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Contribution of specific amino acid and secondary structure to the antioxidant property of corn gluten proteins

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ABSTRACT

The composition, structure, and proper positioning of amino acid in a peptide are closely related to its antioxidant activity. In this study, we purified antioxidant peptides from corn protein hydrolysates (CPH) and identified novel antioxidant peptides from fraction CPH2-III as Ala-Gly-Ile/Leu-Pro-Met (AGI/LPM; 487.62 Da) and His-Ala-Ile/Leu-Gly-Ala (HAI/LGA; 467.53 Da). AGLPM and HALGA exhibited better oxygen radical absorbance capacities than AGIPM and HAIGA did ($P < 0.05$), as assessed using HepG2 cells with the cellular antioxidant activity assay (CAA) and electron spin resonance (ESR) spectroscopy. Finally, the secondary structure was determined using circular dichroism (CD). ESR showed that the AGLPM and HALGA peptides had the strongest abilities to scavenge hydroxyl radicals, by 79.41 ± 1.41% and 75.16 ± 2.26%, respectively. Thus, corn gluten meal could be used as a potential source of antioxidant peptides for food applications. Additionally, the amino acid Leu compared with Ile may be a critical factor contributing to strong antioxidant activity than the Ile in the peptide sequence (not C-terminus or N-terminus) and CD showed that the lower α-helix and random coil are the main causes.

1. Introduction

Usually, when excess free radicals are produced, or when the cell defences fail, the biological molecules are damaged, which is known as oxidative stress. The free radicals in the body are caused by excessive oxidative stress, which thus destroy the internal redox balance and cause various chronic diseases including arteriosclerosis, cardiovascular diseases, neurodegenerative diseases, cancers, and aging-related disorders (Huang, Kaustav, & Wu, 2010). Therefore, free radicals have become a culprit for negatively influencing human health. In order to scavenge superfluous free radicals and maintain the balance of homeostasis in the human body, as well as for prevention and treatment of diseases, dietary intake of antioxidants is necessary. At present, some synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert-butylhydroquinone (TBHQ) are used to scavenge free radicals in foods and biological systems; however, the use of synthetic antioxidants is of concern due to their potential health hazards (Samaranayaka & Li-Chan, 2011). Antioxidants from natural plant and animal sources have potential beneficial effects due to their low molecular weight, easy absorption, and little or no side effects in the human body; hence, these have attracted growing interest (Sarmadi & Ismail, 2010).

In recent years, hydrolyzed proteins from many animal and plant sources such as peanut kernels (Hwang, Shyu, Wang, & Hsu, 2010), sunflower (Megías et al., 2008), frog skin (Qian, Jung, & Kim, 2008),egg yolk (Sakanaka & Tachibana, 2006), and canola (Cumby, Zhong, Naczk, & Shahidi, 2008) have been found to possess antioxidant activity. Corn gluten meal is a by-product of the corn wet-milling process containing 60% (w/w) protein, and has low water solubility and a severely imbalanced amino acid composition, which makes it difficult to be used as a food additive (Zhuang, Tang, & Yuan, 2013). However, the corn gluten meal protein contains considerable amounts of hydrophobic amino acids such as leucine, alanine, and phenylalanine. Therefore, it is thought to be a good source of antioxidant peptides. Corn gluten meal peptides are food-derived bioactive peptides obtained via amylolysis technology (Suh, Whang, Kim, Bae, & Noh, 2003). It is known that the antioxidant activities of these peptides are closely related to their amino acid composition, structure, and proper localization (Sarmadi & Ismail, 2010). Research on the sequences of corn antioxidant peptides is indispensable for providing a better understanding of the structure–activity relationship. The purpose of this study was to: (i) isolate and identify the antioxidant peptides from corn gluten meal; (ii) determine the antioxidant activities of these peptides

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using the oxygen radical absorbance capacity assay (ORAC), cellular antioxidant activity assay (CAA), and electron spin resonance (ESR) measurements; and (iii) obtain more information about the contribution of specific amino acid and secondary structure on the antioxidant activity of corn gluten meal using circular dichroism (CD).

2. Materials and methods

2.1. Materials and reagents

Corn gluten meal (protein content of 79.4%) was purchased from Dacheng Co. (Changchun, China). HepG2 cells were purchased from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). The 1,1-diphenyl-2-picrylhydrazy(DPPH),6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 3-(4, 5-dimethylthiazol-2-y1)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide, quercetin,2′,7′-dichlorofluorescin diacetate (DCFH-DA), fluorescein sodium salt (FL), 2,2-azobis (2-methylpropionamidine) dihydrochloride (AAPH), 5,5′-dimethyl-1-pyrroline-N-oxide(DMPO), ethylenediaminetetraacetic acid disodium (EDTA-2Na),2,2′-azobis(2-amidinopropane) dihydrochloride (ABAP) and Sephadex G-25 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), phosphate-buffered saline (PBS), L-glutamine,penicillin-streptomycin combination, and fetal bovine serum (FBS) were purchased from Gibco BRL Life Technology (Grand Island, USA). Alcalase (2.0×10^5 U/g) was purchased from Jiangsu Ruiyang Biotech Co., Ltd. (Wuxi, China). Methanol and all other chemical reagents were purchased from Peking Chemical Plant (Beijing, China) and were all of analytical grade purity.

2.2. Preparation of corn protein hydrolysate(CPH)

Antioxidant peptides from CPH were prepared as previously described by Wang et al. (2012). Corn gluten meal was dispersed in distilled water with a meal/distilled water ratio of 1:20 (w/v). The solution was mixed using a magnetic spectroscopy stirring apparatus for 5 min in order to ensure complete mixing. The solution was then heated to 90 °C to denature the protein, and hydrolyzed for 150 min using Alcalase (enzyme/substrate ratio 9.13%, 50 °C, and pH 8.6). After completion of hydrolysis, the hydrolysate was taken out, followed by 10 min of heating in a boiling water bath to inactivate the enzymes. Then, the hydrolysate was centrifuged at 10,000 × *g* for 20 min at 4 °C. The supernatant was lyophilized in a freeze drier and stored at − 20 °C until further use.

2.3. Ultrafiltration isolation

CPH was fractionated by ultrafiltration equipment (Millipore MiniTan system, Millipore, Bedford, MA, USA) using a UF membrane with cut-off molecular weights (MW) of 30 kDa, 10 kDa, 3 kDa, and 1 kDa, respectively. CPH was mainly categorized into two fractions: CPH1, MW < 1 kDa; and CPH2, 10 kDa < MW < 30 kDa. Each fraction was freeze-dried and stored in a desiccator for further assay of its antioxidant activities by testing the DPPH and ORAC radical-scavenging activities.

2.4. DPPH radical-scavenging activity test

The DPPH free radical-scavenging activity was determined according to the method by Lin et al. (2013) with some modifications. The peptide or protein was dissolved in deionized water to obtain a concentration of 10 mg/mL. DPPH radicals were dissolved in methanol to 0.1 mM, and then 100 μL of DPPH radicals, 100 μL of peptide or protein solution, and 100 μL of methanol were combined in a 96-well microplate, shaken vigorously, and incubated for 1 h in a 37 °C water bath in the dark. The absorbance was then measured at 517 nm using a Synergy HT microplate reader (BioTek Instruments, Inc., Winooski, VT, USA). Water was used instead of the sample in the blank group. The DPPH radical-scavenging activity was calculated using the following equation:

$$\text{DPPH radical scavenging activity}\ (\%) = \left(1 - \frac{A_{sample}}{A_{blank}}\right) \times 100$$

2.5. Oxygen radical absorbance capacity (ORAC) assay

The antioxidant capacity was measured by the oxygen radical absorbance capacity-fluorescein (ORAC-FL) test according to previous studies (Ou, Hampsch-Woodill, & Prior, 2001), in which the samples were diluted with 75 mM phosphate buffer (pH 7.4) to 10 mg/mL. Each 50 μL aliquot of the diluted sample solution was mixed with a solution of 50 μL of 50 mM fluorescein FL solution in a 96-well microplate, and then the content of each well was rapidly added into the well with 150 μL of 0.4 M AAPH. To create a blank decay curve and a Trolox standard decay curve, phosphate buffer was used to replace the sample solution, and the Trolox standard was used to calibrate the solution. After shaking for 30 s, the microplate was immediately placed into the Synergy™ microplate reader (Bio-Tek Instruments), and recorded every minute for 180 min. The fluorescence filters of the plate reader were set at 485 nm with a tolerance of ± 20 nm for the excitation wavelength, and set at 530 nm with a tolerance of ± 20 nm for the emission wavelength. The temperature of the plate reader was set at 37 °C. Each ORAC value of the extracts was calculated using a regression equation between the Trolox concentration and the net area under the fluorescence decay curve (AUC). The net AUC corresponding to a sample was calculated by subtracting the AUC corresponding to the blank. ORAC-FL values were expressed as mmol Trolox equivalent (TE)/mg of dried sample.

2.6. Gel filtration chromatography

Fractions of 10–30 kDa of CPH (CPH2) with the strongest antioxidant capacity were purified using Sephadex gel filtration chromatography. CPH solution (3.0 mL, 30 mg/mL) was filtered using a 0.22-μm membrane and added to a pre-equilibrated Sephadex G-25 column (1.6 × 70 cm). The column was fixed at 4 °C in a thermostatic chromatography system, which was eluted with distilled water at a flow rate of 1.0 mL/min. Then, the elution was detected at 280 nm using an online UV HD-21-2 spectrophotometer (Qingpu Instruments Co., Shanghai, China) to obtain a sample elution profile. Each peak of the effluent fractions was pooled and collected for freeze-drying and stored in desiccators. The antioxidant activity was measured using the DPPH and ORAC assays. The fraction exhibiting the highest activity was lyophilized, and subjected to the next step of experiment.

2.7. Determination of the amino acid sequence by mass spectrometry (MS)

An electrospray linear ion trap mass spectrometer (LTQ XL; Thermo Fisher, USA) equipped with a thermal spray ionization source was used for determination of amino acids. The mode was set to positive ion, the spray voltage to 5 KV, the capillary temperature to 250 °C, and the cone voltage to 120 V. The scanning range was set at *m/z* 100.00–2000.00 and the sample flow rate was 5 μL/min.

2.8. Synthetic peptides

The peptide sequences derived from corn gluten meal were synthesized with an AAPPTEC 396 Automated Peptide Synthesizer (Advanced Automated Peptide Protein Technologies, Kentucky, USA) using Fmoc protected amino acid synthesis methods (Yu et al., 2011; Yu, Yin, Zhao, Liu, & Chen, 2012). These peptides were provided by China Peptides Co., Ltd. (Hefei, China). The synthesized peptides were