Force regulated conformational change of integrin $\alpha_V\beta_3$

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Abstract

Integrins mediate cell adhesion to extracellular matrix and transduce signals bidirectionally across the membrane. Integrin $\alpha_V\beta_3$ has been shown to play an essential role in tumor metastasis, angiogenesis, hemostasis and phagocytosis. Integrins can take several conformations, including the bent and extended conformations of the ectodomain, which regulate integrin functions. Using a biomembrane force probe, we characterized the bending and unbending conformational changes of single $\alpha_V\beta_3$ integrins on living cell surfaces in real-time. We measured the probabilities of conformational changes, rates and speeds of conformational transitions, and the dynamic equilibrium between the two conformations, which were regulated by tensile force, dependent on the ligand, and altered by point mutations. These findings provide insights into how $\alpha_V\beta_3$ acts as a molecular machine and how its physiological function and molecular structure are coupled at the single-molecule level.

Introduction

Integrin $\alpha_V\beta_3$ is a member of the integrin family and is widely expressed on endothelial cells, osteoclasts and blood cells. It acts as a bridging molecule between the cell and the extracellular environment, and mediates cell adhesions and mechanosignaling [24,27,42]. The over-expression of integrin $\alpha_V\beta_3$ in certain tumor cells facilitates tumor development, angiogenesis, and metastasis [31].

Integrins are heterodimers of non-covalently associated α and β subunits with an extracellular domain, a transmembrane segment, and a cytoplasmic region [32]. Integrins can adopt multiple conformations, including a bent or extended ectodomain, a joined or separated tailpiece and a closed or opened β subunit hybrid domain, which have been visualized by electron microscopy and crystallography [1,14,32,37,55–57] and detected by conformation-specific antibodies [59]. Specifically, integrin $\alpha_V\beta_3$, the subject of the present paper, has been observed in both bent and extended ectodomain conformations [52,56,57]. In the bent conformation, the N-terminal headpiece of the integrin (including the β-propeller and thigh domains of the α subunit and βA and hybrid domains of the β subunit) is bent towards and contacts the C-terminal tailpiece (including Calf-1 and Calf-2 domains of the α subunit and I-EGF-1 to I-EGF-4 domains and β tail domain of the β subunit). Upon extension, the headpiece flips upwards around the N-terminus of Calf-1 and I-EGF-1 domains and becomes more aligned with the tailpiece.

Integrin conformation is known to correlate with integrin activity: the bent conformation correlates with low affinity for ligands whereas the extended conformation correlates with high affinity. Integrin activation is accompanied by ectodomain extension, as shown by

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studies with extracellular activators, such as divalent cations (e.g. Mg^{2+}, Mn^{2+}), activating antibodies (e.g. CBR LFA1/2), and ligand-mimicking peptides, and with intracellular activators such as overexpressed talin head domain [11,37,46,49,52,58]. Several activation-associated mutations also result in more extended conformations compared to the wild-type (WT) [59]. Molecular dynamics (MD) simulations have suggested that mechanical forces may induce integrin conformational changes [8,40,44,54,60]. Using a single-molecule force technique, the biomembrane force probe (BFP), we characterized bending and unbending conformational changes of single $\alpha_L\beta_2$ integrins on a living cell, which has demonstrated a role for mechanical force to regulate integrin conformational changes [7].

Building upon these recent studies, here we used a BFP to investigate bending and unbending conformational changes of single $\alpha_V\beta_3$ integrins on living cells. Characterization of the conformational change dynamics revealed the effects of mutation, force and ligand engagement.

Results

Real-time observation of single integrin $\alpha_V\beta_3$ conformational changes on living cells

We used a BFP to characterize the conformational dynamics and binding kinetics of human integrin $\alpha_V\beta_3$ expressed on mouse lung endothelial cells (mLECs), including WT and two gain-of-function (GOF) mutants (MT), D723R and L138I (see Experimental procedures). D723R, located at the N-terminus of the β_3 cytoplasmic domain (Fig. 1A), disrupts interactions between the α- and β-subunit transmembrane domain-tail to induce integrin tail separation and functional activation [29,39,51]. L138I, a point mutation in the β_3 βA domain, promotes extracellular domain extension and also activates the integrin [39]. Cells grown to confluence were detached from a culture flask by EDTA, washed, and injected into the cell chamber on the microscope stage. A mLEC was aspirated by the right micropipette and served as the target (Fig. 1B right). Two ligands and two anti-β_3 antibodies were tested. Most experiments used a biotinylated fibronectin module III domain 7–10 ($FN_{III7–10}$) containing both the integrin-binding RGD sequence in the domain 10 and the synergy site in the domain 9 [38]. The biotin-$FN_{III7–10}$ was immobilized on a streptavidin (SA) covalently coupled glass bead that served as the probe (Fig. 1B left). Some experiments used fibrinogen (Fg) or antibody (Ab) covalently coupled to the probe bead together with SA for attachment to the biotinylated RBC (Fig. 1B left). The covalent linking protocol resulted in random distribution of non-clustered SA, Abs or Fg on the bead surface, an important condition to ensure binding events of different ligands to be independent and to follow the Poisson statistics at small numbers. Binding specificity was confirmed using the adhesion frequency as an assay (see Experimental procedures). Target cells were driven to repeatedly contact the probe bead for 2 s then retracted, and the number of no-adhesion (Fig. 1C, ①) and adhesion (Fig. 1C, ②) events were counted. At appropriately adjusted ligand density, bead binding to mLECs expressing WT, D723R or L138I $\alpha_V\beta_3$ exhibited 30–40% frequencies; in comparison, binding to untransfected cells was ~five-fold lower, ensuring the observed binding events were predominately mediated by $\alpha_V\beta_3$ (Fig. 1D). Contacts between SA-bearing beads and mLECs expressing WT $\alpha_V\beta_3$ also yielded a very low level of adhesion frequency, confirming the binding specificity of the ligand $FN_{III7–10}$ (Fig. 1D).

Position-clamp experiments (see Experimental procedures) were performed to quantify $\alpha_V\beta_3$–$FN_{III7–10}$ bond dissociation and the integrin bending and unbending conformational changes under force. The contact time was shortened to lower the adhesion frequency to ~20%, a necessary but not sufficient condition to ensure binding between the bead and the cell to be mediated predominately (~90%) by a single bond [13]. Upon retraction to a desired force level, the mLEC was clamped at a fixed position to allow measurement of the lifetimes of the bonds (if present) and observe the subsequent bending or unbending event. Under such a position-clamp condition, the force signal often remained stable around the initial value until dissociation (Fig. 1C, ③). The lifetime (Fig. 1C, indicated) reflects the sustainability of the bond at a given force. However, in some lifetime events, a spontaneous decrease (Fig. 2A and C) or increase (Fig. 2B and D) in the mean force signal was observed with a concurrent decrease (Fig. 2E) or increase (Fig. 2F) in their fluctuations, indicating that the integrin under interrogation underwent an unbending or bending conformational change, respectively [7]. The force change was due to the lengthening or shortening of the distance between the ligand-binding site and the membrane anchor point of the integrin, which partly decreased or increased pulling on the RBC, respectively. The change in the signal fluctuation results from the change in the integrin molecular stiffness, which is softer in the bent and stiffer in the extended conformation (see below) [7].

In rare cases the cell and the bead were linked by more than one bond. Because of the random distribution of $FN_{III7–10}$, these bonds tended to be spatially separated and formed independently from each other. As such, their dissociations during the clamping phase should occur independently, most likely one at a time rather than two or more simultaneously, resulting in sequential drops in the BFP force signal (Fig. 2G). Although Fig. 2A and G appear to look alike, a bond dissociation event could

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