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Recent Estimates of the Structure of the Factor VIIa (FVIIa)/Tissue Factor (TF) and Factor Xa (FXa) Ternary Complex

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Abstract

The putative structure of the Tissue Factor/Factor VIIa/Factor Xa (TF/FVIIa/FXa) ternary complex is reconsidered. Two independently derived docking models proposed in 2003 (one for our laboratory: CHeA and one from the Scripps laboratory: Ss) are dynamically equilibrated for over 10 ns in an electrically neutral solution using all-atom molecular dynamics. Although the dynamical models (CHeB and Se) differ in atomic detail, there are similarities in that TF is found to interact with the γ-carboxyglutamic acid (Gla) and Epidermal Growth Factor-like 1 (EGF-1) domains of FXa, and FVIIa is found to interact with the Gla, EGF-2 and serine protease (SP) domains of FXa in both models. FVIIa does not interact with the FXa EGF-1 domain in Se and the EGF domains of FVIIa do not interact with FXa in the CHeB. Both models are consistent with experimentally suggested contacts between the SP domain of FVIIa with the EGF-2 and SP domains of FXa.

Keywords

Factor VIIa; Factor Xa; ternary complex; Molecular Dynamics simulation

Introduction

In both the conventional blood coagulation cascade [1] and in the more recent cell-based cascade [2,3], the ternary complex of FVIIa/TF/FXa is thought to play an important role in providing free FXa for the formation of prothrombinase (Factor Va, FXa, negatively charged phospholipids and calcium ions). While the structure of FVIIa/sTF has been determined in several laboratories and under several different conditions [4–6], the structure of the ternary complex remains unsolved. A static docking model [7] and a solution-equilibrated model [8] were generated in 2003; both models were derived independently and while globally similar, showed differences in the details of the structures. Significant gains in computational power have been realized since 2003, as well as additional experiments that probe the possible structure of the complex are now available [9]. Thus we wished to revisit these two models and provide a more extensive, current “best-guess” solution-equilibrated model.

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Conflict of Interest Statement
All authors declare no conflicts of interest to disclose.

Methods

We will use the following labels to describe the various models: Ss =Scripps static, Se=Scripps solution-equilibrated (14.2 ns), CHeA=original Chapel Hill solution-equilibrated (3.6 ns) and CHeB=current Chapel Hill solution-equilibrated for 10.5 ns. The Se model derives from the Scripps static model (PDB code: 1NL8) [7] with several modifications: Glu39 was modified to a Gla39 residue, residues 159–162 in TF were added, and calcium ions were placed on Gla (γ-carboxyglutamic acids) residues Gla32 and Gla39 to be consistent with our prior simulation (CHeA) [8]. The CHeB model derives from the starting docking model (the chirality of several residues was corrected) that led to CHeA [9], but which has undergone a simulation time sufficient for equilibration.

The details of the setup for the solvent equilibration of the ternary complex models (CHeB and Se) are given in the Supplementary Information of Ref. 10. The essentials are that the docked complexes are surrounded with layers of water using periodic boundary condition so that images in surrounding boxes do not interact, the systems are carefully equilibrated at the starting conformation and the particle mesh Ewald (PME) method [11] is used to compute the electrostatic interactions. The AMBER9 [12] program was employed along with the ff99SB force field, the TIP3P water model [13] and the dynamics code PMEMD9. The importance of using the PME method for macromolecular simulations has been discussed [14]. Both CHeB and Se were simulated for sufficient times (>10 ns) that the overall RMSD of the simulation to the starting structures was relatively constant with time.

Results and Discussion

A fair question is “How good is molecular dynamics (MD) for predicting the structures of macromolecular complexes in solution?” An optimistic view of the usefulness of MD to drug design has been given recently [15]. Likewise, the improvement of docked protease-inhibitor binding energies (experimental to predicted) for a large number of HIV-1-inhibtor complexes was improved considerably by use of molecular dynamics as opposed to protein-rigid docking without dynamics [16]. Significant overlap between essential spaces of proteins defined by NMR ensembles and molecular dynamics has also been shown [17]. In our hands, we were able to refine an NMR structure of the factor IX Gla domain obtained with significant denaturing agent present and locate the ω-loop (residues 1–13) and positions of the calcium ions to obtain a refined whole Gla domain that compared closely to similar domains of prothrombin and factor VIIa [18].

The goal of this work was to solution-refine two independently derived docking models for the FVIIa/TF/FXa complex and thereby arrive at an up-to-date estimates of the solution structure. The same force field, water model, integration method and treatment of electrostatic forces were employed for both models. Fig. 1 shows the Se model for orientation purposes. Table 1 provides a comparison of the contacts derived, TF/FVIIa with FXa, from the simulations (10.5 ns for CHeB and 14.2 ns for Se) and also gives a comparison to the experimental contacts [20–32]. Eight of the contacts are similar in both models, of these five have partial agreement with experiment. Both models bury about the same area in binding FXa, 4150 (CHeB) and 3921(Se) $Å^2$. The TF/FVIIa units have an RMSD (backbone) of 2.92 Å between the models. For comparison, Se is 2.72 Å and CHeB is 2.78 Å RMSD to the X-ray crystal structure of TF/FVIIa in Ref. [4]. On the other hand, the TF/FVIIa/FXa (des-Gla) units have an RMSD of 5.53 Å. Both models are consistent with experimentally suggested contacts between the SP domain of FVIIa with the EGF-2 and SP domains of FXa. FVIIa does not, however, interact with the FXa EGF-1 domain in the Se model and the EGF domains of FVIIa do not interact with FXa in the CHeB model. Overall, the differences in the models appear to

trace back to the relative orientation of the EGF-1 in the original docking models. These models, which can be obtained on request, should be useful for comparison for new experiments.

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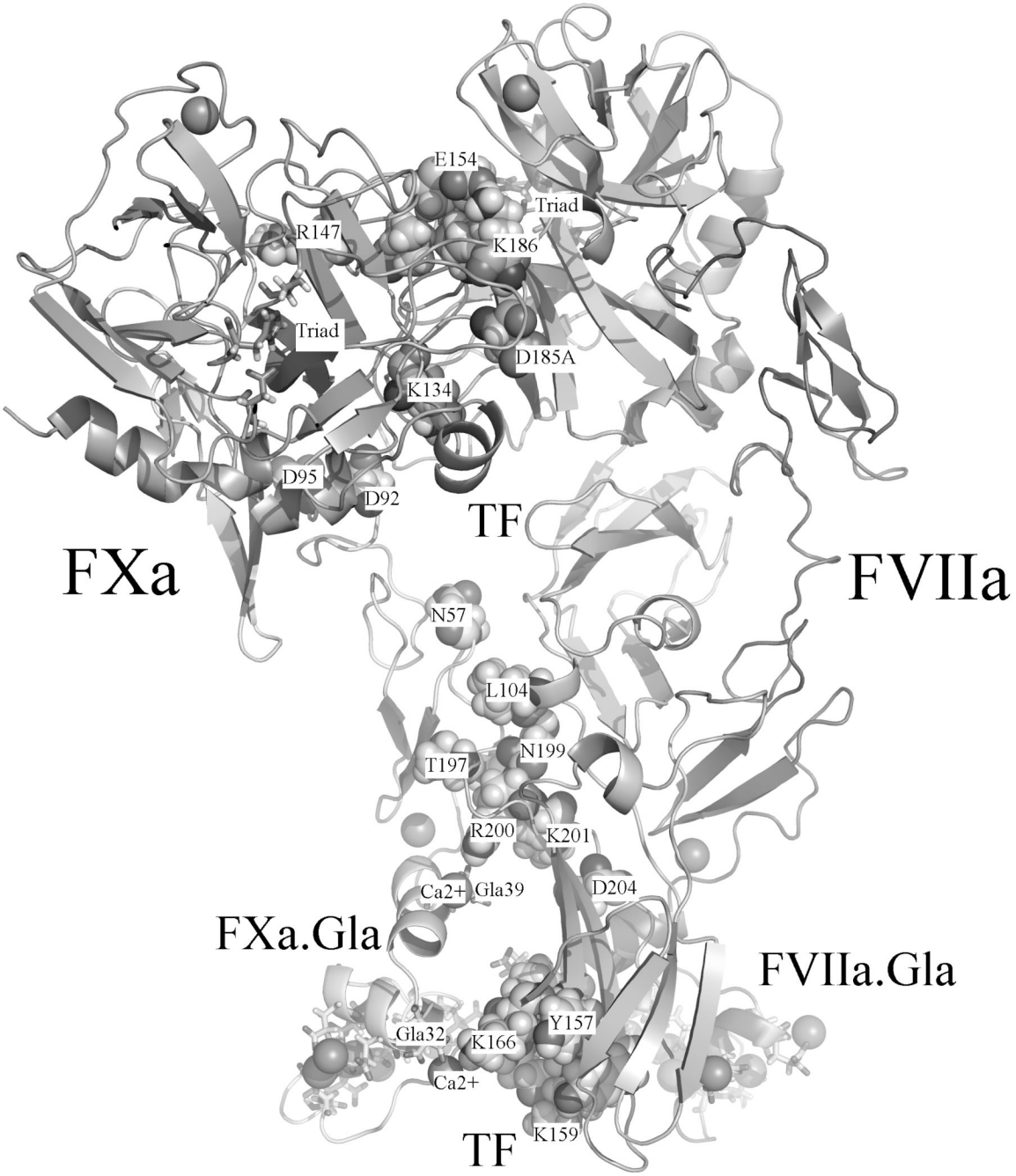

Fig. 1.

Residues involved in experimental contacts are mapped onto the Se model. **TF**: (Lys165, Lys166) [20–25], (Tyr157, Lys159, Ser163, Gly164) [23,25], Tyr185 [24,25], (Asn199, Asp204) [24], Arg200 [9,24,26,27], Lys201 [9,27], (Leu104, Thr197) [27]; **FVIIa**: Arg 36 [28], SP((Val21, Glu154, Met156)[29,30], Leu144 [30] Ala152 [30,31], Arg147 [32]); **FXa** [7]: Glu51, Asn57, Asp92, Asp95, SP(Lys134, Asp185A, Lys186).

Table 1

Comparison of predicted and experimental contacts from the Se and CHeB models. Interactions were computed with default setting in the Protein Interactions calculator server [19]. Chymotrypsin numbering system as in 1NL8 (pdb of Ss) is used for SP domains of FVIIa and FXa. **Bold** residues correspond to experimental contacts [20–32]. Abbreviations: HI, hydrophobic; HB, hydrogen bond; MS, main chain-side chain; SS, side chain-side chain; LC, light chain of FVIIa and FXa for residues over EGF-2 domain in light chain. *Italic* residues designate residues adjacent to residues involved in experimental contact.

	CHeB	Se
TF/fXa	Glu24^{TF}@OE1,OE2 :: Gly66$^{fXa.EGF-1}$@O (MS-HB)	
	Lys41^{TF} :: Glu77$^{fXa.EGF-1}$ (Ionic interaction)	
		Thr70^{TF}@OG1 :: *Gln58$^{fXa.EGF-1}$*@OE1 (SS-HB)
	Glu99^{TF}@N :: Glu77$^{fXa.EGF-1}$@OE1 (MS-HB)	Glu99^{TF}@OE1:: *Gln58$^{fXa.EGF-1}$*@NE2(SS-HB)
	Glu105^{TF} :: Lys79$^{fXa.EGF-1}$ (Ionic interaction)	
	Glu105^{TF}@N :: Lys79$^{fXa.EGF-1}$@OE2 (MS-HB)	
	Lys165^{TF}@NZ :: Asp35$^{fXa.Gla}$@OD2 (SS-HB)	
	Lys165^{TF} :: Asp35$^{fXa.Gla}$ (Ionic interaction)	
		Lys166^{TF} :: Gla32$^{fXa.Gla}$ (Ionic interaction)
		Thr197^{TF}@OG1:: Cys61$^{fXa.EGF-1}$@N (MS-HB)
	Val198^{TF} :: Pro54$^{fXa.EGF-1}$ (HI)	
		Asn199^{TF}@OD1:: Ser53$^{fXa.EGF-1}$@OG (SS-HB)
		Arg200^{TF} :: Gla39$^{fXa.Gla}$ (Ionic interaction)
		Arg200^{TF} :: Asp48$^{fXa.EGF-1}$ (Ionic interaction)
	Lys201^{TF}@NZ :: **Glu51$^{fXa.EGF-1}$**@OE2 (SS-HB)	
	Lys201^{TF} :: Glu51$^{fXa.EGF-1}$ (Ionic interaction)	
	Asp204^{TF} :: Lys43$^{fXa.Gla}$ (Ionic interaction)	
	Asp204^{TF}@OD1,OD2 :: Lys43$^{fXa.Gla}$@NZ (SS-HB)	
fVIIa/fXa		Ala34$^{fVIIa.Gla}$ :: Met18$^{fXa.Gla}$ (HI)
		Gla35$^{fVIIa.Gla}$ :: Lys36$^{fXa.Gla}$ (Ionic interaction)
		Gla35$^{fVIIa.Gla}$@N :: Met18$^{fXa.Gla}$@SD (MS-HB)
		Arg36$^{fVIIa.Gla}$ :: Gla14$^{fXa.Gla}$ (Ionic interaction)
		Arg36$^{fVIIa.Gla}$@NH1,NH2 :: Gla14$^{fXa.Gla}$@OE1,OE2 (SS-HB)
	Leu39$^{fVIIa.Gla}$ :: Phe31$^{fXa.Gla}$ (HI)	
	Leu39$^{fVIIa.Gla}$ :: Phe40$^{fXa.Gla}$ (HI)	
	Trp41$^{fVIIa.Gla}$ :: Met18$^{fXa.Gla}$ (HI)	
	Ile42$^{fVIIa.Gla}$ :: Met18$^{fXa.Gla}$ (HI)	
	Ile42$^{fVIIa.Gla}$ :: Phe40$^{fXa.Gla}$ (HI)	
	Ile42$^{fVIIa.Gla}$ :: Tyr44$^{fXa.Gla}$ (HI)	
	Gln150$^{fVIIa.LC}$@O :: **Lys134$^{fXa.SP}$**@NZ(MS-HB)	
	Arg152$^{fVIIa.LC}$@O :: **Lys134$^{fXa.SP}$**@NZ (MS-HB)	

	CHeB	Se
	Lys20$^{fVIIa.SP}$@NZ :: Tyr162$^{fXa.SP}$@O (MS-HB)	*Lys20*$^{fVIIa.SP}$ :: Glu159$^{fXa.SP}$ (Ionic interaction)
		Lys20$^{fVIIa.SP}$@NZ :: Glu159$^{fXa.SP}$@OE1 (MS-HB)
	Val21$^{fVIIa.SP}$ :: Pro161$^{fXa.SP}$ (HI)	
	Val21$^{fVIIa.SP}$ :: *Tyr185*$^{fXa.SP}$ (HI)	**Val21$^{fVIIa.SP}$ ::** *Tyr185*$^{fXa.SP}$ (HI)
		Glu26$^{fVIIa.SP}$@OE1 :: *Thr185B*$^{fXa.SP}$@OG1 (SS-HB)
		Asp72$^{fVIIa.SP}$ **:: Lys186$^{fXa.SP}$** (Ionic interaction)
	Glu75$^{fVIIa.SP}$ :: Lys223$^{fXa.SP}$ (Ionic interaction)	
		Leu145$^{fVIIa.SP}$ :: Ile137$^{fXa.SP}$ (HI)
		Leu145$^{fVIIa.SP}$ :: Met157$^{fXa.SP}$ (HI)
		Leu145$^{fVIIa.SP}$ :: Tyr207$^{fXa.SP}$ (HI)
		Asp146$^{fVIIa.SP}$ :: Arg202$^{fXa.SP}$ (Ionic interaction)
		Arg147$^{fVIIa.SP}$@NH1,NH2 :: Glu138$^{fXa.LC}$@OE1,OE2 (SS-HB)
		Arg147$^{fVIIa.SP}$ :: Glu138$^{fXa.LC}$ (Ionic interaction)
		Leu153$^{fVIIa.SP}$@N :: Gln20$^{fXa.SP}$@OE1 (MS-HB)
	Glu154$^{fVIIa.SP}$@OE1,OE2 :: *Tyr185*$^{fXa.SP}$@OH (SS-HB)	
	Glu154$^{fVIIa.SP}$ :: Lys186$^{fXa.SP}$ (Ionic interaction)	**Glu154$^{fVIIa.SP}$ :: Lys186$^{fXa.SP}$** (Ionic interaction)
	Glu154$^{fVIIa.SP}$@OE1 **:: Lys186$^{fXa.SP}$**@OH (SS-HB)	**Glu154$^{fVIIa.SP}$**@OE2 **:: Lys186$^{fXa.SP}$**@NZ (SS-HB)
	Lys170D$^{fVIIa.SP}$ :: Glu74$^{fXa.EGF-1}$ (Ionic interaction)	
	Lys170D$^{fVIIa.SP}$@NZ :: Glu74$^{fXa.EGF-1}$@OE1,OE2 (SS-HB)	
	Lys170D$^{fVIIa.SP}$ **:: Asp92$^{fXa.EGF-2}$** (Ionic interaction)	Arg170C$^{fVIIa.SP}$ **:: Asp92$^{fXa.EGF-2}$** (Ionic interaction)
	Lys170D$^{fVIIa.SP}$ **:: Asp95$^{fXa.EGF-2}$** (Ionic interaction)	Arg170C$^{fVIIa.SP}$ **:: Asp95$^{fXa.EGF-2}$** (Ionic interaction)
		Arg170C$^{fVIIa.SP}$@NH1,NH2 **:: Asp95$^{fXa.EGF-2}$**@OD1 (SS-HB)
		Tyr184$^{fVIIa.SP}$ **:: Lys134$^{fXa.SP}$** (Cation-Pi interaction)
		Asp186$^{fVIIa.SP}$ **:: Lys134$^{fXa.SP}$** (Ionic interaction)
		Asp186$^{fVIIa.SP}$@OD1 **:: Lys134$^{fXa.SP}$**@NZ (SS-HB)
	Ser188A$^{fVIIa.SP}$@OG :: Lys204$^{fXa.SP}$@N (MS-HB)	Ser188A$^{fVIIa.SP}$@O :: Arg202$^{fXa.SP}$@NH2 (MS-HB)