Available at www.sciencedirect.com

ScienceDirect

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

Isolation and identification of antioxidative peptides from hydrolysate of threadfin bream surimi processing byproduct

Chompoonuch Wiriyaphan[a], *Benjamart Chitsomboon*[b], *Sittiruk Roytrakul*[c], *Jirawat Yongsawadigul*[a,*]

[a]*School of Food Technology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand*
[b]*School of Biology, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand*
[c]*National Center for Genetic Engineering and Biotechnology, Pathumthani 12120, Thailand*

ARTICLE INFO

Article history:
Received 14 December 2012
Received in revised form
6 July 2013
Accepted 8 July 2013
Available online 2 August 2013

Keywords:
Antioxidant
Peptides
Mode of action
Threadfin bream
Surimi byproduct

ABSTRACT

The objective of this study was to determine the antioxidant activity and possible mode of action of partially purified peptides derived from threadfin bream surimi byproduct. The frame, bone and skin (FBS) byproduct, which was obtained from a deboning process, was hydrolyzed by *Virgibacillus* sp. SK33 proteinase and fractionated using anion exchange and size exclusion chromatography. Three fractions, i.e., B1, B2 and B3, were obtained, and the amino acid sequences of the peptides in all 3 fractions were determined using LC-MS/MS. Fractions B2 and B3 contained higher amounts of Trp, Met, Cys and Tyr residues than fraction B1. Fraction B3 exhibited high ABTS radical scavenging activity and ferric reducing antioxidant power (FRAP), while metal chelation and hydroxyl radical scavenging ability were principle modes of action of peptides in fraction B2 and B3. In addition, fraction B1 and a synthetic peptide selected from the pooled *de novo* peptides of fraction B3, FLGSFLYEYSR, had a cellular radical scavenging effect when HepG2 cells were treated with H_2O_2.

1. Introduction

In aerobic organisms, reactive oxygen species (ROS), such as superoxide radical ($O_2^{-\bullet}$), hydroxyl radical ($HO^{\bullet}$) and hydrogen peroxide (H_2O_2), are produced during normal physiological processes. These radicals are very unstable and rapidly react with other groups or substances in the body, leading to cell or tissue injury (Ames, Shigena, & Hagen, 1993). In addition, metal ions are known to induce free radical formation through a variety of processes, including the Fenton reaction (Halliwell, Aeschbach, Loliger, & Aruoma, 1995). Under normal conditions, cells are protected against oxidation by both enzymatic and non-enzymatic antioxidant pathways. However, excessive ROS can damage DNA, proteins, and lipids, thereby contributing to many serious diseases, such as liver disease and cancer (Leanderson, Faresjo, & Tagesson, 1997; Vitaglione, Morisco, Caporaso, & Fogliano, 2004). Therefore, natural antioxidants that enhance antioxidant defenses in the body have been continually sought.

Various food protein hydrolysates have been reported to exhibit antioxidant activity, such as porcine myofibrillar protein (Saiga, Tanabe, & Nishimura, 2003), porcine collagen (Li, Chen, Wang, Ji, & Wu, 2007), tuna backbone protein (Je, Qian, Byun, & Kim, 2007), hoki frame protein (Kim, Je, & Kim, 2007), potato protein (Kudo, Onodera, Takeda, Benkeblia, & Shiomi, 2009), royal jelly protein from the honeybee (Guo, Kouzuma,

* *Corresponding author*. Tel.: +66 44224359; fax: +66 44224387.
E-mail address: jirawat@sut.ac.th (J. Yongsawadigul).
1756-4646/$ - see front matter
http://dx.doi.org/10.1016/j.jff.2013.07.009

& Yonekura, 2009), Nile tilapia scale protein (Ngo, Qian, Ryu, Park, & Kim, 2010), rapeseed protein (He, Girgih, Malomo, Ju, & Aluko, 2013), and tilapia protein (Sun, Zhang, & Zhuang, 2013). These hydrolysates were composed of 2–16 amino acids, and their activities depended on size, amino acid composition, and sequence. In addition, different modes of antioxidant activity, namely the donation of electrons/hydrogen atoms, direct scavenging of free radicals, and sequestration of pro-oxidative metal ions, have been observed in protein hydrolysates (Samaranayaka & Li-Chan, 2011).

Threadfin bream (*Nemipterus* spp.) is a major raw material for tropical surimi production. Approximately 50,000–60,000 MT/year of solid wastes, including frame, bone and skin (FBS), are typically generated during surimi production. These wastes are usually converted to low-value fish meal. Our previous study revealed that an FBS hydrolysate with antioxidant activity can be obtained with *Virgibacillus* sp. SK33 proteinase (Wiriyaphan, Chitsomboon, & Yongsawadigul, 2012). However, the antioxidant mode of action of the peptides in the FBS hydrolysate is still unknown. Therefore, the objectives of this study were to fractionate and identify the antioxidant peptides from the FBS hydrolysate prepared from *Virgibacillus* sp. SK33 proteinase and to elucidate the possible mode of action of the antioxidant peptides in both cellular and non-cellular oxidative systems.

2. Materials and methods

2.1. Materials

The mixture of frame, bone, and skin (FBS), which was obtained from a deboning process of threadfin bream surimi production, was donated by the Andaman Surimi Industry (Samutsakorn, Thailand). FBS was packed in a polystyrene foam box filled with ice and transported to a laboratory at the Suranaree University of Technology. FBS was dried, ground, and defatted as described by Wiriyaphan et al. (2012). Defatted FBS powder contained 41.70 ± 2.68% crude protein, 47.90 ± 1.86% ash, 0.30 ± 0.03% crude fat, and 7.30 ± 0.74% carbohydrates on a dry matter basis. *Virgibacillus* sp. SK 33 isolated from one-month-old Thai fish sauce mashes was used as the source of proteinase. Details of *Virgibacillus* proteinase production were described by Wiriyaphan et al. (2012). *Virgibacillus* sp. SK33 proteinase activity was assayed following the method of Sinsuwan et al. (2008) using Suc-Ala-Ala-Pro-Phe-aminomethylcoumarin (AMC) as substrate, at 50 °C and pH 8.One unitof activity is theamount of enzyme that releases 1 nanomole of AMC per min.

The compounds 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 2, 4, 6-tripyridyl-S-triazine (TPTZ) were purchased from BioChemika (Buchs, Switzerland). 6-Hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox), xanthine, 2-deoxy-D-ribose, and xanthine oxidase from bovinemilk were purchased fromSigma Chemical Co. (St. Louis, MO, USA). The HepG2 human hepatoma cell line was obtained from the American Type Culture Collection (Manassas, VA, USA). Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM-F12) was purchased from Gibco BRL, Life Technologies (Gaithersburg, MD, USA). Other chemicals and reagents used were of analytical grade.

2.2. Production ofa defatted FBS hydrolysate

Ten grams of defatted FBS powder were added 0.1 M phosphate buffer at pH 8.0 (10%, w/v) and hydrolyzed with *Virgibacillus* sp. SK33 proteinase at 13.5 U/g powder at 65 °C, which was found to be the optimal temperature for FBS hydrolysis, for 8 h. The hydrolysate was heated at 90 °C for 10 min to stop the reaction and centrifuged at 10,000×*g* for 20 min at 4 °C. The supernatants were lyophilized and stored at −20 °C until further use. The peptide content was determined using the Lowry method with tyrosine as a standard. Degree of hydrolysis was determined as described by Adler-Nissen (1979).

2.3. Purification of the antioxidant peptides from the FBS hydrolysate

2.3.1. Ion exchange chromatography

The lyophilized FBS hydrolysate (0.33 g) was dissolved in deionized water (8 ml). One millilitre of peptide solution was applied onto a DEAE–Sephacel ion exchange column (2.6 × 6.5 cm, GE Healthcare, Piscataway, NJ, USA) equilibrated with 50 mM Tris–HCl buffer (pH 8.0). The elution was performed using an AKTA Purifier (GE Healthcare, Piscataway, NJ, USA) with a linear gradient of NaCl (0–1.0 M) at a flow rate of 1 ml/min. The eluate was monitored at 215 nm and collected in 5 ml-volume fractions. Ion exchange chromatography was repeated 5 times with a total loading volume of 5 ml. Fractions with high absorbance at 215 nm from each injection were pooled and lyophilized. The peptide content was determined using the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951) with tyrosine as a standard. The antioxidant activity of each pooled fraction was measured using the ABTS radical scavenging activity and FRAP assays. The pooled fraction with the highest antioxidant activity was used for further purification.

2.3.2. Size exclusion chromatography (SEC)

The lyophilized powder obtained from DEAE-Sephacel ion exchange chromatography (1.2 g) was dissolved in deionized water (15 ml). Three millilitres of reconstituted peptide were applied onto a Superdex 30 prep grade column (1.6 × 100 cm, GE Healthcare, Piscataway, NJ, USA) equilibrated with deionized water. The elution was performed using an AKTA Purifier (GE Healthcare, Piscataway, NJ, USA) with an isocratic mode with deionized water at a flow rate of 0.5 ml/min. The eluate was monitored at 215 nm and collected in 3 ml-volume fractions. The purification was repeated 5 times with the total loading volume of 15 ml. The pooled fractions were then lyophilized and determined peptide content as described above. The antioxidant activity of each lyophilized fraction at varied concentrations was measured as described in Section 2.6.

2.4. LC-MS/MS

The amino acid sequences of the fractionated peptides were determined using an Ultimate 3000 LC System (Dionex Ltd., USA) coupled to an ESI-Ion Trap MS (HCT Ultra PTM Discovery System, Bruker, Daltonic, Germany) with electrospray ionization. The peptides were separated in a nanocolumn (Acclaim