Identification of a Novel PSD-95/Dlg/ZO-1 (PDZ)-like Protein Interacting with the C Terminus of Presenilin-1*

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Presenilin-1 (PS-1) is the most causative Alzheimer gene product, and its function is not well understood. In an attempt to elucidate the function of PS-1, we screened a human brain cDNA library for PS-1-interacting proteins using the yeast two-hybrid system and isolated a novel protein containing a PSD-95/Dlg/ZO-1 (PDZ)-like domain. This novel PS-1-associated protein (PSAP) shares a significant similarity with a Caenorhabditis elegans protein of unknown function. Northern blot analysis revealed that PSAP is predominantly expressed in the brain. Deletion of the first four C-terminal amino acid residues of PS-1, which contain the PDZ domain-binding motif (Gln-Phe-Tyr-Ile), reduced the binding activity of PS-1 toward PSAP 4-fold. These data suggest that PS-1 may associate with a PDZ-like domain-containing protein in vivo and thus may participate in receptor or channel clustering and intracellular signaling events in the brain.

To date, familial forms of Alzheimer’s disease have been linked to mutations in three different genes. The first is the amyloid precursor protein (APP)† gene located on chromosome 21 (1). The other two genes are presenilin-1 (PS-1) and presenilin-2 (PS-2), located on chromosomes 1 and 1, respectively (2, 3). Mutations in all three genes lead to either increased total amounts of amyloid β-peptide (Aβ) or longer (42–43 amino acids) and hence more amyloidogenic Aβ formation. This supports the hypothesis that the aberrant processing of APP and its β-deposition are the primary pathogenic events in Alzheimer’s disease development (for review see Ref. 4). However, the mechanism by which the mutant APP, PS-1, and PS-2 alter both APP processing and the normal function of these proteins remains obscure.

On the basis of the predicted multiple transmembrane structure, it has been suggested that presenilin proteins may function as signal receptors, form channels, or participate in protein trafficking (2). Evidence supporting a signaling function for PS-1 and PS-2 comes from studies showing that both wild-type PS-1 and PS-2 cDNAs can complement Sel-12, a Caenorhabditis elegans homologue of PS-1 (PS-2), which facilitates Notch/Lin-12 signaling and cell fate specification during development (5). The involvement of PS-1 in Notch-mediated signaling during mammalian embryogenesis has also been suggested by studies using knockout mice (6, 7). Furthermore, mutations in PS-1 and PS-2 have been implicated in apoptotic cell death (8, 9). Protein topological studies suggest that PS-1 and PS-2 proteins have six to eight transmembrane domains (2, 10–12). Despite the diversity, it is notable that in all the topological models, the C-terminal domain is oriented toward the cytoplasm. This suggests that the C-terminal domain might mediate protein-protein interactions. Furthermore, it has been shown that the C-terminal fragment of PS-2 functions as a dominant negative mutant in T-cell receptor-induced apoptosis (8). This role might be explained by competitive binding of the C-terminal fragment to an unknown factor, resulting in the inhibition of signal transduction mediated by PS-2.

We have used the C-terminal fragment of PS-1 as a probe to screen a human brain cDNA library for PS-1-interacting protein(s). In this report, we describe the molecular cloning and characterization of a novel molecule, PSAP (PS-1-associated protein), which interacts with the C terminus of PS-1. Sequence analysis revealed that PSAP contains several putative protein kinase C and tyrosine kinase phosphorylation sites and shares a significant similarity with a C. elegans protein of unknown function. Interestingly, it was also found that PSAP contains a PDZ domain-like structure, which may account for its binding to the C-terminal Phe-Tyr-Ile-COOH motif of PS-1, a consensus sequence of the PDZ domain-binding site.

**MATERIALS AND METHODS**

*Yeast Two-hybrid and cDNA Library Screening—*All yeast strains, plasmids, and the human brain cDNA library used in the two-hybrid experiments were from CLONTECH as components of the Matchmaker two-hybrid system. The C-terminal 79 amino acids of PS-1 (PS1C79) were fused to the GAL4 DNA binding domain by subcloning the polymerase chain reaction-amplified corresponding coding region into the pAS2-1 vector (pAS1C79). Yeast strain Y190 was co-transformed with the plasmid pAS1C79 and a human brain cDNA library fused to the GAL4 transcription activation domain in pACT2 vector. Two-hybrid screening was carried out according to the manufacturer’s instructions (CLONTECH).

Galactosidase Assay—The following plasmids were used in yeast mating experiments. Blank vectors pAS2-1 and pACT2, pVA3-1 (5p3 gene in pAS2-1), pTD1-1 (SV40 large T antigen in pACT2), and pLAM5-1 (the lamin C gene in pAS2-1) from CLONTECH were used as controls. Plasmid pACPSAP was isolated from one of the two positive clones. The pAS1C79 plasmid was constructed as described above. Plasmids pASILoop (amino acids 302–376 of PS-1), pASILPH7-8 (amino acids 263–407 of PS-1), deletion mutant pAS1C79 (Δ4), which lacks the last C-terminal 4 amino acids of PS-1, and pAS2C79, which contains the last 79 amino acids of PS-2, were also constructed in the pAS2-1 vector. Plasmids based on the pAS1-2 vector were used to transform the...
yeast strain Y187. Plasmids based on the pACT2 vector were used to transform the yeast strain Y190. The mating experiments and color development assays were performed according to the manufacturer’s instructions (CLONTECH). For qualitative evaluation of the interaction between PSIC79 and PSAP, galactosidase filter assay selections were performed. For quantitative studies, galactosidase activity was determined using the chemiluminescent Galacton-Star detection kit (CLONTECH).

**Immunoprecipitation and Western Blot—**PS-1 with an N-terminal FLAG tag was subcloned into pCEP4. PSAP tagged with a Myc tag at the C terminus was subcloned into pCDNA3.1. HEK293 cells were stably transfected with a PSAP-expressing vector. To determine the interaction of PSAP with PS-1 in intact cells, cells stably expressing PSAP were further transiently transfected with PS-1 expression vector. The total cell lysate for immunoprecipitation was obtained as 18,000 g supernatant after cell lysis in lysis buffer (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.5% Triton X-100, 5 mM EDTA) containing protease inhibitors on ice for 30 min. The supernatants were incubated with α-Myc (Invitrogen) or AD3L or anti-L (specific to the C-terminal fragment of PS1-1 (10, 13)) and protein A-Sepharose overnight at 4 °C. The immunoprecipitates were separated by 10–16% Tris/glycine SDS-polyacrylamide gel electrophoresis (PAGE) and probed with the appropriate antibody. The blots were visualized by ECL-Plus (Amersham Pharmacia Biotech).

**Northern Blot Analysis—**Multiple tissue Northern blot (CLONTECH) containing 2 μg of poly(A)+ mRNA isolated from a variety of human tissues was probed with the 1.2-kb cDNA corresponding to the PSAP coding region. Northern hybridization was performed according to the manufacturer’s instructions (CLONTECH). The blot was also probed with radiolabeled β-actin cDNA (CLONTECH) as an indicator of RNA loading.

**RESULTS**

**PS-1 Interacts with a PDZ-like Domain-containing Protein—**Of 3.1 × 10⁶ total clones screened using PSIC79 as a probe, two positive clones, 46 and 299, were isolated. To eliminate false positives, yeast-mating experiments were carried out. As shown in Table I, only the transformant pair that bears the bait plasmid pAS1C79 and the plasmid pACPSAP from clone 46, as well as the transformant pair that bears the positive control plasmids containing murine p53 and SV40 large T, were positive for the His3 phenotype and for β-galactosidase activity, indicating a true positive interaction between PSIC79 and PSAP. Plasmid from clone 299 was also tested in the mating experiment, and the same results were observed (data not shown).

Restriction mapping and DNA sequencing analysis indicated that these two clones are identical and contain a 1.9-kb cDNA. We have performed the 5′-RACE (rapid amplification of cDNA ends) reaction using human brain whole cDNA (CLONTECH), and no DNA fragment with a 5′-end longer than the insert in the two positive clones has been isolated. The putative open reading frame of 1113 base pairs encodes a polypeptide of 371 amino acids with a predicted mass of 39.9 kDa, designated as PSAP (Fig. 1A). Sequence analysis of PSAP using “Prosite” revealed six consensus sites for phosphorylation by protein kinase C, three by casein kinase II, one by cAMP-dependent kinase, and one by tyrosine kinase (Fig. 1A). A BLAST search of GenBank™ revealed that PSAP is novel but shares homology (28% identity, 65% similarity) with a C. elegans protein, F43E2.7, of unknown function (14). It was also found that PSAP contains a GLLGF sequence preceded by a basic amino acid lysine that is similar to the conserved motif, R/KXXXXXGLGF, found in most PDZ proteins and as seen in the binding pocket of a typical PDZ protein, PSD-95 (15). The overall homology between the PDZ-like domain (amino acids 222–304) of PSAP and typical PDZ domains is lower but significant, 12–14% identical in 80–95 amino acids (Fig. 1B). PSAP, like the Veil/Homer family protein (16, 17), may represent another unique member of the PDZ protein superfamily.

**PSIC79 Interacts with PSAP in a Specific Manner—**To determine the specificity of the interaction of PSIC79 with PSAP, we tested whether other fragments of PS-1, including the large loop between the sixth and seventh transmembrane domains, interact with PSAP in the yeast two-hybrid system. As shown in Table I, none of the tested fragments interacts with PSAP. To de

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**Table I**

| Binding domain protein in pAS52-1 | Activation domain protein in pACT2 | Galactosidase filter assay | His3 phenotype selection |
|----------------------------------|----------------------------------|--------------------------|-------------------------|
| PSIC79                           | pACT2                            | Blue++                   | Good growth             |
| PS1C79                           | pACT2                            | Blue++                   | Good growth             |
| PSIC79                           | pACT2                            | Blue++                   | Good growth             |
| PS2C79                           | pACT2                            | Blue++                   | Good growth             |
| PS1loop                          | pACT2                            | Blue++                   | Good growth             |
| PS1loop                          | pACT2                            | Blue++                   | Good growth             |
| Lamin C                          | pACT2                            | Blue++                   | Good growth             |
| p53                              | SV40 large T                     | Blue+++                  | Good growth             |

**Fig. 1. Sequence analysis of PSAP.** A, amino acid sequence of PSAP. Putative sites of phosphorylation by protein kinase C are circled, those by casein kinase are boxed, that by tyrosine kinase is shown in bold underlined letters, and that by cAMP-dependent kinase activity is shown in bold letters. B, amino acid sequence alignment between the PDZ-like domains of PSAP and other PDZ domain proteins. Residues identical to or similar to the PSAP sequence are heavily shaded or lightly shaded, respectively.

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79-residue region of PS-2, which is highly conserved between PS-1 and PS-2, interacts with PSAP. As shown in Table I, PS2C79 failed to interact with PSAP in the yeast two-hybrid assay.

PSAP Interacts with Full-length PS-1 in Intact Cells—HEK293 cells stably expressing Myc-tagged PSAP were transiently transfected with plasmid encoding FLAG-tagged PS1. As a control, cells stably expressing Myc-tagged LacZ were also transiently transfected with PS-1. Twenty-eight hours later, cells were lysed and then co-immunoprecipitated, and Western blot analysis was performed. As shown in Fig. 2, PS-1 was specifically co-immunoprecipitated with PSAP by anti-Myc antibody. This result indicates that PSAP interacts with PS1 in intact cells.

**PS1C79-PSAP Interaction Requires the C-terminal Gln-Phe-Tyr-Ile-COOH Motif of PS-1**—Ligand interaction studies have revealed that PDZ domains bind to a consensus sequence in the C terminus of the target protein, which contains a hydrophobic or an aromatic amino acid as the last amino acid (18). On the basis of the primary sequences, two large groups of unique C-terminal motifs of target proteins are recognized by two PDZ domain classes. Class I PDZ domain binds to a C-terminal motif with the sequence Ser/Thr-X-Val/Ile-COOH. In contrast, class II PDZ domains have a preference for Phe/Tyr-X-Phe/Val/Ale?-COOH (18). We examined PS-1 for this motif and found a sequence, Gln-Phe-Tyr-Ile-COOH, at the extreme C terminus of PS-1. The glutamine residue at the 4 position was also shown to influence the interaction (18). To determine whether the sequence Gln-Phe-Tyr-Ile-COOH is the PSAP-binding motif of PS1, we deleted this sequence in PS1C79. This mutant, PS1C79(–4), interacts poorly with PSAP and the binding activity was reduced by 4-fold compared with native PS1C79 (Fig. 3 and Table I). This result indicates that the sequence Gln-Phe-Tyr-Ile-COOH in PS-1 is required for optimal interaction with PSAP.

**DISCUSSION**

In the present study, we identified a novel protein, PSAP, that interacts with the C terminus of PS-1 and contains a PDZ-like domain, a motif thought to mediate protein-protein interactions and to be involved in cellular junction formation, receptor or channel clustering, and cellular signaling events. PS-1 has been implicated in the Notch/Sel-12 signaling pathway; thus, the direct interaction of PSAP with the C terminus of PS-1 may play a regulatory role in PS-1-mediated Notch and/or wingless signal transduction by analogizing the function of Dsh, which contains one PDZ domain and is required for signal transduction from seven-transmembrane receptors, frizzled and Dfz2, in the wingless signal pathway in Drosophila (for review see Ref. 19).

One of the important functions of PDZ domain-containing proteins is in the clustering and localization of specific classes of ion channels at synapses and possibly other sites of membrane specialization in neurons. Recently, it has been reported that the overexpression of PS-1 and PS-2 up-regulates functional K⁺ channel expression either by directly associating with K⁺ channel pore-forming subunits or by indirectly increasing the synthesis, assembly, and/or transport of these subunits to the plasma membrane (20). The mechanism underlying the effects of presenilins on ion channels is not understood. Studies employing cell culture systems or transgenic mice have shown that Alzheimer mutant presenilins influence APP processing in a manner that elevates production of the longer and more amyloidogenic Aβ (4). However, whether this aberrant Aβ production results from the alteration of protein trafficking caused by mutant presenilin remains to be established. By analogizing the function of the known PDZ proteins,
further investigation on the role of the direct interaction between PSAP and PS-1 will provide important information on these issues.

In a recent study, we found that PS-1 has a regulatory effect on the GTPase activity of Go and may function as a G protein-coupled receptor (21). It is notable that about 30% of known G protein-coupled receptors contain the PDZ-binding sequence (22). In Drosophila, a PDZ domain-containing protein, InaD, serves as a scaffold to assemble different signaling molecules of the Go-regulated phototransduction cascade (23). By analogy, this implies a potential role for PSAP in the Go-regulated signaling pathway mediated by PS-1.

PSAP contains only one PDZ-like domain and no other modular domains such as Src homology 2 (SH2), SH3, or pleckstrin homology (PH). However, PSAP contains several potential protein kinase phosphorylation sites by various kinases. These phosphorylation sites may be important for the regulation of the function of PSAP by the kinase activities and/or in mediating the interaction of PSAP with other molecules.

It is also noteworthy that PSAP specifically interacts with PS-1 but not PS-2. This may be because of the substitution of Leu for Phe at position -3 in PS-2. This result suggests that PS-1 and PS-2 may be involved in different signaling pathways. The differences in the physiological regulation and/or function of PS-1 and PS-2 were also suggested by the observations that PS-1 and PS-2 possess different phosphorylation patterns (24, 25).

In conclusion, we have identified a novel molecule, PSAP, a unique PDZ domain-containing protein that specifically binds to PS-1. Mutational analysis revealed that the C-terminal Gln-Phe-Tyr-Ile-COOH, motif of PS-1 is required for the interaction with PSAP. PSAP is predominantly expressed in the brain and may function as an adaptor molecule that links PS-1 to an intracellular signal transduction pathway. This finding may open a new avenue for determining the normal and pathological functions of PS-1 and the mechanism by which PS-1 is involved in Alzheimer's disease.

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