Thrombosis Research 184 (2019) 129–135

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

Prediction of binding characteristics between von Willebrand factor and platelet glycoprotein Ibα with various mutations by molecular dynamic simulation☆

Shinichi Goto[a,e], Hideki Oka[a], Kengo Ayabe[a], Hiroto Yabushita[a], Masamitsu Nakayama[a], Terumitsu Hasebe[b], Hideo Yokota[c], Shu Takagi[d], Motoaki Sano[e], Aiko Tomita[f], Shinya Goto[a,*]

[a] Department of Medicine (Cardiology), Tokai University School of Medicine, Metabolic Disease Research Center, Tokai University Graduate School of Medicine, Isehara, Japan
[b] Department of Radiology, Tokai University Hachioji Hospital, Tokai University School of Medicine, Hachioji, Tokyo, Japan
[c] Image Processing Research Team, Center for Advanced Photonics Extreme Photonics Research, RIKEN, Wako, Japan
[d] Department of Mechanical Engineering, The University of Tokyo, Tokyo, Japan
[e] Department of Cardiology, Keio University School of Medicine, Tokyo, Japan
[f] Department of Clinical Pharmacology, Tokai University School of Medicine, Isehara, Japan

ARTICLE INFO

Keywords:
Platelet
Thrombosis
von Willebrand factor
Platelet glycoprotein
Molecular dynamic

ABSTRACT

Introduction: Binding of platelet glycoprotein (GP)Ibα with von-Willebrand factor (VWF) exclusively mediates the initial platelet adhesion to injured vessel wall. To understand the mechanism of biomedical functions, we calculated the dynamic fluctuating three-dimensional (3D) structures and dissociation energy for GPIbα with various single amino-acid substitution at G233, which location is known to cause significant changes in platelet adhesive characteristics.

Material and methods: Molecular dynamics (MD) simulation was utilized to calculate 3D structures and Potential of Mean Force (PMF) for wild-type VWF bound with wild-type, G233A (equal function), G233V (gain of function), and G233D (loss of function) GPIbα. Simulation was done on water-soluble condition with time-step of 2×10^{-15} s using NAnoscale Molecular Dynamics (NAMD) with Chemistry at HARvard Molecular Mechanics (CHARMM) force field. Initial structure for each mutant was obtained by inducing single amino-acid substitution to the stable water-soluble binding structure of wild-type.

Results: The most stable structures of wild-type VWF bound to GPIbα in wild-type or any mutant did not differ. However, bond dissociation energy defined as difference of PMF between most stable structure and the structure at 65 Å mass center distances in G233D was 4.32 kcal/mol (19.5%) lower than that of wild-type. Approximately, 2.07 kcal/mol energy was required to dissociate VWF from GPIbα with G233V at mass center distance from 48 to 52 Å, which may explain the apparent "gain of function" in G233V.

Conclusion: The mechanism of substantially different biochemical characteristics of GPIbα with mutations in G233 location was predicted from physical movement of atoms constructing these proteins.

1. Introduction

Platelets play crucial roles for hemostasis, thrombosis, and local regulation of vessel function [1–4]. The initial interaction of platelet with vessel wall is mediated exclusively by platelet glycoprotein (GP) Ibα binding with von Willebrand factor (VWF) [5]. This binding is known not only to initiate platelet adhesion but also activates intercellular signaling eventually leading to platelet activation [6]. Thus, the clarification of the biochemical characteristics of this binding process is important for understanding the mechanism of the initiation of platelet cell interaction with vessel wall and subsequent clot formation. Currently available antiplatelet agents such as aspirin/$P2Y_{12}$ inhibitor target platelet activation but do not influence initial platelet adhesion at site of endothelial injury caused by atheroma rupture. Thus,

☆ The work was done at Tokai University School of Medicine/Tokai University Graduate School of Medicine.

* Corresponding author at: Department of Medicine (Cardiology), Metabolic Disease Research Center, Tokai University Graduate School of Medicine, 143 Shimokasuya, Isehara 259-1193, Japan.
E-mail address: shinichi@is.icc.u-tokai.ac.jp (S. Goto).

https://doi.org/10.1016/j.thromres.2019.10.022
Received 8 June 2019; Received in revised form 12 October 2019; Accepted 21 October 2019
Available online 24 October 2019

interactions of GPIbα with VWF are still an important potential target for new generation antiplatelet agents.

Previous biological experiments revealed the importance of A1 domain of VWF [7–9] and N-terminus domain of GPIbα [10–12] for their bindings. The binding of VWF and GPIbα become stable only under specific conditions such as in the presence of ristocetin [13–17], botrocetin [16,18], under de-natured conditions [19], with specific single amino acid mutation either in VWF [20,21] or GPIbα [22], and so on. It is of note that the interaction of GPIbα and VWF become apparent under blood flow conditions [23]. Some investigators suggest the modifying effect of shear force for the three-dimensional (3D) conformations in either VWF [24] or GPIbα [25], while others show that the stable binding of VWF to platelet could be seen only in the presence of activated GPIIb/IIIa [26]. The precise understanding of the structure mediated binding function of platelet GPIbα and VWF is crucial to dissect the process of platelet interaction with vessel wall.

Single amino-acid mutations in GPIbα were shown to lead to clinical phenotype, such as bleeding, by changing the adhesion characteristics of platelets [27–30]. Of them, the amino-acid mutation in 233 location of GPIbα has been studied thoroughly in *in-vitro* analysis and is known to shows different binding characteristics with different amino-acid even in the same location: G233A (equal function), G233V (gain of function), and G233D (loss of function) [13]. Both loss of function and gain of function for GPIbα binding to VWF, are known to cause bleeding. The loss of function reduces the interaction of platelet with VWF, which is necessary to initiate clot formation while the gain of function causes spontaneous binding of VWF to platelet through GPIbα which results in reduced plasma concentration of VWF. The binding of GPIbα with VWF is a protein-protein interaction. Studying the 3D structural bases for the change in binding characteristics for these mutations may give us a clue about the process of structure mediated binding function of these proteins.

X-ray crystallography is a strong tool to understand the 3D structures and functions of various proteins including GPIbα and VWF. Indeed, the stable binding structures of N-terminus domain of GPIbα bound with VWF both in wild-type and in various mutants were published previously [31–33]. However, dynamic structural fluctuations of GPIbα bound with VWF in the presence of water molecules were hard to be predicted by crystallographic analysis. Nuclear magnetic resonance (NMR) spectroscopy is applicable to predict water soluble structure of VWF [34], but both technologies are too expensive to apply for large numbers of mutations. Therefore, the structural details are still to be elucidated.

Recently progressed high performance computing technology allows us to predict dynamic fluctuations of protein structures calculated from the physical movement of atoms [35]. The most important breakthrough enabling this prediction is the combination of Newton's mechanics and quantum mechanics using force fields. Karplus M, et al. established the force field implementing simplified quantum mechanics which can be integrated in Newton's mechanics calculation, namely Chemistry at HARvard Molecular Mechanics (CHARMM) [36]. Dynamic three-dimensional structural fluctuation and dissociation force of wild-type GPIbα bound with wild-type VWF was predicted with the use of CHARMM-22 force field previously [37]. The validity of prediction of this molecular dynamics calculation on the binding characteristics of these proteins was confirmed by actual measurement of dissociation force between GPIbα and VWF detected by atomic force microscopy [38] and by optical tweezers [39]. The same technology can be applied to predict the 3D structure and binding energy of mutants on these proteins. Of note, binding force between the mass center of GPIbα and VWF is also predictable by molecular dynamic simulation. This provides us with means to predict large numbers of 3D binding structures for various mutants at various mass center distances.

Here we report the dynamic 3D structures and binding energies of VWF and GPIbα with various single amino acid substitution at 233, showing apparently different phenotypes, calculated using molecular dynamics simulation. Theoretical basis of substantial difference in phenotypic of “equivalent function” in G233A, “gain of function” in G233V and “loss of function” in G233D mutant will be provided.

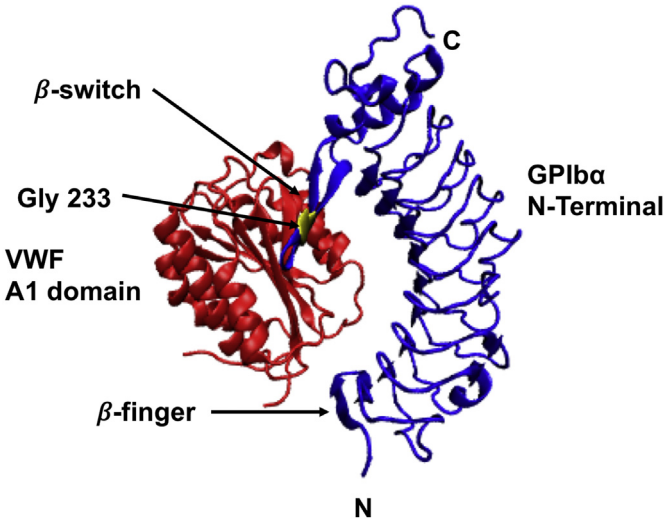

Fig. 1. Crystal structure of N-terminus domain of wild-type GPIbα binding with A1 domain of VWF and the location of Gly 233 in the structure.
Structure of GPIbα is shown in blue and VWF is shown in red. α-Helices are shown as coiled ribbons, β-stands as ribbons with arrows, and loops as tubes. Gly 233 is located in the β-switch region of GPIbα. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

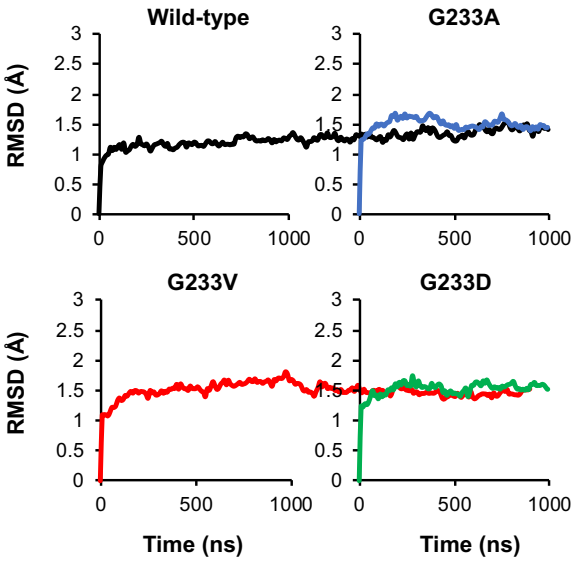

Fig. 2. Root mean square deviation (RMSD) of atoms constructing A1 domain of VWF and N-terminus domain of GPIbα in wild type and mutants of G233A, G233V, and G233D.
The RMSD of all atoms which constitute the A1 domain of VWF and N-terminus domain of GPIbα in wild-type (black line), G233A (blue line), G233V (red line) and G233D (β-finger region) starting from the initial structure constructed from the stable soluble structures of wild-type (time 0) to 1000 ps are shown. The RMSD become stabilized after 400 ps (400×10^3 step) of calculation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

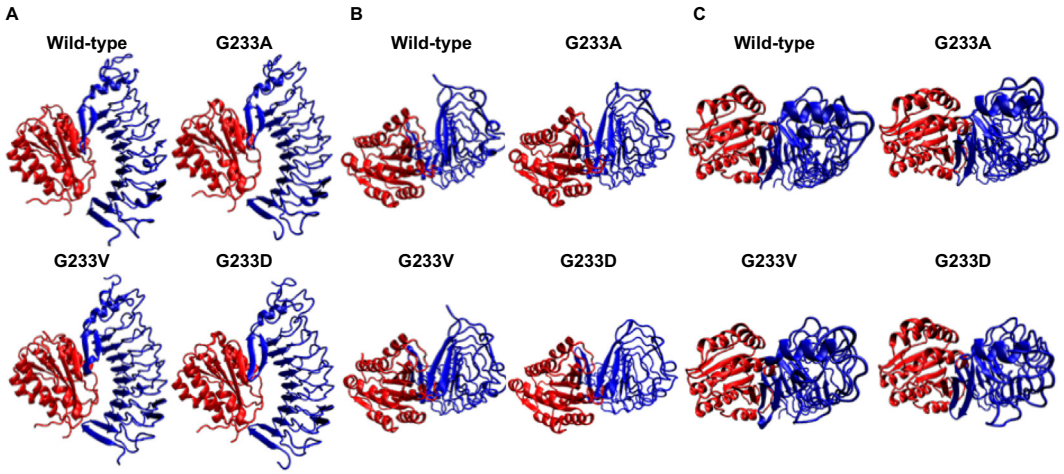

Fig. 3. The predicted most energetically stable structures of VWF bound with GPIbα in wild type, G233A, G233V, and G233D.
Comparison of the calculated most stable structure in the presence of water molecules. View showing the binding site horizontally (A), view from the N-terminus of GPIbα (B) and view from the C-terminus of GPIbα (C) are shown. The VWF binding N-terminus site of GPIbα and A1 domain of VWF are shown as blue and red, respectively. α-Helices are shown as coiled ribbons, β-stands as ribbons with arrows, and loops as tubes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. Material and methods

2.1. Molecular dynamic simulation

Molecular dynamic simulation on the three-dimensional structure of A1 domain of wild-type von Willebrand factor (VWF: residues ASP^{506}-PRO^{703}) interacting with N-terminus domain of platelet glycoprotein Ibα (GP: residues HSE^{1}-PRO^{265}) with amino acid substitution of G233A, G233V, and G233D were conducted according to the methods previously published [37].

Briefly, the Newton's second law; F (force) = m (mass) × a (acceleration) were solved in all atoms constructing VWF and GPIbα along with water molecules surrounding them. As the force field, CHARMM-22 was used. The initial structure of each mutant was obtained by inducing the amino-acid substitution against the previously published stable structure in water-soluble condition of wild-type VWF bound to wild-type GP1bα using mutate residue plugin of Visual Molecular Dynamics (VMD) version 1.9.3. The water molecules in wild-type structure was removed and re-solvated in the same condition as the other structure with mutations. The water molecules were modeled as transferable intermolecular potential water molecules (TIP3P) [40]. The position and velocity vector of each atom and water molecule were calculated in each 2.0 fs (1×10^{-15} s.) Similar to the previous publication, Particle mesh Ewald (PME) summation [41] with a cut off length of 12 Å for the direct interactions was used for predicting long range electrostatic interaction.

All the results were visualized with VMD version 1.9.3. The hydrogen bond was predicted on the most stable binding structures for wild-type and each mutant using VMD with distance cutoff of 4.0 Å and angle cutoff of 30°. The root mean square deviation (RMSD) of all atoms, excluding the water molecules, was calculated in each 10 ps. Calculation was assumed to be stabilized when RMSD stopped changing with extension of calculation time.

The multi-dimensional calculations of Newton's second law were conducted with the software of NAnoscale Molecular Dynamics (NAMD) compiled on the following high-performance computers; K (Riken, Kobe, Japan), Oak Forest-PACS (Tokyo University, Tokyo), and Hokusai (Riken, Wako, Japan). Other software packages such as the Amber Molecular Dynamics Package were tried, but the NAMD were fastest in these super computers. Supplemental calculations were conducted on rack mount servers in our institute equipped with Xeon Phi™ 7210 (Intel, US) or TITAN Xp (NVIDIA, US).

2.2. Selection of target mutants in GPIbα

The biological characteristics of GPIbα regarding the interaction with VWF differ substantially by the presence of single amino acid mutation in various bleeding disorders including platelet type von Willebrand diseases as previously published [27,28]. Fig. 1 shows the crystal structure of GPIbα binding with VWF A1 domain. As shown in this figure, Glycine at 233 of GPIbα is located in a structure called "β-switch". This "β-switch" structure is known to be an important position for the binding [13,42]. Of the mutation in this location, G233A, G233V, and G233D, were selected for calculation target because each three of them has specific biological characteristics, namely "equivalent function" in G233A, "gain of function" in G233V and "loss of function" in G233D as reported by Matsubara et al. [13]. They have shown that "equivalent function" G233A GPIbα bound to VWF with ristocetin concentration of 1.0 mg/ml, which was similar to wild-type. The "loss of function" G233D GPIbα was bound to approximately 20% lower numbers of VWF in the same ristocetin concentration. The "gain of function" G233V GPIbα started binding to VWF in ristocetin concentration as low as 0.11 mg/ml. The apparently different bio-chemical characteristics of GPIbα molecules in regard to VWF binding was attempted to be clarified in the current analysis.

2.3. Dissociation energy of the bond between A1 domain of VWF and N-terminus domain of GPIbα with various mutants

The potential of mean force (PMF) of the binding structure of wild-type VWF and GPIbα molecule with 3 different mutation at residue 233 (G233A, G233V, and G233D) along with the wild-type were calculated with the methods previously published [37]. Briefly, PMF was calculated as free energy surface along the mass center distances between GPIbα and vwf using the umbrella sampling methods with weighted histogram analysis method (WHAM) [43–45]. PMF expressed as kcal/mol were calculated in VWF interacting with wild-type, G233A, G233V and G233D GPIbα with restraints in mass center of VWF and GPIbα each 0.5 Å from the distance of 25 Å to 65 Å. The mass centers were calculated separately for GPIbα and VWF, as the unique point where the weighted relative position of the distributed mass involving all the atoms excluding water. The dissociation energy of the bond between VWF and GPIbα was calculated as the difference of PMF between the most stable structure and the structure at center distances of 65 Å.

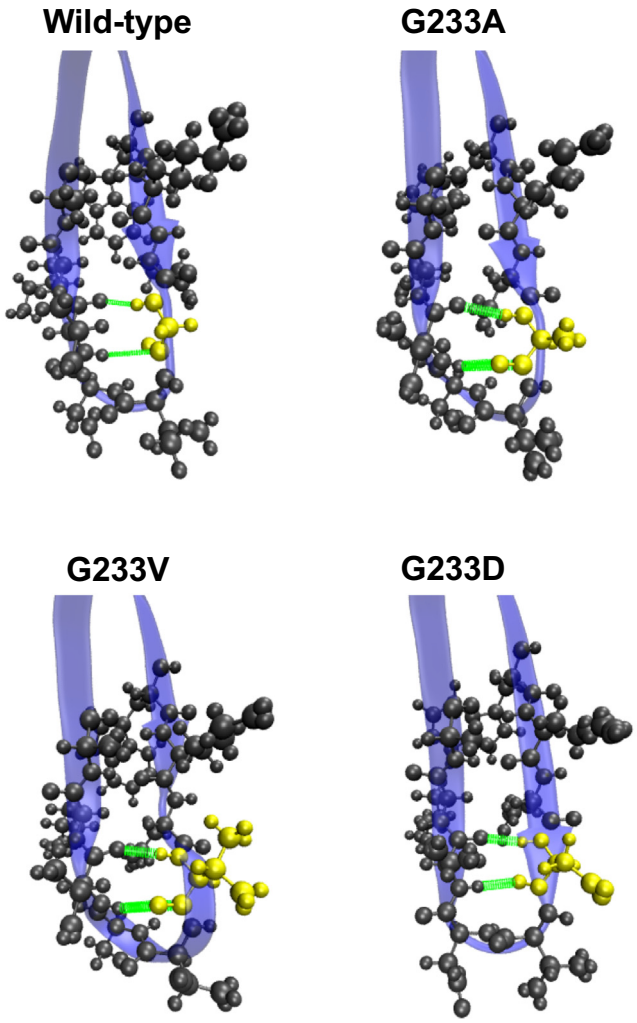

Fig. 4. Hydrogen bond formed between the amino-acid at 233 of GPIbα in wild type, G233A, G233V, and G233D at stable structure.
Representative structure of amino-acid at 230 to 238 of GPIbα for wild type, G233A, G233V and G233D are shown as ball-and-stick. The hydrogen bond between amino-acid at 233 and other amino-acid are shown as green line. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3. Results

3.1. Stable binding structure

To obtain the stable binding structure for wild-type VWF and GPIbα with G233A, G233V, G233D mutation or wild-type, molecular dynamics simulation was performed in water-soluble condition starting from initial structures, produced by inducing single amino-acid substitution in previously published water-soluble stable binding structure. As shown in Fig. 2, the binding structures in water of A1 domain of VWF and GPIbα with 3 mutations and wild-type had large fluctuation as indicated by large change in RMSD in the initial phase (up to 20 ps of calculation) but stabilized to RMSD change within 0.5 Å after calculation of 400 ps or longer. We defined the most stable binding structure as the one after 1000 ps of calculation and compared them as shown in Fig. 3. The most stable binding structure of GPIbα and VWF in water-soluble condition did not differ substantially even in the presence of various mutations. Of note, none of the mutant including the loss-of function (G233D) showed destructive protein unfolding. We further analyzed the stable structure focusing on hydrogen bond around the amino-acid at 233 of GPIbα. There was no direct hydrogen bond formed between the amino-acid at this location with any amino-acid of VWF. Hydrogen bonds formed between the amino-acid at 233 and other amino-acid in GPIbα are shown in Fig. 4. There was no marked change in hydrogen bond formation observed in the mutants.

3.2. Dynamic 3D structural change of the binding structure of VWF and GPIbα with mutants by changing the distance between mass center of both molecules

When mass center between A1 domain of VWF and GPIbα increased as 27.5, 30.0, 40.0, and 65.0 Å, wild-type (Fig. 5A), G233A mutant (Fig. 5B) and G233V mutant (Fig. 5C) showed similar changes in three-dimensional structures. Structural change in response to the mass center distance change was markedly different in G233D mutant. Indeed, VWF binding with the β-finger region of GPIbα was already separated at 40.0 Å while other mutants and wild-type retained this bond (Fig. 5D). These results suggest that G233D mutant may have unstable binding characteristics around this center distances and may have weaker binding energy between VWF due to early loss of bond with β-finger region. Dynamic fluctuation of 3-dimensional structure of VWF bound with GPIbα in each 2×10^{-15} s at distance of mass center between GPIbα and VWF at 27.5, 30.0, 40.0, and 65.0 Å, in wild type, G233A, G233V, and G233D were shown as Supplemental movie WTR, G233AR, G233VR, and G233DR. These video supports that the changes in the dynamic structure at 40.0 Å is not only a snap-shot captured by chance but the structure in G233D is actually different.

3.3. Dissociation energy of the bond between VWF and GPIbα with mutants

To calculate the dissociation energy for the bond between GPIbα and VWF in each mutant and wild-type, PMF were calculated along various mass center distances between GPIbα and VWF. We assumed that the PMF of the structure at 65 Å will reflect the energy of unbound state and that the PMF at most stable binding structure will reflect the energy of complete binding. Therefore, we defined the dissociation energy of the bond between GPIbα and VWF as the difference of PMF between the most stable binding structure and the structure at 65 Å mass center distance. Fig. 6 shows the PMF for the binding structure of VWF and GPIbα plotted against mass-center distances of GPIbα and VWF for wild-type and the 3 mutation at residue 233. Predicted dissociation energy between VWF was lowest in G233D mutant, which was 4.32 kcal/mol (19.5%) lower compared with wild-type. The difference in dissociation energy was slightly smaller in G233V mutant (difference with wild-type: 0.48 kcal/mol) or G233A mutant (difference with wild-type: 0.63 kcal/mol). However, the magnitude of the difference in dissociation energy compared with wild-type was approximately 1/10 of that of G233D. In G233A mutant, there was small drop of PMF at distance of mass center between 40 and 50 Å. With G233V mutant, there was small increase of PMF at mass center distances between 48 and 53 Å.

4. Discussion

We show here the similarity of predicted stable structure of A1 domain of VWF bound with N-terminus VWF binding site of GPIbα with various amino acid substitution of residue 233 despite apparently different biological functions of “equivalent function”, “gain of function”, or “loss of function”. When mass centers of VWF and GPIbα were spread, markedly different structures appeared in G233D mutant. These drastic structural changes, in addition to smallest dissociation energy provide potential explanation of “loss of function” in G233D mutant. Our result suggests that even without significant changes in stable binding structure, a mutation can cause a dramatic effect and raises an argument that dynamic structures visible only upon very specific conditions, such as right before dissociation, is important.

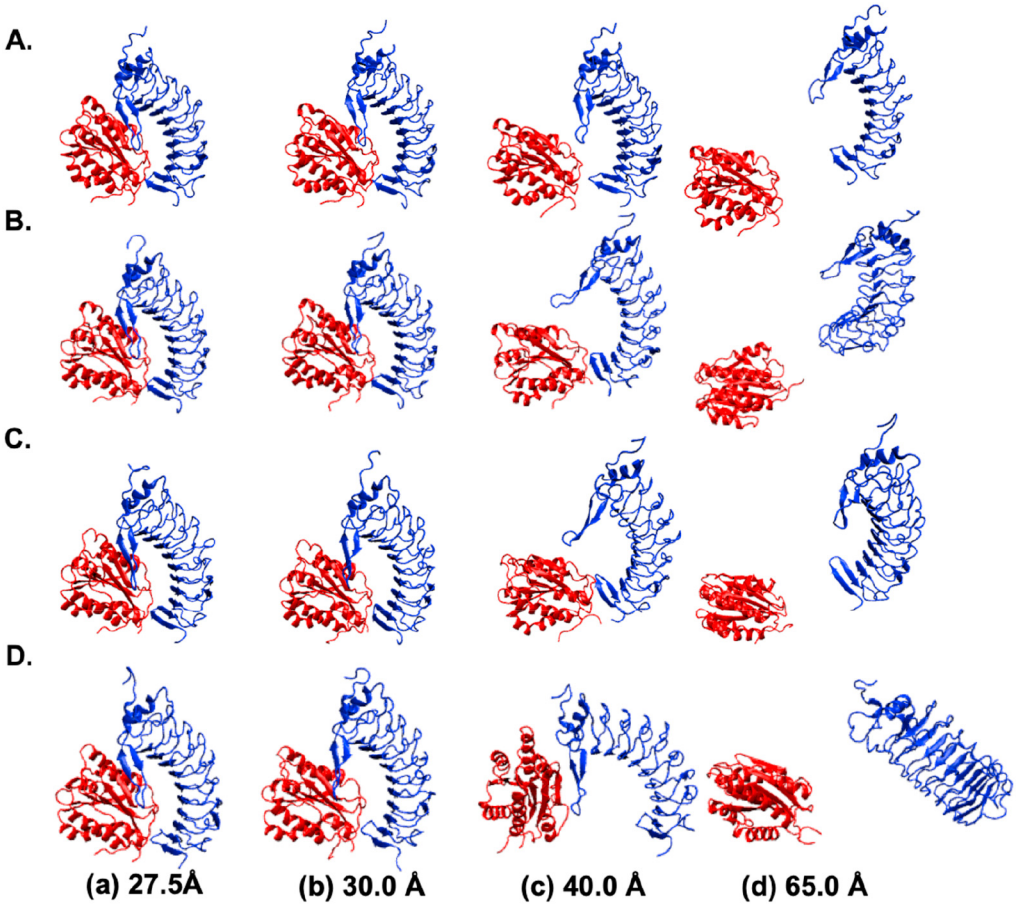

Fig. 5. Structures of A1 domain of VWF bound with N-terminus VWF binding domain of GPIbα in wild type (A), G233A (B), G233V (C), and G233D (D) at the distance of mass center of 27.5, 30.0, 40.0, and 65.0 Å.
Representative stable structure of VWF bound with GPIb*α* in wild type, G233A, G233V and G233D are shown at the fixed distance of mass center of 27.5, 30.0, 40.0, and 65.0 Å. *α*-Helices are shown as coiled ribbons, β-stands as ribbons with arrows, and loops as tubes.

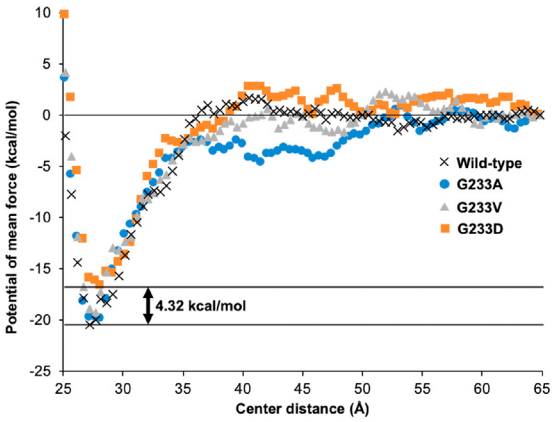

Fig. 6. Potential of Mean Force (PMF) for A1 domain of VWF bound with N-terminus GPIbα.
Potential of mean forces for binding between A1 domain of VWF and N-terminus domain of GPIb*α* were calculated as kcal/mol in every 0.5 Å of the distance of the center of mass of both molecules in wild type, G233A, G233V, and G233D are shown.

Platelet adhesion at the site of vessel injury under blood flow condition is mediated solely by GPIb*α* binding with VWF [5] no matter whether platelets are activated or not [46]. The specific biochemical characteristic of VWF binding with GPIb*α* is that the binding occurs transiently, but dissociate rapidly in the absence of specific substances such as ristocetin [11,18,47] or botrocetin [12,16]. Under high shear flow conditions, VWF binding to platelet and VWF-mediated platelet aggregation is known to occur. But, the stable VWF binding on platelet could only be seen when activated GPIIb/IIIa are involved [26]. The transient nature of VWF binding with GPIb*α* make the biochemical assessment of their interaction more difficult compared to other proteins stably binding to each other. Crystal structure of VWF bound with GPIb*α* provides important information for their interaction. However, the dynamic analysis is necessary to dissect the process of transient nature of the binding between GPIb*α* and VWF. Molecular dynamic simulation enabled to predict the binding force between VWF and GPIb*α* to be 62.3 pN. This predicted value was in agreement with actual measurement by atomic force microscopy [38] and optical tweezers [39]. In the current analysis, we applied this method to study the structure and dissociation energy for GPIb*α*-VWF binding in various GPIb*α* mutation. This approach allowed us to predict the stable binding structure for 3 mutants of GPIb*α* including the one that does not stably bind to VWF. Another strength of our method is the ability to predict the dynamic structure of GPIb*α* binding with VWF just before dissociation. It is impossible to obtain a crystal for this structure. Thus, these structures cannot be solved by X-ray crystallography. Obtaining these dynamic structures may provide us with a new approach of drug development. Since GPIb*α* binding with VWF only transiently capture platelet at site of endothelial injury, small molecule, that is able to bind either GPIb*α* or VWF in this structure just before dissociation, may act as antiplatelet agents. We applied the validated methods to understand the substantial differences in the phenotype of 3 mutant at G233 in GPIb*α* and obtained results applicable to understand the biochemical characteristic of these mutants. Substantial difference in the structure of GPIb*α* bound with VWF only at mass center difference of 40 Å support our hypothesis that small molecules, that binds either GPIb*α* or VWF just before dissociation, could be strong enough antiplatelet agents even if they do not bind to the stable structure.

Our result provides possible explanation for the “loss of function” of G233D by lowest dissociation energy of the bond between VWF and

GPIbα with G233D mutant compared to wild-type and other mutants. Unstable binding structure from mass center distance of 30 to 40 Å may also be a reason not to bind even in the presence of ristocetin. Biologically "equivalent function" in G233A and "gain of function" in G233V are more difficult to explain by our calculation. Relatively lower binding energy in G233A and VWF as compared to wild-type did not contribute to apparent biological difference. The "gain of function" in G233V mutant for VWF binding might be explained by energy increase from the mass center distance from 48 to 53 Å which may create an energy barrier for dissociation around this distance. However, the energy barrier is small and difference in the distance dependent change in structure was not markedly different from wild-type. Further works, especially the calculation in the presence of ristocetin, is necessary to clarify the reason for functional difference in various mutants.

High performance computer and advanced information technology allow us to understand dynamic fluctuation of various biological molecules under various conditions. The molecular dynamic simulation is a strong technology for constructive understanding of the complicated biological functions of various molecules from the atomic movement. Newton's second law should be applicable for determining the position and velocity of various freely moving atoms placed apart. However, quantum mechanics is necessary to understand the forces and position of atoms when they share electrons to make chemical bond. CHARMM force field is a strong tool to simplify quantum mechanics to incorporate into molecular dynamic calculation [36,40,48]. It is of note that there are several potential limitations in our methods in regard to apply it to understand the biological mechanism. Matsubara et al. previously demonstrated the importance of differences in hydrogen bonds between oxygen atoms of main-chain of amino-acid at 233 position *GPIbα* with computer modeling that potentially changes the stability of *β*-hairpin structure [13]. In our method, using molecular dynamics with CHARMM force field, we could not confirm this finding. Indeed, CHARMM force field simplifies various forces between atoms. It could not directly account for dynamic changes in covalent bond. Other method such as quantum mechanics may solve this remaining issue.

To overcome potential limitations, validation of predicted results with biological or biochemical experiments are necessary. For the VWF binding with *GPIbα*, quantitative validation was conducted previously by comparison of the predicted binding force between VWF and GPIbα in wild-type and actually measured ones by atomic force microscopy [38] and optical tweezer [39]. Strong limitation in our study is that the quantitative validation was conducted only in wild-type, but not for variants. Although our results are in good agreement with previously published biological experiments [13], further quantitative validation is needed for mutant molecules.

In conclusion, we have shown the similar stable structure of GPIbα bound with VWF even in the presence of biologically "equivalent function" (G233A), "gain of function" (G233V), or "loss of function" (G233D) mutations. Unstable structure and smaller binding energy in binding with VWF may be the reason of loss of biological function in G233D. Small energetic and structural difference in molecules may be the cause of substantial functional difference in single amino acid mutants.

Each movie provided the calculation results of the position and velocity of all atoms constructing the A1 domain of VWF bound with N-terminus VWF binding domain of GPIbα with wild-type (WTR.mov), G233A (G233AR.mov), G233V (G233VR.mov), and G233D (G233DR.mov) in each 2×10^{-15} s. Despite similar stable structure around mass center distance between VWF and GPIbα at 27.5 Å, marked change in binding structure was observed in G233D mutant. Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2019.10.022.

Acknowledgement and potential conflict of interests

Shinichi Goto has nothing to disclose. Hideki Oka has nothing to disclose. Kengo Ayabe has nothing to disclose. Hiroto Yabushita has nothing to disclose. Terumitsu Hasebe acknowledges the financial support from MEXT/JSPS KAKENHI 18H01726. Hideo Yokota has nothing to disclose. Shu Takagi has nothing to disclose. Motoaki Sano has nothing to disclose. Aiko Tomita has nothing to disclose. Shinya Goto acknowledges the financial support from MEXT/JSPS KAKENHI 17K19669, partly by 18H01726 and 19H03661. Shinya Goto acknowledges grant support from The Vehicle Racing Commemorative Foundation and Nakatani Foundation for Advancement of Measuring Technologies in Biomedical Engineering. Shinya Goto acknowledges the financial support from Bristol-Myers Squibb from their independent research support project (33999603). Shinya Goto received research funding from Sanofi, Pfizer, and Ono. Shinya Goto received a modest personal fee from Bayer. Shinya Goto is an associated Editor for Circulation, an associate Editor for Journal of Biorheology, an associate Editor for Archives of Medical Science, section Editor for Thrombosis and Hemostasis.

References

[1] Z.M. Ruggeri, Platelets in atherothrombosis, Nature Medicine. 8 (2002) 1227–1234.
[2] S. Goto, Understanding the mechanism and prevention of arterial occlusive thrombus formation by anti-platelet agents, Current Medicinal Chemistry Cardiovascular and Hematological Agents. 2 (2004) 149–156.
[3] D. Sibbing, D.J. Angiolillo, K. Huber, Antithrombotic therapy for acute coronary syndrome: past, present and future, Thrombosis and Haemostasis. 117 (2017) 1240–1248.
[4] D.D. McManus, J.E. Freedman, MicroRNAs in platelet function and cardiovascular disease, Nature Reviews Cardiology. 12 (2015) 711–717.
[5] S. Goto, T. Hasebe, T.S. Platelets, Small in size but essential in the regulation of vascular homeostasis - translation from basic science to clinical medicine, Circulation Journal: Official Journal of the Japanese Circulation Society. 79 (2015) 1871–1881.
[6] Kroll MH, Harris TS, Moake JL, Handin RI and Schafer AI. von Willebrand factor binding to platelet GpIb initiates signals for platelet activation. The Journal of clinical investigation. 1991;88:1568-1573.
[7] Z.M. Ruggeri, Von Willebrand factor, Current Opinion in Hematology. 10 (2003) 142–149.
[8] S. Vasudevan, J.R. Roberts, R.A. McClintock, J.A. Dent, R. Celikel, J. Ware, K.I. Varughese, Z.M. Ruggeri, Modeling and functional analysis of the interaction between von Willebrand factor A1 domain and glycoprotein Ibalpha, The Journal of Biological Chemistry. 275 (2000) 12763–12768.
[9] J. Ware, J.A. Dent, H. Azuma, M. Sugimoto, P.A. Kyrle, A. Yoshioka, Z.M. Ruggeri, Identification of a point mutation in type IIB von Willebrand disease illustrating the regulation of von Willebrand factor affinity for the platelet membrane glycoprotein Ib-IX receptor, Proceedings of the National Academy of Sciences of the United States of America. 88 (1991) 2946–2950.
[10] M. Handa, K. Titani, L.Z. Holland, J.R. Roberts, Z.M. Ruggeri, The von Willebrand factor-binding domain of platelet membrane glycoprotein Ib. Characterization by monoclonal antibodies and partial amino acid sequence analysis of proteolytic fragments, The Journal of Biological Chemistry. 261 (1986) 12579–12585.
[11] V. Vicente, P.J. Kostel, Z.M. Ruggeri, Isolation and functional characterization of the von Willebrand factor-binding domain located between residues His1-Arg293 of the alpha-chain of glycoprotein Ib, The Journal of Biological Chemistry. 263 (1988) 18473–18479.
[12] M. Murata, J. Ware, Z.M. Ruggeri, Site-directed mutagenesis of a soluble recombinant fragment of platelet glycoprotein Ib alpha demonstrating negatively charged residues involved in von Willebrand factor binding, The Journal of Biological Chemistry. 266 (1991) 15474–15480.
[13] Y. Matsubara, M. Murata, K. Sugita, Y. Ikeda, Identification of a novel point mutation in platelet glycoprotein Ibalpha, Gly to Ser at residue 233, in a Japanese family with platelet-type von Willebrand disease, J Thromb Haemost. 1 (2003) 2198–2205.
[14] J.P. Frontroth, E.J. Favaloro, Ristocetin-induced platelet aggregation (RIPA) and RIPA mixing studies, Methods in Molecular Biology (Clifton, NJ). 1646 (2017) 473–494.
[15] M.A. Cruz, T.G. Diacovo, J. Emsley, R. Liddington, R.I. Handin, Mapping the glycoprotein Ib-binding site in the von willebrand factor A1 domain, The Journal of Biological Chemistry. 275 (2000) 19098–19105.
[16] Y. Fujimura, Y. Usami, K. Titani, K. Niinomi, K. Nishio, T. Takase, A. Yoshioka, H. Fukui, Studies on anti-von Willebrand factor (vWF) monoclonal antibody NMC-4, which inhibits both ristocetin- and botrocetin-induced vWF binding to platelet glycoprotein Ib, Blood. 77 (1991) 113–120.
[17] T. Matsushita, J.E. Sadler, Identification of amino acid residues essential for von Willebrand factor binding to platelet glycoprotein Ib. Charged-to-alanine scanning mutagenesis of the A1 domain of human von Willebrand factor, The Journal of Biological Chemistry. 270 (1995) 13406–13414.
[18] J.F. Dong, M.C. Berndt, A. Schade, L.V. McIntire, R.K. Andrews, J.A. Lopez, Ristocetin-dependent, but not botrocetin-dependent, binding of von Willebrand factor to the platelet glycoprotein Ib-IX-V complex correlates with shear-dependent

interactions, Blood. 97 (2001) 162–168.
[19] S. Miyata, S. Goto, A.B. Federici, J. Ware, Z.M. Ruggeri, Conformational changes in the A1 domain of von Willebrand factor modulating the interaction with platelet glycoprotein Ibalpha, The Journal of Biological Chemistry. 271 (1996) 9046–9053.
[20] A.I. Woods, A.C. Kempfer, J. Paiva, A. Sanchez-Luceros, E. Bermejo, R. Chuit, M.F. Alberto, A.N. Blanco, M.A. Lazzari, Phenotypic parameters in genotypically selected type 2B von Willebrand disease patients: a large, single-center experience including a new novel mutation, Seminars in Thrombosis and Hemostasis. 43 (2017) 92–100.
[21] K.A. Cooney, D. Ginsburg, Comparative analysis of type 2b von Willebrand disease mutations: implications for the mechanism of von Willebrand factor binding to platelets, Blood. 87 (1996) 2322–2328.
[22] M. Othman, C. Notley, F.L. Lavender, H. White, C.D. Byrne, D. Lillicrap, D.F. O'Shaughnessy, Identification and functional characterization of a novel 27-bp deletion in the macroglycopeptide-coding region of the GPIBA gene resulting in platelet-type von Willebrand disease, Blood. 105 (2005) 4330–4336.
[23] B. Savage, F. Almus-Jacobs, Z.M. Ruggeri, Specific synergy of multiple substrate-receptor interactions in platelet thrombus formation under flow, Cell. 94 (1998) 657–666.
[24] C.A. Siedlecki, B.J. Lestini, K.K. Kottke-Marchant, S.J. Eppell, D.L. Wilson, R.E. Marchant, Shear-dependent changes in the three-dimensional structure of human von Willebrand factor, Blood. 88 (1996) 2939–2950.
[25] H. Geng, G. Xu, Y. Ran, J.A. Lopez, Y. Peng, Platelet glycoprotein Ib beta/IX mediates glycoprotein Ib alpha localization to membrane lipid domain critical for von Willebrand factor interaction at high shear, The Journal of Biological Chemistry. 286 (2011) 21315–21323.
[26] S. Goto, D.R. Salomon, Y. Ikeda, Z.M. Ruggeri, Characterization of the unique mechanism mediating the shear-dependent binding of soluble von Willebrand factor to platelets, The Journal of Biological Chemistry. 270 (1995) 23352–23361.
[27] T.A. Doggett, G. Girdhar, A. Lawshe, J.L. Miller, I.J. Laurenzi, S.L. Diamond, T.G. Diacovo, Alterations in the intrinsic properties of the GPIbalpha-VWF tether bond define the kinetics of the platelet-type von Willebrand disease mutation, Gly233Val, Blood. 102 (2003) 152–160.
[28] J.L. Miller, Z.M. Ruggeri, V.A. Lyle, Unique interactions of asialo von Willebrand factor with platelets in platelet-type von Willebrand disease, Blood. 70 (1987) 1804–1809.
[29] A.S. Tait, S.L. Cranmer, S.P. Jackson, I.W. Dawes, B.H. Chong, Phenotype changes resulting in high-affinity binding of von Willebrand factor to recombinant glycoprotein Ib-IX: analysis of the platelet-type von Willebrand disease mutations, Blood. 98 (2001) 1812.
[30] M. Othman, J. Emsley, Gene of the issue: GP1BA gene mutations associated with bleeding, Platelets. 28 (2017) 832–836.
[31] M.A. Blenner, X. Dong, T.A. Springer, Structural basis of regulation of von Willebrand factor binding to glycoprotein Ib, The Journal of Biological Chemistry. 289 (2014) 5565–5579.
[32] E.G. Huizinga, S. Tsuji, R.A. Romijn, M.E. Schiphorst, P.G. de Groot, J.J. Sixma, P. Gros, Structures of glycoprotein Ibalpha and its complex with von Willebrand factor A1 domain, Science (New York, NY). 297 (2002) 1176–1179.
[33] J.C. Campbell, A. Tischer, V. Machha, L. Moon-Tasson, B. Sankaran, C. Kim, M. Auton, Data on the purification and crystallization of the loss-of-function von Willebrand disease variant (p.Gly1324Ser) of the von Willebrand factor A1 domain, Data in Brief. 7 (2016) 1700–1706.
[34] H.M. Knott, M.C. Berndt, A.V. Kralicek, S.I. O'Donoghue, G.F. King, Determination of the solution structure of a platelet-adhesion peptide of von Willebrand factor, Biochemistry. 31 (1992) 11152–11158.
[35] A.D. MacKerell, D. Bashford, M. Bellott, R.L. Dunbrack, J.D. Evanseck, M.J. Field, S. Fischer, J. Gao, H. Guo, S. Ha, D. Joseph-McCarthy, L. Kuchnir, K. Kuczera, F.T. Lau, C. Mattos, S. Michnick, T. Ngo, D.T. Nguyen, B. Prodhom, W.E. Reiher, B. Roux, M. Schlenkrich, J.C. Smith, R. Stote, J. Straub, M. Watanabe, J. Wiorkiewicz-Kuczera, D. Yin, M. Karplus, All-atom empirical potential for molecular modeling and dynamics studies of proteins, The Journal of Physical Chemistry B. 102 (1998) 3586–3616.
[36] Brooks BR, Brooks CL, 3rd, Mackerell AD, Jr., Nilsson L, Petrella RJ, Roux B, Won Y, Archontis G, Bartels C, Boresch S, Caflisch A, Caves L, Cui Q, Dinner AR, Feig M, Fischer S, Gao J, Hodoscek M, Im W, Kuczera K, Lazaridis T, Ma J, Ovchinnikov V, Paci E, Pastor RW, Post CB, Pu JZ, Schaefer M, Tidor B, Venable RM, Woodcock HL, Wu X, Yang W, York DM and Karplus M. CHARMM: the biomolecular simulation program. Journal of computational chemistry. 2009;30:1545-614.
[37] S. Shiozaki, S. Takagi, S. Goto, Prediction of molecular interaction between platelet glycoprotein Ibalpha and von Willebrand factor using molecular dynamics simulations, J Atheroscler Thromb. 23 (2016) 455–464.
[38] H. Tobimatsu, Y. Nishibuchi, R. Sudo, S. Goto, K. Tanishita, Adhesive forces between A1 domain of von Willebrand factor and N-terminus domain of glycoprotein Ibalpha measured by atomic force microscopy, Journal of Atherosclerosis and Thrombosis. 22 (2015) 1091–1099.
[39] J. Kim, C.Z. Zhang, X. Zhang, T.A. Springer, A mechanically stabilized receptor-ligand flex-bond important in the vasculature, Nature. 466 (2010) 992–995.
[40] S. Boonstra, P.R. Onck, E. Giessen, CHARMM TIP3P water model suppresses peptide folding by solvating the unfolded state, The Journal of Physical Chemistry B. 120 (2016) 3692–3698.
[41] M.J. Abraham, J.E. Gready, Optimization of parameters for molecular dynamics simulation using smooth particle-mesh Ewald in GROMACS 4.5, Journal of Computational Chemistry. 32 (2011) 2031–2040.
[42] Z. Chen, J. Lou, C. Zhu, K. Schulten, Flow-induced structural transition in the beta-switch region of glycoprotein Ib, Biophysical Journal. 95 (2008) 1303–1313.
[43] L.S. Stelzl, A. Kells, E. Rosta, G. Hummer, Dynamic histogram analysis to determine free energies and rates from biased simulations, Journal of Chemical Theory and Computation. 13 (2017) 6328–6342.
[44] M. Yang, A.D. MacKerell Jr., Conformational sampling of oligosaccharides using Hamiltonian replica exchange with two-dimensional dihedral biasing potentials and the weighted histogram analysis method (WHAM), Journal of Chemical Theory and Computation. 11 (2015) 788–799.
[45] S. Kumar, J.M. Rosenberg, D. Bouzida, R.H. Swendsen, P.A. Kollman, Multidimensional free-energy calculations using the weighted histogram analysis method, Journal of Computational Chemistry. 16 (1995) 1339–1350.
[46] S. Goto, Y. Ikeda, E. Saldivar, Z.M. Ruggeri, Distinct mechanisms of platelet aggregation as a consequence of different shearing flow conditions, The Journal of Clinical Investigation. 101 (1998) 479–486.
[47] H. Azuma, M. Sugimoto, Z.M. Ruggeri, J. Ware, A role for von Willebrand factor proline residues 702–704 in ristocetin-mediated binding to platelet glycoprotein Ib, Thrombosis and Haemostasis. 69 (1993) 192–196.
[48] J. Huang, A.D. MacKerell Jr., CHARMM36 all-atom additive protein force field: validation based on comparison to NMR data, Journal of Computational Chemistry. 34 (2013) 2135–2145.