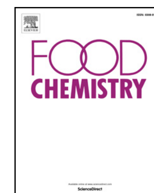
Food Chemistry 260 (2018) 27–36

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Antityrosinase and antioxidant properties of mung bean seed proanthocyanidins: Novel insights into the inhibitory mechanism

Wei-Ming Chai*, Chong Ou-Yang, Qian Huang, Mei-Zhen Lin, Ying-Xia Wang, Kai-Li Xu, Wen-Yang Huang, Dan-Dan Pang

College of Life Science and Key Laboratory of Ministry of Education, Jiangxi Normal University, Nanchang, Jiangxi 330022, China

ARTICLE INFO

Keywords:
Mung bean
Proanthocyanidins
MALDI-TOF MS
ESI-Full-MS
HPLC-ESI-MS
Tyrosinase inhibitor
Antioxidant

ABSTRACT

This study investigated the structure, antioxidant activity, antityrosinase activity and mechanism of proanthocyanidins from mung bean seed [*Vigna radiata* (L.) Wilczek]. The structural composition were characterized by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), electrospray ionization-full-mass spectrometry (ESI-Full-MS), and high-pressure liquid chromatography-electrospray ionization-mass spectrometry (HPLC-ESI-MS) techniques. The mung bean seed proanthocyanidins were composed of procyanidins, prodelphinidins, and their rhamnosides. According to enzyme kinetic analysis, these compounds were potent, reversible, and mixed-type inhibitors of tyrosinase. They inhibited the enzyme activity by interacting with enzyme as well as substrates. The results of molecular docking showed that the interaction between mung bean seed proanthocyanidins and tyrosinase was driven by hydrogen bond, hydrophobic and electrostatic interactions. In addition, mung bean seed proanthocyanidins were demonstrated as powerful antioxidants. Therefore, this study confirmed a novel tyrosinase inhibitor and would lay a scientific foundation for their utilization in pharmaceutical and food industries.

1. Introduction

Tyrosinase (EC 1.14.18.1) is a rate-limiting enzyme of melanogenesis (Seo, Sharma, & Sharma, 2003). It catalyzes tyrosine to dihydroxyphenylalanine ($_L$-DOPA), and $_L$-DOPA to o-quinones (Korner & Pawelek, 1982). Its abnormal expression brings about various dermatological diseases (Kim & Uyama, 2005). In the food industry, tyrosinase induces enzymatic browning, which generally leads to significant losses of nutritional quality and market value of many fruits and vegetables (Loizzo, Tundis, & Menichini, 2012). These phenomena have encouraged researchers to search new potent tyrosinase inhibitors for their use in the medical, food, and agricultural industries.

Autoxidation in food and biological systems results in a multitude of adverse effects in food preservation as well as in human health (Shahidi & Zhong, 2010). Antioxidants can stop the oxidization of polyphenolic compounds in the fruit and vegetables. They play a crucial role in preventing or delaying autoxidation and have attracted much attention as food preservative, dietary supplements and natural health products (Kaur & Kapoor, 2001; Shahidi & Zhong, 2010). In addition, the intake of food containing high amounts of antioxidative nutraceuticals helps to reduce the risks of various diseases (Kaur & Kapoor, 2001; Shahidi & Ambigaipalan, 2015). Therefore, there is an increasing interest in searching natural antioxidants (e.g., flavonols, flavonoids, procyanidins, tannins, anthocyanins) existed in dietary plants (Chai, Shi et al., 2014a; Kaur & Kapoor, 2001).

Proanthocyanidins are polyphenol compounds naturally present in legume seeds. They have attracted significant research and practical attention because of their various biological activities (Chai, Chen et al., 2014b; Li et al., 2016). These compounds are formed of flavan-3-ol monomers, which are polymerized into B-type proanthocyanidins (mainly) and A-type proanthocyanidins (Santos-Buelga & Scalbert, 2000) (Fig. 1 in supplementary materials). The proanthocyanidins have a high structural diversity that is based on the monomer units, substituent, interflavan linkage, and degree of polymerization (DP) (Dixon, Xie, & Sharma, 2005). Thus, there is still difficult in the characterization of polymerized proanthocyanidins.

Mung bean [*Vigna radiata* (L.) Wilczek] is one of the most significant edible legumes grown widely throughout the world. Many bioactive substances including flavonoids (eg. quercetin-3-glucoside, quercetin, myricetin, and kaempferol), phenolic acids (eg. p-hydroxybenzoic, protocatechuic, and gallic acid), organic acids, and polysaccharides have been identified from it in the past decades (Tang, Dong, Ren, Li, & He, 2014). However, there is no relevant report about the mung bean proanthocyanidins, including their structure and bioactivity. This

* Corresponding author.
E-mail address: chaiweiming@jxnu.edu.cn (W.-M. Chai).

https://doi.org/10.1016/j.foodchem.2018.04.001
Received 5 December 2017; Received in revised form 8 March 2018; Accepted 1 April 2018
Available online 02 April 2018
0308-8146/ © 2018 Elsevier Ltd. All rights reserved.

research therefore aimed to study the structure, antityrosinase and antioxidant activities of these compounds and provided a scientific foundation for their uses in food, nutritional, cosmetic and medical industries.

2. Experimental

2.1. Chemicals and materials

We purchased mushroom tyrosinase, $_{\text{L}}$-tyrosine, $_{\text{L}}$-3,4-dihydroxyphenylalanine ($_{\text{L}}$-DOPA), Sephadex LH-20, high performance liquid chromatography (HPLC) standards, benzyl mercaptan, formic acid, caesium chloride (CsCl), 2,5-dihydroxybenzoic acid, gallic acid, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-S-triazine (TPTZ), Trolox, ascorbic acid (Vc), and butylated hydroxyanisole (BHA) from Sigma-Aldrich (St. Louis, MO, USA). Sinopharm (Shanghai, China) was the supplier of all analytical grade solvents (acetone, petroleum ether, ethyl acetate, and methanol) as well as HPLC grade CH_3CN for analysing high performance liquid chromatography electrospray ionization mass spectrometry (HPLC-ESI-MS). Mung bean seeds (0.5 kg) were purchased from Wal-Mart in July 2014 (Zhonglv No. 1, Nanchang, China) and selected without any physical damage. They were immediately cleaned, freeze-dried, and then ground to fine powders. At last, these powders were preserved at −20 °C before use.

2.2. Extraction and purification of the proanthocyanidins

The process of extraction and purification was implemented on the basis of our previous report (Chai et al., 2017). Briefly, powders of mung bean seed (10 g) were dissolved in 7:3 (v/v) acetone–water solution (250 mL, 0.5% acetic acid was added into solution to prevent autoxidation of proanthocyanidins) and then ultrasound-assisted extracted three times (30 min at a time). Petroleum ether (3 × 150 mL) and ethyl acetate (3 × 150 mL) were selected as extractants to acquire crude proanthocyanidins. The crude proanthocyanidins were then chromatographed on an LH-20 column (Pharmacia Biotech, Uppsala, Sweden), which was eluted with methanol-water (60:40, v/v) and acetone-water (7:3, v/v) orderly. For the latter, the water-soluble fraction was freeze-dried to obtain purified proanthocyanidins after the acetone was eliminated by rotary evaporation.

2.3. Determination of total phenolics and extractable proanthocyanidins content

0.1 g of mung bean seed dry powders were extracted with 7:3 (v/v) acetone-water solution (5 mL) three times, the supernatants were combined and diluted with distilled water to 50 mL to obtain sample solution. Total phenolics content of mung bean seed was determined in accordance with Folin-Ciocalteu method assay. In brief, 0.2 mL of the sample solution, 0.3 mL of distilled water, 0.5 mL of Folin-Ciocalteu reagent, and 2.5 mL of Na_2CO_3 solution were added into test tube in order and shaken well. The absorbance, temperature and time were selected as 725 nm, 25 °C, and 40 min, respectively. Gallic acid was used as a standard and the result was indicated as mg gallic acid equivalents per g mung bean seed dry weight. Total extractable proanthocyanidins content was determined based on Butanol-HCl method. In brief, 1 mL of the sample solution and 6 mL of butanol-HCl (v:v, 95:5) were mixed. The mixture was measured at 550 nm after bathed in the boiling water for 75 min. Purified proanthocyanidins fraction of mung bean seed was used as the standard [proanthocyanidins content = 100%, assessed by liquid chromatography-mass spectrometry (LC-MS) after thiolysis] (Desrues, Mueller-Harvey, Pellikaan, Enemark, & Tharnsborg, 2017) and the result was represented as mg purified proanthocyanidins equivalents per g mung bean seed dry weight (Zhou et al., 2011).

2.4. Structural analysis of mung bean seed proanthocyanidins

2.4.1. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis of mung bean seed proanthocyanidins

We performed the MALDI-TOF MS experiment on a Bruker Reflex III instrument (Bruker-Franzen, Bremen, Germany) and detected the mass spectrum in the reflection mode with Cs^+ as adducts. The irradiation source was a pulsed nitrogen laser (337 nm), and the duration of the laser pulse was 3 ns. The sample (5 mg mL^{-1}), matrix (2,5-Dihydroxybenzoic acid, 10 mg mL^{-1}) and CsCl (1.5 mg mL^{-1}) solution were mixed at a volumetric ratio of 1:3:1. The final volume of mixture for the mass spectrometry (MS) test was 1 µL (Chai et al., 2012).

2.4.2. Electrospray ionization-full-mass spectrometry (ESI-Full-MS) analysis of mung bean seed proanthocyanidins

The ESI-Full-MS analysis was executed on a Xevo G2 QT of MS (Waters, Milford, USA) in the negative mode (ESI^-). The capillary and the sampling cone voltage were 2000 V and 100 V, respectively. In the experiment, the employed source and the desolvation temperature were 120 °C and 400 °C, respectively. The desolvation gas flowrate was set at 600 L/h. Injection volume was 200 µL, and detection flow rate was 20 µL/min. The scan range of molecular weight was from 500 to 2000 *m/z*. MS spectrum was analyzed by using MassLynx 4.0 (Waters).

2.4.3. Reversed-phase HPLC-ESI-MS assay of mung bean seed proanthocyanidins followed thiolysis

Proanthocyanidins (0.5 mg dissolved in 100 µL methanols, 50 µL) were mixed with 50 µL of hydrochloric acid-methanol solution (3.3:96.7, v/v) and 100 µL of benzyl mercaptan-methanol solution (5:95, v/v). The solution was filtered through a membrane filter with an aperture size of 0.45 µm, and then 20 µL of sample solution was analyzed by HPLC-ESI-MS. The high performance liquid system was an Agilent 1200 system (Agilent, Santa Clara, CA, USA) equipped with a diode array detector and a quaternary pump (280 nm). The thiolysis medium was further analyzed using QTRAP 3200 mass spectrometer (Applied Biosystems, Foster, USA). Two solvents, namely A = 0.5% (v/v) formic acid in aqueous and B = CH_3CN, were used. The linear gradient elution process was performed as follows: 12–80% CH_3CN (0–45 min); 80–12% CH_3CN (45–50 min) (Gu et al., 2003). The column temperature was controlled at 25 °C. The flow rate was set at 1 mL min^{-1}. Degradation products were determinated on the basis of their retention time on chromatogram and corresponding mass.

2.5. Enzyme assay of mung bean seed proanthocyanidins

2.5.1. Inhibitory effect of mung bean seed proanthocyanidins on the monophenolase activity

In this study, $_{\text{L}}$-tyrosine was served as substrate for determinating monophenolase activity. In short, $_{\text{L}}$-tyrosine (2 mM), Na_2HPO_4-NaH_2PO_4 buffer (50 mM, pH 6.8), mung bean seed proanthocyanidins (0.1 mL) with different concentrations (0, 20, 40, 80, 120, 160, and 200 µg mL^{-1}), 1.55 mL H_2O and mushroom tyrosinase solution (0.2 mg mL^{-1}) were well mixed and constituted a reaction system with a total volume of 3 mL. The reaction processes were traced by using a Beckman UD-800 spectrophotometer (Beckman coulter, Pasadena, California, USA) at 475 nm.

2.5.2. Inhibitory effect of mung bean seed proanthocyanidins on the diphenolase activity

In current study, we used $_{\text{L}}$-DOPA as diphenolase substrate. $_{\text{L}}$-DOPA (5 mM), Na_2HPO_4-NaH_2PO_4 buffer (50 mM, pH 6.8), 1.8 mL H_2O, and mung bean seed proanthocyanidins with different concentrations (0, 20, 40, 60, 80, and 100 µg mL^{-1}) were well mixed. Finally, the enzyme solution (0.2 mg mL^{-1}) was added into the mixture (the final volume was 3 mL). Controls, with equal amount of water replacing mung bean

Download English Version:

https://daneshyari.com/en/article/7584942

Download Persian Version:

https://daneshyari.com/article/7584942

Daneshyari.com