Structural basis of kindlin-mediated integrin recognition and activation

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Kindlins and talins are integrin-binding proteins that are critically involved in integrin activation, an essential process for many fundamental cellular activities including cell-matrix adhesion, migration, and proliferation. As FERM-domain-containing proteins, talins and kindlins, respectively, bind different regions of β-integrin cytoplasmic tails. However, compared with the extensively studied talin, little is known about how kindlins specifically interact with integrins and synergistically enhance their activation by talins. Here, we determined crystal structures of kindlin2 in the apo-form and the β1- and β3-integrin bound forms. The apo-structure shows an overall architecture distinct from talins. The complex structures reveal a unique integrin recognition mode of kindlins, which combines two binding motifs to provide specificity that is essential for integrin activation and signaling. Strikingly, our structures uncover an unexpected dimer formation of kindlins. Interrupting dimer formation impairs kindlin-mediated integrin activation. Collectively, the structural, biochemical, and cellular results provide mechanistic explanations that account for the effects of kindlins on integrin activation as well as for how kindlin mutations found in patients with Kindler syndrome and leukocyte-adhesion deficiency may impact integrin-mediated processes.

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Significance

Kindlins proteins play crucial roles in the integrin-signaling pathway by directly interacting with and activating integrins, which mediate the cell-extracellular matrix adhesion and signaling. Mutations of kindlins lead to diseases, such as Kindler syndrome, associated with skin blistering and atrophy; leukocyte adhesion deficiency; and cancers. However, the molecular basis underlying kindlin-mediated integrin activation remains to be determined. Here, we report the structural basis of the specific interaction between kindlins and integrins. Furthermore, we demonstrate that kindlins synergize integrin activation by forming a dimer, providing a model for understanding integrin signaling. Finally, we interpret disease-causing mutations found in kindlins at the atomic level, which can be useful for understanding and treating these diseases.


The authors declare no conflict of interest.

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Data deposition: The atomic coordinates and structure factors have been deposited in the Protein Data Bank, www.pdb.org (PDB ID codes 5XQ1, 5XQ2, 5XQ9, and 5XQ1).


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We attempted to characterize the integrin activation by kindlin-2. To ensure a strict 1:1 stoichiometry in the complex, we set out to solve the kindlin-2 (MPT) and the membrane distal tail (MDT) for the binding of talins and kindlins, respectively (Fig. 2 A and B). How can kindlins specifically recognize the NPxY motif in the MDT rather than the one in the MPT? To address this question, we set out to solve the structure of the kindlin-β-tail complex. Isothermal titration calorimetry (ITC)-based assays showed that both the full-length kindlin2 and kindlin2Δ bind to the β-tail with a similar affinity (Kₐ of ~20–30 μM) at a 1:1 ratio (Fig. 2 D and E). Because the binding is relatively weak, we fused the β1-MDT to the C terminus of kindlin2Δ to ensure a strict 1:1 stoichiometry in the complex. By using these strategies, we determined the kindlin-2β/β1-MDT complex structure (SI Appendix, Table S1).

In the resulting complex, kindlin2 adopts a conformation essentially identical to the apo-form (the rmsd values of 0.7 Å for the overall structure and of 0.5 Å for the F3 lobe), indicating that the β1-MDT binding does not induce obvious conformational changes. The β1-MDT folds as a β-strand to bind with a groove (termed the αβ-groove, as formed mainly by α1₂β₃ and β5₃) at the F3 lobe (Fig. 2B), which is a well-characterized binding groove in FERM domains (25–30). The 796NPXY798 sequence in the β1-MDT adopts a turn-like shape in which N792β₁ and P793β₁ insert their side-chains into the αβ-groove, and Y795β₁ closely packs with K613ε₃ (Fig. 2C). Mutating the interacting residues diminishes their interaction (8, 31, 32) (Fig. 2E). This NPXY-binding mode was also found in the talin-FERM/β1-MPT interactions (33, 34) (Fig. 2B and SI Appendix, Fig. S5), indicating that sequences other than NPXY are required for the β1-MDT’s specific binding to kindlins.

Previous studies have suggested that several residues at the N-terminal to NPXY are involved in the kindlin-β-tail interaction (35). Consistently, the conserved 778TVTS790 sequence in the β1-MDT binds with the F3 lobe in a sequence-dependent manner (Fig. 2 A and C). Specifically, T788β₁ and T789β₁, respectively, form H-bonds with W619ε₃ and N616ε₃, which are strictly conserved in kindlins but not in the corresponding position in talins (SI Appendix, Fig. S4B); although not previously reported as an interacting residue, V790β₁ interacts with a hydrophobic patch in the αβ-groove. Interestingly, the hydrophobic patch is formed by residues from not only α1₂β₃ and β5₃ but also the α2ε₃ helix, which is, to the best of our knowledge, found only in kindlins (Fig. 2B and SI Appendix, Fig. S3). Thus, W619ε₃ and N616ε₃ together with L675ε₃ in α2ε₃ provide a highly specific binding environment for kindlins.
the TTV motif. Because the T788p1-corresponding residue in the β1-MPT is an Asp (SI Appendix, Fig. SSC), it is unlikely to be accommodated by the hydrophobic βp-groove. Consistent with our structural analysis, mutations in the TTV motif or the TTV-interacting site in kindlin2 disrupt the binding of kindlin2 to the β1-tail (Fig. 2 D and E). The interface residues are highly conserved in both kindlins and several β-integrin isoforms (Fig. 24 and SI Appendix, Fig. S1), indicating that the β-integrin binding mode found in the kindlin2Δαβ1-MDT structure is common to all kindlins. Consistently, the binding of kindlin1 to the β1-tail shows a similar affinity of ~20 μM (SI Appendix, Fig. S6). Furthermore, by determining the kindlin2Δαβ3-MDT structure, we confirmed that the β3-tail binds to kindlin2 using the same mode as β1-MDT (SI Appendix, Fig. S3D).

In addition, although the very last three nonconserved residues (798EGK798) in the β1-tail were not well assigned (SI Appendix, Fig. S5), deletion of the EGK sequence abolishes the binding of integrins to kindlins in both our and others’ observations (Fig. 2E) (36, 37). Because the amide of E796p1 forms a main-chain/main-chain H-bond with the β4p3/β5p3 loop in kindlin2 (Fig. 2C), deletion of EGK eliminates the H-bond, thereby disrupting the interaction, which aligns with previous findings that the binding of the β1-tail to kindlin2 requires at least one residue to the C terminus of Y795p1 in a sequence-independent manner (36).

The Binding of Kindlin2 to the TTV Motif in β1-Integrin Is Required for Integrin Signaling. Previously, we and others reported that kindlin2 is localized to FA and that the kindlin/integrin interaction is required for the FA localization of kindlin2 (7, 8, 35). Therefore, we used the localization of kindlin2 to FA as an assay to examine the TTV-binding pocket in kindlin2. As expected, kindlin2 localizes well to FAs in either wild-type or kindlin2 knockout HT1080 cells (Fig. 3A and SI Appendix, Fig. S7). In contrast, either the W619Q or L675E mutation, which disrupts the TTV-binding pocket, abolishes kindlin2 FA localization, suggesting that the TTV-mediated interaction is indispensable for kindlin2 to localize to FA. To further test whether the kindlin2 mutants affect cell function, we performed a cell-spreading assay using kindlin2 knockout cells. As expected, the reduced cell area of the knockout cells was largely recovered by expressing wild-type, but not TTV-binding deficient mutants (SI Appendix, Fig. S8).

Because kindlins function as coactivators of integrin in the presence of talin-FERM (9, 11), we coexpressed talin-FERM with various kindlin2 constructs (the wild-type or the TTV-binding deficient mutants) in αβ1β2 integrin-expressing CHO A5 cells and performed an integrin activation assay. Consistent with previous findings, wild-type kindlin2 synergized with talin to activate integrin (Fig. 3B). The two TTV-binding deficient mutants, however, showed little synergistic enhancement in integrin activation (Fig. 3B).

Taken together, the above structural, biochemical, and cellular characterizations clearly demonstrate that the binding of kindlin2 to the TTV motif of β1-integrin is crucial for kindlin-mediated integrin activation and signaling.

Kindlin2 Forms a Domain-Swapped Dimer via its F2 Lobe. Because we used the monomeric protein for crystallization, finding a dimeric kindlin2 structure in the crystals was unexpected. The symmetric dimer is mediated by the F2 lobe in a domain-swapped manner (Fig. 1B). Unlike talin-F2, the F2 lobe in the kindlin2 dimer adopts an extended, “open” conformation (SI Appendix, Fig. S4A). Instead of forming two separated helices as found in talin-F2, the corresponding part in kindlin2 becomes a long, continuous helix (α4–2). The rigidity of α4–2 prevents the region between α2–2 and α4–2 from forming an intramolecular interaction with the hydrophobic pocket formed by α1–2, α2–2, and α2–1, but promotes the intermolecular interaction with the same structural elements in the other molecule (Fig. 4A). Interestingly, although the dimeric conformation was preferred in most crystallization conditions that formed crystals, the monomeric structure was found under a few conditions. By further modifying the deletion boundary of the PH domain (residues 367–512), we obtained a structure of the monomeric kindlin2Δαβ1 (SI Appendix, Table S1). Although most parts remain the same as the dimer, the monomeric structure shows the “closed” conformation of the F2 lobe (Fig. 4A and SI Appendix, Fig. S9). Specifically, the α4–2 helix is
bent into two helices, α4N_{F2} and α4C_{F2}, resulting in a folding back of α3_{F2} and its preceding loop, which occupy the same hydrophobic pocket involved in dimerization (SI Appendix, Fig. S10). Therefore, the switch between the closed and open conformation of the F2 lobe controls the monomer–dimer exchange of kindlin2. In addition, the C-terminal part of α2_{F2} and the middle region of α4_{F2} are involved in forming another specific dimer interface (SI Appendix, Fig. S10), which may facilitate the monomer-to-dimer transition.

Next, we tried to confirm the dimer conformation in solution. The freshly purified kindlin2 protein is monomeric as shown by analytical gel filtration (Fig. 4B). Interestingly, the dimer was detected in a sample placed at 4 °C for several days, and the dimer population increased over time (Fig. 4B and C). In comparison, no similar dimer formation was observed in talin-FERM protein (SI Appendix, Fig. S11A). To rule out the possibility that the dimer is formed nonspecifically by partially unfolded protein, we measured the binding of the dimer to β-integrin. The dimer showed essentially the same binding affinity as the monomer (Fig. 2E).

Furthermore, we used a site-specific chemical cross-linking approach to probe the dimer state. In the kindlin2 dimer structure, V522 (in one protomer) and A318 (in another protomer) are close to each other in the dimer interface (Fig. 4A). If the dimer in solution adopts the same conformation found in the crystal structure, the respective substitution of V522 and A318 with Cys in the two protomers of the dimer would specifically promote the formation of a disulfide bond-mediated dimerization. Consistently, the dimerization process was greatly enhanced by mixing the V522C and A318C mutants, whereas neither the V522C nor the A318C mutant alone showed accelerated dimerization (Fig. 4D).

To investigate kindlin2 dimer formation in cells, we cotransfected GFP-tagged W619Q kindlin2, which shows abolished FA localizations, with Flag-tagged wild-type kindlin2. The dimerization of kindlin2 would presumably bring the W619Q mutant to FA by interacting with the wild-type protein. Indeed, we detected the partial FA localization of W619Q by cotransferring it with wild-type kindlin2 (Fig. 4E).

Because kindlin family proteins are highly conserved (SI Appendix, Fig. S1), it would be interesting to know whether other kindlins also form dimers. By using analytical gel filtration, we observed the similar dimerization process of kindlin1 (SI Appendix, Fig. S11B). Consistent with this, the kindlin homolog in Caenorhabditis elegans, UNC112, was also shown to have the potential to form a dimer (38). Thus, the monomer–dimer transition is likely an evolutionarily conserved property of kindlins.

The Monomer–Dimer Transition Is Important for Kindlin-Mediated Integrin Activation. To understand the functional roles of the dimer formation of kindlins, we set out to design mutations that disrupt the monomer–dimer transition in kindlin2. As talin-FERM keeps a monomeric state stably (SI Appendix, Fig. S11A), substitution of...
certain sequences from talins may lock kindlin2 into a monomer. Through structural and sequence comparison, we found that formation of the closed conformation of talin-F2 largely relies on two turns, formed by Φ248GP and Q542GP, respectively (Fig. 5A). The two GP mutants show decreased integrin activation activity, compared with the wild-type kindlin2. Data were collected and analyzed using the same method as indicated in Fig. 3B. **P < 0.01; ***P < 0.001.

Structural Implications of Kindlin Mutations in Diseases. Defective mutations of kindlins lead to various human genetic diseases, such as Kindler syndrome and LADIII (40). More than 80 disease-causing mutations in kindlins have been identified (SI Appendix, Table S2). Most of these mutations are truncation or frame-shift mutations, thereby producing defective proteins (SI Appendix, Fig. S1A). Five missense mutations in kindlins have been reported (41–44). By fitting them in the kindlin2 structure, we analyzed potential effects on protein folding or target binding due to these mutations (SI Appendix, Fig. S1A). The R297G and W559R mutations found in Kindler syndrome likely disrupt the F2 folding as well as the F1/F2 interaction, whereas the Q595P mutation is likely to distort the β5α2 conformation and thereby disrupts the kindlin/integrin interaction. Consistent with our structural analysis, the corresponding mutations in kindlin2 either disrupt the folding or abolish the integrin binding (SI Appendix, Fig. S1A). Thus, the genetic mutations found in kindlins are expected to alter their structures at different levels and thereby impair proper functions of kindlins.

Discussion

In this study, we have solved the crystal structures of kindlin2, a ubiquitously expressed member of the kindlin family, as well as the kindlin2/β-tails complex structures. The structures provide a major advancement in understanding the structural basis of the kindlin/integrin interaction as well as the genetic diseases associated with kindlin mutations.

The monomer–dimer transition of kindlins described here provides insight into the mechanism of integrin signaling. Integrins form clusters upon full activation. There is evidence that kindlins activate integrins by promoting the clustering of talin-activated integrin and increasing integrin binding to multivalent ligands.
(45). The dimerization of kindlins provides a mechanistic explanation of how kindlins promote integrin clustering (Fig. 6). In this model, kindlins in either monomeric or dimeric forms are recruited to adhesion sites through interacting with the β-tails, whereas only dimeric kindlins can bridge talin-activated integrins to promote the clustering. Interestingly, talins are also dimerized antiparallelly, which is not mediated by the FERM domains but by the dimerization domain in their C terminus (46), which may further enhance the integrin clustering (Fig. 6). In line with our model, disruption of the monomer–dimer transition of kindlin2 by the GP mutations led to impaired integrin activation and FA formation, but had little impact on the FA localization of kindlin2 (Fig. 5C and SI Appendix, Figs. S12 and S13). The in vitro dimerization process occurs spontaneously yet slowly (Fig. 4B). Therefore, in living cells, dimer formation needs to be accelerated locally for fast and efficient integrin signaling. The regulatory factors for the monomer–dimer transition remain to be identified. Because kindlins link integrin-mediated adhesion to the cytoskeleton via interacting with other actin cytoskeleton regulators (ILK, migfilin, paxillin, etc.) as well as actin (7, 47–49), it would be interesting to investigate whether dimeric kindlins impact these interactions or vice versa.


Materials and Methods

All proteins used in this study were expressed in Escherichia coli and purified by Ni2+–NTA affinity chromatography followed by size-exclusion chromatography. Crystals were obtained by the sitting drop vapor diffusion method at 16 °C. An extended description of the methods for protein preparation, crystallography, and biochemical and cellular assays is included in SI Appendix.

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