Characterization of the Interactions between PDZ Domains of the Protein-tyrosine Phosphatase PTPL1 and the Carboxyl-terminal Tail of Fas*

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The intracellular protein-tyrosine phosphatase PTPL1 has five PDZ domains and one of them, PDZ 2, has previously been shown to interact with the C-terminal tail of Fas, a member of the tumor necrosis factor receptor family. Using a peptide binding assay, we show that not only PDZ 2 but also PDZ 4 of PTPL1 interacts with high affinity with peptides derived from the C terminus of Fas. The five most C-terminal amino acid residues of Fas influence the affinity of the interaction. Whereas the glutamine and isoleucine residues in the 4th and 5th positions from the C terminus affect the interaction in a negative and positive manner, respectively, the three C-terminal amino acid residues (SLV) are necessary and sufficient for a high affinity interaction to occur. Both the carboxyl group and side chain of the valine residue at the C terminus of Fas are essential; both the carboxyl group and side chain of the valine residue at the C terminus of Fas are essential, and the leucine and serine residues in the 2nd and 3rd positions, respectively, from the C terminus are important for the interactions with PDZ 2 and PDZ 4 of PTPL1.

Intracellular protein-tyrosine phosphatases (PTPs)† are a diverse group of proteins involved in signal transduction (reviewed in Ref. 1). They contain a conserved PTP domain, which regulates their subcellular localization and activity (reviewed in Ref. 2). For example, the SH2 domains of the intracellular PTPs SHP-1 and SHP-2 enable these signaling molecules to localize to and interact with growth factor receptors (2). The PTP domains usually have broad substrate specificity, and a correct subcellular localization of these molecules is therefore important to assure specificity in their action.

PTPL1 (3), also called PTP-BAS (4), hPTP1E (5), and FAP-1 (6), is a ubiquitously expressed intracellular PTP. Besides the C-terminal catalytic PT domain, several domain structures have been identified: an N-terminal leucine zipper motif followed by a domain that has similarity to the Band 4.1 superfamily (7) and five PDZ domains. The PDZ domain is a conserved motif of about 90 amino acid residues, which originally was identified in PSD-95 (8), Dlg-A (9), and ZO-1 (10), each of which contains three such domains; the motif has also been called GLGF repeat or DHR. PDZ domains have subsequently been identified in many different proteins (11). The crystal structure of the third PDZ domain of PSD-95 in complex with a peptide shows that the last four C-terminal amino acid residues of the peptide bind to a cleft in the domain (12), consistent with the notion that PDZ domains interact with C-terminal tails of target proteins (reviewed in Ref. 13).

Fas is a member of the tumor necrosis factor receptor family (14), the activation of which induces apoptosis in many cell types (reviewed in Ref. 15). The intracellular part of Fas contains a “death domain” necessary for the transduction of apoptotic signals (16). Deletion of the last 15 amino acid residues in the C terminus of Fas leads to a potentiated apoptotic response (16), indicating that this region is involved in negative regulation of signal transduction via Fas. Using a two-hybrid system in yeast, it was shown that PDZ 2 of PTPL1 could interact with the C-terminal region of Fas (Ref. 6; note that in this article PDZ 2 was referred to as GLGF3). Furthermore, PTPL1 was shown to have an inhibitory effect on Fas-induced apoptosis in a T-cell line (6), indicating that the interaction between Fas and PTPL1 is of physiological importance.

We have characterized the interactions between the PDZ domains of PTPL1 and the C terminus of Fas. Both PDZ 2 and PDZ 4 were shown to bind to short peptides derived from the C-terminal tail of Fas. The five C-terminal amino acid residues are important for the affinity and specificity of binding, and the C-terminal valine residue has a critical role in the interaction.

MATERIALS AND METHODS

Construction and Production of GST Fusion Proteins—DNA fragments coding for PDZ domains of PTPL1 were obtained by polymerase chain reaction and subcloned into the GST fusion protein expression vector pGEX1AT (Pharmacia Biotech Inc.): GST–PDZ 1, amino acid residues 1066–1166 of PTPL1; GST–PDZ 2–3, residues 1340–1579; GST–PDZ 4–5, residues 1669–1864; GST–PDZ 4–5, residues 1762–1866; and GST–PDZ 5, residues 1856–1960. These expression vector constructs were transformed into Escherichia coli. The GST fusion proteins were produced and purified essentially as described previously (17).

Preparation and Immobilization of Peptides—Peptides were synthesized in an Applied Biosystems 430A peptide synthesizer using Fmoc (9-fluorenylmethoxycarbonyl) chemistry. All of the peptides were made with C-terminal carboxyl groups, except QSLV-amide, which was amidated in the C terminus. The peptides were coupled to Affi-Gel 15 beads (Bio-Rad; 0.2 μmol of peptide/ml of swollen beads) following the manufacturer’s instructions.

In Vitro Peptide Binding Assay—Peptide-coupled beads (10 μl) were incubated with GST–PDZ fusion proteins (50 nM) at 4 °C for 2 h in binding buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 10 mM EDTA, 0.5% Triton X-100, 0.5% deoxycholate, 1 mM dithiobetrol; total volume, 500 μl). The beads were washed four times in binding buffer, and bound fusion proteins were eluted by boiling for 5 min in SDS-sample buffer (100 mM Tris-HCl, pH 8.8, 0.01% bromphenol blue, 3% glycerol, 4% SDS, 10 mM dithiobetrol) and subjected to SDS–gel electrophoresis (18) using 11% polyacrylamide gels. After electrophoresis, the proteins were transferred to nitrocellulose membranes (Hybond C Extra, Amer- sham Corp.), and the membranes were incubated with a rabbit anti- serum raised against recombinant GST expressed in bacteria. Thereafter, the membranes were incubated with 125I-labeled protein A followed by quantification of the bands using a Fuji image analyzer. For figures,
PDZ Domain-mediated Interactions between PTPL1 and Fas

RESULTS

Regions of PTPL1 cDNA corresponding to the various PDZ domains were cloned into the bacterial expression vector pGEX1AT (Fig. 1). The GST fusion proteins were produced in E. coli and then subjected to SDS-gel electrophoresis. Pure preparations of fusion proteins with expected sizes were obtained (results not shown).

Peptides corresponding to the C-terminal end of Fas were synthesized and coupled to Affi-Gel beads via their N termini. The beads were incubated with the different GST-PDZ fusion proteins and washed, and the bound material was eluted and analyzed by immunoblotting with an antiserum raised against GST. The fusion proteins containing PDZ 2 and PDZ 4 (GST-PDZ 2–3, GST-PDZ 4, and GST-PDZ 4–5) of PTPL1, but not GST-PDZ 1, GST-PDZ 3, and GST-PDZ 5, bound to a peptide corresponding to the last 15 amino acid residues in the C terminus of PTPL1 (residues 1762–1960; and GST-PDZ 5, residues 1856–1960).

Bound antibodies were visualized using enhanced chemiluminescence (Amersham Corp.), according to the manufacturer’s instructions.

![Schematic illustration of the domain structure of PTPL1 and the design of GST fusion proteins of the PDZ domains of PTPL1 used in this study.](image)

Motifs and domains indicated in the figure are: L, leucine zipper motif; Band 4.1, a domain of 300 amino acid residues with homology to the Band 4.1 superfamily; P, PDZ domain; PTP, protein-tyrosine phosphatase catalytic domain; GST-PDZ 1, amino acid residues 1066–1166 of PTPL1; GST-PDZ 2–3, residues 1340–1579; GST-PDZ 3, residues 1469–1579; GST-PDZ 4, residues 1762–1864; GST-PDZ 4–5, residues 1762–1960; and GST-PDZ 5, residues 1856–1960.

![Binding of GST-PDZ fusion proteins to a C-terminal peptide of Fas.](image)

**TABLE I**

Summary of data obtained from binding experiments

These experiments were performed as described under “Materials and Methods,” using a set of immobilized peptides derived from the C terminus of human Fas. +++, more than 25% of the added fusion protein was bound to the immobilized peptide; + +, 10–25% binding; +, 2–10% binding; −, no detected binding (less than 2%). Each experiment was performed at least three times. PDZ 1, PDZ 3, or PDZ 5 did not interact with any of the peptides.

| PDZ 2–3 | PDZ 4 | PDZ 4–5 |
|---------|-------|---------|
| DSENSNFRNEIQSLV | +++ | +++ | +++ |
| RNEIQSLV | +++ | +++ | +++ |
| NEIQSLV | +++ | +++ | +++ |
| EIQLSV | +++ | +++ | +++ |
| IQSLV | +++ | +++ | +++ |
| QSLV | +++ | +++ | +++ |
| SLV | +++ | +++ | +++ |
| QSLV | +++ | +++ | +++ |
| QSLV-amide | − | − | − |
| QSLA | − | − | − |
| QSLV-amide | − | − | − |
| QSLV | − | − | − |
QSSLw were made. No interaction between these peptides and the PDZ domains of PTPL1 could be detected. Furthermore, PDZ 1, PDZ 3, or PDZ 5 had no detectable affinity for any of the tested peptides. Together, these results show that the C-terminal valine residue of Fas has a critical role in the interaction with PDZ 2 and PDZ 4 of PTPL1 and that the five C-terminal amino acid residues of Fas determine the affinity of the interactions.

**DISCUSSION**

Several conserved domain structures have been identified in proteins that participate in signal transduction pathways, including SH2 domains, SH3 domains, PTB domains, and WW domains (19, 20). The function of these domains appears to be to mediate protein-protein interactions; SH2 and PTB domains bind to regions containing phosphorylated tyrosine residues, and SH3 and WW domains bind to proline-rich regions. The PDZ domain is a novel example of a domain involved in protein-protein interactions. PDZ 1 and 2 of the membrane-associated synaptic protein PSD-95 have been shown to interact with the C-terminal tails of the N-methyl-d-aspartate receptor and Shaker-type K+ channels (21, 22), and PDZ domains of p55 and hDLG, proteins similar in structure to PSD-95, have been reported to bind to the C-terminal parts of the erythrocyte membrane protein glycoporphin C and the adrenomatous polyposis coli tumor suppressor gene product APC, respectively (23, 24). The third PDZ domain of InaD binds to the C terminus of the Ca2+ channel TRP, involved in visual signal transduction in Drosophila (25).

Here, we have shown that fusion proteins containing PDZ 2 and PDZ 4 of PTPL1 bind with high affinity to a peptide corresponding to the last 15 amino acid residues in the C-terminal end of Fas. Similar results were obtained using the C-terminal pentapeptide IQSLV. However, if this peptide was modified at any position, its affinity for PDZ domains was altered, indicating that the last 5 amino acid residues in the C terminus are involved in the interaction. These results are consistent with the previous observation that PDZ domains function as C-terminal peptide binding modules.

Using peptide libraries, the binding specificities of certain PDZ domains, including PDZ 2 and PDZ 4 of PTPL1, have been investigated (Ref. 26; PDZ 2 and PDZ 4 were in this report referred to as PDZ3 and PDZ5 of PTBPAS, respectively). In conformity with our data this report indicated that a serine (or threonine) residue at the 3rd position from the C terminus is preferred by PDZ 2 and that a valine residue at the C terminus is preferred by both PDZ 2 and PDZ 4. However, data obtained with the peptide library approach suggested that PDZ 2 and PDZ 4 of PTPL1 can interact with peptides containing, not only valine residues, but also isoleucine or leucine residues at the C terminus, whereas our data indicated that no such interactions occur. Moreover, the peptide library data suggested that PDZ 2 does not have any clear amino acid preferences at the 2nd position from the C terminus, whereas we have shown that an alanine residue at this position totally blocks the interaction. The reason for these discrepancies remains to be elucidated.

The crystal structure of the third PDZ domain of PSD-95 in complex with a peptide showed that the last four C-terminal amino acid residues of the peptide bound to a cleft of the domain with the C-terminal valine buried in a shallow pocket (12). Interestingly, the side chain of the second residue (serine) of the bound peptide did not interact with the PDZ domain. Since our results show that the interaction does not occur if the penultimate residue is an alanine residue, which is smaller than the leucine residue found at this position in Fas, it is likely that the side chain of the leucine residue is involved in the interaction between PDZ 2 and PDZ 4 of PTPL1 and Fas. PDZ 1, PDZ 3, or PDZ 5 of PTPL1 does not interact with the C terminus of Fas, and binding specificities for these domains remain to be determined. Of the five PDZ domains of PTPL1, PDZ 2 and PDZ 4 display the highest sequence similarity (3), consistent with our finding that these domains have in common the ability to interact with Fas; however, their amino acid identity is rather low (35%), and additional structural information is needed before the amino acid residues involved in the interaction with Fas can be identified.

The available information suggests that all PDZ domains bind C-terminal sequences in proteins and that aliphatic residues are preferred at the most C-terminal position. PTPL1 is able to interact not only with Fas but also with several other proteins via its five PDZ domains and can thus potentially form multimeric protein complexes. We have recently identified and cloned a novel GTPase-activating protein for Rho that specifically interacts with PDZ 4 of PTPL1 through its C terminus.2 Further identification and characterization of proteins able to interact with PTPL1 will be an important step toward an understanding of its physiological role.

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