Structural Basis of the Na\textsuperscript{+}/H\textsuperscript{+} Exchanger Regulatory Factor PDZ1 Interaction with the Carboxyl-terminal Region of the Cystic Fibrosis Transmembrane Conductance Regulator*

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The PDZ1 domain of the Na\textsuperscript{+}/H\textsuperscript{+} exchanger regulatory factor (NHERF) binds with nanomolar affinity to the carboxyl-terminal sequence QDTRL of the cystic fibrosis transmembrane conductance regulator (CFTR) and plays a central role in the cellular localization and physiological regulation of this chloride channel. The crystal structure of human NHERF PDZ1 bound to the carboxyl-terminal peptide QDTRL has been determined at 1.7-Å resolution. The structure reveals the specificity of the interaction.

The cystic fibrosis transmembrane conductance regulator (CFTR) is an ATP-regulated chloride channel that determines the rate of electrolyte and fluid transport in the apical membrane of epithelial cells (1–3). Abnormal CFTR function is associated with the pathogenesis of cystic fibrosis and secretory diarrhea (1–3). The CFTR activity is modulated through interactions with other proteins; however, the regulatory mechanisms remain unknown. One protein that interacts with the carboxyl terminus of CFTR is the Na\textsuperscript{+}/H\textsuperscript{+} exchanger regulatory factor (NHERF), a cytoplasmic protein originally cloned as an essential cofactor for the cAMP-dependent protein kinase-mediated inhibition of the Na\textsuperscript{+}/H\textsuperscript{+} exchanger 3 (4–6). NHERF is also known as EBP50 (ezrin-radixin-moesin-binding phosphoprotein-50), a membrane-cytoskeleton linking protein that binds to membrane proteins through its two PDZ (PSD-95/Discs-large/ZO-1) domains and to the cortical actin cytoskeleton through its carboxyl-terminal domain (7). The NHERF PDZ1 and PDZ2 domains (Fig. 1A) bind with nanomolar affinity to the CFTR carboxyl-terminal sequence QDTRL and play a critical role in the regulation of channel gating (8–10). In addition, the NHERF-related protein, NHERF2, also binds to the carboxyl-terminal tail of CFTR through its two PDZ domains (11) (Fig. 1A). Interestingly, CAP70, the murine homolog of the PDZK1 protein (12, 13), also interacts with the CFTR carboxyl terminus through its PDZ3 domain and modulates the channel activity (12). These findings corroborate previous studies in establishing the essential role of the CFTR carboxyl-terminal motif DTRL for the functional expression of this channel in the apical plasma membrane (14–16).

PDZ domains are protein modules that mediate specific interactions between proteins and participate in the assembly of membrane receptors, ion channels, and other signaling molecules into specific signal transduction complexes (17, 18). PDZ domains bind to short carboxyl-terminal peptides and have been categorized into two classes based on target sequence specificity. Class I domains bind to peptides with the consensus sequence (S/T)(V/I/L)X (X denoting any amino acid), whereas class II domains recognize the motif (F/Y)(F/W/V/A) (19). The PDZ fold comprises a six-stranded antiparallel \(\beta\)-strand to the PDZ \(\beta\)-sheet, and an extensive network of hydrogen bonds and hydrophobic interactions stabilize the complex. Remarkably, the guanido group of arginine at position 1 of the CFTR peptide forms two salt bridges and two hydrogen bonds with PDZ1 residues Glu\textsuperscript{29} and Asn\textsuperscript{22}, respectively, providing the structural basis for the contribution of the penultimate amino acid of the peptide ligand to the affinity of the interaction.

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The atomic coordinates and structure factors (code 1i92) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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1 The abbreviations used are: CFTR, cystic fibrosis transmembrane conductance regulator; NHERF, Na\textsuperscript{+}/H\textsuperscript{+} exchanger regulatory factor; PDZ, PSD-95/Discs-large/ZO-1 homology.

EXPERIMENTAL PROCEDURES

Protein Purification and Crystallization—A DNA fragment encoding the human NHERF PDZ1 (residues 11–94), and having the carboxyl-terminal extension Q\textsuperscript{p}DTRL\textsuperscript{29} that corresponds to residues 1476–1480 of human CFTR (1), was amplified using the polymerase chain reaction and cloned in the vector pGEX-2T DL (27). PDZ1 was expressed in Escherichia coli BL21 (DE3) cells as a glutathione S-transferase fusion protein, purified using glutathione-Sepharose 4B resin, and the PDZ1 was released by digestion with thrombin, as described previously (27). PDZ1 protein (18 mg/ml) was crystallized using the sitting drop vapor
diffusion method in 0.1 M sodium acetate, pH 4.6, 2 M sodium chloride, at 20 °C. Diffraction data were collected at room temperature using an R-AXIS IV detector and CuKα radiation. The data were processed using the programs DENZO and SCALEPACK (29) (Table I). Crystals belong to space group P3121 with unit cell parameters a = 51.7 Å, b = 51.7 Å, c = 67.0 Å, and one molecule in the asymmetric unit.

Structure Determination and Refinement—The structure was solved by molecular replacement using the program MOLREP (30) and the human NHERF PDZ1 (Protein Data Bank code 1g9o) as the search model. The rotation function search in the 20–3 Å resolution range produced a clear solution with a peak height of 5.9 sigma. The translation function indicated that the correct space group was P3121 with a correlation coefficient of 0.36 and an Rcryst of 50.5%, compared with its enantiomorphic mate P3221, which had a correlation coefficient of 0.23 and an Rcryst of 56.9%. The model was built using the program O (31) and was refined by the maximum likelihood method using REFMAC5 (32). The structure is well ordered except for the loop regions 31–35 and 81–85, which are disordered. The PDZ1 also contains at its amino terminus the vector-derived residues GSSRM, from which only methionine is ordered and included in the final model.

RESULTS AND DISCUSSION

Structure Determination—We recently determined the crystal structure of the human NHERF PDZ1 domain (residues 11–99) at 1.5-Å resolution (27). The crystal structure produced a dimeric arrangement of PDZ1 domains with the carboxyl-terminal region T95DEQL99 of one PDZ1 molecule bound to a neighboring PDZ1 because of its resemblance to the PDZ1 ligand consensus (27). We exploited this intermolecular association of NHERF PDZ1 in the crystalline state to facilitate the co-crystallization of this domain with the CFTR ligand by converting the PDZ1 sequence T95DEQL99 to Q95DTRL99, which corresponds to the CFTR carboxyl-terminal tail. Recombinant NHERF PDZ1 was crystallized, and its structure was determined by molecular replacement. The model was refined to an Rcryst of 18.7% and an Rfree of 21.7% (Table I), and the evaluation of its stereochemistry using PROCHECK (33) showed that 89.2% of the residues are in the most favored, 8.1% in the additional allowed, and 2.7% in the generously allowed regions.

Overview of the Structure—The present NHERF PDZ1 crystal structure produces infinite head-to-tail polymers of PDZ1 molecules along the z axis, with the carboxyl-terminal extension Q95DTRL99 of one PDZ1 molecule serving as a ligand for a neighboring PDZ1 (Fig. 1B). The overall topology of NHERF PDZ1 is similar to other PDZ structures (20–27), consisting of six β-strands (β1–β6) and two α-helices (α1 and α2) (Fig. 1, A).

**FIG. 1. Structure of the NHERF PDZ1 domain bound to the CFTR sequence QDTRL.** A, sequence comparison of PDZ domains that bind to CFTR. The indicated PDZ domains from human NHERF (5), human NHERF2 (8), and murine PDZK1/CAP70 (12) were aligned using MACAW (36). Absolutely conserved residues are shown as white letters on blue background. Identical residues in four domains are shaded in cyan. The secondary structure of NHERF PDZ1 is indicated at the top. Conserved acidic residues proposed to interact with Arg-1 of the CFTR ligand are denoted by an asterisk. B, stereo view of the NHERF PDZ1 crystal packing. Each carboxyl terminus serves as a ligand for a neighboring PDZ1 molecule. C, ribbon diagram of the NHERF PDZ1 domain bound to the QDTRL peptide. The strands β1–β6 are shown in yellow, and the helices α1 and α2 are shown in green. The peptide ligand QDTRL is shown in pink. The figure was made using MOLSCRIPT (37) and Raster3D (38). D, surface topology of the NHERF PDZ1 bound to the peptide QDTRL. The figure was generated using GRASP (39).
TABLE I

Statistics of structure determination and refinement

\[ R_{\text{sym}} = \frac{\sum I - \langle I \rangle}{\sum I}, \text{where } I \text{ is the observed integrated intensity,} \]
\[ \langle I \rangle \text{ is the average integrated intensity obtained from multiple measurements, and the summation is over all observed reflections.} \]

\[ R_{\text{free}} = \frac{\sum |F_{\text{calc}}| - |F_{\text{obs}}|}{\sum |F_{\text{calc}}|}. \]

| Data collection | Resolution (Å) | 26.8–1.7 |
|-----------------|----------------|----------|
| Total number of observed reflections | 110,148 |
| Total number of unique reflections | 10,950 |
| Overall completeness | 92.6 |
| Completeness in outer shell (1.76–1.70 Å) (%) | 40.0 |
| \( R_{\text{sym}} \) (%) | 5.3 |
| \( R_{\text{sym}} \) outer shell (%) | 30.0 |
| Multiplicity | 9.9 |
| Average \( \langle I/\sigma(I) \rangle \) | 27.6 |

| Refinement | Resolution range (Å) | 26.8–1.7 |
|-----------------|----------------------|----------|
| Number of reflections in working set | 9,885 |
| Number of reflections in test set | 1,065 |
| \( R_{\text{free}} \) (%) | 18.7 |
| Number of non-hydrogen protein atoms | 700 |
| Number of water molecules | 57 |
| Chlorine atoms | 2 |
| Bond lengths (Å) | 0.018 |
| Bond angles (degrees) | 1.8 |

\( ^{a} \text{Bond lengths and angles are root-mean-square deviations from ideal values.} \)

A and C).

Structural Basis for the Specificity of the NHERF PDZ1-
CFTR Interaction—The peptide ligand Q0DTRL98 inserts into
the PDZ1 binding pocket antiparallel to the \( \beta \)-sheet and
extends the \( \beta \)-sheet of PDZ1 (Fig. 1, C and D). In this arrange-
ment, the invading pentapeptide is highly ordered, as indicated
by the high quality electron density map (Fig. 2A). The
arginine residue in the other PDZ structures where this function is mediated by an
amino acid residue (see also Table I) is involved in hydrogen bonding with the similar
side chain and carboxylate group of Leu 0 enter into a
deep cavity formed by Tyr24, Gly25, Phe26, Leu28, Val76, and
Leu78 residues (Fig. 1D). The C^α atom of Leu 0 makes hydrophobic contacts with the atoms C^α of Phe25 and C^α of Leu28, and the C^α atom of Asp – 3 forms a salt bridge with N^ε of Arg40 (Fig. 2, B and C). Similarly, the amide nitrogen and carbonyl oxygen of
Thr –2 hydrogen bond with the carboxyl oxygen and amide nitrogen of Leu28, respectively, while the O^ε atom of Thr –2 hydrogen bonds with the N^ε atom of the conserved His72 (Fig. 2, A–C).

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phobic character of the cavity would likely exclude polar and charged side chains. It therefore appears that the volume, shape, and hydrophobicity of the PDZ pocket provide the structural determinants for the selection of stereochemically complementary hydrophobic carboxyl-terminal side chains for high affinity binding.

The Importance of Arg –1 for the Affinity of the NHERF PDZ1-CFTR Interaction—Strikingly, the guanido group of Arg –1 forms two salt bridges with Oδ2 of Glu43 and two hydrogen bonds with the carbonyl oxygen of Asn22 (Fig. 2, A–C). This finding was unexpected because the residue –1 of the peptide has been considered to be unimportant for the PDZ-ligand interaction. Indeed, in other PDZ structures the side chain of the penultimate residue is oriented toward the solution and does not interact with PDZ residues (20, 22). Nevertheless, previous biochemical studies demonstrated that arginine is the preferred residue at position –1 for optimal binding to NHERF PDZ1 (8, 9). Affinity selection experiments showed that NHERF PDZ1 selected almost exclusively ligands with arginine at position –1 from random peptides (9). Furthermore, point mutagenesis of the penultimate arginine to alanine, phenylalanine, leucine, or glutamic acid decreased the affinity of binding to NHERF PDZ1 (8, 9). Affinity selection experiments presented toward the solution and does not interact with PDZ residues (20, 22). Nevertheless, previous biochemical studies demonstrated that arginine is the preferred residue at position –1 for optimal binding to NHERF PDZ1 (8, 9). Affinity selection experiments showed that NHERF PDZ1 selected almost exclusively ligands with arginine at position –1 from random peptides (9). Furthermore, point mutagenesis of the penultimate arginine to alanine, phenylalanine, leucine, or glutamic acid decreased the affinity of binding to NHERF PDZ1 (8, 9).

Perspective—The present work reveals the specificity and affinity determinants of the NHERF PDZ1-CFTR interaction and provides insights into carboxyl-terminal leucine recognition by class I PDZ domains, particularly those of NHERF, NHERF2, and PDZK1/CAP70. The sequence similarity shared among the aforementioned PDZ domains (Fig. 1A) suggests similar modes of interactions with CFTR. Elucidation of the molecular mechanisms underlying the interaction between these proteins and CFTR may facilitate the design of potent and specific modulators of CFTR activity with important clinical applications in the treatment of secretory diarrhea and cystic fibrosis.

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