Structural characterization of dioscorin, the major tuber protein of yams, by near infrared Raman spectroscopy

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Abstract. As very little is known about the molecular structure of dioscorin, the major storage protein of yam tuber, we report here FT-Raman spectroscopic investigation of this yam protein isolated from D. alata L., for the first time. According to a series of purification and identification by ion-exchange chromatography, gel chromatography, SDS-PAGE, and MALDI-TOF-MS, it shows that the major storage protein is made up of dioscorin A (M.W. ~33 kDa) and dioscorin B (M.W. ~31 kDa). Raman spectral results indicate that the secondary structure of dioscorin A is major in \( \alpha \)-helix, while dioscorin B belongs to anti-parallel \( \beta \)-sheet. It also shows that the microenvironment of major amino acids including tyrosine, phenylalanine, tryptophan, and methionine, and cysteine exhibit explicit differences between these two components. The conformation of disulfide bonding in dioscorin A predominates in Gauche-Gauche-Trans form, while Gauche-Gauche-Gauche and Trans-Gauche-Trans share the conformation in dioscorin B. Structural resemblance between dioscorin A and crude yam proteins implies that dioscorin A exhibits structural preference even though its content is lower than dioscorin B.

1. Introduction

As an important staple food in many tropical countries, yam, the tuber of Dioscorea spp., also receives much attention on its functional properties and pharmaceutical potential.1,2 Yams have been used as a health food and herbal medicine in Chinese society. Several beneficial properties of yams have been reported recently.3-7 Yam extracts shows antioxidative activity and modified serum lipid levels in human.3 Hou et al.4 show that the dioscorins purified from D. batatas, D. alata, and D. pseudojaponica all exhibit carbonic anhydrase activity. The dioscorin from D. batatas also possesses antioxidant properties, with dehydroascorbate reductase and mono-dehydroascorbate reductase activities, and an ability to scavenge against both 1,1-diphenyl-2-picrylhydrazl radicals and hydroxyl radicals.5,6 Besides, dioscorin has been demonstrated to inhibit angiotensin-converting enzyme activity, which is a target for pharmacological agents used in the treatment of hypertension.7 To unveil the remarkable biological properties of yam proteins, a number of studies have been focused on their sequence determination and functional properties exploration.8,11 However, very little is known about the structure of yam proteins to date. It is of great importance to determine the molecular structure of dioscorin since protein structure plays a key role in directing relative biological functions; for example, protein folding–unfolding process is critical to enzymatic activity. Generally, x-Ray crystallography is used to determine the three-dimensional structure of 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 limits x-ray’s application. Since there is little article reported on the structural characteristics of major yam proteins, we report here the structural characterization of dioscorins isolated from *Dioscorea alata L.* by near infrared Fourier transform Raman spectroscopy.

2. **Experimental Section**

Fresh yams tubers of *D. alata* L. was purchased from 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 dodecylsulfate (SDS), protein standard reagents were purchased from 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). Ethanol was obtained from Taiwan Wine & Tobaco Inc. (Chiayi, Taiwan). All the chemicals for chromatography were analytical grade, and others except for electrophoresis were reagent grade.

2.1. Isolation and purification of dioscorin from yam tuber

Sample purification following those of Harvery and Boulter with some modifications was carried out at 4°C. Yam tubers were washed with water, peeled and cut into pieces right before dioscorin extraction. Pieces of yam were first macerated with acetone or 95% ethanol to remove mucous material and then were filtrated with cotton clothe. The residual was weighed and homogenized with 5 volumes (w/v) of 50mM Tris-HCl buffer (pH 8.3) and forwarded to centrifugation at 23,130g for 30 min. After centrifugation, the crude yam protein in the supernatant was salting out with 0 to 70 % ammonium sulfate. The precipitate from the ammonium sulfate solution was dialyzed against deionized and distilled water, lyophilized, and saved for further purification.

Crude yam protein was further purified by ion exchange chromatography and then analyzed with gel chromatography by a Hitachi D-7000 HPLC system (Hitachi, Ltd., Tokoy, Japan) equipped with a TSK gel SW guard column (4 cm x 8mm) and a TSK G3000 SW (30 cm x 8 mm) (TosoHaas, Japan). The system was equipped with Model L-7100 pump, Model L-7420 UV-VIS detector, and Rheodyne Model 7725 injector. Peaks were detected at wavelength 280nm, and acquisition and processing of data were completed by Hitachi B-7000 software with AID interface. 0.1M Sodium phosphate with 0.05% sodium azide driven by the pump system was prepared as mobile phase. Buffer solution was sonicated to deaeration right before employing. Each sample was filtered using 0.45μm sterile units (Millipore, US), and 10μL was injected in the chromatographic system with an optimal flow rate. A typical analysis could be completed in 40 minutes with the flow rate of 0.6 mL/min.

Electrophoresis on sodium dodecyl sulfate polyacrylamide slab gel (5% stacking and 14% resolving gel) was performed in a Mini-Protean II Dual Slab Cell (Bio-Rad Lab, Richmond, CA) as described by Laemmli with some modifications. The polyacrylamide gels were formed by copolymerization of acrylamide and bis-acrylamide with the aid of initiator TEMED and catalyst ammonium persulfate. The final buffers of the stacking and resolving gels were 0.125M Tris-HCl (pH 6.8), and 0.375M Thris-HCl (pH 8.9), respectively, and 0.1% SDS. The electrode buffer (pH 8.6) consisted of 0.1% SDS, 0.1% 2-mercaptoethanol, 0.19M glycine, 0.025M Tris-HCl, and 1mM EDTA. The sample buffer contained the final concentration of 0.1% SDS buffer, 10% sucrose, 0.05% bromophenol blue and 20mM dithiothreitol. The sample solutions (~15μL) in vials were mixed thoroughly by microcentrifuge at 1200 rpm (Hettich GmbH & Co., Tuttinglen, Germany) and heated in Dry-Bath (Dubuque, Iowa, USA) for 1 minute before applying to the gels. Electrophoresis was carried out with a fixed voltage of 160 V for 50 minutes. Gel after electrophoresis were stained with 0.25% Coomallie brilliant blue in 12.5% trichloroacetic acid, 20% methanol, and 7.0% acetic acid for 20 minutes and destained with 20% methanol and 7.0% acetic acid overnight.

2.2. Raman measurement
FT-Raman spectra of yam proteins were obtained by using a Bruker RFS-100 FT-spectrophotometer (Bruker Optik GmbH, Lubeck, Germany). Sample was put into the tiny hole of a stainless steel holder for Raman measurement. Continuous wave near infrared excitation at 1064 nm was provided by a diode laser pumped Nd:YAG laser (Coherent Lubeck GmbH, Lubeck, Germany). The laser light with 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 which 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 time about 15 minutes.

3. Results and discussion

Yams (*Dioscorea* spp.) are classified as monocotyledonous but are considered to be closely related to dicotyledonous plants. Harvey and Boulter⁹ reported that a major group of proteins with molecular mass of around 31 kDa was present at yam tuber. Colans *et al.*¹⁰ reported the cloning of cDNA for the major tuber proteins from *D. cayenensis*, of which proteins encoded by the clones are estimated to have masses of 28-29 kDa. Two classes of cDNA encoding dioscorin A and B, respectively, are identified and the proteins share 69% sequence similarity with each other.¹⁰ Our results, based on chromatographic and electrophoretic analysis, also indicated that the major storage protein of *D. alata* L. is made up of dioscorin A (M.W. ~33 kDa) and dioscorin B (M.W. ~31 kDa). Fig.1 shows the FT-Raman spectra of crude protein, dioscorin A, and dioscorin B, respectively. The secondary structure of dioscorin A is mainly in α-helix as clearly proved by the vibrational mode of amide I at 1657 cm⁻¹ and amide III at 1271 cm⁻¹ (Fig.1c). The locations of amide I at 1668 cm⁻¹ and amide III at 1240 cm⁻¹ as shown in Fig.1b indicate that the secondary structure of dioscorin B belongs to anti-parallel β-sheet. The flattening of amide III band in *D. alata* L. may indicate the complexity of H-bonds.¹²-¹³ The fluctuation of Raman peaks at 1317 cm⁻¹ and 1338 cm⁻¹ which is assigned to CH₂ deformation also marks the difference in the secondary structure between dioscorin A and dioscorin B. Structural difference between both components can also be easily obtained by examining the vibrational modes of FT-Raman profile such as 621 cm⁻¹ for phenylalanine, 643 cm⁻¹, 828 cm⁻¹, and 853 cm⁻¹ for tyrosine, 759 cm⁻¹ for tryptophan. Variation in the Raman intensity of 621 cm⁻¹, 643 cm⁻¹, 759 cm⁻¹, 828 cm⁻¹, and 853 cm⁻¹ clearly indicated the different behaviors of major amino acids in different dioscorins. According to the variation of vibrational modes around 500-540 cm⁻¹, the conformation of disulfide bonding in dioscorin A predominates in Gauche-Gauche-Trans form; while Gauche-Gauche-Gauche and Trans-Gauche-Trans share the conformation in dioscorin B. Structural resemblance between dioscorin A and crude yam proteins implies that dioscorin A exhibits more structural preference than dioscorin B. The differences in the Raman profile between dioscorin A and dioscorin B maybe due to their intrinsically compositional differences and distinct spatial arrangement.

In summary, dioscorins isolated from *D. alata* L. was purified and identified as a dioscorin A (M.W. ~33 kDa) and dioscorin B (M.W. ~31 kDa). The major storage protein is made up of dioscorin A (M.W. ~33 kDa) and dioscorin B. Raman spectral results indicate that the secondary structure of dioscorin A is major in α-helix, while dioscorin B belongs to anti-parallel β-sheet. It also shows that the microenvironment of major amino acids including tyrosine, phenylalanine, tryptophan, and methionine, and cysteine exhibit explicit differences between these two components. The conformation of disulfide bonding in dioscorin A predominates in Gauche-Gauche-Trans form, while Gauche-Gauche-Gauche and Trans-Gauche-Trans share the conformation in dioscorin B. Structural resemblance between dioscorin A and crude yam proteins implies that dioscorin A exhibits structural preference even though its content is lower than dioscorin B.

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