Screening for antihypertensive activity of protein hydrolysates prepared by proteolytic digestions of velvet bean protein

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Abstract

This research screened the antihypertensive activity of protein hydrolysates prepared by proteolytic digestion of seed protein from velvet bean (*Mucuna pruriens* (L.) DC.). Seed protein extract was hydrolyzed by alcalase, papain, pronase and trypsin using an enzyme/substrate ratio of 1/10 (w/w) at the optimum conditions of each enzyme for 1, 2 and 3 h. Results revealed that the protein hydrolysates exhibited antihypertensive activity measured by ACE-I inhibition. The optimum hydrolysis times for hydrolysates prepared by alcalase, papain, pronase and trypsin were 3, 1, 2, and 3 h which inhibited ACE-I at the highest values of 58.20, 47.24, 62.72 and 51.94% at 500 μg protein used, respectively. Each protein hydrolysate showed ACE-I inhibitory activity equivalent to captopril, an antihypertensive agent, as values of 10.6, 8.9, 11.8 and 9.7 nmol per 1 mg protein, respectively. The protein hydrolysate prepared by pronase digestion had the highest inhibitory activity towards ACE-I. Results suggested that protein hydrolysates of velvet bean seeds showed interesting potential application as food supplements for blood pressure control.

Keywords: ACE-I inhibitory activity, protein hydrolysate, *Mucuna pruriens* (L.) DC.

Introduction

Hypertension is the cause of many illnesses such as cardiovascular disease, strokes and myocardial infarctions, and end-stage renal disease [1]. One cause of hypertension results from an angiotensin-I converting enzyme (ACE-I) which converts inactive angiotensin I to the potent vasopressor angiotensin II. Nowadays, synthetic drugs inhibitors of ACE-I such as captopril and enalapril are used for treatment of hypertension. However, these synthetic drugs have side effects [2]. ACE-I inhibitors from food proteins and protein hydrolysates have recently gained interest due to their less potent side effects than synthetic drugs. Food derived ACE-I inhibitory peptides was reported in cheese whey [3], casein [4], and corn gluten [5]. Legumes such as green bean and soybean are especially interesting because of their high protein content. Many crude proteins of beans were hydrolyzed with proteolytic enzymes to yield peptides with antihypertension activity. These hydrolysates included soybean hydrolyzed with pepsin-pancreatin [6], black bean and navy bean hydrolyzed with alcalase-flavozyme [7]. This research screened for antihypertensive activity of crude proteins and protein hydrolysates from velvet bean seeds (*Mucuna pruriens* (L.) DC.). Results can be utilized for further research and development of velvet protein as bioactive peptides for blood pressure control.

Materials and methods

Crude protein extraction

Velvet bean dry seeds (*Mucuna pruriens* (L.) DC.) were imbibed in distilled water overnight. The shells were then removed by hand and the seeds were homogenized with distilled water at a seed/water ratio of 1: 3 (w/v). The crude protein slurry was filtered through a double layer cheesecloth and centrifuged. The collected supernatant was freeze-dried and *stored* at 4ºC. The crude protein content was determined by the Lowry method [8].

Preparation of protein hydrolysates

Four kinds of proteases (alcalase, papain, pronase and trypsin) were used for hydrolysis of velvet bean crude protein under optimal conditions (Table 1). An enzyme/substrate ratio of 1:10 (w/w) was used and hydrolysis time was 1, 2 and 3 hours. After hydrolysis, the reaction mixture was heated at 100ºC for 10 min to inactivate the enzyme. Centrifugation was

performed for removal of non-hydrolysate proteins, and the supernatant was collected and stored at -20ºC until required for used.

Table 1. Optimum conditions for preparation of velvet bean protein hydrolysates

Enzyme	Buffer	pH	Temperature (ºC)
Alcalase	50 mM Sodium phosphate	8.0	50
Papain	50 mM Sodium phosphate	6.5	60
Pronase	50 mM Tris-HCl	7.5	40
Trypsin	50 mM Sodium phosphate	8.0	37

Protein profile analysis

Crude protein and protein hydrolysates profile were analyzed according to the method of Laemmli [9]. A 5% stacking and 15% separating gel of SDS-PAGE was run and stained with *Coomassie* Brilliant Blue (*R-250*).

Assay of ACE-I inhibitory activity

ACE-I inhibitory activity was performed according to the method of Cushman and Cheung [10] with slight modifications, using Hippuryl-Histidyl-Leucine hydrate (HHL) as a substrate and ACE-I from rabbit lung. After hydrolyzing HHL by ACE-I the hippuric acid product was measured at 228 nm. In the presence of the inhibitor the product decreased, compared to the control with no inhibitor. ACE-I inhibitory activity was calculated according to the equation;

$$\text{ACE-I inhibition } (\%) = \frac{\text{Abs}_{\text{Control}} - (\text{Abs}_{\text{Inhibitor}} - \text{Abs}_{\text{Inhibitor blank}})}{\text{Abs}_{\text{Control}}} \times 100$$

Statistical analysis

Results were presented as mean ± standard deviation to determine significant statistical differences among the samples by One-way Analysis of Variance (ANOVA) using SPSS version 18. The differences in means were determined at the confidence level $P < 0.05$.

Results and discussion

Protein hydrolysate preparation

Crude protein extracts of *M. pruriens* (L.) DC. were analyzed for protein profiles by SDS-PAGE after hydrolysis with alcalase, papain, pronase and trypsin (Figure 1). The protein profile of the protein hydrolysate prepared by alcalase (Figure 1A), revealed that alcalase digested the crude protein completely within 1 h. The protein profile of the protein hydrolysate prepared by papain (Figure 1B) revealed incomplete digestion during 3 h of incubation, and the major protein band at about 20.1 – 30.0 kDa remained. Similar protein profiles were observed in protein hydrolysates prepared by pronase and trypsin (Figure 1C and 1D). Protein remaining in the hydrolysate fractions might be a trypsin inhibitor as its molecular weight was around 20.1 – 30.0 kDa which was in the same range of a protein corresponding to soybean trypsin inhibitor in a previous report [11].
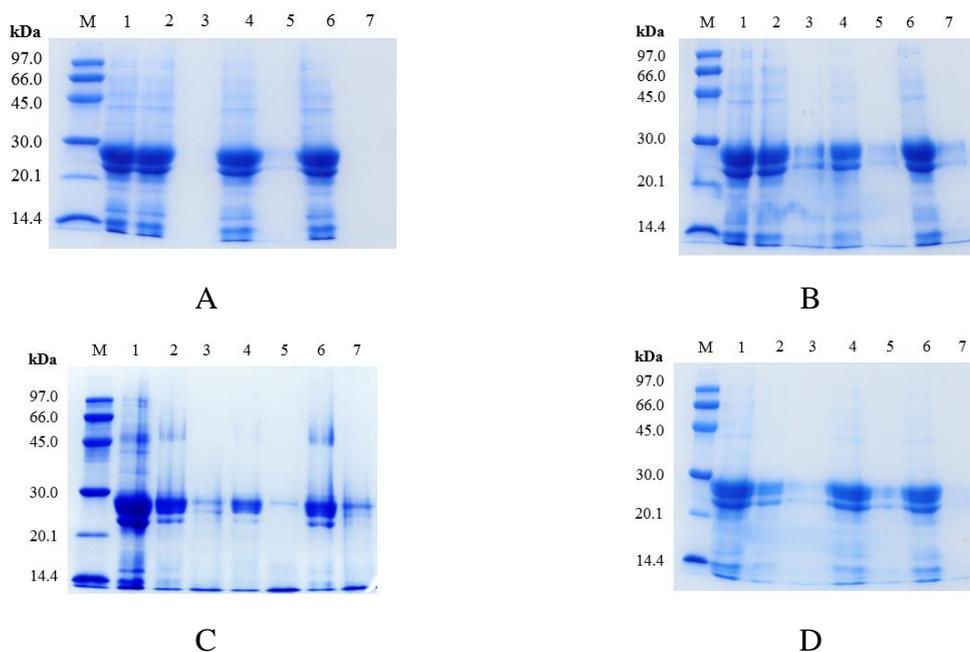

Figure 1. 15% SDS-PAGE of protein and protein hydrolysates of *Mucuna pruriens* (L.) DC. seeds prepared by proteolytic enzymes at the difference periods. (A) alcalase, (B) *papain,* (C) pronase and (D) trypsin. M = Protein molecular weight marker; Lane 1 = Crude protein from *Mucuna pruriens* (L.) DC. seeds; Lane 2, 3 = Control, Test at 1 h; Lane 4, 5 = Control, Test at 2 h; Lane 6, 7 = Control, Test at 3 h (protein content of 20 µg/well)

ACE-I inhibitory activity of crude protein and its protein hydrolysates

The crude protein and its protein hydrolysate of *Mucuna pruriens* (L.) DC. seeds prepared by four types of proteolytic enzyme digestion were measured for ACE-I inhibitory activity according to the method described above. Results determined that both the crude protein and its protein hydrolysates inhibited   ACE-I activity, but to different extents. The optimum hydrolysis times for each protein hydrolysate prepared by alcalase, papain, pronase and trypsin were at 3, 1, 2, and 3 h which inhibited ACE-I at the highest value of 58.20, 47.24, 62.72 and 51.94%, respectively, when 500 μg protein was used, respectively (Figure 2).

The most potent ACE-I inhibitory activity of each protein hydrolysate was compared to the inhibitory activity of the standard antihypertension agent captopril, and reported as nmol captopril equivalent per mg protein. Each protein hydrolysates prepared by alcalase, papain, pronase and trypsin showed captopril equivalent of 10.6, 8.9, 11.8 and 9.7 nmol per mg protein, respectively (Table 2). The ACE-I inhibition of each *M. pruriens* (L.) DC. seed protein hydrolysates was significantly different ($P < 0.05$). When comparing ACE-I inhibition of protein hydrolysates between groups, ACE-I inhibitory activity was significantly different at the value of 0.05. The results indicated that the protein hydrolysate prepared by pronase at 2 h gave the highest ACE-I inhibitory activity which was similar to the bovine skin gelatin hydrolysate previously reported [12].

Table 2. The highest captopril equivalent of each *Mucuna pruriens* (L.) DC. protein hydrolysates

Enzyme	Captopril equivalent (nmol/mg protein)
Alcalase 3 h	10.6 ± 0.058^{b}
Papain 1 h	8.9 ± 0.153^{d}
Pronase 2 h	11.8 ± 0.208^{a}
Trypsin 3 h	9.7 ± 0.252^{c}

All values are mean ± standard deviation from triplicate experiments.

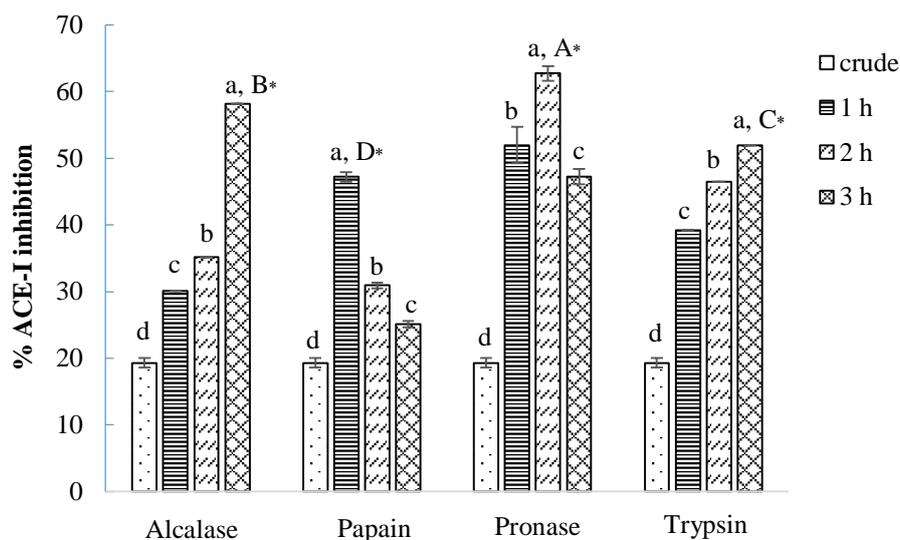Values with different letter superscripts are significantly different at $P < 0.05$.

Figure 2. The ACE-I inhibitory activity of crude protein and protein hydrolysate of *Mucuna pruriens* (L.) DC. seeds prepared by proteolytic enzymes. The small letters indicate significant difference within the groups and the capital letters indicate significant difference between the groups ($P < 0.05$).

Conclusions

Crude protein of velvet bean (*M. pruriens* (L.) DC.) seeds and its protein hydrolysates prepared by hydrolysis with alcalase, papain, pronase and trypsin exhibited interesting ACE-I inhibitory activity. Results suggested that velvet bean seed is potential protein source for the development of food supplementary products to alleviate blood pressure for patients with hypertension.

Acknowledgements

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