Integrin $\alpha_v\beta_8$ Adopts a High Affinity State for Soluble Ligands Under Physiological Conditions

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ABSTRACT

It has been proposed that integrins adopt a low affinity conformation under physiological conditions. Integrin can either be activated through cytoplasm or by binding of cations such as Mn$^{2+}$ to the head domain. The cytoplasmic activation pathway, that is, inside-out signaling has been regarded as the physiological pathway for integrin activation. Integrin $\beta_8$ is important for neuron vascular development. However, due to the highly divergent cytoplasmic domain, this integrin probably does not rely on inside-out signaling for affinity regulation. We therefore hypothesized that the $\beta_8$ integrin uniquely assumes a constitutively high affinity state under physiological conditions. We discovered that $\beta_8$ indeed exhibited high binding to soluble vitronectin in the presence of Ca$^{2+}$ and the ligand binding could not be further enhanced by addition of Mn$^{2+}$. The lower ectodomain stalk of the integrin, which is comprised by the integrin epidermal growth factor-like (I-EGF) domains and $\beta$TD domain, is critical for this high affinity conformation. In addition, we found that unlike other integrins, Mg$^{2+}$ at low concentration inhibited $\beta_8$ ligand binding. Mutagenesis studies indicated that $\beta_8$ integrin possesses a unique cation binding site which might contribute to the ligand binding affinity. Our study showed that both the $\beta_8$ lower ectodomain stalk and the head domain play an important role in its high affinity state under physiological conditions. J. Cell. Biochem. 118: 2044–2052, 2017. © 2016 Wiley Periodicals, Inc.

KEY WORDS: INTEGRIN; HIGH AFFINITY CONFORMATION; SIGNALING; METAL ION BINDING; SITE AFFINITY REGULATION

Integrins are $\alpha$-$\beta$ heterodimeric cell surface adhesive receptors that play important roles in many biological functions including cell migration, proliferation, differentiation, and survival [Hynes, 2002]. At least twenty-four integrins, which are composed of eighteen $\alpha$ and eight $\beta$ subunits, have been identified in vertebrates [Humphries et al., 2006]. Different integrins have diverse functions due to distinct submembrane linkers to cytoskeleton and intracellular proteins as well as different ligand-binding specificities and affinities. Integrin $\alpha_v\beta_8$, is distinctive among the integrins. This integrin is almost exclusively expressed in the central and peripheral nervous systems and placenta. The $\beta_8$ integrin expression is correlated with central nervous system (CNS) development [Chernousov and Carey, 2003]. $\alpha_v\beta_8$ also regulates TGF-$\beta$1 activation and uniquely aids in the repair of neural cells, especially Schwann cells, by binding to ECM ligands and inducing growth, differentiation, and healing. The integrin is essential for embryonic development as $\beta_8$ knock-out mice exhibited embryonic or perinatal lethality with abnormal vascular development [Zhu et al., 2002; Proctor et al., 2005]. Lack of interaction between latent TGF-$\beta$1 and $\alpha_v\beta_8$, which is required for TGF-$\beta$1 activation, has been proposed to be the cause of these defects [Mobley et al., 2009]. Despite its interactions with vitronectin, the $\beta_8$ integrin was reported as a non-adhesive receptor [Nishimura et al., 1994]. Later studies found that the $\beta_8$ cytoplasmic domain is completely different from other $\alpha_v$ partners, including $\beta_1$, $\beta_2$, $\beta_5$, and $\beta_6$, which may explain why this integrin does not mediate cell adhesion like its siblings.

Integrins are considered as special adhesive receptors because their adhesiveness can be dynamically regulated through a process termed inside-out signaling. On the cell surface, integrins normally adopt a low affinity state, which can be switched into high affinity state through inside-out signaling. Binding of the integrin extracellular ligands in turn transmits signals inward, a process called inside-out signaling, which exerts significant influences on cell mobility, proliferation, and differentiation, etc. [Ginsberg et al., 2005]. It has been shown that many integrins, including the $\beta_3$ integrin, can transmit these bidirectional signals across the plasma membrane. However, the $\beta_8$ cytoplasmic domain lacks all the protein–protein interaction sites that have been identified on the $\beta_3$ cytoplasmic domain and shows little homology to other $\alpha_v$ pairing partners. This fact implicates that either the $\beta_8$ integrin does not rely on inside-out signaling as other integrins or it uses a completely different set of machinery for cytosolic activation.
Likewise, the integrin probably mediates a distinct outside-in signaling.

It has been proposed that integrin affinity is regulated through global conformational changes of the integrin extracellular domains [Luo and Springer, 2006; Luo et al., 2007]. Many studies suggest that integrins exist on the cell surface as three different conformational states: bent, extended-closed, and extended-open. In the crystal structures of the full length ectodomains of αvβ3 [Xiong et al., 2001, 2002, 2009; Dong et al., 2012], αinβ3 [Zhu et al., 2008], and αvβ3 [Xie et al., 2010], integrins are all in the bent conformation with the α- and β-subunit C-termini only a few angstroms apart, consistent with association of the α- and β-subunit transmembrane (TM) domains. It was hypothesized that this bent conformation represents a resting, low affinity state, and inside-out activating signals induce separation of the integrin TM and cytoplasmic domains followed by separation of two ectodomain lower stalks and extension of these two legs [Wang and Luo, 2010; Ye et al., 2011; Hu and Luo, 2013]. Integrin extension then results in swing-out of the β-subunit hybrid domain, which causes the downward movement of the α7 helix of the β-I domain, leading to exposure of the ligands binding site and high affinity for soluble ligand binding [Luo et al., 2007]. Even though the activating model has been supported by numerous experimental data, it is still unclear whether every integrin assumes a bent conformation with low affinity for ligands under physiological conditions and goes through activation during integrin signaling. Based on previous studies [Mu et al., 2002], we proposed that the βa integrin might be an exception, which could adopt a conformation with high affinity for soluble ligands under physiological conditions.

In addition to global conformational changes, cations also play a critical role in regulating integrin affinity by interacting with the three ion binding sites [Luo et al., 2007; Campbell and Humphries, 2011]. The metal ion depend adhesion site (MIDAS) sits in the middle and is flanked by adjacent to MIDAS (ADMIDAS) and synergistic metal ion binding site (SyMBS) [Campbell and Humphries, 2011]. The MIDAS functions as a key integrating site for integrin ligands as metal ion binding site (SyMBS) [Campbell and Humphries, 2011]. The metal ion depend adhesion site (MIDAS) sits in the middle and is flanked by adjacent to MIDAS (ADMIDAS) and synergistic metal ion binding site (SyMBS) [Campbell and Humphries, 2011]. The MIDAS functions as a key integrating site for integrin ligands as metal ion binding site (SyMBS) [Campbell and Humphries, 2011].

EXPERIMENTAL PROCEDURES

CELLS AND PLASMIDS

HEK293T cells (American Type Culture Collection, Manassas, VA) were maintained in DMEM medium (Life Technologies, Grand Island, NY) supplemented with 10% FBS, 1% Non-Essential Amino Acids, 1% L-Glutamine, 1% Sodium Pyruvate, and 1% Penicillin-Streptomycin (all from Life Technologies). Plasmids containing wild-type human integrin αv and β3 were described previously [Takagi et al., 2002]. Wild-type βa integrin was generously gifted by Dr. Nishimura [Nishimura et al., 1994]. All of the chimera constructs were created via overlap PCR and the mutants were created by QuikChange kit (Stratagene, La Jolla, CA).

CHIMERIC INTEGRIN CONSTRUCTION

The integrin chimeras were generated by replacing different regions of β3 with the corresponding regions on βa. The chimera β3β8Leg was constructed by replacing the β3 residues 461–788 with βa 468–769 (whole lower leg swapping). Briefly, the DNA fragment coding the β3 integrin head domain (PSI, βI, and hybrid domains) was produced by using a forward primer: AGACTGACTACGC-CAGGCCGGGCCCG and an overlapping reverse primer which contains part of the βa EGF1 domain: GTCCCTGTGGTCTCTCA-CACTGACAGTCAAAATCAAGGGTACCTGGACG. Likewise, the leg domain fragment of βa was created using an overlapping forward primer which contains part of the βa hybrid domain: CGTCCAGGT-CACTTTGTATTGACTGTCGACTTGGAGCAAAGGAGGAC and a reverse primer of βa: GGAATCTTTGAAGTTCGACCTTGAAGTTCGACCTTGAAGT. The PCR reaction was performed by using Phusion® high-fidelity DNA polymerase (New England Biolabs, Ipswich, MA). The two fragments were then fused together and amplified by adding both the forward primer of β3 and the reverse primer of βa. The chimeric cDNA was then purified and subcloned into pCDNA3.1 vector.

Similarly, the β3β8 TM-Cyt chimera was created by replacing β3 717–788 with βa 680–769 using following primers: TATATGAAAGATAATTTTCTAAGTGCTGAGGAG and AGAGGCATGTTGATGCTTGTTACGTGACGAGACGAGC. The β3β8EGF-βTD chimera was created by replacing β3, 461–717 with βa 468–680 and following primers were used: CTAGAGACACACACAGGTAGTGGCTGAGGAGAACATCT-TGAGAT and AACTCGAAGATTTTCTCCAGCCGACAGATCTGGGT GTCCGTCTCAG.

TRANSIENT TRANSFECATION

 Constructs were transiently transfected into HEK293T cells using X-tremegene 9 transfection kit (Roche Diagnostics, Indianapolis, IN).
The expression levels of the wild-type integrins, the chimeras, and the mutants were determined by flow cytometry staining with monoclonal antibody AP3 (anti-β3 mAb, American Type Culture Collection), MAb1980 (anti-αv mAb, EMD Millipore Corporation, Billerica, MA), and 37E1 (anti-β8 mAb, a kind gift from Dr. Nishimura [Nishimura et al., 1994]).

**SOLUBLE LIGAND BINDING ASSAY**

Soluble ligand binding of Alexa Fluor 488-labeled human vitronectin and fibronectin (both from Sigma-Aldrich, St. Louis, MO) was determined as previously described [Wang et al., 2010]. Briefly, transfected cells were suspended in 20 mM HEPES-buffered saline (pH 7.4) (HBS) supplemented with 5.5 mM glucose and 1% BSA were pre-blocked with 1 μg anti-β1 antibody (EMD Millipore Corporation, Thermo Scientific Pierce, Rockford, IL). For fibronectin binding, cells were incubated with Alexa Fluor 543-labeled AP3 on ice for 30 min. Binding activity was presented as the percentage of the mean fluorescence intensity (MFI) of ligands staining relative to the MFI of MAb1980 or AP3 staining.

**RESULTS**

**EXPRESSION AND SOLUBLE VITRONECTIN BINDING OF INTEGRIN αvβ8 ON THE CELL SURFACE**

It has been proposed that integrins normally assume a bent conformation with low affinity for ligands on the cell surface whereas extracellular stimuli activate integrin from cytosol and induce extension with high affinity toward soluble ligands [Luo et al., 2007; Shattil et al., 2010; Wang and Luo, 2010; Kim et al., 2011; Hu and Luo, 2013]. However, based on the fact that there is no binding site on the β8 cytoplasmic domain for the inside-out activators, we proposed that αvβ8 integrin may adopt a different conformation with high affinity to its ligands under physiological conditions. To verify our hypothesis, we first expressed integrins αvβ3 and αvβ8 in the 293T cells, and used antibody staining to confirm their expression on the cell surface. As shown in Figure 1A, αv-specific MAb 1980 bound both transfecants well. The anti-β3 mAb AP3 bound to 293T cells expressing αvβ3 only, but not αvβ8 and control cells, confirming previous observation that there was no endogenous αvβ8 expression in 293T cells. By contrast, the anti-β8 mAb 37E1 bound cells transfected with αvβ8, indicating efficient expression and proper folding of the integrin on the cell surface (Fig. 1A).

To determine the ligand binding capacity, soluble ligand binding assay was performed using Alexa-488 labeled recombinant human vitronectin (Fig. 1B). As expected, αvβ3 bound little soluble vitronectin in the presence of Ca2+, whereas the presence of Mn2+ increased the binding significantly. By contrast, integrin αvβ8 bound soluble vitronectin in the presence of Ca2+ at maximal level, even better than that of αvβ3 in Mn2+. Addition of Mn2+ did not further increase binding affinity, suggesting that most population of this integrin has already adopted a high affinity state under physiological conditions.

We noticed that in a recent publication, a purified disulfide-linked extracellular fragment of αvβ8 integrin expressed marginal affinity for vitronectin [Ozawa et al., 2016] which is inconsistent with our observation and the previous report [Nishimura et al., 1994]. We believe that this contradictory may be caused by the artificially introduced disulfide bond which restricted separation of the β8 leg from the αv subunit, leading to a structural alteration on the ligand binding head domain.

**THE ECTODOMAIN LOWER STALK OF β8 IS IMPORTANT FOR THE HIGH AFFINITY STATE**

This high affinity of β8 integrin could be caused by the ligand binding head domain or the lower leg or by both. We therefore created a chimera swapping the β8 lower leg with that of the β3 to determine whether this region plays important role in the constitutively high affinity state. The chimera showed about 30% lower binding in comparison to the wild-type β8, indicating this region’s critical role in maintaining high affinity conformation (Fig. 1C).

The lower leg of β integrin is composed by four EGF-like domains (I-EGF1–4), a BTD domain, a TM domain, and a short cytoplasmic tail. We created ββ3 chimeras in which the whole β3 lower leg or only the TM-cytoplasmic domains were swapped with the corresponding β8 domains (Fig. 2A). The inherent low affinity of β3 integrin at resting state can provide a better sensitivity in studying the roles of β8 lower leg domains. Soluble ligand binding assay was performed to test ligand binding ability of the chimeras (Fig. 2B). We used human fibronectin labeled with Alexa-488 instead of vitronectin because fibronectin is more affordable, well studied, and more importantly, unlike vitronectin as shown in Figure 1, the basal binding of integrin αvβ3 to soluble fibronectin is very low under the physiological Ca2+ condition. Therefore, fibronectin is more sensitive to identify potential change of the ligand binding. As expected, the wild-type αvβ3 bound soluble fibronectin poorly in the presence of Ca2+, whereas the addition of Mn2+ significantly increased the binding capability. By contrast, the chimera containing the whole β8 lower leg (β3β8Leg) bound soluble fibronectin with much higher capability in the presence of Ca2+, comparable to the wild-type αvβ3 in Mn2+ (Fig. 2B), implicating a potent activating effect of the region. In contrast, the chimera with the β8 TM and cytoplasmic domains (β3β8TMT-Cyt) showed only moderately increased binding to soluble fibronectin under Ca2+ condition. The results suggested a critical role of the β8 lower ectodomain stalk, which consists of four EGF domains, in high ligand binding affinity. Indeed, swapping in only the lower stalk (β3β8EGF–BTD) was sufficient to promote ligand binding to a similar level as the whole leg swapping chimera (Fig. 2C). We also noticed that although both β3β8Leg and β3β8EGF–BTD expressed high affinity for soluble ligands, the binding could be further enhanced by addition of Mn2+. This suggests that swapping in the β8 lower stalk may shift the conformation equilibrium toward high affinity state, whereas Mn2+ activated the remaining integrin population that resides in low affinity state. Taking together, these discoveries strongly indicated that the β8
Fig. 1. Expression and soluble ligand binding of integrin $\alpha_8$ and $\beta_3\beta_3$ chimera. A. HEK293T cells were transfected with either $\alpha_8\beta_3$ or $\alpha_8\beta_8$ and stained with MAb1980 (anti-$\alpha_8$ antibody), AP3 (anti-$\beta_3$ antibody), and 37E1 (anti-$\beta_8$ antibody), respectively. Bold-solid line: untransfected HEK293T cells; thin-dashed line: transfected cells. B. Binding of Alexa A488 labeled vitronectin to HEK293T cells transiently transfected with either $\alpha_8\beta_3$ or $\alpha_8\beta_8$ in the presence of EDTA (5 mM), $\text{Ca}^{2+}$ (5 mM), or $\text{Mn}^{2+}$ (1 mM) as indicated. C. HEK293T cells were transiently transfected with the chimera containing a $\beta_8$ ligand binding head domain and a full length $\beta_3$ lower leg ($\beta_8\beta_3\text{Leg}$) and tested for Alexa A488 conjugated vitronectin binding together with wild-type $\beta_3$ and $\beta_8$ in the presence of EDTA (5 mM), $\text{Ca}^{2+}$ (5 mM), or $\text{Mn}^{2+}$ (1 mM) as indicated. Error bars represent standard deviation (S.D.) from at least three independent assays.
lower ectodomain stalk is important for the overall high affinity conformation.

**Ca2⁺ IS ESSENTIAL FOR INTEGRIN αVβ8 MEDIATED LIGAND BINDING, WHEREAS Mg2⁺ INHIBITS LIGAND BINDING AT LOW CONCENTRATIONS BUT ENHANCES IT AT HIGHER CONCENTRATIONS**

Previous studies indicated that Mn²⁺ and Mg²⁺ are positive regulators of integrin ligand binding for most integrins [Mould, 1996], by the interaction of the cations with MIDAS on the αI or βI domain and by competition of Mn²⁺ against Ca²⁺ at ADMIDAS, a negatively regulatory site when occupied by Ca²⁺, respectively [Zhang and Chen, 2012]. However, these studies have all been carried out in integrins expressing low affinity under physiological conditions. We therefore tested affinity states of integrin αVβ8 with Alexa 488 labeled vitronectin under various ion conditions and used αVβ3 as a comparison (Fig. 3a). The results clearly suggested a dramatically different role of Mg²⁺ on αVβ8 regulation. Addition of Mg²⁺ at 1 mM concentration reduced ligand binding by about 30% from the level of Ca²⁺ only condition for αVβ8. In contrast, the ligand binding of αVβ3 did not change after addition of Mg²⁺. Furthermore, chelating Ca²⁺ with 1 mM EGTA in the presence of Mg²⁺ completely ablated ligand binding of the αVβ8 integrin. We further studied the effects of Mg²⁺ in a concentration dependent manner (Fig. 3b). The results indicated that low concentration of Mg²⁺ could reduce ligand binding in αVβ8. However, appearance of Mg²⁺ at high...
concentration (>5 mM) restored the αvβ8 ligand binding to a level similar to the Ca²⁺ only condition. Our results suggested that Mg²⁺ at low concentration inhibits interaction between αvβ8 and its ligand, but this inhibitory effect could be reversed by high concentration of Mg²⁺. Therefore, we propose that there is another Mg²⁺ binding site other than MIDAS in the β8 integrin. The site has a lower affinity for Mg²⁺ and can only be occupied at high ion concentration, and the occupancy of Mg²⁺ at this site could promote affinity of the β8 integrin toward its ligand. More study is needed for a better understanding of this potential ion binding site.

THE UNIQUE ADMIDAS CONTRIBUTES TO HIGH AFFINITY FOR SOLUBLE LIGANDS OF β8 INTEGRIN

Due to the importance of the cation regulation of integrins, it is possible that the high affinity of αvβ8 is also caused by the ion coordinating sites. By comparing the primary sequences of beta integrin subunits, we found that the ADMIDAS domain on β8 is unique among all the beta integrins (Fig. 4A). The two Asp residues which are universal at ADMIDAS in all other integrins are replaced with two Asn (Asn120 and Asn121). Since Asn is polar instead of negatively charged under physiological conditions, this unique ADMIDAS likely plays a distinct role in integrin affinity regulation. We therefore designed two mutants: β3Asp126_127Asn (β3N) and β8Asn120_121Asp (β8D), to study the effect of this specific residue replacement (Fig. 4B). The results indicated that mutating β3 ADMIDAS did not cause significant change, and Mn²⁺ could increase ligand binding of the β3N mutant similar to the wild type. By contrast, replacing the two Asn with Asp at β8 ADMIDAS reduced ligand binding and this negative effect was more prominent in the presence of Mg²⁺. Our result suggests that the lower negativity of the β8 ADMIDAS may play an important role on stabilizing the integrin in high affinity conformation.

We further tested the effects of Mg²⁺ in β8 mediated ligand binding with several chimeric/mutant β8 constructs. The β8β3Leg is

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**Fig. 4.** The unique ADMIDAS plays a critical role in β8 integrin mediated ligand binding. A. Sequence alignment of MIDAS (Bold), ADMIDAS (Bold and underlined), and SyMBS (Underlined) between all β integrins. Note that the β8 ADMIDAS is distinct from others. B. Binding of Alexa A488 labeled vitronectin to HEK293T cells transiently transfected with different αvβ3 or αvβ8 constructs in the presence of different ions as indicated. Error bars represent standard deviation (S.D.) from at least three independent assays.
We identified the magnesium binding sites have been identified as the two highly conservative, negatively charged Asp are replaced with Asn at β8 ADMIDAS. Our mutagenesis study suggested that this difference may contribute to the high affinity conformation of α5β8.

It has been shown that many integrins adopt a bent conformation with low affinity for ligands in resting state and can be converted into extended, high affinity conformation upon inside-out activation [Luo and Springer, 2006; Kim et al., 2011]. Our study indicated that the β8 integrin is an exception. This integrin lacks all the identified binding sites of the integrin cytosolic activators, indicating that the integrin is unable to be activated from the cytosol. In this study, our experiments showed that β8 integrin expressed high affinity for soluble ligands even under physiological conditions. Therefore, this integrin may not necessarily rely on intracellular signal-induced conformational change to switch into a high affinity state and become functional. This is the first integrin that has been shown to exist in an active state under physiological conditions. The discovery has many important implications for the mechanism of the integrin conformational regulation as well as the relationship between the integrin signal transduction and diverse biological functions.

The β integrin lower leg is comprised by several domains. Previous studies have shown that the disruption of the cytoplasmic domain association and afterward dissociation of the TM domain are essential in integrin activation, making the TM/cytoplasmic domains the most important regulatory sites during integrin activation [Hughes et al., 1996; Zhu et al., 2007; Hu and Luo, 2015]. However, our results suggested that in β8 integrin, the TM domain and cytoplasmic tail do not play a key role in maintaining high affinity conformation. In contrast, the ectodomain lower stalk, which is comprised by the EGF domains and βTD, is sufficient to transform the β3β8 chimera into high affinity state. On the plasma membrane, integrins are proposed to dynamically adopt three conformational states: bent-closed, extended-closed, and extended-open [Luo and Springer, 2006]. These three states are believed to coexist in a dynamic equilibrium whereas ligand interaction or inside-out activation alters the equilibrium toward extended-open conformation [Chen et al., 2010; Springer and Dustin, 2012]. It is plausible that the lower leg of β8 integrin shifts the equilibrium toward extended-open state. The lower β leg is believed to bend sharply between the EGF1 and EGF2 domains under physiological conditions, forming a β knee which is extended and straightened during integrin activation [Zhu et al., 2008]. Since the EGF domains are crucial in integrin conformational change, it is possible that the β8 EGF domains, especially the EGF 1–2, are important in stabilizing the high affinity conformation.

Metal ions have been shown to play a critical role in integrin affinity regulation [Luo et al., 2007; Zhang and Chen, 2012]. Three ion binding sites have been identified on beta integrin head: MIDAS, ADMIDAS, and SyMBS. Cation occupancy in MIDAS is known to direct ligands binding through coordinating negatively charged carboxyl group of the ligands. In contrast, it has been reported that ADMIDAS can only be bound by Ca2+ and Mn2+, and the ligand binding ability can be inhibited by Ca2+ occupancy or promoted by Mn2+ occupancy [Chen et al., 2003; Mould et al., 2003; Pesho et al., 2006]. However, the previous studies had been carried out in integrins

**DISCUSSION**

Two β3 integrins (αβ3β3 and αβ3β3) have been widely studied as models for integrin bidirectional signaling. On the contrary, little is known about the β8, which also associates with αv integrin and belongs to arginine–glycine–aspartic acid receptor family. Its affinity and conformational states still remain elusive. In this study, we identified integrin β8 as the first integrin to express high affinity for soluble ligands under physiological conditions. We also discovered that both the head and the leg of the integrin contribute to this high affinity state. By using the chimeras that combined the ligand binding head domain of the β3 integrin with different domains of the β8 integrin leg, we revealed an important role of β8 ectodomain lower stalk in the high affinity conformation. We also discovered that the ADMIDAS on β8 integrin is strikingly different from all other β integrins, as the two highly conservative, negatively charged Asp are replaced with Asn at β8 ADMIDAS. Our mutagenesis study suggested that this difference may contribute to the high affinity conformation of α5β8.

Fig. 5. β8 ADMIDAS is a potential regulatory site of Mg2+. Binding of Alexa A488 labeled vitronectin to HEK293T cells transiently transfected with different α5β8 constructs under gradually increasing Mg2+ concentrations as indicated. Note that the ADMIDAS mutation greatly jeopardized the ability of high concentration Mg2+ to promote ligand binding. Error bars represent standard deviation (S.D.) from at least three independent assays.

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[Chen et al., 2003; Mould et al., 2003; Pesho et al., 2006] has shown that many integrins adopt a bent conformation with low affinity for ligands in resting state and can be converted into extended, high affinity conformation upon inside-out activation [Luo and Springer, 2006; Kim et al., 2011]. Our study indicated that the β8 integrin is an exception. This integrin lacks all the identified binding sites of the integrin cytosolic activators, indicating that the integrin is unable to be activated from the cytosol. In this study, our experiments showed that β8 integrin expressed high affinity for soluble ligands even under physiological conditions. Therefore, this integrin may not necessarily rely on intracellular signal-induced conformational change to switch into a high affinity state and become functional. This is the first integrin that has been shown to exist in an active state under physiological conditions. The discovery has many important implications for the mechanism of the integrin conformational regulation as well as the relationship between the integrin signal transduction and diverse biological functions.

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adopting low affinity conformation at resting state. We have shown that β₈ integrin adopts a high affinity state in the presence of Ca²⁺ and therefore, the metal ions may regulate the integrin in a different manner. Indeed, our study showed that the β₈ ADMIDAS is critical for high affinity conformation of the integrin. We also found that low concentration of Mg²⁺ inhibited β₈ integrin for ligand binding, whereas high concentration of Mg²⁺ promoted ligand binding. The discovery suggests the existence of another Mg²⁺ binding site rather than MIDAS on the β₈ head domain. The unique β₈ ADMIDAS might be occupied by Mg²⁺ at high concentration and this occupancy can promote β₈ mediated ligand binding. The extracellular calcium with mM concentration plays a vital role in nervous system [Simons, 1988; Burgoyne and Haynes, 2012]. Peripheral nerve is exposed to extracellular fluid which contains about 1–2 mM Ca²⁺ whereas central neurons are bathed in an intracellular fluid with about 1 mM Ca²⁺. Changes in the calcium concentration have a profound effect on neuronal functions. On the other hand, magnesium may have more complex effects on neuronal functions [Lee et al., 2010]. It is possible that the β₈ ADMIDAS might have an essential role in sensing a fluctuating environmental ion condition as this integrin lacks a cytosolic “on/off” switch.

In conclusion, our research revealed for the first time that the β₈ integrin adopts a constitutively high affinity state toward its soluble ligands under physiological conditions. Our study showed that both the β₈ ectodomain lower stalk and its unique ADMIDAS are important for this high affinity conformation. One of the most important biological roles of the β₈ integrin is mediating maturation and activation of TGF-β1. This is achieved through binding to latent TGF-β1 and introducing membrane type 1 metalloprotease dependent active TGF-β1 release [Mu et al., 2002]. Since the β₈ cytoplasmic tail lacks binding sites for all the known integrin cytosol activators, it is most likely that the only way for this integrin to efficiently bind latent TGF-β1 is to maintain high affinity state under physiological conditions. In fact, a recent study showed that recombiant integrin α₁β₈ extracellular domain indeed strongly interacts with latent TGF-β1 [Ozawa et al., 2016]. However, more research is required to confirm if the high affinity conformation is indispensable for β₈ to function properly.

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