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Short Communication

Pilot and plant scaled production of ACE inhibitory hydrolysates from *Acetes chinensis* and its *in vivo* antihypertensive effect

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Abstract

The angiotensin-I-converting enzyme (ACE) inhibitory oligopeptide-enriched hydrolysates from *Acetes chinensis* by treatment with the protease from *Bacillus* sp. SM98011 were produced at pilot scale (100 L) and plant scale (1000 L). The pilot and plant scaled hydrolysate products almost had the same properties as that at laboratory scale. Spray-drying had little effect on the peptide composition and bioactivity of the hydrolysates. The plant scaled hydrolysates were used to study its blood pressure-depressing effect *in vivo*. It caused reduce of 18.3–38.6 mmHg of the blood pressure of spontaneously hypertensive rats in dose-dependent manner in the range of 100–1200 mg/kg/day. Histopathologic study showed that the pathologic changes of heart and brain in SHRs got obvious alleviation after treatment of the hydrolysates.

Keywords: Hydrolysates; Angiotensin-I-converting enzyme (ACE); ACE inhibitory peptide; Spontaneously hypertensive rats (SHRs); Histopathology

1. Introduction

Enzymatic hydrolysis of proteins is a well known method to modify protein physical properties and, more importantly, to improve their nutritional properties. The application of enzyme technology to recover modified seafood protein may produce a broad spectrum of food ingredients or industrial products (Shahidi et al., 1994; Jérôme et al., 2002; Tsai et al., 2006). Recent advances in biotechnology have demonstrated the capacity of enzymes to produce novel food products, modified foodstuffs and improved waste management (Afonso and Borquez, 2002). These trends cater to consumer preferences for 'natural' products. Among many functional peptides, ones possessing hypotensive activity are thought to be useful as functional food materials for high blood pressure patients. This activity is mainly due to the inhibition of the angiotensin-I-converting enzyme (ACE). ACE has been classically associated with the renin–angiotensin system, which regulates peripheral blood pressure via conversion of angiotensin-I to angiotensin-II. Inhibition of ACE may have an antihypertensive effect as a consequence of a decrease in blood pressure (Skeggs et al., 1957). Although synthetic ACE inhibitors, including captopril, enalapril and listinopril, are remarkably effective as antihypertensive drugs, they inevitably cause adverse side effects (Atkinson and Robertson, 1979). Many ACE inhibitory peptides have been reported in hydrolysates from diverse food proteins digested with different proteases (Meisel, 1998). Most of these peptides were identified from hydrolytic products of terrestrial food proteins but less from marine proteins. The composition and primary amino acid sequences of marine proteins are different from those of terrestrial proteins; therefore, marine proteins may become important resources for the exploitation of novel ACE inhibitory peptides by enzymatic hydrolysis.

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Acetes chinensis growing in Bo Hai Gulf of China is an underutilized shrimp species with a low commercial value, as a consequence of low consumption by humans. Every year, about 300,000 tones of *A. chinensis* are made into dried small shrimps or shrimp sauce that have low adding value. The advanced utilizing process of this marine resource is a significant topic in marine biologic study. Because of its high content of muscle proteins and chitin, it is a valuable raw material for further processing. Our previous studies had shown that hydrolysates of *A. chinensis* digested with the crude protease from *Bacillus* sp. SM98011 possessed ACE inhibitory activity and three novel ACE inhibitory peptides had been found (He et al., 2006a; He et al., 2006b). In the present work, the enzymatic hydrolysis of the shrimp *A. chinensis* was scaled up to pilot scale and plant scale. The peptide compositions, ACE inhibitory activities, and *in vivo* anti-hypertensive effect in SHRs of the hydrolysates were also investigated.

2. Methods

2.1. Experimental materials and strains

Acetes chinensis was purchased from Chinese Dayudao Group and stored at cold store. Protease-producing bacterium *Bacillus* sp. SM98011 was preserved in our laboratory.

2.2. Protease preparation and activity assay

The strain *Bacillus* sp. SM98011 was fermented in 200 L fermenter at 28 °C for 36 h, and the activity of protease SM98011 in ferment broth reached 4000 U/ml. Then, the ferment broth was centrifuged at 10,000*g* for 20 min, and the supernatant was collected and used for protein hydrolysis. The activity of protease was determined with the method as before (He et al., 2004). The protease SM98011 was crude serine protease and optimum hydrolysis temperature and pH of protease was 50 °C and pH 7.5.

2.3. Acetes chinensis hydrolysates production at pilot scale and plant scale

The hydrolysis was carried out at laboratory scale (500 ml Erlenmeyer flask), pilot scale (100 L thermostatically stirred-batch reactor) and plant scale (1000 L thermostatically stirred-batch reactor), respectively. In the batch experiments, minced shrimp was mixed with water at a ratio of 1:1 (w/w) by continuous stirring and the enzyme was added at a ratio of 400,000 U/kg (U/w, enzyme/protein substrate). All reactions were performed at pH 7.5 and 50 °C for 5 h with constant agitation (200 rpm). The reactions were stopped by heating the solution to 90 °C for 15 min, assuring the complete inactivation of the enzyme. The resulted slurry was centrifuged at 9000*g* for 20 min and the supernatant was the hydrolysates of *A. chinensis*. The supernatant of laboratory scale was freeze-dried and kept at 4 °C. Hydrolysates of pilot and plant scale were condensed with ball vacuum concentration tanker (RD500, GuanYi Mechanical Equipment Co., Ltd, China) and were dried with Spray Dryer (BoDa Co., China) at a 10 kg h^{-1} flow rate with a 170 °C inlet temperature and 90 °C outlet temperature (Kapel et al., 2006).

2.4. Assay of content of peptides in hydrolysates

Content of peptides in the hydrolysates was assayed with the previous method (He et al., 2006a).

2.5. ACE-inhibition activity assay

ACE inhibitory activity of the hydrolysates and the IC_{50} value was assayed with the previous method (He et al., 2007).

2.6. RP-HPLC analysis

Analysis of peptide composition of the hydrolysates was carried out by RP-HPLC (Waters alliance2695, USA) coupled with Dual Wavelength UV Detector 2487. The separation was performed using a Symmetry C18 column (250×4.6 mm). The eluent was 0.1% trifluoroacetic acid (A) and a mixture of eluent A with acetonitrile (2/3, v/v) (B). A linear A–B gradient from 10% to 60% was applied over a period of 30 min followed by a linear A–B gradient from 60% to 10% for 15 min with flow rate 1 ml/min monitored at 214 nm.

2.7. Antihypertensive activity in SHRs

Forty male SHRs (12 weeks old, Calco, Italy) and 10 age-matched normotensive Wistar-Kyoto (WKY) rats were kept at an ambient temperature of 23 ± 1 °C, with free access to food and water before beginning the experiments. SHRs were divided into five groups ($n = 8$). A negative control group was orally administered with physiological saline, a positive control group was orally administered with captopril at 10 mg/kg/day and three experimental groups were orally administered with the hydrolysates at doses of 100, 500, 1200 mg/kg/day, respectively. Five untreated WKY rats served as normotensive control group. The other five WKY rats were administered with hydrolysates at a dose of 500 mg/kg/day. Dosages were adjusted every week according to the changes of body weight. The systolic blood pressure (SBP) of the rats was measured by a tail-cuff method (model BP-98, Softron, Tokyo, Japan). During a month of treatment, measurements were taken weekly. Several determinations were made for each animal, and the values were considered valid if five consecutive measurements were within 6 mmHg (Jung et al., 2006). Under anaesthesia with sodium pentobarbital (60 mg/kg i.p.), the animals were sacrificed immediately after the end of treatment. The rats' hearts, brains were removed and immediately stored in 10% formalin