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Conformation Prediction of Human Plasmin and Docking on Gold Nanoparticle

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***Abstract*—**Plasmin plays an important role in the human circulatory system owing to its catalytic ability of fibrinolysis. The immediate injection of plasmin in patients of strokes has intrigued many scientists to design vectors that can transport plasmin to the desired location in human body. Here we predict the structure of human plasmin and investigate the interaction of plasmin with the gold-nanoparticle.

Because the crystal structure of plasminogen has been solved, we deleted N-terminal domain (Pan-apple domain) of plasminogen and generate a mimic of the active form of this enzyme (plasmin). We conducted a simulated annealing process on plasmin and discovered a very large conformation occurs. Kringle domains 1, 4 and 5 had been observed to leave its original location relative to the main body of the enzyme and the original doughnut shape of this enzyme has been transformed to a V-shaped by opening its two arms. This observation of conformational change is consistent with the experimental results of neutron scattering and centrifugation.

We subsequently docked the plasmin on the simulated gold surface to predict their interaction. The V-shaped plasmin could utilize its Kringle domain and catalytic domain to contact the gold surface.

Our findings not only reveal the flexibility of plasmin structure but also provide a guide for the design of a plasmin-gold nanoparticle.

***Keywords*—**docking, gold nanoparticle, molecular simulation, plasmin.

I. Introduction

PLASMIN is important in human disease such as heart disease, stroke, bleeding and cancer since plasmin is involved in the fibrinolysis. Plasmin is derived from plasminogen (a zymogen). Plasminogen is composed of seven domains: PAp, (Pan-apple), Kr1 (kringle), Kr2, Kr3, Kr4, Kr5, SP (serine protease). Plasminogen contains 810 amino acids. After plasmin cleavage in K77-K78 and R580-V581, Lys-plasmin becomes active. Based on small-angle neutron scattering, the gyration of radius of plasminogen is about 39 angstrom, while the gyration of radius of Lys-plasmin is about 51 angstrom. The shape of plasminogen is like an ellipsoid and that of Lys-plasmin is like a Debye coil [1]. Crystal structure of plasminogen has been solved independently by two research groups [2], [3] and revealed that the shape of plasminogen is like a doughnut. The packing of domains suggested that the ligand-binding site of KR1 could direct proenzyme recruitment to targets and KR5 detaching from the PAp domain could induce the plasminogen conformational change.

The purpose of this study is to predict the conformation of plasmin and the docking mode of plasmin on the gold nanoparticle.

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II. Methods

A. Molecular Dynamics Simulations

Crystal structure of plasminogen was downloaded from protein data bank (pdb id: 4DUR) [2]. To generate Lys-plasmin, PAp domain was deleted from plasminogen and the coordinates of Lys-plasmin was resulted. Lys-plasmin was put inside a cubic simulation box of dimension (146 Å X 146 Å X 146 Å) including SPC/E water molecules. pH7 was assumed to assign the charge states of the charged residues. Besides four chloride ions, five chloride ions were added into the simulation box to obtain a simulation box of the neutral charge.

The simulations were performed with the Gromacs 5.0 and all atom OPLS force field were used for the protein [4]. The valence bonds were constrained with the LINCS algorithm. First, the system was minimized by using the steepest descents. Subsequent equilibration was conducted in two phases. The first phase was conducted under an NVT ensemble for 100ps (where Number of particles, Volume, and Temperature are constant; isothermal-isochoric" or "canonical) and the second NPT ensemble for 100ps (where the Number of particles, Pressure, and Temperature are constant; isothermal-isobaric" ensemble) (http://www.bevanlab.biochem.vt.edu/Pages/Personal/justin/gmx-tutorials/lysozyme/index.html). To simulate the conformation of plasmin, we conducted an annealing simulation protocol using NPT ensemble for 110 ns as follows [5]: (i) protein and solvent were first coupled to 0 K at 0 ps, the reference temperature was then increased linearly to reach (ii) 300 K after 1 ns (iii) the maximum temperature of 500 K at 2 ns. The temperature (iv) was kept fixed at 500 K until 102 ns and then (v) decreased to 300 K at 103 ns. After this, the temperature (vi) was kept constant at 300 K until the end of the 110 ns simulation. Periodic boundary conditions and the Particle-Mesh-Ewald algorithm for electrostatic force were employed. The integration for 2 fs time step was used. Pressure coupling employs Parrinello-Rahman method. The trajectory was saved for every 50 ps.

B. Plasmin-Nanogold Interaction by Brownian Dynamics Simulations

Docking of nanogold and protein were implemented before [5]-[7]. We follow the protocol suggested by [5] as follows. We

employed the rigid-body docking simulations by using Brownian dynamics (BD) techniques. Implicit-solvent ProMetCS force field parametrized for protein gold surface interactions were used. The calculations were performed using the SDA version 7 software [8], [9]. A gold surface of 400 Å X 400 Å with three atomic layers was constructed to simulate the gold-nanostructure. For nonspecific screening effect on the electrostatic potential of the protein, the salt concentration of 15 mM was included using the APBS 1.4. We put plasmin at a distance 120 Å from the gold surface and 7,000 BD trajectories were computed.

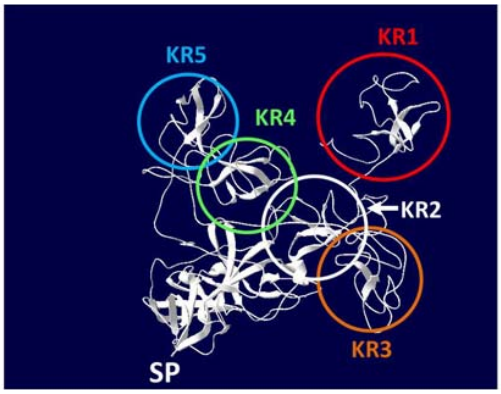
III. Results

A. The Prediction of Plasmin Structure through the Simulated Annealing

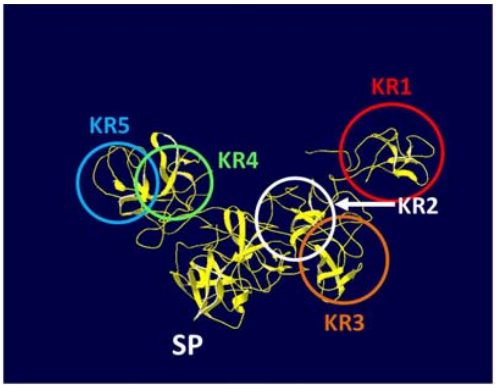
Plasminogen is composed of seven doamains (PAp, Kr1, Kr2, Kr3, Kr4, Kr5, SP) [2]. Deletion of the N-terminal domain (PAp) and the peptide cutting on the C-terminal trypsin-like domain was shown to possess the enzymatic activity (plasmin). Therefore we deleted the N-terminal domain to investigate its conformation change. Since plasminogen is in the circular shape (doughnut), the deleted form of plasminogen (plasmin) still showed the same shape but with a small missing trunk. Because the plasmin conformation was reported to transform from ellipsoid form to Debye coil form [1], we decided to engage plasmin to molecular dynamics (MD) to search for its possible conformation.

After MD of 300K (100 ns) plasmin does not change conformation in a big scale. Plasmin still retains its overall shape except some minor rotation of the two domains (data not shown). We decided to subject plasmin to simulated annealing (300K-500K-300K) and explore the possible conformation of plasmin (plasmin-500K) (Figs. 1, 2). Plasmin-500K has adopted a totally different shape by transforming from a doughnut form to a high-angle V-shape form. The angle of the V is about 100 degrees and plasmin was observed to open two arms. The left arm was formed by domain Kr4 and Kr5 and the right arm by Kr1, respectively. By comparing the structure of plasmin before and after the simulated annealing, we found that most of the conformation change comes from two processes: dissociation and expanding. Kr1, Kr4 and Kr5 were originally next to N-terminal domain. After the deletion of N-terminal domain and simulated annealing, Kr1, Kr4 and Kr5 dissociated from the original relative location. After the dissociation, Kr4 and Kr5 lost the contacts with SP; Kr1 moves away from Sp. But Kr2 and Kr3 still retained its contact with SP. Owing to the spreading out of Kr1, Kr4 and Kr5, the overall conformation of plasmin become a V-shape coil. Several important contacts are listed as follows:

a. In the original plasminogen, D413 and D411 (KR4) interact with R70 and R68 (PAp). D534, D518 and D516 (KR5) interact with K50 (PAp). After the deletion of PAp, these interactions disappear.
b. E350 and L351 (KR3-KR4 linker) interacts with K378, T369, T352, T372 and S371 (KR4) in the original plasminogen. But after MD, these interactions disappear since KR4 had been far away from KR3-KR4 linker.
c. The contact of V355 (KR3-KR4 linker) and E554 (activation loop) still exists after MD since the relative positions between KR3-KR4 linker and the activation loops still remains after MD.
d. E708 (SP) interacts with W235, D219 and E221 (KR2) after MD since the relative position between SP and KR2 remains after MD.



(a)



(b)

Fig. 1 Structure of the plasmin before (a) and after (b) the simulated annealing (500°K) for 100 ns. Domains are indicated as the circled region

The secondary structure does not differ before and after the spreading of two arms. The majority of secondary structures are composed of beta-strand and beta-sheets. The number of amino acids with the beta structure does not change after the simulated annealing, indicating no unfolding process is undergone. But it was worth noting that large amount of “sharp” turn emerged (from 15% to 24%) after the simulated annealing.

Furthermore, residues in KR1, KR4 and KR5 show the apparent increase in the solvent accessibility. For example, the contact between P248 and L160/H168 disappeared after the simulated annealing, resulting into the large increase of the solvent accessibility (48 $Å^2$).

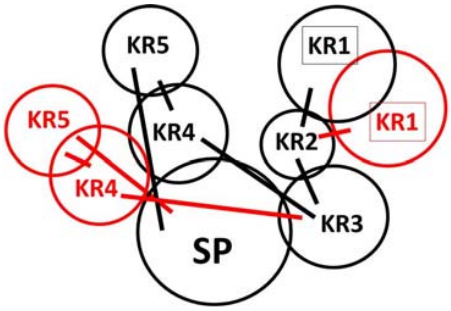


Fig. 2 Description of the conformational change of plasmin after the simulated annealing (500°K) for 100 ns. Domain colored in red indicates the domain after the simulated annealing.

B. Docking of Plasmin on the Gold Surface

About 70% of the docking structures are represented by six representative structures (dubbed complex 1~6). There are three major binding modes: gold surface contacted by either KR1/KR3, or KR5, or SP domain. Both electrostatic and Lennard-Jones interactions are important in determining the binding modes of the plasmin on the gold surface (Tables I, II).

Lennard-Jones interaction and electrostatic interaction are both the highest in the complex #1 among the six complexes. E151 (DR1), E302 (KR3), E490 (KR5), R504 (KR5), R582 (SP), R610 (SP) are the important residues responsible for the electrostatic interaction between plasmin and gold slab. Pi electron from F583 (SP) is important for the stacking of plasmin on the gold slab (Figs. 3-5).

TABLE I
TOTAL ENERGY AND THE CONTACT RESIDUES FOR THE COMPLEXES FROM THE RIGID-BODY BROWNIAN DYNAMICS DOCKING OF PLASMIN TO AN AU (111) SURFACE

Complex	Total interaction energy of the complex (in kT)	Relative Population (%)	contactdomain	contact residues
1	-45.23	20.92	KR1, KR3	E151,Q278,H279,S281,E302,T319
2	-21.44	0.13	KR3	T276,Q278,E302,T319
3	-14.28	18.14	SP	R582,F583,M585,R610
4	-9.60	10.88	KR5	E490,P503,R504
5	-9.45	0.02	KR3	T276,Q278,T319,S321,Q322
6	-9.33	21.52	KR5	E490,P503,R504

TABLE II
INTERACTION ENERGY FOR THE COMPLEXES FROM RIGID-BODY BROWNIAN DYNAMICS DOCKING OF PLASMIN TO AN AU (111) SURFACE

Complex	electrostatic interaction energy between plasmin and gold (in kT)	electrostatic desolvation energy of plasmin (in kT)	hydrophobic desolvation energy of plasmin (in kT)	Lennard-Jones interaction energy between plasmin and gold (kT)
1	-40.25	22.72	-5.47	-22.23
2	-38.26	22.37	-4.74	-0.82
3	-29.05	19.76	-2.97	-2.01
4	-10.89	8.57	-2.81	-4.48
5	-12.64	13.78	-4.12	-6.47
6	-8.66	8.38	-2.85	-6.20

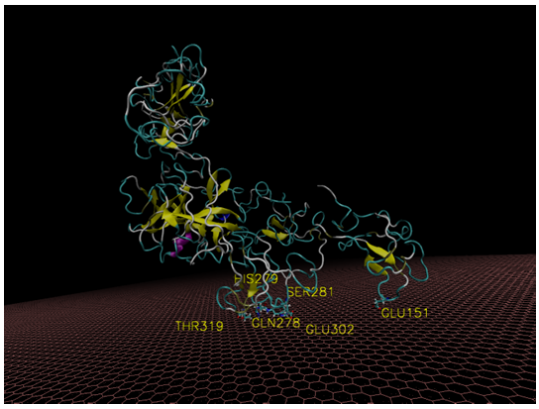


Fig. 3 Complex structure #1, showing the contact residues

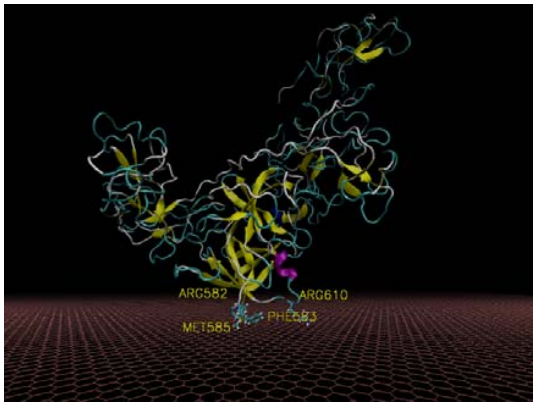


Fig. 4 Complex structure #3, showing the contact residues

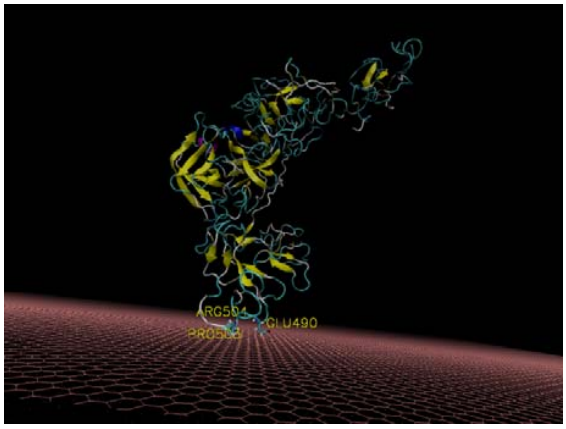


Fig. 5 Complex structure #4, showing the contact residues

IV. Discussion

We have demonstrated the large degree of conformational change of plasmin without disrupting secondary structure. We have also shown that the docking of plasmin on gold slab is through both electrostatic and van der Waals forces. However, there are several aspects that should be taken into consideration in the future study.

A. Deletion of PAp domain triggers the conformational change. But it is also possible that the conformation of plasminogen will spend some time in the "expanded" form similar to what is observed in plasmin. More studies like those utilizing "umbrella sampling" could help in exploring the possible conformational space for both plasminogen and plasmin.
B. The conformation of plasmin after docking could change. More molecular dynamics study on the plasmin on gold nanoparticle will certainly help to clarify this issue. It was observed that the catalytic activity of plasmin was decreased about half after the binding of plasmin on gold nanoparticle (unpublished data). How do gold atoms abolish the activity of plasmin? Is it possible to restore the activity of plasmin? Modification on the gold nanoparticle and plasmin are two possible directions toward a new design of the plasmin-gold nanoparticle complex.

V. Conclusion

We have demonstrated that plasmin conformation could deviate from the original conformation of plasminogen to a great extent and this conformational change is consistent with the experimental data. Furthermore, we employed Brownian Dynamics to dock plasmin on the gold slab, a mimic of gold nanoparticle. We found three major binding modes that utilized different domain of plasmin to contact gold slab. This research will facilitate the understanding of the interaction of plasmin and gold nanoparticle and aid the design of the use of gold nanoparticle as a versatile shuttle of plasmin in human body.

Acknowledgment

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