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Biological Activities and Applications of Dioscorins, the Major Tuber Storage Proteins of Yam

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Abstract

Yam tubers, a common tuber crop and an important traditional Chinese medicine in Taiwan, have many bioactive substances, including phenolic compounds, mucilage polysaccharides, steroidal saponins and proteins. Among the total soluble proteins, 80% of them are dioscorins. In the past two decades, many studies showed that dioscorins exhibited biological activities both *in vitro* and *in vivo*, including the enzymatic, antioxidant, antihypertensive, immunomodulatory, lectin activities and the protecting role on airway epithelial cells against allergens *in vitro*. Some of these activities are survived after chemical, heating process or enzymatic digestion. Despite of lacking the intact structural information and the detail action mechanisms in the cells, yam dioscorins are potential resources for developing as functional foods and interesting targets for food protein researchers.

Key words: Allergen, Antihypertensive protein, Antioxidant, Dioscorin, Immunomodulatory, Lectin, Yam tuber storage protein

Overview

Yams (*Dioscorea* spp., *Dioscorea*ceae) are one of important tuber crops in Africa, Asia, and Middle and South America and also staple foods in Caribbean. In the traditional Chinese medicine classic, The Divine Husbandman's Herbal Foundation Canon (神農本草經 shén nóng běn cǎo jīng), the dried slices of *Dioscorea*e Rhizoma (山藥 shān yào; the tuber of *Dioscorea* spp.) are classified as top grade (上岛 shàng pǐn) which are juvenescent with no toxic effect when long-term uses. The yam tubers, like other storage roots, are rich in starch, but they also contain about 1-3% protein in fresh samples. On the dry weight basis, the crude protein content raises to ca. 6-13%. Harvey and Boulter (1983) first isolated the major group of proteins accounted for approximately 85% of the total soluble protein content by alkaline buffers (borate or Tris-HCl buffer, pH 8.3) and anion-exchange chromatography from the tubers of D. rotundata cv Nwapoko. They are assumed to deposit aggregately in vacuoles of the storage cells and share lots of characteristics, such as high amide content and solubility, with other storage proteins. Conlan et al. (1995) first reported two classes of cDNA clones encoding the major tuber storage proteins of the D. cayenensis. The protein coding regions between the two classes, A and B, represent 84.1% similarities and the deduced amino acid sequences are 69.6% similarities with respected to each other, but with no sequence identity with that of patatin or sporamin, the storage protein of potato and sweet potato, respectively. Their findings brought more detail information and the name, dioscorins, was first introduced to represent the yam tuber storage proteins. In this article, we will review

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some of the biological activities of dioscorins and also compare these from different yam species.

Characteristics of Dioscorins

The monomeric molecular weights of dioscorins purified from different species are about 31 kDa (Harvey and Boulter, 1983; Conlan et al., 1995; Hou et al., 2000; Liao et al, 2004). They can be extracted by alkaline buffer (borate or Tris-HCl buffer, pH 8.3) from the tubers and simply purified by weak anionexchange chromatography, ex. DE-52 resin, with the purity > 95%. In different buffer systems, the monomers usually associate to form dimers, tetramers and higher molecular weight polymers by noncovalent or disulphide inter-molecular interactions. Sample preparation in the presence of SDS and 2-mercaptoethanol seems to facilitate the breakdown of dioscorins. By prior treatment of urea or guanidine-HCl, followed by reduction and alkylation, the breakdown phenomenon could be minimized (Harvey and Boulter, 1983; Harvey and Boulter, 1985). Figure 1 shows the alignment of protein sequences of different dioscorins. The precursor of dioscorins contains a signal peptide in their N-terminal, which may be removed by processing protease in the vacuoles, but the detailed mechanism is still unclear. Even the carbohydrates cannot be detected in the dioscorins purified by Harvey and Boulter. The comparison of molecular weight between native protein and protein deduced from cDNAs shows the post-translational modifications occur (Conlan et al., 1995). Further study by Con A-peroxidase analysis system shows that, at least, some of the dioscorins are glycoproteins (Hou et al., 2000). The structural information of dioscorins is really limited. Conlan et al. (1995) showed that the secondary structure contents of dioscorins analyzed by far UV circular dichroism (CD) spectroscopy were about 33% α -helix, 8% β -sheet and 38% β -turn. By using near infrared Fourier transform Raman spectrometry analysis (Liao et al., 2004), the secondary structure of dioscorin from D. alata L. was majorly α -helix, while that from *D. alata* L. var. purpurea was mostly antiparallel β -sheets even their amino acid compositions were similar. Dioscorin from D. japonica, with different amino acid compositions, had a mixed form of α -helix and antiparallel β -sheets. These show that dioscorins from different sources might have different structure and activities.

Biological Activities

α-Carbonic Anhydrase (α-CA) Activity

Carbonic anhydrases (CAs) are zinc metalloenzymes that catalyze the interchange of CO_2 and $HCO_5^{/}$, play a key role in CO₂ fixation for photosynthesis in cyanobacteria and plants. In mammals, CA reaction is involved in respiration and transport of CO₂ between lungs and tissues. To date, at least five genetically unrelated families (α , β , γ , δ and ε) of CAs were reported, the detail information could be found in recent reviews (Supuran, 2008; Gilmour, 2010). cDNA alignment of dioscorins and CAs shows significant sequence similarity to α -CAs, but may fail to have activity because of the alternations in conserved activesite and mismatch in one of three Zn-liganding sites (Hewett-Emmett and Tashian, 1996). Hou et al. (1999b) first report the experimental evidence for the CA activity of dioscorin. They purified dioscorin from D. batatas Decne, the CA activity was determined from the direction of dehydration of sodium bicarbonate by pHstat technique and the autotitration was done with 0.1 M H₂SO₄ to pH 7.1 set as a fixed end point and SDS-PAGE followed by active staining by color change of bromothymol blue. Subsequently, dioscorins from other five cultivars of three D. species were also proved to have CA activity by activity stainings, including D. batatas Decne var. Shoufeng, D. alata L. var. Tainong 1, D. alata L. var. Tainong 2, D. alata L. var. Zhongguochang and D. pseudojaponica var. Keelung (Hou et al., 2000). Interestingly, the CA activities could be found in all dioscorins with no appreciable amount of zinc atom detected and the activities remained after 2-mercaptoethanol, acetazolamide (inhibitor of CAs) or 2,6-pyridinedicarboxylic acid (Zn-chelating agent) treatments. These findings show dioscorins are novel α -CAs, but no further investigations have been made until now.

Antioxidant Activity

The antioxidant is defined as a molecule that has the ability to slow or prevent the oxidation of other molecules. The oxidation damages on physiological substances are highly related to many diseases, such as atherosclerosis, aging, neurodegenerative diseases and cancers (Moon and Shibamoto, 2009). Hou et al. (1999a) showed that dioscorin isolated from *D. batatas* Decne had both dehydroascorbate reductase and monodehydroascorbate reductase activities *in vitro* at the pH close to neutral, by these, dioscorin could signal peptide

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	188	NQ-LC	DDSAY	RYMGSFT	APPCTI	GISWT	VMRKV	ATVSP	RQVLM	LKQAY	NENA	INNARP	VQPTNF	252
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	196	NO-LO	DDSAY	RYMOSET	APPER	GISWT	VIRKV	ATVER	ROVIL	LKOAL	NENA	INNARP	LOPTNE	252
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Figure 1. The sequence alignments of dioscorins from various species. 20 protein sequences of dioscorins from NCBI protein database are aligned by ClustalW2 and modified by Jalview.

26.8

256 R SV FY FEQ L K SK L GV I 253 R SV FY FEQ L K SK L GV I

The arrow denotes the first N-terminal residue sequenced by Conlan et al. (1995) and gaps are presented by dashes. All locations with cysteine residues present are marked with dark-red frames (if you print with grayscale, you can use the asterisk in the bottom of the frame instead). The abbreviations for *Dioscorea* spp. are as follows: Da, *D. alata*; DaTN1, *D. alata* L. cv. Tainong 1; Dc, *D. cayenensis*; Dj, *D. japonica*; DpjK, *D. pseudojaponica* var. Keelung. The accession numbers are signed inside the bracket of the names. The different colors marked for amino acid residues are based on the properties of their side-chain groups: \blacksquare for glycine and proline; \square for aliphatic; \blacksquare for aromatic; \blacksquare for positive charged; \blacksquare for poly, uncharged; \blacksquare for cysteine.

reduce dehydroascorbate and monodehydroascorbate to generate ascorbate in turn to reduce the ROS and increase the scavenger concentration at the same time. Hou et al. (2001) reported that dioscorin exhibited the scavenging activity against both 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radical in a dose-dependent manner *in vitro*. The use of pepsin to simulate dioscorin digestion *in vivo*, it was found that DPPH scavenging activities of peptic hydrolysates were increasing with hydrolyzed time and active fraction of small molecules were revealed by gel permeation chromatography (Liu and Lin 2009). It is noted that some Cys-containing synthetic peptides derived from computer-aided dioscorin hydrolysis show DPPH and hydroxyl radical scavenging activities.

Antihypertensive Activity

Hypertension, a high prevalence chronic disease in the world, is a clear risk factor for several kinds of cardiovascular disease, such as coronary vascular disease (CVD) and stroke. In prehypertension state, lifestyle changes are suitable for improvement. When blood pressure goes up, pharmaceutical treatments will be required. Among all classes of antihypertensive drugs, ACE inhibitors are wildly use in hypertension patients and patients with other complications, ex. diabetes and chronic kidney disease (JNC 7). Angiotensinconverting enzyme (ACE, EC 3.4.15.1), a part of the renin-angotensin-aldosterone (RAA) system, is an exopeptidase that can convert angiotensin I (decapeptide) to the potent vasoconstrictor, angiotensin II (octapeptide). ACE also degrades bradykinin and other vasodilatory peptides (Zaman et al., 2002). Even the major adverse effect of dry cough, the relative low adverse effect rate and renal protection effect make them become the commonly prescribed class. Hsu et al. (2002) first reported that native protein of dioscorin purified from D. alata cv. Tainong No. 1 (TN1dioscorin) and its peptic hydrolysates presented ACE inhibitory activities in dose-dependent manner. The IC₅₀ of dioscorin on ACE in reported assay system was 6.404 µM compared to 0.00781 µM of captopril, the first commercial ACE inhibitor. According to kinetic analysis, dioscorin showed mixed noncompetitive inhibition against ACE. When 31.25 µg discorin was applied, the apparent inhibition constant (K_i) was 2.738 µM. Following this study, Lin et al. (2006) fed the spontaneously hypertensive rats (SHRs) with TN1-dioscorin and its peptic hydrolysates. In shortterm (24h) experiments, both TN1-dioscorin and its

peptic hydrolysates (administrated orally once by the dose 40 mg/kg) showed significant lowing on mean blood pressure (MBP), systolic blood pressure (SBP) and diastolic blood pressure (DBP) and the pressurelowing effect were equal to the captopril group. In the long-term measurement, the TN1-dioscorin was orally administrated once daily for 25 days in the dose of 40 mg/kg. The greatest reduction in BP was appeared on day 9 and the BP-lowing activity remained in the end of experiment. In order to evaluate the BP-lowing activity on human, the instant food (30 g) with and without lyophilized yam powder, containing 140 mg dioscorin determined by ELISA, was made and applied daily on hypertensive subjects for 5 weeks, followed by 1 week washout and then a 5 week crossover. By this double-blind, placebo-controlled trial, the intake of dioscorin-containing meal showed a regulating effect on human blood pressure (Liu et al., 2009a). Liu et al. (2009b) evaluated the BP-lowing different preparations of yam products on SHRs, all of them containing dioscorin, but the amounts were not determined. The results showed that different food processing methods, including alcoholic-insoluble solids from 80% ethanolic extraction, tuber slices by hot-air-drying and steamcook and water extracts by hot water treatment (90°C and 95°C) for 10 minutes, show no significant changes in the BP-lowing activities in the SHR model. Thus, the antihypertensive activity of dioscorins or yam processing products can be easily applied in the development of healthy or functional foods. Several synthetic peptides derived from computer-aided dioscorin hydrolysis show ACE inhibitory activities, and the oral administration of these peptides to SHR is currently undertaken for BP regulations. It is noted that a synthetic peptide derived from computer-aided dioscorin hydrolysis show antihypertensive activity by vasorelaxation effects with or without endothelium by using phenylephrine-contracted thoracic aorta from SD rats.

Immunomodulatory Activity

The innate immunity is the first defense line of the immune system. Many of the involved molecules have the property of pattern recognition and can be divided into to groups of soluble molecules or cell-associated receptors. Toll-like receptors (TLRs), a kind of pathogen recognition receptors (PRRs), play a key role in innate immunity. In human, 10 TLR family members have been identified; each of them has its own pathogenassociated molecular patterns (PAMPs) (Kawai and

Akira, 2011). TN1-dioscorin activates both NF-κB and MAPKs (JNK, p38 and ERK1/2) signaling via TLR-4 and induces the expression of inducible nitric oxide synthase (iNOS) and pro-inflammatory cytokines (TNF- α , IL-1 β and IL-6) in bone marrow cells isolated from C3H/HeN mice (TLR-4 functional) and the RAW 264.7 cells which these effects are eliminated in the TLR-4 dull mice, C3H/HeJ (Fu et al., 2006). In addition, TN1-dioscorin enhances the phagocytosis against E. coli, the oxidative burst activity of RAW 264.7 cells and the proliferation of spleen cells isolated from BALB/c mice (Liu et al., 2007). When oral administration of TN1-dioscorin (2.5 and 20 mg/kg/day) to BALB/c mice for 21 days, the lymphocyte subpopulation in both peripheral blood and splenocytes are changed. The significant enhancement of phagocytosis and natural killer (NK) cell cytotoxic activity are appeared. The Peyer's patches and sIgA in the feces are also increased which shows the immunomodulatory activity might be related to mucosal immune responses (Liu et al., 2009). Lin et al. (2009) compared the immunomodulatory activities of dioscorins from two different species, D. alata cv. Tainong No. 1 and D. japonica (Djdioscorin), in mice. TN1-dioscorin stimulated major on phagocytosis activity of lymphoid cells, whereas the major immunomodulatory activity of Dj-dioscorin was lymphoid cells proliferation. These results show that dioscorins from different species may behave different activities on immune responses.

Lectin Activity

Lectins, a kind of sugar-binding protein with high specificity to their own sugar moieties, play the key role of cells, pathogens and molecules recognition in both animals and plants. They can be applied for cell selection, glycoprotein isolation, sugar moiety identification and so on. In addition, some lectins, like concanavalin A (Con A) and phytohaemagglutinin (PHA), have mitogen activity that can stimulate lymphocytes proliferation in functional immune system (Sharon, 2007; Michiels et al., 2010). Gaidamashvili et al. (2004) isolated four proteins from D. batatas, three of them (DB1, DB3 and DB4) existed lectin activity. The large subunit of the DB3 (DB3L), which was further compose of two 31-kDa subunits linked by disulfide bond, was homologous with class B dioscorins and responsible for the full lectin activity of DB3. But the relationship between lymphocytes proliferation and lectin activity of dioscorins still needs further investigations.

Airway Epithelial Cells Protection

In the airway, the first line of host defenses is the barrier of mucus and epithelial cells. The mucus can trap the pathogens and remove them before they can contact with the epithelial cells, but unfortunately, because of the decrease in secretion cell (type II alveolar epithelial cells and Clara cells), the mucociliary clearance is dramatically impaired. The airway epithelial cells protect against the pathogens by three ways, the first is the secretion of multiple proteases to degrade the pathogens; the second is to secret protease inhibitors which inactive the proteases derived from pathogens and protect themselves against proteolytic effects from host immune cells; the last one is the epithelial tight junctions that prevent pathogens penetrate into subepithelial mucosa. House dust mite (HDM) allergens, the most common allergen in the world, and some fungal allergens containing serine proteases which can destroy the tight junction via lyse the junction-associated proteins, for example, claudin-1, zonaoccludens-1 (ZO-1), occludin, E-cadherin (EC) and desmoplakin (DP). In addition, some of these allergens can directly induce the cytokine releases of epithelial cells that facilitate the permeability of the pathogens (Proud and Leigh, 2011). Ko et al. (2009) demonstrated that dioscorin had the protective ability in A549 cells against damages of dust mite extracts by maintaining the tight junction structure and the expression of junctionassociated proteins, including ZO-1, EC and DP. As the trypsin, a serine-type protease, inhibitory activities of dioscorins were reported (Hou et al., 1999b and 2000), and the authors proposed that the protective effect of dioscorin in airway epithelial cells might be from the antiprotease activity.

Conclusion

In summary, dioscorins are easily purified proteins rich in the tuber of many species of yam. They exhibit antioxidant, antihypertensive, immunomodulatory, lectin activities and can protect airway epithelial cells against dust mite allergen destruction (Figure 2). They also show some enzyme activities, such as α -CA, DHA reductase and MDA activities, and present minor trypsin-inhibitor activity. Even after oral digestion, the antihypertensive and immunomodulatory effects remain. According to these properties, dioscorins are worth developing as healthy or functional foods. Further investigations are required in the studies of the mechanism of actions, structures and findings on new



Figure 2. Summary of biological activity of dioscorins

biological activities.

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