

The role of Ca²⁺ ions in the complex assembling of protein Z and Z-dependent protease inhibitor: A structure and dynamics investigation

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Abstract:

We investigated the solution structure and dynamics of the human anti-coagulation protein Z (PZ) in the complex with protein Z-dependent protease inhibitor (ZPI) to order to understand key structural changes in the presence and absence of Ca²⁺. Structural features of the complete complex of PZ-ZPI are poorly understood due to lack of complete atomic model of the PZ-ZPI complex. We have constructed a model of the complete PZ-ZPI complex and molecular dynamics (MD) simulation of the solvated PZ-ZPI complex with and without Ca²⁺ was achieved for 100ns. It is consider that the Ω-loop of GLA domains interacts with negatively charged biological membranes in the presence of Ca²⁺ ions. The PZ exerts its role as cofactor in a similar way. However, we used solvent-equilibrated dynamics to show structural features of the PZ-ZPI complex in the presence and the absence of Ca²⁺ ions. We observed that the distance between the interacting sites of the ZPI with the PZ and the GLA domain decreases in the presence of Ca²⁺ ions. Further, we postulated that the calculated distance between the dominant plane of the Ca²⁺ ions and Ser196 of the pseudo-catalytic triad of the PZ is similar to the equivalent distance of FXa. This suggests that the central role of the PZ in the blood coagulation may be to align the inhibitory site of the ZPI with the active site of the FXa, which is depends on the interaction of the calcium bound GLA domain of the PZ with the active membrane.

Key words: Protein Z, Protein Z-dependent protease inhibitor, Ca ion, Blood coagulation, Molecular Dynamics simulation

Background:

Protein (PZ) is a Vitamin K-dependent protein with 360 residues and a molecular weight of 62 KD, being homolog to the coagulation factors FVII, FIX, FX, and the PC. The PZ has significant differences to other serine proteases by lack of histidine and serine residues in the catalytic triad, causing loss of proteolytic function [1, 2]. PZ serves as a cofactor, mediating inhibition of the FX by the ZPI in the presence of Ca²⁺ ions and phospholipids [3]. In relation to other coagulation proteins, the PZ has 4 domains, including the GLA domain (residue 1- 46), the EGF1 (47- 83), the EGF2 (85-126), and the SP domain (135-360) with 11 disulfide bonds within and between the domains [1, 2]. The Vitamin K-Dependent (VKD) proteins contain 10-12

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γ- carboxy glutamic acid (Gla) residues in the GLA domain, consisting of three turns of alpha-helix that is conserved in other VKD dependent proteins. Additional feature of PZ is the existence of 13 Gla residues in the GLA domain, which is necessary to bind to Ca²⁺ and membrane. A significant section in the N-terminus of the GLA domain is the ω-loop including 1-11 residues responsible for the high-affinity binding to phospholipid membrane, and for membrane penetrating that is mediated by the so-called "keel" region of hydrophobic residues and dominant plane of Ca²⁺ ions [4-6]. The ω-loop contains two groups of hydrophobic and Gla residues with negative 2 charge. Previous data regarding PZ showed that binding of Ca²⁺ in the BHA make no structural effect, but

neutralize it due to lack of Ca^{2+} network in this domain in opposite another VKD proteins [7]. To date, there is no data about importance and effect of Ca^{2+} in the complex of PZZPI and cofactor role of PZ. ZPI is a serpin with 72 KD molecular weight and 423 amino acid residues. Inhibition of FX by ZPI is PZ-dependent and increases the rate of inhibition to 1000 folds in contrast to free ZPI. It is known that the ZPI inhibit FXIa and FIXa but didn't require PZ. Also it has been suggested that ZPI secretion, localization, clearance, even stabilization has been affected by PZ [3, 8, 9]. The ZPI inhibits factor X, while the FX inhibition takes place by anti-thrombin and the tissue factor pathway inhibitor (TFPI) [10, 11]. PZ binding to ZPI and factor X is accompanied by the SP domain and the GLA domain-calcium ions- on the same phospholipid surfaces, respectively.

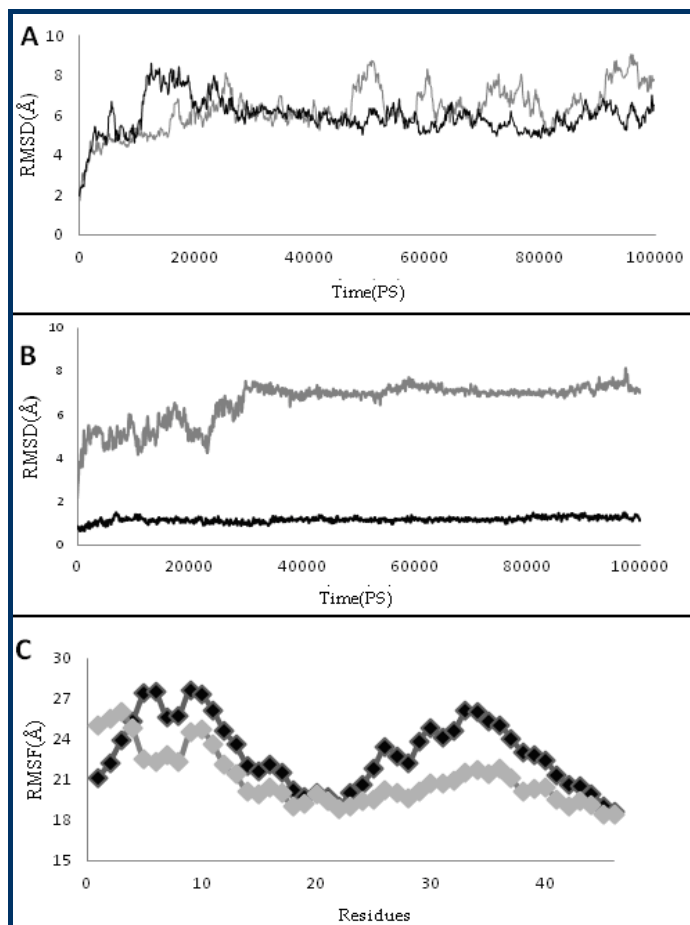


Figure 1: (A) the root mean square deviation (RMSDs) of the backbone atoms of protein Z and ZPI complex in the presence and absence of Ca^{2+} ions; (B) RMSD of GLA domain during 100 ns simulation in the presence and absence of Ca^{2+} ions; (C) Root mean square fluctuation (RMSF) of GLA domain in the presence and absence of Ca^{2+} ions. Gray curve exhibits the PZZPI without Ca^{2+} and Black curve is PZZPI with Ca^{2+} ion.

The Binding of the PZ and the FX decreases the rate of the FXa inhibition by anti-thrombin and enhances the inhibition by ZPI. In the other hand, the binding of the PZ to the ZPI increases inhibition of the FX and then reduces formation of the prothrombinase complex by delay the initiation and diminish generation of thrombin. These support the anticoagulant and critical role of PZ. So this is implied that PZ accelerate inhibition FX by 3 mechanisms, 1) bind and bring ZPI to the membrane for

binding to FX, 2) prevention of FX inhibition by another inhibitor like antithrombin and 3) prevention ZPI to inhibit another coagulation factor. So due to this important effect and role of PZ we prepared and investigated complete complex of PZZPI. The previous study on x-ray crystal structure of PZZPI complex is incomplete due to missing GLA domain [12, 13]. In present work we constructed full model of PZZPI complex and employed molecular dynamics (MD) simulations to investigate structural changes of GLA domain of PZ in the presence and absence of Ca^{2+} and relationship between GLA domain and binding site in ZPI. The results of over 100 ns of MD simulations suggest a new cofactor role for PZ. In addition homology modeling of PZ had been reported in free PZ not in the complex form. While it appears that all PZ are in bound form to ZPI in plasma [14]. Finally this study exhibit more details about structural feature and mechanisms to know more about important cofactor role of PZ.

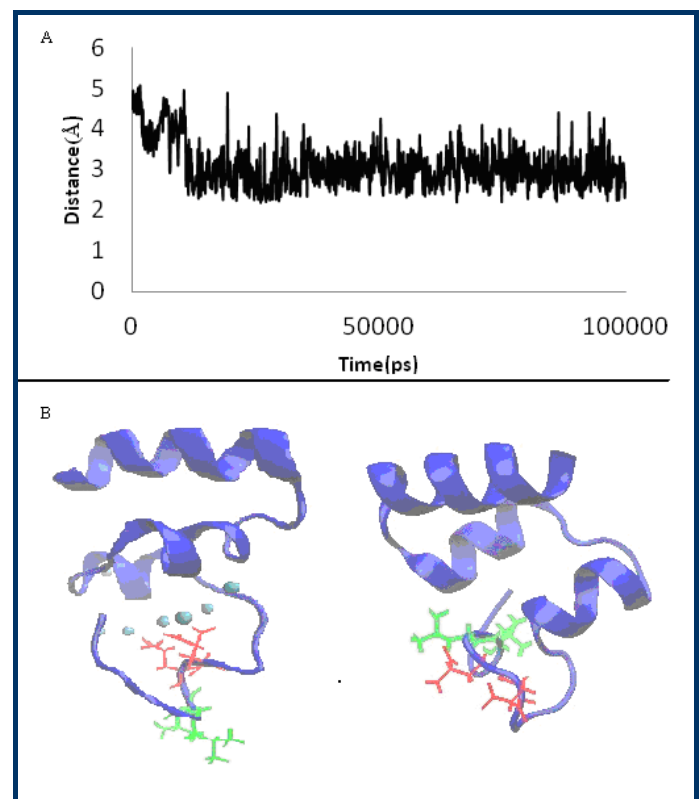


Figure 2: (A) Distance between ala1 (o atom) and cal364 during simulation; (B) Illustration of 3-d structure of gla domain (blue cartoon) shows orientation of gla 7, 8 (red residues) and leu 5, 6 (green residues) in the absence (right picture) and presence of Ca^{2+} ions (cyan sphere, left picture).

Methodology:

We add missing GLA domain of crystal structure PZZPI (3H5C.pdb with resolution 3.26Å) [12], by superimposing residues 49- 86 of full homology model PZ to same segment of crystal structure using visual molecular dynamic (VMD) (Figure 1C) [7, 15]. Then we prepared two system of complex with and without Ca^{2+} for MD simulation. Protein was solvated in a water box with 124, 133, 130Å dimensions. 12 Na^+ and 14 Cl^- ions neutralized system with Ca^{2+} ions but complex of PZZPI without Ca^{2+} neutralized by 23 Na^+ and 3 Cl^- due to lack of Ca^{2+} using VMD. We utilized cutoff of 12 Å for short-range

non-bonded interactions. The system with Ca^{2+} ions contains 11 Ca^{2+} ions, and 57204 water molecules (TIP3W), 183453 atoms. We initially performed energy minimization for two systems (with and without Ca^{2+}) in 10000 step in 20ps. All simulation was performed at NPT at 310k and a pressure of 1 atm and set 2fs for time step. The MD simulation was carried out for 100ns using NAMD Version 2.7 and Charmm 27 force field [16, 17].

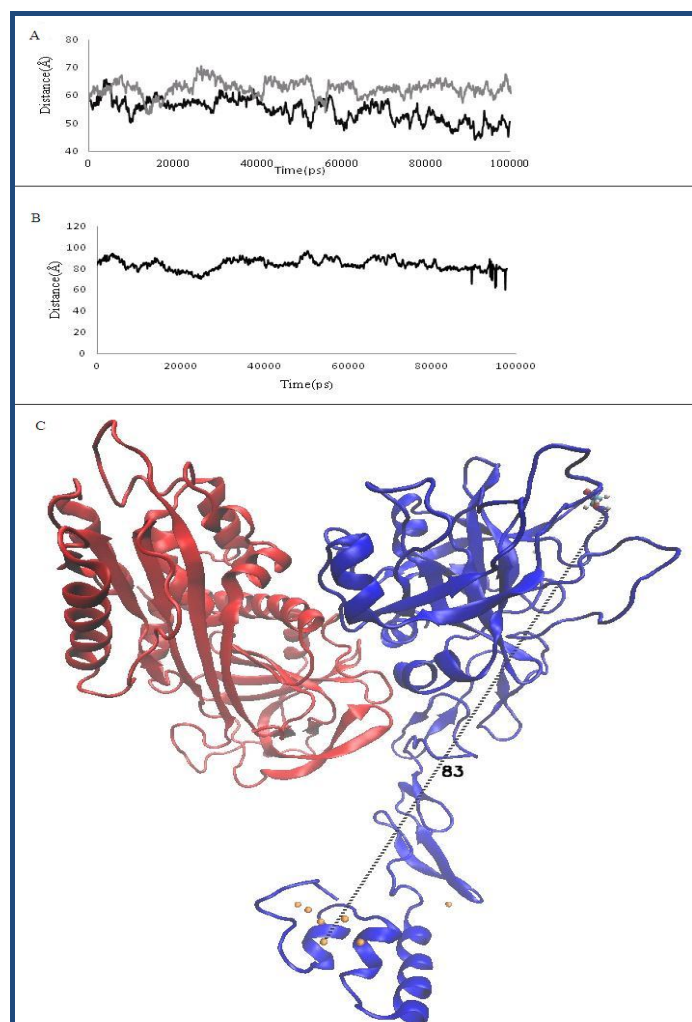


Figure 3: (A) Graph of Distance between center mass of Gla domain and ZPI binding residues. Gray curve is PZZPI without Ca^{2+} and Black curve is PZZPI with Ca^{2+} ion; (B) Plot of distance between Ser 196 to dominant plane of Ca^{2+} ions during simulation of 100ns which it has been calculated with VMD DisRg Plug-in; (C) The illustration shows distance between ser196 and center of mass of Ca^{2+} ions in the PZZPI complex. Protein Z and ZPI have been shown with blue and red color respectively and orange spheres show Ca^{2+} ions.

Discussion:

Prominent differences between Ca^{2+} -bound form and free form structure were established by following methods: **3.1 RMSD** Root mean square deviations (RMSD) of protein Z and Z-dependent protease inhibitor (PZZPI) complex in the presence and the absence of Ca^{2+} shows stability of two complexes. The RMSD ranges to 1 and 0.8 Å for pzzpica and pzzpi, respectively (Figure 1A). The RMSD of the GLA domain exposes in contrast to other domains of protein Z more differences in the presence

of Ca^{2+} (Figure 1B). The Ca^{2+} bound GLA domain attains 0.12 Å, but it makes 0.91 Å in Ca^{2+} free conformation. We postulate therefore that Ca^{2+} affects on the decreasing square deviation (SD) of the GLA domain and increases its stability. **3.2 RMSF:** Root mean square fluctuation (RMSF) plot of the GLA domain of two proteins exhibits different fluctuation curves, excluding the Disulfide Bridge containing regions 18 to 23, which causes rigidity in this region. We suggest further that the presence of Ca^{2+} ions increase the fluctuation curve of the PZZPI. Major fluctuation was observed for residues around the ω -loop. The bound form represents two distinct hot points at around 5, 6, 9, and 10 (Figure 1C). **3.3 Orientation of ω -loop residue** we observed changes in orientation of two hydrophobic and negative charge groups of ω -loop in the presence and in the absence of Ca^{2+} (Figure 2B). Gla residues placed toward Ca^{2+} line within ω -loop and hydrophobic residues like leu5,6 placed to solvent and more exposed area but Gla residues in the absence of Ca^{2+} become more exposed and impair cohesion of the hydrophobic residues. Data of solvent accessible surface area demonstrated these result which Gla 7 and 8 which are key residues in binding to Ca^{2+} have SASA in the absence more than presence of Ca^{2+} . The opposite way, leu5 and 6 have SASA in the presence of Ca^{2+} more than in its absence. The number of SASA differences for these residues is significant and this data is in agreement with Maria et al experimental results about factor X (Table 1) (see supplementary material) [18]. This imply that Ca^{2+} ions give a favorable conformation to ω -loop by making a powerful hydrophobic region anterior and trapping of GLA residues inside of ω -loop, which is necessary for membrane penetration.

3.4 Network of Ala1 The network of Ala1 plays critical role in the conformation of interior loop of the GLA domain due to involving in different H-bond to hydrophobic and Gla residues along with Ca^{2+} ions [19, 20]. We observed that in the presence of Ca^{2+} ions Ala1 keeps binding to Gla 21, 27, an Ile22, but it losses H-bond to the Gla 27 during simulation that has effect on the conformation of the ω -loop (data has not been shown). Further, Ala1 keeps the binding to Ca^{2+} (Figure 2A) and participates in dominant plane in the GLA domain and binding to residue of Ile 21 of first disulfide loop and Gla residues. This would underline the critical role of Ala1 in the ω -loop, and explains how the Ca ions impress the network of H-bond. **3.5 Distance between center mass of Gla domain to ZPI binding residues** Membrane binding of PZ causes the binding of ZPI to the membrane. The distance between the centre mass of the GLA domain and interaction region of ZPI with PZ show remarkable differences. This implies that Ca^{2+} may change the distance by effecting the conformation of the GLA domain (Figure 3A) [10]. The distance between the dominant planes of calcium ions in the GLA domain has been indicated in the active site of factors FX and FVII about 61 and 83 Å [21-24]. In the present study, we calculated the distance between the calcium ion plane and the Ser 196 residue in the pseudo active site of the PZ [25], which was estimated around 83 Å, as it was measured for FX (Figure 3B & 3C). Apparently, the PZ situates the ZPI in a good height to bind to the FX on the surface of membrane.

Conclusion:

Previous studies have been investigated the role of the Ca^{2+} in the coagulation factors FIX, FX, protein S, and FVII [18, 19, 26, 27]. Additionally, we prepared complete PZZPI complex in the presence and in the absence of Ca^{2+} to investigate different

structural features and elaborate the effect of Ca²⁺ ions on structural-functional properties of the mentioned complex. Our data revealed prominent difference between Ca²⁺ bound and Ca²⁺ free form structure. Binding of Ca²⁺ to the coagulation protein is a fundamental process for the membrane attaching of various blood coagulation factors. The GLA domain is a sufficient domain with Gla residues responsible for interacting to Ca²⁺ ions. It has been demonstrated that Ca²⁺ ions play two distinct roles in the biology of coagulation factors. They play a critical role in folding of the GLA domain and in protein anchoring due to direct contacting to lipids [25]. Here, we constructed the full complex of protein Z and protein Z-dependent protease inhibitor with Gla domain, and carried out a MD simulation for the mentioned complex in the presence and in the absence of Ca²⁺. Furthermore, we investigated the effect of Ca²⁺ ions on the PZZPI complex. The presence of Ca²⁺ is apparently essential to the optimal orientation of PZ and ZPI to place in an appropriate height to membrane. The presented model can be utilized for simulating the entire complex at the membrane surface and to study of membrane binding and the inhibitory effect on FX.

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Supplementary material:

Table1: solvent accessible surface area (SASA). SASA of ω -loop (residue 1 to 11 of GLA domain) has been evaluated in the presence and in the absence of Ca^{2+} .

Number	Residue	SASA- Ca	SASA+ Ca
1	ALA	59.47	37.14
2	GLY	51.82	49.39
3	SER	93.87	93.41
4	TYR	176.50	167.85
5	LEU	36.39	149.10
6	LEU	76.77	156.18
7	GLA	117	79.95
8	GLA	156.39	53.61
9	LEU	142.56	133.26
10	PHE	125.77	154.40
11	GLA	164.66	142.75