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Antimicrobial, Dehydroascorbate Reductase, and Monodehydroascorbate Reductase Activities of Defensin from Sweet Potato [*Ipomoea batatas* (L.) Lam. 'Tainong 57'] Storage Roots

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A cDNA encoding a small cysteine-rich protein designated defensin (SPD1) was isolated from sweet potato storage roots. On the basis of the amino acid sequence similarity and conserved residues, it is suggested that SPD1 is a member of the plant defensin family. Recombinant SPD1 protein overproduced in *Escherichia coli* was purified by Ni^{2+}-chelated affinity chromatography. A recombinant protein from the storage root cDNA clone effectively inhibited the trypsin activity in a dose-dependent manner. Both the corresponding mRNA and protein level were found to be highest in the storage roots, followed by sprout. SPD1 reduced dehydroascorbate (DHA) in the presence of glutathione to regenerate L-ascorbic acid (AsA). However, without glutathione, SPD1 has very low DHA reductase activity, and AsA was oxidized by AsA oxidase to generate monodehydroascorbate (MDA) free radical. MDA was also reduced by SPD1 to AsA in the presence of NADH, mimicking the MDA reductase catalyzed reaction. These data suggest that SPD1 has both DHA reductase and MDA reductase activities. SPD1 was also shown to inhibit the growth of both fungi and bacteria. SPD1 is apparently the first reported plant defensin exhibiting DHA and MDA activities in vitro.

KEYWORDS: Sweet potato; defensin; gene expression; antimicrobial activity; dehydroascorbate reductase activity; monodehydroascorbate reductase activity

INTRODUCTION

Plant defensins have been shown to be major constituents of the immune systems of plants. This class of cysteine-rich peptides is usually basic and contains 70–100 amino acids with 4 conserved disulfide bridges (*1*). The diverse biological functions of plant defensins include antifungal activities (*1–3*), inhibition of insect gut α-amylases (*4*) and bovine trypsin (*5*), inhibition of protein synthesis (*6–9*), antibacterial activities (*8, 10*), blockage of the sodium channel (*11*), and a sweet taste (*12*).

Plant defensins were originally termed γ-thionins because they have a similar size (5 kDa) and the same number of disulfide bridges (four) as α- and β-thionins. γ-Thionins are structurally different from α- and β-thionins and, instead, rather similar to insect and mammalian defensins, structurally as well as functionally. Thus, this class of plant peptides was named "plant defensins" (*1*). A variety of plant defensins have been isolated and characterized from many plant species including monocots and dicots (*13–15*). Plant defensin families have been known as potent growth inhibitors of a broad spectrum of fungi and bacteria; however, the antimicrobial activity of the plant defensins has been quite diverse and was classified into two main groups (A and B) sharing only 25% similarity based on amino acid sequence (*16*). Plant defensins have been detected in different organs of plants such as leaves (*17*), flowers (*18*), seeds (*8, 19*), and tubers (*10*). Furthermore, the expression of some defensin genes is developmentally regulated (*15*), whereas that of others is greatly elevated in response to biotic and abiotic external stimuli (*17, 1*). This paper describes cloning, characterization, and biological activities of defensin (SPD1) from sweet potato storage roots.

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MATERIALS AND METHODS

Materials. Fresh storage roots of sweet potato [*Ipomoea batatas* (L.) Lam. 'Tainong 57'] were purchased from a local market. After cleaning with water, the roots were placed in a thermostated (28 °C) growth chamber and sprayed with water twice a day. Sprouted plants were cultivated in the greenhouse to collect roots, stems, fully expanded green leaves, and flowers for experiments. Three pathogenic fungi, *Cladosporium colocasiae*, *Fusarium decemcellulare*, and *Botrytis cinereus*, were kindly provided by Professor Teng-Yung Feng, Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan. Four food pathogenic bacteria, *Escherichia coli* (ATCC 11229, BCRC 11549), *Salmonella enteritidis* (ATCC 13076, BCRC 10744), *Staphylococcus aureus* subsp. *aureu* (BCRC 12652), and *Vibrio vulnificus* (BCRC 15430), were kindly provided by Professor Shann-Tzong Jiang, Department of Food Science, National Taiwan University, Taipei, Taiwan. Dehydroascorbate, ascorbate reductase, ascorbate oxidase, anti-actin (plant) antibody, and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

PCR-Based Subtractive Hybridization and RACE PCR. Total RNA was isolated separately from the storage roots and sprouts of roots of sweet potato according to the method of Sambrook et al. (*20*). Then, mRNA was purified with a purification kit (Promega) and used for the differentially expressed first-strand cDNA synthesis using a PCR-based subtractive hybridization kit (Clontech) following the protocol supplied by the manufacturer. The double-strand cDNAs of the storage roots were subtracted with the sprouts of roots and then ligated to the pGEM-T easy vector for *E. coli* DH5α competent cell transformation. Recombinant plasmids were isolated for DNA sequencing using the ABI PRIZM 337 DNA Sequencer. Nucleotide sequence data were analyzed using the Genetics Computer Group (GCG) programs. Full-length cDNA clone was obtained by performing 5′ and 3′ RACE (5′ and 3′ rapid amplification of cDNA ends) using the Marathon cDNA amplification kit (Clontech) according to the manufacturer's instructions. The gene-specific primer (5′-CCGCT G TGTG TGTAT GTGTG GTGTG T-3′) was used to amplify the double-strand cDNAs.

Expression of Defensin in *E. coli*. Defensin with its pro-sequence (SPD1) was expressed in *E. coli*. The coding sequence was amplified from cDNA SPD1 using an oligonucleotide (5′-AGGAT CCATG GCTTC ATCTC TTCGT TC-3′), with a *Bam*HI site (underlined) at the putative initial Met residue, and an oligonucleotide (5′-GCCTT GCTAA TTCAG TCGAC CGCTG T-3′), with a *Sal*I site at the 3′ end. The PCR fragment was subcloned in pGEM T-easy vector. The plasmid was then digested with *Bam*HI and *Sal*I, and the excised fragments were subcloned in pQE30 expression vector (QIAexpress expression system, Qiagen). The resulting plasmid, termed pQE-SPD1, was introduced into *E. coli* (M15). Cultures of the transformed *E. coli* (M15) overexpressed a protein of the expected molecular mass, which was purified by affinity chromatography in Ni-nitrilotriacetic acid (NTA) columns (Qiagen), according to the manufacturer's instructions. Proteins from *E. coli* carrying empty pQE30 vector and run through the same affinity column described above without binding were used as negative controls.

RNA Isolation and Northern Blot Analysis. Total RNA was extracted from different tissues of sweet potato with TRIzol reagents kit (Invitrogen) according to the manufacturer's instructions. For Northern blotting, 10 μg of total RNA isolated from storage roots, sprouts, sprouted roots, veins, fully expanded green leaves, and flowers were applied to a formaldehyde denaturing gel and then transferred to an Amersham Hybond-N nylon membrane after electrophoresis, according to the method of Sambrook et al. (*20*). The filter was hybridized sequentially with α-^{32}P-labeled defensin full-length cDNA. The procedures for hybridization and autoradiography were according to those of Sambrook et al. (*20*). Visualization of hybridization bands was carried out using X-ray film (Kodak).

Protein Extraction and Electroblotting Analysis of Defensin. All steps were carried out at 4–8 °C. Fully expanded green leaves, mature flower, sprouted roots, veins, and storage roots were cleaned and air-dried and homogenized with 4 volumes (v/w) of 50 mM Tris-HCl buffer (pH 7.5) in a Polytron homogenizer (Luzern, Swiss). The homogenate was filtered through two layers of cheesecloth and then centrifuged in a Sorvall RC-2B with an SS-34 rotor at 10000*g* for 20 min. The protein concentration of the supernatant was determined according to the Bradford dye-binding assay (Bio-Rad, Hercules, CA). The supernatant was saved for electroblotting. The crude extract was subjected to 15% SDS-PAGE according to the method of Laemmli (*21*). After electrophoresis, gels were equilibrated in transfer buffer [25 mM Tris-HCl, pH 8.3, 150 mM glycine, and 10% (w/v) methanol]. The separated proteins were transferred to an Immobilon PVDF membrane (Millipore, Bedford, MA) in transfer buffer at pH 8.3 for 1 h at 100 V. Membranes were blocked for 2 h at room temperature in 5% nonfat dry milk powder and then incubated with polyclonal antibody as the primary antibody against SPD1. The primary antibody was obtained from mouse antiserum. After incubation, membranes were washed in phosphate-buffered saline (PBS) with 0.05% Tween (PBST) three times, 10 min each, then incubated with anti-mouse alkaline phosphatase-conjugated antibody, washed in PBST three times, 10 min each, and developed using NBT (nitroblue tetrazolium)/BCIP (5-bromo-4-chloro- 3-indolylphosphate) (Sigma). The secondary antibody (goat against mouse Fc portion of Ig) was a product of Sigma.

Production of Polyclonal Antibody and Western Blot Hybridization. Expressed SPD1 protein was cut from the 15% polyacrylamide gel, eluted, and mixed with the appropriate amount of pH 7.5 PBS containing 0.1% SDS. The eluted protein was precipitated with acetone containing 10% trichloroacetic acid (TCA) at –20 °C for 2 h. After centrifugation at 13000*g* for 20 min, the pellet was washed with acetone twice and then dried at room temperature. The acetone powder was redissolved in a small amount of PBS containing 0.1% SDS and used as antigen for subcutaneous injections of mouse to prepare the first antibody. The second antigen (goat against mouse Fc portion of Ig) was a product of Sigma. Polyclonal antibodies obtained from mouse antiserum were utilized for Western blot hybridization to study the gene expression of SPD1 in different tissues of sweet potato.

Trypsin Inhibitor Activity (TIA) Assay. TIA was assayed according to the method of Lin and Chen (*22*) using *N*-benzoyl-L-arginine-*p*-nitroanilide (BAPA) as a substrate. In a total volume of 200 μL, different amounts of TI (100 mM Tris-HCl buffer, pH 7.9) were preincubated with trypsin from bovine pancreas (0.15 nmol in 1 mM HCl) at 25 °C for 15 min, and 800 μL of BAPA (0.25 mM BAPA, 100 mM Tris-HCl buffer, pH 7.9) was added for an additional 20 min. The absorbance at 405 nm was determined. Three determinations were averaged for TIA and expressed as micrograms of trypsin inhibited. Statistical significance of the data was tested using a commercial SAS program (SAS 8.0, Cary, NC).

Bioassay of the Defensin (SPD1). Antifungal and antibacterial activities were assessed against several phytopathogenic fungal strains and food pathogenic bacterial strains using previously described methods (*23, 24*). Bacterial cell suspension, fungal spores, or mycelial fragments were used to inoculate the cultures. Absorbance was measured at 595 nm with a microplate reader after an appropriate incubation period.

DHA Reductase Activity Assay. The DHA reductase activity of SPD1 was assayed according to the method of Huang et al. (*25*), with some modifications. Ten milligrams of DHA was dissolved in 5.0 mL of 100 mM phosphate buffer with pH 6.0 or 7.0. The reaction was carried out at 30 °C by adding 100 μL of SPD1 solution (100 μg of protein) to 0.9 mL of DHA solution with or without 4 mM glutathione. The increase of absorbance at 265 nm was recorded for 5 min. Nonenzymatic reduction of DHA in phosphate buffer was measured in a separate cuvette at the same time. A standard curve was plotted using 0–50 nmol of AsA. SPD1 solution was replaced with empty pQE30 vector proteins for negative controls.

Monodehydroascorbate (MDA) Reductase Activity Assay. The MDA reductase activity of SPD1 was assayed according to the method of Huang et al. (*26*), by following the decrease in absorbance at 340 nm due to NADH oxidation. MDA free radicals were generated by AsA oxidase (EC 1.10.3.3) in the assay system (*27*). The reaction mixtures contained 50 mM phosphate buffer (pH 6.0 and 7.0), 0.33 mM NADH, 3 mM AsA, AsA oxidase (0.9 unit), and 200 μL of SPD1 solution (200 μg of protein) in a final volume of 1 mL. SPD1 solution was replaced with empty pQE30 vector proteins for negative controls. The concentration of MDA was determined from the absorbance at

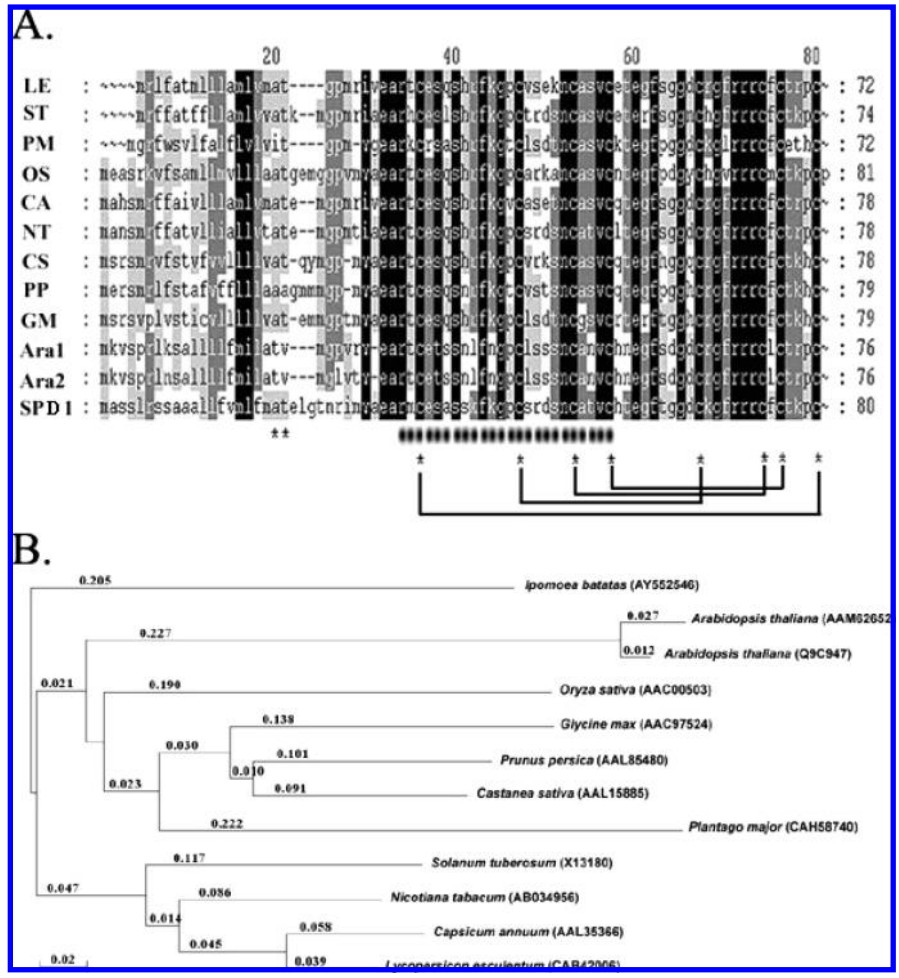

Figure 1. Multiple alignments of plant defensin proteins. (**A**) The sequences are from *Lycopersicon esculentum* γ-thionin protein (LE, CAB42006), *Solanum tuberosum* proteinase inhibitor (ST, X13180), *Plantago major* defensin protein (PM, CAH58740), *Oryza sativa* proteinase inhibitor (OS, AAC00503), *Capsicum annuum* defensin protein precursor (CA, AAL35366), *Nicotiana tabacum* thionin-like protein (NT, AB034956), *Castanea sativa* putative γ-thionin protein (CS, AAL15885), *Prunus persica* defensin protein 1 (PP, AAL85480), *Glycine max* protease inhibitor (GM, AAC97524), *Arabidopsis thaliana* putative low molecular weight cysteine-rich protein LCR66 precursor (Ara1, Q9C947), *A. thaliana* protease inhibitor II (Ara2, AAM62652), and *Ipomoea batatas* defensin (SPD1, AY552546). The conserved cysteine residues are marked. The proteins were aligned using the GCG program. Black shading indicates the same amino acid at that position among all sequences. Gray shading shows those amino acids with similar side-chain properties. The numbers at the end of the right-hand side of each line stand for the cumulative total number of amino acids in each line of each γ-thionin sequence. The numbers above all sequences stand for the positions of the amino acids within individual proteins corresponding to the numbering system of *I. batatas* defensin (AY552546). (**B**) Phylogenetic analysis of defensins based on their amino acid sequences.

340 nm, assuming an absorbance coefficient of 3.3 mM^{-1} cm^{-1}. One unit of MDA reductase was defined as the amount of enzyme required to oxidize 1 μmol of NADH min^{-1}.

Statistical Analysis. Means of triplicates were measured. Student's *t* test was used for comparison between two treatments. A difference was considered to be statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

Isolation and Nucleotide Sequence of Defensin cDNA Clones from Sweet Potato Storage Roots. Defensin cDNA clones from sweet potato storage roots were isolated. We have completed the sequencing of the clones, which were named SPD1 (GenBank accession no. AY552546). The open reading frame in this cDNA encodes a pro-protein of 80 amino acids with a predicted molecular mass of 8643 Da (p*I* 8.93). A comparison of the deduced amino acid sequence of SPD1 with other precursor proteins indicates 75% identity. **Figure 1A** shows a multiple alignment of the sweet potato SPSPD1 proteins and other homologous plant precursor proteinase inhibitor and defensin proteins available in GenBank.

A BLAST search in the GenBank protein database suggested that the predicted amino acid sequence has considerable homology to plant proteinase inhibitor proteins, defensins or γ-thionins, that are representative plant antifungal/antibacterial polypeptides (**Figure 1A**). The eight cysteine residues of SPD1 forming four disulfide bridges were determined as Cys36−Cys80, Cys47−Cys67, Cys53−Cys74, and Cys57−Cys76. The amino acid sequences of the SPD1 share a high degree of similarity with the related plant γ-thionins. Although the SPD1 sequence was rather divergent from other defensins (**Figure 1B**), most residues were conserved as in other plant defensins. These results indicate that the structural similarities among the different γ-thionins extend far beyond the primary structure and possibly concern the secondary structure and the general folding of the entire γ-thionin family. These characteristics suggested that

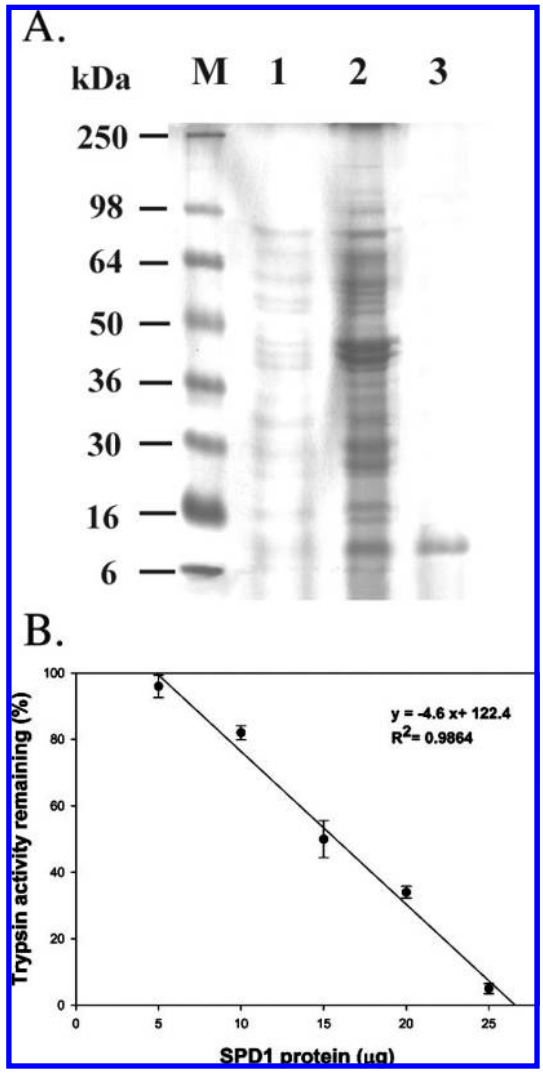

Figure 2. Purified recombinant sweet potato defensin. (**A**) 15% SDS-PAGE analysis. Crude extracts (5 μg of protein) from *E. coli* (M15) transformed with pQE30 (lane 1) or with pQE30-SPD1 (lane 2) were analyzed by 15% (w/v) SDS-PAGE, and then the gels were stained with Coomassie brilliant blue G-250. Molecular masses of standard proteins are indicated at the left of the figure. His-tagged SPD1 was purified by Ni^{2+}-chelated affinity chromatography (lane 3). (**B**) Trypsin inhibitor activity analysis. The experiments were done twice, and a representative one is shown. "M" indicates the blue prestained markers for SDS-PAGE.

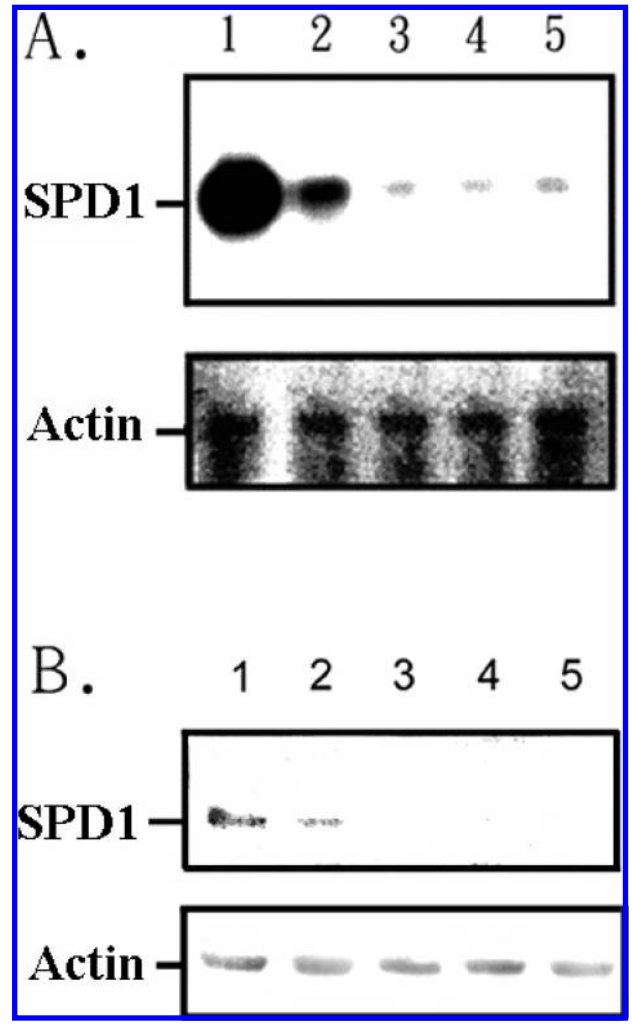

Figure 3. Northern (**A**) and Western (**B**) blot detections of sweet potato defensin gene. (**A**) Samples (10 μg each) of total RNA were isolated from different tissues of sweet potato, and actin (AY905538) was utilized as an internal control of mRNA from sweet potato. Blots were hybridized to α-^{32}P-labeled 3′ specific cDNA probes. (**B**) Ten micrograms of crude extracted proteins from sweet potato was analyzed by 15% (w/v) SDS-PAGE, and then the gels were transferred onto PVDF membranes that were probed with a 1:1000 (v/v) dilution of mouse antibodies raised against SPD1 using goat anti-mouse alkaline phosphatase as the secondary antibody. Lanes: 1, storage roots; 2, sprout; 3, veins; 4, sprouted roots; 5, fully expanded green leaves. Actin was used as a control. The experiments were done twice, and a representative one is shown.

SPD1 encodes a plant defensin polypeptide and that SPD1 was a novel defensin.

A putative cleavage site to the hydrophobic N-terminal signal peptide for targeting to ER was predicted between Ala-20 and Thr-21 with the highest probability, which required further investigation (*28, 29*).

Expression of Defensin in *E. coli*. SDS-PAGE analysis of SPD1 crude extracts from the transformed *E. coli* (M15) showed high amounts of a polypeptide with the expected molecular mass (ca. 9 kDa) (**Figure 2**). This polypeptide was found as a soluble protein in the supernatant (**Figure 2A**, lane 2) and was absent in protein extracts obtained from *E. coli* transformed with pQE-30 vector (**Figure 2A**, lane 1). The expressed protein was highly purified from crude extracts as His-tagged SPD1 (**Figure 3A**, lane 3). According to Huang et al. (*29*), this polypeptide was analyzed by trypsin inhibitor activity assay. As shown in **Figure 2B**, the SPD1 protein inhibited the trypsin activity. These results suggested that the SPD1 has a weak trypsin inhibitor activity in a dose-dependent manner (25 μg of SPD1 protein was needed to inhibit 4 μg of trypsin). The mechanism of weak trypsin inhibitor activity of SPD1 remains to be established in further studies.

Defensin mRNA and Protein Levels Were Developmentally Regulated. The presence and amounts of different sweet potato *SPD1* mRNAs were examined in various organs and tissues by Northern blot analysis. **Figure 3A** shows that *SPD1* probe hybridized to mRNA species of approximately 0.5 kb. SPD1 mRNA levels were the highest in the storage roots, followed by that in sprouts, whereas they were lowest in sprouted roots, vein, and fully expanded green leaves. Western blot hybridization using SPD1 polyclonal antibody from mouse antiserum was used for the gene expression analysis of SPD1 in crude extracts from different sweet potato tissues (**Figure**

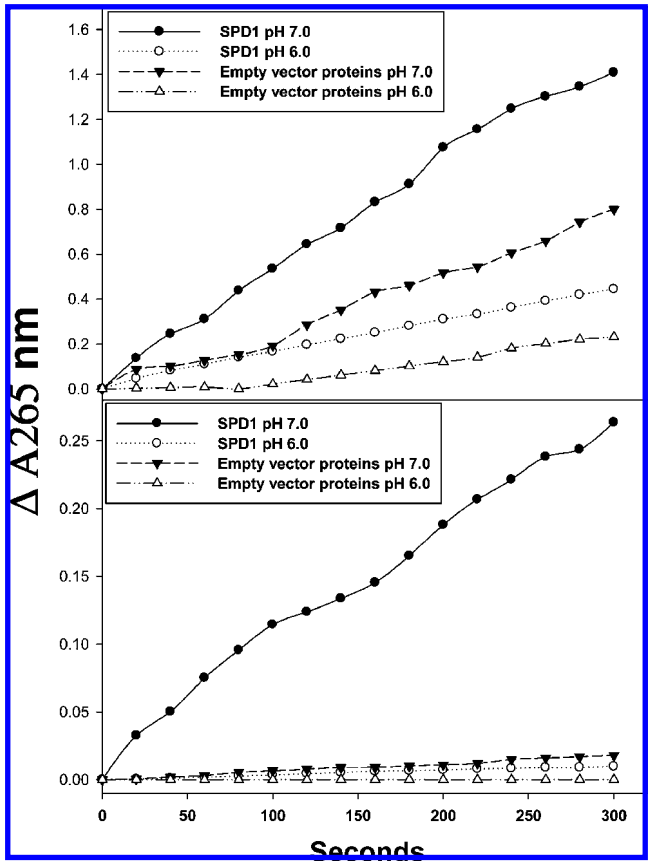

Figure 4. Effect of pH (6.0 and 7.0) on dehydroascorbate reductase activity. Purified recombinant protein of SPD1 was with (**A**) or without (**B**) 4 mM glutathione in the reaction mixtures. The reaction was carried out at 30 °C by adding 100 μL of SPD1 solution (100 μg of protein, 100 mM phosphate buffer, pH 7.0 and 6.0) to 0.9 mL of DHA solution with or without 4 mM glutathione. The increase of absorbance at 265 nm was recorded for 5 min. Proteins from *E. coli* carrying empty pQE30 vector and run through the same affinity column [used for transformed *E. coli* (M15)] without binding were used as negative controls (100 μg of protein each). The experiments were done twice, and a representative one is shown.

3B). SPD1 levels were highest in the storage roots followed by sprouts. No signal at all was found in sprouted root, vein, and fully expanded green leaves. Actin was used as a control.

Effect of pH (6.0 and 7.0) on Dehydroascorbate Reductase Activity of SPD1. The purified SPD1 were used to examine DHA reductase activity. **Figure 4** shows AsA regeneration (ΔA = 265 nm) from DHA at both pH 6.0 and 7.0 with (**A**) or without (**B**) glutathione. **Figure 4A** shows that SPD1 exhibited DHA reductase activity and could reduce DHA back to AsA. The specific activities of DHA reductase for SPD1 in the presence of glutathione were 5.68 and 26.32 nmol of AsA produced/min/mg of protein at pH 6.0 and 7.0, respectively. However, in the absence of glutathione, very low DHA reductase activities of SPD1 were found (**Figure 4B**): only 0.065 and 3.42 nmol of AsA produced/min/mg of protein at pH 6.0 and 7.0, respectively. SPD1 acts as a GSH-dependent DHA reductase (**Figure 5**), and the rate of reduction was closely proportional to the concentration of GSH. Expression of DHA reductase in plant, responsible for regenerating AsA from an oxidized state, regulates the cellular AsA redox state, which in turn affects cell responsiveness and tolerance to environmental reactive oxygen species. Because of its role in AsA recycling, we examined whether DHA reductase is important for plant growth (*30*).

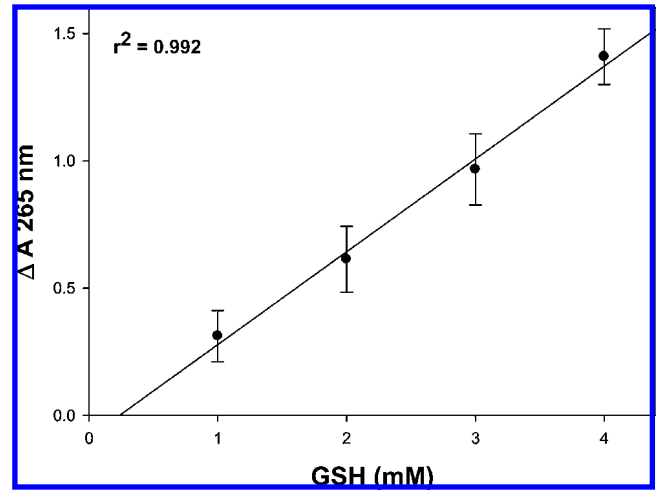

Figure 5. Dependence of dehydroascorbate reductase activity of SPD1 on GSH concentration. The reaction was carried out at 30 °C by adding 100 μL of SPD1 solution (100 μg of protein, 100 mM phosphate buffer, pH 7.0) to 0.9 mL of DHA solution with different concentrations of glutathione. The increase of absorbance at 265 nm was recorded for 5 min. Each data point shows the mean ± SD of one experiment performed in triplicate.

Table 1. N-Terminal Sequences Compared between DHA Reductase, Kunitz-Type Protease Inhibitor Homologue (AAB32051), and Defensin (SPD1) (SPD1, AY552546)

protein	N-terminal (partial) sequence
DHA reductase and Kunitz-type protease inhibitor homologue	**FV**LDNEGNP**L**ENGGTY
SPDI	**FV**MLFMATE**L**GTNRIM

There are two possible reasons to explain the apparent low DHAR activity of SPD1. Trümper et al. (*31*) reported that oxidized soybean trypsin inhibitors (native) had no DHAR activity but reduced form (with GSH presence) had a DHAR activity of about 250 nmol/min/mg of protein. The partial, yet not quantitative, mutual conversion between the two states in vitro remains incomplete in the absence of catalysts (e.g., protein disulfide isomerase). Therefore, SPD1 may be less reduced by GSH resulting in low DHAR activity compared to other DHARs. The second possibility is that the prosequence may have an inhibitory effect on the DHAR activity of SPD1. The active domains (cysteine regions) remain to be established in future studies (*31*). N-Terminal sequences of DHA reductase and Kunitz-type protease inhibitor homologue (AAB32051) were compared with that of SPD1 (AY552546) by CLUSTALX 1.81 software (**Table 1**). However, the above results already permit a chemically and physiologically sensible rationale in which the same protein functions as DHA reductase in the reduced (thiol) state, a compartment characterized by reducing conditions, and in the oxidized (disulfide) form as protease inhibitor. Whether the other portions of the molecules exhibit sequence homology remains to be established in further studies.

Effect of pH (6.0 and 7.0) on Monodehydroascorbate Reductase Activity of SPD1. MDA was reduced to AsA in coupling with NADH oxidation (ΔA = 340 nm) at pH 6.0, and 7.0 when SPD1 was used as MDA reductase. The SPD1 exhibited MDA reductase activity at both pH 6.0 and 7.0 (**Figure 6**), with higher activity at pH 6.0 than at pH 7.0. Therefore, the specific MDAR activities of SPD1 were 4.65 and 1.86 units/mg of protein at pH 6.0 and 7.0, respectively.

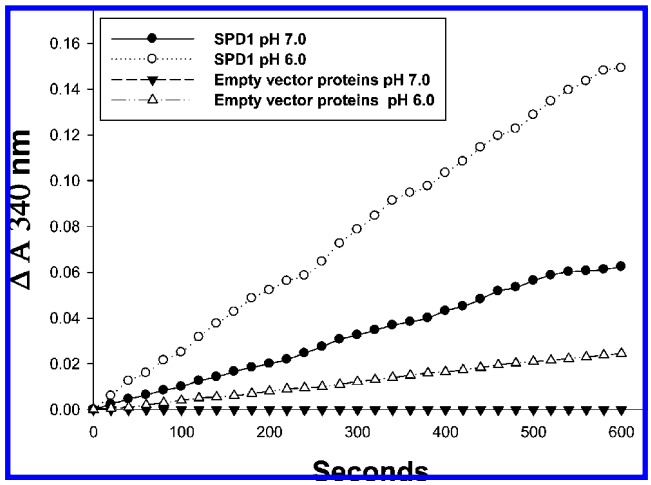

Figure 6. Effect of pH (6.0 and 7.0) on monodehydroascorbate reductase activity of SPD1. The reaction mixtures contained 50 mM phosphate buffer (pH 6.0 and 7.0), 0.33 mM NADH, 3 mM AsA, AsA oxidase (0.9 unit), and 200 μL of SPD1 solution (200 μg of protein) in a final volume of 1 mL. SPD1 solution was replaced with distilled water for blank. Proteins from *E. coli* carrying empty pQE30 vector and run through the same affinity column [used for transformed *E. coli* (M15)] without binding were used as negative controls (200 μg each). The experiments were done twice, and a representative one is shown.

Table 2. Antimicrobial Activity of Defensin (SPD1)

organism	IC_{50}[a] (μg/mL)
fungus[b]	
Cladosporium colocasiae	30.76 ± 0.92
Fusarium decemcellulare	28.56 ± 0.86
Botrytis cinereus	27.28 ± 0.82
bacterium[c]	
Staphylococcus aureus	25.67 ± 0.77
Escherichia coli	>500
Salmonella enteritidis	>500
Vibrio	>500

[a] Concentration of defensin required for 50% inhibition was defined as IC_{50}, which was determined from dose−response curve (percent growth inhibition vs protein concentration). Data show the mean ± SD of one experiment performed in triplicate. [b] Relative growth was expressed as the ratio of OD495 with filter-sterilized SPD1 (0–100 μg/mL in final concentrations) to OD495 without SPD1 after 48 h of incubation at 24 °C. [c] Relative growth was expressed as the ratio of OD595 with filter-sterilized SPD1 (0–500 μg/mL in final concentrations) to OD595 without SPD1 after 16 h of incubation at 30 °C.

Antimicrobial Activity of SPD1. The purified SPD1 was assayed for antifungal and antibacterial activity. The antimicrobial activity was expressed as IC_{50}, which represents the concentration of SPD1 required for 50% inhibition of fungal or bacterial growth. SPD1 was shown to inhibit the growth of both fungi and bacteria (**Table 2**). IC_{50} values of antifungal activity were in the range of 27–31 μg/mL, and the IC_{50} value of antibacterial activity was about 26 μg/mL. Most members of the plant defensin family are either inactive or less active against bacteria. *Vigna angularis* (VaD1) is active against fungi in the 30–53.2 μg/mL range and is also active against bacteria in the range of 36.6–143.4 μg/mL. *Clitoria ternatea* plant defensin (Ct-AMP1) is active against fungi in the 2–20 μg/mL range and is also active against the Gram-positive bacterium *Bacillus subtilis* at 15 μg/mL (*8*). Thus, SPD1, like VaD1 and Ct-AMP1, has both antifungal and antibacterial activities.

Anticrobial activities of plant defensins have been the subject of intensive study. However, little is known about whether defensins also have DHA and MDA activity in vitro. Thus, the new defensin SPD1 cloned and characterized appears to possess antimicrobial activity. Hence, SPD1 is a suitable candidate for transforming plants to improve resistance against microbial diseases. It seems also beneficial for people who consume sweet potato roots.

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