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Chungho Kim,1 Tong-Lay Lau,2 Tobias S. Ulmer,2 and Mark H. Ginsberg1

1Department of Medicine, University of California San Diego, La Jolla; and 2Department of Biochemistry and Molecular Biology and Zilkha Neurogenetic Institute, Keck School of Medicine, University of Southern California, Los Angeles

Clustering and occupancy of platelet integrin αβ3 (GPIIb-IIIa) generate biologically important signals: conversely, intracellular signals increase the integrins' affinity, leading to integrin activation; both forms of integrin signaling play important roles in hemostasis and thrombosis. Indirect evidence implicates interactions between integrin α and β transmembrane domains (TMDs) and cytoplasmic domains in integrin signaling; however, efforts to directly identify these associations have met with varying and controversial results. In this study, we develop mini-integrin affinity capture and use it in combination with nuclear magnetic resonance spectroscopy to show preferential heterodimeric association of integrin αββ3 TMD-tails via specific TMD interactions in mammalian cell membranes and in lipid bicelles. Furthermore, charge reversal mutations at αβ(R995)ββ(D723) confirm a proposed salt bridge and show that it stabilizes the TMD-tail association; talin binding to the β3 tail, which activates the integrin, disrupts this association. These studies establish the preferential heterodimeric interactions of integrin αββ3 TMD-tails in mammalian cell membranes and document their role in integrin signaling. (Blood. 2009;113:4747-4753)

Introduction

Integrin-mediated cell adhesion modulates signaling pathways that control many biologic functions, and these signals involve both integrin clustering and integrin occupancy; the latter changes integrin conformation, leading to allosteric rearrangements that propagate across the membrane and modify intracellular interactions. A second form of integrin signaling, initiated intracellularly, leads to increased affinity for ligands, a process termed integrin activation. Both forms of integrin signaling are central to the functions of platelet integrin αIIbβ3 (GPIIb-IIIa) in hemostasis and thrombosis. Integrin activation is also essential for functions such as inflammation and assembly of the extracellular matrix. Integrins are noncovalent α-β heterodimeric, type-1 transmembrane (TM) receptors formed from combinations of 18 α and 8 β subunits. Many studies indirectly implicate interactions between integrin α and β TM domains (TMDs) and cytoplasmic domains (tails) in both forms of integrin signaling5-11; however, studies to directly identify such interactions have met with contradictory results.

Mutational studies of platelet integrin αIIbβ3, based on sequence alignments, suggested an interaction between β3 Asp723 and αIIb Arg995.5 Charge reversal mutation of either residue resulted in constitutive bidirectional integrin signaling (ie, integrin activation and constitutive phosphorylation of pp125FAK); however, a double-charge reversal in αIIb(R995D)β3(D723R) did not exhibit constitutive signaling, suggesting that a salt bridge between these residues was a defined structural constraint that limited the integrin signaling.5 A spontaneous activating mutation (β3(D723H)) found in patients also suggests the importance of the β3 Asp723 residue for stabilization of the inactive state of the integrin.12 This constraint gained support from elegant protein engineering studies in which clamping the cytoplasmic domains or TMDs together inhibited integrin activation,9 and joining of the α and β TMDs with disulfide bonds limited bidirectional integrin signaling.7 Despite this mutational data, our initial efforts to identify interactions of isolated α and β integrin tails by nuclear magnetic resonance (NMR) spectroscopy were unsuccessful in aqueous solution.13 Two laboratories reported NMR studies of these tails that suggested the existence of such interactions, albeit different structures for the αβ dimer were reported.14,15 Structures of the individual αIIb and β3 TMDs in phospholipid bicelles16-17 show that the β3 TMD adopts an elongated, tilted membrane helix17 and the αIIb TMD folds into a short, straight helix, followed by a surprising backbone reversal that packs Phe992 and Phe993 against the TM helix.16 This structure demonstrated an unexpected complexity in the αIIb TMD and membrane-proximal cytoplasmic domain that was not observed in the studies of cytoplasmic domains in aqueous solution.14,15 thus calling NMR studies that suggested the interactions into question.

 Whereas mutational studies and molecular modeling have strongly suggested that integrin αβ TMD interactions are important in regulating integrin signaling,8,10,11,18,19 efforts to identify direct interactions between α and β TMDs (either biochemically or genetically) have had contradictory results.20-23 Indeed, αα and ββ interactions were proposed to make a major contribution to the clustering that occurs in integrin signaling22; however, later studies have strongly challenged this idea.8,11 Furthermore, a 20 Å reconstruction of detergent-solubilized integrin αIIbβ3 revealed a cylindrical density interpreted as a TM segment that suggests that the TMDs are associated in a parallel, α-helical coiled-coil24; however, as noted above, higher resolution structures of the individual αIIb β3 TMDs in a lipid environment are not consistent with a purely coiled-coil architecture. In this study, we use a novel mini-integrin affinity capture approach to establish the preferential heteromeric interaction of integrin αIIb and β3 TMDs and cytoplasmic domains


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in mammalian cells, and show by NMR spectroscopy that heterodimerization takes place analogously in small bicelle model membranes. Thus, we show that integrin TMDs and cytoplasmic domains preferentially interact in a heterodimeric rather than homodimeric fashion in mammalian cell membranes, interaction between the highly conserved β3 Asp723 and αb Arg906 stabilizes this interaction, and talin binding to the β3 cytoplasmic domain disrupts it.

Methods

Plasmids, antibody, and cell lines

Tac-αbTM, Tac-β3TM were generated by ligation of polymersase chain reaction (PCR)–generated fusion sequences consisting of extracellular domain of Tac and TM tail regions of each integrin subunit (Figure 1A) into pcDNA3.1 (Invitrogen, Carlsbad, CA). For the construction of αbTM-TAP (tandem affinity purification), the sequences of the preprotrypsin leader tag for purification and an N-terminal FLAG tag for detection. Tαcβ3TM and Tacβ3TM were made by fusion of αbTM and β3TM, respectively, with Tal extracellular domain. CHO cells were transiently transfected with αbTM-TAP (TAP) and TacαbTM or Tacβ3TM (preys), and cells were lysed and incubated with calmodulin beads to capture the baits. Bound Tac constructs were analyzed by Western blot using anti-Tac antibody (top panels). Expression of Tac preys (middle panel) and captured αbTM-TAP (bottom panel) were verified by Western blot using anti-Tac antibody and anti-FLAG antibody, respectively. The arrows indicate mature cell-surface proteins, and the arrowheads incompletely glycosylated intracellular proteins. Open symbols represent Tac-β3TM, and closed symbols represent TacαbTM. CHO cells were transiently transfected with baits and preys, as indicated, and cell-surface proteins were biotinylated before cell lysis. Ten percent of the lysates were incubated with NeutrAvidin beads to determine the input of biotinylated proteins in the lysates (middle and bottom panels). The remaining lysates were first incubated with calmodulin beads to capture the baits, and the bound proteins were eluted with 10 mM EDTA. The eluates were then incubated with NeutrAvidin beads to capture the biotinylated surface proteins and the presence of Tac preys was analyzed with Western blot using anti-Tac antibody (top panel). (E) αbTM-TAP constructs containing deletion of GFFKR motif or mutations of 2 Gly in GXXGXG motif to Leu were tested for their binding to Tacβ3 as in (C).}

Affinity capture

Twenty-four hours after transfection, cells were lysed with CHAPS [3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate] lysis buffer (20 mM HEPES [N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid] pH 7.4, 1% CHAPS, 150 mM NaCl, 2 mM CaCl2, and EDTA [ethylenediaminetetraacetic acid]–free protease inhibitor mixture [Roche, Basel, Switzerland]) and clarified by centrifugation at 14 000 rpm for 15 minutes, and then the clarified lysates were incubated with calmodulin Sepharose (GE Healthcare, Piscataway, NJ) for 2 hours at 4°C. Bound proteins were eluted with sodium dodecyl sulfate (SDS) reducing sample buffer, subjected to SDS–polyacrylamide gel electrophoresis (PAGE), and analyzed by Western blot. For capturing biotinylated intact integrin subunits, CHO cells were transiently transfected with baits and preys, as indicated, and cell-surface proteins were biotinylated before cell lysis. Ten percent of the lysates were incubated with NeutrAvidin beads to determine the input of biotinylated proteins in the lysates (middle and bottom panels). The remaining lysates were first incubated with calmodulin beads to capture the baits, and the bound proteins were eluted with 10 mM EDTA. The eluates were then incubated with NeutrAvidin beads to capture the biotinylated surface proteins and the presence of Tac preys was analyzed with Western blot using anti-Tac antibody (top panel). (E) αbTM-TAP constructs containing deletion of GFFKR motif or mutations of 2 Gly in GXXGXG motif to Leu were tested for their binding to Tacβ3 as in (C).
being lysed with CHAPS lysis buffer. Lysates were incubated with calmodulin-beads to capture TAP constructs for 2 hours, bound proteins were eluted with 10 mM EDTA, and then the eluates were further incubated with NeutrAvidin agarose resin (Thermo Scientific) overnight to capture biotinylated proteins. Bound proteins were eluted with SDS reducing sample buffer, subjected to SDS-PAGE, and analyzed by Western blot.

**NMR spectroscopy**

Peptides encompassing human integrin αIIb(Ala593-Pro608) and β3(Pro685-Phe727) residues, respectively, including β3(Cys687-Ser), were prepared as described previously. In brief, an αIIb peptide incorporating Arg969Ala and a β3 peptide with an Asp727Ala substitution were prepared analogously. The peptides were reconstituted in 385 mM 1,2-dihexanoyl-sn-glycero-3-phosphocholine, 83 mM 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, 41 mM 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho- (H)NaOH, pH 7.4, 6% D2O, and 0.02% wt/vol NaN3. Transverse relaxation optimized–heteronuclear single quantum coherence (TROSY-HSQC) NMR experiments were conducted on a cryo-probe-equipped Bruker Avance 700 spectrometer at 23°C.

**Flow cytometry**

PAC1-binding assay and fibronectin-binding assay were performed essentially as described. In brief, 1 day after transfection, suspended cells were incubated with 7G7B6 (anti-Tac) in combination with either PAC1 or Tac3-B3 (Figure 1D left panels) and only the top band was observed. Conversely, bait containing the TMD and tail of β3 preferentially bound the Tac-c3TM rather than Tac-β3Tm on the cell surface, although subtle β3 homomeric interactions were also observed (Figure 1D right panels). Thus, we observed preferential heteromeric interactions between αIIb and β3 TMD-tail in both cell surface and intracellular membranes.

The foregoing results showed preferential heteromeric interactions between baits and preys containing the TMDs and cytoplasmic domains of integrin αIIbβ3, and that the interaction required the β3 TMD. To test the role of the αIIb TMD, we changed 2 Gly in the αIIb TMD GXXG motif to Leu (Figure 1A). This motif is buried in the hydrophobic core of the lipid bilayer and is predicted to be important in αβ TMD packing. As a second test of specificity, we deleted the membrane-proximal GFFKR motif; both this deletion and the Leu substitutions activate integrin αIIbβ3. Both of these mutations markedly reduced interaction of αIIb bait with β3 prey (Figure 1E). Thus, the bait-prey interactions detected in this study are mediated by specific packing interactions of the TMDs. The strong preference for heteromeric αβ interactions in mammalian cell membranes is in sharp contrast to the homodimeric and trimeric interactions previously observed in detergent micelles, in electrophoresis, and in the Escherichia coli inner membrane. Schneider and Engelman did observe promiscuous weak heterodimeric interactions of central hydrophobic 17-residue fragments of the integrin TMDs using transcriptional repression of LacZ by dimer formation in E. coli membranes; those authors observed extensive homo-oligomerization as well. Thus, the present data provide the first direct evidence for the heteromeric association of αIIbβ3 TMD-tails in mammalian cell membranes.

**Results**

**Integrin αIIb and β3 TMDs mediate heterodimeric interactions in mammalian cell membranes**

To test whether the TMDs of αIIb and β3 interact with each other, we constructed an αIIb mini-integrin containing the TMD and cytoplasmic tail of αIIb (Figure 1A) joined to an N-terminal flag tag for detection and a C-terminal TAP tag for rapid and efficient purification (Figure 1B). We expressed this αIIbTM-TAP bait in combination with preys comprising the extracellular domain of the Tac (interleukin-2 receptor α) joined to the TMD and tail of αIIb (Tac-αIIb TM) or β3 (Tac-β3TM, Figure 1B). The cells were lysed, and baits were captured using calmodulin beads. In the reaction, approximately 20% of expressed baits were usually captured. We detected bound prey by Western blotting with anti-Tac antibody and found that αIIbTM bait bound preferentially to Tac-β3TM rather than Tac-αIIbTM (17-fold more in quantification; Figure 1C). This interaction required the β3 TMD, because a prey containing the Tac extracellular and TMD joined to the β3 tail failed to bind to the αIIbTM bait (Figure S1A, available on the Blood website; see the Supplemental Materials link at the top of the online article). We noted multiple bands for each Tac construct and enzymatic deglycosylation and cell-surface biotinylation experiments showed that the top band observed in Figure 1C is a glycosylated form found predominantly on the cell surface, whereas the bottom band is a nonglycosylated intracellular form (Figure S1B,C). When we surface labeled the cells, we again found that αIIbTM-TAP bait interacts with Tac-β3TM (Figure 1D left panels) and only the top band was observed. Conversely, bait containing the TMD and tail of β3 preferentially bound the Tac-αIIbTM rather than Tac-β3TM on the cell surface, although subtle β3 homomeric interactions were also observed (Figure 1D right panels). Thus, we observed preferential heteromeric interactions between αIIb and β3 TMD-tail in both cell surface and intracellular membranes.

**Integrin αIIb and β3 TMD interaction is stabilized by interaction of αIIb Arg969 and β3 Asp723**

The plasma membrane is a complex environment; that is, it contains many proteins and lipids. Whereas this complexity is representative of the environment of integrin αIIbβ3 in blood platelets, it raises the possibility of indirect signals, as exemplified for αIIbβ3 binding to Tac-β3TM. This interaction is stabilized by interaction of αIIbArg969 and β3Asp723. This second set of resonances arises from αIIb-β3 association (heterodimerization), which creates new chemical environments for all αIIbβ3 TMD resonances (Figure S2A) as a consequence of direct αIIb-β3 contacts and indirect, propagated effects.
For example, $\beta_2(G708)$ is expected to form the dimerization interface, whereas $\beta_3(G702)$ is peripheral and most likely experiences predominantly indirect, next-neighbors changes. To test the existence of the proposed $\alpha_{IIb}(R995)-\beta_3(D723)$ salt bridge and verify the specificity of the $\alpha_{IIb}-\beta_3$ interaction, wild-type $\alpha_{IIb}$ TMD peptide was substituted with $\alpha_{IIb}(R995A)$. This mutant $\alpha_{IIb}$ peptide, whose backbone fold is indistinguishable from wild-type $\alpha_{IIb}$ (Figure S2B), failed to induce a significant second set of $\beta_3$ signals (Figure 2D), demonstrating that heterodimerization weakened considerably. Analogously, mutant $\beta_3(D723A)$ peptide did not induce a second set of $\alpha_{IIb}$ resonances in contrast to wild-type peptide (data not shown). Thus, in the defined model membrane environment of phospholipid bicelles, specific TM $\alpha_{IIb}-\beta_3$ interactions, leading to heterodimerization, were observed and were dependent on $\alpha_{IIb}(R995)$ and $\beta_3(D723)$ electrostatic interactions.

To assess whether $\alpha_{IIb}(R995)-\beta_3(D723)$ electrostatic interaction also takes place in cell membranes, we introduced charge reversal mutations in these residues creating $\alpha_{IIb}(R995D)$ bait and $\beta_3(D723R)$ prey. The combination of $\alpha_{IIb}(R995D)$ bait with $\beta_3(D723R)$ prey, or of $\beta_3(D723R)$ prey with $\alpha_{IIb}$ bait, resulted in marked reduction in the interaction (Figure 2E). The $\alpha_{IIb}$ interaction was largely rescued when a combination of $\alpha_{IIb}(R995D)$ bait and $\beta_3(D723R)$ prey was used (Figure 2E). Thus, both NMR in synthetic membranes and affinity capture in mammalian cell membranes confirm the importance of the $\alpha_{IIb}(R995)-\beta_3(D723)$ interaction in stabilizing the association of the $\alpha_{IIb}-\beta_3$ TMD-tails. Our previous inability to detect specific $\alpha_{IIb}(\text{Arg}^{995})-\beta_3(\text{Asp}^{723})$ contacts in aqueous solution indicates their weakness in the absence of a lipid milieu that provides a lowered dielectric constant and reduced solvent water concentration.

Talin binding to the $\beta_3$ tail disrupts the interaction of the $\alpha_{IIb}$ and $\beta_3$ TMD-tails

Talin binding to integrin $\beta$ cytoplasmic domains is a final step in integrin activation. Transfection of cells with the talin head domain (THD) inhibits Förster resonance energy transfer (FRET) between donors and acceptors fused to the cytoplasmic tails of $\alpha_L$ and $\beta_2$ integrins, indicating a change in the orientation and/or distance between the tails. Having developed a direct measure of the association of the integrin $\alpha$ and $\beta$ TMD-tails, we asked whether talin binding can inhibit the interaction. THD reduced the $\alpha_{IIb}^{\text{TM}}$-TAP-Tac-$\beta_3^{\text{TM}}$ interaction by 40% relative to a $\beta_3$ binding-defective mutant THD, THD(W395A) (Figure 3A). Conversely, THD did not block binding of Tac-$\beta_3^{\text{TM}}(Y747A)$, a mutation that inhibits talin binding, to $\alpha_{IIb}^{\text{TM}}$-TAP (Figure 3B). In the absence of THD, Tac-$\beta_3^{\text{TM}}(Y747A)$ also showed increased association with $\alpha_{IIb}^{\text{TM}}$-TAP, probably due to the presence of endogenous talin in the CHO cells (data not shown). Thus, talin binding to the $\beta_3$ tail inhibits the interaction of the $\alpha_{IIb}$ and $\beta_3$ TMD-tails. The partial dissociation of the $\alpha$ complex by THD may be due to variation in the ratio of THD to Tac-$\beta_3^{\text{TM}}$ and $\alpha_{IIb}^{\text{TM}}$-TAP on a per cell basis in these triple transfection experiments.

It is notable that mutations in the $\alpha_{IIb}$ GXXXG motif, located near the outer leaftlet of the membrane, and mutations that disrupt the salt bridge or talin binding, which would act near the inner leaftlet of the membrane, both decrease the TMD-tail association. These results raise the intriguing possibility that both outer membrane interaction (including the $\alpha_{IIb}$ GXXXG motif) and inner membrane interaction (involving $\alpha_{IIb}$ F992, 933 and R995) and $\beta_3(D723)$ are required for stabilization of the TMD interface and the inactive state of the integrin.
were tested at least 3 times, and representative data are shown. The amount of Tac-β3, leading to integrin activation. Indeed, overexpression of Tac-αβ3 induced a dose-dependent increase in the binding of an activation-specific anti-αβ3, PAC1, whereas a construct lacking the αβ3 TMD (Tac-αβ3) exhibited no such effect (Figure 4A). The Tac-αβ3 failed to activate αβ3 (Figure S3), supporting the integrin class specificity of interactions of integrin TMDs. Additional evidence for specificity of this effect was provided by mutants that disrupt the TMD-tail interaction of integrin αβ3 and β3; Tac-αβ3 constructs in which we deleted the GFFKR motif or mutated the 2 Gly in the GXXXG motif failed to activate αβ3β3 (Figure 4B).

Expression of the free β3 cytoplasmic domain inhibited integrin activation (Figure 4C) probably due to sequestration of endogenously expressed talin. Addition of the β3 TMD to the cytoplasmic domain led to reversal of the inhibitory effect and integrin activation at higher levels of expression (Figure 4C), possibly resulted from combination of inhibitory effect by sequestering talin and activating effect by binding to the TMDs of the intact integrin. Furthermore, the β3(Y747A) mutation, which blocks talin binding, increased the ability of the β3 TMD-tail to activate integrin αβ3β3 and reduced the inhibitory effect (Figure 4C). Thus, overexpression of the TMD-tail of either αβ3 or β3 activated the native integrin, and activation depended on the presence of the TMDs.

The TMD-tail of αβ3 or β3 activates integrin αβ3

We reasoned that these TMD-tails interact with native αβ3β3, then they might disrupt the interaction of the endogenous αβ3 and β3, leading to integrin activation. Indeed, overexpression of Tac-αβ3 induced a dose-dependent increase in the binding of an activation-specific anti-αβ3, PAC1, whereas a construct lacking the αβ3 TMD (Tac-αβ3) exhibited no such effect (Figure 4A). The Tac-αβ3 failed to activate αβ3 (Figure S3), supporting the integrin class specificity of interactions of integrin TMDs. Additional evidence for specificity of this effect was provided by mutants that disrupt the TMD-tail interaction of integrin αβ3 and β3; Tac-αβ3 constructs in which we deleted the GFFKR motif or mutated the 2 Gly in the GXXXG motif failed to activate αβ3β3 (Figure 4B).

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**Figure 3.** Talin binding to the β3 tail inhibits the αβ TMD-tail interaction. (A) αβ3 TAP and Tac-β3 TAP were cotransfected with THD or THD(W359A) mutant into CHO cells, and their interaction was analyzed, as described in Figure 1C. Ratio of the amount of Tac-β3 TAP bound to αβ3 TAP in presence of wild-type THD to that in the presence of THD W359A is shown as a bar graph. The data are the mean ± SE of 3 experiments. (B) αβ3 TAP and THD were cotransfected with Tac-β3 TAP or Tac-β3(Y747A) mutant into CHO cells and their interaction was analyzed, as described in panel A. The data are mean ± SE of 3 experiments.

**TMD-tail of αβ3 interacts with native αβ3β3 integrin by binding the β3 subunit**

To examine the interaction of αβ3 TMD-tail with native αβ3β3, we captured the αβ3 TAP bait, resulting in isolation of native αβ3β3 (Figure 5A), whereas deletion of the GFFKR motif inhibited the association (Figure 5A). We also examined the association of Tac-αβ3 with activated αβ3β3 in cells. Most PAC1-positive clusters of activated αβ3β3 also contained the Tac-αβ3 TMD-tail (Figure 5B). Cells transfected with Tac-αβ3, which lacks the TMDs, failed

![Figure 4](image-url)
to stain with PAC1; however, when the α₁bβ₃ was activated with an activating antibody, anti-LIBS6,¹⁴ Tac-α₁b was not associated with the clusters of activated integrin (Figure 5B). Thus, the α₁b TMD-tail interacts with native α₁bβ₃, and this physical association activates the integrin.

Consistent with the findings described above, a peptide containing a partial sequence of α₁b TMD (Trp⁹⁶⁸-Lys⁹⁸⁹) can bind and activate α₁bβ₃; however, this effect was ascribed to a homodimeric interaction of the α₁b peptide with the full-length α₁b. To assess which subunit of full-length α₁bβ₃ binds to the α₁b TMD-tail, we used EDTA to dissociate the subunits and verified the dissociation by the loss of binding of a complex-specific antibody (Figure 5C). Because the EDTA treatment can affect only cell surface–expressed integrin, we surface biotinylated cells expressing α₁bβ₃ in combination with α₁b™-TAP to exclude the intracellular integrin from this assay, treated the cells with EDTA to dissociate the α₁bβ₃ on cell surface, and then captured α₁b™-TAP with immobilized calmodulin (Figure S4). Biotinylated surface proteins were then affinity purified with NeutrAvidin beads and detected by antibody against α₁b (PMI-1) and β₃ (Rb8053). We found that β₃ subunit was captured by α₁b™-TAP (Figure 5D top, lane 2), and the LXXXL and ΔGFFKR mutations markedly reduced the association (Figure 5D top, lanes 3, 4). In sharp contrast, only nonspecific (as judged by equal interactions of wt, LXXXL, or ΔGFFKR baits and by the ratio of captured [Figure 5D lanes 2-4] to input of the cell surface–expressed integrin subunits [Figure 5D lanes 6-8]) were observed with the α₁b subunit (Figure 5D middle panel). This result shows that the α₁b™-TAP preferentially captures full-length integrin β₃ in the presence of an equimolar amount of native full-length α₁b. Thus, the unpaired α₁b™-TMD-tail induces equilibrium shift in favor of activation of integrin α₁bβ₃ by interacting with the β₃ subunit and separating the TMD-tail interaction of the intact integrin (Figure 5E).

Discussion

Bidirectional TM integrin signaling is central to integrin-mediated biological functions. Mutational studies suggested that this signaling involves heterodimeric interactions of the TMD-tails; frustratingly, efforts to directly demonstrate these interactions produced conflicting results. In this study, we took advantage of the high efficiency and rapidity of TAP tag purification to show preferential biological functions. Mutational studies suggested that this signal-
fact that we used a full-length cytoplasmic domain and included the GFFKR motif, shown in this study to be critical for stabilization of the association of the TMD-tail mini-integrins. Similarly, previous studies in detergent micelles suggested primarily homomeric αIib and β3 TMD-tail interactions; however, phospholipid bicelles are a more accurate representation of the bulk mammalian plasma membrane because they contain lipids in a bilayer arrangement. Indeed, embedding in dodecylphosphocholine micelles distorts the helical structure of the β3 membrane-proximal domain, a region important in regulating integrin signaling. The remarkable agreement of biochemical and biophysical methods in identifying heterodimeric associations, combined with previous mutational data, provides compelling evidence for preferential heterodimeric TMD-tail interactions of αIibβ3 in the lipid environment and the mammalian cell membrane. Furthermore, we now provide direct proof that talin binding disrupts the TMD-tail association and that an αIib(R995)β3(D723) interaction stabilizes it. Moreover, the new approaches described in this study now enable analysis of TMD-tail interactions among many type I membrane proteins, such as tyrosine kinase growth factor receptors, immunoreceptors, or cytokine receptors that transduce signals as homo- and heteromultimers.

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Authorship
Contribution: C.K. designed and performed the mammalian cell experiments and wrote the paper; T.L.L. designed and performed the NMR experiments; T.S.U. designed the NMR experiments and edited the paper; and M.H.G. designed the mammalian cell experiments and wrote the paper.
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Correspondence: Dr Mark H. Ginsberg, Department of Medicine, University of California San Diego, 9500 Gilman Dr, La Jolla, CA 92039; e-mail: mhginsberg@ucsd.edu.

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