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Research paper

Structural transitions during prothrombin activation: On the importance of fragment 2

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ABSTRACT

Prothrombin is activated to thrombin by the prothrombinase complex through sequential cleavage at two distinct sites. This occurs at sites of vascular injury in a highly regulated cascade of serine protease and cofactor activation, where activated platelets provide a suitable surface for protease/cofactor/substrate assembly. The precise structural and conformational changes undergone during the transition from prothrombin to thrombin have been studied for decades, and several structures of prothrombin fragments along the activation pathway have been solved. Here we present a new structure analyzed in context of other recent structures and biochemical studies. What emerges is an unexpected mechanism that involves a change in the mode of binding of the F2 domain (fragment 2) on the catalytic domain after cleavage at Arg320, and a subsequent reorientation of the linker between the F2 and catalytic domain to present the Arg271 site for cleavage.

1. Introduction

A critical event in hemostasis is the conversion of prothrombin from an inactive zymogen to the active enzyme thrombin. This is the end-point of the blood coagulation cascade and is regulated by many factors [1,2]. Once formed, thrombin uses two anion-binding exosites (I and II) and its active site to recognize several substrates (Fig. 1) [3,4]. Maintenance of prothrombin as a zymogen requires the encryption of these three sites to prevent premature interactions. Catalytic inactivity is a general feature of zymogens of serine proteases, but encryption of the two exosites is equally important in prothrombin to prevent the dysregulation of hemostasis. If exosite I were 'competent' then prothrombin could saturate thrombomodulin on the vascular endothelium, thereby precluding binding by active thrombin and preventing protein C activation. It could also bind to fibrinogen and potentially block fibrin formation, and similarly bind to circulating fV and fVIII thereby preventing their activation [5–9]. Exosite II exposure would localize prothrombin to the intact vascular surface via heparan sulfate binding and to platelets via GpIbα binding [10–13].

Most serine protease zymogens are converted to active proteases through the cleavage of a single scissile bond between residues 15 and 16 (chymotrypsin numbering). The structural rearrangements that lead to the development of a functional active site cleft is well understood, and involves the 'insertion' of the new N-terminus (normally Ile16) into the so-called 'activation pocket' [14,15]. However, it can also be effected in the absence of cleavage by peptides and certain virulence factors such as staphylocoagulase [16–18]. This results in the formation of the principal substrate-binding pocket (S1) and the oxyanion hole. Prothrombin is an unusual zymogen in the sense that cleavage of the Arg15-Ile16 (Arg320-Ile321 in prothrombin numbering) peptide bond does not result in a fully functional enzyme, but in the disulfide-linked enzyme intermediate meizothrombin (mIIa) [19]. Complete activation to thrombin requires an additional cleavage at residue Arg271 (prothrombin numbering) to release the catalytic domain from the N-terminal fragment 1.2 (F1.2), composed of the Gla domain and two kringle domains (Fig. 2). Two potential pathways for prothrombin activation are available depending on which of these two cleavage events occurs first. With factor Xa alone, cleavage at Arg271 is kinetically favored, and cleavage at Arg320 progressing relatively slowly. Initial cleavage at Arg271 results in formation of the zymogen precursor prethrombin-2 (pre2) and F1.2 [20]. In the presence of factor Va and phospholipid membranes, prothrombinase cleaves first at the Arg320 site, followed by cleavage at Arg271 [21,22]. The ordered cleavage of Arg320

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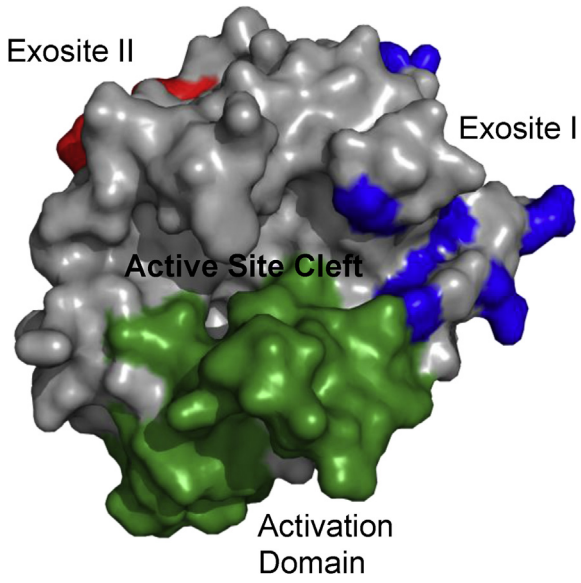

Fig. 1. Structural features of thrombin. Surface representation of thrombin in gray in the standard orientation showing the positions of exosite I (in blue), exosite II (in red), the activation domain (in green), and the active site cleft.

followed by Arg271 of prothrombin by prothrombinase has been referred to as 'ratcheting' because the zymogen to protease conformational change seems to present the second cleavage site to the active site of fXa [23]. A recent study concluded that cleavage by prothrombinase through the meizothrombin intermediate may be attributed to the use of synthetic phospholipids and that activation of prothrombin on platelets proceeds via the pre2 intermediate [24]. However, it is clear that the meizothrombin pathway predominates provided prothrombin is anchored to the same surface as the prothrombinase complex [25], and this must thus be considered the physiological pathway, in spite of the use of synthetic phospholipid vesicles.

Exosite I exists in a flexible, low-affinity state in prothrombin that is ordered upon conversion to thrombin [26]. Exosite II is occupied in prothrombin by the second kringle domain, fragment 2 (F2), blocking exosite II mediated interactions, and effectively encrypting this exosite in prothrombin. F2 binds 2-fold more weakly to thrombin than to the zymogen pre2, and F1.2 binds to thrombin 20-fold more weakly than to pre2 [27]. This suggests that formation of meizothrombin through cleavage at Arg320 may help release the catalytic domain from its interaction with F2. It is unclear how the covalent linkage of the F1 domain affects the binding of F2 to the catalytic domain, but a direct interaction between the F1 and F2 domains is unlikely. The conformational changes in the zymogen activation domain upon cleavage at Arg320 matures exosite I, although meizothrombin is still somewhat deficient in its ability to bind to exosite I-dependent ligands [28]. Thus, cleavage at both Arg320 bond and removal of F1.2 by cleavage at Arg271 is required for the formation of fully competent exosites on thrombin. Here we present a new structure of the pre2/F2 complex in context of other structures of prothrombin fragments containing at least the F2 and catalytic domains, and investigate how conformational change might contribute to prothrombin activation.

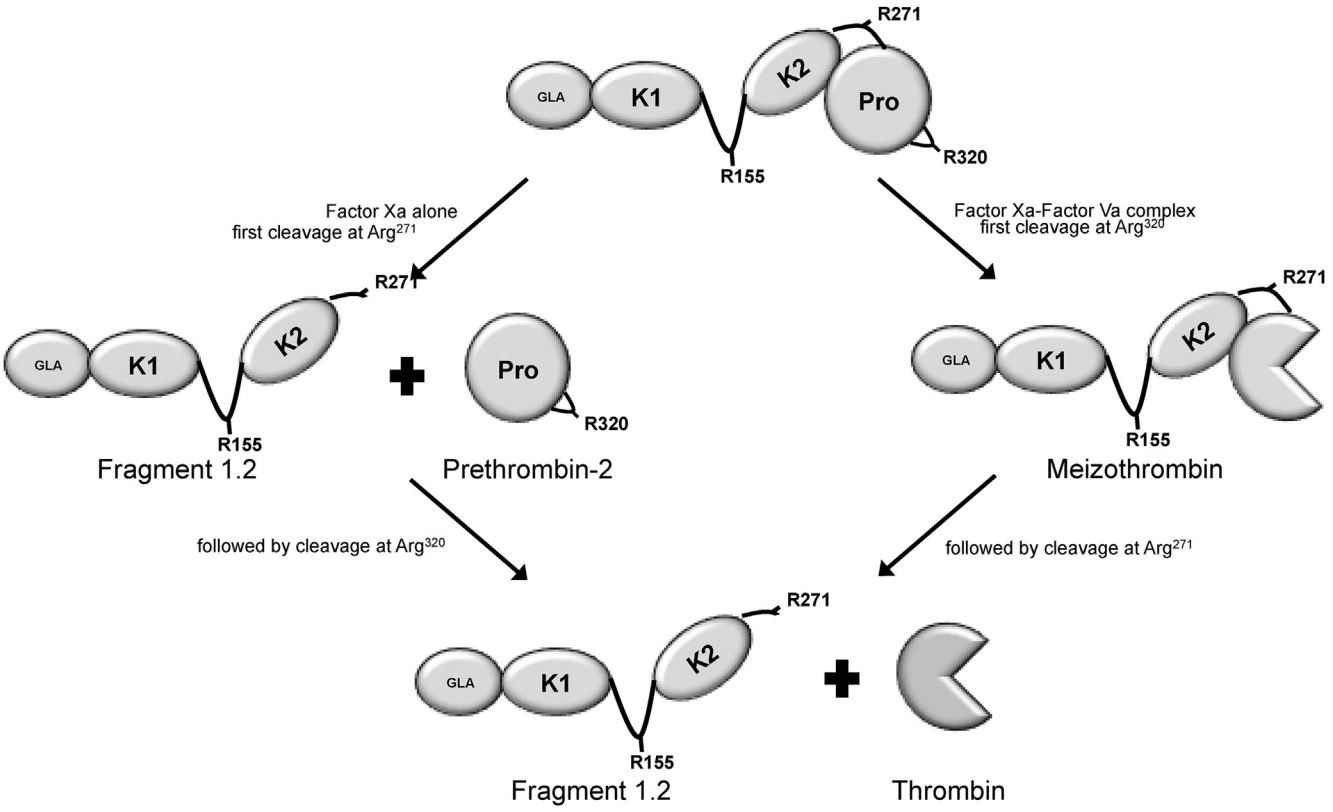

Fig. 2. Prothrombin activation pathways. The proteolytic conversion of the zymogen prothrombin to the enzyme thrombin is the result of proteolytic cleavage at Arg271 and Arg320. In the presence of factor Xa alone, initial cleavage at Arg271 produces F1.2 and pre2 (left pathway), followed by cleavage at Arg320 generating active thrombin. In the presence of factor Va and phospholipids, factor Xa cleaves Arg320 producing the active intermediate meizothrombin, which is further processed at Arg271 to release thrombin from F1.2 (right pathway). The Gla domain (GLA), kringle 1 (K1), kringle 2 (K2), and protease domains (Pro) are shown as ovals showing linkers between each as lines.