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A novel antioxidant and ACE inhibitory peptide from rice bran protein: Biochemical characterization and molecular docking study

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ABSTRACT

Rice bran protein was hydrolyzed using trypsin. The hydrolysate (RBPH) was then further separated by membrane bioreactor system, gel filtration and reversed phase high-performance liquid chromatography (RP-HPLC). A novel antioxidant and angiotensin I-converting enzyme (ACE) inhibitory peptide named as F2-a, which exhibited high DPPH• free radicals scavenging activity, reducing power and ACE inhibitory activity (IC_{50} of 76 μM) was isolated. The amino acid sequence, Tyr-Ser-Lys (Mw: 395.0 Da), was identified by Quardrupole Time-of-flight Mass Spectrometer (Q-TOF-MS) with an electro-spray ionization (ESI) source. The molecular docking study revealed that the ACE inhibition of Tyr-Ser-Lys was mainly attributed to forming very strong hydrogen bonds with the active pockets of human ACE. These results indicate that rice bran is a potential source of bioactive peptides possessing antioxidant and ACE inhibitory activities.

1. Introduction

Rice (*Oyaza sativa*) is one of the most staple diets for human especially in Asian countries. About 610 million metric tons of rice is produced annually. This huge amount of production results in commensurate amount of rice by-products (Sereewatthanawut et al., 2008). Rice bran is the outer component of raw rice that is obtained as a by-product during the rice milling process and has 12–15% protein content and possesses a powdery consistency (Kaewka, Therakulkait, & Cadwallader, 2009). Rice bran protein is a high-quality and hypoallergenic protein that may be useful in infant formulations (Helm & Burks, 1996) and it has also been reported to have anti-cancer activity (Kawamura, Muramoto, Waldron, Johson, & Fenwick, 1993) and dipeptidyl peptidase IV inhibitory activity (Hatanaka et al., 2012).

Bioactive peptides are specific and small protein fragments that are inactive within the sequence of their parent protein. These peptides are 2–9 amino acids in size and typically possess specific amino acid sequences, mainly comprised of hydrophobic groups in addition to proline, arginine, and lysine (Dizuba, Minkiewicz, & Nalcez, 1999; Kitts & Weiler, 2003). Several bioactive peptides exhibit antioxidant, anti-hypertensive and anti-obesity activities (Sarmadi & Ismail, 2010; Li, Le, Shi, & Shrestha, 2004; Kim, Bae, Ahn, Lee, & Lee, 2007) and the ACE inhibitory activity are often shown by antioxidant peptides simultaneously (Intarasirisawat, Benjakul, Wu, & Visessanguan, 2013; Torruco-Uco, Chel-Guerrero, Martínez-Ayala, Dávila-Ortíz&Betancur-Ancona, 2009). Some studies indicated that rice residue protein had antioxidant activity (Yan, Huang, Sun, Jiang, & Wu, 2015) and rice protein had ACE inhibitory activity (Fu, Chen, Dong, Zhang, & Zhang, 2010). However, there is scanty information about the antioxidant and ACE inhibitory activities of rice bran protein or its hydrolysate.

For better understanding of antioxidant and ACE inhibitory activities of hydrolysate from rice bran, the peptides contributing to those activities should be examined. The aim of this study was to isolate and characterize peptides possessing ACE inhibitory activity and antioxidative properties from protein hydrolysate of rice bran. In the present study, trypsin (25×10^4 U/g, EC3.4.21.4) was used to hydrolyze rice bran protein (RBP). The ultrafiltration, gel filtration chromatography and reversed-phased high performance liquid chromatography (RP-HPLC) were used to purify the antioxidant and ACE inhibitory peptide. The sequence of purified peptide was identified using Quardrupole Time-of-flight Mass Spectrometer (Q-TOF-MS). Furthermore, the binding interaction of purified peptide within the active site of human ACE was also determined using Auto Dock 4.0 software which was recently reported to be the most

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popular docking program with high accuracy and versatility (Dong, Liao, & Wei, 2011).

2. Materials and methods

2.1. Materials

Rice bran was obtained from Suichang Shuige Rice Milling (Zhejiang, China). Trypsin (25×10^4 U/g, EC3.4.21.4) was procured from Tianjin Hongqiao Chemical Engineering Co. (Tianjin, China). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Hippuryl-histidyl-leucine (HHL), angiotensin-converting enzyme of rabbit lung (ACE) and Sephadex G-25 were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A). Spontaneously hypertensive rats (SHRs) (Production certificate: 11400700073450), weighing 240 ± 20 g, 12-week-old, male, were purchased from Beijing Weitonglihua Laboratory Animal Technology Co., Ltd. (Beijing, China). All other chemicals and reagents used were of analytical grade.

2.2. Production of rice bran protein (RBP)

Rice bran powder was defatted with petroleum ether. The defatted flour was dispersed in deionized water at ratio 1:10 (w/v). The suspension pH was adjusted to 9 by using 1 M NaOH. After stirring for 2.5 h, the suspension was centrifuged at 3000×*g* for 15 min. The supernatant was adjusted to pH 4 using 1 M HCl to precipitate the protein, centrifuged again at 3000×*g* for 15 min. The precipitate was lyophilized using a freeze-dryer (FD-1-50, Beijing Boyikang Experimental Instrument Co., Ltd, Beijing, China) and stored in polyethylene bag at −4 °C until used.

2.3. Preparation of rice bran protein hydrolysate (RBPH)

The hydrolysis process was carried out according to our previous study and the method of Yan, Huang, Sun, Jiang, &Wu (2015) with some modifications. Based on our previous studies, trypsin-generated rice bran protein hydrolysate exhibited highest antioxidant activity among the three commonly used proteases, including neutral protease, alkaline protease and trypsin. In this study, the trypsin was chosen for the preparation in the hydrolysis process. RBP was dissolved in distilled water at a concentration of 50 mg/mL and hydrolyzed for 2 h using trypsin (enzyme–substrate ratio, 1500 U/mg) at pH 8 and 37 °C with stirring. The reaction was stopped by heating the mixture in a boiling water bath for 10 min. Then, the solution were centrifuged at 6000×*g* for 10 min and the supernatant was lyophilized and stored at −4 °C until used.

2.4. Assay of antioxidant activities

2.4.1. Scavenging activity against DPPH• free radicals

Scavenging activity on DPPH• free radicals was according to our previous studies (Fu, Chen, Dong, Zhang, & Zhang, 2010). Briefly, 100 μL of samples aqueous solution with different concentration was mixed with 2.9 mL of 0.12 mmol/L DPPH• solution, and then the mixture was incubated in the dark at 37 °C for 30 min. The absorbance of the mixture was measured at 517 nm. The scavenging activity was explained by the following equation and the relative half inhibition concentration (IC_{50}) was calculated.

$$I\% = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100\% \quad (1)$$

2.4.2. Reducing power assay

The ability of samples to reducing iron (III) was determined according to Chen et al. (2009).Briefly, 100 μL of samples aqueous solution with different concentration (30 mg/mL and 10 mg/mL, respectively before and after gel filtration) were mixed with 0.7 mL of 0.2 mol/L sodium phosphate buffer and 2 mL of 30 mmol/L potassium ferricyanide aqueous solution. The mixture was incubated at 50 °C for 20 min, followed by addition of 2 mL of 10% trichloroacetic acid solution. Then, the mixture was centrifuged at 3000×*g* for 10 min. Finally, 1 mL supernatant was mixed with 3 mL of 1.7 mmol/L ferric chloride aqueous, and absorbance was measured at 700 nm. Reducing power was proportional to the absorbance of the reaction mixture.

2.5. Assay of ACE-inhibitory activity

The ACE inhibitory activity was measured according to Cushman and Cheung (1971) with some modifications. Briefly, 30 μL of samples were mixed with 80 μL of 5 mmol/L HHL and 30 μL of 0.1 mol/L boric acid buffer (pH 8.3), and the mixture was pre-incubated at 37 °C for 5 min. The reaction was started by the addition of 10 μL of 0.1×10^3 U/L ACE, which was incubated at 37 °C for 30 min. Finally, the mixture was added 150 μL of 1 mol/L HCl to terminate reaction. The control was boric acid buffer instead of samples. The reaction mixture was analyzed by HPLC after membrane filtration. It was separated from HHL by RP-HPLC on Kromasil 100-5C18 column (250 mm × 4.6 mm, 5 μm, Eka Chemicals AB, Bohus, Sweden) by isocratic elution with 15% methanol at a flow rate of 0.4 mL/min and detected at 228 nm to evaluate the ACE inhibitory activity of samples. The experiments were carried out by triplicate. The inhibition activity was calculated using the following equation and the relative IC_{50} value was calculated.

$$\text{ACE inhibition } \% = \frac{A' - A}{A'} \times 100\% \quad (2)$$

where A′ is the peak area of control and A is the peak area of sample.

2.6. Animals and measurement of systolic and diastolic blood pressure

SHRs were housed individually in steel cages in a room kept at 24 °C with a 12 h light-dark cycle, and fed with tap water and a standard laboratory diet (No. 11003800004211, Beijing HFK Bioscience Co., China). A total of 16 SHRs, were divided into four groups (4 SHRs each group) namely the control group, positive control group and two peptide groups. The peptides were dissolved in physiological saline and were administered orally using a flexible sonde at a dose of 50 mg/kg of body weight (1 mL/rat). The same volume of physiological saline was administered to the control group. The positive control group (captopril group) was administered orally using the dosage of 10 mg/kg of body weight (1 mL/rat). The lowering efficacy of samples on systolic blood pressure (SBP) and diastolic blood pressure (DBP) was compared with that of captopril. The SBP and DBP of the rats were measured at 0, 2, 4, 6, 8, 12 and 24 h after administration by the tail cuff method with a BP 2010 blood pressure meter (Softron Biotechnology Co. Ltd, China) after the rat had been warmed in a chamber maintained at 30 °C for 10 min.

2.7. Purification of antioxidant and ACE inhibitory peptide

2.7.1. Ultrafiltration

For purification of antioxidant and ACE inhibitory rice bran