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Patatin, the Tuber Storage Protein of Potato (*Solanum tuberosum* L.), Exhibits Antioxidant Activity in Vitro

YEN-WENN LIU,[†,§] CHUAN-HSIAO HAN,[#,§] MEI-HSIEN LEE,[#] FENG-LIN HSU,[*,#] AND WEN-CHI HOU[*,#]

School of Pharmacy and Graduate Institute of Pharmacognosy, Taipei Medical University, Taipei 110, Taiwan, Republic of China

The potato (*Solanum tuberosum* L.) tuber storage protein, patatin, was purified to homogeneity with a molecular mass of 45 kDa. The purified patatin showed antioxidant or antiradical activity by a series of in vitro tests, including 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (half-inhibition concentration, IC_{50}, was 0.582 mg/mL) scavenging activity assays, anti-human low-density lipoprotein peroxidation tests, and protections against hydroxyl radical-mediated DNA damages and peroxynitrite-mediated dihydrorhodamine 123 oxidations. Using electron paramagnetic resonance (EPR) spectrometry for hydroxyl radical detections, it was found that the intensities of the EPR signal were decreased by the increased amounts of patatin added (IC_{50} was 0.775 mg/mL). Through modifications of patatin by iodoacetamide or *N*-bromosuccinimide, it was found that the antiradical activities of modified patatin against DPPH or hydroxyl radicals were decreased. It was suggested that cysteine and tryptophan residues in patatin might contribute to its antioxidant activities against radicals.

KEYWORDS: Antioxidant; 1,1-diphenyl-2-picrylhydrazyl (DPPH); electron paramagnetic resonance (EPR); hydroxyl radical; *Solanum tuberosum*; storage protein

INTRODUCTION

Active oxygen species (or reactive oxygen species) and free radical-mediated reactions are involved in degenerative or pathological processes such as aging (*1*), cancer, coronary heart disease, and Alzheimer's disease (*2*, *3*). There are several reports concerning natural compounds in fruits and vegetables with regard to their antioxidant activities in vitro, including water extracts of roasted *Cassia tora* (*4*), phenolic compounds (*5*), the storage proteins of sweet potato root (*6*) and yam tuber (*7*), and whey proteins (*8*, *9*).

Patatin is the trivial name given to a family of glycoproteins that make up >40% of the total soluble protein in potato (*Solanum tuberosum*) tubers and serves as a storage protein. It was proved that patatin exhibited both lipid acyl hydrolase and acyltransferase activities, which might be involved in tuber tissue in the response to wounding (*10*). Al-Saikhan et al. (*11*) showed that different potato cultivars exhibited different antioxidant activities, which were higher than those of bell pepper, carrot, and onion. They found that different components in potato, including chlorogenic acid (300 μg/mL), glutathione (100 μg/mL), ascorbic acid (320 μg/mL), quercetin (15 μg/mL), and patatin (33 mg/mL), had antioxidant activities against the coupled oxidation of β-carotene and linoleic acid. In this work we report that purified patatin had different antioxidant activities in comparison with chemicals such as butylated hydroxytoluene (BHT) or reduced glutathione in a series of in vitro tests. We also used chemicals for amino acid side-chain modifications to identify key positions involved in the antioxidant activities of patatin.

MATERIALS AND METHODS

Materials. Tris, 2-thiobarbituric acid (TBA), and electrophoretic reagents were purchased from E. Merck Inc. (Darmstadt, Germany). Peroxynitrite was obtained from Calbiochem-Novabiochem Co. (Darmstadt, Germany). Calf thymus DNA (activated, 25 A_{260} units/mL) was purchased from Amersham Biosciences (Uppsala, Sweden). Hydrogen peroxide (33%) was from Wako Pure Chemicals Industry (Osaka, Japan). BHT, reduced glutathione, dihydrorhodamine 123 (DHR), 1,1-diphenyl-2-picrylhydrazyl (DPPH), human low-density lipoprotein (LDL), iodoacetamide, *N*-bromosuccinimide (NBSI), phenylmethanesulfonyl fluoride (PMSF), 2-deoxyribose, peroxynitrite, and other chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO).

Patatin Extractions and Purifications. Fresh potato (*S. tuberosum*) tubers were purchased from a wholesaler. After washing and peeling, the tubers were cut into strips for patatin extraction and purification. After extraction and centrifugation, patatins were purified from crude extracts successively by a DEAE-Sepharose CL-6B ion exchange column and a Con A affinity column according to the methods of Racusen and Foote (*12*). The eluted fraction was collected and concentrated with Ultrafree-4 (molecular weight cutoff is 5 kDa, Millipore Co., Bedford, MA). The concentrated patatin solution was

* Authors to whom corrrespondence should be addressed at the Graduate Institute of Pharmacognosy, Taipei Medical University, No. 250 Wu-Hsing Street, Taipei 110, Taiwan, ROC [fax 886 (2) 2378-0134; e-mail (W.-C.H.) wchou@tmu.edu.tw or (F.-L.H.) hsu0320@tmu.edu.tw].
† School of Pharmacy.
§ Equal contributions.
# Graduate Institute of Pharmacognosy.

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dialyzed against deionized water overnight and lyophilized for further use.

Protein Staining on SDS-PAGE Gels. Sixteen microliters of patatin solution was mixed with 4 μL of sample buffer, namely, 60 mM Tris-HCl buffer (pH 6.8) containing 2% SDS, 25% glycerol, and 0.1% bromophenol blue without 2-mercaptoethanol, and heated in boiling water for 5 min followed by electrophoresis according to the method of Laemmli (*13*). Coomassie brilliant blue R-250 was used for protein staining (*14*).

Scavenging Activity of DPPH Radical by Spectrophotometry. The scavenging activity of purified patatin against DPPH radical was measured according to the method of Hou et al. (*6*, *7*, *15*). Every 0.3 mL of patatin solution [from 0.1 mg/mL (2.22 nmol) to 0.7 mg/mL (15.54 nmol)] was added to 0.1 mL of 1 M Tris-HCl buffer (pH 7.9) and then mixed with 0.6 mL of 100 μM DPPH in methanol to the final concentrations of 60 μM for 20 min under light protection at room temperature. The absorbance at 517 nm was measured. Deionized water was used as blank experiment, and BHT [from 1 μg/mL (4.54 nmol) to 5 μg/mL (22.7 nmol)] and reduced glutathione [from 1 μg/mL (3.25 nmol) to 12.5 μg/mL (40.68 nmol)] were used as positive controls. The scavenging activity of DPPH radicals (%) was calculated with the equation $(A_{517,\text{blank}} - A_{517,\text{sample}}) \div A_{517,\text{blank}} \times 100\%$. IC_{50} identifies the concentration of half-inhibition.

Scavenging Activity of Hydroxyl Radicals by EPR Spectrometry. The hydroxyl radical was generated by Fenton reaction according to the method of Kohno et al. (*16*). The total 500-μL mixture included 45 kDa patatin (0.194, 0.388, 0.775, and 1.55 mg/mL), 5 mM 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), and 0.05 mM ferrous sulfate. After mixing, the solution was transferred to an EPR quartz cell and placed at the cavity of the EPR spectrometer, and then hydrogen peroxide was added to a final concentration of 0.25 mM. Deionized water was used instead of sample solution for blank experiments. After 40 s, the relative intensity of the signal of the DMPO−OH spin adduct was measured. All EPR spectra were recorded at the ambient temperature (298 K) on a Bruker EMX-6/1 EPR spectrometer equipped with WIN-EPR SimFonia software version 1.2. The conditions of EPR spectrometry were as follows: center field, 345.4 ± 5.0 mT; microwave power, 8 mW (9.416 GHz); modulation amplitude, 5 G; modulation frequency, 100 kHz; time constant, 0.6 s; scan time, 1.5 min.

Modified Patatin against DPPH and Hydroxyl Radicals. The 250 μL of purified patatin (10 mg/mL) was modified by different chemicals as follows. (1) The cysteine residues were alkylated by 10 μL of 200 mM iodoacetamide to the final concentrations of 200 μM (*17*) at Tris buffer (pH 8.3), 37 °C for 2 h, and then dialyzed against deionized water overnight. (2) The tryptophan residues were modified by 0.6 mL of 1 mM NBSI (*18*) in 0.1 M acetate buffer (pH 4.0) to the concentration of 150 μM at room temperature for 1 h and then dialyzed against deionized water overnight. The purified patatin with or without chemical modifications was used for scavenging activity assays against DPPH and hydroxyl radicals described aboved.

Protection against Cu^{2+}-Induced LDL Peroxidation by Patatin. The capacity of purified patatin (0.2−1.2 mg/mL) against Cu^{2+}-induced human LDL oxidation in a total 1.1-mL sample volume was measured by thiobarbituric acid reactive substances (TBARS) assay at a wavelength of 532 nm (*19*). The LDL (0.5 mg of protein/mL) was incubated at 37 °C under air in 10 mM phosphate buffer (pH 7.4) containing 10 μM $CuSO_4$ for 24 h with or without purified patatin. The peroxidation reaction was stopped by adding 100 μM EDTA. The TBARS value of 24-h LDL peroxidation was assumed as 100%. BHT was used as a positive control.

Protection against Hydroxyl Radical-Induced Calf Thymus DNA Damage by Patatin. The hydroxyl radical was generated by Fenton reaction according to the method of Kohno et al. (*16*). The 45-μL reaction mixture included 45 kDa patatin (0.182, 0.364, 0.910, 1.82, and 3.64 mg/mL), 15 μL of calf thymus DNA (25 A_{260} units/mL), 18 mM $FeSO_4$, and 60 mM hydroxygen peroxide at room temperature for 15 or 30 min. Then 10 μL of 1 mM EDTA was added to stop the reaction. Only calf thymus DNA was used for blank test, and the control test was without patatin additions. After agarose electrophoresis, the treated DNA solutions were stained with ethidium bromide and observed under UV light.

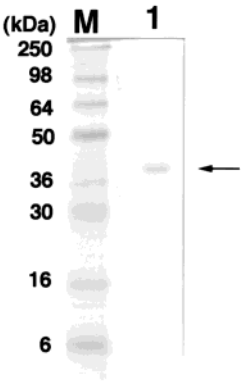

Figure 1. Protein stainings of patatin on an SDS-PAGE gel after ion exchange column and Con A affinity purifications. M indicates the Seeblue prestained markers of SDS-PAGE. Two micrograms of protein was loaded in each well.

Protection against Peroxynitrite-Mediated DHR Oxidation by Patatin. The protection of peroxynitrite-mediated DHR oxidation was according to the methods of Kooy et al. (*20*). The total 180-μL reaction mixture included different amounts of 45 kDa patatin (22.22, 55.56, 111.11, and 277.78 μg/mL), 0.9 mM DHR, and 5 μL of peroxynitrite in 50 mM phosphate buffer (pH 7.4) containing 90 mM NaCl. After 5 min of reaction, the fluorescent intensity was measured at the excitation and emission wavelengths of 500 and 536 nm, respectively, and excitation and emission slit widths of 2.5 and 3.0 nm, respectively. The control test was without patatin additions.

Statistics. 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

Patatin Purification from Potato Tuber. The purity of patatin was determined by an SDS-PAGE gel. **Figure 1** showed the protein stainings of patatin. A single band (lane 1) with a molecular mass of 45 kDa was found. This result was the same as that of Racusen and Foote (*12*). This purified patatin was lyophilized for further investigations.

Antiradical Activity of Patatin on DPPH. The effects of different concentrations of purified patatin or the nanomole basis of purified patatin on the scavenging activities of DPPH radicals with spectrophotometry are shown in **Figure 2**. BHT and reduced glutathione were used as positive controls. It was found that patatin exhibited dose-dependent scavenging activity against DPPH radicals from 0.1 to 0.7 mg/mL for 12−61%, respectively. The IC_{50} for DPPH radical scavenging activity was 0.582 mg/mL (**Figure 2A**). The anti-DPPH radical capacities of purified patatin were about $^1/_{140}$ that of BHT or $^1/_{56}$ that of reduced glutathione; however, under the nanomole basis, the anti-DPPH radical capacities of purified patatin were similar to that of BHT and higher than that of reduced glutathione (**Figure 2B**).

Scavenging Activity of Patatin against Hydroxyl Radical Determined by EPR Spectrometry. The hydroxyl radical was generated by Fenton reaction and was trapped by DMPO to form the DMPO−OH adduct. The intensities of the DMPO−OH spin signal in EPR spectrometry were used to evaluate the scavenging activity of 45 kDa potato patatin against hydroxyl radical. **Figure 3** shows the scavenging activity against the hydroxyl radical with different amounts of 45 kDa patatin: (A) blank, (B−E) 0.194, 0.388, 0.775, and 1.55 mg/mL, respectively, purified patatin. The effect of 45 kDa patatin as a scavenger of hydroxyl radical was evident as decreased intensities of DMPO−OH signals. EPR signals were significantly decreased

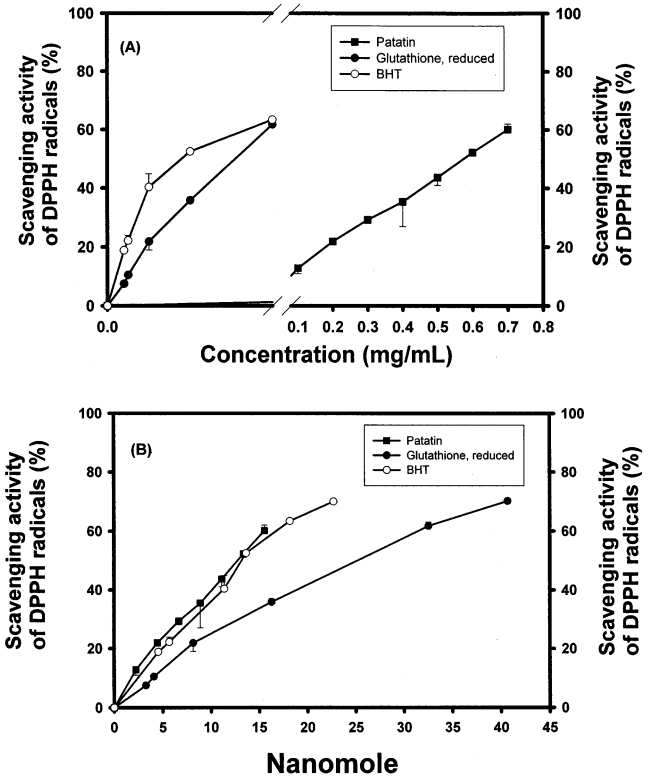

Figure 2. Effects of different concentrations of (A) 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 mg/mL of purified patatin or (B) 2.22, 4.44, 6.66, 8.88, 11.1, 13.32, and 15.54 nmol of purified patatin on the scavenging activities of DPPH radicals with spectrophotometry. BHT [(A) 1, 1.25, 2.5, 3, 4, and 5 μg/mL corresponding to (B) 4.54, 5.67, 11.35, 13.62, 18.16, and 22.7 nmol, respectively] and reduced glutathione [(A) 1, 1.25, 2.5, 5, 10, and 12.5 μg/mL corresponding to (B) 3.25, 4.07, 8.14, 16.27, 32.54, and 40.68 nmol, respectively] were used as positive controls. The scavenging activity of DPPH radicals (%) was calculated with the following equation: $(A_{517,blank} - A_{517,sample}) \div A_{517,blank} \times 100\%$.

by 0.194−1.55 mg/mL of 45 kDa patatin compared to the blank with a positive correlation between two parameters ($r = 0.971$). On the basis of DMPO−OH signal intensities, there were about 23, 39, 50, and 69% reductions, respectively, by 0.194, 0.388, 0.775, and 1.55 mg/mL of patatin compared to controls (**Figure 3B−E**). The IC_{50} for hydroxyl radical scavenging activity was 0.775 mg/mL. **Figure 3** provides the first piece of evidence that potato patatin exhibited scavenging activity against hydroxyl radical as shown by EPR spectrometry.

Scavenging Activity of Modified Patatin against DPPH and Hydroxyl Radicals. Chemicals were used for amino acid side-chain modifications to identify key positions involved in antioxidant activities of patatin. From the results of **Figure 4**, it was found that all modifications could apparently scavenge activities against DPPH and hydroxyl radicals. Yan et al. (*21*) reported that tryptophan exhibited anti-hydroxyl radical activities. Free cysteine residues in whey proteins (*8*, *9*) were also reported to have antioxidant activities. These findings mean that cysteine and tryptophan residues in potato patatin participated in the antiradical activities.

Effect of Purified Patatin on Protecting Cu^{2+}-Induced Human LDL Peroxidation by TBARS Assay. LDL peroxidation has been reported to contribute to atherosclerosis development (*22*). Therefore, delay or prevention of LDL peroxidation is an important function of antioxidants. With the TBARS assay, the degrees of Cu^{2+}-induced human LDL peroxidation could be revealed. From the results of **Figure 5**,

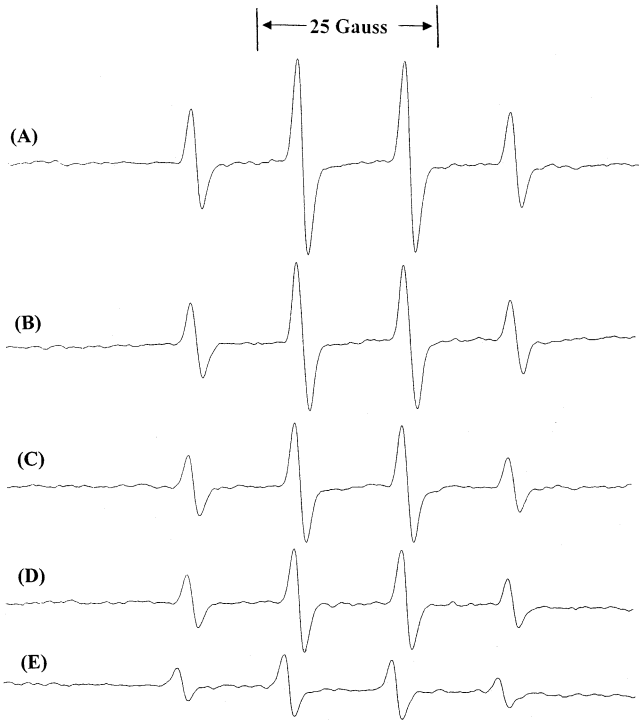

Figure 3. Scavenging activities of purified patatin against hydroxyl radicals by EPR spectrometry: (A) deionized water as a blank; (B) 0.194 mg/mL patatin; (C) 0.388 mg/mL patatin; (D) 0.775 mg/mL patatin; (E) 1.55 mg/mL patatin. The signal intensities of the DMPO-OH adduct were determined.

the protection effect of purified patatin against LDL peroxidation was dose-dependent. The protection capacities of purified patatin were 9, 9, 10, 22.5, 30, and 45% for 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mg/mL, respectively. Significant difference was observed among the oxidized LDL, oxidized LDL + 0.8 mg/mL purified patatin ($p < 0.05$), and oxidized LDL + 1.0 mg/mL purified patatin or 1.2 mg/mL purified patatin ($p < 0.01$).

Protecting Hydroxyl Radical-Induced Calf Thymus DNA Damage by Patatin. Free radicals could damage macromolecules in cells, such as DNA, protein, and lipids in membranes (*23*). **Figure 6** shows that purified patatin protected against hydroxyl radical-induced calf thymus DNA damages. Only calf thymus DNA was used for the blank test, and the control test was without patatin additions. Compared to the blank test and control test, it was found that the added patatin above 0.364 mg/mL could protect against hydroxyl radical induced calf thymus DNA damages during 15-min (**Figure 6A**) or 30-min (**Figure 6B**) reactions.

Protecting Peroxynitrite-Mediated DHR Oxidation by Patatin. Peroxynitrite is formed from nearly diffusion limited reaction between nitric oxide and superoxide and an initiator of potentially harmful oxidation reaction (*24*). From the results of **Figure 7**, it was found that the protective effect of peroxynitrite-mediated DHR oxidation of purified patatin was dose-dependent. The protection capacities of purified patatin were 8.36, 32.78, 41.07, and 46.57% for 22.22, 55.56, 111.11, and 277.78 μg/mL, respectively. Significant difference was observed between the peroxnitrite, peroxnitrite + 22.22 μg/mL purified patatin ($p < 0.05$), and peroxnitrite + 55.56 μg/mL patatin or 111.11 μg/mL patatin or 277.78 μg/mL patatin ($p < 0.01$).

In conclusion, the results from in vitro experiments, including DPPH radical (**Figure 2**) and hydroxyl radical (**Figure 3**) scavenging activity assays, anti-human LDL oxidation analysis (**Figure 5**), protection against hydroxyl radical-induced calf

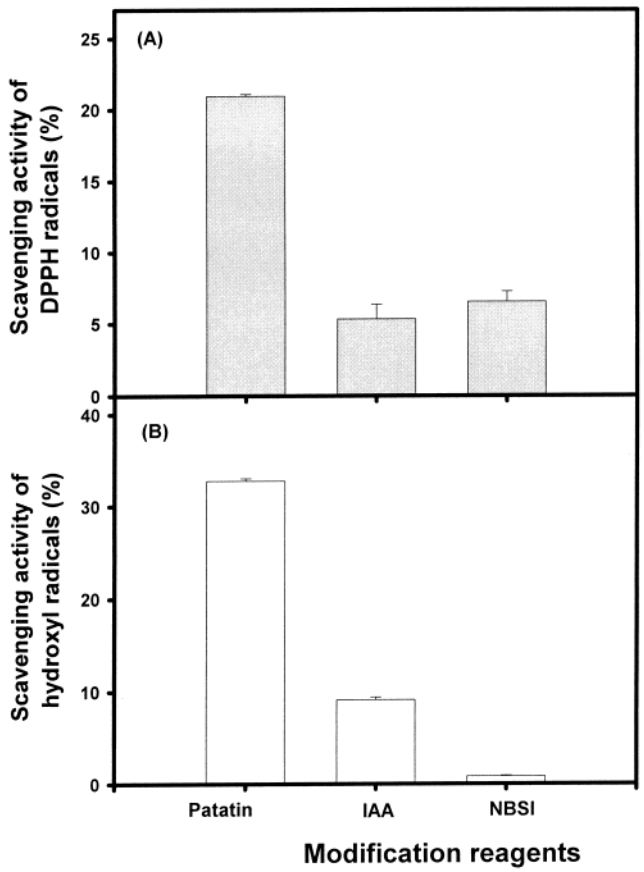

Figure 4. Purified patatin (10 mg/mL) was modified by iodoacetamide or *N*-bromosuccinimide. Patatin with or without chemical modifications was used for scavenging activity assays against DPPH (A) and hydroxyl radicals (B).

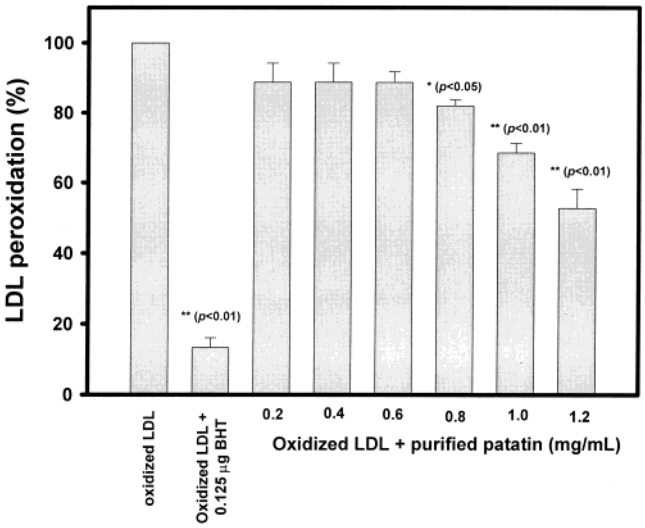

Figure 5. TBARS assay of purified patatin (0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mg/mL) against Cu^{2+}-induced human LDL peroxidation. The TBARS of 24-h LDL peroxidation was recognized as 100%. BHT (0.125 μg) was used as a positive control. Student's *t* test was used for the comparison among the oxidized LDL and other treatments. A difference was considered to be statistically significant when $p < 0.05$ (*) or $p < 0.01$ (**).

thymus DNA damage (**Figure 6**), and protection against peroxynitrite-mediated DHR oxidadtion (**Figure 7**), demonstrated that purified patatin exhibited antioxidant activities. It was also demonstrated that cysteine and tryptophan residues in patatin were involved in antiradical activity against DPPH and

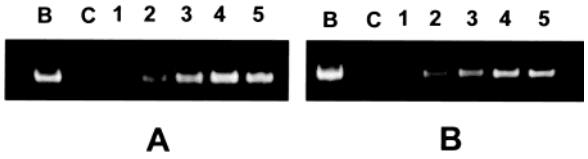

Figure 6. Purified patatin protected against hydroxyl radical-induced calf thymus DNA damage: (A) 15-min reaction; (B) 30-min reaction. Lanes 1–5 were 0.182, 0.364, 0.910, 1.82, and 3.64 mg/mL purified patatin additions. Only calf thymus DNA was used for blank test, and the control test was without patatin additions.

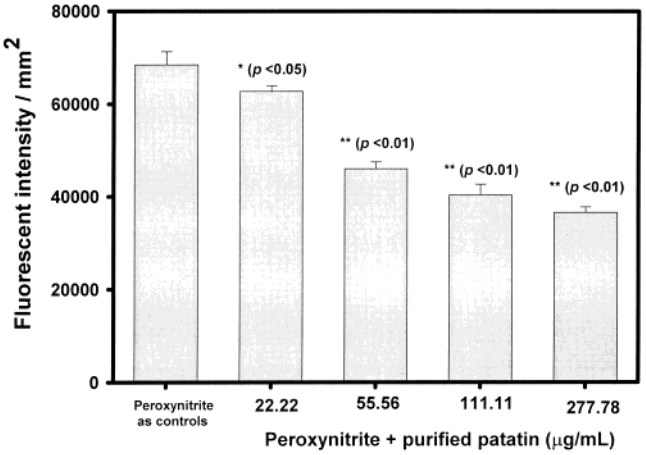

Figure 7. Purified patatin (22.22, 55.56, 111.11, and 277.78 μg/mL) protected against peroxynitrite-mediated dihydrorhodamine 123 oxidation. The total 180-μL reaction mixture included different amounts of purified patatin, 0.9 mM DHR, and 5 μL of peroxynitrite in 50 mM phosphate buffer (pH 7.4) containing 90 mM NaCl. After 5 min of reaction, the fluorescent intensity was measured. The control test was without patatin additions. A difference was considered to be statistically significant when $p < 0.05$ (*) or $p < 0.01$ (**).

hydroxyl radicals. Al-Saikhan et al. (*11*) reported that patatin at 33 mg/mL exhibited an antioxidant activity that was very similar to that in potato extracts. From the above experimental results, it is revealed that 45 kDa patatin could capture radicals in aconcentration-dependent manner and may play a role as an antioxidant in potato tubers, which accounted for up 40% of the total soluble proteins, and may be beneficial when it is consumed for its nutritional and antioxidant activity. Some in vivo experiments will need for further investigations.

LITERATURE CITED

(1) Harman, D. Role of antioxidant nutrients in aging: overview. *Age* **1995**, *18*, 51−62.
(2) Ames, B. N. Dietary carcinogens and anticarcinogens: oxygen radicals and degenerative diseases. *Science* **1983**, *221*, 1256−1264.
(3) Gey, K. F. The antioxidant hypothesis of cardiovascular disease: epidemiology and mechanisms. *Biochem. Soc. Trans.* **1990**, *18*, 1041−1045.
(4) Yen, G. C.; Chuang, D. Y. Antioxidant properties of water extracts from *Cassia tora* L. in relation to the degree of roasting. *J. Agric. Food Chem.* **2000**, *48*, 2760−2765.
(5) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Antioxidant properties of phenolic compounds. *Trends Plant Sci.* **1997**, *2*, 152−159.
(6) Hou, W. C.; Chen, Y. C.; Chen, H. J.; Lin, Y. H.; Yang, L. L.; Lee, M. H. Antioxidant activities of trypsin inhibitor, a 33 kDa root storage protein of sweetpotato (*Ipomoea batatas* (L.) Lam cv. Tainong 57). *J. Agric. Food Chem.* **2001**, *49*, 2978−2981.

(7) Hou, W. C.; Lee, M. H.; Chen, H. J.; Liang, W. L.; Han, C. H.; Liu, Y. W.; Lin,Y. H. Antioxidant activities of dioscorin, the storage protein of yam (*Dioscorea batatas* Decne) tuber. *J. Agric. Food Chem.* **2001**, *49*, 4956−4960.

(8) Allen, J. C.; Wrieden, W. L. Influence of milk proteins on lipid oxidation in aqueous emulsion I. Casein, whey protein and α-lactalbumin. *J. Dairy Res.* **1982**, *49*, 239−248.

(9) Allen, J. C.; Wrieden, W. L. Influence of milk proteins on lipid oxidation in aqueous emulsion II. Lactoperoxidase, lactoferrin, superoxide dismutase and xanthine oxidase. *J. Dairy Res.* **1982**, *49*, 249−263.

(10) Andrews, D. L.; Beames, B.; Summers, M. D.; Park, W. D. Characterization of the lipid acyl hydrolase activity of the major potato (*Solanum tuberosum*) tuber protain, patatin, by cloning and abundant expression in a baculovirus vector. *Biochem. J.* **1988**, *252*, 199−206.

(11) Al-Saikhan, M. S.; Howard, L. R.; Miller, J. C., Jr. Antioxidant activity and total phenolics in different genotypes of potato (*Solanum tuberosum*, L.) *J. Food Sci.* **1995**, *60*, 341−343, 347.

(12) Racusen, D.; Foote, M. A major soluble glycoprotein of potato tubers. *J. Food Biochem.* **1980**, *4*, 43−52.

(13) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680−685.

(14) Neuhoff, V.; Stamm, R.; Eibl, H. Clear background and highly sensitive protein staining with Coomassie blue dyes in polyacrylamide gels: a systematic analysis. *Electrophoresis* **1985**, *6*, 427−448.

(15) Hou, W. C.; Hsu, F. L.; Lee, M. H. Yam (*Dioscorea batatas* Decne) tuber mucilage exhibited antioxidant activities *in vitro*. *Planta Med.* **2002**, *68*, 1072−1076.

(16) Kohno, M.; Yamada, M.; Mitsuta, K.; Mizuta, Y.; Yoshikawa, T. Spin-trapping studies on the reaction of iron complexes with peroxides and the effects of water-soluble antioxidants. *Bull. Chem. Soc. Jpn.* **1991**, *64*, 1447−1453.

(17) Wen, L.; Miao, Z. W.; Qing, W. D. Chemical modification of xylanase from *Trichosporon cutaneum* shows the presence of carboxyl groups and cysteine residues essential for enzyme activity. *J. Protein Chem.* **1999**, *18*, 677−686.

(18) Uhteg, L. C.; Lundblad, R. L. The modification of tryptophan in bovine thrombin. *Biochim. Biophys. Acta* **1977**, *491*, 551−557.

(19) Yan, L. J.; Droy-Lefaix, T.; Packer, L. *Ginkgo biloba* extract (Egb 761) protects human low-density lipoproteins against oxidative modification mediated by copper. *Biochem. Biophys. Res. Commun.* **1995**, *212*, 360−366.

(20) Kooy, N. W.; Royall, J. A.; Ischiropoulos, H.; Beckman, J. S. Peroxynitrite-mediated oxidation of dihydrorhodamine 123. *Free Radical Biol. Med.* **1994**, *16*, 149−156.

(21) Yan, X.; Suzuki, M.; Ohnish-Kameyama, M.; Sada, Y.; Nakanishi, T.; Nagata, T. Extraction and identification of antioxidants in roots of yacon (*Smallanthus sonchifolius*). *J. Agric. Food Chem.* **1999**, *47*, 4711−4713.

(22) Steinbrecher, U. P. Oxidation of human low-density lipoprotein results in derivatization of lysine residues of apolipoprotein B by lipid peroxide decomposition products. *J. Biol. Chem.* **1987**, *262*, 3603−3608.

(23) Halliwell, B. Food-derived antioxidants. Evaluation their importance in food and in vivo. *Food Sci. Agric. Chem.* **1999**, *1*, 67−109.

(24) Brannan, R. G.; Connolly, B. J.; Decker, E. A. Peroxynitrite: a potential initiator of lipid oxidation in food. *Trends Food Sci. Technol.* **2001**, *12*, 164−173.

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