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Storage proteins of two cultivars of sweet potato (*Ipomoea batatas* L.) and their protease hydrolysates exhibited antioxidant activity in vitro

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Abstract

The major root storage proteins of sweet potato (*Ipomoea batatas* Lam.), trypsin inhibitors (TIs), were purified from tuberous roots of two cultivars, Tainong 57 (T57) and T65, by trypsin-Sepharose 4B affinity column. The major protein was 28 kDa TI from two sweet potato cultivars. The purified TIs from T57 and T65 showed dose-dependent DPPH and hydroxyl radical-scavenging activities with glutathione as a control, and casein or bovine serum albumin were used for comparisons. In average, scavenging capacity for superoxide radical of 100 μg TN57 TIs and TN65 TIs were equivalent to 10.49 and 6.13 units of recombinant human superoxide dismutase, respectively. TN65 TIs had activity in the protection of Cu^{2+}-induced human LDL peroxidation, about three-fold that of TN57 TIs. The capacity of TN65 TIs to protect calf thymus from hydroxyl radical-induced DNA damage was about 10-fold that of TN57 TIs. The TN57 TIs showed dose-dependent (1–8 mg/ml) protection effect on dihydrorhodamine 123 (DHR) against peroxynitrite-mediated oxidation. Modifications of denatured TIs by iodoacetamide, or *N*-bromosuccinimide showed that Trp (tryptophan) residues mainly contributed scavenging activity against hydroxyl radical, while Cys (cysteine) residues mainly contributed scavenging activity against DPPH radical. Putting the above data together we propose that the 3D conformations of native molecular forms of both TN65 TIs and TN57 TIs are important factors that determine the relative capacities of the samples when different antioxidant test systems with different pH, ionic strength, chemical composition and temperature are used. Pepsin treatment or sequential treatments of pepsin and chymotrypsin for various times showed that the DPPH radical-scavenging activity of TI hydrolysates increased during hydrolysis.
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Keywords: Antioxidant; 1,1-Diphenyl-2-picrylhydrazyl (DPPH); Hydroxyl radical; *Ipomoea batatas*; Low-density lipoprotein; Peroxynitrite; Superoxide radical

1. 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 for their antioxidant activities in vitro, including both ethylacetate and butanol extracts of *Sideritis* species [4], ether, ethanol and hot water extracts of *Polyginum cognatum* [5], yam tuber proteins [6], whey proteins [7] and potato tuber proteins [8].

Sohonie and Bhandarker [9] reported for the first time the presence of trypsin inhibitors (TIs) in sweet potato. TIs in sweet potato roots accounted for about 60% of total water-soluble proteins and could be recognized as storage proteins [10]. Maeshima et al. [11] identified sporamin as the major storage protein in sweet potato root, which accounted for 80% of total proteins in root. Lin [12] proposed that the sporamin should be one form of TIs in sweet potato, which was confirmed later by Yeh et al. [13]. In this work, we

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reported that purified TIs from two sweet potato cultivars had different antioxidant activities in a series of in vitro tests that were compared to those of glutathione, casein or bovine serum albumin. We also used chemicals for amino acid side-chain modifications to identify key amino acid residues involved in antioxidant activities of TIs. Pepsin or chymotrypsin hydrolysates of TI were also used for antioxidant tests to simulate biological effects of TI metabolites after in vivo gastrointestinal digestion.

2. Materials and methods

2.1. Extraction and purification of sweet potato trypsin Inhibitors

Storage roots of two cultivars, Tainong 57 (T57) and T65, of sweet potato (*Ipomoea batatas* (L.) Lam.) were purchased from a wholesaler. After washing and peeling, the roots were cut into strips for TI extraction and purification. After extraction and centrifugation, the crude extracts were loaded directly onto a trypsin-Sepharose 4B affinity column. The adsorbed TIs were eluted by pH changes with 0.2 M KCl (pH 2.0) according to Hou et al. [14]. The extracts were desalted and concentrated with Centricon 10 and then lyophilized for further use. The protein and TI activity stainings on 12.5% SDS-PAGE gels were according to Hou et al. [14].

2.2. Scavenging activity of DPPH radical by spectrophotometry

Sample solution of 0.3 ml 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 for 20 min under light protection at room temperature [14]. The TN57 TI (7.14, 14.29, 28.57, and 35.71 nmol corresponding to 200, 400, 800, and 1000 μg, respectively), TN65 TI (3.57, 7.14, 10.71, and 14.29 nmol corresponding to 50, 100, 200, and 400 μg, respectively), bovine casein (4.24, 8.48, 16.95, and 25.43 nmol corresponding to 100, 200, 400, and 600 μg, respectively), glutathione (1, 3, 5, 10, and 20 nmol) and bovine serum albumin (BSA) (0.78, 1.56, 3.12, 6.25, and 9.375 nmol corresponding to 50, 100, 200, 400, and 600 μg, respectively) were used to scavenge DPPH radical. Glutathione was used as a positive control and both casein and BSA were used for comparisons. The absorbance at 517 nm was measured after the reactions. Deionized water was used as a blank. The decrease of absorbance at 517 nm was calculated and expressed as ΔA_{517} nm for activity of scavenging DPPH radical.

2.3. Scavenging activity of hydroxyl radical by spectrophotometry

The hydroxyl radical was determined by the deoxyribose method of Halliwell et al. [15]. Each 0.5 ml sample was added to 1.0 ml of solution of 20 mM potassium phosphate buffer (pH 7.4) containing 2.8 mM 2-deoxy-ribose, 104 μM EDTA, 100 μM $FeCl_3$, 100 μM ascorbate and 1 mM hydrogen peroxide. The mixtures were incubated at 37 °C for 1 h. After incubation, equal volume of 0.5% TBA in 10% trichloroacetic acid was added and the mixtures were boiled at 100 °C for 10 min. The T57 TI (9.1, 18.2, and 36.4 nmol), TN65 TI (2.27, 4.55, 6.82, and 9.1 nmol), bovine casein (2.12, 4.24, 6.36, and 8.48 nmol), glutathione (20, 40, and 60 nmol) and BSA (0.78, 1.56, 2.34, and 3.12 nmol) were used to scavenge the hydroxyl radical. Deionized water was used as a blank. Glutathione was used as a positive control and both casein and BSA were used for comparisons. The decrease of absorbance at 532 nm was calculated and expressed as ΔA_{532} nm for activity of scavenging hydroxyl radical.

2.4. Scavenging activity of superoxide radical by spectrophotometry

The superoxide radical was determined by the phenazine methosulfate (PMS)–NADH generating system [16]. Each 0.5 ml sample containing different amounts of TN57 (5–250 μg/ml) and TN65 (50–300 μg/ml) TIs was added in sequence to 0.5 ml of 630 μM nitroblue tetrazolium, 0.5 ml of 33 μM PMS and 0.5 ml of 156 μM NADH in 100 mM phosphate buffer (pH 7.4). The commercial recombinant human superoxide dismutase (rhSOD, 20, 40, and 60 units) was used as a positive control. Deionized water was used as a blank. The changes of absorbance at 560 nm (ΔA_{560} nm) were recorded during 2 min and expressed as ΔA_{560} nm/min for the activity of scavenging superoxide radical.

2.5. Preventing Cu^{2+}-iduced low-density lipoprotein peroxidation by TIs

The capacity of purified TIs of TN57 (3–18 mg/ml) or TN65 (1–6 mg/ml) to prevent Cu^{2+}-induced human low-density lipoprotein (LDL) oxidation in a total of 1.0 ml sample was measured by thiobarbituric acid reactive substances (TBARS) assay using absorbance at 532 nm [17]. The LDL (0.5 mg 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 TIs. EDTA with a final concentration of 100 μM was added to stop the reaction. The TBARS value of 24-h LDL peroxidation served as a control and LDL only served as a blank.

2.6. Protecting calf thymus DNA from hydroxyl radical-induced damages by TIs

The hydroxyl radical was generated by Fenton reaction according to the method of Kohno et al. [18]. The 45 μl reaction mixture including TIs of TN57 (250–1000 μg) or TN65 (10–200 μg), 15 μl of calf thymus DNA (25 A260 units/ml), 18 mM $FeSO_4$, and 60 mM hydrogen

peroxide was incubated at room temperature for 15 min. Then, 10 μl of 1 mM EDTA was added to stop the reaction. The calf thymus DNA was used as the blank, and the control contained all reaction components except TIs. After agarose electrophoresis, gels were stained with ethidium bromide and observed under UV light.

2.7. Modified TIs against 1,1-diphenyl-2-picrylhydrazyl and hydroxyl radicals

The 250 μl of purified TIs (100 or 150 μg) was reacted with different chemicals: (1) TIs were reduced by 10 mM dithiothreitol (DTT), and then were alkylated by 10 μl of 200 mM iodoacetamide with a final concentration of 200 μM [19] in 100 mM Tris–HCl buffer (pH 8.3), at 37 °C for 2 h, and then dialyzed against deionized water overnight; (2) tryptophan residues of TIs were modified by 0.6 ml of 0.5 or 1.0 mM *N*-bromosuccinimide [20] in 100 mM acetate buffer (pH 4.0) at room temperature for 1 h and then dialyzed against deionized water overnight. Purified TIs with or without chemical modifications were used for scavenging activity assays against DPPH and hydroxyl radicals described above.

2.8. Protecting dihydrorhodamine 123 from peroxynitrite-mediated oxidation by TIs

The protection of dihydrorhodamine 123 (DHR) against peroxynitrite-mediated oxidation was detected according to the method of Kooy et al. [21]. The total 180 μl reaction mixture included different amounts of TN57 TIs (1–4 mg/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 reaction, the fluorescence intensity was measured at the excitation and emission wavelengths of 500 and 536 nm, respectively, with excitation and emission slit widths of 2.5 and 3.0 nm, respectively. The control contained all reaction components except TIs.

2.9. 1,1-Diphenyl-2-picrylhydrazyl radical-scavenging activity of trypsin Inhibitor hydrolysates by pepsin or by sequential treatment of pepsin and chymotrypsin

TN57 TIs (4 mg) were dissolved in 1 ml of 100 mM KCl–HCl buffer (pH 2.0) and 0.1 ml of pepsin (0.8 mg) was added to allow hydrolysis for 8 h at 37 °C. After hydrolysis, 0.5 ml of 0.5 M Tris–HCl buffer (pH 8.3) was added and heated at 100 °C for 5 min to stop enzyme reaction. Then, 0.1 ml of chymotrypsin (0.8 mg) was added for another 4, 8 and 12 h hydrolysis. The chymotrypsin reaction was stopped by heating at 100 °C for 5 min. Pepsin was heated before TI hydrolysis for zero-hour reaction data. The changes of free amino groups were determined by measuring absorbance at 570 nm using ninhydrin method [22]. The DPPH radical-scavenging activity of TI hydrolysates was determined and expressed as ΔA_{517} nm.

2.10. Statistics

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

2.11. Chemicals

Tris, 2-thiobarbituric acid (TBA) were purchased from E. Merck Inc. (Darmstadt, Germany). Peroxynitrite was obtained from Calbiochem Novabiochem Co. (Darmstadt,

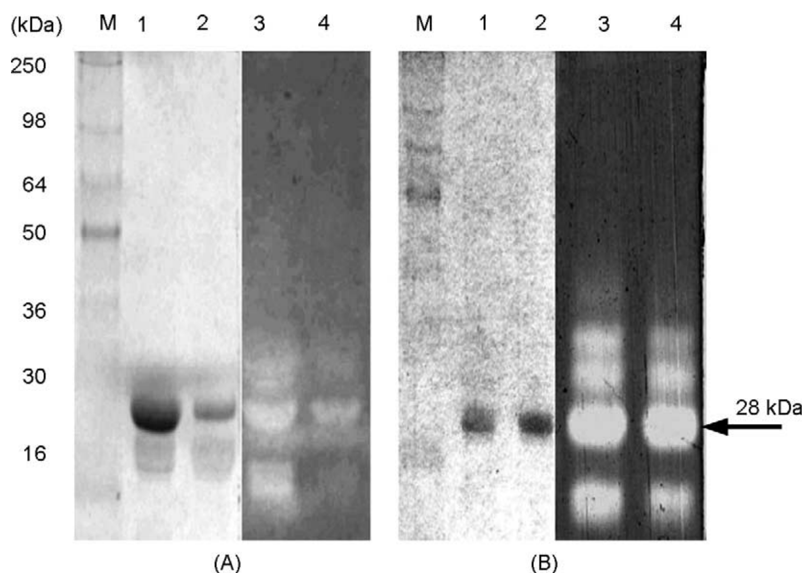

Fig. 1. Protein staining (lane 1, without heating at 100 °C; lane 2, heating at 100 °C for 10 min) and TI activity staining (lane 3, without heating at 100 °C; lane 4, heating at 100 °C for 10 min) of purified TIs from sweet potato roots by trypsin affinity column of TN57 (A) or TN65 (B) on 12.5% SDS-PAGE gels. Five micrograms of protein was loaded in each well. M indicated the sea blue prestained markers of SDS-PAGE. Arrow indicated the position of 28 kDa TI.

Germany). Calf thymus DNA (activated, 25 A260 units/ml) was purchased from Amersham Biosciences (Uppsala, Sweden). Hydrogen peroxide (33%) was from Wako Pure Chemical Industry (Osaka, Japan). Dihydrorhodamine 123 (DHR), DPPH, human LDL, iodoacetamide, *N*-bromosuccinimide (NBSI), and other chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

3. Results and discussion

3.1. Purification of sweet potato TIs

Storage proteins of sweet potato storage root were purified by trypsin affinity column. Fig. 1 showed the protein staining (lanes 1 and 2) and TI activity staining (lanes 3 and 4) on SDS-PAGE gels of TN57 (Fig. 1A) and TN65 (Fig. 1B). The blank zones (white ones) showed positions where the trypsin inhibitory activities locate. Several TI molecular forms from two cultivars of sweet potato were found after the affinity column purification, and the major protein band was the 28 kDa TI (indicated by an arrow). Compared with protein staining, purified proteins from TN57 and TN65 showed trypsin inhibitory activities. Heat treatment (100 °C for 10 min, lane 4, Fig. 1A and B) did not destroy all trypsin inhibitory activities, and TIs from TN65 showed higher heat stability. These purified TIs from TN57 and TN65 were used for scavenging activity assays.

3.2. Scavenging activity of TIs against 1,1-diphenyl-2-picrylhydrazyl radical

Our previous reports revealed that sweet potato TIs exhibited both activities of dehydroascorbate reductase and monodehydroascorbate reductase [23], and one form (33 kDa) of purified TIs also exhibited glutathione peroxidase-like activities [24]. Therefore, the purified TIs were used to test the antioxidant or antiradical activities against reactive oxygen species or reactive nitrogen species.

The DPPH radicals were widely used in the model system to investigate the scavenging activities of several natural compounds. The color changes from purple to yellow, and its absorbance at wavelength 517 nm decreases. Fig. 2 showed the results of scavenging activity of TIs against DPPH radical. TIs purified from TN57 and TN65, which showed dose-dependent DPPH radical-scavenging activities. Under the same nanomole basis, both glutathione and BSA exhibited the highest capacity. TN65 TIs exhibited next higher scavenging activity, followed by that of bovine casein or TN57 TIs.

3.3. Detecting hydroxyl radical clearance by spectrophotometry

Fig. 3 shows the results of clearance of hydroxyl radical by TIs. TIs purified from TN57 and TN65 also showed dose-dependent hydroxyl radical-scavenging activities. Different from the results of Fig. 2, under the same nanomole basis, glutathione exhibited the lowest hydroxyl radical-scavenging activity, while TN65 TIs exhibited the highest one.

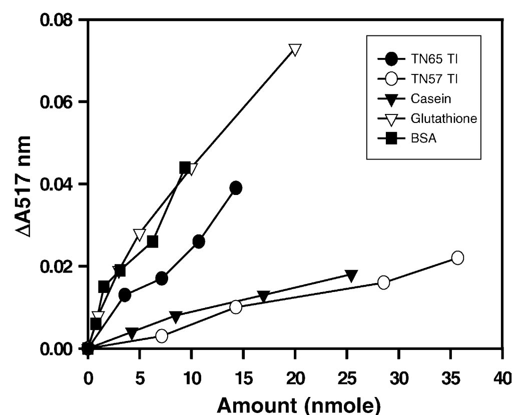

Fig. 2. Scavenging activity of TN57 TI (7.14, 14.29, 28.57, and 35.71 nmol), TN65 TI (3.57, 7.14, 10.71, and 14.29 nmol), bovine casein (4.24, 8.48, 16.95, and 25.43 nmol), glutathione (1, 3, 5, 10, and 20 nmol) and bovine serum albumin (BSA) (0.78, 1.56, 3.12, 6.25, and 9.375 nmol) against DPPH radicals. The decrease of absorbance at 517 nm was calculated and expressed as ΔA_{517} nm for DPPH radical-scavenging activity.

3.4. Scavenging activity of trypsin Inhibitors against superoxide radical

PMS–NADH generating system was used to produce superoxide radical [16]. TIs from two cultivars of sweet potato (T57 and T65) were used to scavenge superoxide radical with rhSOD as a positive control (Fig. 4). From the results of Fig. 4 (lower), the ΔA_{560} nm/min was negatively correlated ($r^2 = 0.998$) with rhSOD added (20, 40, and 60 units, respectively, for 19.79, 42.98, and 61.28% scavenging activity). TIs from TN57 (2.5, 5, 25, 50,

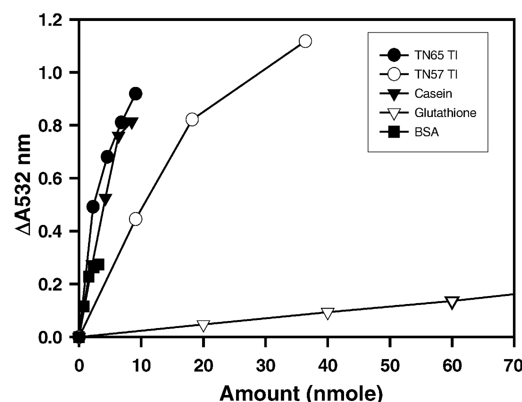

Fig. 3. The scavenging activity of TN57 TI (9.1, 18.2, and 36.4 nmol), TN65 TI (2.27, 4.55, 6.82, and 9.1 nmol), bovine casein (2.12, 4.24, 6.36, and 8.48 nmol), glutathione (20, 40, and 60 nmol) and bovine serum albumin (BSA) (0.78, 1.56, 2.34, and 3.12 nmol) against the hydroxyl radical. The decrease of absorbance at 532 nm was calculated and expressed as ΔA_{532} nm for hydroxyl radical-scavenging activity.

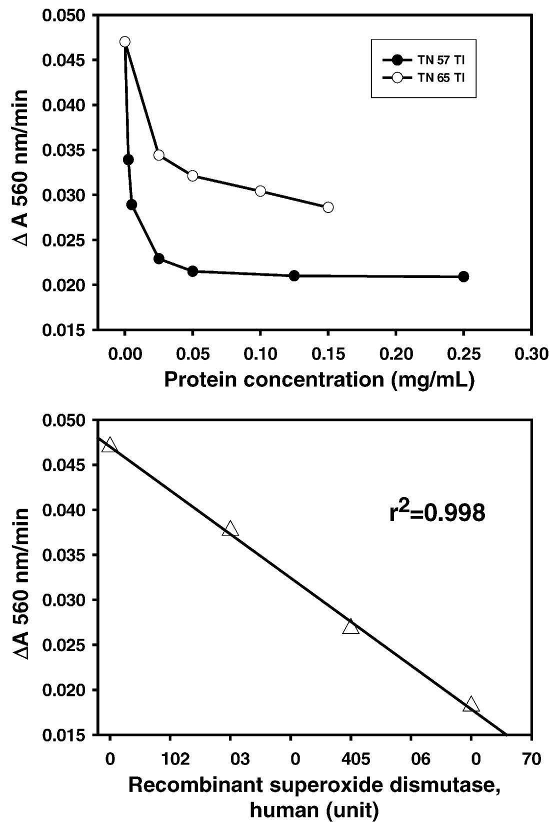

Fig. 4. The scavenging activity of TIs purified from TN57 (2.5–250 μg/ml) or TN65 (25–150 μg/ml) against superoxide radical (upper). The commercial recombinant human superoxide dismutase (20, 40, and 60 units) was used as a positive control (lower). The changes of absorbance at 560 nm (ΔA_{560} nm) were recorded during 2 min and expressed as ΔA_{560} nm/min for superoxide radical-scavenging activity.

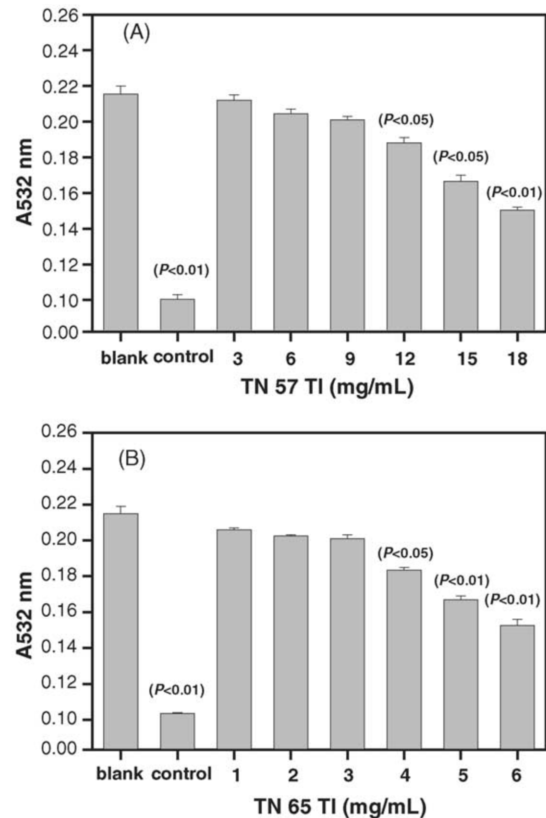

Fig. 5. The TBARS assay of purified TIs from (A) TN57 (3, 6, 9, 12, 15, and 18 mg/ml) or from (B) TN65 (1, 2, 3, 4, 5, and 6 mg/ml) against Cu^{2+}-induced human LDL peroxidation. The TBARS of 24-h LDL peroxidation was set as 100%. LDL alone served as a control. Student's *t*-test was used for the comparisons of the oxidized LDL among treatments. A difference was considered statistically significant when $^{*}P < 0.05$ or $^{**}P < 0.01$.

125, and 250 μg/ml, respectively, for 27.87, 38.51, 51.28, 54.26, 55.32, and 55.53% scavenging activity) or TN65 (25, 50, 100, and 150 μg/ml, respectively, for 26.81, 31.70, 35.32, and 39.15% scavenging activity) showed dose-dependent superoxide radical-clearance ability (Fig. 4 (upper)). On an average, 100 μg each of TN57 TIsand TN65 TIs are equivalent to 10.49 and 6.13 units recombinant human superoxide dismutase, respectively.

3.5. Protecting human low-density lipoprotein from Cu^{2+}-induced peroxidation by TIs using TBARS assay

LDL peroxidation has been reported to contribute to the atherosclerosis development [25]. Therefore, delay or prevention of LDL peroxidation is an important function of antioxidants. Using TBARS assay, the degrees of Cu^{2+}-induced human LDL peroxidation could be measured. Results of Fig. 5 show that the protection effects of both TN57 and TN65 TIs against LDL peroxidation were dose-dependent. From Fig. 5A, TIs from TN57 showed the significant differences between each of the oxidized LDL, oxidized LDL + 12 mg/ml TIs, oxidized LDL + 15 mg/ml TIs ($P < 0.05$) or oxidized LDL + 18 mg/ml TIs ($P < 0.01$). While, in Fig. 5B, TIs from TN65 showed the significant differences between each of the oxidized LDL, oxidized LDL + 4 mg/ml TIs ($P < 0.05$), and oxidized LDL + 5 mg/ml TIs or 6 mg/ml TIs ($P < 0.01$). From calculations, the capacity to protect human LDL against Cu^{2+}-induced peroxidation of TN65 TIs was about three times that of TN57 TIs.

3.6. Protecting calf thymus DNA against hydroxyl radical-induced damage by TIs

Free radicals could damage macromolecules in cells, such as DNA, protein and lipids in membranes [26]. Results of Fig. 6 (upper) show that the added TIs from TN57 above 0.5 mg (lane 2) could protect calf thymus DNA against hydroxyl radical-induced damages. While results of Fig. 6 (lower) show that the added TIs from TN65 above 0.05 mg (lane 2) could protect calf thymus DNA from hydroxyl radical-induced damages. The capacity of TN65 TIs to protect calf thymus DNA against hydroxyl radical-induced damage was about 10-folds that of TN57 TIs.

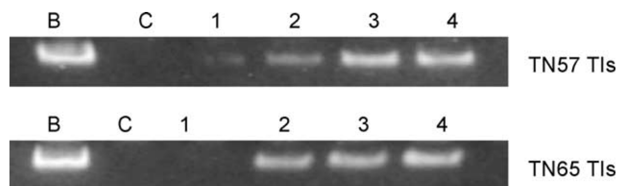

Fig. 6. The purified TIs from TN57 (250, 500, 750, and 1000 μg, respectively, lanes 1–4, (upper)) or from TN65 (10, 50, 100, and 200 μg, respectively, lanes 1–4, (lower)) protected calf thymus DNA against hydroxyl radical-induced damages. Calf thymus DNA alone served as a blank, and the control contained all reaction components except TIs. After agarose electrophoresis, the treated DNA solutions were stained with ethidium bromide and observed under UV light.

3.7. Protecting dihydrorhodamine 123 from peroxynitrite-mediated oxidation by TIs

Peroxynitrite is formed from nearly diffusion-limited reaction between nitric oxide and superoxide and acts as an initiator of potentially harmful oxidation reaction [27]. Results of Fig. 7 show that the protecting effect of TN57 TIs against peroxynitrite-mediated DHR oxidation was dose-dependent. The protection capacities of 1, 2, 4, and 8 mg/ml TN57 TIs were 12.68, 29.24, 40.32, and 42.39%, respectively. Significant difference was observed between peroxynitrite and peroxynitrite + 2 mg/ml, peroxynitrite + 4 mg/ml purified TIs ($P < 0.05$), or peroxynitrite + 8 mg/ml purified TIs ($P < 0.01$).

3.8. Scavenging activity of modified TIs against 1,1-diphenyl-2-picrylhydrazyl and hydroxyl radicals

Chemicals were used for modifications of amino acid side chains to identify key amino acid residues involved in antioxidant activities of DTT-denatured TIs. After reduction

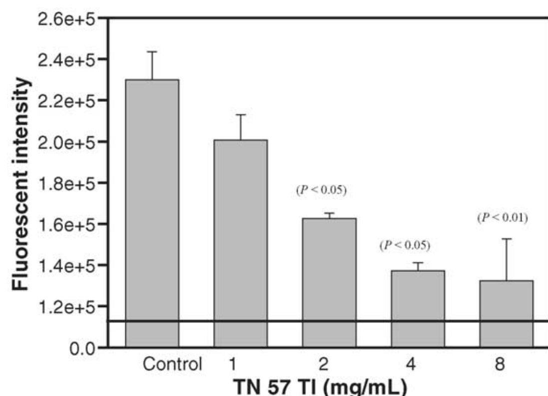

Fig. 7. The purified TIs of TN57-protected dihydrorhodamine 123 (DHR) against peroxynitrite-mediated oxidation. The reaction mixture (a total of 180 μl) included different amounts of TN57 TIs (1–8 mg/ml), 0.9 mM DHR and 5 μl peroxynitrite in 50 mM phosphate buffer (pH 7.4) containing 90 mM NaCl. After 5 min reaction, the fluorescence intensity was measured. The control contained all reaction components except TIs. A difference was considered statistically significant when $^{*}P < 0.05$ or $^{**}P < 0.01$.

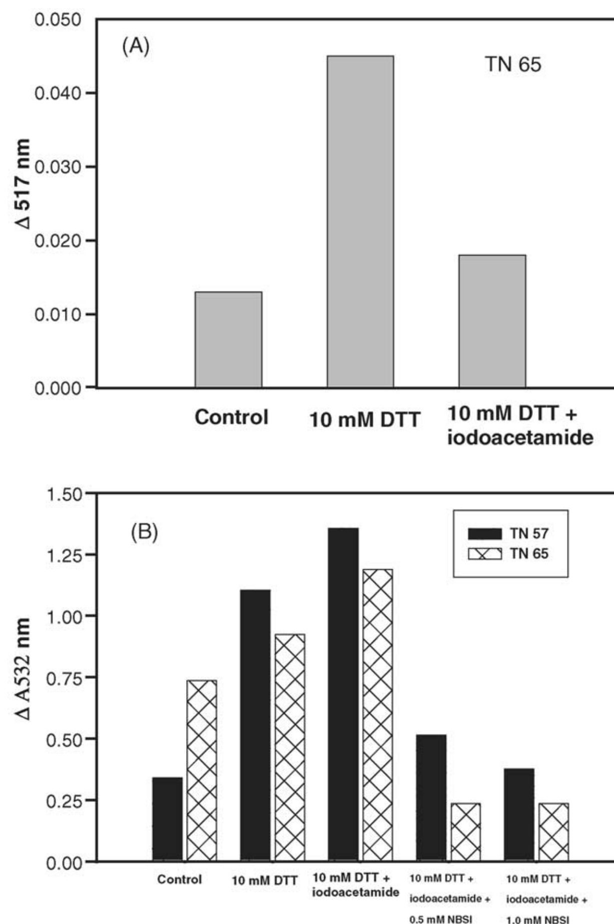

Fig. 8. Purified TIs (100 or 150 μg) in 250 μl were modified by different chemicals and then used for scavenging activity assays against DPPH (A) and hydroxyl (B) radicals.

by DTT (Fig. 8A), the DPPH scavenging activity of TIs from TN65 was increased to about three times that of controls; however, alkylation of reduced TIs decreased the scavenging activities to the control level. Free cysteine (Cys) residues in whey proteins were reported to have antioxidant activities [7]. Results of Fig. 8A also showed that Cys residues in TIs contributed scavenging activities against DPPH radical. Fig. 2 also shows that under the same nanomole basis, glutathione exhibited the highest DPPH radical-scavenging capacity. Results of Fig. 8B show that although the hydroxyl radical-scavenging activity of TIs from either TN57 or TN65 was increased after reduction by DTT (column 2), alkylation of reduced TIs did not affect the scavenging activity (column 3). This result revealed that free Cys in TIs (from TN57 and TN65) contribute little to hydroxyl radical scavenging, which explains why in Fig. 3, based on the same nanomole basis, glutathione exhibits the lowest hydroxyl radical-scavenging activity. While the reduced TIs were treated with either 0.5 mM or 1.0 mM NBSI to modify (trypsin) Trp residues, the hydroxyl radical-scavenging activity was significantly decreased to the control level in TIs of both cultivars. It was clear that Trp residues in TIs contributed largely against hydroxyl radical, which is in agreement with

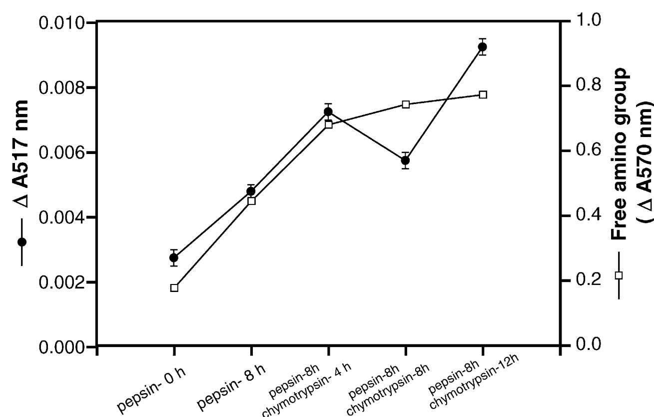

Fig. 9. The changes of free amino groups and scavenging activity against DPPH radical of TN57 TI hydrolysates by pepsin treatment or by serial treatment of pepsin and chymotrypsin.

report by Yan et al. [17] that Trp exhibited antihydroxyl radical activity. We propose that after reduction by DTT, the Trp residues in the inner regions of TIs were exposed and then could scavenge hydroxyl radical.

3.9. 1,1-Diphenyl-2-picrylhydrazyl radical-scavenging activity of TI hydrolysates by pepsin treatment or by sequential treatment of pepsin and chymotrypsin

The TI hydrolysates by pepsin treatment or sequential treatment of pepsin and chymotrypsin at different time points were used to simulate biological function of TI metabolites after in vivo digestion. Results of Fig. 9 show that free amino groups of TN57 TIs were increased after hydrolysis by pepsin or by both pepsin and chymotrypsin in series. The DPPH scavenging activity was increased and showed in parallel with TI hydrolysates. Wallner et al. [28] reported that the α-amino group of L-arginine contributed its antioxidant effects. The increased free amino groups and inner region of amino acids of TN57 TIs after enzyme hydrolysis (Fig. 9) might contribute to the increased DPPH scavenging activities. It might be beneficial for antioxidant effects as peptide forms after being ingested.

In conclusion, the major storage proteins of sweet potato were reported to account for over 80% of the total proteins [11]. The sweet potato ranked in the third position of tuber crop productions in 1999. From a series of antioxidant or antiradical experiments in vitro, native forms of TIs from both cultivars exhibited dose-dependent antioxidant capacities, but their effect levels are different depending on the assay systems used. Since we have already known (Fig. 1) that the main TI of TN65 is more heat-stable than that of TN57 as shown on both protein staining and TI activity staining on SDS-PAGE gels, we may propose that TN65 TI and TN57 TI display differential antioxidant activities in various systems due to their unique 3D conformations, which change in different reaction conditions. In general, the conformation of a particular protein in native form might contribute to its antihydroxyl radical activity in addition to its competent surface amino acids such as Trp reported by Yan et al. [17].

The in vivo mimic hydrolysis increased DPPH radical-scavenging activity of TN57 TIs based on the same weight basis. It is beneficial for health when people consume sweet potato as far as the antioxidant effects are concerned.

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