Structural Basis and Kinetics of Force-Induced Conformational Changes of an αA Domain-Containing Integrin

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Abstract

Background: Integrin αβ2 (lymphocyte function-associated antigen, LFA-1) bears force upon binding to its ligand intercellular adhesion molecule 1 (ICAM-1) when a leukocyte adheres to vascular endothelium or an antigen presenting cell (APC) during immune responses. The ligand binding propensity of LFA-1 is related to its conformations, which can be regulated by force. Three conformations of the LFA-1 αA domain, determined by the position of its α7-helix, have been suggested to correspond to three different affinity states for ligand binding.

Methodology/Principal Findings: The kinetics of the force-driven transitions between these conformations has not been defined and dynamically coupled to the force-dependent dissociation from ligand. Here we show, by steered molecular dynamics (SMD) simulations, that the αA domain was successively transitioned through three distinct conformations upon pulling the C-terminus of its α7-helix. Based on these sequential transitions, we have constructed a mathematical model to describe the coupling between the αA domain conformational changes of LFA-1 and its dissociation from ICAM-1 under force. Using this model to analyze the published data on the force-induced dissociation of single LFA-1/ICAM-1 bonds, we estimated the force-dependent kinetic rates of interstate transition from the short-lived to intermediate-lived and from intermediate-lived to long-lived states. Interestingly, force increased these transition rates; hence activation of LFA-1 was accelerated by pulling it via an engaged ICAM-1.

Conclusions/Significance: Our study defines the structural basis for mechanical regulation of the kinetics of LFA-1 αA domain conformational changes and relates these simulation results to experimental data of force-induced dissociation of single LFA-1/ICAM-1 bonds by a new mathematical model, thus providing detailed structural and kinetic characterizations for force-stabilization of LFA-1/ICAM-1 interaction.

Citation: Xiang X, Lee C-y, Li T, Chen W, Lou J, et al. (2011) Structural Basis and Kinetics of Force-Induced Conformational Changes of an αA Domain-Containing Integrin. PLoS ONE 6(11): e27946. doi:10.1371/journal.pone.0027946

Editor: Donald Gullberg, University of Bergen, Norway

Received June 14, 2011; Accepted October 28, 2011; Published November 28, 2011

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Funding: This work was supported by Natural Science Foundations of China under 31070827 (to J. L.) by the Knowledge Innovation Program of the Chinese Academy of Sciences (to J. L.) and by a National Institutes of Health (NIH) grant AI44902 (to C.Z.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Integrins are a family of heterodimeric transmembrane receptors composed of an α and a β subunit that involve in a wide variety of physiological processes such as cell adhesion, cell migration and immunoresponse [1]. They usually bear forces upon binding to ligands in cell-cell and cell-extracellular matrix adhesions, which are crucial to mechanosensing and mechanotransduction of cells [2,3]. Of the 24 known human integrins, 10 of them, including the integrin αβ2 or lymphocyte function-associated antigen 1 (LFA-1) studied here, have an additional αA (or αL) domain in the β-propeller domain of the α subunit, where the ligand binding site resides [4]. By binding intercellular adhesion molecule 1 (ICAM-1), LFA-1 mediates adhesion of leukocytes to the blood vessel wall or antigen presenting cells (APC), and sustains forces generated by the blood flow or the cell’s motile machinery [1,5].

In response to various biochemical [3,4,6] and mechanical signals [7,8], integrins change conformations and ligand binding affinities. In physiological condition, they may assume a bent conformation and have a low ligand binding affinity. Inside-out signaling or changes in the metal ion conditions from Ca2+/Mg2+ to Mn2+ result in integrin conformational change to an extended form, with a closed or swung-out hybrid domain, accompanied by a higher ligand binding affinity [Fig. 1A, 1B] [3,4,9].

In addition to global conformational changes in the whole ectodomain and in the hybrid domain, the αA domain conformation also controls the affinity of αA-containing integrins such as LFA-1 [5,9]. Several αA domains, including that of LFA-1, have been crystallized [10–15], revealing as many as three
conformations termed closed, intermediate and open, depending on the position of the C-terminal α7-helix [5] (Fig. 1C). As measured by surface plasmon resonance [5] and micropipette adhesion frequency assay [6], LFA-1 with the αA domain locked in the intermediate and open conformations have hundreds and thousands folds higher affinities for ICAM-1, respectively, than that locked in the closed conformation. A study of molecular dynamics (MD) simulations of αA domains with implicit water suggested that the fractions of these three conformation states are sensitive to the force applied to the C-terminus. A domain. We used constant-force steered molecular dynamics (SMD) simulations to observe the sequential transitions of the α7-helix position, we simulated structure and its initial “up” position, which corresponds to the “closed” conformation of the αA domain [16]. Pulling the α7-helix C-terminus in the first 3.6 ns only increased the RMSD slightly, indicating the stability of the “up” position (Fig. 2A, 2B). A sudden increase of the RMSD from 3 to 6 Å was then observed during 3.4–4 ns simulations, suggesting state transitions. Zooming in this transition phase with a magnified time scale, a stable “intermediate” α7-helix position with a 4.5 Å RMS was observed (Fig. 2A inset, 2C). This “intermediate” α7-helix position is linked to the “intermediate” conformation of the αA domain. After two abrupt increments, the RMSD was stabilized at around 8 Å for the next 10 ns, corresponding to a “down” position of the α7-helix and the “open” conformation of the αA domain (Fig. 2A, 2D). After the pulling force was removed at the 15 ns time point, the α7-helix returned back from the “down” position to the “up” position in a few nanoseconds and remained up within the next 20-ns simulations (Video S1).

Besides the α7-helix position, another remarkable difference between the open and closed conformation of LFA-1 αA domain revealed by structural studies is the metal ion position at the metal ion dependent adhesion site (MIDAS). It was observed that in the open conformation, the MIDAS metal ion underwent inward movement for about 2 Å. Previous implicit water molecular dynamics simulations suggested that the movement of α7-helix and that of the MIDAS metal ion were coupled. Hence, we measured the RMSD of the MIDAS metal ion and other important residues between the simulated structures and the open or closed conformations (Figure S1). These included residues S139, S141, T206, and D239 that coordinated the MIDAS metal ion and residues L289, F292, and L295 that formed a “ratchet”-like structure to define the position of the α7-helix. In the simulations, although the pulling force induced movements of the α7-helix, no movements of the MIDAS metal ion were observed (Figure S1B), nor were their coordinating residues (Figure S1C–F). Nevertheless,

**Results**

**SMD-simulated force-induced conformation transitions of LFA-1 αA domain**

To study the force-induced conformational transitions of the LFA-1 αA domain, we used constant-force SMD simulations to pull the C-terminus of its α7-helix, as the position of the tension-bearing α7-helix determines the αA domain conformation [5,16].

Unlike the previous implicit water simulations [16], our simulations included physiologically relevant water molecules. To observe the sequential transitions of the α7-helix position, we quantified the root mean square distance (RMSD) between the simulated structure and its initial “up” position, which corresponds to the “closed” conformation of the αA domain [16]. Pulling the α7-helix C-terminus in the first 3.6 ns only increased the RMSD slightly, indicating the stability of the “up” position (Fig. 2A, 2B). A sudden increase of the RMSD from 3 to 6 Å was then observed during 3.4–4 ns simulations, suggesting state transitions. Zooming in this transition phase with a magnified time scale, a stable “intermediate” α7-helix position with a 4.5 Å RMS was observed (Fig. 2A inset, 2C). This “intermediate” α7-helix position is linked to the “intermediate” conformation of the αA domain. After two abrupt increments, the RMSD was stabilized at around 8 Å for the next 10 ns, corresponding to a “down” position of the α7-helix and the “open” conformation of the αA domain (Fig. 2A, 2D). After the pulling force was removed at the 15 ns time point, the α7-helix returned back from the “down” position to the “up” position in a few nanoseconds and remained up within the next 20-ns simulations (Video S1).

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we did observe the relevant “ratchet”-like movements on residues L289, F292, and L295 (Figure S1G–I), which followed the movements of the $\alpha_7$-helix. Residue D239 coordinated directly with the MIDAS metal ion in the closed conformation as observed in the crystal structures [11,12]. On the other hand, in the open conformation, D239 might not coordinate with metal ion directly but through a water molecule. In our pulling simulation, it seemed that the strong ionic interaction between D239 and the metal ion constrained the metal ion at its closed (outward) position, thus preventing the inward movement from being observed within the short timescale of the simulation. To test this hypothesis, we performed a set of three simulations. These simulations started from the structures generated from the above pulling simulations. The snapshots at 0, 3.7 and 16 ns were taken as the respective new starting points. Among them, the 0 ns configuration represented the “up” position of the $\alpha_7$-helix, the 3.7 ns configuration represented the “middle” position and the 16 ns one represented the “down” position. In these free dynamics simulations, the applied force was released. To prevent the $\alpha_7$-helix from returning back to the “up” position in the simulations starting from 3.7 and 16 ns snapshots, we constrained the Cα atoms of the $\alpha_7$-helix in addition to the original constraint residues. Firstly, 30 ns free dynamics simulations were performed followed by 20 ns free dynamics simulations with the point charges of the two oxygen atoms of D239 carboxyl group reduced by 0.5e each. As shown in Fig. 3 with the RMSD time courses of the MIDAS ion between the simulated structure and its closed or open positions, in all three simulations, the MIDAS ions fluctuated around their closed position without any tendency to move towards the open position before the point charges were reduced. By comparison, after the point charges of the D239 carboxyl oxygen were reduced, in the simulations starting from 3.7 ns ($\alpha_7$-helix at middle position) and 16 ns ($\alpha_7$-helix at down position) (Fig. 3B and 3C), the metal ion showed strong tendencies to move inward towards the open position, with the RMSD to the closed position reduced and that to the open position increased. For the simulation starting from 0 ns ($\alpha_7$-helix at down position) (Fig. 3A), the movement was also possible (30–32 ns and 44–46 ns in Fig. 3A), but the duration was short. The simulated structure fluctuated around the closed position for the majority of simulation times. These simulations confirm that the position of the metal ion is related to the position of the $\alpha_7$-helix, consistent with the generally accepted contention that the position of the metal ion determines the ligand binding affinity of the $\alpha A$ domain.

These results support the hypothesis that the closed, intermediate and open conformations of LFA-1 $\alpha A$ domain represent stable states and that sequential transitions from the closed to intermediate and from intermediate to open conformations can be induced by pulling the $\alpha_7$-helix.

Mathematical model for force-induced interstate transition of LFA-1 and ICAM-1 dissociation

Our SMD simulations suggested that the LFA-1 $\alpha A$ domain transitioned from the closed, intermediate and open conforma-
tions successively by applied force (Fig. 2). To incorporate such conformational change kinetics into the kinetics of force-induced ligand dissociation, we constructed a mathematical model for the BFP force-clamp experiment in which single LFA-1/ICAM-1 bonds were pulled with a constant force until rupture [9]. This simple model considers two interstate transition steps: from state \( C_1 \) to state \( C_2 \) and from state \( C_2 \) to state \( C_3 \) (Fig. 4) as well as three ligand dissociation steps from each of the three states. Each of these steps is assumed irreversible, which seems reasonable under force, as force drives unidirectionally both the interstate transition and ligand dissociation.

The model results in a set of coupled, linear, first-order, ordinary differential equations (Equations 1–3, Materials and Methods) governing the changes of the probabilities of the LFA-1/ICAM-1 bond in the three states in time, with constant coefficients (functions of force but not time): two interstate transition rates, \( k_{12} \) and \( k_{23} \), as well as three reverse-rates \( k_{r1} \), \( k_{r2} \) and \( k_{r3} \). The equations were solved analytically (Equations 7–9, Materials and Methods). The solution was fit to the data of the BFP force-clamped experiments [9] to obtain three apparent dissociation rate constants \( k_1 \), \( k_2 \), \( k_3 \) and their associated apparent fractions \( v_1 \), \( v_2 \), \( v_3 \) (summarized in Tables S1, S2, S3, S4, S5, S6). The intrinsic parameters are expressed as functions of the apparent parameters (Equations 20–24, Materials and Methods) and evaluated at different forces. Since the unique force-stabilizing catch-bond behavior of LFA-1/ICAM-1 interaction occurred at the force regime of about 10 pN, only the data below 20 pN were analyzed and shown (Figs. 5, 6, 7).

Analysis of force-dependent ICAM-1 dissociation reveals characteristics of three LFA-1 states

The intrinsic reverse-rates, \( k_{r1}–k_{r3} \), of ICAM-1 dissociation from the three LFA-1 states were plotted versus force in Fig. 5 in the range analyzed. They follow trends similar to the apparent off-rates determined previously [9], but are quantitatively different. Interestingly, ICAM-1 dissociated from state \( C_1 \) with the highest but least force-sensitive reverse-rate \( k_{r1} \) (Fig. 5A), from state \( C_2 \) with an intermediate reverse-rate \( k_{r2} \) that has an intermediate force sensitivity (Fig. 5B), and from state \( C_3 \) with the lowest but most force-sensitive reverse-rate \( k_{r3} \) (Fig. 5C). Although the model assumes that all bonds start from state \( C_1 \) and then proceed successively to states \( C_2 \) and \( C_3 \), the \( k_{12}–k_{23} \) values were evaluated from data without assuming a priori their relative values and relative sensitivities to force. It is therefore gratifying that our analysis of the previous BFP experimental data [9] with the present model returns the results that state \( C_1 \) is short-lived, state \( C_2 \) is intermediate-lived, and state \( C_3 \) is long-lived. These results indicate a correlation between the experimentally observed short-, intermediate- and long-lived states of LFA-1/ICAM-1 bonds and the SMD-simulated closed, intermediate, and open conformations of the LFA-1 \( \alpha \)A domain (Fig. 2).

The force dependencies of all these intrinsic reverse-rates follow the Bell model [17], as indicated by the linear reverse-rates vs. force semi-log plots. They were indifferent to cation conditions \( \text{Ca}^{2+}/\text{Mg}^{2+} \), \( \text{Mg}^{2+}/\text{EGTA} \) or \( \text{Mn}^{2+} \), suggesting that the initial parameters are expressed as functions of the apparent parameters (Equations 20–24, Materials and Methods) and evaluated at different forces. Since the unique force-stabilizing catch-bond behavior of LFA-1/ICAM-1 interaction occurred at the force regime of about 10 pN, only the data below 20 pN were analyzed and shown (Figs. 5, 6, 7).

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global conformation of the LFA-1 before it was liganded and stressed did not affect the intrinsic dissociation rates.

Force-dependent kinetics of LFA-1 transitions from short- to intermediate- to long-lived states and inhibition by XVA143

Interestingly, our kinetic analysis found that the transition rate $k_{12}$ from the short- to intermediate-lived states of LFA-1/ICAM-1 bonds (Fig. 6) was zero at zero force but increased with force in the range studied (Fig. 6A). Force also enhanced the transition rate $k_{23}$ from the intermediate- to long-lived states of LFA-1/ICAM-1 bonds from its zero value at zero force (Fig. 6B), but to a lesser extent (compare the two Fig. 6 panels). In the force regime studied (<20 pN), the force-dependent interstate transition rates were indifferent to the cation conditions Ca$^{2+}$/Mg$^{2+}$, Mg$^{2+}$/EGTA, or Mn$^{2+}$, thus were not affected by the initial global conformation of the LFA-1 molecule before it was liganded and stressed.

With XVA143, a small molecule antagonist that blocks the interaction between the $\alpha$A and $\beta$A domains [18,19], the force-dependent $k_{12}$ was suppressed (Fig. 6A, red). The transition from the intermediate- to long-lived states of LFA-1 was nearly completely blocked by XVA143, as shown by the zero $k_{23}$ in the force range studied (Fig. 6B, red). A possible explanation for this result may be that the force applied on the $\alpha_7$-helix to induce the conformational changes has to be transmitted through the main chain of the protein.

Figure 5. Force-dependent reverse-rates of three states under different cation conditions. Intrinsic reverse-rates $k_r1$ (A), $k_r2$ (B) and $k_r3$ (C) of ICAM-1 dissociating from respective LFA-1 states C1, C2, and C3 (see Fig. 3) were estimated by fitting the experimental data from Ref. [9] with our kinetic model (equations 22–24) and plotted versus force at indicated cation conditions.

doi:10.1371/journal.pone.0027946.g005

Figure 6. Force-dependent interstate transition rates. (A) The rate of transition of ICAM-1-bound LFA-1 from short- to intermediate-lived states ($k_{12}$) is accelerated by force. This force-accelerated transition rate is suppressed by XVA143 (red). (B) The transition rate of ICAM-1-bound LFA-1 from intermediate- to long-lived states ($k_{23}$) is accelerated by force, which is nearly completely blocked by XVA143 (red). The force-dependency of transition rates is not affected by cation conditions.

doi:10.1371/journal.pone.0027946.g006
connection between αA and βA domains. Further, the suppression and blocking effects of XVA143 on k12 and k23 were not affected by the cation conditions.

Force decelerates LFA-1 dissociation from ICAM-1 by accelerating LFA-1 activation

With the intrinsic parameters $k_{12}$, $k_{23}$, and $k_{11}$–$k_{3}$ estimated, we used equations 1–3 to study the dynamic evolution of LFA-1/ICAM-1 bonds and of individual conformation states and their overall behavior. As shown with representative model predictions for the Mg$^{2+}$/EGTA condition, ligand dissociation manifests as decrease in time of the total survival probability of an LFA-1/ICAM-1 bond in all three states (Fig. 7A and B). The decay of the curve is decelerated by force from 0 to 5.9 pN (Fig. 7A). This is not surprising since this force range corresponds to the experimentally observed catch-bond regime where the bond lifetimes are prolonged by force [9]. As force further increases, the decay of the bond survival probability is accelerated by force (Fig. 7B), corresponding to the slip-bond regime where the bond lifetimes are shortened by force, also observed experimentally [9]. Similar trends are predicted for other cation conditions (data not shown).

Remarkably, our analysis predicts that as force increases, the probability vs. time curves of the long-lived LFA-1/ICAM-1 bonds (C3 state) are left-shifted, as the slope of the initial phase is increased and the time needed to reach the maximal probability is shortened by 10-folds, from $\geq 3$ s to $\sim 0.3$ s (Fig. 7C). Since LFA-1 with an open αA domain binds ligand with the highest affinity [5] and the lowest reverse-rate (Fig. 5C), this result indicates that force accelerates the activation of LFA-1/ICAM-1 bond by increasing the interstate transition rates.

Discussion

As primary force-bearing molecules governing cell-cell and cell-matrix adhesions [2,5], integrins are tightly regulated biochemically [3,4,6] and mechanically [7,8] via their dynamic conformational changes. The closed, intermediate and open conformations of the integrin LFA-1 αA domain metal ion dependent adhesion site (MIDAS) have been observed crystallographically to couple with the up, middle and down positions of its $\alpha_7$-helix position [5]. The distribution among these conformations has been observed by MD simulations to depend on force [16]. The present work has added to this body of literature by defining the sequential process of the force-induced conformational changes of the LFA-1 αA domain and modeling the coupled kinetics of interstate transition between, and ligand dissociation from, different LFA-1 states.

Unlike the previous implicit water SMD study that analyzed the cluster distribution of αA domain conformations at the end of force application [16], our explicit water SMD simulations have observed the sequential transitions of the αA domain under force: Upon pulling the LFA-1 αA domain C-terminus, the $\alpha_7$-helix successively moved from the up to middle and down positions (Fig. 2). Our reduced charge simulations suggest that when $\alpha_7$-helix stays in middle or down position, the MIDAS ion has a strong tendency to move inward to its open position, which binds ligand with high affinity. These simulations indicate that applied force results in successive changes from the closed to intermediate and open conformations.

The force-induced transition of the three αA domain conformations observed in our simulations correlates with the force-dependent three-state dissociation observed in our previous BFP experiment [9]. Another interesting simulation result is that the $\alpha_7$-helix relaxed back to the up position after force removal (Fig. 2A), suggesting that force is required to maintain its intermediate and down conformations under the simulation conditions. This also correlates with the experimental observation that the LFA-1/ICAM-1 reverse-rate at zero-force was indifferent to changes in cation conditions and XVA143 treatment [9]. These correlations support our hypothesis that while the ICAM-1 association on-rate depends on the global conformations of LFA-1, the ligand dissociation off-rate is primarily determined by the αA domain conformation, which has been supported by experiment [9].

We constructed a mathematical model to further test this hypothesis, by examining how the three αA domain conformational transition may be related to the three-state dissociation kinetics. The model assumes force-induced successive transitions from C1 to C2 and C3 states (Fig. 4), in accordance with the SMD results. Comparing to the previous phenomenological treatment, which fitted the force-dependent lifetime distributions by three apparent off-rates and their associated static fractions [9], the present mechanistic model treats the coupled kinetics of both

Figure 7. Predicted time courses of LFA-1/ICAM-1 bond survival probability at different forces. (A and B) The total survival probability of LFA-1/ICAM-1 bond (sum of all three states) decayed slower as the force increased from 0 to 5.9 pN (A) and decayed faster as force increased further (B). (C) The force-dependent time courses of the survival probability of the long-lived state (C3). The presence of C3 state was induced by force, indicating activation of LFA-1 by force applied via the ICAM-1 bond. As force increased from 0 to 17.1 pN, the time needed to reach the maximal probability was shortened with the maximum level increased, indicating shorter activation time with higher activity. Data obtained in Mg$^{2+}$/EGTA were taken as representative parameters for the model prediction.

doi:10.1371/journal.pone.0027946.g007
interstate transition and ligand dissociation. This new model advances our knowledge in several aspects.

First, analyzing the previous BFP experiments [9] with this model has shown that the stability of LFA-1/ICAM-1 bonds are lower at $C_1$, intermediate at $C_2$, and highest at $C_3$ states, suggesting a correspondence of the short-, intermediate- and long-lived states with the closed, intermediate, and open conformations, respectively. Incorporating other forms of integrin conformational changes and relating them to functionality will be an important subject of future studies.

Second, the previously proposed allosteric mechanism for the LFA-1/ICAM-1 catch-slip bond [9] can be fully accounted for using the newly evaluated intrinsic parameters. Indeed, although the force-dependent dissociation of ICAM-1 from each of the three states behaves as slip bonds (Fig. 5), force accelerates transition from $C_2$ to $C_3$ more than it does dissociation from $C_2$ to R+L (compare Figs. 5A and 6A). Force also increases transition rate $k_{23}$ from $C_2$ to $C_3$ comparably to it does dissociation rate $k_{21}$ from $C_2$ to R+L (compare Figs. 5B and 6B). This interplay between force-accelerated interstate transition and dissociation gives rise to the LFA-1/ICAM-1 catch bond at low forces (Fig. 7A) and slip bond at higher forces (Fig. 7B), as observed experimentally [9].

Third, our model reveals that XVA143 suppresses the transition from $C_1$ to $C_2$ and inhibits the transition from $C_2$ to $C_3$ without altering the intrinsic reverse-rates $k_{12}$, $k_{21}$ for dissociation from the three LFA-1/ICAM-1 bond states. This result has elucidated the mechanism for XVA143 to covert the LFA-1/ICAM-1 catch-slip bond to slip-only bond. Because both interstate transitions are induced by force (Fig. 6), our data indicate that XVA143 significantly weakens the force transmission from the $\alpha$A to $\beta$A domains by blocking the binding of the intrinsic ligand of the $\alpha$A domain.

Fourth, the new model has allowed us to estimate the time scale for integrin activation by force. Integrin activation has been suggested to be almost instantaneous [3], but data from different experiments are variable. Binding of fluorochrome-labeled ligands suggested to be almost instantaneous [3], but data from different experiments are variable. Binding of fluorochrome-labeled ligands

### Methods

#### Molecular dynamics simulations

The LFA-1 $\alpha$A domain was modeled from the crystal structure 1LFA (residues 128–292) [12] except for the distorted $\alpha_7$-helix (residues 293–308), which was from another crystal structure 1ZON [11]. The MIDAS $\text{Mg}^{2+}$ and all crystallized waters in 1LFA were retained. The modeled structure was soaked in an 80×80×80 Å$^3$ water box with periodic boundary conditions, which included 3 Na$^+$ and 2 Cl$^-$ to neutralize the system. The NAMD package [25] and CHARMM22 all-atom force field [26] were used for energy minimization and molecular dynamics simulations. A 12-Å cutoff was used for van der Waals interactions and Particle Mesh Ewald summation was used to calculate the electrostatic interactions. Energy was minimized in multi-steps with careful treatments of the interactions to avoid any clashes between the $\alpha_7$-helix and other portion of the $\alpha$A domain. The energy-minimized system was then equilibrated for $6\text{ ns}$ with temperature controlled at 310 K by Langevin dynamics with damping coefficient $\sim 1\text{ ps}^{-1}$ and pressure controlled at 1 atm by Langevin piston method. At the end of equilibration, the RMSD of the system converged and the $\alpha_7$-helix reached a position that aligned well with that observed in the up position of the Mac-1 $\alpha$A domain structure 1JLM [10]. A 15-ns free dynamics simulation was performed with the equilibrated structure to generate initial conformations for SMD simulations. Two constant-force SMD simulations were performed, starting respectively from 10 and 15 ns of the free dynamics simulations, with the C$\alpha$ atoms of residues 131–135, 167–172, 177–181 and 232–234 of the $\beta_1$–$\beta_3$ strands harmonically constrained by springs with a spring constant $\sim 140\text{ pN/Å}$. A 250-pN force was applied at the C-terminal residue Val308 to pull the $\alpha_7$-helix along its axis to the down position suggested by the Mac-1 $\alpha$A domain structure 1D0 [10].

The backbone hydrogen-bonding atoms in the $\alpha_7$-helix were constrained to prevent it from unfolding such that the constraint forces would be added if the distance between the hydrogen-bond pair exceeded 3.5 Å through a spring with a spring constant of $\sim 700\text{ pN/Å}$.

With the snapshots obtained from the SMD simulations at 0, 3.7, and 16 ns as respective starting points, we performed additional 50-ns free dynamics simulations for each case, with the C$\alpha$ atoms of the $\alpha_7$-helix residues constrained. At 30 ns, the PSF input file for NAMD was modified such that the point charges of the two carboxyl oxygen atoms of residue D239 were changed from $-0.76e$ to $-0.26e$, and the point charge of one Na$^+$ atom far
away from the protein was changed to 0 to maintain charge neutral of the system.

**Mathematical modeling**

We constructed a mathematical model to describe the coupled kinetics of force-induced successive interstate transitions from the three states of LFA-1/ICAM-1 bonds and dissociation from these states (Fig. 4). The three states are denoted as C1, C2 and C3, with interstate transition rates \( k_{12} \) and \( k_{23} \) (Fig. 4). Under tensile force, each transition step is assumed to be unidirectional and irreversible, for there was no observable reverse transition of the \( \alpha_7 \)-helix position when pulling force was applied (Fig. 2).

The dissociation of the LFA-1/ICAM-1 bond can occur at any of the C1, C2 and C3 states, with intrinsic reverse-rates \( k_1, k_2 \) and \( k_3 \), respectively. Dissociation from each state is also assumed unidirectional and irreversible. This is reasonable because in the BFP force-clamped experiments [9], once a bond was rupture by tensile force, its component receptor and ligand were pulled apart and no longer able to rebind under the applied force.

Let \( p_1, p_2 \) and \( p_3 \) denote the respective probabilities of ICAM-1 bound with LFA-1 at C1, C2 and C3 states, respectively. The kinetic equations governing the time evolution of the system can be formulated as:

\[
\frac{dp_1}{dt} = -(k_{11} + k_{12})p_1 + k_{12}p_2
\]  
(1)

\[
\frac{dp_2}{dt} = k_{12}p_1 - (k_{21} + k_{23})p_2 + k_{23}p_3
\]  
(2)

\[
\frac{dp_3}{dt} = k_{23}p_2 - k_{31}p_3
\]  
(3)

Equations 1–3 can be expressed in a matrix form:

\[
\frac{dp}{dt} = -Ap,
\]

where \( \mathbf{p} = \begin{pmatrix} p_1 \\ p_2 \\ p_3 \end{pmatrix} \), \( \mathbf{A} = \begin{pmatrix} (k_{11} + k_{12}) & 0 & 0 \\ -k_{12} & (k_{21} + k_{23}) & 0 \\ 0 & -k_{23} & k_{31} \end{pmatrix} \).

Let \( k_1, k_2, \) and \( k_3 \) be the eigen-values of \( \mathbf{A} \) with corresponding eigen-vectors \( \mathbf{v}_1, \mathbf{v}_2 \) and \( \mathbf{v}_3 \), respectively. It can be found that:

\[
k_1 = k_{11} + k_{12}
\]  
(4)

\[
k_2 = k_{21} + k_{23}
\]  
(5)

\[
k_3 = k_{31}
\]  
(6)

and \( v_{11} = v_{31} = v_{32} = 0 \), where \( v_{ij} \) is the \( j \)th component of the vector \( \mathbf{v}_i \). Therefore, the general solution of equations 1–3 can be expressed as:

\[
p_i = a_i \exp(-k_it)
\]  
(7)

where \( a_1, a_2, a_3 \) are nonzero constants. By substituting equations 7–9 into equations 1–3 and compare the corresponding coefficients, we have:

\[
p_1 = a_1 \exp(-k_1t) + b_2 \exp(-k_2t)
\]  
(8)

\[
p_2 = a_2 \exp(-k_1t) + b_3 \exp(-k_2t) + c_1 \exp(-k_3t)
\]  
(9)

where \( a_1, a_2, a_3, b_2, b_3, c_3 \) are nonzero constants. By substituting equations 7–9 into equations 1–3 and compare the corresponding coefficients, we have:

\[
a_2 = a_2 \left( \frac{k_{12}}{k_2 - k_1} \right)
\]  
(10)

\[
a_3 = a_3 \left( \frac{k_{23}}{k_3 - k_1} \right)
\]  
(11)

\[
b_3 = b_3 \left( \frac{k_{23}}{k_3 - k_2} \right)
\]  
(12)

In addition, because both the experimental data [9] and our SMD simulations (Fig. 2) showed that the transition from C1 to C2 and C3 did not happen without force applied, the initial condition can be set as:

\[
p(t = 0) = \begin{pmatrix} 1 \\ 0 \\ 0 \end{pmatrix}
\]

Applying this initial condition to equations 7–9, we have:

\[
a_1 = 1
\]  
(13)

\[
a_2 + b_2 = 0
\]  
(14)

\[
a_3 + b_3 + c_3 = 0
\]  
(15)

Taking equations 10–15 together, each of \( a_1, a_2, a_3, b_2, b_3, c_3 \) can be solved as a function of \( k_{12}, k_{23}, k_1, k_2, k_3 \) from this, by letting \( \omega_1 = a_1 + a_2 + a_3 \), \( \omega_2 = b_2 + b_3 \), \( \omega_3 = c_3 \), and taking equations 4–6 into account, we got:

\[
\omega_1 = 1 + \frac{k_{12}}{k_2 - k_1} + \frac{k_{12}k_{23}}{(k_2 - k_1)(k_3 - k_1)}
\]  
(16)

\[
\omega_2 = \frac{-k_{12}}{k_2 - k_1} - \frac{k_{12}k_{23}}{(k_2 - k_1)(k_3 - k_2)}
\]  
(17)

\[
\omega_3 = \frac{k_{12}k_{23}}{(k_3 - k_1)(k_3 - k_2)}
\]  
(18)

Summing equations 7–9 yields:

\[
\sum_{i=1}^{3} p_i = \sum_{i=1}^{3} \omega_i \exp(-k_it)
\]  
(19)
The left hand side of the equation 19 is the total survival probability of the LFA-1/ICAM-1 bond in all states, which corresponds to the measurements from the BFP force-clamped experiments [9]. The format at the right-hand side indicated that $k_1$, $k_2$, $k_3$ should be the apparent off-rates and $\omega_1$, $\omega_2$, $\omega_3$ should be the associated apparent fractions of the three bond states analyzed from the experimental data [9].

With the apparent off-rates $k_1$, $k_2$, $k_3$ and the apparent associated fractions $\omega_1$, $\omega_2$, $\omega_3$ obtained from fitting the experimental data (summarized in Table S1,S2,S3,S4,S5,S6) [9], the intrinsic kinetic parameters $k_{12}$, $k_{23}$, $k_{11}$, $k_{22}$ and $k_{33}$ can be obtained by solving equations 4–6 and 16–18 and expressed as functions of the known apparent kinetic parameters:

$$k_{12} = \omega_2(k_1-k_2)+\omega_3(k_1-k_3)$$

$$k_{23} = \frac{\omega_3(k_3-k_2)(k_3-k_1)}{\omega_2(k_1-k_2)+\omega_3(k_1-k_3)}$$

$$k_{11} = k_1-\omega_2(k_1-k_2)-\omega_3(k_1-k_3)$$

$$k_{22} = k_2-\frac{\omega_3(k_3-k_1)(k_3-k_1)}{\omega_2(k_1-k_2)+\omega_3(k_1-k_3)}$$

$$k_{33} = k_3$$

Supporting Information

Figure S1 RMSD time courses of several key elements between the simulated structure and the equilibrated closed (blue) or proposed open (red) conformations of LFA-1 α4A domain. (A) 7-helix; (B) MIDAS metal ion Mg2+; (C) S139; (D) S141; (E) T206; (F) D239; (G) L289; (H) F292; and (I) L295. The RMSD between the simulated 7-helix structure and the equilibrated structure shown in Fig. 2A is redrawn in (A). S139, S141, T206 and D239 are key residues that coordinate the metal ion. L289, F292 and L295 are “ratchet” residues that locate on β6-β7 loop or on 7-β helix. They have been proposed to be important to the 7-β helix position. (TIF)

Table S1 Model parameters from BFP experiments measured in Mg2+/EGTA condition.

(DOC)

Table S2 Model parameters from BFP experiments measured in Ca2+/Mg2+ condition.

(DOC)

Table S3 Model parameters from BFP experiments measured in Mn2+ condition.

(DOC)

Table S4 Model parameters from BFP experiments measured in Mg2+/EGTA plus XVA143 condition.

(DOC)

Table S5 Model parameters from BFP experiments measured in Ca2+/Mg2+ plus XVA143 condition.

(DOC)

Table S6 Model parameters from BFP experiments measured in Mn2+ plus XVA143 condition.

(DOC)

Video S1 SMD simulation of pulling the 7-helix of the LFA-1 α4A domain. The simulated structures were shown in cyan with the 7-helix shown in green. The equilibrated 7-helix at the up (blue) and down (red) positions are superimposed for comparison. The Mg2+ ion is shown as golden spheres. A 250-pN force was applied to the Cz atom of the residue 308 at the C-terminal of the 7-helix. At 15 ns, the force was released to allow the system to relax. The Cz atoms of residues 131–135, 167–172, 177–181 and 232–234 of the β1–β3 strands were constrained to prevent the rigid body motion of the αA domain. The backbone hydrogen-bonding atoms in the 7-helix were constrained to prevent it from unfolding. (MPG)

Acknowledgments

The supercomputer time used to perform the simulations in this study was provided by NSF TeraGrid via TG-MCA08X014 and by the Supercomputing Center of Chinese Academy of Sciences (SCCAS).

Author Contributions

Conceived and designed the experiments: XX CL TL JL CZ. Performed the experiments: XX CL TL JL. Analyzed the data: XX CL TL JL. Wrote the paper: XX CL TL JL.

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