ISOLATION OF ANTIHYPERTENSIVE PEPTIDES FROM ENZYMATIC HYDROLYSATES OF BEEF SARCOPLASMIC PROTEIN

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Background

It has become clear that dietary proteins are a source of biologically active peptides. These peptides are inactive within the sequence of parent protein and can be released during gastrointestinal digestion or upon enzymatic hydrolysis *in vitro*. Sources of bioactive proteins are milk, egg, meat, gelatin, fish protein and plant proteins such as soy, corn, rice and wheat (Korhonen et al., 1998). Proten hydrolysates are physiologically better than intact proteins because their intestinal absorption appears to be more effective (Ziegler et al., 1998). For dietary use, the protein hydrolysates should be rich in low molecular weight peptides, especially di- and tripeptides, with as little as possible free amino acids. Physiologially active hydrolysates of proteins include the angiotensin I-converting enzyme (ACE) inhibitor, antioxidative peptide, antithrombotic peptides and cholesterol reducers (Kim, 1999). There have been many studies showing that ACE inhibitory peptide was isolated from protein digest of vegetables, fish, collagen and gelatin (Fujita et al., 2000). Recently, Arihara et al.(2001) reported that ACE inhibitory peptides were purified from porcine skeletal muscle proteins. However, very few studies have been done on beef protein.

Objective

The aim of the this study was to identify and purify low molecular bioactive peptides of ACE inhibitory effect from beef sarcoplasmic protein upon the enzymatic hydrolysis.

Methods

Preparation of the protein

In order to obtain sarcoplasmic protein, beef rump muscle was homogenized with 10 volumes of 0.03N phosphate buffer at pH 7.4 in a Ultra-turrax T25 blender for 3min and centrifuged at 12,000 rpm for 20min at 4℃(Toldra et al., 1999). The supernatant was filtered through a 0.45 μm pore size filter in aseptic conditions.

Purification of ACE inhibitory peptides from sarcoplasmic protein hydrolysate

Thermolysin, Protease A, and Protease type XIII fungal (Sigma Chemical Co., St. Louis, MO, U.S.A.) were added in the extracted sarcoplasmic protein(1:100). After 4hrs of digestion at 37℃, the solution was heated for 10min at 98℃. The heated solution was centrifuged and supernatant solution was filtered using ultrafiltation to remove large proteins(m.w. cutoff: 10,000). Then the filtered solution was fractionated by gel filtration (Sephadex G25, Pharmacia Co., Upsala, Sweden). The active elution was purified by HPLC with reversed-phase mode(column: PICO TAG C18 2.1×150mm; Waters, Miliford. MA.USA). Elution was performed with a gradient system from solvent A(0.1% trifluoroacetic acid in distilled water) to solvent B(0.07% trifluoroacetic acid in acetonitrile).

Assay of ACE inhibitory activity

The determination of ACE inhibitory activity was made by the spectrophotometric method described by Nakamura et al.(1995). This assay is based on the liberation of hippuric acid from hippuryl-L-histidyl-Lleucine catalyzed by ACE. The concentration of ACE inhibitor needed to inhibit 50% of ACE activity was defined as the IC_{50} value.

Results & discussion

The comparison of the inhibitory effect of the fractions obtained from various enzymatic hydrolysis of beef sarcoplasmic protein is shown in Table 1. Protein digestion was carried out with 4 enzymes either alone or in combination of two. Those in Table 1 resulted in more than 150 IC_{50} unit. The enzymatic hydrolysates were purified further with ultrafiltration and gel filtration. With the further purification step the ACE inhibitory effect improved remarkably. Of each hydrolysate the fraction with the highest inhibitory activity was selected from gel filtration. Protein hydrolysates by Thermolysin +Protease A resulted in the highest inhibiting activity (Table 1). Fujita et al. (2000) reported that ACE inhibitory peptides were isolated from thermolysin digests of chicken muscle and ovalbumin. The purified fraction with the highest activity of S46 was further purified by RP-HPLC. Three peaks were obtained (Fig. 1). The highest ACE inhibitory activity was observed in fraction peak 2(Table 2). As shown in Fig 1, the peptides were found to be relatively pure. The each fraction of S46 by HPLC was analyzed for amino acid composition (Table 3). Peak 2 having the highest ACE inhibitory activity consisted of 70.64% of lysine. The other two fractions also possessed lysine up to 26.44% and 81.76%, respectively. The result indicated that the most dominant amino acid of the fraction 2 of S46 was lysine, followed by proline. Arihara et al.(2001) suggested that ACE inhibitory activity *in vivo* would be higher in smaller peptides with proline. The fraction of this study seemed to have 13 amino acids. The protein hydrolysates with a high ACE inhibitory activity have been shown to be from dodeca- to dipeptides (Kohmura et al., 1989). In spite of potent ACE inhibitory activities, some of them failed to show antihypertensive activity after oral administration in hypertensive rat (Kim et al., 1990). Recently, Fujita et al.(2000) gave the possible causes responsible for the such conflicting activities. Enzyme-modified proteins are likely to have properties of health benefits, and thus enzymatic hydrolysis would be a viable method to produce bioactive components that can be used for meat products.

Conclusions

Antihypertensive peptides were purified from beef sarcoplasmic protein hydrolysates with various enzymes. The protein hydrolysate by thermolysin + Protease A resulted in the highest angitensin I-converting enzyme inhibition activity. Serial purification using ultrafiltration, gel filtration and reverse phase HPLC improved the activity greatly. The purified peptide with the highest activity had 13 amino acids and lysine as the most abundant amino acid. . From this result, it can be concluded that red meat can be utilized to produce a functional food ingredient for the promotion of its consumption.

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Table1. ACE inhibitory activity of protein hydrolysates with the purification (unit: μg/ml)

Steps / Protein Hydrolysates	Enzymatic hydrolyzates	UF aqueous extract	Gel filtrated fraction
S41	259.34	81.39	38.80
S43	206.60	30.36	26.64
S44	248.99	50.66	34.40
S46	163.08	37.89	9.08

S41: Hydrolysate by thermolysin
S43: Hydrolysate by protease A
S44: Hydrolysate by protease A + protease type XIII fungal
S46: hydrolysate by thermolysin + protease A

Table 2. ACE inhibitory activity of gel filtrated fractions on RP-HPLC(unit: μg/ml)

	Peak No.1	Peak No.2	Peak No.3
S46	8.87	7.50	21.66

Table 3. Amino acid composition of S46 fractions purified by HPLC (unit: %)

	Peak No.1	Peak No.2	Peak No.3
Asp	1.66	1.93	-
Glu	1.17	-	-
Ser	3.70	5.14	2.51
Gly	5.71	3.53	2.27
His	5.55	1.39	2.23
Arg	6.27	0.79	-
Thr	1.89	0.57	-
Ala	8.90	3.41	-
Pro	2.63	7.26	-
Tyr	5.84	1.17	1.92
Val	3.31	1.40	-
Met	7.0	1.33	2.05
Cys	6.42	0.93	-
Ile	2.8	-	-
Leu	-	-	-
Phe	7.72	-	-
Lys	26.44	70.64	81.76

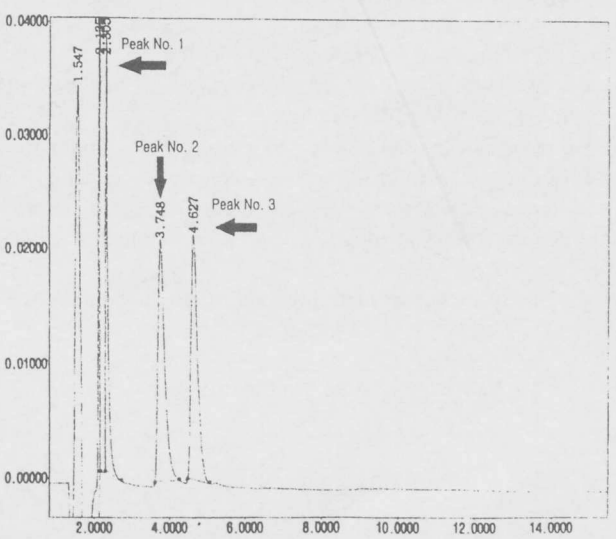

Figure 1. Reverse-phase HPLC chromatograms of gel- filtrated S46.