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Effects of Altered Restraints in $\beta 1$ Integrin on the Force-Regulated Interaction between the Glycosylated I-Like Domain of $\beta 1$ Integrin and Fibronectin III_{9-10}: A Steered Molecular Dynamic Study

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Abstract: Cytoskeletal restraints affect force-regulated integrin function in cell adhesion. However, the structural and molecular basis underlying the effect of cytoskeletal restraints on $\beta 1$ integrin binding to fibronectin is still largely unknown. In this study, we used steered molecular dynamics simulations to investigate the changes in glycosylated $\beta 1$ integrin-fibronectin binding and in conformation and structure of the glycosylated $\beta 1$ I-like domain-FN-III_{9-10} complex caused by altered restraints applied to $\beta 1$ I-like domain. The results revealed that imposition of the increased constraints on $\beta 1$ integrin increased resistance to force-induced dissociation of the $\beta 1$ I-like domain-fibronectin complex. Specifically, the increased constraints enhanced resistance to relative conformational changes in the RGD-synergy site in fibronectin, increased the conformational stability of fibronectin, and prevented losses in hydrogen bond occupancy of each β-strand pair in FN-III_{10} resulting from external force. The increased constraints also resulted in an increase in correlated motion between residues in the $\beta 1$ I-like domain, which may directly affect the interaction of $\beta 1$ integrin with fibronectin. Results from this study provide molecular and structural insights into the effects of altered restraints in $\beta 1$ integrin on the interaction between glycosylated $\beta 1$ Integrin and fibronectin and its induced cell adhesion.

Keywords: altered restraints on integrin; glycosylated $\beta 1$ I-like domain; fibronectin; steered molecular dynamics

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1 Introduction

Cell adhesion, which is based on interactions between cells and the extracellular matrix (ECM), plays an important role in many cellular functions, including cellular growth, migration, differentiation, and proliferation (1-3). Cell adhesion is also an important regulator of both normal and pathophysiological biological processes, such as morphogenesis, wounding healing, and malignant invasion (4-6). Studies have revealed that interactions between the transmembrane glycoprotein $\alpha5\beta1$ integrin and the extracellular matrix protein fibronectin are fundamental for vertebrate development and have been suggested to be involved in cardiovascular events and tumor invasion (7-11). The extracellular matrix protein fibronectin (FN) is a ligand for a large number of different integrins, including $\alpha5\beta1$ integrin (12, 13). For the integrin-FN complex, fibronectin not only provides a substrate for cell anchorage to the ECM, but also serves as a regulatory factor in many cellular processes including cell adhesion, growth, migration, differentiation, and proliferation (1).

Emerging results indicate that integrin activity can also be regulated by other mechanisms, including signal-induced changes in *N*-glycosylation and cytoskeletal restraints, which in turn affect the ability of integrins to bind ligands (14-21). Cytoskeletal plays crucial role in regulating cell adhesion (22-24). $\alpha5\beta1$ integrin has been shown to bind fibronectin (25, 26), forming an interaction that mediates cell adhesion and migration (25). De-sialylation of $\alpha5\beta1$ integrin has been shown to increase the binding affinity of $\beta1$ integrin for fibronectin (15, 27). Additionally, release of cytoskeletal constraints on integrin motion is known to result in rearrangement of integrins in leukocytes for activation of cellular adhesion (17-19). These observations indicate that control of constraints on integrins represent a key regulatory mechanism required for proper cell adhesion that may influence the functional behavior of leukocytes in inflammatory reactions. Several studies have used steered molecular dynamics (SMD) simulations to characterize external force-induced mechanical responses of fibronectin type III that may modulate binding of integrins to fibronectin (28, 29). However, the effects of altered restraints on the force-modulated interaction between the glycosylated I-like domain of $\beta1$ integrin and fibronectin remain unknown.

Integrins are transmembrane glycoproteins that form heterodimers composed of α and β subunits that interact via noncovalent associations. The glycosylated I-like domain of β integrins, a region crucial for ligand binding (30), carries *N*-glycans attached to Asn 192, Asn 249, and Asn 343 (15). *N*-glycosylation of the I-like domain of $\beta1$ integrin is essential for $\alpha5\beta1$ integrin expression, heterodimer formation, and fibronectin-mediated biological functions, including cell adhesion and spreading (31). The I-like domain of $\beta1$ integrin contains critical functional sites

required for ligand binding, including the specificity-determining loop (SDL) (32), the metal ion-dependent adhesion site (MIDAS), which contains a DLSYS motif (33, 34), the $\alpha 1$ and $\alpha 7$ helices (35), and other critical residues (Asn-224, Asp-226, Glu-229, Asp-233, Asp-267, and Asp-295) (36). Fibronectin is a multimodular extracellular protein composed of more than 20 modules per monomer. The structure of fibronectin is composed of three different types of homologous, repeating modules: type I (FN-I), type II (FN-II), and type III (FN-III) (12). The type III module, which contains the RGD (Arg-Gly-Asp) sequence that is specifically recognized by integrins, is the most abundant of the modules. In addition to the RGD sequence, fibronectin also contains a synergy site that selectively enhances binding of specific integrins to the neighboring RGD loop (37). Some integrins, including $\alpha 5\beta 1$, recognize both the synergy site and the RGD loop of fibronectin. The synergy site facilitates preferential binding of integrin $\alpha 5\beta 1$ to fibronectin over other RGD-containing matrix proteins (38-40). A model of $\beta 1$ I-like domain has been constructed based on the crystal structure of the I-like domain of $\beta 3$ integrin (21), and the fully sampled conformations of the associated *N*-linked oligosaccharides with $\alpha 2$-6 sialic acid (ASO) and without $\alpha 2$-6 sialic acid (ANS) have been characterized (20). The crystal structure of the type III modules of fibronectin (FN-III) that includes modules FN-III_7 through FN-III_{10} has been solved (PDB ID: 1FNF) (41). The FN-III_{10} module contains the integrin-specific binding recognition site (RGD), and the FN-III_9 module contains the "synergy" region that is involved in preferential enhancement of integrin binding (41). Based on these structures, a validated glycosylated $\beta 1$ I-like domain-FN-III_{9-10} complex was constructed (20) and has been used to characterize the structural and thermodynamic bases for the role of altered sialylation of $\beta 1$ I-like domain in the binding of fibronectin to $\beta 1$ integrin (20, 21). The available structure of the glycosylated $\beta 1$ I-like domain-FN-III_{9-10} complex provides a basis for characterizing the effects of altered cytoskeletal restraints on force-regulated interaction of the glycosylated I-like domain of $\beta 1$ integrin with fibronectin III_{9-10}.

In this study, we have examined the effects of altered restraints in $\beta 1$ integrin on force-regulated interaction of the glycosylated $\beta 1$ I-like domain with fibronectin III_{9-10} using SMD simulations. SMD simulation results characterizing the effect of de-sialylation of the $\beta 1$ I-like domain on $\beta 1$ integrin-fibronectin binding were validated using experimental results (15, 27). We then evaluated the effects of altered constraints in $\beta 1$ integrin on dissociation of the $\beta 1$ I-like domain-fibronectin complex. We further characterized the mechanical responses and the conformational and structural changes of the glycosylated $\beta 1$ I-like domain-fibronectin complex with the altered restraints on $\beta 1$ integrin under external force applied to fibronectin. Results from this study provide molecular and structural insights into the effect of

altered restraints in $\beta 1$ integrin on the interaction between glycosylated $\beta 1$ integrin and fibronectin that regulates cell adhesion.

2 Materials and Methods

2.1 Steered molecular dynamics simulations

In this study, we adopted steered molecular dynamics (SMD) simulations to determine the effects of altered restraints in $\beta 1$ integrin on force-regulated interaction of the glycosylated I-like domain of $\beta 1$ integrin with fibronectin III_{9-10}. A relatively larger restraint that mimics the degree of cytoskeletal restrain and a much smaller restrains that mimic the membrane-anchoring effect on $\beta 1$ integrin were separately applied to the $\beta 1$ I-like domain. A total of eight glycosylated $\beta 1$ I-like domain-fibronectin complexes with altered constraints in $\beta 1$ integrin and altered glycosylation states were simulated under either constant velocity (cv-SMD) or constant force (cf-SMD) (Table 1). SMD simulations were performed using the NAMD 2.6 MD package (42) with the AMBER 10 force field, which includes the GLYCAM06 force field for carbohydrates (43). The validated glycosylated $\beta 1$ I-like domain-fibronectin III_{9-10} complex obtained from a previous study (20) was solvated with TIP3P water molecules (44) and 150 mM physiological NaCl concentration in a periodic box. One nm of solvent between the protein and the box boundaries was ensured to reduce potential artifacts arising from periodicity. Prior to SMD simulations, energy minimization and equilibration of the water molecules was performed, and the simulated system was warmed using a standard protocol similar to that of our previous molecular dynamics (MD) simulation studies (20, 21, 45-47). A standard energy minimization of 5000 steps was applied to the solvent and the glycosylated $\beta 1$ I-like domain-fibronectin complex in order to relax internal constraints. After energy minimization, equilibration of water was performed for 10 ps at constant number-pressure-temperature (NpT) with the protein constrained. Thermalization was achieved by heating the system to 300K at 50K increments under constant number-volume-temperature (NvT) over a total of 60 ps at 2 fs intervals. SMD simulations were performed for the system at 300K in order to determine the effects of altered restraints on force-regulated interaction of the glycosylated I-like domain of $\beta 1$ integrin with fibronectin III_{9-10}.

To examine the effect of altered restrains in $\beta 1$ integrin on the interaction of the glycosylated I-like domain of $\beta 1$ integrin with fibronectin III_{9-10}, a harmonic constraint with a force constant of 1 Kcal/mol*Å and 0.01 Kcal/mol*Å was separately applied to the I-like domain of $\beta 1$ integrin at the Asn 303, Asp 120, and Ser 359 residues, which have firm contacts with the adjacent hybrid domain in the integrin structure (48). During the SMD simulations, the pulling force applied to FN-III_{9-10}

Table 1: Simulated systems in SMD study

System	I-like domain constrained (with relatively larger restrain to mimic cytosketal constrains)	I-like domain unconstrained (with much smaller restrain to mimic membrane-anchoring effect on the $\beta 1$ integrin)
Constant Force pulling		
1	Glycosylation without sialic acid (3ANS)	
2		Glycosylation without sialic acid (3ANS)
3	Glycosylation with sialic acid (3ASO)	
4		Glycosylation with sialic acid (3ASO)
Constant Velocity pulling		
5	Glycosylation without sialic acid (3ANS)	
6		Glycosylation without sialic acid (3ANS)
7	Glycosylation with sialic acid (3ASO)	
8		Glycosylation with sialic acid (3ASO)

was similar to that used in previous studies (28, 29, 49-51), which mimics atomic force microscopy (AFM). The pulling force was applied to the N-terminal C_a atom of FN-III_{9-10}, while the C-terminal C_a atom was constrained to a fixed position. The pulling direction was assigned along the vector from the C-terminal C_a to the N-terminal C_a, the so-called extension vector (Figure 1). The pulling force was applied by pulling under constant force (cf-SMD) and constant velocity (cv-SMD) separately. The conformational and structural changes in fibronectin and in the $\beta 1$ I-like domain were observed by cf-SMD simulation. The force in the cf-SMD simulation was set to 14.42 kcal/mol/Å, equivalent to 1000 pN (49), and a total of 225 ps simulations were performed for each case. The pulling force chosen in SMD simulation is several orders higher than the real pulling force in the AFM experiment because of the limitations in simulation timescale. The real pulling in AFM experiment is several orders of longer than SMD simulation (29). Based on the previous SMD simulation study (52), in which the pulling force is from several hundred to thousand, the 1000 pN pulling force was chosed in this SMD study.

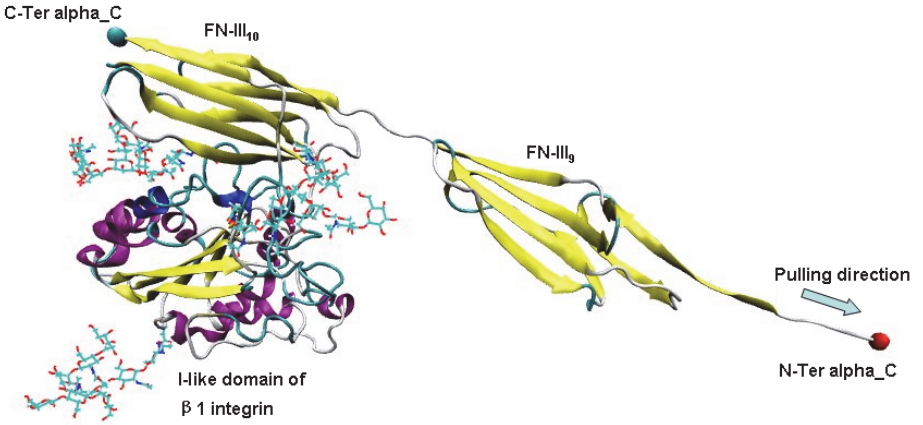


Figure 1: The pulling force was applied to the N-terminal Ca (Gly) of FN-III9-10 in the fibronectin-$\beta 1$ I-like domain complex in constant force SMD simulations. The image was generated with the support of VMD software.

The cv-SMD simulation was performed to calculate the detaching force between the I-like domain and fibronectin, which was used to assess the binding affinity of $\beta 1$ integrin for fibronectin under altered constraints in $\beta 1$ integrin. The pulling speed in the cv-SMD simulation was set to 0.0005nm/ps in the same direction used for cf-SMD simulations over a total of 250 ps. The force experienced by the C_a

atom of the pulled residues was calculated using Equation (1) (50):

$$F = K(vt - x) \tag{1}$$

where K is the spring constant of 7kcal/mol/?^{2} . (53), v is the pulling velocity, t is time, and x is the displacement of the pulled C_a atom from its original position. The force profiles of the glycosylated $\beta 1$ I-like domain-fibronectin complex and the distance profiles between the binding sites of the $\beta 1$ I-like domain and fibronectin over time in the presence of different sialylation states and altered constraints were calculated. Calculated results regarding the effects of altered sialylation of $\beta 1$ integrin on the binding of the $\beta 1$ I-like domain-fibronectin complex were compared with experimental data (15, 27) for validation. The effects of altered constraints in $\beta 1$ integrin on the interaction between the glycosylated $\beta 1$ I-like domain and fibronectin were then further characterized.

2.2 Conformational and structural analyses

$\alpha 5\beta 1$integrin binds to both the RGD and the synergy sites of fibronectin, and the synergy site facilitates preferential binding of $\alpha 5\beta 1$ integrin to fibronectin over other RGD-containing matrix proteins (37-40, 54). Relative conformational changes between the RGD loop and the synergy site in fibronectin play a critical role in the interaction between $\alpha 5\beta 1$ integrin and fibronectin (55-57). Therefore, we calculated the relative rotational orientation and the distance between the RGD loop and the synergy site for the glycosylated $\beta 1$ I-like domain-fibronectin complexes under altered constraints in $\beta 1$ integrin during the cf-SMD simulations. Similar to previous studies (20, 51), the relative rotational orientation was defined as the dihedral angle formed by the $C_a$atoms of residues Arg 1379 in FN-III_9, Arg 1493 in FN-III_{10}, and the centers of mass of FN-III_9 and FN-III_{10}. The distance between the RGD loop and the synergy site was defined as the distance between the $C_a$atoms of Arg 1379 in the synergy site and Arg 1493 in the RGD loop of fibronectin as described in our previous study (20). Changes in the hydrogen bond occupancy of each β-strand pair in FN-III_{9-10} were also calculated for the glycosylated $\beta 1$ I-like domain-fibronectin complexes under altered constraints in $\beta 1$ integrin over the cf-SMD simulations. These results were used to characterize the effects of altered constraints in $\beta 1$ integrin on resistance to structural changes of fibronectin under external force. The I-like domain of $\beta 1$ integrin contains critical functional sites required for ligand binding (32-36). Therefore, we also analyzed the dynamical cross-correlation map between residues of the $\beta 1$ I-like domain for systems with altered constraints. Changes in the degree of correlated motion between residues within the I-like domain could directly affect binding of the $\beta 1$ I-like domain to fibronectin.

3 Results & Discussion

3.1 Effects of altered constraints in $\beta 1$ integrin on interaction of the glycosylated $\beta 1$ I-like domain with fibronectin.

We first validated SMD simulation results regarding the effects of altered sialylation of the $\beta 1$ I-like domain on binding of the $\beta 1$ I-like domain to fibronectin using experimental results (15, 27). The distance between the RGD and SDL regions, as defined in Figure 2A, was used to determine the time at which fibronectin dissociated from the integrin under constant velocity SMD simulations. Under constant velocity SMD simulations, the force required to break the binding of fibronectin from $\beta 1$ I-like domain represents a quantitative parameter that can be used to evaluate the binding affinity between the two proteins. Under SMD simulations with a constant velocity of 0.0005nm/ps, the distance between the RGD and SDL regions began to increase at approximately 190 ps for systems containing glycans without sialic acid (3ANS) and at approximately 175 ps for systems containing glycans with sialic acid (3ASO) under the different constraints in $\beta 1$ integrin, indicative of the initiation of dissociation of the $\beta 1$ I-like domain-fibronectin complex (Figure 2B). Under SMD simulations with a constant velocity of 0.0005nm/ps, for systems with larger constraints, the force required to dissociate the glycosylated $\beta 1$ I-like domain-fibronectin complex was 8511 pN for the glycosylated system without sialic acid (3ANS) and 7546 pN for the glycosylated system with sialic acid (3ASO) (Figure 2C). For the systems without smaller constraints, the force required to dissociate the glycosylated $\beta 1$ I-like domain-fibronectin complex was 7616 pN for the 3ANS system and 6409 pN for the 3ASO system (Figure 2C). These data indicate that de-sialylation of $\beta 1$ integrin increased the resistance of the $\beta 1$ integrin unbinding from fibronectin under external pulling. This result was consistent with experimental results showing that de-sialylation of $\beta 1$ integrin increased binding of $\beta 1$ integrin to fibronectin (15, 27). Before the dissociation of $\beta 1$ I-like domain with fibronectin for 3ANS system or 3ASO system, the distance between the binding sites showed constant and stable (Figure 2B), however, the system experiences the peak of the force at $\sim$ 15 ps and 60 ps (Figure 2C). The peak of the force experienced in the systems under external pulling is directly related to the breakage of the secondary structure of fibronectin and possibly the secondary structure of $\beta 1$ I-like domain. Results from simulations assessing the distance between the RGD and SDL regions (Figure 2B) over constant velocity SMD simulations also indicated that the systems with larger constraints that mimic cytoskeletal restrains were more resistant to dissociation of the $\beta 1$ I-like domain-fibronectin complex under external pulling in comparison to systems with smaller constraints that mimic the membrane anchoring of the integrin irrespective of the presence or absence of sialic

acid-containing glycans. Force calculations (Figure 2C) revealed that the force required for dissociation of the $\beta 1$ I-like domain-fibronectin complex was larger for the system with larger constraints compared to the system with smaller constraints for both the 3ANS and 3ASO systems. These data demonstrate that the larger constraints in $\beta 1$ integrin enhance resistance to dissociation of the $\beta 1$ I-like domain-fibronectin complex by external force.

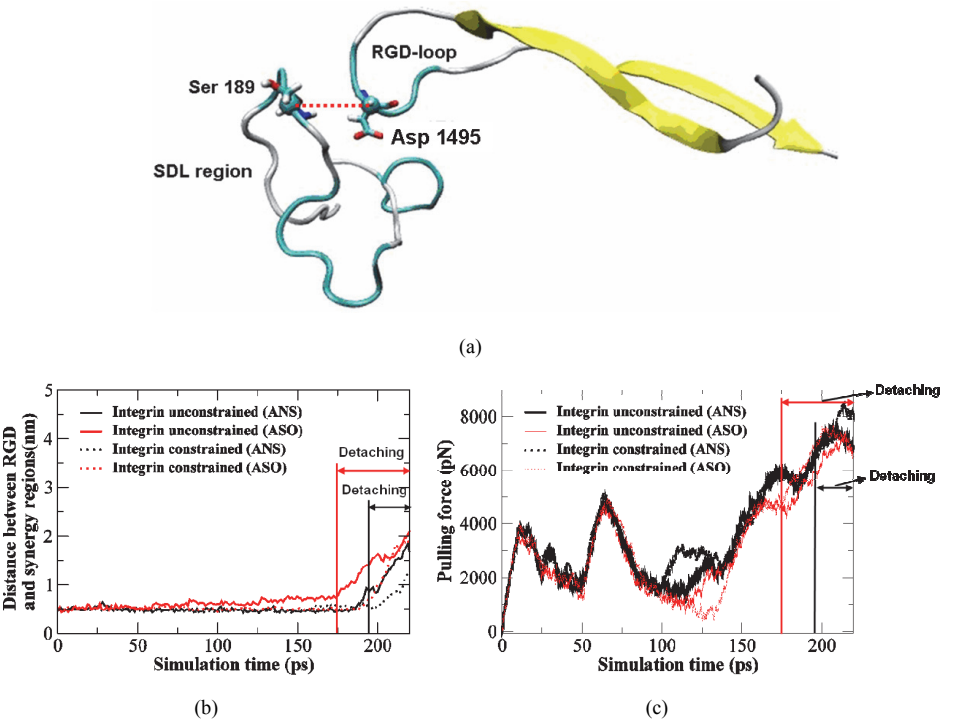


Figure 2: Distance between the binding sites and force experienced in the glycosylated $\beta 1$ I-like domain-fibronectin complexes over constant velocity SMD simulations. (A) Definition of the distance between the RGD and SDL regions. Distance between the RGD and SDL regions was defined as the distance between the Ca atom of Ser 189 in the SDL region of the $\beta 1$ I-like domain and the Ca atom of Asp 1495 in the RGD loop of Fn-III10. (B) Distance between the RGD and SDL regions for complexes glycosylated without sialic acid (3ANS) and with sialic acid (3ASO) with the altered constraints in $\beta 1$ integrin. (C) Force experienced by $\beta 1$ I-like domain-fibronectin complexes glycosylated without sialic acid (3ANS) and with sialic acid (3ASO) with the altered constraints in $\beta 1$ integrin.

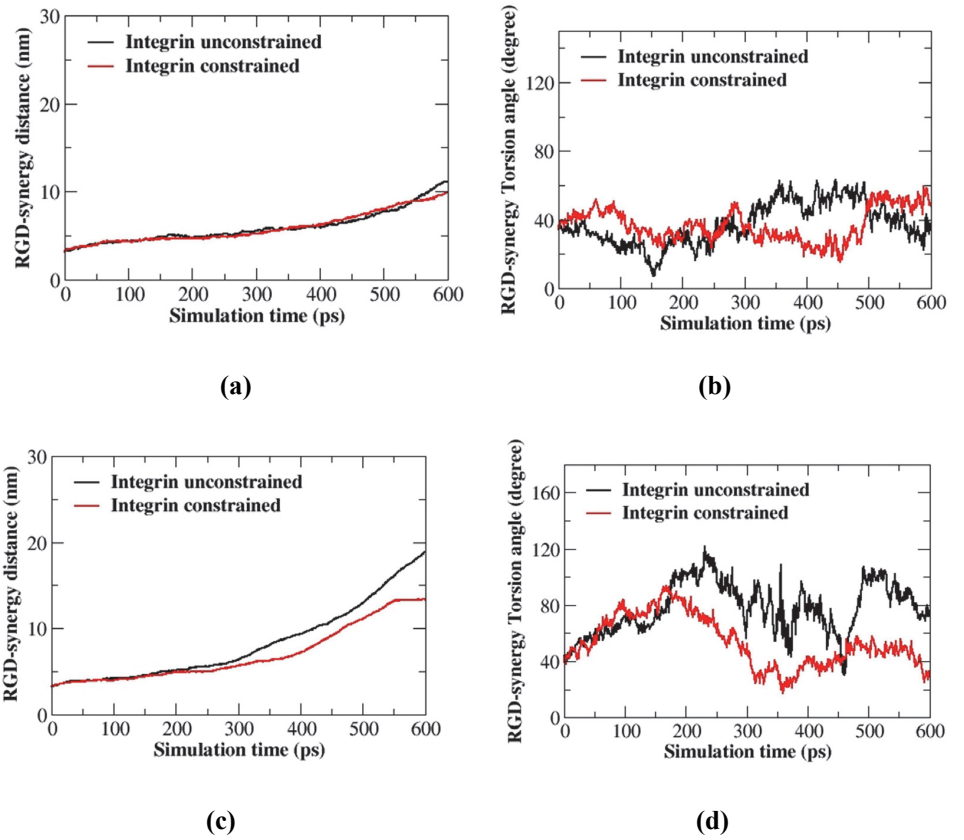


Figure 3: Effects of sialylation and constraints in $\beta 1$ integrin on the conformation of fibronectin. (A) Distance between the RGD loop and the synergy site in the unsialylated $\beta 1$ I-like domain-fibronectin complex (3ANS). (B) Torsional orientation between the RGD loop and the synergy site in the unsialylated $\beta 1$ I-like domain-fibronectin complex (3ANS). (C) Distance between the RGD loop and the synergy site in the sialylated $\beta 1$ I-like domain-fibronectin complex (3ASO). (D) Torsional orientation between the RGD loop and the synergy site in the sialylated $\beta 1$ I-like domain-fibronectin complex (3ASO).

4 Conformational and structural changes in fibronectin induced by altered cytoskeletal constraints.

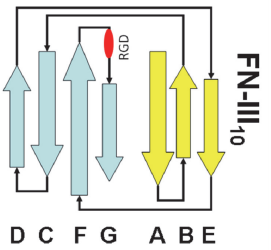
4.1 Interactive conformational changes in the RGD-synergy site.

Studies have suggested that the relative conformational changes between the RGD loop and synergy site in fibronectin play a critical role in the interaction between

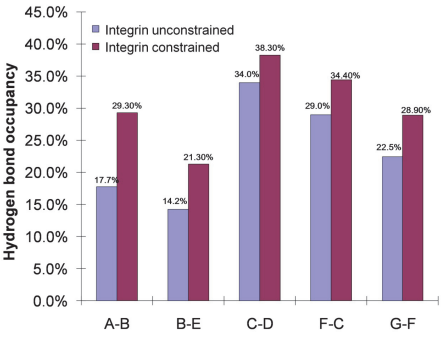
$\alpha 5\beta 1$ integrin and fibronectin (55-57). Using constant force SMD simulations, we calculated the relative rotational orientation and the distance between the RGD loop and the synergy site of the glycosylated $\beta 1$ I-like domain-fibronectin complex in the altered constraints for both the 3ANS and 3ASO systems (Figure 3). These results showed that changes in the distance and orientation between the RGD loop and the synergy site were much smaller in the $\beta 1$ I-like domain-fibronectin complex with unsialylated glycosylation (3ANS) compared to the complex with sialylated glycosylation (3ASO) at the difference degree of constrains in $\beta 1$ integrin under external force (Figure 3). These data indicate that de-sialylation of $\beta 1$ integrin enhances the resistance to relative conformational changes in the RGD-synergy site under external force. These results are consistent with experimental observations showing that de-sialylation of $\beta 1$ integrin increases binding of $\beta 1$ integrin to fibronectin (15, 27). We also evaluated the effects of altered constraints in $\beta 1$ integrin on changes in the distance and orientation between the RGD loop and the synergy site under the cf-SMD simulations. For the glycosylated system without sialic acid (3ANS), changes in the constraints did not significantly affect the interactive conformational changes in the RGD-synergy site (Figure 3A, B). This observation could be related to a potential role for de-sialylation of $\beta 1$ integrin in stabilizing the relative conformational change in the RGD-synergy site under external force. However, for the glycosylated system with sialic acid (3ASO), the larger constraints resulted in a slower rate of increase in the distance between the RGD loop and the synergy site (Figure 3C) and a smaller torsional angle between the RGD loop and the synergy site under external force (Figure 3D). These data indicate that the larger constraints in $\beta 1$ integrin enhance the resistance to relative conformational changes in the RGD-synergy sites under external force. Due to the important role of relative conformational changes between the RGD loop and the synergy site in the $\beta 1$ integrin–fibronectin interaction, we concluded that the altered constraints in $\beta 1$ integrin may directly affect force-regulated binding of $\beta 1$ integrin to fibronectin.

4.2 Characterization of the hydrogen bond occupancy of each β-strand pair in FN-III$_{10}$.

The FN-III$_{10}$ structural model shows that FN-III$_{10}$ is constructed of a series of parallel β-strands (Figure 4A) (41) . The $\beta 1$ integrin binding site in the RGD loop of FN-III$_{10}$ connects the F and G β-strands to form the F-G β-strand pair (Figure 4). The hydrogen bonds formed between these β strands play an important role in the structural stability of fibronectin. Therefore, we next analyzed the occupancy of the hydrogen bonds between the β-strands in FN-III$_{10}$ in constant force SMD simulations in order to evaluate the secondary structural stability of fibronectin in



(a)



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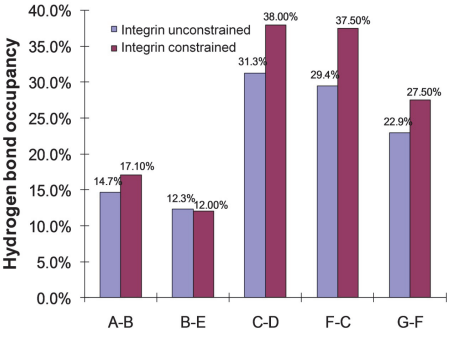
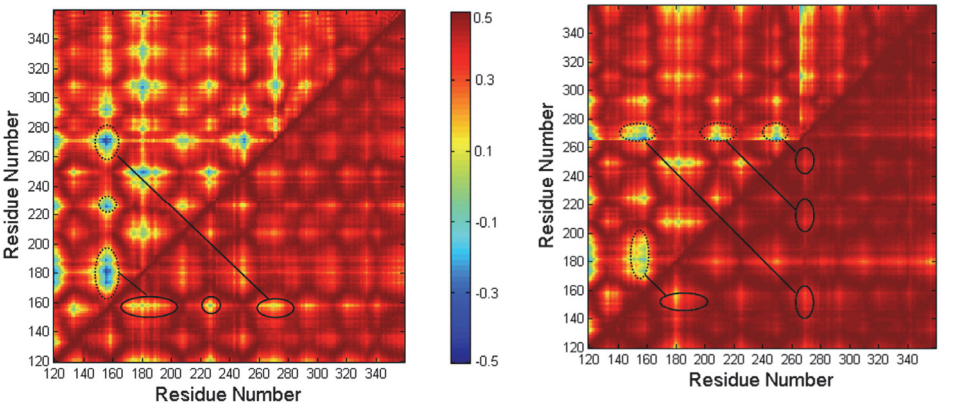


(d)

Figure 4: Effects of sialylation and cytoskeletal constraints on the hydrogen bond occupancy of β-strands in fibronectin. (A) The secondary structure of FN-III10 [20]. (B) Hydrogen bond occupancy of each β-strand pair in fibronectin over the constant force SMD simulation for complexes containing unsialylated glycans (3ANS) with the altered constraints in $\beta 1$ integrin. (C) Hydrogen bond occupancy of each β-strand pair in fibronectin over the constant force SMD simulation for complexes containing sialylated glycans (3ASO) with the altered constraints in $\beta 1$ integrin.

the glycosylated $\beta 1$ I-like domain-fibronectin complex under altered constraints in $\beta 1$ integrin (Figure 4). The occupancy of each hydrogen bond was calculated based on the percentage of time that the hydrogen bond existed over the entire simulation time. The hydrogen bond occupancy of each β-strand pair in FN-III$_{10}$ was considered to be the average occupancy of the hydrogen bonds of each β-strand pair. Results from this analysis revealed that during the constant force SMD simulations, a higher percentage of hydrogen bond occupancy was observed for each β-strand pair in FN-III$_{10}$ for systems with larger constraints compared to those



(a) (b)

Figure 5: Dynamical cross-correlation maps comparing the degree of correlated motion of residues in the glycosylated I-like domain with the altered constraints in $\beta 1$ integrin. Red indicates correlation between residues, and blue indicates anti-correlation between residues. (A) Comparison of correlated motion in the I-like domain with smaller constraints (top left) and the I-like domain with larger constraints (bottom right) of $\beta 1$ I-like domain-fibronectin complexes containing unsialylated glycans (3ANS). B. Comparison of correlated motion in the I-like domain with smaller constraints (top left) and the I-like domain with smaller constraints (bottom right) of $\beta 1$ I-like domain-fibronectin complexes containing sialylated glycans (3ASO).

with smaller constraints both in the presence and absence of sialic acid (3ASO and 3ANS, respectively; Figure 4B, C). These results indicate that the larger constraints on $\beta 1$ integrin increase the conformational stability of fibronectin, protect against changes in the secondary structure of fibronectin, and prevent the dissociation of the glycosylated $\beta 1$ integrin-fibronectin complex by external force.

Based on the calculated results regarding the effects of altered constraints on the interactive conformational changes in the RGD-synergy site and the changes in hydrogen bond occupancy of each β-strand pair in FN-III_{10}, we concluded that larger constraints in $\beta 1$ integrin enhances resistance to alterations in the secondary structure of fibronectin, maintains a more stable interactive conformation in the RGD-synergy site, and promotes a more stable integrin-fibronectin complex. These data provide molecular insight into the role of altered restrains in $\beta 1$ integrin in modulation of force-regulated binding between $\beta 1$ integrin and fibronectin during cell adhesion, as observed in experimental studies (17-19).

4.3 Dynamical motion changes in the $\beta 1$ I-like domain induced by altered constraints in $\beta 1$ integrin.

In addition to characterizing the conformational and structural changes in fibronectin induced by altered constraints applied to the integrin-fibronectin complex using constant force SMD simulations, we also evaluated the dynamical motion changes in the $\beta 1$ I-like domain induced by alterations in constraints with constant force SMD simulations. These motion changes could directly contribute to binding of $\beta 1$ integrin to fibronectin (Figure 5). Figure 5 shows dynamical cross-correlation maps that compare the degree of correlated motion of the residues of the I-like domain of β-integrin under different constraints (red: correlation between residues; blue: anti-correlation between residues; top left: with smaller restrains constraints; bottom right: with larger constraints). For both the unsialylated (3ANS; Figure 5A) and sialylated (3ASO; Figure 5B) systems, application of larger constraints resulted in increased correlated motion between the residues in the $\beta 1$ I-like domain compared to that of systems with smaller constraints (Figure 5). Multiple changes in correlated motion between the key residues in the $\beta 1$ I-like domain were induced by altered constraints, including changes between res 150-161 and res 170 to 197 (SDL region), res 150-161 and res 260-280, and res 150-161 and res 220-230 for the 3ANS system (Figure 5A), and changes between res 150-161 and res 170 to 197, res 150 to 161 and res 260-300, res 205-220 and res 260-300, and res 260-300 and res 340-360 for the 3ASO system (Figure 5B). Changes in correlated motion of the $\beta 1$ I-like domain induced by alterations in constraints in $\beta 1$ integrin could directly affect binding of $\beta 1$ integrin to fibronectin.

5 Conclusion

In this study, we characterized the effects of altered restraints on force-regulated interaction of the glycosylated $\beta 1$ I-like domain with fibronectin III_{9-10} using SMD simulations. We performed both constant velocity and constant force SMD simulations for $\beta 1$ I-like domain-fibronectin complexes with variations in glycosylation under different constraints. The calculated results from these analyses showed that de-sialylation of $\beta 1$ integrin enhances the resistance to relative conformational changes in the RGD-synergy site and decreases dissociation of fibronectin from $\beta 1$ integrin with the different degree of constraints applied to the integrin under external force. These results are consistent with experimental results showing that de-sialylation of $\alpha 5\beta 1$ integrin increases the binding affinity of $\beta 1$ integrin for fibronectin (15, 27). The calculated results also revealed that application of larger constraints to $\beta 1$ integrin enhanced the resistance to dissociation of the $\beta 1$ I-like domain-fibronectin complex under external force. The larger constraints applied

on β1 integrin enhanced resistance to relative conformational changes in the RGD-synergy site in fibronectin, increased the conformational stability of fibronectin, prevented losses in hydrogen bond occupancy of each β-strand pair of FN-III$_{10}$ under external force, and inhibited dissociation of the glycosylated β1 integrin-fibronectin complex under external force. The larger constraints in β1 integrin also resulted in the increased levels of correlated motion between residues in the β1 I-like domain compared to those of systems with smaller constraints, which could directly affect binding of β1 integrin to fibronectin. These data provide molecular and structural insight into the role of altered constraints in β1 integrin in mediating the force-regulated interaction of β1 integrin with fibronectin in cell adhesion, as observed in experimental studies (17-19). These results also suggest that modulation of the constraints on the integrin through force or other methods could be used as a strategy to regulate the β1 integrin-fibronectin complex and its cellular activities.

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