme-producing active center region has obvious differences near the catalytic triad, but the backbone atoms of the catalytic triad residues are similar in both forms. The S1-specific capsular bag has significant differences in the two structural forms, and the active-site capsular bag is wider than the active-site capsular bag, and more solvents are exposed to the zymogen.

Recent studies of possible interactions between several domains of these two proteins have shown that the GLA domains of FX and VIIa interact. For example, mutation studies have shown that VII36's Arg36 plays a key role in substrate interactions. Similarly, the GLA domain of FX is also related to the interaction of the TF-VIIa complex, which is based on the study of several mutants in the FX GLA domain.^[22,23] First structural model for the membrane-bound state of FX-GLA is established and two putative PS-specific binding sites based on the simulation results are proposed. Small molecules for potential drug targets can block lipid-specific binding of FX-GLA domains and then achieve the goal of diminishing thrombosis.^[24] In the ternary TF-VIIa-FX complex, TF residues Lys165 and Lys166 have been shown to facilitate protein-FX interactions.^[25] The His263-Lys276 fragment in FXa is related to the specific binding of factor V/Va in the prothrombin complex.^[20] In the current model of FX enzyme production, the peptidyl cleavage site of the Arg194-Ile195 bond is 84 Å on the Ca^{2+} binding surface of the GLA domain. In the x-ray crystal structure, the distance between Ser344 (Ser195) and Ca^{2+} in the triad of the VIIa catalysis is 85°. Therefore, the substrate (FX) and enzyme complex (TF-VIIa) have the optimal length from the corresponding GLA surface to the cleavage site and the active site residues.

Conflict of Interest

The authors declare no conflict of interest.

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Received November 29, 2019

Accepted December 17, 2019