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Involvement of Transmembrane Domain Interactions in Signal Transduction by α/β Integrins*

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The α and β subunits of α/β heterodimeric integrins function together to bind ligands in the extracellular region and transduce signals across cellular membranes. A possible function for the transmembrane regions in integrin signaling has been proposed from structural and computational data. We have analyzed the capacity of the integrin α_2, α_{IIb}, α_4, β_1, β_3, and β_7 transmembrane domains to form homodimers and/or heterodimers. Our data suggest that the integrin transmembrane helices can help to stabilize heterodimeric integrins but that the interactions do not specifically associate particular pairs of α and β subunits; rather, the α/β subunit interaction constrains the extramembranous domains, facilitating signal transduction by a promiscuous transmembrane helix-helix association.

Integrins are eukaryotic cell surface receptors consisting of heterodimers of α and β subunits. Each subunit contains a large extracellular domain, a single transmembrane (TM)[1] -spanning helix, and a small cytoplasmic domain (for recent reviews see Refs. 1–3). In humans, eight different α chains interact with at least 16 different β subunits to form no fewer than 22 different α/β heterodimers with different functions (4). By spanning the membrane, the α/β heterodimeric integrins form a dynamic linkage between the cytoplasm and the extracellular space, transducing signals across the cell membrane to mediate cell growth, differentiation, gene expression, motility, and apoptosis (5). Some of the functions are not fulfilled by the heterodimeric integrins alone but by larger complexes formed on the cell membrane by the interaction of integrins with other membrane-anchored receptors (6).

Inside-out signal transduction via integrins, in which cytoplasmic events are communicated to the outside, is believed to be mediated by a rearrangement of the α and β subunits relative to each other and by separation of the cytoplasmic integrin domains (1, 7, 8), which alters the affinity of the integrins for extracellular ligands.

The high-resolution structure of the soluble extracellular domain of the α_V/β_3 integrin reveals that its 12 domains are assembled in one "head" and two "tail" regions (9). Although the structure shows an extensive interface region of the two domains within the head region, the two tails were shown to be associated as a single stalk in the membrane proximal region. A three-dimensional model of the human α_{IIb}/β_3 integrin derived from electron cryomicroscopy and single particle analysis shows a cylindrical transmembrane density ~30 Å long connecting the small cytoplasmic domains to the extracellular part (10). The shape of the rods suggests a parallel crossed α-helical structure of the two TM helices, which seem to associate in the membrane. NMR measurements show a direct association of the cytoplasmic domains (11), and it was shown that the membrane proximal residues of the cytoplasmic β_3 integrin domain form an α-helix (12). This α-helical structure is most likely the extension of an α-helical transmembrane domain, because the amino acids located N-terminal to this stretch are highly hydrophobic. A multiple sequence alignment of the predicted TM-spanning regions of various integrin sequences reveals that a G*XXX*G-like motif can be found in this region (13). The G*XXX*G motif was previously found to be important for the dimerization of GpA (14–16), and selection of a library of random TM sequences for homodimerization showed that the G*XXX*G motif is sufficient for strong helix-helix interactions and that it provides a more general framework for the formation of homodimers (17). Additionally, a statistical analysis of a data base containing over 13,000 TM sequences showed that the G*XXX*G motif is highly overrepresented in TM domains (18), supporting the importance of the G*XXX*G motif in transmembrane helix-helix interactions. The study also showed that other combinations of small residues (Gly, Ala, Ser) are overrepresented. For the homodimerization of the ErbB receptor family, combinations of small residues other than the G*XXX*G motif can also mediate TM helix-helix interactions (19). A global search of possible integrin TM helix-helix interactions (13) as well as modeling of the integrin TM domains in the electron cryomicroscopy map of the $\alpha_{II}b\beta_3$ integrin structure (10) revealed that the TM helices are most likely packed in a crossed helix structure similar to the structure of GpA (20); the authors in Refs. 10 and 13 suggested that the G*XXX*G-like motif is involved in stabilization of the integrin TM helix interactions. Though it was shown that the interaction of the α and β subunits of the α_{IIb}/β_3 integrin is driven by the large extracellular domain (21), the dimerization efficiency is lower if the intracellular and TM domains are missing (22). The membrane proximal parts of the individual subunits of the α_{IIb}/β_3 integrin interact and can inactivate the integrin (23). NMR analysis of the interaction of the small cytoplasmic domains failed to show any interaction (12), and a study of the cytoplasmic and transmembrane part of the α_{IIb}/β_3 integrin demonstrated only homo-oligomerization of the subunits rather than the formation of hetero-oligomers (24). These results are in contrast to a variety

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[1] The abbreviations used are: TM, transmembrane; WT, wild type.

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of data on the whole proteins showing interaction of at least the cytosolic, membrane proximal parts of the integrins, and it was argued that the missing observation of hetero-oligomers was caused by the experimental conditions used (13). Using Förster resonance energy transfer measurements with αL and β2 proteins fused to cyan or yellow fluorescent proteins, respectively, it was shown that the cytoplasmic domains of integrins get separated during transmembrane signaling (7), and large-scale rearrangements of the purified extracellular domains of integrin α and β subunits associated with integrin activation were additionally shown by NMR and electron microscopic studies (25, 26).

In this study we examine the possible role of the integrin TM domains in the formation and stabilization of the α/β heterodimeric integrin structure. The integrin TM segments were determined to consist of about 30 residues (27), and structural data show a parallel crossed α-helical structure of the two TM helices within the membrane (10). To elucidate the possible function of the TM core residues, and especially the G*XXX*G-like motif, that are most likely involved in the formation or stabilization of oligomeric integrins, we concentrated on a hydrophobic stretch of 16–17 amino acids not involving membrane integral residues localized at the ends of the TM helices and thus probably not involved in interaction. The TM core domains of the α_2, α_{IIb}, α_4, β_1, β_3, and β_7 integrins were expressed as chimeric proteins, and their capacity to form homo-oligomers as well as α_2/β_1, α_{IIb}/β_3, α_4/β_1, and α_4/β_7 hetero-oligomers was measured using the GALLEX assay (28). We show that most TM domains homo-oligomerize to some extent and that they all form hetero-oligomers, although the strength of the hetero interaction is weak when compared with the interaction of the GpA TM domain. Introduction of single point mutations in the G*XXX*G-like motif of the α_4/β_7 integrin destabilized the oligomeric structures, so this motif is most likely involved in the stabilization of heterodimeric integrins. Our data together with recently published results indicate that the TM domains have a function in stabilizing the transmembrane region in the low affinity and/or intermediate affinity state of integrins (1). The observation that most TM domains also homo-oligomerize argues against a function of the TM domains as the sole mediators of specific α/β interactions.

MATERIALS AND METHODS

Construction of GALLEX Chimera and β-Galactosidase Assay—The construction of the plasmids pBLM100 and pALM148 and of the chimera of GpA has been described (28). To create the chimeras for the integrin TM regions, synthetic oligonucleotide cassettes encoding for the integrin TM domains were ligated into the SpeI/SacI restriction-digested plasmids.

To measure homo-association, plasmids containing GALLEX chimeras were transformed in *Escherichia coli* SU101. For the measurement of hetero-association, the plasmids were transformed in *E. coli* SU202 (29). Cells were grown in the presence of antibiotics and 0.01 mM isopropyl-1-thio-β-D-galactopyranoside overnight and diluted in the same medium to $A_{600} = 0.1$ the next morning. Cells were harvested at $A_{600} = 0.6$, and β-galactosidase activity was measured as described in Ref. 30.

Whole cell lysates were used to estimate expression levels of chimeras. Chimeric proteins were detected by Western analysis using anti-LexA-antibodies (Invitrogen), and blots were developed using nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate.

Membrane insertion of the chimeric proteins was tested by NaOH extraction of lysozyme-treated cells (31). To test for the orientation of the chimera in the *E. coli* membrane, plasmids were transformed into *E. coli* NT326 cells, which are *malE*-deficient, and grown on M9 medium with maltose as the only carbon source. Only if the MalE domain of the chimeric proteins is located in the periplasm are the cells able to complement for the absence of endogenous MalE and the topology is established.

RESULTS

Homo-association of Integrin TM Helices—The GALLEX system measures the oligomerization of given TM helices by the repression of a reporter gene (*lac*Z) activity in the *E. coli* strains SU101 or SU202 (Fig. 1). In SU101 the wt promoter/operator region of the *lexA* gene controls the expression of the *lacZ* reporter gene; if two TM helices form a homo-oligomer the dimeric DNA-binding domain can bind to the promoter region and repress the reporter gene activity (28, 29). Incorporation of the chimeric proteins into the *E. coli* membrane was tested by NaOH extraction; the orientation of the chimera in the membrane was tested by a complementation assay as described under "Materials and Methods" and in Ref. 28 (results not shown).

To test the ability of the α_2, α_{IIb}, α_4, β_1, β_3, and β_7 TM domains to form homo-oligomers, synthetic oligonucleotide cassettes encoding the hydrophobic core regions of integrin TM helices (Fig. 2*A*) were cloned into the plasmid pBLM (28) and homo-oligomerization was measured using the GALLEX system (28). As internal standards, the homodimerization capacity of GpA wt and the disruptive G83I mutation was also measured. The TM domain of the wt GpA forms a strong homodimer as shown in many studies (15, 16, 32), and the introduction of the G83I mutation strongly disrupts this dimer (14, 15). We used the GpA wt and G83I mutant to set the repression scale, with wt set as "strong interaction" and G83I as "weak interaction."

The results shown in Fig. 2*B* demonstrate that the integrin α_2, α_{IIb}, α_4, and β_1 TM domains have only a slight tendency to form homo-oligomers when compared with the GpA TM domain, with a homodimerization capacity only slightly higher than for GpA G83I. The integrin β_3 TM domain forms only very weakly associating oligomers, whereas the integrin β_7 TM domain interacts with itself as strongly as GpA wt.

Heterodimerization of Integrin TM Helices—Most available data have indicated that integrins form α/β heterodimeric complexes, an inference strongly supported by the recently published structure of the α_{IIb}/β_3 integrin (10). It is known that the extracellular domains alone can hetero-associate (22). Nevertheless, although the extracellular domains of α and β subunits are able to form a heterodimer, this is accomplished with a lower efficiency in the absence of the transmembrane and cytoplasmic domains compared with the full-length proteins (22).

To determine the capacity of two different TM helices to form hetero-oligomers, the two chimeric proteins are expressed from the two different plasmids, pBLM100 and pALM148, that encode wt and mutated LexA DNA-binding domains, respectively (28). In the *E. coli* reporter strain SU202, only a dimeric LexA DNA-binding domain, comprising one wt and one mutated LexA domain, can efficiently repress the reporter gene activity (29). Using the GALLEX approach, we tested the TM domains for the ability to form α_2/β_1, α_{IIb}/β_3, α_4/β_1, and α_4/β_7 hetero-oligomers, respectively. For any pair of integrin TM helices, two measurements were carried out: one with the α TM domain expressed from pBLM100 and the β TM domain from pALM148, and in the complementary experiment the individual TM domains were each expressed as chimera from the opposite plasmid.

The results shown in Fig. 3 indicate that the integrin TM domains of all tested combinations form hetero-oligomers, although the interactions are not as strong as the GpA wt homodimerization. The capacity of the integrin α/β pairs to hetero-associate is somewhat higher than the weak homodimerization capacity of the GpA G83I mutant.

Sequence Analysis of Human Integrins—A recent sequence alignment of integrin sequences from a variety of organisms revealed that a G*XXX*G-like motif is conserved in most integrin

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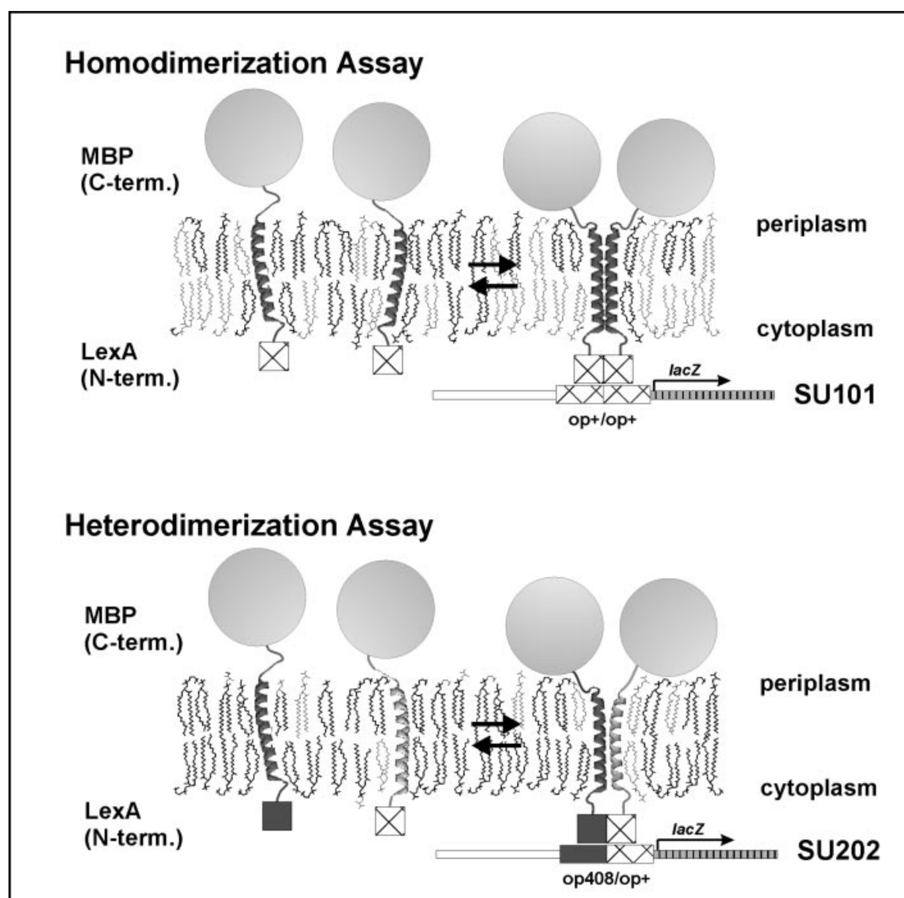

FIG. 1. **The GALLEX system measures homo- and hetero-association of single TM helices.** TM domains are expressed as chimeras with an MBP domain at the C terminus and the LexA DNA-binding domain coupled to the N terminus. If two TM domains interact, the dimeric LexA DNA-binding domain can bind to the promoter/operator region repressing the activity of the reporter gene *lacZ*. In the reporter strain SU101 the wt *lexA* promoter/operator can be recognized by a homodimeric wt LexA DNA-binding domain (*top*), whereas in the strain SU202 only a heterodimeric protein, composed of one wt and one mutated LexA DNA-binding domain, can bind to the promoter/operator (*bottom*). The wt LexA fusion is expressed from the plasmid pBLM100; the LexA408 chimera is expressed from pALM148.

TM domains (13). A sequence alignment of the human integrin sequences in shown in Fig. 4. The alignment shows that in most cases a motif can be found that consists of one small residue (Gly, Ser, Ala), three variant amino acids, and one other small residue.

The G*XXX*G motif is a central component of the dimerization motif of the human GpA sequence, and it has been shown that in some cases at least one Gly residue can be replaced by another small residue (19). Although all of the human integrin α subunits and most of the β subunits contain a G*XXX*G-like motif at similar positions in the predicted TM regions, the integrin β_4 sequence has no such motif and the β_8 sequence contains a G*XXX*V motif.

Importance of the GXXXG-like Motif for Heterodimer Formation—Because most of the integrin TM domains contain a G*XXX*G-like motif and it was proposed that this motif mediates the association of the two α and β subunits in a GpA-like manner (10, 13), we tested for the significance of the small residues in mediating the interaction of the two TM domains. For this purpose the $\alpha_4\beta_7$ integrin was selected because this pair forms the strongest hetero-oligomer (Fig. 3) and changes in the interaction capacity of two TM-helices after introduction of single point mutations can easily be monitored.

In Fig. 5 the homo-association capacity of the wt and mutated α_4 and β_7 integrin TM domains is shown. In the α_4 sequence, the S*XXX*G motif was changed to G*XXX*G, I*XXX*G, or S*XXX*I, respectively. In the β_7 sequence the two Gly residues were mutated to Ile separately. A replacement of the Gly-83 residue in the dimerization motif of GpA by Ile was shown to be highly disruptive (15). The exchange of the small Gly by the bulky Ile residue changes the interacting surface, resulting in only a very weak interaction of the monomers.

The results show that the substitution of the Ser in the α_4 sequence by both Gly or Ile leads to a decreased interaction capacity of the TM helix. Also, the mutation of the Gly residues to Ile strongly decreases the tendency of the TM helices to form homo-oligomers. The effect on the homo-oligomerization capacity after mutating the Gly residues to Ile is stronger in the integrin β_7 case. The substitution of either of the two Gly by Ile leads to a strong decrease in the interaction capacity, and the effect is stronger for Gly-11 than for Gly-7.

The effect of introducing the single point mutations on the hetero-oligomerization capacity of the heterodimeric α_4/β_7 integrin was tested in *E. coli* SU202. The results, shown in Fig. 6, indicate that all mutations influence the capacity of the two TM domains to associate, and the substitution of either of the two Gly residues in the integrin β_7 sequence has the strongest effect on the hetero-oligomer formation. In all cases the expression level of the chimera was approximately the same as tested by Western analysis. We observed an asymmetry in the results depending on which TM domain was expressed from which plasmid. If the α_4 wt and mutated sequences are expressed from the plasmid pALM148, differences can be observed that are in good agreement with the data obtained in the homo-association assay. If the β_7 TM domain is expressed from the plasmid pALM148, basically no differences between the wt

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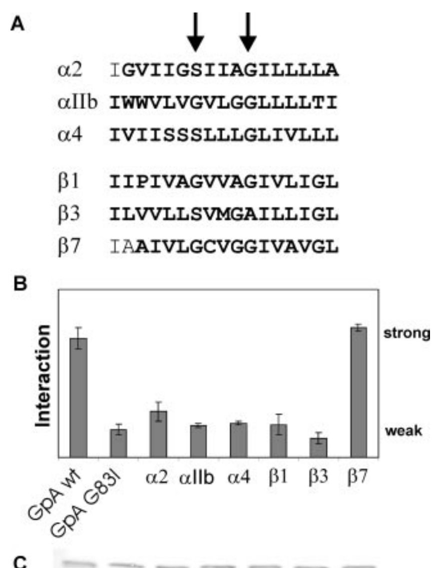

FIG. 2. **Homo-association of integrin TM domains.** *A*, the sequences of the integrin TM domains used for the GALLEX measurements are shown. The sequences are numbered 1 to 17. Polar amino acids at the ends of the putative TM sequences were replaced by unpolar residues, indicated by *light letters*. *B*, the capacity of the integrin α_2, α_{IIb}, α_4, β_1, and β_3, β_7 integrin TM domains to form homo-oligomers was measured using the GALLEX system (28). As internal standards the capacity of the wt and G83I mutated GpA TM domains to form homodimers was measured. The relative association capacity of each TM domain was determined by measuring the β-galactosidase activities of three independent clones; the activities are shown relative to GpA wt. The expression level of the chimeric proteins was checked by Western analysis using anti-LexA-antibodies (*C*).

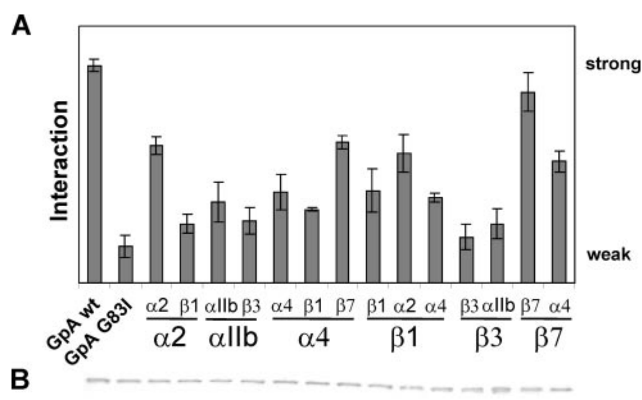

FIG. 3. *A*, heterodimerization of the α_2/β_1, α_{IIb}/β_3, α_4/β_1, and α_4/β_7 hetero-oligomers. *Bars* represent the S.D. of three independent measurements. The association capacities are shown relative to the strain expressing GpA wt from both plasmids. Integrin sequences shown *below the black bar* are expressed from pALM148, the ones *above* the bar are expressed from pBLM100. *B*, expression level of the chimera in each strain was tested by Western analysis using anti-LexA antibodies.

hetero-oligomer and the oligomers containing one mutated subunit can be observed. This kind of asymmetry was observed in other studies (28, 33) and is most likely caused by the slightly different expression levels from the two plasmids used in this study. Nonetheless, the putative interaction motifs are implicated by the preponderance of the mutation evidence.

DISCUSSION

Formation of Heterodimeric α/β Integrins—Biochemical and biophysical data strongly indicate the presence of α/β heterodimeric integrins (2). The large extracellular domain of integrins is not only important for ligand binding but also for subunit association and stabilization of the heterodimer (21). Analysis of the cytoplasmic domain alone (12) as well as of the cytoplasmic and the TM domains (24) failed to show any importance of these domains for the formation of α/β hetero-oligomeric integrins. Li *et al*. (24) identified strong homo-oligomers; a function of these homo-oligomers in integrin signaling processes *in vivo* was postulated (34).

The data presented in our study clearly show that some of the integrin TM helices form homo-oligomers. Though these oligomers do not interact as strongly as GpA wt, the result could explain the observed formation of homo-oligomers (24). For the β_3 TM domain we could not see any homo-oligomerization, as recently shown by analytical ultracentrifugation of the cytoplasmic and TM domains (24). Because the GALLEX assay is highly sensitive to the localization of the interacting surface of the TM helices with respect to the DNA-binding domain (28), the results presented here are not necessarily contradictory to the recent findings and the formation of heterotrimers driven by any other surface, but the G*XXX*G side cannot be excluded. The results only show that the G*XXX*G motif is not involved in the formation of strong integrin β_3 homodimers.

Mechanism of Interaction/Importance of the GXXXG-like Motif—It has been suggested that the G*XXX*G-like motif found in most integrin TM sequences is involved in the formation of α/β heterodimeric integrins (13). It was proposed that this motif drives the interaction to form a dimeric structure that is similar to the structure of GpA, and a global search of possible TM helix-helix interactions showed that the TM domains of the α_{IIb} and β_3 integrins are capable of forming a structure similar to the structure of the homodimeric GpA (20). The recently published structure of the α_{IIb}/β_3 integrin from cryoelectron microscopy also indicates that the TM domains are most likely associated as a right-handed dimer with a crossing angle of about 40° similar to the GpA structure (10).

In the study presented here we investigated the role of the TM domains in the formation of an integrin structure with interacting TM domains. The function of the G*XXX*G-like motif for the formation of the heterodimeric integrins was investigated using the α_4/β_7 integrin. *In vivo* this α/β heterodimeric integrin can mostly be found on leukocytes, where it mediates lymphocyte adhesion (4). Although most integrins contain a G*XXX*G-like motif with single substitutions of Ala or Ser, a precise G*XXX*G motif can only be found in the human α_{IIb}, α_E, β_1, and β_7 integrins (Fig. 4). In the cases of the α_D and the β_5 integrins, both Gly positions are filled by Ala and Ser.

The GALLEX measurements of the integrin α_4 and β_7 TM domains show that single point mutations affect the formation of homodimers (Fig. 5). In the case of the α_4 integrin the differences between the interaction capacity of the wt and mutated sequences were not as strong as in the case of the β_7 integrin. Because the wt α_4 integrin TM domain shows only a small tendency to form homodimers, this effect is not surprising. Interestingly, the replacement of Ser-7 by the smaller Gly residue did not result in a stronger interaction but also destabilized the existing homodimer (Fig. 5), although not as much as the introduction of an Ile at this position. Because a smaller Gly residue would allow a tighter packing of two TM helices, this result indicates that the interaction is not only stabilized by tight packing but that the Ser residues may have an additional role. Ser residues can contribute to the formation of homo-oligomers by forming hydrogen bonds (35), and it is possible that the formation of hydrogen bonds is also important for the interaction studied here.

The replacement of any of the two Gly residues in the G*XXX*G motif of the β_7 integrin TM domain highly disrupted the homologous interaction, as shown in Fig. 6.

A measurement of the capacity of the wt and integrin α_4 and β_7 sequences with single point mutations showed that the

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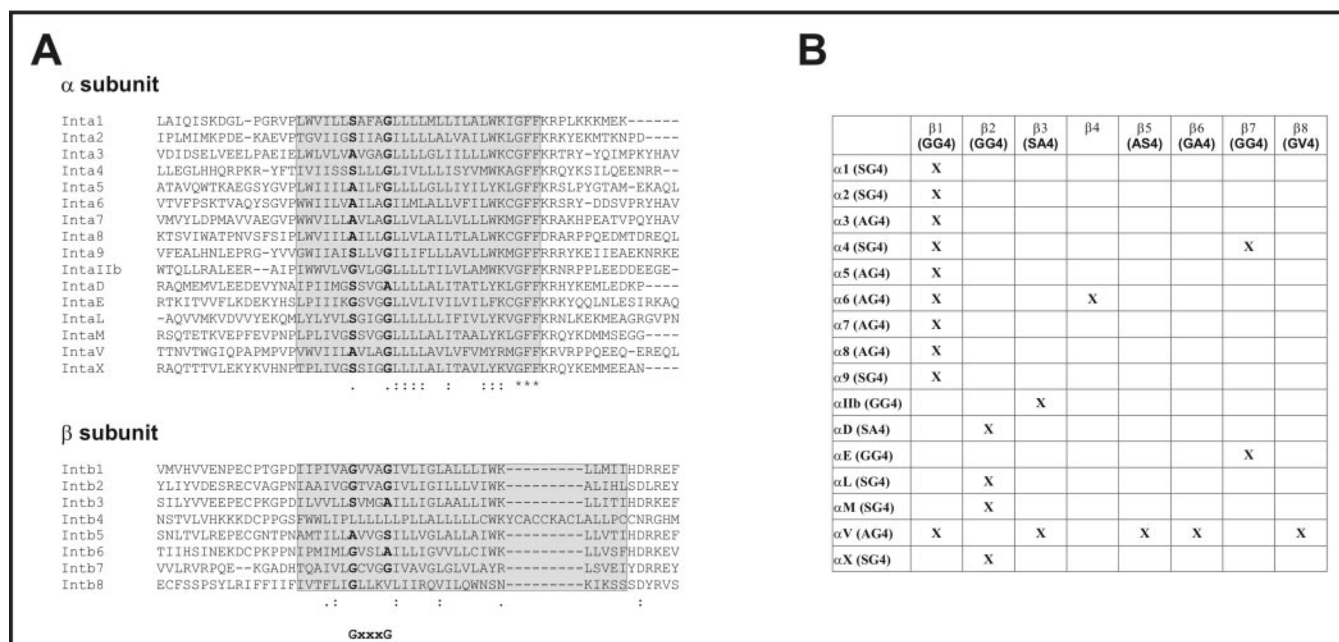

	β1 (GG4)	β2 (GG4)	β3 (SA4)	β4	β5 (AS4)	β6 (GA4)	β7 (GG4)	β8 (GV4)
α1 (SG4)	X							
α2 (SG4)	X							
α3 (AG4)	X							
α4 (SG4)	X						X	
α5 (AG4)	X							
α6 (AG4)	X			X				
α7 (AG4)	X							
α8 (AG4)	X							
α9 (SG4)	X							
αIIb (GG4)			X					
αD (SA4)		X						
αE (GG4)							X	
αL (SG4)		X						
αM (SG4)		X						
αV (AG4)	X		X		X	X		X
αX (SG4)		X						

FIG. 4. *A*, sequence alignment of human integrin α and β subunits. The TM regions are aligned using the program Clustal X (44) with default settings. The TM domains are highlighted in *gray*, and the possible G*XXX*G-like dimerization motif is indicated. *B*, integrin α/β subunit combinations in humans. *Empty boxes* represent subunit interactions that have not been examined. The exact composition of the G*XXX*G-like motifs is given in *parentheses* behind each integrin subunit.

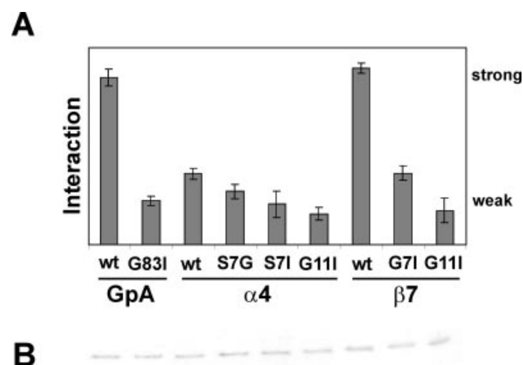

FIG. 5. **Homo-association of integrin TM domains with single point mutations in the G*XXX*G-like motif.** *A*, the point mutations are indicated in the sequences of the α_4 and β_7 integrin TM domains. *B*, capacity of the wt and mutated integrin TM domains to form homo-oligomers. As internal standards the capacity of the wt and G83I mutated GpA TM domains to form homodimers was measured. *Bars* represent the S.D. of three independent measurements. Oligomerization capacities are shown relative to GpA wt. The expression level of the chimeric proteins was checked by Western analysis using anti-LexA antibodies (*C*).

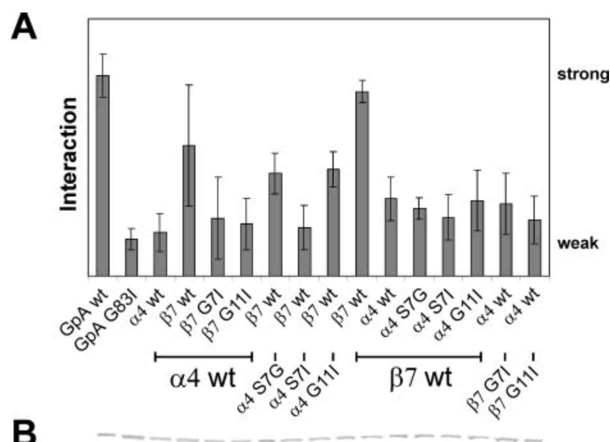

FIG. 6. **Heterodimerization of the α_4/β_7 hetero-oligomer with single point mutations.** *A*, *bars* represent the S.D. of three independent measurements. Activities are shown relative to the strain expressing GpA wt from both plasmids. Integrin sequences shown *below the black bar* are expressed from pALM148; the ones *above the bar* are expressed from pBLM100. *B*, expression level of the chimera in each strain tested by Western analysis using anti-LexA antibodies.

G*XXX*G-like motif indeed seems to be important for the interaction. The observed effects in the capacity to form heterodimers are not as strong as in the case of the homo-oligomers. In the case of the measurement of hetero-oligomers only one small residue is changed on one TM helix, whereas in the homo-oligomerization measurement the same residue is replaced on both TM helices. As one would expect, the effect of a mutation to disrupt a dimeric structure is more dramatic if the residue is replaced on both helices (homodimer), whereas the disrupting effect can partially be compensated if a small residue remains on one helical interface (28).

We observed an asymmetry in the interaction capacity depending on which TM sequence was expressed from which plasmid (pALM148 or pBLM100). Such an asymmetry has been noted in earlier studies (28, 33); it is most likely caused by the different copy numbers of the two plasmids used, resulting in different amounts of chimeric proteins. Because we showed that most TM domains not only hetero- but also homodimerize, the concentration of free monomers to form a heterodimer is also lowered if these sequences can form homodimers. Taken together, the data presented here indicate that the G*XXX*G-like motif is important for the formation of heterodimeric integrins.

GXXXG = (small)XXX(small)?—The G*XXX*G motif can drive a strong interaction of two TM helices (17), and this motif is highly overrepresented in TM domains (18). The G*XXX*G motif is not only important for the packing of TM proteins but also of soluble proteins (36). It is believed that the two Gly residues allow two helices to come together in close contact to optimize interfacial packing between them. Although the G*XXX*G motif has been extensively studied in the case of GpA, it has not yet been shown whether the Gly residues can be substituted by other small residues to obtain a similar packing effect. The members of the ErbB receptor family of tyrosine kinases also

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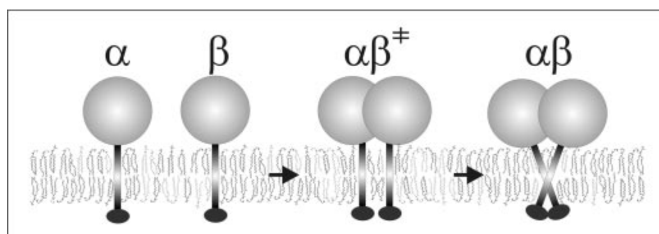

FIG. 7. **Model for formation and stabilization of α/β heterodimeric integrins.** After membrane incorporation of the individual subunits, specific interactions between the α and β subunits are mediated by the extracellular domain. After formation of a transition heterodimeric α/β integrin state ($\alpha\beta^{\neq}$), the bent and/or extended conformation with closed headpiece (45) is organized in the bilayer region by an interaction of the TM domains that is driven by a G*XXX*G-like motif.

contain G*XXX*G-like motifs in their transmembrane domains, and it was recently shown that homodimerization of the TM domains involves these motifs (19). Besides the classical G*XXX*G motif the authors showed that an S*XXX*G and an A*XXX*G motif can mediate transmembrane helix interactions. The results presented here show also that an S*XXX*G motif, which can be found in the α_2 and α_4 integrin TM domains, can mediate homo- as well as heterodimerization of TM helices. The results with the β_3 integrin indicate that an S*XXX*A motif does not mediate strong homodimerization of TM helices (Fig. 2) but can contribute to the formation of heterodimeric structures. Interestingly, in most cases of the known human heterodimeric integrins at least one of the Gly positions in the G*XXX*G-like motif is occupied by a Gly residue, whereas on the other helix an Ala or Ser residue can be found. In most cases of integrin α/β hetero interactions on either of the two TM helices, at least one Gly residue is present on the positions of the G*XXX*G motif (Fig. 4). This observation suggests that to stabilize a dimeric structure it seems to be sufficient if a Gly residue is present on one helix and another small residue is on the other. In the case where neither helix contains a Gly residue (α_V/β_3, α_V/β_5), a Ser residue is present.

The finding that substitution of the Ser residues in the S*XX*G motif of the α_4 integrin by Gly results in a lowered association capacity indicates that the interaction is optimized for a particular small residue. Also, especially in the case of Ser residues, hydrogen bonding could be involved in stabilization of the dimer. The fact that most but not all of the integrin TM helices contain a typical G*XXX*G-like motif further indicates that other packing motifs have evolved mediating the interaction of integrin TM domains.

Function of the TM Domains for the Formation and/or Stabilization of the α/β Integrins—The role of the TM domains in the formation and/or stabilization of α/β heterodimeric integrins is still not understood in detail. It is known that interaction of the extracellular domain of integrins is sufficient to drive dimerization (21), but the oligomerization efficiency of the mutants is lowered (22). The soluble extracytoplasmic domains are known to form heterodimers that display the same ligand-binding characteristics as the full-length proteins (37, 38). Analysis of isolated cytoplasmic domains of the α_{IIb}/β_3 integrin by NMR failed to show a direct interaction (12), and a study of the cytoplasmic and TM domains of the same integrin by NMR also did not show any interaction (24). In the latter study the authors only described the formation of homo-oligomeric structures. However, Lu *et al.* (23) demonstrated that the membrane proximal parts of the α_{IIb}/β_3 integrin interact and inactivate the integrin, indicating that at least that part is involved in the formation or stabilization of heterodimeric integrins, which is strongly supported by other biochemical and spectroscopic data (39–41).

An association of the α_{IIb} cytoplasmic domain to a membrane can induce the formation of a helical structure (42) so that the absence of a biological membrane could result in the absence of a structural requirement for the subunit interactions. The fact that Li *et al.* (24) could not detect any heterodimers can be explained by the findings of this study. Most integrin TM helices investigated in this study have a higher propensity to form homodimers than heterodimers (Figs. 2 and 3). If the formation of homodimeric integrins missing the extracellular part is thermodynamically favored, it is difficult to observe heterodimerization using the described approach. The GALLEX system described here measures the interaction of TM helices in a biological membrane and allows measurement of hetero-association even in the presence of background homo-association. Our data clearly indicate that the TM domains can form heterodimeric structures driven by the G*XXX*G-like motif, although formation of homo-oligomers was also measured.

The observation of the homo-oligomer formation in this study and in Ref. 24 argues against a dominant role of the TM regions in mediating specific α/β hetrodimeric integrin interactions. It is more likely that the specificity of any integrin α/β subunit association is given by the interaction of the extracellular domains and the TM domains can organize the TM region of a preformed heterodimer (Fig. 7). This model for assembly is in good agreement with the recent finding that the α/β integrin cytoplasmic and transmembrane domains get separated during integrin signaling, whereas the headpieces of the extracytoplasmic domains stay in contact (7). Because the strength of the integrin subunit interaction changes during signaling and the TM helices become separated, a very strong interaction of the two TM domains in any state might not be desirable (43). The results shown in Figs. 2 and 3 demonstrate that although the TM domains form heterodimers, the interaction is not as strong as the interaction in the GpA homodimer. A very strong interaction might lock the integrins in one state and abolish any further signal transduction event. Thus, the specificity of TM interactions may provide a way to organize the effects of the ectodomain interactions to specifically transmit signals across the bilayer but in the context of sufficiently small interaction energy to permit plasticity.

An alternative model for the activation of the integrin $\alpha_{II}\beta_3$ by modulation of the transmembrane helix interaction was proposed (34), suggesting that homo-oligomerization of integrin TM domains drives the activation of the $\alpha_{II}\beta_3$ integrin. Though our data show that heterodimeric interactions between different α/β subunits are possible and most biochemical and structural data indicate the presence of heterodimers, we cannot completely exclude a possible role of homodimeric interactions. Kim *et al.* (7) were not able to observe any significant homo-oligomerization or clustering of integrins during signaling *in vivo*. While we could measure homo-oligomerization of most integrin TM domains, we were also able to measure hetero-interactions of α and β subunits that do not form heterodimers *in vivo* (data not shown). These results argue against a predominant role of the TM domains for the formation of oligomeric integrin structures but may favor a model in which the constraint of helix ends by ectodomain interactions results in helix association using relatively weak interactions, transmitting the signal through the membrane (7).

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