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Tissue Factor/Factor VIIa Complex: Role of the Membrane Surface

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Abstract

Blood clotting is triggered when the plasma serine protease factor VIIa binds to the cell-surface protein, tissue factor (TF); the resulting TF:FVIIa complex activates factors IX (FIX) and X (FX) by limited proteolysis. FVIIa, FIX and FX all bind reversibly to membranes via their gamma-carboxyglutamate-rich (GLA) domains, while TF is an integral membrane protein. Removing these proteases from the membrane surface is known to render them thousands of times less active, although the mechanisms by which blood clotting proteins bind to membranes—and the contributions of membranes to catalysis—remain very incompletely understood. Our recent and ongoing studies use a combination of nanoscale membrane bilayers (Nanodiscs), solid-state NMR and all-atom molecular dynamics (MD) simulations, enabling detailed insights into how GLA domains bind to phospholipid bilayers and how specific phospholipids enhance the catalytic activity of the TF:FVIIa complex.

Introduction

Blood clotting is triggered when plasma comes into contact with tissue factor (TF) on cell surfaces. TF binds the plasma serine protease, factor VIIa (FVIIa), to form the TF:FVIIa complex, which then activates factor IX (FX) and factor X (FX) by limited proteolysis [1]. TF is an integral membrane protein while FVIIa, FIX and FX bind reversibly to membrane surfaces via their gamma-carboxyglutamate-rich (GLA) domains. Removing these proteins from the membrane surface renders them thousands of time less active [2], and in fact, almost every step in the blood clotting cascade is obligatorily tied to the presence of membrane surfaces containing exposed phosphatidylserine (PS) moieties [3]. In spite of their importance, however, the mechanisms by which protein-membrane interactions contribute so profoundly to blood clotting reactions are very incompletely understood. This brief review will discuss new technologies we are bringing to bear to investigate protein-membrane interactions in blood clotting, with a special emphasis on understanding how the membrane so dramatically accelerates FX activation by TF:FVIIa.

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Conflicts of interest statement
J.H.M. and S.G.S are co-inventors on patents covering some of the technologies mentioned in this article.

Nanoscale phospholipid bilayers (Nanodiscs): a unique platform for examining protein-membrane interactions, including FX activation by TF:FVIIa

We have developed the Nanodisc system, a soluble nanobilayer for studying protein-membrane interactions while allowing strict control over local membrane composition [4]. Nanodiscs consist of a nanoscale discoidal bilayer ringed and stabilized by "Membrane Scaffold Protein" (MSP). Nanodiscs self-assemble from mixtures of phospholipid, MSP and detergent [5], yielding monodisperse preparations whose bilayer size and phospholipid composition are under strict experimental control. The physical properties of Nanodisc bilayers have been extensively documented and are comparable to conventional liposomes, including phospholipid bilayer thickness, surface area, phase transition temperature, metal ion interactions and ability to support protein-membrane interactions [4, 6–12]. A "standard" Nanodisc encompasses an ~8 nm-diameter phospholipid bilayer (with ~70 phospholipids per leaflet), while longer versions of MSP have been engineered to allow formation of larger Nanodiscs [5].

Integral membrane proteins included in Nanodisc self-assembly reactions embed into these nanoscale bilayers just as they do in conventional liposomes [6–10, 13–15]. We successfully incorporated TF into Nanodiscs in which we varied the bilayer composition from 0 to 90% PS, with the balance being phosphatidylcholine (PC). TF:FVIIa complexes were assembled on these nanobilayers and had catalytic activities very similar to TF:FVIIa on conventional liposomes [15]. In surface plasmon resonance studies using Nanodiscs, we found that the affinity of FX for membranes increased monotonically with PS content, reaching maximal affinities at about 80% PS. The number of FX binding sites per nanobilayer also increased with PS content, with one FX binding site per 7 to 8 PS molecules. This is consistent with the notion that FX binds to a nanocluster of PS molecules on the bilayer surface.

Maximal rates of FX activation by TF:FVIIa on Nanodiscs required about 70% PS [15]; by comparison, TF:FVIIa complexes on liposomes require ~30% PS for maximal activity [16]. This is also consistent with the notion that FX activation by TF:FVIIa occurs preferentially on PS-rich nanoclusters on the membrane surface. Solution-phase FX has been proposed to bind directly to TF:FVIIa; alternatively, membrane-bound FX has been proposed to laterally diffuse (or skip) on the membrane surface in order to encounter TF:FVIIa [3, 17–19]. The Nanodisc surface can bind at most 5 or 6 FX molecules, which the TF:FVIIa complex will activate to FXa within a few seconds. However, we observed linear rates of FX activation over 20-minute time courses [15], demonstrating that TF:FVIIa is not dependent on a large, preexisting pool of membrane-bound FX substrate.

Solid-state NMR studies of protein-membrane interactions in blood clotting

The binding of blood clotting proteins to membrane surfaces via their GLA domains is Ca^{2+}-dependent. It is well known that Ca^{2+} binds to PS and this is also thought to induce PS clustering. To examine Ca^{2+}-PS interactions in detail, we synthesized PS with ^{13}C and ^{15}N in the serine headgroup for use in solid-state NMR (ssNMR) studies [20]. Ca^{2+} induced two distinct conformations of PS headgroups in PS/PC nanobilayers, which were well resolved in two-dimensional ^{13}C-^{13}C, ^{15}N-^{13}C and ^{31}P-^{13}C ssNMR spectra and which were compared to detailed predictions from MD simulations [20].

We have recently isotopically labeled the isolated extracellular domain of TF (sTF) with ^{13}C and ^{15}N and have reported the assignment of nearly all the backbone ^{1}H, ^{13}C and ^{15}N resonances by solution NMR [21]. We have also used ssNMR to complete most of the ^{1}H, ^{13}C and ^{15}N resonance assignments for nanocrystalline sTF and for membrane-

anchored TF embedded in Nanodiscs containing $^{13}C,^{15}N$-labeled PS (unpublished observations). Combined with detailed biochemical/mutagenesis studies and molecular dynamics (MD) simulations, these studies should afford detailed insights into protein-membrane and protein-protein interactions within the TF:FVIIa:membrane complex.

Computational studies of protein-membrane interactions in the TF:FVIIa complex

Although x-ray crystal structures are now available for many clotting proteins, none include a membrane bilayer so they cannot provide a detailed structural understanding of how these proteins interact with membranes. Recent advances in MD methods are permitting new insights to be gained into clotting protein-membrane interactions, including the binding of the FVIIa GLA domain to membranes [22]. We showed that GLA domains penetrate relatively deeply into membrane bilayers, with the GLA domain's tightly bound Ca^{2+} ions residing at about the level of the phosphates of PS headgroups [22].

Our more recent studies have produced the first all-atom MD simulation of the TF:FVIIa complex assembled on PS-containing bilayers [23]. FVIIa in solution exhibited large hinge motions between the boundaries of the first EGF domain, while sTF was more rigid. When sTF bound to FVIIa it significantly restricted FVIIa's motions, resulting in a fairly rigid sTF:FVIIa complex positioned almost perpendicular to the membrane surface [23], in excellent agreement with our previously published fluorescence resonance energy transfer studies [24–26]. Positioning the active site of FVIIa site relative to the membrane may be important in aligning it with membrane-bound substrates (FIX and FX), and in fact we recently showed that perturbing this alignment compromises the ability of TF:FVIIa to activate FX [27].

The "ABC hypothesis" to explain phospholipid contributions to FX activation by TF:FVIIa

Detailed simulations of the binding of FVIIa's GLA domain to PS bilayers revealed two major types of phospholipid-protein interaction [22]. The first represents a PS binding site that was virtually identical to that observed in the x-ray crystal structure of lyso-PS bound to the prothrombin GLA domain [28]. We propose that this represents a single, phospho-L-serine-specific binding site in each GLA domain. The second type of binding interaction seen in the MD simulations consists of multiple instances in which the GLA domain's bound Ca^{2+} ions participate in coordination complexes with the phosphates of PS molecules; in these cases, the PS headgroups actually fold away from the GLA domain.

These observations prompted us to examine, in detail, how phosphatidylethanolamine (PE) "synergizes" with PS to support FX activation by TF:FVIIa. Liposomes composed of PE and PC support little to no clotting activity, but when PE is incorporated into PS/PC liposomes, it dramatically decreases the PS requirement for optimal activity of TF:FVIIa complex [16]. PE is much more abundant than PS in plasma membranes, so mixtures of small amounts of PS plus larger amounts of PE (with the balance being PC) represents a more physiologic environment for triggering blood clotting than do 30% PS/70% PC liposomes. In a recent study, we developed a novel "Anything But Choline" (ABC) hypothesis to explain PE/PS synergy, that might also explain how GLA domains bind to phospholipid bilayers in general [29]. The ABC hypothesis proposes a single "phospho-L-serine-specific" binding site per GLA domain plus five or six "phosphate-specific" interactions per GLA domain, in which phospholipid headgroups bend away to allow their phosphates to enter into coordination complexes with tightly bound Ca^{2+}. We propose that PS can provide both types of interaction, while PC can provide neither because its bulky

choline headgroup sterically hinders access of GLA domains to its own phosphate. PE can provide the phosphate-specific but not the phospho-L-serine-specific interactions, explaining why PE/PC bilayers poorly support clotting while PE synergizes strongly with small amounts of PS. A strong prediction of the ABC hypothesis is that there is nothing unique about the ethanolamine headgroup in PE, other than that it is not as bulky as PC and therefore would allow access to its phosphate moiety. We showed that almost any glycerophospholipid other than PC strongly synergized with PS to promote FX activation by TF:FVIIa, and we also provided ssNMR data showing that binding of GLA domains to isotopically labeled PS in Nanodiscs induced a novel, third PS headgroup conformation [29]. Taken together, these findings support a new, detailed view of how GLA domains engage the phospholipid bilayer, which can also explain why the combination of PS and PE accelerates FX activation by TF:FVIIa.

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