Biochemical and Biophysical Research Communications **270,** 222–229 (2000)
doi:10.1006/bbrc.2000.2417, available online at http://www.idealibrary.com on IDEAL®

FYVE-DSP1, a Dual-Specificity Protein Phosphatase Containing an FYVE Domain

Runxiang Zhao, Ying Qi, and Zhizhuang Joe Zhao[1]
Division of Hematology/Oncology, Department of Medicine, Vanderbilt–Ingram Cancer Center, Vanderbilt University, Nashville, Tennessee 37232-6305

Received February 26, 2000

Dual-specificity protein phosphatases (DSPs) dephosphorylate proteins at Ser/Thr and Tyr. FYVE domain is a double zinc finger motif which specifically binds phosphatidylinositol(3)-phosphate. Here, we report a novel dual specificity phosphatase that contains a FYVE domain at the C-terminus. We designate the protein FYVE-DSP1. Molecular cloning yielded three isoforms of the enzyme presumably derived from alternate RNA splicing. Sequence alignment revealed that the catalytic phosphatase domain of FYVE-DSP1 closely resembled that of myotubularin, while its FYVE domain has all the conserved amino acid residues found in other proteins of the same family. Recombinant FYVE-DSP1 is partitioned in both cytosolic and membrane fractions. It dephosphorylates proteins phosphorylated on Ser, Thr, and Tyr residues and low molecular weight phosphatase substrate *para*-nitrophenylphosphate. It shows typical characteristics of other DSPs and protein tyrosine phosphatases (PTPs). These include inhibition by sodium vanadate and pervanadate, pH dependency, and inactivation by mutation of the key cysteinyl residue at the phosphatase signature motif. Finally, PCR analyses demonstrated that FYVE-DSP1 is widely distributed in human tissues but different spliced forms expressed differently.

Key Words: **FYVE domain; protein phosphatase; cloning; characterization.**

Protein phosphorylation is one of the most fundamental regulatory mechanisms in living cells. This process is controlled by coordinate action of protein kinases and phosphatases (1). In the protein phosphatase superfamily, protein tyrosine phosphatases (PTPs) and dual specificity phosphatases (DSPs) consist of a wide class of proteins that have a crucial role in signal transduction pathways regulating cell proliferation, differentiation, and transformation (2–6). It has been estimated that PTPs and DSPs correspond about half of the total 1000 protein phosphatases in human genome (1, 4). DSPs were initially known as the VH1-like phosphatase (7). PTPs and DSPs are characterized by the presence of a highly conserved signature motif (I/V)HCxAGxxR(S/T)G, but the sequences outside the catalytic domains are highly diversified. Some of the enzymes contain other protein domains, for example, transmembrane segments together with extracellular domains, endoplasmic reticulum localization motifs, nuclear signal sequences, SH2 domains, cytoskeleton-like domains, and cellular retinaldehyde binding protein-like domains. These sequences flanking the catalytic domain direct the enzymes to specific location and regulate their activity (8).

FYVE (Fab1, YGLO23, Vps27, and EEA1) domain is a double zinc finger motif of ~70 amino acids and is conserved in several proteins involved in vesicular traffic (reviewed in 9). Like the PH domains found in protein kinases PDK1 and PKB, FYVE domain specifically binds 3-phosphorylated phosphoinositides. FYVE domains coordinate 2 Zn^{2+} ions via 8 cysteine/histidine residues spaced in a specific manner (CX2CX9-39CX1-3(C/H)X2-3CX2CX4-48CX2C) (10, 11). This protein motif also contains a basic amino acid patch adjacent to the 3rd cysteinyl residue, which is critical for binding of acidic phosphatidylinositol(3)-phosphate (12). EEA1, a core component of the endosome-docking apparatus, is one of the FYVE domain–containing proteins that have been extensively studied (13–16). The FYVE domain of EEA1 is essential for its correct targeting and endocytic function in cells since mutagenesis of the conserved (R/K)(R/K)HHCR motif disrupted the process (12).

In the present study, we report molecular cloning and characterization of a novel dual specificity protein phosphatase that contain a FYVE domain at the N-ter-

This work was supported by Grants HL-57393, CA75218 (to Z.J.Z.), and CA-68485 (to Vanderbilt-Ingram Cancer Center) from the National Institutes of Health.

Abbreviations used: PTP, protein tyrosine phosphatase; DSP, dual specificity phosphatase; PI, phosphatidylinositol; PI3P, phosphatidylinositol 3-phosphate; PI3K, phosphoinositide 3-kinase.

[1] To whom correspondence and reprint requests should be addressed at 547, MRB II, 2220 Pierce Avenue, Nashville, TN 37232-6305. Fax: (615) 936-3853. E-mail: joe.zhao@mcmail.vanderbilt.edu.

0006-291X/00 $35.00

minus. As a potential target of phosphatidylinositol(3)-phosphate, FYVE-DSP1 may have a crucial role in cell signaling.

EXPERIMENTAL PROCEDURES

Materials. Human 293 cells were obtained from the American Type Culture Collection. Monoclonal anti-Xpress tag antibodies were purchased from Invitrogen. Human brain Marathon-ready cDNA library was from Clontech. Pervanadate was made by mixing equal moles of sodium vanadate and H_2O_2 and incubating at room temperature for 20 min before use (18). [γ-^{32}P]-ATP and *para*-nitrophenylphosphate (pNPP) were purchased from Pharmacia/Amersham and Sigma Chemical Company, respectively.

Molecular cloning of FYVE-DSP1. A search of the GenBank database for proteins with the (I/V)HCxAGxxR(S/T)G signature motif of protein tyrosine phosphates by using the Blast program resulted in identification of a putative dual specificity protein phosphatase with a designation of KIAA0371. This sequence was deposited by the Kazusa DNA Research Institute, Chiba, Japan (19), and its identity has not been revealed. We thus designed PCR primers with sequences of 5′-CCTCTTGTCATGGATGAAGAGACTCGG-3′ and 5′-GGCTCAGTGAGTCCTTGCTCC-3′ to amplify a human brain Marrathon-ready cDNA library. The PCR was run for 30 cycles with Pfu Turbo (Stratagene) at an annealing temperature of 63°C, and the products were cloned into *Eco*RV-digested pBluescript KS vector (Stratagene). A number of clones were chosen for DNA sequence analyses by using the automatic DNA sequencing facility at the Vanderbilt-Ingram Cancer Center.

Expression and immunoprecipitation of FYVE-DSP1. FYVE-DSP1 constructs were subcloned in-frame into the pCDNA3.1/His C vector (Invitrogen) that has an Xpress tag and a 6xHis tag at the N-terminus. The Cys-to-Ser mutant form of FYVE-DSP1 was made by mutating the cysteine in the phosphatase signature motif to serine through PCR with appropriate primers. The mutation was confirmed by sequencing analysis. The DNA constructs were used to transfect 293 cells by using the FuGENE6 cell transfection system (Boehringer Mannheim). Briefly, 293 cells were grown to ~25% confluency in DMEM supplemented with 10% fetal calf serum and 50 μg/ml each of streptomycin and penicillin. For each 150-mm plate of cells, 60 μg of DNA and 45 μl of the FuGENE6 reagent were used. The transfected cells were cultured for 48 hr before harvesting. The cells were lysed in Buffer A (25 mM β-glycerophosphate, pH 7.3, 2 mM β-mercaptoethanol, 1.0 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 20 μg/ml leupeptin, 1 μM pepstatin A, and 1 μg/ml aprotinin) supplemented with 0.1 M NaCl and 1% Triton X-100. Extracts were cleared by centrifugation. For immunoprecipitation, cell extracts were incubated overnight with the anti-Xpress antibody pre-bound to protein A-Sepharose. The beads were washed three times with the above extraction buffer and used for phosphatase assays with ^{32}P-labled substrates as described below.

Cell fractionation and purification of FYVE-DSP1. Human 293 cells were transfected with pCDNA3/HisC-FYVE-DSP1a as described above. The cells were broken up with a Dounce glass homogenizer in Buffer A. Following homogenization, 0.1 M NaCl was added, and the homogenates were centrifuged at 800*g* for 20 min to spin down nuclei. The nuclear pellets were washed once with the extraction buffer and then dissolved in SDS gel sample buffer. The postnuclear supernatants were further centrifuged at 100,000*g* for 60 min. The clear supernatant was referred to as the cytosolic extracts. The pelleted membrane was washed once with Buffer A plus 0.1 M NaCl followed by centrifugation as above and then dissolved in Buffer A supplemented with 1% Triton X-100 and was referred to as the membrane extracts. For purification of FYVE-DSP1, the cytosolic extracts were loaded onto an NDA-Ni-agarose column (Qiagen) equilibrated with Buffer B (50 mM NaH_2PO_4/Na_2HPO_4, pH 7.0, 2 mM 2-mercaptoethanol). The column was washed with Buffer B supplemented with 0.3 M NaCl and 25 mM Imidazole-HCl, pH 7.0 and then eluted with a buffer containing 0.2 M Imidazole-HCl (pH 7.0) and 2 mM 2-mercaptoethanol. Control cells and cells transfected with the Cys-to-Ser mutant form of FYVE-DSP1 were treated with the same procedure.

Phosphatase assays. To determine the substrate specificity, we employed immuno-purified FYVE-DSP1a. Three ^{32}P-labeled protein substrates were prepared as previously described (20). Briefly, glycogen phosphorylase was phosphorylated on serine by phosphorylase kinase. Bovine lipocortin I was phosphorylated on theronine by protein kinase C. The intracellular domain of the EGF receptor (sEGF-R) was autophosphorylated on tyrosine. The dephosphorylation reactions with FYVE-DSP1a were performed in a buffer containing 25 mM Tris-HCl (pH 7.0) and 2 mM dithiothreitol for 30 min at 37°C. The extents of the reactions were analyzed by SDS–PAGE followed by autoradiography. Control experiments were performed with immunoprecipitates obtained from cells transfected with the plain vector. To analyze the phosphatase activity with a commonly used low-molecular-weight phosphatase substrate p-NPP, NDA-Ni-agarose-purified enzyme was used. The reactions were carried out at different pH in the presence or absence of effectors (20).

Determination of the tissue distribution of FYVE-DSP1. To determine the expression level of FYVE-DSP1 in various tissues, we used the Rapid-Scan Gene Expression Panels from OriGene Technologies, Inc. This was performed by PCR with Pfu Turbo polymerase (Stratagene). The PCR primers were 5′-CTTCCAATCCCAGTAGATGCAAAAGT-3′ and 5′-GGCTCAGTGAGTCCTTGCTCC-3′ which covers 1102 bp of the 3′ region of FYVE-DSP1a. The PCR was run for 35 cycles, and the conditions were 94°C for 30 s, 61°C for 30 s, and 72°C for 1.5 min. The products were analyzed on 1% agarose gel and detected by ethidium bromide.

RESULTS

DNA and protein sequence analysis. We have searched the GenBank database for proteins with the (I/V)HCxAGxxR(S/T)G signature motif of protein tyrosine phosphates by using the Blast program and identified a putative dual specificity protein phosphatase with a designation of KIAA0371 (GenBank Accession No. AB002369). We thus designed specific PCR primers to amply human brain cDNA library to obtain the coding region of the cDNA. The PCR products were cloned into the pBluescript KS vector, and we isolated two types of clones with cDNA inserts of 3511 and 3484 bp. We designate them FYVE-DSP1a and FYVE-DSP1b, respectively. FYVE-DSP1a differs from FYVE-DSP1b by a 27 bp insert at 3′ region. Both are essentially identical to the sequences of KIAA0371 in the GenBank database except that the latter has a 111 bp insert at the 3′-end compared with FYVE-DSP1b. These cDNAs likely represent alternately spiced products of a single gene. We tentatively designate KIAA0371 as FYVE-DSP1c. The nucleotide sequences of FYVE-DSP1a, FYVE-DSP1b, and FYVE-DSP1c have been deposited in the GenBank database under accession numbers AF233436, AF233437, and AF233438, respectively. The amino acid sequences deduced from the three DNA sequences are presented in Fig. 1. The open reading frame of FYVE-DSP1a encodes a protein of 1170 amino acid residues,

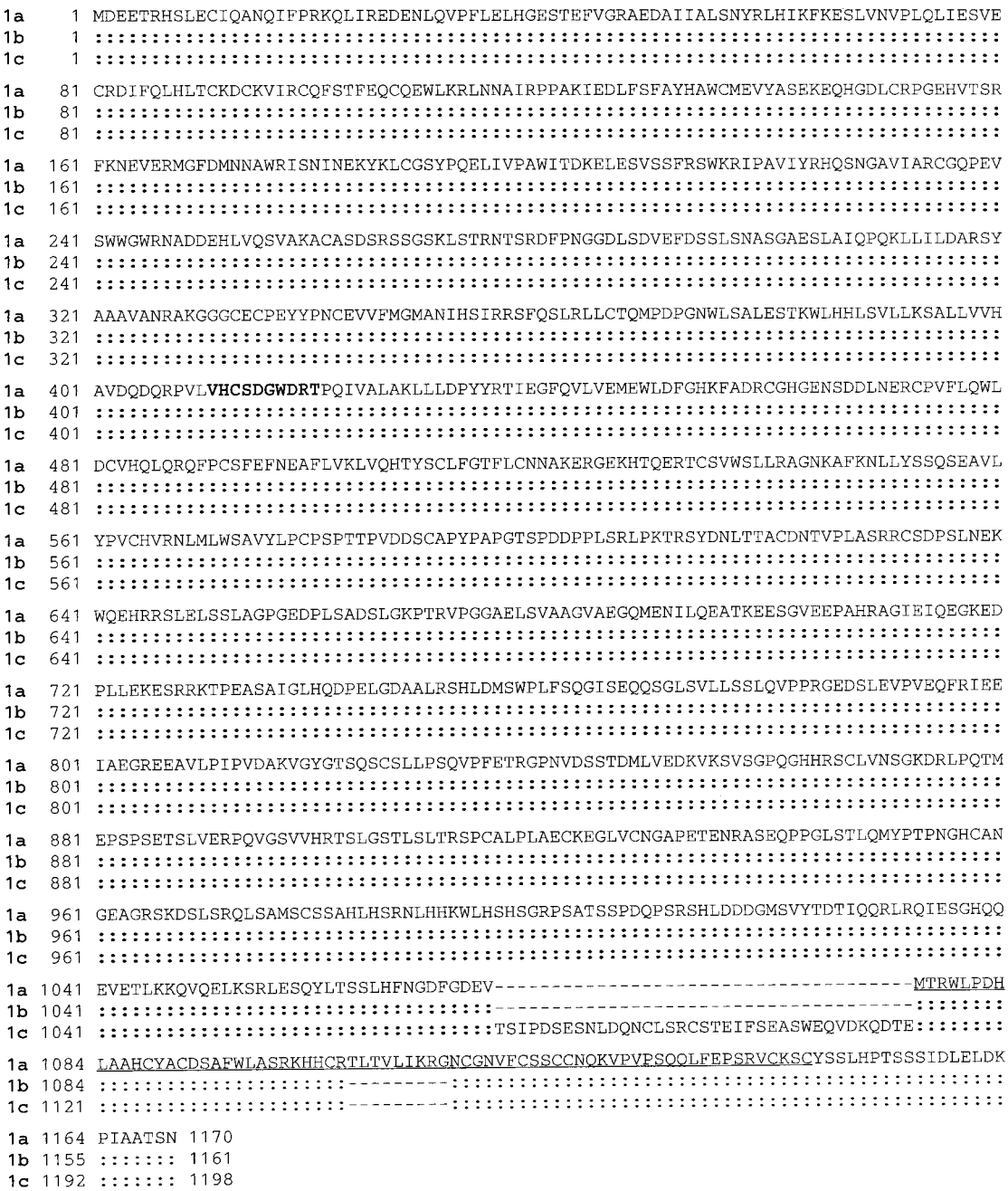

FIG. 1. Amino acid sequences of the three forms of FYVE-DSP1. The entire sequence of FYVE-DSP1a was shown the top. ":" denotes identical amino acid residues, and "−" represents gaps. The PTP and DSP signature motif is boldface and the FYVE domain is underlined.

while FYVE-DSP1b has 1161 amino acid residues. KIAA0371 (FYVE-DSP1c) encodes a protein of 1198 amino acid residues. Except for certain gaps and inserts at the C-terminus, the three protein sequences are identical. At the +4 and −3 positions from the predicted ATG initiation codon of FYVE-DSP1 cDNA are G nucleotides that conforms well to requirements for efficient translation as defined by Kozak (21). In addition, the ATG codon is preceded by an in-frame termination codon at nucleotide −18, further supporting validity of the ATG being the translation initiator of FYVE-DSP1 gene products. Protein sequence analysis revealed that the FYVE-DSP1 gene products contained no hydrophobic transmembrane or signal sequences. Therefore, they likely represent nontransmembrane intracellular proteins. Sequence analysis also revealed that FYVE-DSP1 contained the signature motifs for PTPs and DSPs. Comparison of

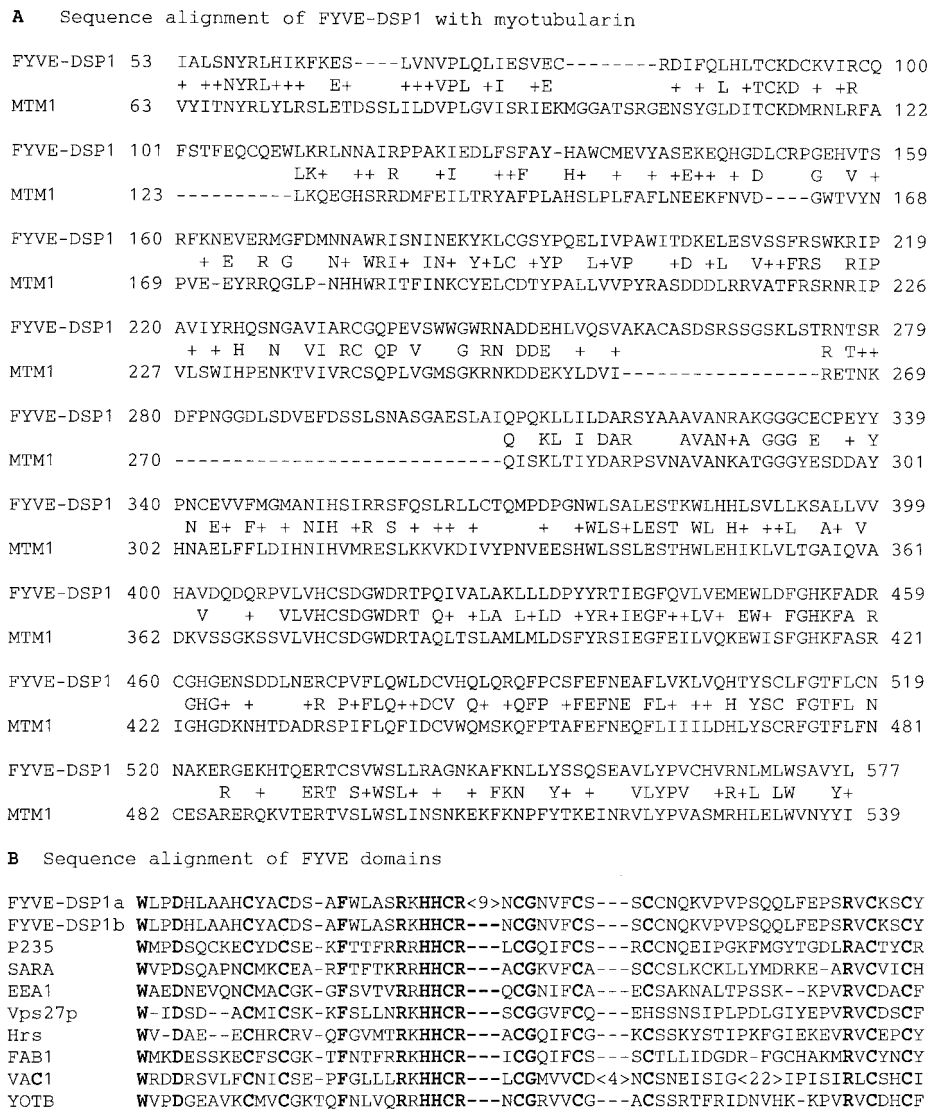

FIG. 2. (A) Sequence alignment of FYVE-DSP1 with myotubularin. Identical amino acid residues are shown in the middle. "+" denotes similar residues. (B) Sequence alignment of FYVE domains. Conserved amino acid residues are boldface.

FYVE-DSP1 protein sequences with those in the protein databanks by using the BLAST program revealed that FYVE-DSP1 showed ~37% sequence identity (out of 525 amino acid residues) to MTM1gene product myotubularin (22), but it had no significant overall sequence homology to other PTPs and DSPs (Fig. 2A). More interestingly, the C-terminus of the FYVE-DSP1 contained a motif resembles the recently defined as FYVE domain. Figure 2B shows the sequence alignment of the FYVE domain of FYVE-DSP1 with those of P235 (23), SARA (24), EEA1 (13), Vps27 (25), Hrs (26), Vac1 (27), FAB1 (28), and YOTB (29) in the region. The FYVE domains of FYVE-DSP1 shares the highest sequence identity (40%) with that of P235 (23), a recently cloned phosphoinositide kinase, and it has all the conserved amino residues required for the FYVE domain. Note that FYVE-DSP1a has a 9 amino acid insert in the region.

Substrate specificity of FYVE-DSP1. Our sequence analysis revealed that FYVE-DSP1 is a putative DSP sharing significant sequence homology with myotubularin. Myotubularin has been shown to hydrolyze both phosphotyrosine and phosphoserine residues *in vitro* (30, 31). To confirm the activity of FYVE-DSP1, we expressed FYVE-DSP1a as a recombinant protein in human 293 cells. The recombinant protein was immunopurified through the Xpress tag located at the N-terminus. We used three protein substrates to analyze its phosphatase activity. As shown in Fig. 3, FYVE-DSP1a was able to efficiently dephosphorylate serine-phosphorylated glycogen phosphorylase, threo-

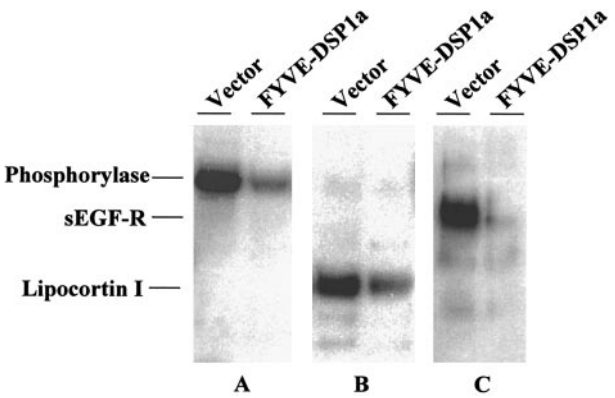

FIG. 3. Substrate specificity of FYVE-DSP1. Immunopurified FYVE-DSP1a on beads were incubated with ^{32}P-labeled glycogen phosphorylase (A), lipocortin I (B), and sEGF-R (C). Dephosphorylation of the proteins was determined by autoradiography after separation on SDS gel.

nine-phosphorylated lipocortin I, and tyrosine-phosphorylated sEGF-R. We also obtained similar results with the enzyme purified from NDA-Ni-agarose column (data not shown). These data indicate that FYVE-DSP1a behaves as a typical DSP, displaying activity toward Ser/Thr- and Tyr-phosphorylated proteins.

Subcellular fractionation and purification of FYVE-DSP1. Fractionation of lysates of 293 cells transfected with FYVE-DSP1a followed by Western blot analysis revealed that the enzyme was distributed in the cytosolic and membrane fractions with a ratio of approximately 6:4 (Fig. 4A). The enzyme was absent in the nucleus. The membrane localization might be attributable to the FYVE domain. Since the recombinant protein had a 6× His tag attached to the N-terminus, the enzyme in the cytosolic fraction was purified by using a single NDA-Ni-agarose column. The purity is over 90% with a yield of 50 μg from 4 plates (150 mm) of transfected cells (Fig. 4B). The enzyme in the membrane fraction was also separated by the same procedure, but the purity and yield were significantly lower (data not shown). Cytosolic extracts from cells transfected with FYVE-DSP1b and Cys-to-Ser mutant forms of FYVE-DSP1a and 1b went through the same chromatographic procedure and gave rise to a similar degree of purification (not shown). The purified cytosolic enzymes were used for the preliminary characterization as described below. Samples obtained through same procedure from plain vector-transfected cells were used as control. No detectable phosphatase activity was obtained with the control samples.

Biochemical characterization of FYVE-DSP1. We used the NDA-Ni-agarose-purified enzyme for biochemical characterization of FYVE-DSP1. We employed the most commonly used protein phosphatase substrate, pNPP. Figure 5A shows the pH-dependence of FYVE-DSP1a. Most of PTPs and DSPs which have been characterized displayed optimal pH of around 5 with pNPP as a substrate (20, 32–36). This also applies to FYVE-DSP1a that had sharp pH optima of 4.5. Another common feature of DSPs and PTPs are their sensitivity to sodium vanadate and pervanadate. As expected, FYVE-DSP1a activity was nearly abolished by 1 mM Na_3VO_4 or 0.1 mM pervanadate (Fig. 5B). In contrast, microcystin, which is able to sufficiently inhibit pp1 and pp2a at nanomolar concentrations, failed to show any inhibitory effects at a concentration of as high as 20 μM. All these data suggest that FYVE-DSP1a has the typical biochemical properties of common DSPs and PTPs. The cysteinyl residue at the signature motif of PTPs and DSPs is involved in the formation of a thiophosphate intermediate in the catalytic reactions (2, 3). Mutation of the residue causes total loss of enzymatic activity. This is proven to be true for FYVE-DSP1 also. Mutation of the cysteinyl residue to a seryl residue resulted in total inactivation of FYVE-DSP1a and FYVE-DSP1b (Fig. 5C).

Distribution of FYVE-DSP1 in human tissues. To study the tissue expression of FYVE-DSP1, we used PCR to amplify single-strand cDNAs from 24 human tissues. These included brain, heart, kidney, spleen, liver, colon, lung, small intestine, muscle, stomach, testis, placenta, salivary gland, thyroid gland, adrenal gland pancreas, ovary, uterus, prostate, skin, peripheral blood, bone marrow, fetal brain, and fetal liver (Fig. 6). FYVE-DSP1 was express in most of the tissues checked except for salivary gland and pancreas. In most cases, the PCR products had the same size as that

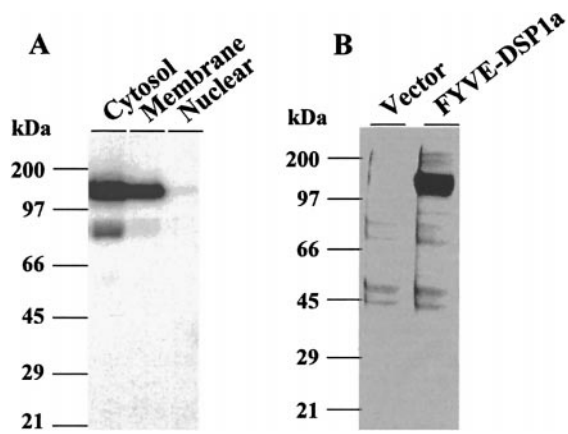

FIG. 4. Subcellular fractionation and purification of recombinant FYVE-DSP1a. Human 293 cells were transfected with the pCDNA3.1/HisC-FYVE-DSP1a plasmid. (A) Cytosolic, membrane, and nuclear extracts were obtained as described under Experimental Procedures. Equal proportions of the extracts were separated on SDS gel, and expression of FYVE-DSP1a were detected by Western blotting with anti-Xpress antibody. (B) SDS gel separation of NDA-Ni-agarose-purified samples from cytosolic extracts of 293 cells transfected with control vector or with the FYVE-DSP1a construct. Gels were stained with Coomassie brilliant blue R-250.

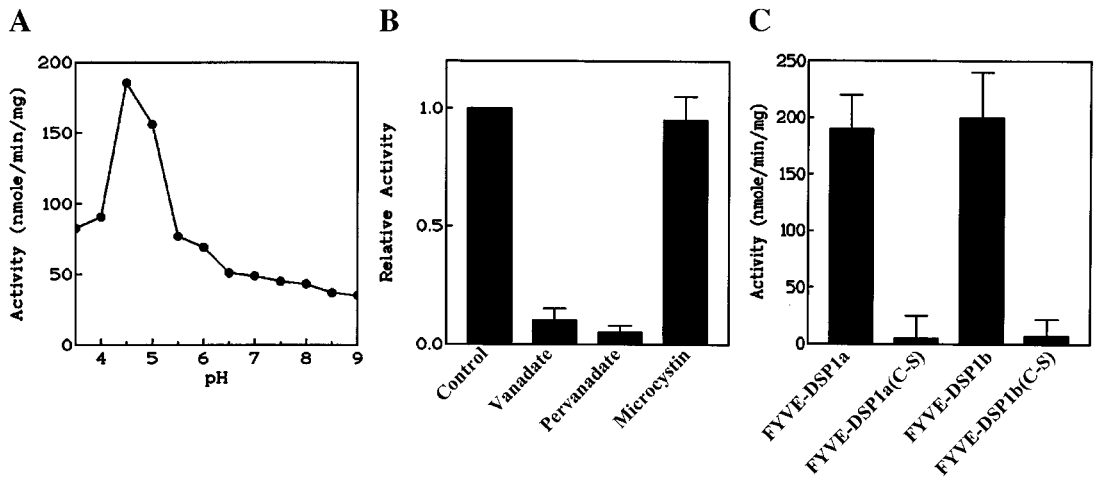

FIG. 5. Factors affecting activity of FYVE-DSP1. NDA-Ni-agarose-purified FYVE-DSP1a was analyzed with 10 mM pNPP at different pH (A) or at pH 5.0 in the presence of 1 mM Na_3VO_4, 0.1 mM pervanadate, or 20 μM microcystin (B). (C) Comparison of the activities of FYVE-DSP1a, FYVE-DSP1b, and their C-to-S mutant forms determined at pH 5.0 with 10 mM pNPP as a substrate. All the proteins were purified from NDA-Ni-agarose with similar degrees of purity.

obtained with the FYVE-DSP1a cDNA plasmid. Sequence analyses of the PCR products confirmed that the products indeed corresponded to FYVE-DSP1a. FYVE-DSP1b was also present in the products, but as a minor form which is hardly detected by sequencing. Interestingly, unlike rest of the tissues, testis and prostate gave rise to PCR products with a slightly larger molecular size. DNA sequencing revealed that all the products in testis and most of products in prostate corresponded to the FYVE-DSP1c isoform with a minor

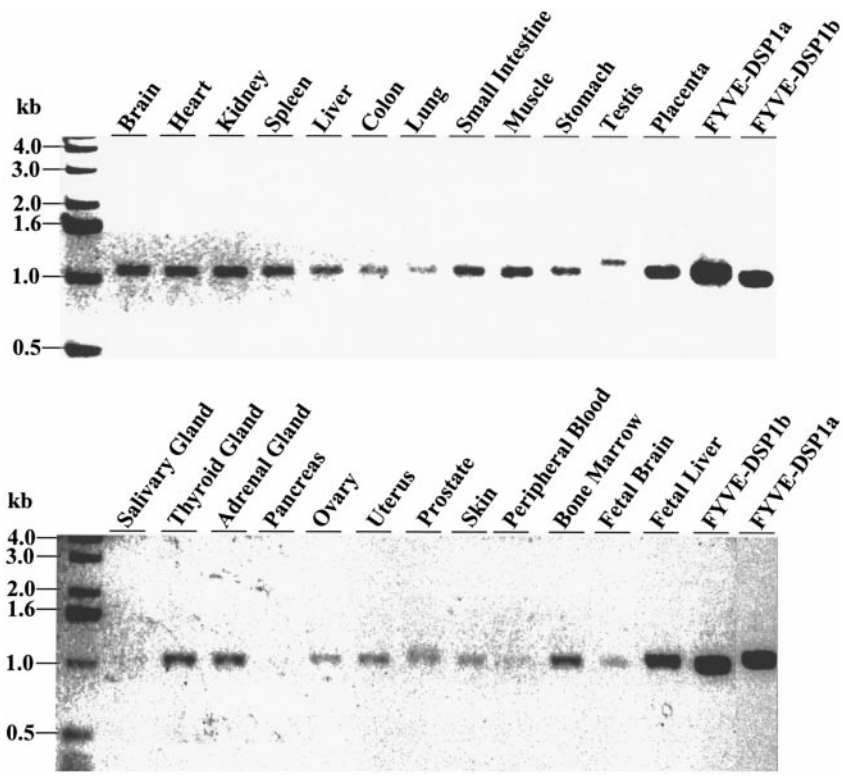

FIG. 6. Distribution of FYVE-DSP1 in 24 human tissues. First-strand cDNAs at a concentration of 100X (OriGene Technologies, Inc.) were amplified by PCR with a pair of specific primers derived from FYVE-DSP1. PCR products were resolved on 1% agarose gel, and DNA products were detected with ethidium bromide. Reversed photo images were shown.

FYVE-DSP1a form in prostate. Although the level of FYVE-DSP1 in these tissues is hard to be ascertained since PCR is not necessarily quantitative, the data at least indicate that FYVE-DSPs is widely expressed and alternately spliced forms of the enzyme are differently expressed. This is indicative of the general importance of the enzyme in cell function.

DISCUSSION

We have identified a novel dual specificity phosphatase which we designate FYVE-DSP1. FYVE-DSP1 has a highly conserved signature motif found in PTPs and DSPs. Our preliminary characterization of recombinant FYVE-DSP1 expressed in human 293 cells revealed that it has all the typical biochemical properties of DSPs. FYVE-DSP1 shares significant overall sequence homology with myotubularin in the catalytic domain but has essentially no sequence identity with other DSPs outside of the PTP/DSP signature motif. Therefore, FYVE-DSP1 and myotubularin form a distinct subfamily of DSPs. Unlike the common PTPs which have a conserved stretch catalytic domain of ~230 amino acid residue with several highly conserved regions including the HCSAGXGRXG signature motif and the WPD loop, DSPs seem to have very limited sequence identity outside the signature motif (2–6). The enzymes acting on the MAP kinase superfamily forms so far the largest subfamily of DSPs. These include VHR (37), CL100 (MKP-1) (38, 39), PAC1 (40), MKP-2 (hVH2, TYP-1) (41), hVH3 (B23) (42), hVH5 (M3/6) (43), MKP-3 (Pyst1, rVH6) (44, 45), Pyst2 (46), and MKP-4 (Pyst3) (47), MKP-5 (48). They share over 35% sequence identity in their catalytic domains. PTEN and Cdi-1 make up of another subgroup of DSPs (49). PTEN has been shown to dephosphorylate both protein and phosphoinositide substrates (50, 51), and more importantly, it acts as a tumor suppressor (52) Myotubularin has been shown to be a DSP and is responsible for X-linked recessive myotubular myopathy (MTM1) which is characterized by severe hypotonia and generalized muscle weakness with impaired maturation of muscle fibers (22). The important role of FYVE-DSP1 as a DSP is to be explored.

A distinct structural feature of FYVE-DSP1 is that it has a FYVE domain at the C-terminus. FYVE domain is a recently described double zinc finger motif which specifically binds phosphatidylinositol(3)-phosphate (9). Phosphoinositides phosphorylated at the 3 position of inositol plays key roles in receptor signaling at the plasma membrane and in membrane trafficking within the cell (53–56). Phosphatidylinositol (3,4)bisphosphate [$PI(3,4)P_2$] and phosphatidylinositol (3,4,5)trisphosphate (PIP_3) produced by class I and II phosphoinositide 3-kinases (PI3Ks) and type II phosphatidylinositol phosphate kinases (PIPKs) (54). They are best known for their roles in receptor signaling by activating the protein kinases PDK1 and PKB through binding to the pleckstrin homology (PH) domains of the kinases (53, 55). Phosphatidylinositol 3-phosphate (PI3P), which is the product of Vps34p and related class III PI3Ks (54), have a pivotal role in regulating membrane trafficking (56). PI3P has also been shown to play a role in signal transduction in insulin regulation of adipocytes (23) and in action of TGF-β (24). FYVE domain is the principal cellular receptors for the singly phosphorylated PI, PI3P (9, 11). Specific binding of PI3P by FYVE domains has recently been demonstrated in many studies and was shown to be important for the correct targeting and functions of the FYVE domain-containing proteins (reviewed in 9). The structural feature of FYVE-DSP1 suggests that it is a target of PI3P. Activation of PI3K accompanied by production of 3-phosphorylated phosphoinositides consists of a major signaling pathway. Protein phosphorylation on Ser/Thr as well on Tyr is a major event in the process. Undoubtedly, as a DSP, FYVE-DSP1 should have a crucial role in regulating the process.

Most of the previously described DSPs have been shown localized in cytosol and/or in the nucleus. For example, CL100/MKP-1, PAC1, MKP-2/hVH2/TYP-1, and hVH3/B23 localize in the nucleus (38–42), whereas MKP-3/Pyst1/rVH6, Pyst2, and MKP-4/Pyst3 localize in the cytosol (43–47). MKP-5 localized both in the cytosol and the nucleus (48). These enzymes have distinct substrate specificity, tissue distributions, and response to different stimuli. Our study showed that FYVE-DSP1 is distributed in both cytosolic and membrane fractions. This makes FYVE-DSP1 a unique player that may have an important role on cell membranes. The membrane localization is presumably attributable to the FYVE domain that potentially binds phosphoinositides. While MPK family DSPs seem to act on the MAP kinase superfamily, its FYVE domain naturally links FYVE-DSP1 to the PI3K signaling pathway.

REFERENCES

1. Hunter, T. (1995) *Cell* **80,** 225–236.
2. Fischer, E. H., Charbonneau, H., and Tonks, N. K. (1991) *Science* **253,** 401–406.
3. Walton, K. M., and Dixon, J. E. (1993) *Annu. Rev. Biochem.* **62,** 101–120.
4. Tonks, N. K., and Neel, B. G. (1996) *Cell* **87,** 365–368.
5. Fauman, E. B., and Saper, M. A. (1996) *Trends Biochem. Sci.* **21,** 413–417.
6. Martell, K. J., Angelotti, T, and Ullrich, A. (1998) *Mol. Cells* **8,** 2–11.
7. Guan, K. L., Broyles, S. S., and Dixon, J. E. (1991) *Nature* **350.** 359–362.
8. Mauro, L. J., and Dixon, J. E. (1994) *Trends Biochem. Sci.* **19,** 151–155.
9. Wurmser, A. E., Gary, J. D., and Emr, S. D. (1999) *J. Biol. Chem.* **274,** 9129–9132.
10. Misra, S., and Hurley, J. H. (1999) *Cell* **97,** 657–666.

11. Stenmark, H., Aasland, R., Toh, B. H., and D'Arrigo, A. (1996) *J. Biol. Chem*. **271,** 24048–24054.
12. Burd, C. G., and Emr, S. D. (1998) *Mol. Cell* **2,** 157–162
13. Mu, F. T., Callaghan, J. M., Steele-Mortimer, O., Stenmark, H., Parton, R. G., Campbell, P. L., McCluskey, J., Yeo, J. P., Tock, E. P., and Toh, B. H. (1995) *J. Biol. Chem*. **270,** 13503–13511.
14. Patki, V., Virbasius, J., Lane, W. S., Toh, B.-H., Shpetner, H. S., and Corvera, S. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 7326–7330.
15. Simonsen, A., Lippe, R., Christoforidis, S., Gaullier, J.-M., Brech, A., Callaghan, J., Toh, B.-H., Murphy, C., Zerial, M., and Stenmark, H. (1998) *Nature* **394,** 494–498.
16. Christoforidis, S., McBride, H. M., Burgoyne, R. D., and Zerial, M. (1999) *Nature* **397,** 621–626.
17. Burd, C. G., and Emr, S. D. (1998) *Mol. Cell* **2,** 157–162.
18. Zhao, Z., Tan, T., Diltz, C. D., You, M., and Fischer, E. H. (1996) *J. Biol. Chem*. **271,** 22251–22255.
19. Nagase, T., Ishikawa, K., Nakajima, D., Ohira, M., Seki, N., Miyajima, N., Tanaka, A., Kotani, H., Nomura, N., and Ohara, O. (1997) *DNA Res.* **4,** 141–150.
20. Zhao, Z., Bouchard, P., Diltz, C. D., Shen, S. H., and Fischer, E. H. (1993) *J. Biol. Chem*. **268,** 2816–2820.
21. Kozak, M. (1989) *J. Cell. Biol.* **108,** 229–241.
22. Laporte, J., Hu, L. J., Kretz, C., Mandel, J. L., Kioschis, P., Coy, J., Klauck, S. M., Poutska, A., and Dahl, N. (1996) *Nature Genet.* **13,** 175–182.
23. Shisheva, A., Sbrissa, D., and Ikonomov, O. (1999) *Mol. Cell. Biol.* **19,** 623–634.
24. Tsukazaki, T., Chiang, T. A., Davison, A. F., Attisano, L., and Wrana, J. L. (1998) *Cell* **95,** 779–791
25. Goffeau, A., Barrell, B. G., Bussey, H., Davis, R. W., Dujon, B., Feldmann, H., Galibert, F., Hoheisel, J. D., Jacq, C., Johnston, M., Louis, E. J., Mewes, H. W., Murakami, Y., Philippsen, P., Tettelin, H., and Oliver, S. G. (1996) *Science* **274,** 546.
26. Asao, H., Sasaki, Y., Arita, T., Tanaka, N., Endo, K., Kasai, H., Takeshita, T., Endo, Y., Fujita, T., and Sugamura, K. (1997) *J. Biol. Chem*. **272,** 32785–32791.
27. Weisman, L. S., and Wickner, W. (1992) *J. Biol. Chem*. **267,** 618–623.
28. Yamamoto, A., DeWald, D. B., Boronenkov, I. V., Anderson, R. A., Emr, S. D., and Koshland, D. (1995) *Mol. Biol. Cell* **6,** 525–539.
29. Wilson, R. et al. (1994) *Nature* **368,** 32–38.
30. Cui, X., De Vivo, I., Slany, R., Miyamoto, A., Firestein, R., and Cleary, M. L. (1998) *Nature Genet.* **18,** 331–337.
31. Laporte, J., Blondeau, F., Buj-Bello, A., Tentler, D., Kretz, C., Dahl, N., and Mandel, J. L. (1998) *Mol. Genet.* **7,** 1703–1712.
32. Tonks, N. K., Diltz, C. D., and Fischer, E. H. (1988) *J. Biol. Chem*.**263,** 6731–6737.
33. Zhang, Z.-Y., Clemens, J. C., Schubert, H. L., Stuckey, J. A., Fischer, M. W. F., Hume, D. M., Saper, M. A., and Dixon, J. E. (1992) J. Biol. Chem. 267, 23759–23766.
34. Zhao, Z., Larocque, R., Ho, W.-T., Fischer, E. H., and Shen, S.-H. (1994) *J. Biol. Chem*. **269,** 8780–8785.
35. Denu, J. M., and Dixon, J. E. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 5910–5914.
36. Denu, J. M., Zhou, G., Wu, L., Zhao, R., Yuvaniyama, J., Saper, M. A., and Dixon, J. E. (1995) *J. Biol. Chem*. **270,** 3796–3803.
37. Ishibashi, T., Bottaro, D. P., Chan, A., Miki, T., and Aaronson, S. A. (1993*) Proc. Natl. Acad. Sci. USA* **89,** 12170–12174.
38. Keyse, S. M., and Emslie, E. A. (1992) *Nature* **359,** 644–646.
39. Sun, H., Charles, C. H., Lau, L. F., and Tonks, N. K. (1993) *Cell* **75,** 487–493.
40. Rohan, P. J., Davis, P., Moskaluk, C. A., Kearns, M., Krutzsch, H., Siebenlist, U., and Kelly, K. (1993) *Science* **259,** 1763–1766.
41. Guan, K.-L., and Butch, E. (1995) *J. Biol. Chem*. **270,** 7197–7203.
42. Kwak, S. P, and Dixon, J. E. (1995) *J. Biol. Chem*. **270,** 1156–1160.
43. Martell, K. J., Seasholtz, A. F., Kwak, S. P., Clemens, K. K., and Dixon, J. E. (1995) *J. Neurochem*. **65,** 1823–1833.
44. Muda, M., Boschert, U., Dickinson, R., Martinou, J.-C., Martinou, I., Camps, M., Schlegel, W., and Arkinstall, S. (1996) *J. Biol. Chem*. **271,** 4319–4326.
45. Mourey, R. J., Vega, Q. C., Campbell, J. S., Wenderoth, M. P., Hauschka, S. D., Krebs, E. G., and Dixon, J. E. (1996) *J. Biol. Chem*. **271,** 3795–3802.
46. Dowd, S., Sneddon, A. A., and Keyse, S. M. (1997) *J. Cell Sci.* **111,** 3389–3399
47. Muda, M., Boschert, U., Smith, A., Antonsson, B., Gillierson, C., Chabert, C., Camps, M., Martinou, I., Ashworth, A., and Arkinstall, S. (1997) *J. Biol. Chem*. **272,** 5141–5151.
48. Tanoue, T., Moriguchi, T., and Nishida, E. (1999) *J. Biol. Chem*. **274,** 19949–19956.
49. Van Huijsduijnen, R. H. (1998) *Gene* **225,** 1–8.
50. Maehama, T., and Dixon, J. E. (1998) *J. Biol. Chem*. **273,** 13375–13378.
51. Li, L., Ernsting, B. R., Wishart, M. J., Lohse, D. L., and Dixon, J. E. (1997). *J. Biol. Chem*. **272,** 29403–29406.
52. Cantley, L. C., and Neel, B. G. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 4240–4245.
53. Toker, A., and Cantley, L. C. (1997) *Nature* **387,** 673–676.
54. Fruman, D. A., Meyers, R. E., and Cantley, L. C. (1998). *Annu. Rev. Biochem*. **67,** 481–507.
55. Corvera, S., and Czech, M. P. (1998). *Trends Cell Biol.* **8,** 442–446.
56. De Camilli, P., Emr, S. D., McPherson, P. S., and Novick, P. (1996) *Science* **271,** 1533–1539.