Novel Mode of Ligand Recognition by the Erbin PDZ Domain*

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Erbin contains a class I PDZ domain that binds to the C-terminal region of the receptor tyrosine kinase ErbB2, a class II ligand. The crystal structure of the human Erbin PDZ bound to the peptide EYLGLDVVPV corresponding to the C-terminal residues 1247–1255 of human ErbB2 has been determined at 1.25 Å resolution. The Erbin PDZ deviates from the canonical PDZ fold in that it contains a single α-helix. The isopropyl group of valine at position −2 of the ErbB2 peptide interacts with the Erbin Val1351 and displaces the peptide backbone away from the α-helix, elucidating the molecular basis of class II ligand recognition by a class I PDZ domain. Strikingly, the phenolic ring of tyrosine −7 enters into a pocket formed by the extended β2-β3 loop of the Erbin PDZ. Phosphorylation of tyrosine −7 abolishes this interaction but does not affect the binding of the four C-terminal peptidic residues to PDZ, as revealed by the crystal structure of the Erbin PDZ complexed with a phosphotyrosine-containing ErbB2 peptide. Since phosphorylation of tyrosine −7 plays a critical role in ErbB2 function, the selective binding and sequestration of this residue in its unphosphorylated state by the Erbin PDZ provides a novel mechanism for regulation of the ErbB2-mediated signaling and oncogenicity.

PDZ2 (PSD-95/DLG/ZO-1) domains are protein interaction modules that play fundamental roles in the assembly of membrane receptors, ion channels, and other molecules into signal transduction complexes known as transducisomes (1–3). The PDZ fold comprises a six-stranded antiparallel β-barrel capped by two α-helices (1–6). PDZ domains interact with C-terminal peptides and are currently classified into two major categories based on their target sequence specificity. Class I domains bind to peptides with the consensus X-(S/T)-X-Φ (Φ denoting any amino acid and Φ representing a hydrophobic residue), whereas class II domains recognize the motif X-Φ-X-Φ (1–3). The residues at positions 0 and −2 of the peptide (position 0 referring to the C-terminal residue) play a critical role in the specificity and affinity of the interaction, whereas it is believed that amino acids upstream of the −5 position do not interact with PDZ (1–7). However, the structural determinants of ligand selectivity by PDZ domains are more complex than initially thought. For example, recent studies established an important contribution of the penultimate peptidic residue in the PDZ-ligand interaction (5, 6). Furthermore, several PDZ domains have sequence specificities that do not fall into the two classes implying the existence of more categories, whereas others bind both class I and II ligands, suggesting an intrinsic flexibility in these modules to accommodate both polar and non-polar side chains at position −2 (1–3).

Erbin was originally identified as a protein that interacts with the receptor tyrosine kinase ErbB2 (also known as HER-2 or Neu) and plays a role in its localization at the basolateral membrane of epithelial cells (8, 9). Recent studies have shown that Erbin is also highly concentrated at neuronal postsynaptic membranes and neuromuscular junctions, where it interacts with ErbB2 (10). Erbin contains a class I PDZ domain that binds with high affinity to the sequence DSWV present at the C termini of δ-catenin, ARVCF, and p0071 (11, 12). Notably, the ErbB2 sequence EYLGLDVVPV that is recognized by the Erbin PDZ (8, 13), is a class II ligand, posing an interesting structural problem regarding the molecular mechanisms underlying the dual ligand specificity of this domain. The Erbin PDZ binds preferentially to the ErbB2 tail having an unphosphorylated tyrosine at position −7 (corresponding to Tyr1148 in full-length human ErbB2), whereas phosphorylation of this residue reduces significantly the affinity of the Erbin-ErbB2 interaction (8). This preference for an unphosphorylated tyrosine is intriguing, because a PDZ interaction with the peptide residue −7 has not been observed in previous structural studies (1–7). Importantly, phosphorylation of Tyr1148 following ErbB2 activation is a critical event for the mitogenic signaling and oncogenicity of this receptor (14–16). Moreover, Tyr1148 plays an important role in the basolateral localization of ErbB2 (17).

Here, we present the crystal structure of the Erbin PDZ bound to the ErbB2 C terminus. The structure reveals a novel interaction of the peptidic Tyr −7 with the extended β2-β3 loop of the Erbin PDZ. A second crystal structure of this domain bound to a phosphotyrosine-containing ErbB2 peptide shows that phosphorylation of Tyr −7 abrogates its interaction with the β2-β3 loop. These results suggest new mechanisms for regulation of the ErbB2-mediated signaling through its dynamic interaction with the Erbin PDZ.

EXPERIMENTAL PROCEDURES

Protein Crystallization—A DNA fragment encoding the human Erbin PDZ domain (residues 1280–1371) was amplified from Quick-Clone cDNA (Clontech) using the polymerase chain reaction and cloned into a modified pGEX-2T vector. The Erbin PDZ was expressed in Escherichia coli BL21(DE3) cells as a glutathione S-transferase fusion, purified on glutathione-Sepharose 4B, released with thrombin digestion, and fur-
Structural Basis of the Erbin-ErbB2 Interaction

Table I

| Crystal 1<sup>a</sup> | Crystal 2<sup>a</sup> | Crystal 3<sup>a</sup> |
|---------------------|---------------------|---------------------|
| Data collection and phasing | Data collection and phasing | Data collection and phasing |
| Data set | Native 1 | Native 2 | MAD λ1 | MAD λ2 | MAD λ3 |
| Wavelength (Å) | 1.5418 | 0.9786 | 0.9789 | 0.9686 | 0.9791 |
| Resolution (Å) | 1.88 | 1.25 | 1.4 | 1.4 | 1.4 |
| Unique reflections | 7,068 | 24,313 | 17,583 | 17,586 | 17,680 |
| Completeness (%)<sup>b</sup> | 95.0 (90.2) | 92.7 (91.4) | 97.8 (89.2) | 98.2 (89.2) | 98.5 (92.9) |
| R<sub>merge</sub> (%) | 2.8 (9.6) | 2.5 (3.7) | 4.9 (7.3) | 3.7 (7.3) | 4.1 (7.5) |
| (Å<sup>2</sup>/σ<sup>2</sup>) | 44.7 (13.3) | 34.7 (16.8) | 48.1 (23.4) | 48.1 (23.2) | 47.6 (22.5) |
| Refinement | | | | | |
| Resolution range (Å) | 30.6–1.88 | 25–1.25 | | | |
| Reflections in working/test set | 6,737/330 | 23,073/1,240 | | | |
| R<sub>cryst</sub> (%) | 16.7 | 12.8 | | | |
| R<sub>free</sub> (%) | 21.6 | 18.4 | | | |
| Allowed/additional/generous (%) | 88.2/29.2/2.6 | 90.8/66.2/6.2 | | | |
| regions in Ramachandran plot | | | | | |

<sup>a</sup> Crystals 1 and 2 refer to crystals of the wild-type Erbin PDZ bound to the phosphorylated and unphosphorylated ErbB2 peptides, respectively. Crystal 3 refers to the SeMet-substituted PDZ/V1366M bound to the unphosphorylated peptide.

<sup>b</sup> Numbers in parentheses refer to the highest resolution shell (1.93–1.88 Å for Native 1, 1.29–1.25 Å for Native 2, and 1.45–1.4 Å for MAD).

Erbin PDZ lacks the short glycine and two proline residues that create a bent platform (designated here as P1), where they are stabilized through a hydrogen bond. The isopropyl group of Val 0 enters into the carboxylate-binding pocket of the Erbin PDZ (Fig. 1 A, B, and C). The isopropyl group of Val −2 makes hydrogen bonds with Val<sup>1134</sup>, which appear to cause a displacement of the peptide backbone away from the α-helix (Fig. 1 B), providing an explanation for the ability of Erbin PDZ to recognize a class II ligand. The peptide is further stabilized at this position through an interaction of Asp −3 with Thr<sup>1316</sup> (Fig. 1 D), whereas Leu −4, Gly −5, and Leu −6 do not bind to PDZ. Interestingly, the imidazole ring of the conserved His<sup>1347</sup>, which is the hallmark of class I PDZ domains and plays a critical role in the selection of the residue −2, points away from Val −2, where it hydrogen bonds with the carbonyl oxygen of Gly. The β2-β3 Loop of Erbin PDZ Interacts with Tyr −7 of the ErbB2 Ligand—Strikingly, the phenolic ring of Tyr −7 folds back in a direction parallel to the peptide backbone and enters a pocket, designated P<sub>0</sub>, which is formed by Ser<sup>1296</sup> in the β2 strand and Gly<sup>1305</sup>, Asn<sup>1304</sup>, and Pro<sup>1305</sup> in the β2-β3 loop (Fig. 1 A, B, and C). This represents the first structural evidence for a direct interaction of the PDZ domain with the peptide residue −7. The β2-β3 loop of Erbin PDZ is considerably longer than that of PDZs with known structure (Fig. 1 A) and contains five glycine and two proline residues that create a bent platform against which Tyr −7 is stacked. The phenolic ring of Tyr −7 is stabilized primarily by hydrogen bonds and is well ordered, as indicated by the high quality electron density map (Fig. 1 E). The hydroxyl group of Tyr −7 hydrogen bonds through two ordered water molecules with Asp −3 (Fig. 1 D).

Phosphorylation of Tyr −7 Abolishes Binding to the P<sub>2</sub> Pock-
et—Because phosphorylation of Tyr1248 plays a critical role in ErbB2 signaling (14–16), we also determined the crystal structure of the Erbin PDZ bound to the peptide EpYLGLDVPV. No electron density is observed for the peptidic residues 7 to 8 and the P2 pocket is empty (Fig. 2A). In contrast, Val 0, Pro –1, Val –2, Asp –3, and Leu –4 are well ordered inside the ligand-binding groove (Fig. 2A). The integrity of the peptide in the crystallized complex was verified by mass spectroscopic analysis (data not shown), indicating that the invisible portion of the peptide is disordered and faces toward the solution.

Isothermal titration calorimetry experiments showed that the native ErbB2 peptide binds to the Erbin PDZ with a $K_d$ of $50 \mu$M, whereas the phosphotyrosine-containing peptide binds to PDZ with a $K_d$ of $128 \mu$M. The 2.5-fold reduction in the affinity of Erbin PDZ for the phosphorylated ErbB2 peptide is attributed to the loss of the hydrophobic interactions and hydrogen bonds stabilizing the phenolic ring of Tyr 7 inside the P2 pocket.

Superposition of the Erbin PDZ structures with the PSD-95 PDZ3 (4) reveals that Val 0, Pro –1, Val –2, and Asp –3 are superposed extremely well in both Erbin complexes, whereas the ErbB2 backbone is displaced away from the $\alpha$-helix as compared with PSD-95 PDZ3 (Fig. 2B). These results indicate that the displacement of the ErbB2 peptide is due to the Val 2 interaction with Val1351 rather than the Tyr 7 binding to P2. Only small differences are observed in the backbone positions of the Erbin $\beta_2$-$\beta_3$ loops (overall root-mean-square deviation 0.26 Å for residues 1299–1311), indicating that the P2 site is preformed and does not undergo major conformational changes upon Tyr 7 binding. By contrast, the $\beta_2$-$\beta_3$ loops of the Erbin PDZ and PSD-95 PDZ3 occupy completely different positions and are not superimposable.

Structural and Functional Implications—The property of the newly discovered pocket P2 to discriminate between the phosphorylation states of Tyr 7 indicates that it may play a regulatory role in ErbB2 signaling and suggests an attractive
that have not been shown to interact with peptidic residues (4–6), the extended β2-β3 loops of the PSD-95 PDZ1, PSD-95 PDZ2, and PTP1E PDZ2 domains are involved in ligand interactions (7, 25–28). Importantly, alternative spliced isoforms of PTP1E PDZ2 with different β2-β3 loop lengths have entirely different binding affinities for the C-terminal region of the tumor suppressor protein APC (29), providing further evidence for an important role of P2 in PDZ-ligand interactions. These observations, taken together with the present structures of Erbin PDZ, demonstrate that the P2 site is a hitherto unrecognized important structural element with possible regulatory function, at least for a subset of PDZ domains. Moreover, the emerging complexity of PDZ selectivity mechanisms points to the need for new PDZ classification schemes that will take into consideration the β2-β3 loop length, the specificity of the P2 ligand interaction, and the structural determinants underlying the dual ligand specificity of these versatile protein modules.

**Fig. 2. Structure of the Erbin PDZ bound to the phosphorylated ErbB2 peptide.** A, stereo view of the Erbin PDZ bound to the peptide EpYLGLDVPV. A weighted 2Fo-Fc electron density map calculated at 1.88-Å resolution and contoured at 1.0 is superimposed on the ErbB2 peptide. B, superposition of the Cα backbone traces of Erbin PDZ-peptide (pink), Erbin PDZ-phosphopeptide (blue), and PSD-95 PDZ3-peptide (yellow) (Protein Data Bank code 1BE9). Side chains of the peptidic residues, Erbin His1317 and Val1321, and PSD-95 His272 are shown as stick models.

model for this regulation. Conceivably, during the inactive state of ErbB2, Tyr–7 is buried inside P2 and is inaccessible for phosphorylation and interaction with other proteins. Activation of ErbB2 triggers the release of Tyr–7 from P2, possibly through conformational changes induced in Erbin and/or the cytoplasmic domain of ErbB2. Notably, Erbin becomes phosphorylated by ErbB2 following receptor activation (8), raising the intriguing possibility that this may represent a step preceding the dissociation of Tyr–7. Subsequently, the released tyrosine is primed for phosphorylation and interaction with phosphotyrosine-binding domains (e.g., PTB or SH2) of downstream signaling proteins (14, 15). Following signal transduction, dephosphorylation of Tyr–7 restores its original position inside P2. Importantly, in contrast to the regulatory site P2 that oscillates between bound and unbound states, P1 interacts constitutively with the last four residues of ErbB2 securing the continuous participation of Erbin and ErbB2 in the same macromolecular complex at the basolateral membrane throughout the activation-inactivation cycles of the receptor. This model also allows for simultaneous binding of the Erbin PDZ and either PTB or SH2 domains to the phosphorylated ErbB2 C-terminal region, because these modules have non-overlapping recognition motifs.

Do other PDZ domains have a P2 pocket? In contrast to the short β2-β3 loops of PSD-95 PDZ3 and NHERF PDZ1 (Fig. 1A)