8-P14

Angiotensin I-converting enzyme inhibitors derived from muscle proteins

Keizo Arihara, Takao Mukai and Makoto Itoh

Faculty of Animal Science, School of Veterinary Medicine and Animal Sciences,
Kitasato University, Towada-shi 034-8628, Japan

KEY WORDS: Angiotensin I-converting enzyme inhibitor, peptide, porcine muscle protein, myosin

BACKGROUND

Angiotensin I-converting enzyme (ACE), which is a dipeptidylcarboxypeptidase, plays an important physiological role in regulating blood pressure. ACE converts an inactive form of decapeptide, angiotensin I, to a potent vasoconstrictor, octapeptide angiotensin II, and inactivating bradykinin, which has depressor action.

Several inhibitors of ACE have been discovered to be effective as antihypertensive pharma ceuticals. The ACE-inhibitory activity in foods has been also studied (Meisel et al., 1997). ACE inhibitory peptides derived from food proteins such as milk casein and cheese whey protein have been reported to show the antihypertensive activity (Abubakar et al., 1998). Some of these peptides have been utilized for developing new functional foods in Japan. Research has also been conducted to characterize the ACE-inhibitory activity derived from fish muscle and fish products (Astawan et al., 1995). However, little is known about derivation of the ACE-inhibitory activity from muscle proteins of domestic animals.

OBJECTIVES

In this study, we describe ACE inhibitory activities derived from porcine muscle proteins. Efforts were also directed to purify ACE inhibitory peptides from enzymatic hydrolysates of myosin.

MATERIALS & METHODS

PREPARATION OF WATER SOLUBLE AND INSOLUBLE PROTEIN FRACTIONS OF MUSCLE

Porcine skeletal muscle (Longissimus dorsi) was homogenized in two volumes of distilled water. The homogenate was centrifuged, and the supernatant was filtered. The filtrate was dialyzed against distilled water and lyophilized (water soluble protein fraction). The homogenate removed the supernatant was further washed with distilled water and lyophilized (water insoluble protein fraction).

DIGESTION OF MUSCLE PROTEINS WITH PROTEASES

Five kinds of proteases (trypsin, chymotrypsin, pronase E, proteinase K, thermolysin) were used for the digestion of muscle proteins. Proteins were dissolved in distilled water and digested at 37C for 24 h, then each sample was heated at 100C for 10 min to inactivate the protease. After removal of insoluble materials, supernatant solution was used for the measurement of the ACE-inhibitory activity.

PURIFICATION OF ACE-INHIBITORY PEPTIDES

Porcine skeletal muscle myosin (Sigma) was digested by thermolysin and heated to inactivate the protease. After enzymatic digestion and removal of insoluble materials, supernatant solution was fractionated by HPLC with reversed-phase mode (column: CAPCELL PAK C_{18} UG120 4.6 x 150mm; Shiseido, Tokyo). Elution was performed with a linear gradient system from solvent A (0.1% trifluoroacetic acid in distilled water) to solvent B (0.1% trifluoroacetic acid in CH_3CN). Further purification of peptides was carried out using the same system except for elution solution (solvent A: 0.015% ammonia in distilled water, solvent B: 0.015% ammonia in CH_3CN).

ASSAY OF ACE INHIBITORY ACTIVITY

The ACE-inhibitory activity was measured by a spectrophotometric assay according to Cushman and Cheung (1971). This assay is based on the liberation of hippuric acid from hippuryl-L-histidyl-L leucine catalyzed by ACE.

RESULTS & DISCUSSION

Table 1 shows the ACE-inhibitory activity of enzymatic digests of porcine skeletal muscle protein fractions. Before the enzymatic digestion, each solution of fractions showed no inhibitory activity. The results indicated that a combination of thermolysin and water insoluble muscle proteins was most suitable for induction of the high ACE-inhibitory activity.

Next, porcine skeletal muscle myosin was digested by three kinds of proteases (Table 2). Thermolysin digest showed highest inhibitory activity. The digest of myosin was fractionated by reversed-phase HPLC (Figure 1). Since ACE-inhibitory activities were widely distributed in fractions, many ACE-inhibitory peptides were induced from myosin by thermolysin digestion. The fraction with highest inhibitory activity (25-30 min) was further purified by same conditions (Figure 2). From two active peaks in Figure 2, two peptides with high ACE-inhibitory activity were obtained through another chromatography (data not shown). Structural analysis of these peptides is now in progress in our laboratory. It is also required to measure in vivo antihypertensive activities.

Since we have detected ACE-inhibitory peptides in several commercial fermented meat products (e.g., Lomo from Spain) and model sausages fermented with lactic acid bacteria (data not shown), it seems likely that ACE inhibitory peptides can be easily induced from muscle proteins of domestic animals.

Table 1. ACE-inhibitory activity of enzymatic hydrolysates of porcine skeletal muscle protein fractions.

Protease	ACE-inhibitory activity (%)	
	Muscle protein fraction[1)]	
	Soluble	Insoluble
Trypsin	32.1	51.2
Cymotrypsin	26.9	67.2
Pronase E	47.5	81.1
Proteinase K	70.5	89.4
Thermolysin	73.8	93.3

1) Water soluble and insoluble protein fractions of muscle were used for enzymatic hydrolysis.

Table 2. ACE-inhibitory activity of enzymatic hydrolysates of porcine skeletal muscle myosin.

Protease	ACE-inhibitory activity (%)
Pronase E	12.5
Proteinase K	24.1
Thermolysin	40.5

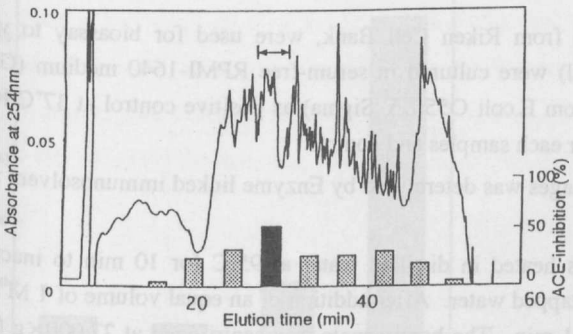

Figure 1. Distribution of ACE-inhibitory activity in reversed-phase HPLC fractions from thermolysin digest of porcine skeletal muscle myosin. Fractions were collected at 5 min intervals.

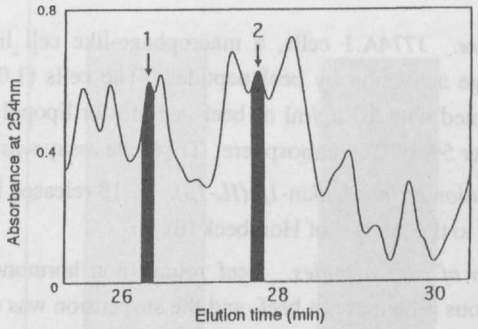

Figure 2. Distribution of ACE-inhibitory activity in reversed-phase HPLC peaks from the 25-30 min fraction of Figure 1. Arrows indicate active peaks.

CONCLUSIONS

ACE-inhibitory activities can be generated from muscle proteins by enzymatic hydrolysis. Also, protease-digestion of myosin gives ACE-inhibitory peptides. The results from this study suggest that ACE-inhibitory activities derived from muscle proteins would be utilized to develop functional foods.

REFERENCES

Abubakar, A., Saito, T., Kitazawa, H., Kawai, Y. and Itoh, T. 1998. Structural analysis of new antihypertensive peptides derived from cheese whey protein by protease K digestion. J. Dairy Sci., 81 : 3131-3138.

Astawan, M., Wahyuni, M., Yasuhara, T., Yamada,K., Tadokoro, T. and Maekawa, A. 1995. Effects of angiotensin I-converting enzyme inhibitory substances derived from indonesian dried-salted fish on blood pressure of rats. Biosci.Biotech.Biochem., 59:425-429.

Cushman, D. W. and Cheung, H. S. 1971. Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. Biochem. Pharmacol., 20 : 1637-1648.

Meisel, H., Goepfert, A., and Gunther, S. 1997. ACE-inhibitory activities in milk products. Milchwissenschaft, 52 : 307-311.