Archives of Advances
in Biosciences

Original Article:

In-Silico Investigation of PAI-1 Structural Changes in Active Forms of The Protein

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Cite this article as: Zaheriani, D., Eslanmi, H., Saeedian, S., & Haji-Allahverdipoor, K. (2024). In-Silico Investigation of PAI-1 Structural Changes in Active Forms of The Protein: In-Silico Analysis of Structural Alterations in Active Forms of PAI-1. *Archives of Advances in Biosciences*, *15*(1), 1–8. https://doi.org/10.22037/aab.v15i1.45593

https://journals.sbmu.ac.ir/aab/article/view/45593

Article info:
Received: 15 Jun 2024
Accepted: 24 Nov 2024
Published: 26 Nov 2024

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Abstract

Introduction: Plasminogen activator inhibitor-1 (PAI-1) is a protein that plays an important role in regulating the fibrinolytic system, which is responsible for breaking down blood clots. Dysregulation of PAI-1 has been implicated in various diseases, including thrombosis, atherosclerosis, and cancer. Hence, expanding our knowledge of the PAI-1 structure is important for developing new therapies for these diseases. In this study, we aimed to compare the active form of Neovison and Human PAI-1 structures to rationalize pharmaceutical inhibitors in future studies.

Materials and Methods: The Neovison PAI-1 protein model was generated using MODELLER, and the validity of the constructed model was confirmed through various validation servers. Subsequent analyses, including root mean square deviation (RMSD), root mean square fluctuation (RMSF), and solvent-accessible surface area (SASA), were performed over a 40 ns molecular dynamics (MD) simulation.

Results: The data analysis of MD simulations and superimposed structures revealed that the stability of the Neovison PAI-1 protein is slightly higher than that of the Human PAI-1 protein; however, this difference is negligible. These findings were corroborated by the average RMSD, RMSF, and SASA plot values.

Conclusion: These results suggest a new landscape for the design of inhibitors and enable a more accurate identification of the PAI-1 structure.

Keywords: Plasminogen activator inhibitor-1, Molecular Dynamic Simulation, Homology Modeling

1. Introduction

After vascular damage, platelets and proteins accumulate at the site of injury to form a stable blood clot that prevents excessive blood loss [1]. Blood coagulation is a crucial mechanism within the hemostatic system, involving complex interactions between proteases, enzymes, and cofactors leading to thrombin production and the formation of a fibrin-rich clot. To restore blood flow, it is necessary to break down this clot through the fibrinolytic system. This system is the primary mechanism for clot removal via the proteolytic action of plasmin on fibrin [2]. Fibrinolysis is initiated by the release of plasminogen activators, such as tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA), which convert the inactive proenzyme plasminogen into its active form, plasmin [3]. The well-known glycoprotein plasminogen activator inhibitor-1 (PAI-1) is a member

of the serine protease inhibitor (Serpin) family and acts as the main physiological inhibitor of tPA and uPA. Consequently, PAI-1 plays a crucial role in regulating the fibrinolytic system [4]. In addition to the role of anti-fibrinolysis, it also has a positive regulation in angiogenesis. Indeed, the up-regulation of PAI-1 leads to a variety of disorders, e.g., cardiovascular disease, cancer, aging, tissue fibrosis, and inflammation [5-7]. The expression and release of PAI-1 are strongly regulated by various factors, including growth factors, inflammatory cytokines, hormones, glucose, and endotoxins[8-9].

PAI-1 is a single-chain glycoprotein, approximately 50 kDa that is composed of 379-402 residues (3 β-sheets and 9 α-helices) [10-11]. Structural analysis showed that PAI-1 exists in three conformations: active, latent, and cleaved [12-14]. PAI-1 circulates in an active form in plasma and consists of two main domains: the first domain named reactive center loop (RCL) which is comprised of 26 amino acids from Ser 343 to Arg 368, acting as a protease (tPA and uPA) binding region, and exposed on the PAI-1 surface [14, 15]. When uPA binds and cleaves the P1–P1´site (Arg346–Met347) within the RCL, two structural changes occur: (1) the N-terminal portion of the RCL inserts into the central β-sheet A of PAI-1, ultimately forming s4A strand, and (2) uPA is translocated to the opposite pole of the PAI-1 molecule. Unlike the active form, the latent form is unable to interact with uPA because the N-terminal part of RCL is internalized in the β-sheet A without prior cleavage [14]. The next domain is the vitronectin binding site located in a region containing helices hD, hE, and hF, collectively known as the flexible joint region (residue 13 to 147). The flexible joint region of PAI-1 under physiological conditions (in plasma and the extracellular matrix) binds to the somatomedin domain of vitronectin with high affinity; thereby this interaction causes a) stabilizes PAI-1, b) delaying the forming latent conformation, c) amplifying its anti-uPA ability [12,16-18].

Detailed structural insight into the PAI-1 protein is essential for the development of therapeutic targets to optimally design inhibitors against active PAI-1. The optimal binding site for an inhibitor may vary across different orthologs, necessitating precise structural knowledge to ensure effective inhibition [19]. The conformational plasticity of native PAI-1 makes it more difficult to identify and develop modulators of PAI-1 activity. Moreover, no PAI-1 inhibitors have been approved to date for therapeutic use in humans [20]. Therefore, obtaining more information about the structural variations of this protein in different animal models is valuable for designing effective PAI-1 inhibitors to manage diseases associated with its dysregulation. Additionally, it is crucial to compare the impact of amino acid changes in the PAI-1 structure across different orthologs, including both human and animal models. This comparison, facilitated by bioinformatics tools, ensures that the protein retains its activity under physiological conditions. In this study, the structural changes of active PAI-1 in the animal model Neovison (American mink) have been investigated in comparison with the those of the human model by bioinformatics tools to advance the rational design of pharmaceutical inhibitors in future studies.

2. Materials and Methods

Protein sequence alignment

To compare the Neovison PAI-1 and Human PAI-1 protein sequences, pairwise sequence alignment was performed utilizing Clustal Omega: https://www.ebi.ac.uk/Tools/msa/clustalo/.

Protein Structure Prediction

The structural model of wild-type Neovison PAI-1 was generated using MODELLER 9V15, with human PAI-1 X-ray crystal structures (PDB entry 1DVM) serving as a template. Following model completion, the model with the lowest discrete optimized energy (DOPE score) was selected from among 10,000 calculated models for the active form of Neovison PAI-1 protein. Subsequently, the PAI-1 model was successfully submitted to the Protein Model Database (PMDB) accessible at :

http:// srv00.recas.ba.infn.it/PMDB/.

Molecular Dynamics Simulation

Molecular dynamics simulations of the active forms of Neovison PAI-1 and Human PAI-1 were conducted using Gromacs 5.0.2 software, employing the Amber ff99SB-LIDN force field. Each initial structure was centered in a cubic box with a 1.0 nm distance from the box edge. The box was subsequently filled with the appropriate number of TIP3P water molecules. To ensure electrical neutrality, counter-ions were added by replacing water molecules using the genion tool available in the Gromacs package [21]. Energy minimization was conducted utilizing the steepest descent algorithm. The energy-minimized system underwent equilibration through position-restrained simulations under the canonical ensemble (NVT) and subsequently the isothermal isobaric ensemble (NPT) for 500 and 1000 ps, respectively. Initial velocities were generated from a Maxwell-Boltzmann distribution at 300 K. Productive unrestrained MD

simulations were performed in the NPT ensemble for 40 ns, with bond lengths constrained using the LINCS algorithm. Integration of the equation of motion was achieved using the leapfrog algorithm with a 2-fs time step. Conditions of constant pressure (1 bar) and temperature (300 K) were maintained via the Parinello-Rahman barostat and Nosé-Hoover thermostat, respectively. The particle mesh Ewald (PME) method was employed to calculate long-range electrostatic interactions. Analysis of MD simulation trajectories was conducted using Gromacs 5.0.2 utilities such as gmx rms, gmx rmsf, and gmx sasa [22-23].

Results

Sequence and structural analysis

The amino acid sequence homology between human PAI-1 and Neovison PAI-1 is 84.96% (identical residue: 322aa) [Figure 1]. The superimpose comparison of these two structures indicates that in some regions (for instance: Arg101- Phe123) the Neovison PAI-1 structure is more rigid and has less motion than human PAI-1 [Figure 2].

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PAI1_HUMAN      VHHPPSYVAHLASDFGVRVFQQVAQASKDRNVVFSPYGVASVLAMLQLTTGGETQQQIQA   60
PAI1_NEOVI      SYLHETRAAELATDFGVKVFKQVAQASKDRNMVFSPYGLASVLAMLQLTTAGETRQQIQE   60
                 :   : .*.**:****:**:**********:******:**********.***:****

PAI1_HUMAN      AMGFKIDDKGMAPALRHLYKELMGPWNKDEISTTDAIFVQRDLKLVQGFMPHFFRLFRST   120
PAI1_NEOVI      AMRFQIDEKGMAPALRQLYKELMGPWNKDEISTADAIFVQRDLKLVHGFMPYFFRLFQTT   120
                ** *:**:********:***************:***********:****:*****::*

PAI1_HUMAN      VKQVDFSEVERARFIINDWVKTHTKGMISNLLGKGAVDQLTRLVLVNALYFNGQWKTPFP   180
PAI1_NEOVI      VKQVDFSEVERARFIINDWVKRHTKGMIGDLLGRGTVDQLTRLMLVNALYFNGQWKTPFP   180
                ******************** ******.:***:*:*******:*****************

PAI1_HUMAN      DSSTHRRLFHKSDGSTVSVPMMAQTNKFNYTEFTTPDGHYYDILELPYHGDTLSMFIAAP   240
PAI1_NEOVI      KSGTHHRLFHKSDGSTVSVPMMAQTNKFNYTEFSTPEGRYYDILELPYHGDTLSMFIAAP   240
                .*.**:****************************.**:*:**********************

PAI1_HUMAN      YEKEVPLSALTNILSAQLISHWKGNMTRLPRLLVLPKFSLETEVDLRKPLENLGMTDMFR   300
PAI1_NEOVI      YEKDVPLSALTNILDAQLISQWKGNMTRRLRLLVLPKFSLESEVNLRGPLENLGMTDMFR   300
                ***:**********.*****:*******  **********:**:** ***********

PAI1_HUMAN      QFQADFTSLSDQEPLHVAQALQKVKIEVNESGTVASSSTAVIVSARMAPEEIIMDRPFLF   360
PAI1_NEOVI      PNQADFSSLSDQEALYVSQALQKVKIEVNESGTVASSSTAIIVSARMAPEEIIMDRPFLF   360
                  ****:****** *:*:**********************.**********************

PAI1_HUMAN      VVRHNPTGTVLFMGQVMEP     379
PAI1_NEOVI      VVRHNPTGTVLFMGQVMEP     379
                ******************
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Figure 1. Amino acid sequence alignment of Neovison and human PAI-1.

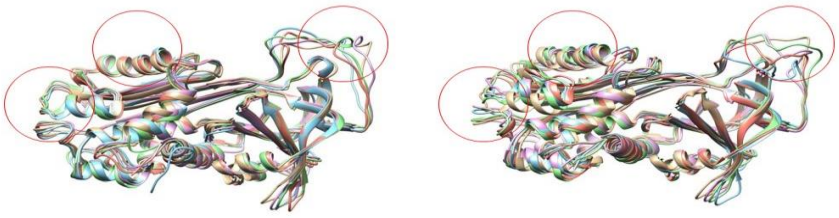


Figure 2. The superimposition of Neovison PAI-1 (a) and Human PAI-1 (b) structures during the 40 ns MD simulation indicates differences in motion, as highlighted by red circles. Notably, the protein-modeled Neovison PAI-1 exhibits less mobility compared to Human PAI-1.

Protein Modeling

Since there is no experimentally solved 3D structure for Neovison PAI-1, the structure was modeled based on the x-ray structure of Human PAI-1 through homology modeling and using the program MODELLER. Finally, it was illustrated in Chimera software [Figure 3].

Molecular Dynamics Simulation

Root Mean Square Deviation (RMSD)

To evaluate the stability of the protein modeled Neovison PAI-1 in comparison with the Human PAI-1, RMSDs of the Cα atoms after 40 ns simulations were calculated. The RMSD of all Cα atoms was evaluated to study the convergence of protein structure through the simulations. The average and standard deviation of RMSD values for both proteins have been presented in Table 1. Accordingly, we could find out that Neovison PAI-1 is more stable in comparison with Human PAI-1. Therefore, the RMSD plot of these proteins has been shown in [Figure 4a].

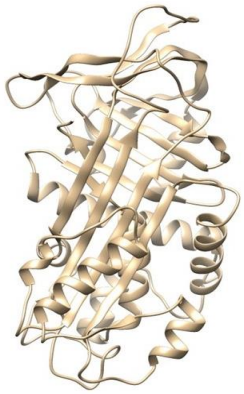
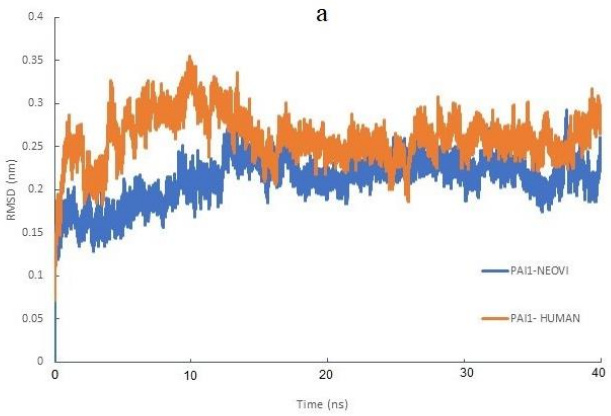


Figure 3. Model of active Neovison PAI-1.



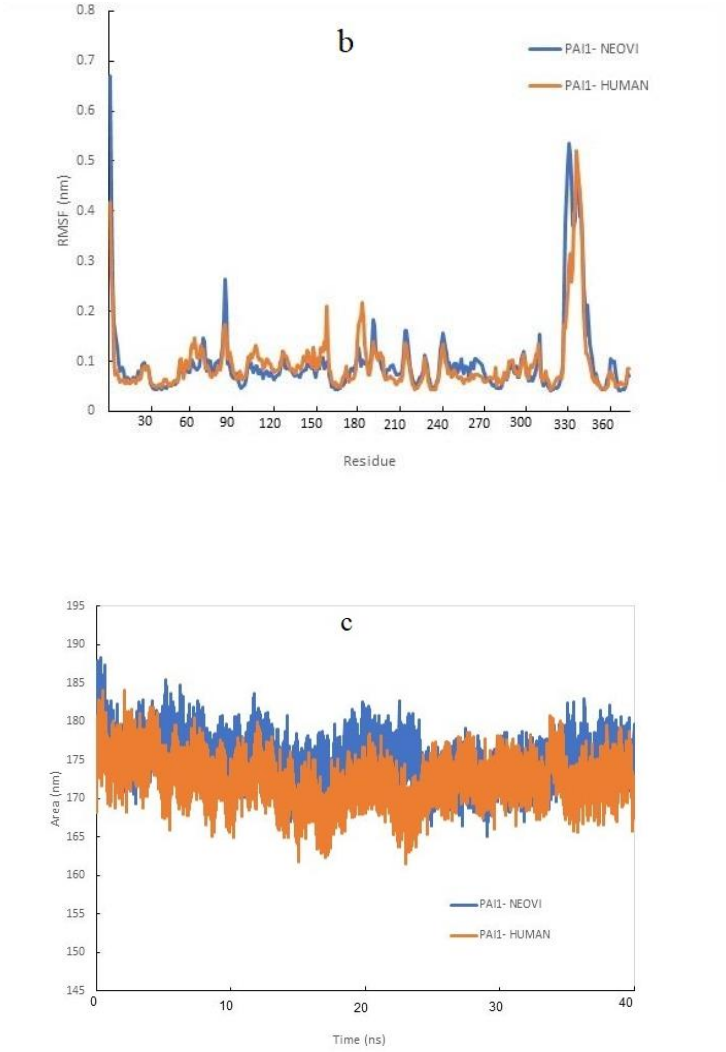


Figure4. RMSD, RMSF, and SASA plots are presented. The results for Neovison PAI-1 are depicted in blue, while those for Human PAI-1 are illustrated in orange. a) The plots of Cα RMSD, b) The plots of RMSF, and c) the Plots of SASA.

Table 1. Summarized parameters of 40 ns molecular dynamics simulations at 300 K

protein	RMSD*(nm)	RMSf†(nm)	SASA§(nm2)
Human PAI-1	0.260 ± 0.028	0.093 ± 0.061	172.323 ± 2.873
Neovison PAI-1	0.211 ± 0.025	0.097 ± 0.079	175.219 ± 2.814

* Root mean square deviation
† Root mean square fluctuation
§ Solvent accessible surface area

Root Mean Square Fluctuation (RMSF)

One of the important indicators to determine the general fluctuations of the protein structure is the RMSF index. Comparing the diagrams, the average and standard deviation of RMSF values between these proteins indicated that both of them have the same fluctuation at simulation conditions except for some regions which were illustrated in superimposition structure. [Figure 4b; Table 1]. The C-terminal of PAI-1 contains RCL and is highly flexible in its interaction with t-pa and uPA protease.

Solvent Accessible Surface Area (SASA)

The compactness of the hydrophobic core of protein was analyzed by the solvent-accessible surface area (SASA). The average values of SASA indicated that despite more motion of human PAI-1 in comparison with Neovison PAI-1, packaging and core formation in human PAI-1 were conducted a bit better. [Table 1] The profile of SASA of these protein structures is illustrated [Figure 4c)].

4. Discussion

Once protease binds to the RCL, the protease/PAI-1 complex undergoes a huge conformational change [24]. This structural flexibility makes targeting PAI-1 for the therapeutic effect of its inhibition challenging [25]. In a study, existing five inhibitors were evaluated for their toxicity, binding affinity, and bioactivities using an in-silico methodology. Five non-toxic inhibitors were identified, with N-acetyl-D-glucosamine demonstrating notable binding affinity with PAI-1 [26]. Nevertheless, none of the inhibitors currently have received approval for therapeutic application in humans, primarily because of concerns related to selectivity and toxicity [20, 27]. The purpose of this study was to answer these questions: (1) how much is the structure fluctuation of PAI-1 in other organisms? (this study: Neovison); and (2) Do these fluctuations and or structural differences affect protein activity? In this study, therefore, the conformational changes of PAI-1 protein in humans and Neovison were performed under computational simulation conditions, to evaluate the structural tolerance level of this protein. Our results show that despite some amino acid substitution (Figure 1), human and Neovison structures are very similar and the molecular dynamics simulation parameters showed only small differences. In addition, if the simulation continues after 40 nanoseconds, these parameters will become more and more close to each other. The pairwise rms deviation between these two structures is 0.049 nm, indicating these structures are highly similar. Elsewhere, RMSD data of three structures of mutants of human PAI-1 in the active conformation (PDB entry 1B3K, 1DB2, 1DVM) showed high similarity [15, 28, 29]. Less mobility in some regions of Neovison PAI-1 than Human PAI-1 indicated that, the rigidity causes more stability but it appears that rigidity is not necessary for function, and despite the high fluctuation in human PAI-1, meanwhile, PAI-1 is still active. According to the RMSF data, the greatest differences in protein fluctuation and movement were in the areas of amino acids 334, 335 (RCL region), 185, 159, and 85 with a difference of 0.255, 0.243, 0.115, 0.111 and 0.090 nm, respectively (Figure 4b), which indicates a higher level of tolerance for changes in these areas compared to the areas where the fluctuation difference is less, so in the areas where the fluctuation difference is more, it is a suitable candidate for protein engineering. In this respect, binding small molecule TM5484 to the flexible joint region in PAI-1 might restrict the structural flexibility of this region which is typically necessary for the proper functioning PAI-1. In addition, the observed mechanism further indicates that TM5484 could potentially reduce the functional half-life of PAI-1 [30].

According to these results, we found that PAI-1 can remain active in a wide range of fluctuations and Neovison PAI-1 preserved biochemical activity. On the contrary, structural differences between human and mouse PAI-1 caused biochemical differences in DeWilde et al.'s study [19]. Higher flexibility in the gate region of mouse PAI-1 compared to human PAI-1 made it more stable; the mutations created in this region significantly increased the half-life of human PAI-1 compared to mouse PAI-1 [19].

5. Conclusion

Overall, this study showed that the molecular dynamics simulation parameters of PAI-1 did not change significantly and we predicted that the biochemical properties are preserved. In addition, more accurate identification of PAI-1 structure will help us to design new and effective inhibitors. Hence, structure evaluation of other creatures such as Pig, Rat, and Bovin is recommended.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

Funding

The paper was extracted from the MSc thesis from the Department of Biology, Payame-Noor University.

Author's contributions

Davood Zaheriani: Writing-Original draft preparation. Shahriar Saeedian and Habib Eslami: Reviewing and Editing.
Kaveh Haji-Allahverdipoor: Conceptualization, Methodology, Software, Visualization, Supervision.

Conflict of interest

Authors declare no competing interests.

Acknowledgments

We sincerely thank Koosha Rokhzadi for his invaluable technical assistance throughout the Molecular Dynamics Simulation process, which greatly contributed to the success of this work.

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