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Consumption of Purple Sweet Potato Affects Post-Translational Modification of Plasma Proteins in Hamsters

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Supporting Information

ABSTRACT: A high level of intake of vegetables is strongly associated with the prevention of chronic diseases. Because post-translational modifications (PTMs) have been shown to be the important biomarkers of the change in physiological functions, this study aimed to explore the changes in PTMs of plasma proteins when purple sweet potato (PSP), a root vegetable, was incorporated into the daily diet. Male Syrian hamsters were maintained on a rice diet (50% rice) or PSP diet (25% rice and 25% PSP) for 12 weeks. Plasma proteins were fractionated by electrophoresis, digested by trypsin, and then separated by nano-liquid chromatography and tandem mass spectrometry. The TurboSequest algorithm was used to identify peptide sequence against the hamster database in Universal Proteins Resource Knowledgebase, and in-house PTM finder programs were used for identification and quantification of PTMs. The results indicated that 95 plasma proteins were identified and 28 PTM sites on 26 of these 95 proteins were affected by consumption of PSP ($p < 0.05$). Methylation accounted for the largest percentage of affected modifications (35.71%). This study also showed that incorporation of purple sweet potato into the diet significantly lowered blood and liver lipids ($p < 0.05$). The results of this study provide a basis for prospective studies evaluating the effects of dietary intervention on modifications of proteins.

KEYWORDS: *purple sweet potato, post-translational modification, plasma proteins*

INTRODUCTION

Post-translational modifications (PTMs) are site specific amino acid substitutions and/or side chain modifications that take place after the translation of mRNA into a protein. They affect the folding, conformation, and functions of proteins. More than 300 different types of PTMs have been characterized to date; the most common types are methylation, phosphorylation, glycosylation, acylation, ubiquitination, sulfation, and prenylation.[1] PTMs provide diverse and complex roles for proteins, playing crucial roles in physiological processes and disease progression. It is irrational to infer protein function without having the information about the PTMs it undergoes. Exploration of changes in PTMs of plasma or tissue proteins will help to elucidate the physiological effects of nutrients and foods.

Sweet potatoes (*Ipomoea batatas* L.) are rich in vitamins, minerals, dietary fibers, and phytochemicals.[2] Among all the cultivars of sweet potatoes, anthocyanin-rich dark purple-flesh sweet potato (PSP) has the highest antioxidant activity compared with those of potatoes with white, cream, yellow, and orange flesh.[3] Intake of PSP flakes improved the antioxidant status in rats fed either a cholesterol-rich diet or a regular diet.[4,5] Intake of 250 mL of PSP beverages (containing 400 mg of anthocyanins) daily for 8 weeks decreased the serum levels of the hepatic biomarker in healthy men with borderline hepatitis.[6] Ethanol and water extracts of PSP inhibited cupric ion-mediated low-density lipoprotein (LDL) oxidation and the glycation of apolipoprotein A-I, showing anti-atherosclerotic and anti-diabetic potential.[7] When a differentiated 3T3-L1 cell was treated with a PSP extract, the expression of inflammatory and lipogenic factors was suppressed.[8] Recent findings suggest that PSP attenuates domoic acid-induced cognitive deficits by promoting estrogen receptor-α-mediated mitochondrial biogenesis signaling in mice.[9]

A high level of intake of plant foods is strongly recommended for the prevention of chronic diseases.[10] Phytochemicals, the bioactive non-nutrient plant compounds, significantly contribute to the health benefits of plant foods.[11,12] Anthocyanins are phenolic phytochemicals that are responsible for the purple, red, and blue colors of many plant foods and have been reported to exhibit various biological activities.[13] Phytochemicals, including anthocyanins, are best acquired through whole-food consumption instead of dietary supplements, because the additive and synergistic effects of various phytochemicals are responsible for the biological activities of plant foods.[14]

Plasma is a complex biological system that contains numerous proteins. Although the physiological effects of PSP have been studied, its effect on post-translational modification of plasma proteins has not been investigated. This study aimed to explore the changes in PTMs of plasma proteins by a mass spectrometry (MS)-based approach when purple sweet potato, a root vegetable, was incorporated into the daily diet for 12 weeks.

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This study will provide a basis for prospective studies evaluating the effects of dietary intervention on modifications of proteins.

MATERIALS AND METHODS

Plant Materials. White rice (TNG67) and the root of purple-flesh sweet potato (*I. batatas* L.) variety Quandow were obtained from Taiwan Agricultural Research Institute (Wufeng, Taiwan). The rice was oven-dried, and the roots (skins and flesh) were freeze-dried before being ground into fine powder with a cyclotec mill (Rose Scientific, Edmonton, AB).

Animals and Diets. Eight-week-old Syrian hamsters were purchased from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). The animals were maintained at 22 ± 2 °C with a 12 h light−dark cycle and had free access to water. After being acclimated for 1 week, hamsters were randomly assigned to either the rice group or the purple sweet potato (PSP) group (n = 12). Rice instead of regular sweet potato was used as control because it is the most widely consumed staple food through the world. The PSP was incorporated into the diet by replacing half of the rice in the diet. The diets (Table 1) and water were provided *ad libitum*, and body weights were measured once a week. At the end of 12 weeks, all animals were sacrificed. The collected blood was divided into two parts for the preparation of serum and plasma. The protocol for this study was approved by the Shih Chien University Animal Care and Use Committee.

Table 1. Animal Diet (grams per kilogram of diet)

	rice	PSP
casein	200	200
soybean oil	100	100
rice	500	250
purple sweet potato	0	250
sucrose	100	100
cellulose	50	50
AIN-76 vitamin mix	10	10
AIN-76 mineral mix	35	35
L-methionine	3	3
choline bitartrate	2	2
total anthocyanins	0	0.73
total flavonoids	0.20	0.50
total phenolics (gallic acid equivialents)	0.60	2.75

Measurement of Anthocyanins, Phenolics, and Flavonoids. Each sample was extracted with HCl-containing methanol (1:99, v/v) at a ratio of 1:10 at 4 °C for 24 h. The extract was filtered through a 0.45 μm membrane filter and concentrated before being analyzed. The concentrated sample was analyzed by high-performance liquid chromatography (model 2996, Waters, Milford, MA) with an ODS column (Inertsil ODS-3 column, 4.6 mm × 250 mm, 5 μm; precolumn, Inertsil ODS-3 column, 4.6 mm × 33 mm, 5 μm) (Inertsil, Kumamoto, Japan). The flow rate of the H_2O/acetic acid/methanol mobile phase (69:10:21, v/v/v) was set at 1 mL/min, and the temperature of the column was set at 30 °C. The absorption of eluted compounds was monitored at 530 nm. Commercial cyanidin and peonidin (Extrasynthase, Genay, Cedex, France) were used to generate calibration curves by injecting 0.05−1 μg of pure compound in 20 μL of 1% HCl-methanol. For the measurement of total phenolics, 1 g of sample was extracted with 20 mL of 80% methanol (v/v) for 15 min, and the volume of the filter was increased to 20 mL with extraction reagent. Five hundred microliters of extract was mixed with 600 μL of 50% Folin-Ciocatlteu reagent. Five minutes later, 1.2 mL of 20% Na_2CO_3 was added to the mixture, and the solution was then incubated at room temperature while being shaken for 10 min. After centrifugation at 150g for 10 min, the absorbance was obtained at 730 nm, and the total amount of phenolics in the extracts was determined as gallic acid equivalents. For the measurement of total flavonoids, 0.5 mL of extract was mixed with 2.8 mL of H_2O, 0.1 mL of 1 M potassium acetate, 0.1 mL of 10% aluminum chloride (w/v), and 1.5 mL of 95% ethanol (v/v) before being incubated at room temperature for 40 min. The absorbance of the mixture was taken at 415 nm. Quercetin was used to construct the standard curve.

Determination of Serum and Liver Lipids. Serum triglyceride and cholesterol were determined by using a commercial kit (Randox, Antrim, United Kingdom). Liver lipids were measured according to the method of Folch et al.[15] with modification. Briefly, livers were homogenized with Folch reagent [2:1 (v/v) chloroform/methanol] at a ratio of 20:1. The homogenates were centrifuged at 865g for 10 min, and the organic solvent in the supernatant was removed before the lipids were measured with a commercial kit (Randox).

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS−PAGE) and In-Gel Digestion. Plasma proteins were fractionated by SDS−PAGE. Fifty micrograms of total protein from each sample was loaded onto the sodium dodecyl sulfate−polyacrylamide gel. After fractionation by electrophoresis, the gel was stained with Coomassie Brilliant Blue G-250 (Bio-Rad, Hercules, CA). Each lane was sliced into 10 fractions based on molecular weight and destained in a solution of 25 mM NH_4HCO_3 and 50% (v/v) acetonitrile (1:1). After being dried in the Speed-Vac (Thermo Electron, Waltham, MA), all gel slices were incubated with 25 mM NH_4HCO_3 and 2% β-mercaptoethanol in the dark for 20 min to reduce the disulfide bonds. Following the reduction, gel slices were incubated with an equal volume of 10% 4-vinylpyridine in 25 mM NH_4HCO_3 and 50% acetonitrile for 20 min to alkylate cysteine residues. After the slices had been soaked in 25 mM NH_4HCO_3 for 10 min and dried in the Speed-Vac for 20 min, in-gel digestion was initiated by addition of 100 ng of modified trypsin (Promega, Mannheim, Germany) in 25 mM NH_4HCO_3 at 37 °C overnight. The digested peptides were removed from the gel, dried with the Speed-Vac, and then kept at −20 °C until further analysis. The peptides were resuspended in 0.1% formic acid immediately before being used.[16]

Mass Spectrometric Analysis. The tryptic peptides were injected into a nanoflow high-performance liquid chromatography system (1200 series, Agilent Technologies, Waldbronn, Germany) coupled to an LTQ-Orbitrap Discovery hybrid mass spectrometer with a nanoelectrospray ionization source (Thermo Electron). An analytical column (100 mm × 0.075 mm, 3.5 μm, Agilent C18) was operated at a flow rate of 0.5 μL/min. The elution solvent system was employed using gradient elution using solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile). The course of gradient elution was programmed as a 30 min linear gradient from 5 to 35% solvent followed by 95% solvent B for a duration of 10 min. Following each full scan in the m/z range of 200−2000, a data-dependent acquired MS/MS scan for precursor ions was selected on the basis of conventional MS spectra recorded at high resolution (M/DM, full width ay half-maximum of 60000). The accurate mass and the charge state of the precursor ions were obtained from each spectrum. Following MS_1, the fragment ions

Table 2. Concentrations of Serum and Liver Lipids in Hamsters[a]

		rice	PSP[b]
body weight		104.38 ± 9.04	103.13 ± 8.43
serum			
	triglyceride (mg/dL)	51.61 ± 11.72	25.01 ± 7.23[c]
	cholesterol (mg/dL)	122.91 ± 22.20	83.48 ± 7.23[c]
	HDL-C (mg/dL)	91.18 ± 15.50	66.13 ± 8.45[c]
	LDL-C (mg/dL)	21.40 ± 7.02	12.35 ± 4.07[c]
	LDL-C/HDL-C	0.23 ± 0.06	0.19 ± 0.07[c]
liver			
	triglyceride (mg/g)	16.13 ± 2.87	14.92 ± 2.65
	cholesterol (mg/g)	6.70 ± 1.84	3.91 ± 0.61[c]

[a]Values are mean ± the standard deviation. [b]Purple sweet potato replaced half of the rice in the diet (25% rice and 25% PSP). [c]Significantly different from the value for the rice group (α = 0.05).

Table 3. Post-Translational Modifications of Plasma Proteins Affected by Consumption of Purple Sweet Potato

	PTM	protein	molecular function	site	modified peptide[a]	positions[b]	*x*-fold[c]
1	methylation	albumin, A6YF56	binding protein	Glu, Asn	(−) SHCLSE#VE#N#DDLPADLPSLAADFVEDK	311−337	100
		maltase-glucoamylase, G3HU95	CHO binding and hydroxylase	Glu	(−)RQDPVSWDEAFE#DISR	1360−1375	100
		pregnancy protein 60 kDa, Q60552	endopeptidase inhibitor	Arg	(−)MQQLESSLQPETLR#R#	282−296	6.8
		complement C3, G3HCL6	endopeptidase inhibitor	Glu	(−)IWDVVE#KADIGCTPGSGK	589−606	5.38
				Glu, Asp	(−)VMIE#D#GLGE#AVLKR	265−278	5
		plasminogen, G3HGQ6	endopeptidase	Lys	(−)MRDVILFEK#K#	88−97	3.5
		anionic trypsin 2, G3HL18	endopeptidase	Gln, Asp	(−) M*ASPSSTLINCLFSFPISIQ#VAFPID#D#DK	1−29	2.7
		β-Ala-His dipeptidase, G3I691	metallopeptidase	Arg	(−)FILEGMEEAGSVALEELVR#	116−134	2.67
		apolipoprotein A-1, G3I7Q1	binding protein	Glu, Asn	(−)Q#M*N#LNLLEN#WDTLGSTVGR	64−82	2.19
		roquin, G3GZL3	metal ions and nucleic acids binding	Asp, Asn, Gln	(−)FD#TISEKTSD#Q#IHFFFAKLN#CR	1094−1115	−5
2	ubiquitination	cationic trypsin 3, G3HUA1	endopeptidase	Lys	(−)M*KTLIFLAFLGAAGDDTKDK#	1−20	100
		inter-*α*-trypsin inhibitor heavy chain 4 (ITIH4), G3GSF1	endopeptidase inhibitor	Lys	(−)LQDQGLDVLSAKISGQM*HK#	497−515	5.95
3	phosphorylation	60S ribosomal protein L5, G3H732	rRNA and ATP binding	Thr	(−)VGLTNYAAAYCT#GLLLAHR	40−58	2.17
		serine protease inhibitor A3N, G3HBC6	peptidase and endopeptidase inhibitor	Tyr	(−)TVMVPMM*NIEDLTTPY#LR	236−253	−100
4	*O*-(2-aminoethylphosphoryl) serine	receptor-type tyrosine phosphatase, G3H3N0	phosphatase	Ser	(−-)GPTLVHCS#AGVGRTGTFVALLR	1255−1276	3.48
		coiled-coil *α*-helical rod protein 1, G3HQN6	cell differentiation	Ser	(−)GRSLELRVS#GALSQQAELISR	51−71	3.36
5	4-oxo-2-nonenal	Vit D-binding protein, G3IHJ6	binding protein	Lys	(−)TKTPNASPGDLEDM*VDK#	428−444	5.99
6	carboxylation	albumin, A6YF56	binding protein	Glu	(−)TVLGE#FTAFLDK	570−581	−5.99
7	hydroxylation	inter-*α*-trypsin inhibitor heavy chain 1 (ITIH1), P97278	endopeptidase inhibitor	Asp, Pro	(−)QAVD#TTP#DGVLVK	44−56	−17.25
8	malondialdehyde	serotransferrin, G3GZG6	ferric binding	Lys	(−)EAK#CPETPSSSAAVKWCALSHHER	928−951	2.38
9	imidazole	ITIH1, P97278	endopeptidase inhibitor	Lys	(−)EK#RQAVDTTPDGVLVK#	41−56	5
10	hydroimidazolone	vinculin, G3GWQ1	actin cytoskeleton	Arg	(−)DDILR#SLGEIAALTSKLGDLR#	438−458	2.18
11	S-guanylation-2	BET1-like protein, G3IMD8	protein trafficking	Cys	(−)KLLC#GM*AM*VLIVAFFILYHLLSR	167−189	2
12	N6,N6,N6-trimethyl-5-hydroxylysine	nebulin, G3IJ59	actin binding	Lys	(−)ASEIISEK#K#YR	2097−2107	100
13	nitroalkylation by nitrooleic acid	*β*-2-glycoprotein 1, G3I1K9	binding protein	Cys	(−)KCSYTEEAQC#IDGTLDVPK	306−324	−5
14	*S*-(coelenterazin-3a-yl)cysteine	kininogen-1, G3HYG4	endopeptidase inhibitor	Cys	(−)PSEIVIGQC#KVIATRYSDESQDFR	97−120	4.69
15	aminomalonic acid	fibrinogen *β* chain, G3H5I8	platelet activation	Ser	(−)ASVAELNGNIQSVS#ETSS#TTFQYLTLLK	41−68	2.46
16	4-methyl-*δ*-1-pyrroline-5-carboxy	prothrombin, G3GYJ4	Ca^{2+} binding and endopeptidase	Lys	(−)NIEK#ISM*LEKIYIHPR	434−449	100

[a]Site of modification is marked with a number sign. [b]First and last residues in the accession number. [c]Ratio of PTMs in the PSP group to PTMs in the rice group.

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were further generated from a collision-induced dissociation (CID) spectrum.

Database Search. The spectra generated from nano-liquid chromatography and tandem mass spectrometry (nano-LC−MS/MS) were analyzed with Xcalibur 2.0 SRI (Thermo Electron). The TurboSequest algorithm was used to identify the peptide sequences against the hamster database in the Universal Proteins Resource knowledgebase [Uniprot (http://www.unipro.org/)].[17] A variable modification of the methionine residues (methionine, 16 Da), a fixed modification of the cysteine (carboxyamidomethylation, 57 Da), a peptide mass tolerance at 1.5 Da, and a fragment mass tolerance at 1 Da were not considered as specific PTMs. The peptides were filtered by Xcorr versus charge state with a significance level of 0.05. Xcorr was used for a match with 1.9 for singly charged ions and 3.75 for triply charged ions. The proteins were identified as plasma proteins when more than two peptides from a single protein met the Xcorr score. The mass spectra of candidate biomarkers were analyzed with our in-house PTM finder programs PTM-Miner and PTM-Q for identification of PTM peptide sequences, sites of PTMs, and quantification.[16] MS spectral counts were normalized by the sum of the spectral counts per biological sample in quantitative analyses.[18] The fragmented ions obtained from nano-LC−MS/MS were labeled as b, y, y-NH_3, and b-H_2O ions.

Structural Modeling and Sequence Alignment. Structure modeling, figure building, and sequence alignment of proteins were performed using SWISS-MODEL (http://swissmodel.expasy.org/).[19,20]

Statistical Analysis. Statistical analysis was performed using the Statistical Analysis System (SAS Institute, Cary, NC). A *t* test was used to determine significant differences between the rice group and the PSP group ($\alpha = 0.05$).

RESULTS

Blood and Liver Lipid in Animals. Table 2 shows that the hamsters in the PSP group had significantly ($p < 0.05$) lower levels of serum triglyceride and cholesterol and a lower ratio of LDL to HDL than the animals in the rice group. The triglyceride contents in livers were not statistically different between two groups; however, the cholesterol content was significantly lower in the PSP group. There was no difference in body weight between two groups at the end of the study.

Post-Translational Modification of Plasma Proteins. To examine the effect of the consumption of PSP on PTMs of plasma proteins, plasma proteins were separated by electrophoresis and digested by trypsin before being further analyzed by tandem mass spectrometry. The spectra generated were analyzed by TurboSequest to identify the peptide sequences against the hamster database in UniProt. The results were scored using Xcorr. The proteins were regarded as present in the plasma if more than two peptides from a single protein met the threshold of the Xcorr score. On the basis of this criterion, 95 proteins in plasma were identified (Table 1 of the Supporting Information).

Identification and quantification of PTMs of these 95 proteins were then conducted by our in-house PTM finder programs PTM-Miner and PTM-Q. The analysis indicates that the levels of 16 different types of modifications on 26 of the 95 identified proteins (28 unique modification sites) were significantly ($p < 0.05$) increased or decreased by the consumption of PSP. The identified peptides and corresponding modified sites are listed in Table 3. Methylation accounted for the largest percentage of modifications significantly affected by PSP (34.48%). Ubiquitnation, phosphorylation, and aminoethylphosphorylation accounted for 6.89% of affected modifications (Figure 1A). The other modifications significantly affected by PSP include carboxylation, hydroxylation, malondialdehyde, imidazole, hydroimidazolone, guanylation, trimethyl-5-hydroxylysine, nitroalkylation, aminomalonic acid, and *S*-(coelenterazin-3a-yl)cysteine.

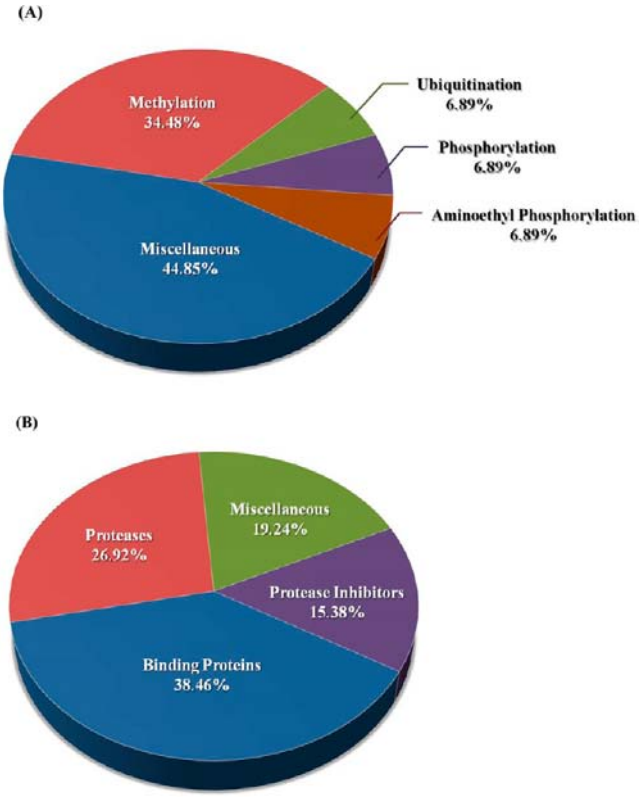

Figure 1. (A) Types of post-translational modifications significantly affected by the consumption of purple sweet potato. (B) Types of plasma proteins affected by the consumption of purple sweet potato.

Among the proteins that exhibited affected PTMs, 38.46% are binding/transport proteins, 26.92% are proteases, and 15.38% are protease inhibitors (Figure 1B).

Fragment Ion Spectrum. Following MS_1, the fragment ions were further generated with a collision-induced dissociation (CID) spectrum. Two representative spectra are shown in Figure 2. Figure 2A is the fragment ion spectrum for the modified peptide 497-LQDQGLDVLSAKISGQMHK$_{UB}$-515 of inter-α-trypsin inhibitor heavy chain H4 (ITIH4). The spacing of b and y ions was used to decipher the sequence of modified peptide. The novel ubiquitination site was detected on the basis of the unmodified b_{15} ion and the mass shift of 383.23 Da for the y_3 ion on the modified peptide, which corresponds to the molecular weight of GGRL cleaved from Lys-515 by trypsin. The matched peaks were labeled according to the Biemann nomenclature. Figure 2B shows the fragment ion spectrum for the modified peptide 428-TKTPNAS-PGDLEDMVDK$_O$-444 of vitamin D-binding protein. The novel 4-oxo-2-nonenal modification site was perceived from the unmodified b_{14} and modified y_1 ions with a mass shift of 154.099 Da.

Structural Modeling and Sequence Alignment. Structural modeling analysis helps to clarify the locations of PTMs on proteins. Figure 3 shows the predicted three-dimensional models of two representative proteins obtained by SWISS-MODEL: inter-α-trypsin inhibitor heavy chain H4 (ITIH4) (Figure 3A) and vitamin D-binding protein (Figure 3B). Most of the PTM sites are labeled. Lys-515 for ubiquitination on ITIH4 and Lys-444 for 4-oxo-2-nonenal on vitamin D-binding protein that were significantly affected by consumption of PSP are marked with asterisks. The sequence alignments of hamster (*Cricetulus musculus*) ITIH4 (Figure 4A) and vitamin D-binding protein (Figure 4B) showed homology with the sequences of rat (*Rattus norvegicus*),

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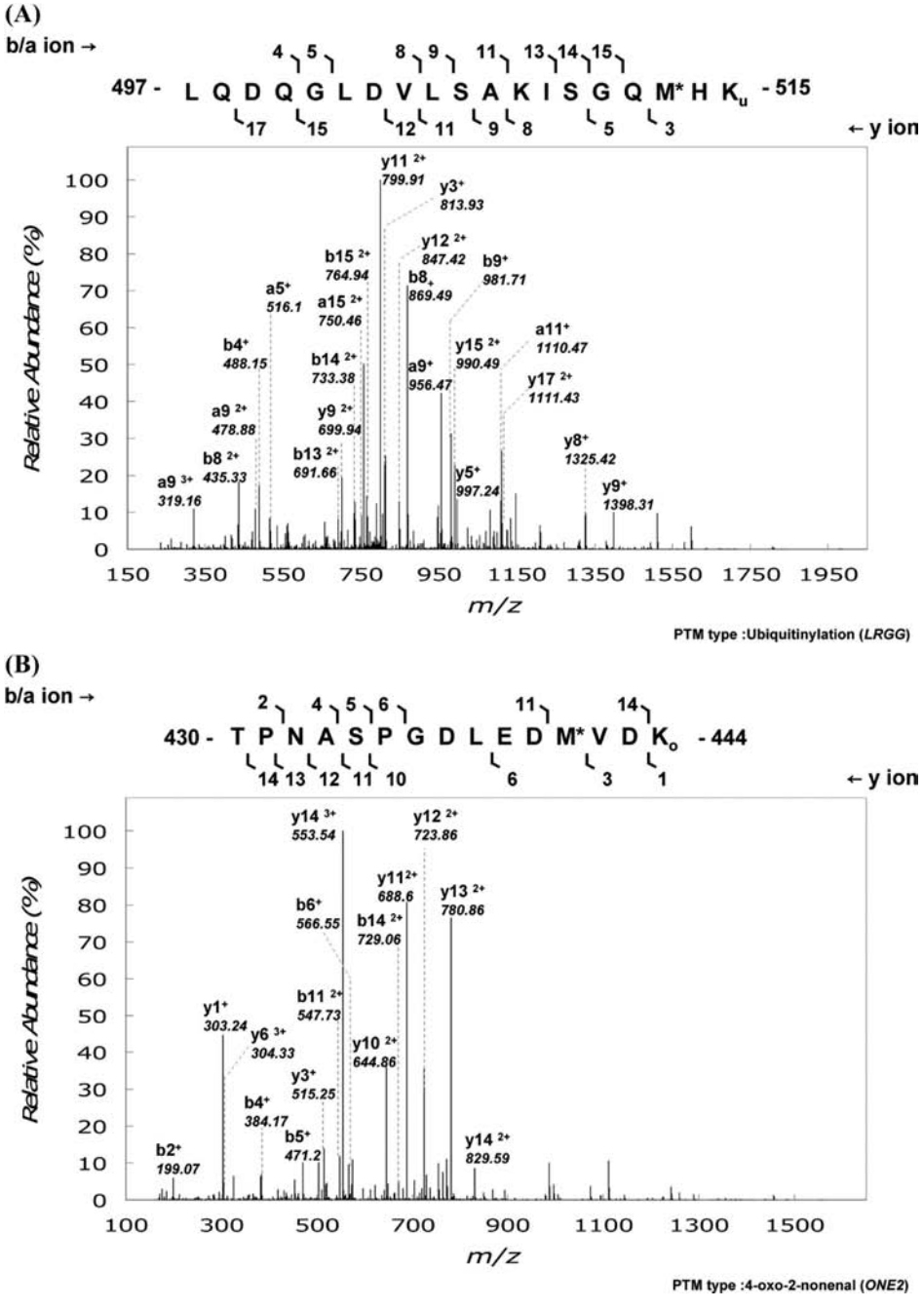

Figure 2. (A) Tandem mass spectrum of the ubiquitination peptide with the sequence 497-LQDQGLDVLSAKISGQM$_{ub}$HK-515 on inter-α-trypsin inhibitor heavy chain H4. (B) Tandem mass spectrum of the 4-oxo-2-nonenal peptide with the sequence 468-TKTPNASPGDLEDMVDK$_{oxo}$-484 on vitamin D-binding protein. The indicated b/a and y ions refer to the number of amino acids counted from the N-terminus and C-terminus, respectively.

mouse (*Mus musculus*), and human (*Homo sapiens*). Most of the residues at modification sites are conserved in the four mammals.

DISCUSSION

The development of MS-based technologies has provided useful platforms for the study of quantitative changes in protein components other than identification of proteins. In this study, we used tandem mass spectrometry to explore the changes in post-translational modification of hamster plasma proteins after the consumption of PSP for 12 weeks.

Plasma is a complex biological system. The plasma proteome contains many high-abundance proteins that have housekeeping functions. Plasma also contains numerous low-abundance proteins that are released into the plasma during necrosis or apoptosis of cells. Post-translational modifications have been shown to be the important biomarkers for the pathological processes of many diseases.[21−23] The discovery of proteomic biomarkers from plasma holds clinical significance. However, analysis of the plasma proteome is a challenging task because of the complexity and multidimensionality of its component. Although post-MS data processing has improved in both throughput and accuracy, detection of many low-abundance proteins is still limited. In this study, 95 plasma proteins were identified on the basis of the "filtering" criterion described in Materials and Methods.

In this study, levels of 16 different types of PTMs on 26 of the 95 identified proteins (28 unique modification sites) were

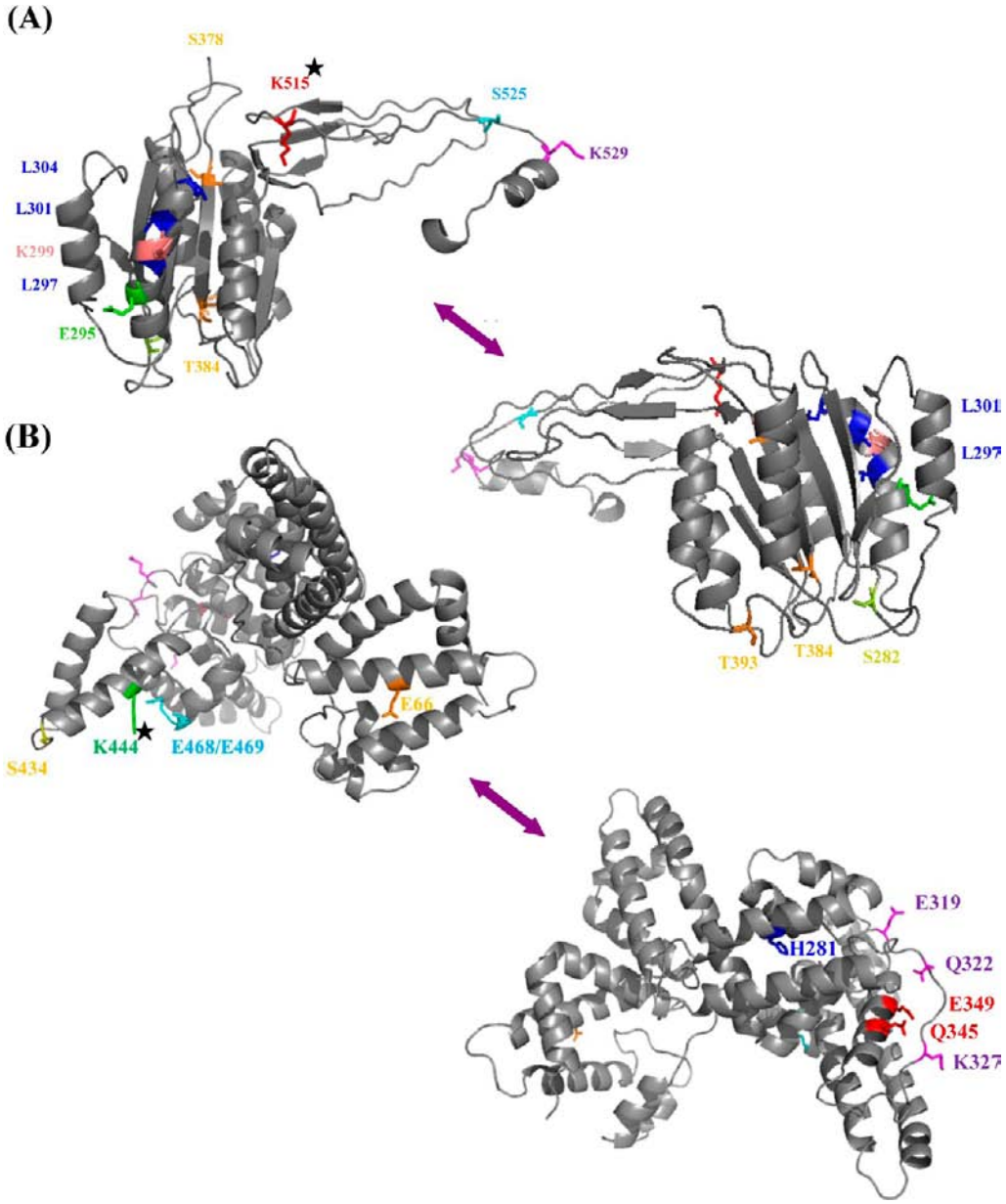

Figure 3. Three-dimensional structures of (A) inter-α-trypsin inhibitor heavy chain H4 (ITIH4) and (B) vitamin D-binding protein predicted by SWISS-MODEL. All PTM sites are labeled. The ubiquitination site (K515) on ITIH4 and the 4-oxo-2-nonenal site (K444) on vitamin D-binding protein were significantly affected by purple sweet potato (marked with asterisks).

significantly ($p < 0.05$) increased or decreased by the consumption of PSP. Ten of the 28 modifications are methylation, and level of only one of them was decreased by PSP. Methylation is a widely observed PTM that involves the transfer of a methyl group from *S*-adenosylmethionine to amino acid side chains. Methylation is generally associated with the activation or inactivation of proteins. While most information regarding methylation focuses on the epigenetic regulation of histones in DNA,[24] little is known about the role of methylation in the function of plasma proteins. Some evidence revealed that methylation may prevent the proteins from turning over by blocking sites of ubiquitination.[25]

Two of the 28 affected modifications are ubiquitination on inter-α-trypsin inhibitor heavy chain 4 (ITIH4) and cationic trypsin 3. Ubiquitination is the attachment of the C-termal glycine of the 76-amino acid polypeptide ubiquitin to lysine residues of target proteins, which labels the proteins for degradation. The covalent ubiquitination of proteins is a widespread regulatory post-translational modification.[26] ITIH4 is a positive acute-phase protein mainly expressed in liver and released into plasma.[27,28] An increased level of ubiquitination on ITIH4 may contribute to the decreased level of ITIH4 in the plasma of animals that consumed PSP (Table 2 of the Supporting Information).

Phosphorylation of proteins regulates all aspects of cell life; however, abnormal phosphorylation leads to many diseases.[29] The main sites of phosphorylation are the hydroxyl groups of serine, threonine, or tyrosine residues, but histidine and lysine residues can also be used. Extracellular phosphorylation is a regulator of plasma proteins, and the degree of phosphorylation changes with age.[30] Phosphorylation may quickly affect the properties of nonenzymatic proteins by modulating their ability to interact with other proteins and their susceptibility to proteolysis.

Hydroxylation involves the introduction of a hydroxyl group into an organic compound and is one of the extensive PTMs that

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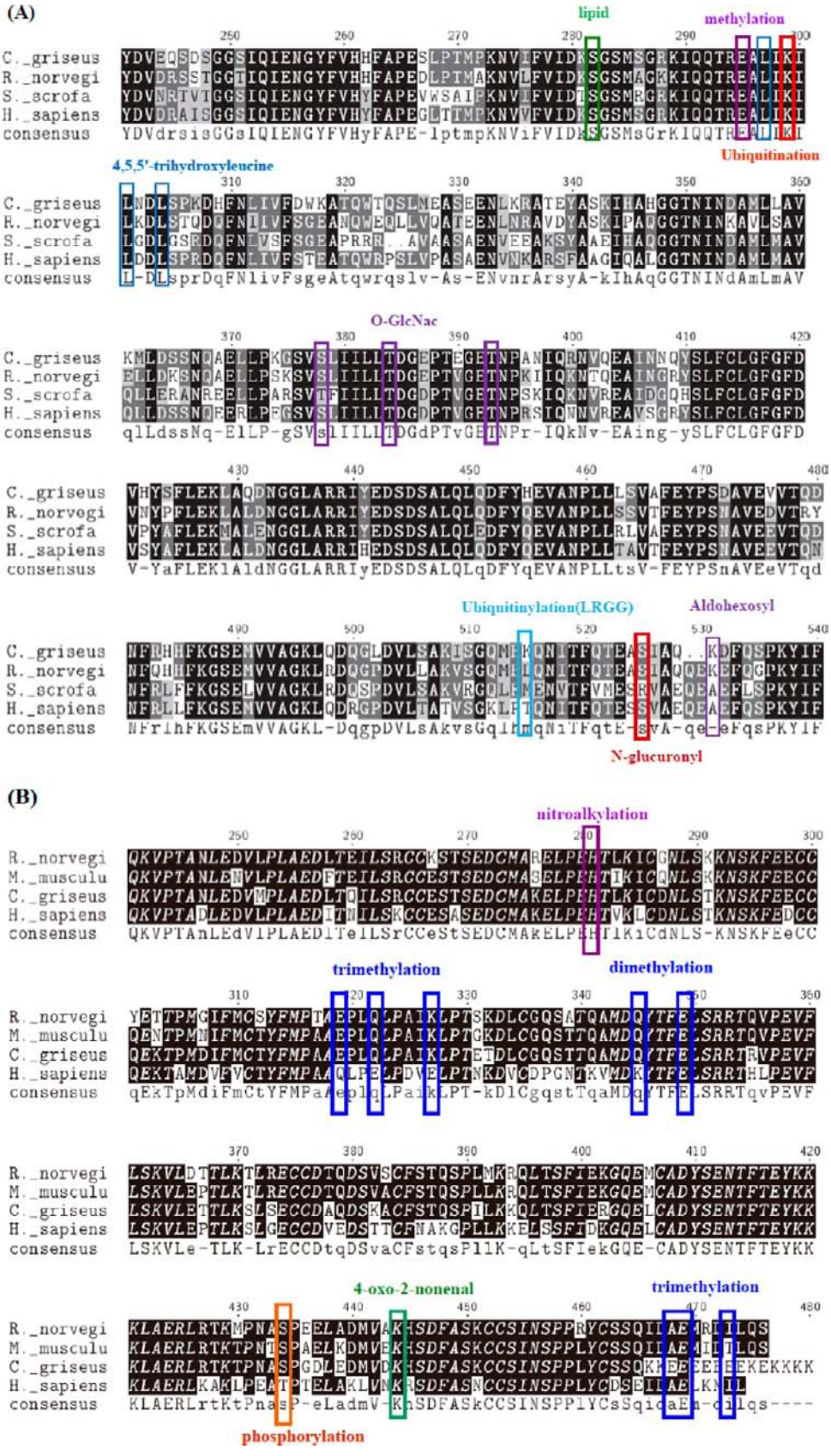

Figure 4. Sequence alignments of (A) inter-α-trypsin inhibitor heavy chain H4 (ITIH4) and (B) vitamin D-binding protein: *R._norvegi*, rat; *M._musculu*, mouse; *C._griseus*, guinea pig; *H._sapiens*, human.

have crucial effects on the stability and metabolism of proteins. The principal residue in proteins to be hydroxylated is proline. Hydroxylation of proline is essential for the physiological roles of many proteins such as collagen and hypoxia inducible factors.[31]

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In addition to hydroxylation of prolines, post-translational hydroxylation of aspartic acids also occurs in plasma proteins.[32]

4-Oxo-2-nonenal is a major product of the Fe^{2+}-mediated breakdown of lipid hydroperoxides.[33] The increased level of 4-oxo-2-nonenal on vitamin D-binding protein may result from the downregulation of hemopexin, which has a strong binding affinity for iron (Table 2 of the Supporting Information).[34] The decreased level of hemopexin increases the number of free irons in the circulation, resulting in more iron-mediated breakdown of lipid hydroperoxides.

Hamsters were used in this study because they and humans have similar responses to dietary components. The results showed that incorporation of PSP into the diet for 12 weeks significantly decreased the levels of blood and liver lipids while body weight was not affected. In addition, the levels of blood and liver lipids observed in this study are lower than those described in other studies.[35−37] Because of the poor absorption of anthocyanin *in vivo*,[38] it is hypothesized that the higher fiber content of PSP (3%) compared to that of rice (0.5%) contributes to the lipid-lowering effect of PSP. Fiber has been shown to decrease the levels of blood and liver lipids by reducing the rate of absorption of lipids in small intestine and increasing the rate of excretion of bile acid.[39,40] According to a meta-analysis of 67 controlled trials, 2−10 g of dietary fiber per day was associated with a small but significant decrease in the levels of total and LDL cholesterol in human plasma.[41]

In conclusion, we have performed a proteomic analysis to reveal the changes in PTM of plasma proteins after PSP had been incorporated into the diet for 12 weeks. Plasma is a complex biological fluid. The changes in modifications of specific proteins by the consumption of PSP may not make sense until the plasma proteome is fully understood. However, the description of changes in PTMs provides a basis for a prospective study that aims to evaluate the physiological effects of dietary intervention.

■ ASSOCIATED CONTENT

Supporting Information

Ninety-five proteins identified in hamster serum (Table 1) and plasma proteins expressed differently in PSP-fed animals (Table 2). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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