Dynamic character of the complex of human blood coagulation factor VIIa with the extracellular domain of human tissue factor: a normal mode analysis

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Abstract As an attempt to investigate the dynamic interactions between plasma serine protease, coagulation factor VIIa (VIIa) and its cofactor, tissue factor (TF), we performed normal mode analysis (NMA) of the complex of VIIa with soluble TF (the extracellular part of TF; sTF). We compared fluctuations of Cα atoms of VIIa or sTF derived from NMA in the VIIa-sTF complex with those of VIIa or sTF in an uncomplexed condition. The atomic fluctuations of the Cα atoms of sTF complexed with VIIa did not significantly differ from those of sTF without VIIa. In contrast, the atomic fluctuations of VIIa complexed with sTF were much smaller than those of VIIa without sTF. These results suggest that domain motions of VIIa molecule alone are markedly dampened in the VIIa-sTF complex and that the sTF molecule is relatively more rigid than the VIIa molecule. This may indicate functions of TF as a cofactor.

Key words: Domain motion; Factor VIIa; Tissue factor; Factor VIIa-tissue factor complex; Normal mode analysis

1. Introduction

Factor VII (VII) is a trypsin-like plasma serine protease that initiates extrinsic blood coagulation. When activated VII (VIIa) forms a complex with its cell surface receptor and cofactor, tissue factor (TF), in the presence of Ca^{2+} and phospholipids, the protease activity of VIIa towards its natural substrates, factors IX and X, is enhanced by a large magnitude. As a result of this process, the extrinsic coagulation cascade is triggered [1–4].

Zymogen VII is a single-chain enzyme precursor with a N-terminal γ-carboxyglutamic acid (Gla) domain (residues 1–39), followed by two epidermal growth factor (EGF) domains, EGF 1 (residues 50–81) and EGF 2 (residues 91–127), and a C-terminal serine protease domain (residues 153–406). Through the limited proteolysis of the Arg-152-Ile-153 peptide bond, zymogen VII is converted to a two-chain form, VIIa, bridged by a disulfide bond (Cys-135-Cys-262), which is composed of a light chain (residues 1–152) with Gla, EGF 1 and EGF 2 domains and a heavy chain serine protease domain (residues 153–406) [5]. However, the TF molecule consists of two immunoglobulin-like extracellular domains, a single membrane spanning region and a C-terminal cytoplasmic tail [6]. The four-domain structure of VIIa and the two-domain structure of TF form a tight complex (Fig. 1).

Despite that the crystal structure of the VIIa-soluble TF (sTF; part of the extracellular domains of TF) complex has been determined [7], the dynamic structure of the complex is not well understood. B-factors derived from X-ray crystallography usually indicate atomic fluctuations. However, it is difficult to identify the internal motion alone for the complex from the B-factors, since B-factors also exhibit external motions, such as lattice disorder. However, a normal mode analysis (NMA) allows for the isolation of only internal motions. In addition, functionally important motions, such as ligand-receptor binding motion [8], enzyme-inhibitor binding motion [9,10] and enzyme-substrate binding motion [11], are also detectable by calculating normal modes of low-frequency vibration. Studies on the dynamic character of the VIIa-sTF complex may lead to an understanding of how the activities of various plasma coagulation enzymes are enhanced by their cofactors. In the present study, a NMA on the complex of VIIa with sTF was performed. The dynamic structure of this molecular complex will be discussed.

2. Materials and methods

2.1. Protein initial coordinates

The original three-dimensional (3D) structure coordinates of human VIIa-sTF complex were obtained from the Brookhaven Protein Data Bank (PDB) [12], (PDB code 1DAN) [7]. Some modifications were made. First, the coordinates of two regions (residues 81–90 and 159–162) in human sTF were constructed via a CHIMERA modeling system [13] based on the X-ray structure of free sTF (PDB code 1BOY) [14], because coordinates of these regions were lost due to limited subtilisin digestion for crystallization [7,15]. Next, on the VIIa structure, as convergence of the fine optimization before NMA (see below) was increased, the Gla domain was modified by replacing all Gla residues with Gln and the calcium ions in the light chain were removed so that the pluses and minuses of electric charges cancel out. It is thought that the Gla domain is a flexible and unstable structure in the absence of calcium ions. This is because the Gla domain has strong electrostatic repulsion in the absence of calcium ions, as a result of two negatively charged carboxyl groups in each Gla residue. It seems that the above modification of the VIIa molecule has a negligible effect on the results. Based upon the assumption that the potential surface is quadratic, the NMA is performed near initial coordinates of the protein structure, which are derived from the VIIa-sTF complex crystal structure. Then, the coordinates for the C-terminal region of the VIIa light chain (residues 143–152) which

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Abbreviations: VII, coagulation factor VII; VIIa, activated coagulation factor VII; Gla, γ-carboxyglutamic acid; EGF, epidermal growth factor; TF, tissue factor; sTF, soluble tissue factor; NMA, normal mode analysis

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were not obtained from the X-ray structure (PDB code 1DAN) were not included in the calculations because of difficulty with homology modeling. Finally, these molecules were refined by energy minimization with an AMBER united-atom force field [16] using the program APRICOT [17]. The program PROCHECK [18] was used to evaluate the stereochemical quality of the model. And then, uncomplexed coordinates of VIIa and sTF were extracted from the modified VIIa-sTF complex coordinates.

2.2. NMA

Thermal fluctuations were carried out using the method described in a previous paper from our laboratory [19]. Before NMA, the above initial structures were finely optimized with a slightly modified force field of AMBER [16] and a threshold of 0.04 kcal/mol Å for the maximal component of atomic gradients. In our calculations, we assumed that the molecules were in vacuo, but a distance-dependent dielectric constant (r/Å) for electrostatic energies was maintained [20]. The electrostatic potential and the van der Waals potential were cut off at 9.0 Å and were switched smoothly and continuously to the value of zero at 10.0 Å. All the torsional angles around the rotatable bonds (except for the peptide C–N bond) were included as independent variables. The fluctuation of atoms was calculated by assuming a temperature of 300 K using normal modes and vibrational frequencies. For the calculation of complex, relative motions (translation and rotation) between constituent molecules were also included. In order to compare the atomic fluctuations of the single molecule with that of the constituent molecules in the complex within the same framework, we focused our attention on the internal fluctuation. If we define the fragmental target part of the system, arbitrary atomic displacements can be separated into internal and external components of that part: $\Delta r_i = \Delta r_i^{int} + \Delta r_i^{ext}$, where Δr_i^{int} contains displacement components which satisfy the local Eckart's condition [21] in the target part and Δr_i^{ext} contains external motions (translation and rotation) as a rigid body in the whole system. The internal fluctuation can be defined as the root mean square of the internal displacement Δr_i^{int}.

3. Results and discussion

3.1. Fluctuations of the VIIa-sTF complex

At first, normal modes were calculated for the VIIa-sTF complex. The number of modes obtained from the NMA was 2537. The lowest and highest frequencies were 3.57 cm^{-1} and 840.45 cm^{-1}. The range of the obtained modes corresponds with a vibrational timescale of 0.04–10 ps. The three lowest frequencies were 3.57, 4.39 and 5.44 cm^{-1}. Fig. 2 shows the fluctuations of Cα atoms obtained from the NMA and B-factors. The lower graph shows the fluctuation obtained from NMA, while the upper graph shows the fluctuation derived from B-factors obtained from X-ray crystallography. The two curves were similar, indicating the same peak pattern. There were some differences, which may be due to non-internal motions, such as lattice disorder. The N-terminal Gla domain of the VIIa light chain and the region of residues 80–90 in the sTF have considerable mobility, even in the VIIa-sTF complex. The former may actually tighten in the event of formation of a complex with TF on the cell surface. The latter is located near the VIIa protease domain, but was not in direct contact with the VIIa molecule, and the biological significance of this flexibility is not clear.

3.2. Comparison of uncomplexed sTF with complexed sTF fluctuations

We next calculated normal modes of uncomplexed sTF and compared them with those of the complexed form. The fluctuations of the complexed sTF with VIIa were extracted from the normal mode calculations of the VIIa-sTF complex in such a way that all external motions of the sTF molecule were eliminated by Eckart's condition [21]. The number of

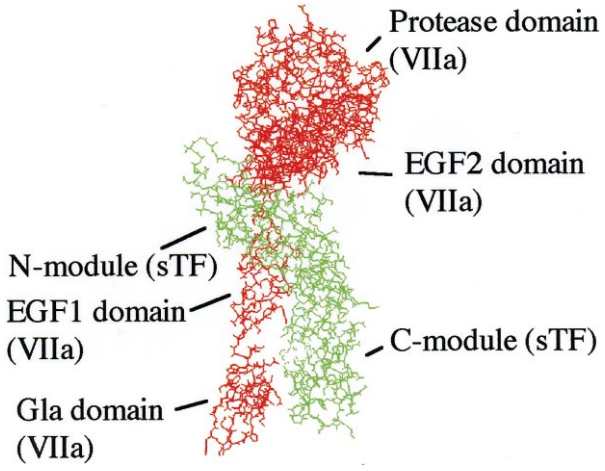

Fig. 1. Representation of the 3D structure of the modified VIIa-sTF complex (see Section 2). Red, VIIa; green, sTF.

modes obtained from the NMA of uncomplexed sTF was 879. The modes covered a frequency range of 7.39–840.45 cm^{-1}. The range of the obtained modes corresponds with a vibrational timescale of 0.04–5 ps. The three lowest frequencies were 7.39, 8.78 and 11.54 cm^{-1}. Fig. 3 shows the fluctuations of Cα atoms derived from the total motions obtained from NMA of uncomplexed sTF (broken line) and those of the total internal motions of sTF extracted from the NMA on the VIIa-sTF complex (continuous line). There was no large difference in the tendencies of the two fluctuations. Human TF is a type I integral membrane glycoprotein with 263 residues. TF has been classified as a member of the cytokine/hematopoietic growth factor receptor family, in which the extracellular domain contains two tandem seven β-strands, called N-module and C-module (residues: Thr-13–Thr-17, Lys-20–Glu-24, Gln-32–Thr-40, Lys-46–Lys-48, Glu-56–Asp-58, Tyr-71–Pro-79, Leu-93–Asn-96 in the N-module and Ile-113–Val-119, Lys-122–Val-127, Ile-152–Trp-158, Lys-166–Thr-170, Glu-174–Asp-178, Cys-186–Val-192 in the C-module; these regions have been identified by X-ray crystallography [7,14]). These strand regions corresponded to the minima

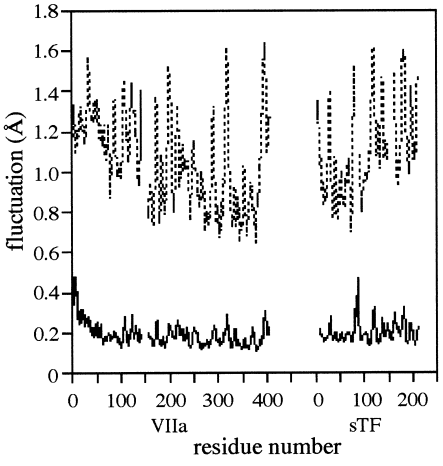

Fig. 2. Fluctuations of Cα atoms obtained from B-factors determined by X-ray crystallography (PDB code 1DAN, broken line) [7] and the calculated fluctuations of Cα atoms derived from the total motions of the NMA (continuous line).

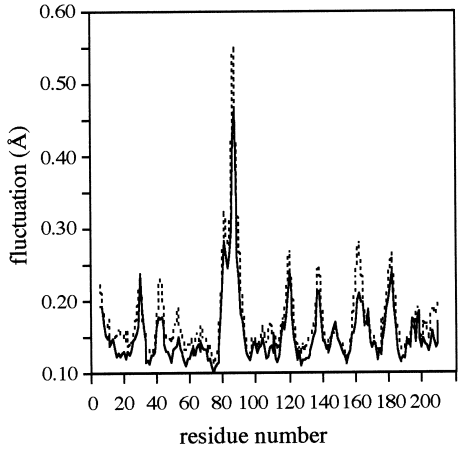

Fig. 3. Fluctuations of Cα atoms derived from the total motions of the NMA of the uncomplexed sTF (broken line) and those of the total internal motions of sTF extracted from the NMA of the VIIa-sTF complex (continuous line).

of the fluctuations of Cα atoms derived from each NMA. These β-strands interact with adjacent strands and form a scaffold. The peaks of fluctuations corresponded to the loop region which connected each strand. The regions where the fluctuations changed corresponded to the regions in contact with the VIIa molecule and were residues: Thr-17–Glu-24, Gln-37–Ser-39, Gly-43–Trp-45, Lys-48–Phe-50, Glu-56–Asp-58, Arg-74–Phe-76, Pro-92–Tyr-94, Gln-110, Arg-131–Leu-133, Phe-140, Trp-158, Ser-163–Lys-165, Cys-186 and Val-207–Cys-209 [7]. In these regions, the fluctuations of uncomplexed sTF became weak when sTF bound to VIIa. However, these changes in fluctuations in the case of uncomplexed or complexed sTF were smaller than in the case of VIIa (discussed below). Fig. 4 shows the atomic displacement vectors of uncomplexed sTF in the two lowest normal modes with frequencies 7.39 cm^{-1} (A) and 8.78 cm^{-1} (B). Hinge bending and twisting motions were found between N-module and C-module in these low-frequency modes. This finding is consistent with the information obtained from X-ray crystallography of the extracellular domain of rabbit TF [22]. These two motions corresponded with a vibrational timescale of approximately 4–5 ps.

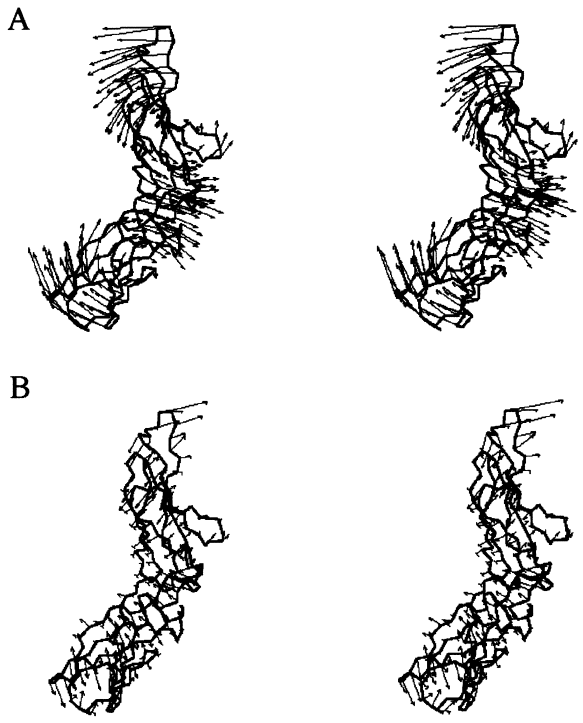

Fig. 4. Stereo drawing of atomic displacement vectors (magnified by a factor 1000) of uncomplexed sTF. (A) The lowest normal mode, with frequency 7.39 cm^{-1}, and (B) the second lowest normal mode, with frequency 8.78 cm^{-1}.

3.3. Comparison of uncomplexed VIIa with complexed VIIa fluctuations

Finally, we calculated normal modes of the uncomplexed VIIa and compared them with those of the complexed form by using Eckart's condition. Also, the internal fluctuations of the VIIa complexed with sTF were extracted from those of the VIIa-sTF complex. The number of modes obtained from the NMA of uncomplexed VIIa was 1652. The range of frequency modes obtained from the NMA was 0.98–941.82 cm^{-1}. This range corresponded to a vibrational timescale of 0.04–34 ps.

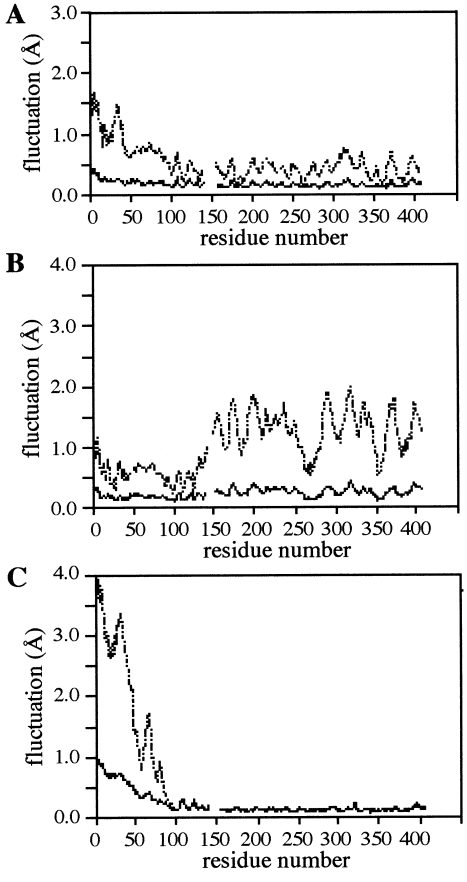

Fig. 5. Fluctuations of Cα atoms derived from the total motions of the NMA for the uncomplexed VIIa (broken line) and the complexed VIIa (continuous line). (A) The NMA was done in such a way that all external motions of the VIIa molecule were eliminated by Eckart's condition [21]. (B) In the case that the motion of the light chain was accompanied only by internal motion and that of the heavy chain involved purely external motion. (C) In the case that the motion of the heavy chain was accompanied only by internal motion and that of the light chain involved purely external motion.

The three lowest frequencies were 0.98, 1.15 and 1.72 cm^{-1}. Fig. 5 shows fluctuations of Cα atoms derived from the total motions of the NMA for uncomplexed VIIa (broken line) and those of complexed VIIa (continuous line). The fluctuations of Cα atoms in the free VIIa were much larger than those in the free sTF in Fig. 3, especially in the light chain region of VIIa. However, these fluctuations markedly diminished when the VIIa molecule was bound to sTF (Fig. 5A). Next, we focused our attention on the light chain of the VIIa molecule. The motions from each normal mode of complexed and uncomplexed VIIa were compared by shifting the center of gravity of the entire VIIa molecule to the center of gravity of only the light chain (Fig. 5B). That is, the pure external motions were projected out around the gravity center of the light chain. Owing to this conversion, the fluctuations of the light chain in the free VIIa were decreased. However, the fluctuations of the heavy chain in the uncomplexed VIIa increased in size. These results signify domain motions between the light chain and heavy chain. Next, we focused our attention on the heavy chain in a similar way (Fig. 5C). In this case, the fluctuations of the heavy chain and EGF 2 region (residues 91–127) in the uncomplexed VIIa were not different from those of the complex form and the fluctuations of only the light chain (except for EGF 2) in the uncomplexed VIIa increased in size. Surprisingly, when we focused on the light or heavy chain, we found the fluctuations in the complexed VIIa to be overwhelmingly smaller than those in the uncomplexed VIIa (Fig. 5B,C). These findings suggest that uncomplexed VIIa has domain motions between the light chain and the heavy chain and that these motions diminish when the VIIa molecule forms a complex with sTF. However, even in an uncomplexed state, the EGF 2 domain in the light chain merged with the protease domain. Also, we found that the three lowest frequencies contributed the most to the total fluctuation (Fig. 6). The frequencies of these modes were 0.98, 1.15 and 1.72 cm^{-1}. These low frequencies did not exist in the modes obtained from the complexed VIIa (in the case of the complex, the lowest frequency was 3.57 cm^{-1}). Fig. 7 shows the atomic displacement vectors of uncomplexed VIIa in the lowest normal modes with frequencies 0.98 cm^{-1} (A) and 1.72 cm^{-1} (B). It is easily understood from these vectors that there

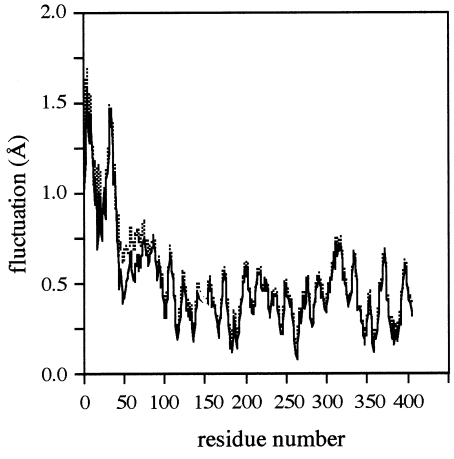

Fig. 6. The calculated fluctuation of the uncomplexed VIIa derived from the NMA. The broken line shows fluctuation of a sum of contributions from all frequency modes. The continuous line shows fluctuation of a sum of contributions from the modes with the three lowest frequencies.

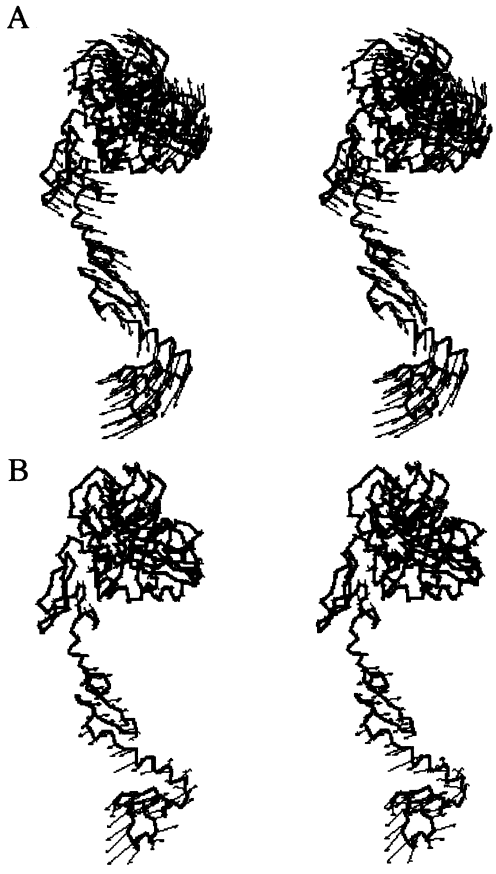

Fig. 7. Stereo drawing of atomic displacement vectors (magnified by a factor 1000) of uncomplexed VIIa in the first and third lowest normal modes with frequencies 0.98 cm^{-1} (A) and 1.72 cm^{-1} (B).

are domain motions between the Gla, EGF 1 and EGF 2 domain merged with the protease domain of uncomplexed VIIa. According to a previous report utilizing fluorescence anisotropy decay experiments [23], VIIa segmental motions are dampened when VIIa binds to sTF. Our calculations have confirmed this.

4. Conclusion

In conclusion, the fluctuations of the sTF molecule without its ligand, VIIa, were not much different from those of sTF in the complex with VIIa. Furthermore, the structure of sTF in the VIIa-sTF complex was very similar to that of uncomplexed sTF [7,14]. These findings suggest that the TF molecule is a rigid scaffold and behaves as if it is anchored to the cell surface when TF traps its ligand, the VIIa molecule. On the other hand, the fluctuations of uncomplexed VIIa were markedly diminished when the VIIa-sTF complex formed. Such diminished fluctuations were composed mainly of the three lowest frequency vibrational modes, which involved domain motions between the Gla, EGF 1 and EGF 2 domain merged with the protease domain of uncomplexed VIIa. These simulations support the suggestion that VIIa segmental motions were dampened when VIIa bound to sTF [23]. This fastening of the conformation of the enzyme VIIa by its cofactor TF is important for the recognition of the natural substrate factors IX and X. This is one of the functions of TF as a cofactor.

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