P-12-06

Purification of novel angiotensin converting enzyme inhibitory peptides from beef myofibrillar proteins and analysis of their effect in SHRs model (#94)

Seung Yun Lee, Da Young Lee, Hea Jin Kang, Rana M. Shohel, Sun Jin Hur

Chung-Ang university, Department of Animal Science and Technology, Anseong-si, South Korea

Introduction

Bioactive peptides derived from hydrolyzed food proteins have been reported to exhibit diverse beneficial effects on human health. Among their beneficial properties, various reports showed that bioactive peptides with ACE inhibitory activity can provide cues for the development of commercial ACE inhibitors.

Spontaneously hypertensive rats (SHRs) are an experimental animal model for hypertension, and are widely used for determination of antihypertensive properties of drugs, because they show similar physiological characteristics as hypertension in humans.

However, no studies in the SHR model have been reported with peptide(s) obtained from beef hydrolysates using cost effective industrial enzymes. In our previous report, we obtained myofibrillar protein hydrolysates with different molecular weights from beef by using cost effective industrial enzymes and determined the ACE inhibitory of hydrolysates under *in vitro* conditions. Therefore, the purpose of this study was to investigate the antihypertensive effect of novel peptides obtained from beef using inexpensive proteases in the SHR model, and to identify the sequence of these peptides.

Methods

Different molecular weight peptides (< 3 and < 10 kDa) were obtained using ultrafiltration. All the experiments were performed in triplicates and statistical analyses were performed using the one-way analysis of variance (ANOVA) using SPSS 20.0 (IBM, Armonk, NY, USA). Tukey's multiple comparisons test was used to determine significant differences between mean values, and evaluations were based on a significance level of $p < 0.05$.

Results

Among the AK3K, AK10K, PA3K and PA10K fractions, the AK3K fraction showed the highest ACE inhibitory activity, followed by inhibitory activities in the following order, PA3K > PA10K > AK10K. The ACE inhibitory activities of the AK3K, AK10K, PA3K, and PA10K were 74.29 ± 1.12%, 60.62 ± 7.89%, 72.93 ± 1.26%, and 69.06 ± 0.55%, respectively (Table 1). Each fraction was pooled and freeze-dried and its ACE inhibitory activity was determined. The ACE inhibitory activity of fractions obtained from AK3K at 20 mg/mL were 65.34± 7.26% for fraction 1 (F1), 36.95 ± 9.74% for fraction 2 (F2), and 52.46 ± 13.95% for fraction 3 (F3), respectively. The ACE inhibitory activities of fractions obtained from PA3K at 20 mg/mL showed ACE inhibitory activities as F1 (38.14 ± 15.74%), F2 (67.05 ± 9.08%), and F3 (43.00 ± 16.53%), respectively (Table 1).

After a 2 h administration of AK3K, SBP of AK3K400 and AK3K800 SHRs showed a decrease in SBP by 17 mmHg and 15 mmHg, respectively when compared with the control group. The SBP of AK3K400 and AK3K800 exhibited a maximal decrement by 28 mmHg and 35 mmHg after 12 h of AK3K administration. There were no differences in the antihypertensive effect in SHR rats between the AK3K and positive control group, which was administered with the antihypertensive drug captopril. Although, the DBP and MAP decreased by about 35 mmHg after 12 h of administration compared to 0 h, no significant differences were observed in the DBP and MAP among the treatment groups in this study.

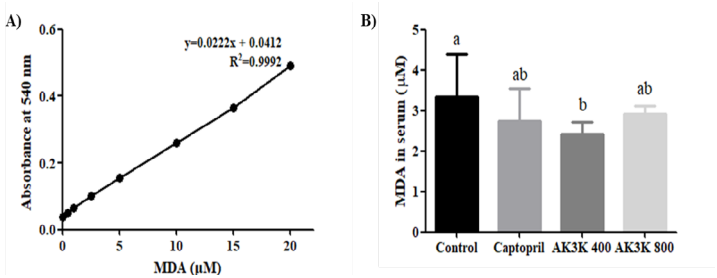
The lipid oxidation levels as measured by the concentration of MDA in the serum were significantly lower in the AK3K at 400 and 800 mg/mL and captopril treated groups than the control group ($p < 0.05$).

Among these fractions, AK3KF1-1 showed the highest ACE inhibitory activity. The amino acid composition of the ACE inhibitory peptide from above F1-1 fraction was determined by LTQ Orbitrap XL mass spectrometer, and the sequence of ACE inhibitory peptide was identified as Leu-Ile-Val-Gly-Ile-Ile-Arg-Cys-Val.

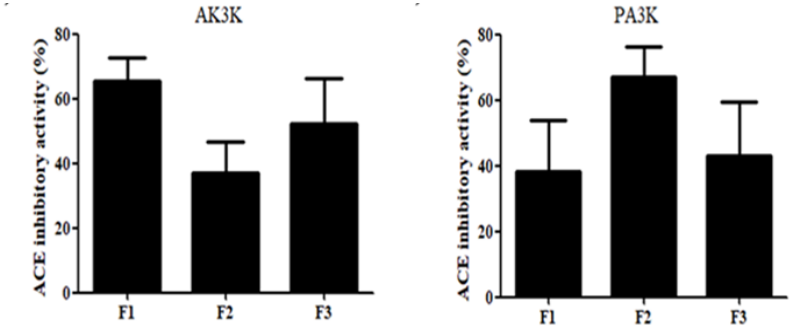
Conclusion

This study determined the antihypertensive effect of novel peptides obtained from beef protein hydrolysates by alkaline-AK and papain digestion in the SHR model. The peptide sequences were identified by FPLC and LC-MS/MS. The < 3 kDa novel peptide from the alkaline-AK fraction had effective antihypertensive activity in SHRs, which could contribute to the reduction of SBP and MDA levels in the serum. Furthermore, the < 3 kDa novel peptide, Leu-Ile-Val-Gly-Ile-Ile-Arg-Cys-Val obtained by alkaline-AK digestion of beef myofibrillar protein was shown to have the highest ACE inhibitory in *in vitro* as compared to the other peptides. This peptide could act as a new potent natural material for treatment of symptoms of cardiovascular disease.

Notes

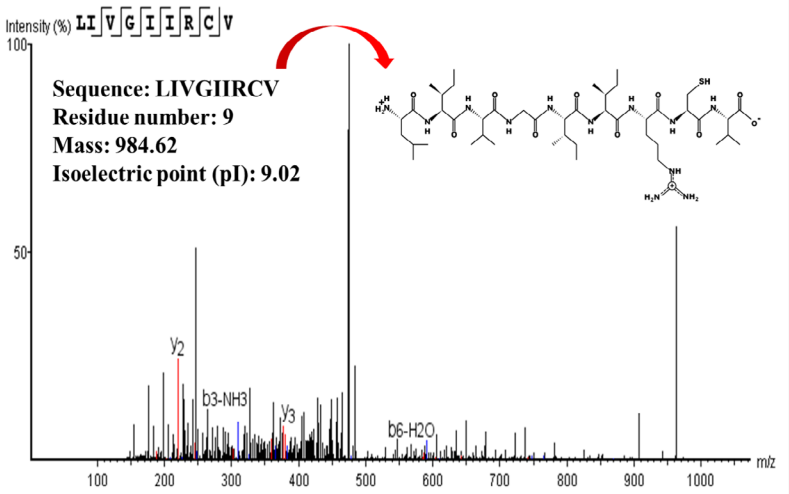


Determination of malondialdehyde (MDA) in SHR serum
TBARS assay results of (A) standard graph for the estimation of lipid peroxidation by malondialdehyde (MDA), and (B) MDA in serum. Values represent mean ± SD (n=3). Different letters indicate statistically significant differences ($p < 0.05$) in organ weight between different treatment groups.



ACE inhibitory activity of the peptides < 3 kDa from beef myofibrillar proteins
ACE inhibitory activity of the peptides < 3 kDa obtained by digestion of beef myofibrillar proteins by alkaline-AK and papain by FPLC on GPC.

Notes



Identification of the ACE inhibitory peptide

Identification of molecular mass and amino acid sequence of ACE inhibitory peptide in peak obtained from AK3KF1-1. MS/MS was performed on a LTQ Orbitrap XL mass spectrometer.

Notes