The structure of Gla-domainless human coagulation Factor VII.

Ashley C.W. Pike, Andrzej M. Brzozowski and Egon Persson1.

York Structural Biology Laboratory, University of York, York YO10 5DD, UK.

1Tissue factor / Factor VII Research, Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Måløv, Denmark.

Blood coagulation plays a crucial role in haemostasis and is controlled by a complex cascade of proteolytic reactions. Factor VII (fVIIa) plays a key regulatory role in this process by triggering the extrinsic coagulation pathway [1]. fVIIa possesses a modular domain organisation with an N-terminal, membrane binding γ-carboxyglutamic (Gla)-domain, two epidermal growth factor (EGF)-like domains and a C-terminal serine protease domain (SPD). Like all serine proteases, fVIIa is activated by the cleavage of an internal peptide bond that facilitates the maturation of its catalytic machinery. fVIIa is unusual, however, in that it remains in a zymogen-like state after this cleavage and only becomes an efficient catalyst when associated with its membrane-bound protein cofactor, tissue factor (TF). The two proteins form a complex at the site of injury, where extravascular TF becomes exposed to the blood, and trigger clot formation by autoactivating fVII and activating factors IX and X. The crystal structure of fVIIa has previously been solved in complex with TF [2]. However, no structure for the free form of fVIIa has been determined to date. In an attempt to unravel the structural changes that underlie the TF-mediated potentiation of fVIIa’s activity we have determined the structure of Gla-domainless fVIIa (gd-fVIIa) in isolation.

A recombinant form of human fVII, that had been treated with cathepsin G to remove the N-terminal Gla-domain (residues 1-44) and covalently inhibited with D-Phe-L-Phe-Arg (FFR) chloromethyl ketone, was used for all the structural studies. Crystals of gd-fVIIa were grown using the sitting drop vapour diffusion method. Drops composed of equal volumes of protein (12mg/ml in 50mM NaCl, 10mM Tris pH8) and reservoir solution (50mM NaCl, 10mM CaCl2, 3.5M sodium formate in 100mM Tris pH8.5). Large tetragonal crystals, belonging to space group \(P_4_3\) with unit cell dimensions \(a=b=117.96\AA \ c=100.44\AA\), grew within 1-2 weeks. A preliminary dataset was collected at room temperature on station 9.5 at the SRS (CLRC Daresbury Laboratory, UK). The crystals initially diffracted to ca. 2.8Å but were highly susceptible to x-ray damage and the resulting dataset, collected from numerous crystals, was good only to 3.1Å. To optimally cryoprotect the crystals, the mother liquor was replaced by a solution containing 50mM NaCl, 10mM CaCl2, 5% (w/v) xylitol, 3.3M sodium acetate in 100mM Tris pH8.5 and the concentration of xylitol was increased to 35% in 5% steps over 3hrs. Crystals treated in this way belong to space group \(P_4_3_2_2\) (\(a=b=115.3\AA \ c=98\AA\)) and have a single molecule of gd-fVIIa per crystallographic asymmetric unit. Data were collected to a resolution of 2.8Å at 100K on a MAR image plate detector mounted on beamline BW7B (\(\lambda=0.83\AA\)) (EMBL Hamburg Outstation, Germany). A total of 238127 reflections were recorded to give a final dataset (representing a 95.4% data coverage between 35Å and 2.8Å) with a merging R-factor of 5.6% (25.3% in 2.85-2.80Å shell). The position of the protease domain was determined by molecular replacement using the coordinates of the corresponding domain in the fVIIa:TF complex (PDB code 1DAN)[2] as a search model. The positions of the two remaining EGF-like domains were revealed by the resulting sigmaa-weighted \(2F_oF_c\) electron density map calculated after 5 cycles of refinement with REFMAC. The coordinates corresponding to each EGF domain (PDB code 1DAN)[2], were manually fitted to the electron density and subjected to rigid-body refinement. The structure was refined with REFMAC incorporating a bulk solvent correction calculated in XPLOR. The final model, comprising 2928 atoms, has an Rcryst and Rfree of 21.5 and 26.7, respectively, for all data between 35 and 2.8Å.

Free fVIIa adopts an extended conformation similar to that observed in complex with TF (Fig. 1). The two EGF-like domains form a narrow stalk about 65Å in length on which the spherical protease domains. However, whilst there is good agreement between the positions of the serine protease and EGF-2 domains in free and complexed fVIIa, the N-terminal EGF-1 domain adopts a different orientation. The EGF-1 domains are related by a ca. 180° rotation about the linker hexapeptide between the two EGF-like modules. This
different relative orientation reflects both the inherent flexibility of the linker region and the environment of this domain in the two structures. Other unexpected differences between the present structure and TF-complexed fVIIa include different binding modes for the FFR covalent inhibitor and a change in conformation of a loop that flanks the active site cleft.

The most striking difference between the two forms of fVIIa is observed for residues Leu305-Glu325 in the protease domain. This region in the TF-complexed fVIIa is composed of a short α-helix followed by a loop that defines the outermost face of the active site cleft’s S4 subsite [2]. The N-terminal portion of the helix forms part of the interface between the protease domain and TF and contains a number of residues that are important for proteolytic function and optimal binding to TF. For these reasons, this region has been proposed to act as the allosteric control site in the TF-mediated activation of fVIIa [2]. In the gd-fVIIa structure, this transmitter helix is distorted and shortened by three residues. The whole region is also shifted by between 0.5Å and 3Å towards the active site cleft relative to its position in the TF-complexed fVII structure. This has a knock-on effect on the positioning of two neighbouring loops in the activation domain that is stabilised on conversion of the zymogen to the protease form of the enzyme. This observation sheds light on the role of TF in the enhancement of fVIIa’s proteolytic activity. It appears as though this region is important for the stabilisation of the protease domain’s activation region - a prerequisite for the expression of proteolytic activity. In the absence of TF, which appears to stabilise both the transmitter helix and, consequently, the activation domain [2], the full proteolytic activity of fVIIa is prevented and therefore ensures a correct temporal and spatial procoagulant response to injury.

References