Identification of Pro-drug Type ACE Inhibitory Peptide from Porcine Myosin B: Antihypertensive Effects of its Hydrolysates *in vivo*

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Abstract

Porcine skeletal myosin B was hydrolyzed with pepsin and the hydrolysates were then applied to various kinds of chromatography to isolate active peptides. The 50% inhibitory concentrations of Lys-Arg-Val-Ile-Gln-Try (M6) and Val-Lys-Ala-Gly-Phe (A5) were 6.1 and 20.3μM, respectively. As a result of a homology search, it was determined that the peptide M6 came from myosin and peptide A5 was an actin origin. M6 is a novel ACE inhibitory peptide, whose activity was the strongest among those of the myosin-originated peptides previously reported. Kinetic evaluations showed that both peptides are competitive inhibitors to ACE. Based on their activity against ACE, M6 was classified as a prodrug conformer and A5 was classified as a substrate conformer. When both peptides were orally administered to spontaneously hypertensive rats (SHR) at doses of 10mg/kg, and temporal hypertensive phenomenon were observed after 6 hrs. This study suggests that M6 and A5 are peptides that may serve several purposes. Based on their remarkable antihypertensive activity, we suggest that M6 and A5 may have potential applications as functional food, which could be used as nutraceutical compounds.

Introduction

High blood pressure is a major cause of human mortality especially among the elderly. More accurately, this disease induces cerebrovascular incidents, heart failure and kidney disease, which could all lead to more complicated dysfunctions of the internal organs. As a large number of individuals suffer from such disease, scientists believe that other methods rather than chemical and pharmacological medication should be identified in the effort to reduce hepertensive diseases. Therefore, potential biological, functional and nutraceutical methods should be utilized as a treatment to minimize the number of individuals who fall into the hypertensive category and have been afflicted with diseases arising from this condition.

The objective of this study was to isolate and identify a novel ACE inhibitory peptide from the hydrolysate of porcine myosin B. This peptide was administered to SHR to determine whether it worked as an antihypertensive substance *in vivo*

Materials and Methods

Myosin B from pork loin muscle (*Longissimus dorsi)* was prepared using a method similar to that described by Katayama et al. (2003). Myosin B (5 mg/ml) was suspended in PBS, and denatured by heating for 10 min at 98 ^{o}C; the pH was adjusted to 2 with 1 M HCl, and pepsin was added in a ratio 1:100 of enzyme to substrate. After 6 hrs of digestion at 37 ^{o}C, the pH of the mixture was again adjusted to 7.5 with 1 M NaOH. The reaction mixture was centrifuged for 20 min at 18,000 *g*, and the supernatant was then

collected for the ACE inhibitory experiment. Purification of ACE inhibitory peptide was carried out according to a method described by Katayama et al. (2008). The amino acid sequences of the active fractions finally obtained were analyzed using a protein sequencer, Procise 492 (Applied Biosystems, Foster City, CA).

Initial velocities of ACE in the presence or absence of inhibitory peptides (KRVIQY and VKAGF, 50 μM) were determined at the various concentrations of HHL (0.0∽2.5). The data obtained were used to produce a double reciprocal plot (Lineweaver and Burk, 1934), using the vertical axis for velocity and the horizontal axis for the concentration of HHL. The peptides were dissolved in distilled water (10 mg/ml) and orally administered to SHRs at a dose of 10 mg/kg body weight (10 ml/kg) with a metal feeding syringe. The control group was administered the same volume of distilled water.

Results and discussion

The IC_{50} of the myosin B hydrolyaste obtained from the pepsin digest was found to be 47 μg/mL. Hydrolysates were then applied to various kinds of chromatography to isolate active peptides. Figure 1 shows the molecular weight of peptides No. 55 and 56, which are considered to be the most active fractions obtained by gel filtration chromatography.

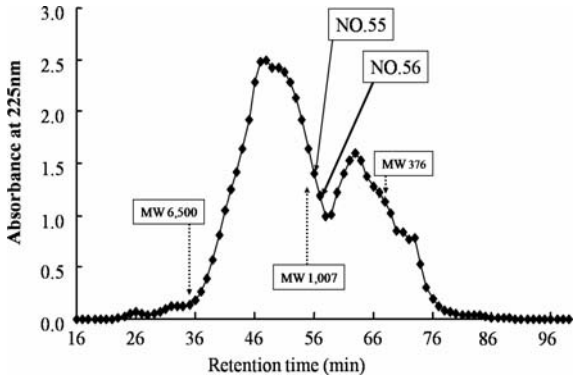

Figure 1. Gel-filtrate chromatogram of peptic hydrolysate of porcine myosin B.

Firstly, by comparison with the standard curve of the molecular weight markers, fractions No. 55 and 56, which were eluted first from the column, were applied to a RP-HPLC 1, eluted with a linear gradient of 0-50% acetonitrile in 0.1% TFA. The final fractions that were collected from RP-HPLC 6 and RP-HPLC 5 of 55 and 56 were named M6 and A5, respectively. Protein sequencing analysis of fractions M6 and A5 revealed their compositions to be KRVITY (MW = 805.97) and VKAGF (MW = 520.62), respectively. The IC_{50} values of M6 and A5 were found to be 6.1 μM (4.9μg/ml) and 20.3 μM (10.6 μg/ml), respectively, and their ACE inhibitory activities were comparable to or higher than that of the oligopeptide from meat. The activity of M6 was found to be greater than that of any myosin-originated peptide previously reported (Katayama et al., 2003a). M6 was found to be an entirely novel ACE inhibitory peptide.

The ACE inhibitory activity of M6 was increased from 4.9 to 1.5μg/ml (IC_{50}) as ACE occurs cleavage action, and the IC_{50} of A5 decreased from 10.6 to 17.7 μg/ml. The pro-drug type peptides are those which

show an increase in ACE inhibitory activity after ACE cleavage. It has also been reported that the substrate type peptides do not affect the blood pressure of SHR but the inhibitor and pro-drug type peptides produce a reduction in blood pressure values.

On the basis of this classification, we would class A5 as a substrate type and M6 as pro-drug type peptide and we therefore suggest that M6 has greater antihypertensive activity than A5 *in vivo*. To elucidate the mechanism of the ACE inhibitory activity of these two synthesized peptides, we represented the results as a Lineweaver-Burk (1934) reciprocal plot (Figure 2). We used two different concentrations of M6 and A5, 5.2 μM/ml and 11.2 μM/ml, respectively and found that both these peptides showed characteristic competitive inhibition.

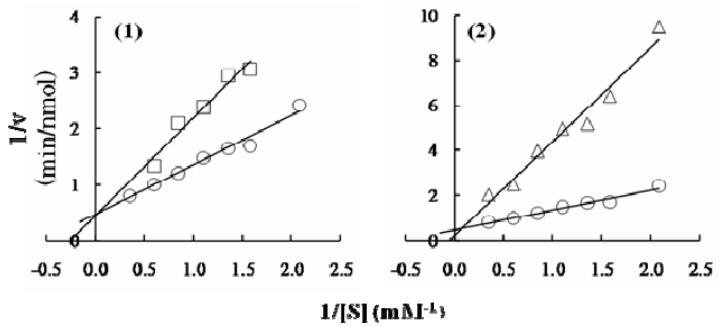

Figure 2. Kinetic evaluation of ACE inhibitory peptides, M6 (1) and A5 (2) evaluated at the concentration of 5.2 mg/ml (square) and 11.2 mg/ml (triangle), respectively. Circle shows data in the absence of peptide. [S]: concentration of HHL, v: initial velocity of ACE.

After the oral administration of M6, the systolic blood pressure of SHR decreased by 12 mmHg in 3 hrs and 23 mmHg in 6 hrs, indicating that M6 has an intense effect on the reduction of blood pressure in mammals (Figure 3). As the maximum reduction in blood pressure occurred between 3 and 6 hours after oral administration, this indicates that M6 is an important peptide in this respect and is thought to be a pro-drug type of inhibitory peptide. However, after oral administration of A5, systolic blood pressure of SHR decreased by 12 mmHg in3 hrs and 17 mmHg in 6 hrs. The reduction in blood pressure produced by A5 was smaller than that produced by M6 as illustrated in which shows that M6 has a greater antihypertensive effect than A5.

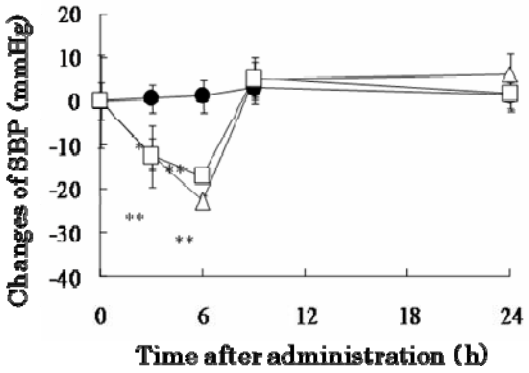

Figure 3. Effect of single oral administration of peptides on SHR. Changes of systolic blood pressure (SBP) from zero time were expressed with means, and the vertical bars represent the standard deviations. Values of controls (Closed circles; distilled water), values of M6 group (triangle; KRVIQY) and values of A5 group (squares; VKAGF), difference from the control: **p*<0.05 and ***p*<0.01.

A5 showed competitive inhibition until it was finally cleaved and therefore requires more time to become fully effective. As described, the ACE peptides isolated in this experiment showed a blood pressure-lowering effect *in vivo* and may be useful in preventing lifestyle-related diseases. These results may considerably enhance the value of meat and lead to increased consumption thereby giving relief to hypertensive individuals over time.

Conclusions

Adding to our previous finding, this study provides evidence that these peptides may have potential for use in hypertensive treatments as well as clinical therapies. We suggest that this research provides adequate evidence that meats contain a considerable number of constituents that could be utilized as functional food and nutraceuticals. Furthermore, this study indicates that even after cooking meat still contains active peptides with considerable antihypertensive activity that play significant roles in reducing blood pressure over time both *in vivo* and *in vitro*.

References

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