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Compositional and Conformational Analysis of Yam Proteins by Near Infrared Fourier Transform Raman Spectroscopy

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Fourier transform (FT)-Raman spectroscopy was employed to study the molecular structure of yam proteins isolated from three commonly consumed yam species including *Dioscorea alata* L., *D. alata* L. var. *purpurea*, and *Dioscorea japonica*. Although *D. alata* L. and *D. alata* L. var. *purpurea* consisted of similar amino acid residues, they still exhibited significant differences in conformational arrangement. The secondary structure of *D. alata* L. was mainly an α-helix, while *D. alata* L. var. *purpurea* was mostly in antiparallel β-sheets. In contrast, *D. japonica*, which belongs to a different species, exhibited explicit differences in amino acid compositions and molecular structures of which the conformation was a mixed form of α-helices and antiparallel β-sheets. FT-Raman directly proved the existence of S−S in yam proteins, implying that oligomer formation in yam proteins might be due to disulfide linking of dioscorin (32 kDa). The microenvironment of aromatic amino acids and the state of S−S in yam proteins were also discussed.

KEYWORDS: Yam protein; FT-Raman; *Dioscorea alata* L.; *Dioscorea alata* L. var. *purpurea*; *Dioscorea japonica*

INTRODUCTION

Yam, the tuber of *Dioscorea* spp., is an important staple food in many tropical countries (*1*, *2*). It receives wide attention due to its functional properties and pharmaceutical potential. In a Chinese society, yams are not only served as a health food but are also used as an important ingredient in Chinese herbal medicines. Several beneficial properties of yams have been reported (*3*−*5*). Yam proteins also demonstrate diversified functional properties and enzymatic activities (*6*−*8*). To unveil the distinct properties of yam proteins, a number of studies have focused on the sequence determination (*9*−*12*). Interestingly, very little is known about the structural relevance of yam proteins to date. It is of great importance to determine the molecular structure of yam protein in order to understand more of its biological functions. Generally, X-ray crystallography is employed to determine the three-dimensional structure of a target protein and is able to figure out the spatial location of certain important amino acid residues. However, the difficulty and complexity of crystallization often limit X-ray crystallography's application. Circular dichroism (CD) maybe serves as a powerful tool in the search of protein secondary structures; however, the requirement for clear samples in CD analysis limits its application to dilute protein solutions or transparent gels. Interference due to the absorbance of various salts and buffer substances in the far-UV region also limits the use of CD spectroscopy in studying the effects of environmental conditions on protein conformation (*13*).

The innovation of spectroscopic techniques receives many merits on structural interpretation of biological interests. Each spectroscopic technique exhibits its unique preference in a certain application, of which Raman spectroscopy has been well-credited for protein structural characterization. With a simple, direct, and noninvasive measurement, Raman spectroscopy can powerfully determine the secondary structure of proteins and disclose the local information of protein side groups such as tryptophan, tyrosine, phenylalanine, and sulfhydryl compounds (*13*−*22*). For example, Yu et al. used the Raman technique to clarify the secondary structure of different biomolecules in solution, crystallization, and lyophilized states (*15*−*18*). Thomas et al. demonstrated the informative Raman results on the α-helix and aromatic and nonaromatic side chains of coat protein units (*21*−*24*). Various techniques have been developed in Raman-related research to enhance its sensitivity and to expand its applications, such as the development of a Fourier transform (FT) Raman spectrometer to circumvent the intrinsic fluorescence interference (*25*−*27*). FT-Raman with multiplex and high throughput properties is able to obtain high quality structural information on proteins at a molecular level. The structural characterization of food proteins by FT-Raman has been successfully reported recently (*13*, *28*−*29*). This further proves that FT-Raman spectroscopy is a powerful technique for elucidating the molecular structure of plant proteins. In the

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current study, we report, for the first time, the structural characterization of yam proteins isolated from three popularly consumed yam cultivars, including *Dioscorea alata* L., *D. alata* L. var. *purpurea*, and *Dioscorea japonica*. FT-Raman results clearly indicate that the secondary structure of yam proteins in *D. alata* L., *D. alata* L. var. *purpurea*, and *D. japonica* is mostly in an α-helix, antiparallel β-sheet, or a mixed form of an α-helix and antiparallel β-sheet, respectively.

MATERIALS AND METHODS

Fresh yam tubers of *D. japonica* were obtained from a local wholesaler, and fresh yam tubers of *D. alata* L. and *D. alata* L. var *purpurea* were purchased from a local agricultural cultivation station. Electrophoresis grade acrylamide, N,N,N,N-tetramethyl ethylenediamine (TEMED), Trizma base, N,N-methylenebisacrylamide, tris-aminomethane, ammonium persulfate, 2-mecaptoethanol, bromophenol blue, coomassie brilliant blue G-250, sodium dodecyl sulfate (SDS), and protein standard reagents were purchased from the Bio-Rad Lab (Richmond, CA). Ammonium sulfate, acetone, glycine, methanol, acetic acid, sodium phosphate, dibasic, 12-hydrate, and sodium sulfate were from Sigma Chem. Co. (St. Louis, MO). All of the chemicals for chromatography were analytical grade, and the rest, except for electrophoresis, were reagent grade.

Isolation and Analysis of Yam Protein. Sample isolation was carried out at 4 °C by following the procedure of Harvey and Boulter (*10*) with some modifications. Yam tubers were washed with water, peeled, and cut into pieces right before protein extraction. The pieces of yam were homogenized with 5 volumes (w/v) of 50 mM Tris-HCl buffer (pH 8.3) and forwarded to centrifugation at 23130*g* for 30 min. After centrifugation, the crude yam proteins in the supernatant were salted out with 0−70% ammonium sulfate. The precipitate from the ammonium sulfate solution was dialyzed against deionized and distilled water, lyophilized, and saved for further analysis.

The analysis of yam protein was carried out on a TSK gel SW guard column (4 cm × 8 mm) and a TSK G3000 SW (30 cm × 8 mm) (TosoHaas, Japan) loaded on a Hitachi D-7000 high-performance liquid chromatography system (Hitachi, Ltd., Tokyo, Japan). The system was equipped with a model L-7100 pump, a model L-7420 UV-Vis detector, and a Rheodyne model 7725 injector. The peaks were detected at wavelength 280 nm, and acquisition and processing of data were completed by Hitachi B-7000 software with an AID interface. A sodium phosphate solution (0.1 M, pH 6.8) with 0.05% sodium azide driven by the pump system was prepared as a mobile phase. The buffer solution was degassed with a Branson 2510 ultrasonic system (Branson Ultransonic Corporation, Danbury, CT) right before it was employed. Each sample (1 mg/mL) was filtered using 0.45 μm sterile units (Millipore Co., Bedford, MA), and 10 μL was injected in the chromatographic system with an optimal flow rate. A typical analysis could be completed in 40 min with a flow rate of 0.6 mL/min.

The molecular mass of each yam protein component was determined by the simple linear regression, correlating elution volume with molecular mass, on the calibration curve. Both high and low molecular mass calibration curves were quickly constructed by measuring the elution volumes of standard compounds, calculating the $*K_{av}$ value for each, and plotting the K_{av} value vs the logarithm of each standard molecular mass. The standard compounds included ribonuclease A (13.7 kDa), chymotrypsinogen A (25.0 kDa), ovalbumin (43.0 kDa), bovine serum albumin (67.0 kDa), aldolase (158 kDa), catalase (232 kDa), ferritin (440 kDa), thyroglobulin (669 kDa), and blue dextran 2000 (2000 kDa).

$$*K_{av} = (V_e - V_o)/(V_t - V_o)$$

where V_e = elution volume of protein, V_o = column void volume, and V_t = total bed volume.

SDS polyacrylamide gel electrophoresis (PAGE) was performed in a vertical mini-gel system (Mini-Protean II Dual Slab Cell, Bio-Rad Lab) as described by Laemmli (*30*) with some modifications. Polyacrylamide gels (5% stacking and 14% resolving gel) were formed by copolymerization of acrylamide and bis-acrylamide with the aid of the initiator TEMED and ammonium persulfate. Buffer solutions for the stacking and resolving gels were prepared from 0.125 M Tris-HCl (pH 6.8) and 0.375 M Thris-HCl (pH 8.9), respectively, incorporating them with 0.1% SDS. The running buffer (pH 8.6) consisted of 0.1% SDS, 0.1% 2-mercaptoethanol, 0.19 M glycine, 0.025 M Tris-HCl, and 1 mM ethylenediaminetetraacetic acid. The sample buffer was composed of 0.1% SDS buffer, 10% sucrose, 0.05% bromophenol blue, and 20 mM dithiothreitol. The samples (10 μL) were well-mixed with the buffer solution by microcentrifuge at 1200 rpm (Hettich GmbH & Co., Tuttlingen, Germany) and heated at 100 °C in a Dry-Bath (Dubuque, IA) for 3 min prior to being loaded to the gels. Electrophoresis was carried out with a fixed voltage of 180 V for 55 min. After electrophoresis, the gel was stained with 0.25% Coomassie Brilliant blue solution containing 12.5% trichloroacetic acid, 20% methanol, and 7.0% acetic acid for 20 min and destained with a solution of 20% methanol and 7.0% acetic acid overnight. Procedures for nonreducing SDS−PAGE electrophoretic analysis were the same as the ones under reducing conditions described above except for the absence of reducing agents such as 2-mercaptoethanol and dithiothreitol in sample and electrophoretic buffer solutions. Protein markers for reduced SDS−PAGE were phosphorylase b (97.6 kDa), bovine serum albumin (66.3 kDa), ovalbumin (45.0 kDa), carbonic anhydrase (31.0 kDa), trypsin inhibitor (21.5 kDa), and aprotinin (6.4 kDa). For SDS−PAGE under nonreducing conditions, markers were obtained from Full Range Rainbow Molecular Weight Markers (Amersham Biosciences Co., Piscataway, NJ), which was a mixture of individually colored recombinant proteins with molecular masses of 250, 160, 105, 75, 50, 35, 30, 15, and 10 kDa.

Amino acid analysis of yam proteins was performed by following the previous method (*31*). The yam protein was vacuumed and hydrolyzed with 6 N HCl solutions at 110 °C for 22 h. The hydrolyzed sample was then subjected to the analysis of amino acid compositions (Beckman 6300 system high-performance amino acid analyzer, Beckman, Inc., Palo Alto, CA). Amino acid standards except tryptophan were prepared as internal references for determining amino acid content, expressed as mg/g, in yam proteins. To enhance the accuracy of amino acid determination, the standard reference vials were loaded in the autosampling tray between every three sample vials. At least three replicates were measured for each cultivar.

Raman Measurement. FT-Raman spectra of yam proteins were obtained by using a Bruker RFS-100 FT-spectrophotometer (Bruker Optik GmbH, Lubeck, Germany). The sample was put into the tiny hole of a stainless steel holder for Raman measurement. Continuous near-infrared wave excitation at 1064 nm was provided by a diode laser pumped Nd:YAG laser (Coherent Lubeck GmbH, Lubeck, Germany). The laser light with a power of 150 mW was introduced and focused on the sample. The scattered radiation was collected at 180° with an ellipsoidal mirror and was filtered, modulated, and reflected back into the highly sensitive GaAs detector that was cooled by liquid nitrogen. Raman spectra were produced over the Raman shift 0−3500 cm^{-1}. Typically, 500 interferograms were coadded at 4 cm^{-1} resolution with a sampling period of about 15 min. The intensity ratio of Raman bands 643 to 621 cm^{-1} ($I_{643/621}$) as well as 855 to 832 cm^{-1} ($I_{855/832}$) was used to evaluate the microenvironment property of tyrosine, and the ratio of 881 to 758 cm^{-1} ($I_{881/758}$) was used for the analysis of tryptophan, respectively (*32*). Each numerical calculation of the Raman intensity ratio was based on the average of triplicate measurements at least. FT-Raman spectra reported in this study were all original and were not smoothed, normalized, or baseline-corrected through data manipulation.

Statistical analysis was conducted with a commercial statistic computing software package (STATISTICA, 1999 edition, StatSoft Inc., Tulsa, OK) with a personal computer. The results were considered statistically significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

D. alata L., *D. alata* L. var. *purpurea*, and *D. japonica* are three widely consumed yams species in Taiwan, and each expresses its specialty in external appearance, texture, coloration, and browning susceptibility during food processing. Several

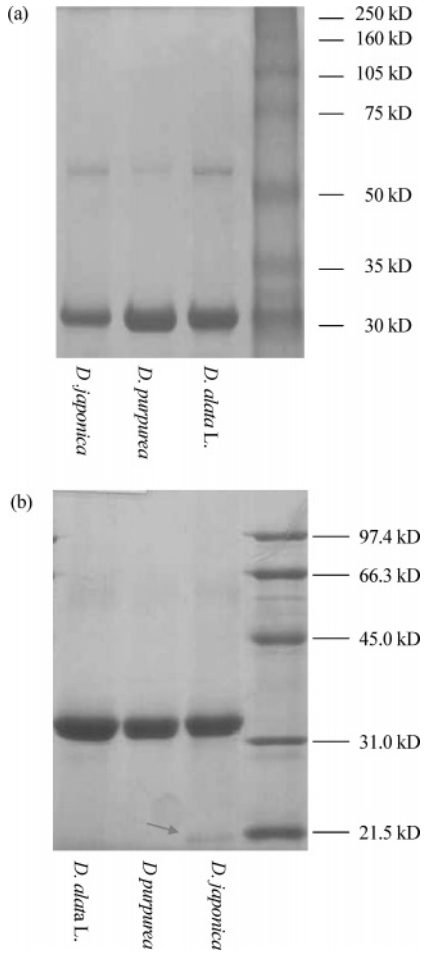

Figure 1. Electrophoretic study of yam proteins isolated from *D. alata* L., *D. alata* L. var. *purpurea*, and *D. japonica*. (**a**) SDS–PAGE under nonreducing conditions; (**b**) SDS–PAGE under reducing conditions. Protein markers for reduced SDS–PAGE were phosphorylase b (97.6 kDa), bovine serum albumin (66.3 kDa), ovalbumin (45.0 kDa), carbonic anhydrase (31.0 kDa), trypsin inhibitor (21.5 kDa), and aprotinin (6.4 kDa), while for nonreduced SDS–PAGE, the markers were Full Range Rainbow Molecular Weight Markers (Amersham Biosciences Co.) with molecular masses of 250, 160, 105, 75, 50, 35, 30, 15, and 10 kDa. The arrow, shown in the reduced SDS–PAGE gel, signals the slight band around 20 kDa in *D. japonica*.

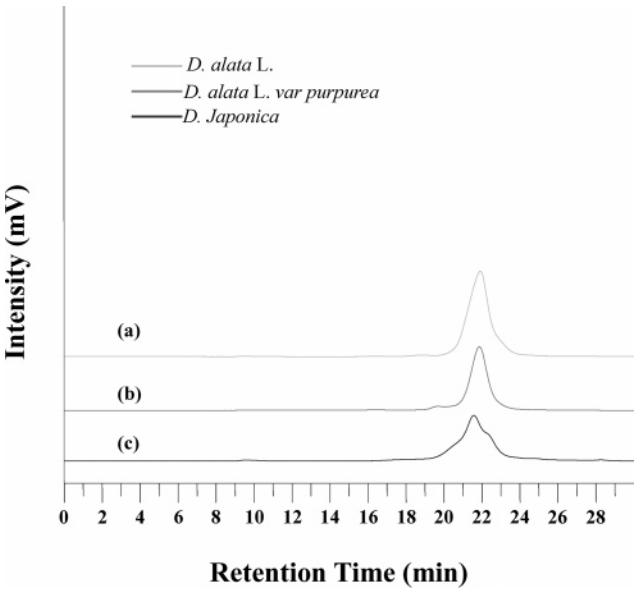

Figure 2. Gel chromatogram of yam proteins isolated from various yams: (**a**) *D. alata* L., (**b**) *D. alata* L. var. *purpurea*, and (**c**) *D. japonica*. Separation conditions are as mentioned in the Materials and Methods.

studies have been reported about their biological and physiological functions (*6*−*8*). Our previous work showed that the chemical composition, physical properties, and antioxidant activities of these yam flours behaved differently (*33*). Here, we further present the compositional and structural analysis of yam proteins by amino acid analyzer and FT-Raman spectroscopy for the first time. **Figure 1** displays electrophoretic results of yam proteins isolated from *D. alata*, *D. alata* L. var. *purpurea*, and *D. japonica*. As the nonreducing SDS−PAGE result has shown in **Figure 1a**, all three yam cultivars exhibit similar gel patterns that show a heavy band with a molecular mass of ∼32 kDa as well as a slight band around 64 kDa. If protein samples are treated with reducing agent such as dithiothreitol or 2-mercaptoethanol, the slight band of ∼64 kDa disappears and only the heavy band of ∼32 kDa presents in the gel for all three yam cultivars as shown in **Figure 1b**. Additionally, a new and slight band with a molecular mass of about 20 kDa is observed in *D. japonica*. According to previous studies (*6*, *7*), the band with a molecular mass of ∼32 kDa was identified as a dioscorin and the molecular mass of ∼64 kDa may come from the interaction of the monomeric dioscorin molecules. The unique band of ∼20 kDa in reduced SDS−PAGE for *D. japonica* illustrates its protein constituent differences as compared to the other two cultivars. This is anticipated as *D. japonica* belongs to different species. **Figure 2** shows the chromatographic profile for yam proteins of *D. alata* L., *D. alata* L. var. *purpurea*, and *D. japonica*, respectively. To make the molecular mass determination more accurate, calibration curves with correlative coefficients of 0.972 and 0.992 for high molecular mass and low molecular mass, respectively, are established by using protein standard compounds. All three cultivars consist of a major protein, eluted at 21.8 min, whose molecular mass is determined to be around 65 kDa, and a shoulder peak in *D. japonica* is about 52 kDa. Referring to SDS−PAGE results in **Figure 1b**, the molecular mass of ∼52 kDa may result from the coupling of dioscorin (32 kDa) with the slight band of 20 kDa, which is only found in the reduced SDS−PAGE of *D. japonica*. Results obtained from electrophoretic methods and chromatographic methods have in common that *D. japonica* is somewhat different from the other two cultivars. This is further proved by the compositional analysis by using the amino acid analyzer as listed in **Table 1**. *D. alata* L. and *D. alata* L. var. *purpurea* show insignificant differences in essential amino acids and most specific amino acids (except cysteine, isoleucine, and lysine). The amino acid compositions of *D. japonica*, however, exhibit conspicuously different characteristics from those of the other two cultivars. This is consistent with our studies on the approximate composition of yam powders, which shows that the content of crude protein is lower in *D. japonica* (8.15 ± 1.10%) than in the other two cultivars (13.31 ± 1.51% for *D. alata* L. and 11.27 ± 1.66% for *D. alata* L. var *purpurea*). Because the protein content in yam cultivar is governed by the genotype and the environmental conditions under which they are cultivated, the significant difference among various yam cultivars shown above is reasonable. As *D. alata* L. and *D. alata* L. var. *purpurea* belong to the same species, both present much resemblance in amino acid compositions. *D. japonica* belongs to a different species; the marked difference in amino acid compositions is expected.

Table 1. Amino Acid Compositions of Yam Protein (mg/g Protein)[a]

	Amino Acids									
cultivars	Asx	Thr	Ser	Glx	Pro	Gly	Ala	Cys	Val	Met
D. alata L.	17.31 ± 0.19[a]	4.59 ± 0.16[a]	6.69 ± 0.03[b]	22.58 ± 0.78[a]	4.46 ± 0.09[a]	2.80 ± 0.08[a]	3.89 ± 0.18[a]	1.18 ± 0.13[a]	4.91 ± 0.24[a]	1.36 ± 0.14[a]
D. alata L. var. *purpurea*	17.85 ± 0.19[a]	4.27 ± 0.03[a]	6.44 ± 0.10[b]	22.27 ± 0.64[a]	4.70 ± 0.18[a]	2.75 ± 0.01[a]	3.69 ± 0.07[a]	0.66 ± 0.10[b]	4.65 ± 0.26[a]	1.44 ± 0.32[a]
D. japonica	14.62 ± 0.70[b]	4.10 ± 0.28[a]	8.71 ± 0.67[a]	20.61 ± 1.02[a]	3.52 ± 0.09[b]	2.31 ± 0.20[a]	4.70 ± 0.51[a]	0.13 ± 0.07[c]	4.01 ± 0.40[a]	0.62 ± 0.13[a]

	Amino Acids								
cultivars	Ile	Leu	Tyr	Phe	His	Lys	Arg	total	EAA[b]
D. alata L.	4.71 ± 0.33[ab]	9.43 ± 0.34[a]	5.18 ± 0.11[a]	11.37 ± 0.32[a]	3.35 ± 0.09[a]	7.02 ± 0.14[a]	22.86 ± 1.30[a]	133.37 ± 4.68[a]	43.39 ± 1.68[a]
D. alata L. var. *purpurea*	4.96 ± 0.08[a]	9.45 ± 0.33[a]	4.92 ± 0.18[a]	10.78 ± 0.03[a]	3.94 ± 0.11[a]	6.70 ± 0.30[ab]	23.10 ± 0.10[a]	132.57 ± 3.03[a]	42.25 ± 1.34[a]
D. japonica	3.57 ± 0.33[b]	6.61 ± 0.62[b]	3.52 ± 0.20[b]	8.57 ± 0.26[b]	2.84 ± 0.07[a]	5.90 ± 1.29[b]	21.48 ± 1.94[a]	115.82 ± 7.67[b]	33.39 ± 2.20[b]

[a] Reported values are the means ± SD ($n = 3$). Data bearing different letters in the same column for each yam variety are significantly different ($P \leq 0.05$) by Duncan's multiple range tests for variables. [b] EEA, essential amino acids.

This is the first report demonstrating FT-Raman spectroscopic study of the molecular structure of yam proteins. Because the variability of proteins relies on the correct spatial placement of amino acid side chains and a defined protein backbone, i.e., a stable three-dimensional structure of proteins, it is important to determine the protein structure at the molecular level before any biological interests can be explored further. Raman spectroscopy is well-credited for structural characterization of proteins. According to previous Raman studies on the molecular structure of various proteins, the secondary structure of yam proteins can be easily determined by examining the vibrational stretching of amide I and amide III (*12*−*18*). **Figure 3a**−**c** shows the FT-Raman spectra, in the range of 800−1800 cm^{-1}, of yam proteins for *D. alata* L., *D. alata* L. var *purpurea*, and *D. japonica*, respectively. For the *D. alata* L., the secondary structure is mostly in an α-helix, as proved by the vibrational mode of amide I at 1662 cm^{-1} and amide III at 1263 cm^{-1}. The secondary structure of *D. alata* L. var *purpurea* is mostly in an antiparallel β-sheet, as evidenced by the amide I at 1668 cm^{-1} and amide III at 1241 cm^{-1}. The locations of amide I at 1667 cm^{-1} and amide III at 1257 cm^{-1} indicate that the secondary structures of *D. japonica* are in a mixed form of an α-helix and antiparallel β-sheet. The sharpening of amide III band in *D. alata L.* var *purpurea* may indicate the uniformity of H-bonds, whereas the flattening of amide III band in *D. alata* L. as well as *D. Japanica* may indicate the complexity of H-bonds (*34*). The differences of Raman peaks at 1317 and 1338 cm^{-1}, which is assigned to CH_2 deformation, also mark the difference in the secondary structure of yam proteins among various yam cultivars. The secondary structure of yam proteins apparently leads to the specific characteristics of each yam cultivars.

The microenvironmental property of major amino acids in yam proteins is easily illustrated by the Raman profile in the low-frequency range, as shown in **Figure 4**, such as 621 cm^{-1} for phenylalanine; 643, 828, and 853 cm^{-1} for tyrosine; and 759 cm^{-1} for tryptophan. As displayed in **Table 2**, different Raman ratios of $I_{643/621}$ (1.46 for *D. alata* L., 0.79 for *D. alata* L. var. *purpurea*, and 1.03 for *D. japonica*) among various yam cultivars reflect their different microenvironmental properties of tyrosine. According to Yu et al.'s studies on enzyme structure (*15*, *17*, *18*), the intensity increases of 644 cm^{-1} are a reflection of tyrosine side chain conformational changes; our results imply that tyrosine residues in different cultivars behave differently upon environmental impacts. The larger intensity ratio of $I_{643/621}$ means that it is more susceptible to external stresses. *D. alata*

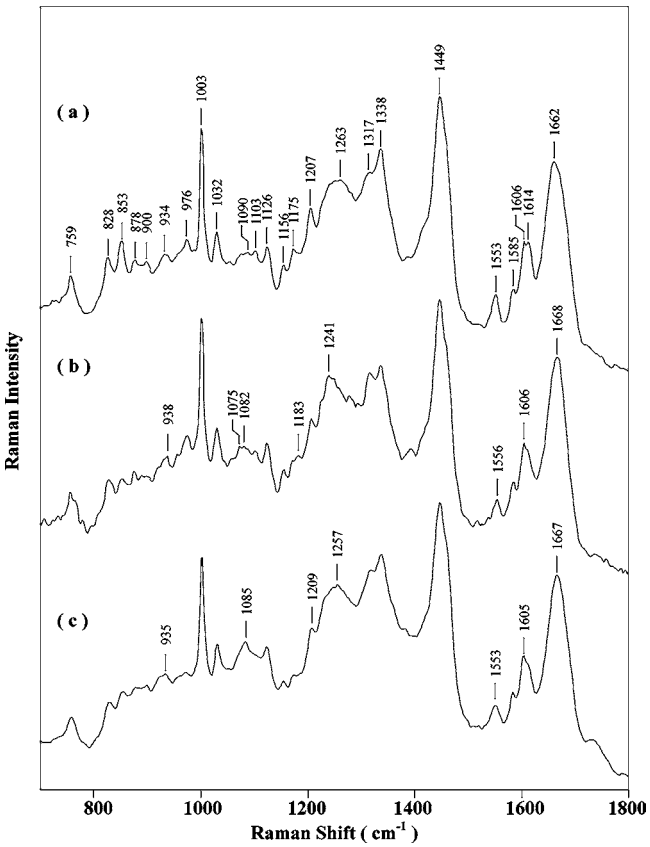

Figure 3. Raman spectra in the 800–1800 cm^{-1} region of yam proteins isolated from various yams: (**a**) *D. alata* L., (**b**) *D. alata* L. var. *purpurea*, and (**c**) *D. japonica*. Data acquisition conditions: excitation wavelength, 1064 nm; laser power, 150 mW; spectral resolution, 4.0 cm^{-1}; and coadded scan, 500 (∼15 min).

L., which has the highest Raman intensity ratio of $I_{643/623}$ among the three cultivars, is most susceptible to environmental changes. Siamwiza et al. (*32*) reported that the tyrosine doublet at 850 and 830 cm^{-1} was sensitive to the nature of the hydrogen bond of the phenol hydroxyl group. If a tyrosine residue is on the surface of a protein in aqueous solution, the phenolic OH will be both an acceptor and a donor of moderate to weak H-bond and the doublet intensity ratio ($I_{850/830}$) will be about 1:0.8 ($I = 1.25$). If the phenolic oxygen is the acceptor atom in a strong H-bond, the intensity ratio will be about 1:0.4 ($I = 2.5$). If the phenolic hydroxyl is the proton donor in a strong H-bond, the

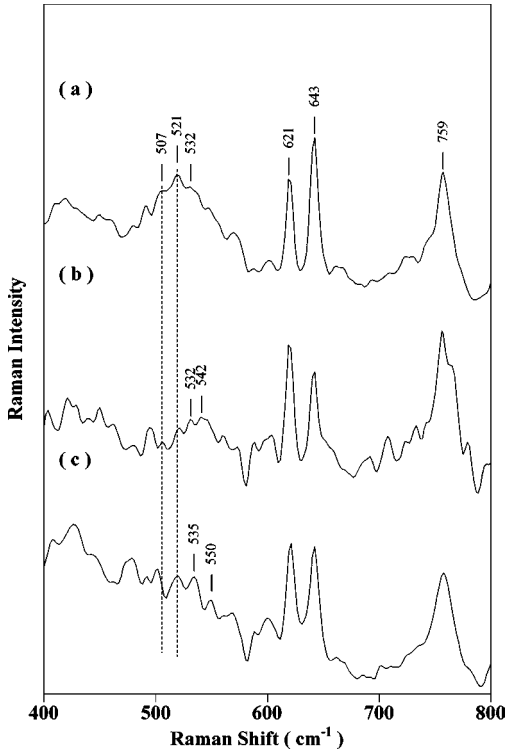

Figure 4. Raman spectra in the 400–800 cm^{-1} region of yam proteins isolated from various yams: (**a**) *D. alata* L., (**b**) *D. alata* L. var. *purpurea*, and (**c**) *D. japonica*. Data acquisition conditions: excitation wavelength, 1064 nm; laser power, 150 mW; spectral resolution, 4.0 cm^{-1}; and coadded scan, 500 (~15 min).

Table 2. Raman Intensity Ratios for Aromatic Amino Acids in Yam Proteins[a]

intensity ratio	cultivars		
	D. alata L.	*D. alata* L. var. *purpurea*	*D. japonica*
$I_{643/621}$	1.46 ± 0.03[a]	0.79 ± 0.01[c]	1.03 ± 0.03[b]
$I_{853/828}$	1.29 ± 0.02[a]	0.95 ± 0.03[c]	1.15 ± 0.02[b]
$I_{878/759}$	0.38 ± 0.01[a]	0.39 ± 0.02[a]	0.23 ± 0.02[b]

[a] Reported values are the means ± SD ($n = 3$). Data bearing different letters in the same raw for each yam variety are significantly different ($P \leq 0.05$) by Duncan's multiple range tests for variables.

intensity ratio will be 1:2 ($I = 0.5$). Accordingly, the current result, as shown in **Table 2**, in which the intensity ratios of $I_{850/830}$ in the yam proteins are about 1:0.78 ($I = 1.29$) for *D. alata* L., 1:1.05 ($I = 0.94$) for *D. alata* L. var. *purpurea*, and 1:0.87 ($I = 1.15$) for *D. japonica*, may indicate that the phenolic hydroxyls of tyrosine are on the surface of yam proteins to be a donor or acceptor to a weak H-bond. The Raman intensity ratios of $I_{643/623}$ and $I_{850/830}$ behave in the same trend and are in increasing order of *D. alata* L. var. *purpurea*, *D. japonica*, and *D. alata* L., which implies that *D. alata* L. is more accessible to externals. Miura et al. (*35*) proposed that the Raman bands at 880 and 1360 cm^{-1} of tryptophan could be used to monitor the strength of H-bonding and the hydrophobicity of the environment of the indole ring. When a tryptophan residue is buried, the relative intensity of the band at 880 cm^{-1} is strong whereas an exposed tryptophan gives a weak feature at 880 cm^{-1}. Hydrophobic interactions between an indole ring of trp residue and the surrounding aliphatic groups cause the 1360 cm^{-1} peak to increase and the 1340 cm^{-1} one to decrease. On the basis of the above description, the present result shows that tryptophan in the yam proteins prefers an exposed state. The intensity ratio of $I_{878/759}$, as shown in **Table 2**, exhibits no significant difference between *D. alata* L. and *D. alata* L. var. *purpurea*, which implies that tryptophan in both cultivars might experience a similar microenvironment. The microenvironment of tryptophan in *D. japonica*, however, is significantly different from them. This is consistent with previous results obtained from amino acid compositional analysis. As *D. alata* L. and *D. alata* L. var. *purpurea* belong to the same species, both present much resemblance in amino acid compositions. *D. japonica* belongs to a different species; it shows a marked difference in amino acid compositions. Raman spectral differences in the range from 600 to 1000 cm^{-1} among various yam proteins revealed their significant differences in the microenvironment of aromatic amino acids. Close review of Raman intensity ratios of $I_{643/621}$, $I_{853/828}$, $I_{878/759}$, and the Raman signal at 1338 cm^{-1} all comes to a consistent conclusion that the aromatic amino acids in *D. alata* L. are more susceptible to external impacts, for example, air oxidation. This may provide a possible explanation for the readily browning development in the flesh of *D. alata* L. during food processing, which would deteriorate its protein quality and acceptability. Earlier studies (*33*) showed that various yam cultivars exhibit different chemical/physical properties and biological functions. Also, it is found that various yam cultivars experienced different degrees of browning development during food processing. The susceptibility of the browning reaction is in the decreasing order of *D. alata* L., *D. japonica*, and *D. alata* L. var. *purpurea*. This trend is in good accordance with the Raman results mentioned above.

The disulfide bond plays an important role in protein folding and assembly. Generally, disulfide bonds are not made and broken in isolation under mild conditions. Hou et al. (*36*) reported that the major yam protein, dioscorin, might aggregate into oligomers via the covalent linking of the intra- and intermolecular disulfide bridges. We here present direct evidence of the presence of disulfide bonds in yam proteins. FT-Raman is capable of providing instructive information on the status of sulfhydryl groups in proteins. Chen et al. (*37*) reported that FT-Raman was sensitive enough to detect the redox properties of sulfhydryl groups in lens crystallins. Here, the presence of Raman stretching at 521 cm^{-1} clearly indicates the disulfide bonding in yam proteins, while the absence of any Raman peaks from 2400 to 2700 cm^{-1} (data not shown) signals the weakness of the S−H bond in yam proteins. This might suggest that the sulfhydryl groups in yam proteins predominate in the form of S−S. Coupled with the electrophoretic and chromatographic results shown in **Figures 1** and **2**, it implies that the high molecular mass of 65 kDa in these yam cultivars may be due to the covalent linkage of disulfide bonds of dioscorin molecules (32 kDa) forming dimers in crude extracts of dioscorin from yam tuber. The intensity of Raman peaks in this area is much stronger for *D. alata* L. than for *D. alata* L. var. *purpurea* and *D. japonica*, meaning that the content of disulfide bonds in *D. alata* L. is much higher than that in *D. alata* L. var. *purpurea* and *D. japonica*. This is completely consistent with the compositional analysis by amino acid analyzer (**Table 1**) showing that the cysteine content in *D. alata* L. is higher than in *D. alata* L. var. *purpurea* and *D. japonica*. Furthermore, the conformation of disulfide bonds in various yam cultivars also exhibits different characteristics, as demonstrated by the variability of Raman profiles in the range of 500−600 cm^{-1}. The conformation of S−S in *D. alata* L. favors in gauche−gauche−trans form, as evidenced by the strong peak at 521 cm^{-1}, while

D. alata L. var. *purpurea* predominates in the trans−gauche−trans conformation, which is proven by the S−S stretching at 542 cm^{-1} (*29*).

In summary, current Raman results clearly indicate that the secondary structure of major yam proteins shares an α-helix and an antiparallel β-sheet with different proportions of each. For the *D. alata* L., the secondary structure of protein was mainly in the α-helix; *D. alata* L. var *purpurea*, however, is mostly in an antiparallel β-sheet, and *D. japonica* is in a mixed form of an α-helix and antiparallel β-sheet. Structural difference among each yam cultivars also can be easily obtained by examining the vibrational modes of the FT-Raman profile at a low-frequency range. Variation in the Raman intensity of 621, 643, 760, 829, 855, and 1338 cm^{-1} clearly indicates the different behaviors of major amino acids among various yam cultivars. This is in good agreement with the result obtained from amino acid analysis. It is apparent that differences in the Raman profile among various yam proteins reflect their intrinsically compositional differences and distinct spatial arrangement. Moreover, different degrees in the susceptibility of browning development for various yams cultivars may be correlated to their distinct structural characteristics.

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