Antihypertensive Peptides from Enzymatic Hydrolysate of Porcine Myosin

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BACKGROUND

Angiotensin I-converting enzyme (ACE) 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 inactivates bradykinin, which has a depressor action. Several inhibitors of ACE have been found to be effective as antihypertensive pharmaceuticals. ACE inhibitory peptides derived from foods, especially milk proteins, have been reported to show antihypertensive effects in spontaneously hypertensive rats by oral administration (Nakamura et al., 1995; Yamamoto et al., 1994; Yamamoto et al., 1999). The antihypertensive effect of a sour milk containing two ACE inhibitory peptides derived from casein was demonstrated in hypertensive patients (Hata et al., 1996). Recently, we have found the derivation of ACE-inhibitory activity from porcine skeletal muscle proteins by enzymatic hydrolysis (Arihara et al., 1999).

OBJECTIVES

In the present study, we identified ACE-inhibitory peptides purified from thermolysin hydrolysate of porcine skeletal muscle myosin. Efforts were also made to investigate the antihypertensive effect of these peptides in spontaneously hypertensive rats. Such peptides could be utilized for producing new healthy meat products, which might open up a new market in the meat industry.

MATERIALS & METHODS

PURIFICATION OF ACE-INHIBITORY PEPTIDES FROM MYOSIN HYDROLYSATE

Porcine skeletal muscle myosin (100 mg) was suspended in distilled water (100 ml), and thermolysin (1 mg) was added. After 18 h of digestion at 37C, the solution was heated for 10 min at 98C. The heated solution was centrifuged and the precipitate was removed. The supernatant solution was fractionated by HPLC with reversed-phase mode (column: CAPCELL PAK C18 UG120 4.6 x 150 mm; Shiseido, Tokyo, Japan). 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). The active fraction was rechromatographed under the same conditions at those described above (second HPLC run). The peptide samples were further purified by HPLC with the same system except for the elution solution (third HPLC run). Elution was performed with a linear gradient (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.

Antihypertensive Effect in Spontaneously Hypertensive Rats

Male spontaneously hypertensive rats (SHR) were housed in cages on a cycle of 12 h of light and 12 h darkness. The SHR were fed a standard laboratory diet, and tap water was freely available. The systolic blood pressure (SBP) of SHR, 15 to 28 week old (280 to 390 g of body weight), were measured. Rats given each sample solution via a gastric metal zonde were put in a thermostatted box at 40C for 15 min, and the SBP were measured by the tail cuff with a programmed electrosphygmomanometer. The phosphate buffered saline was used as a control in SHR.

RESULTS & DISCUSSION

The amino acid sequences of two ACE inhibitory peptides purified by three-step reversed-phase HPLC were determined, and the peptides were named myopentapeptides A and B (Table 1). A search for sequence homology in databases revealed that the same sequences existed in the primary structure of the porcine skeletal muscle myosin heavy chain (Chikuni, K., DDBJ/EMBL/GenBank accession no. AB025260).

Table 1
ACE inhibitory peptides derived from porcine myosin.

Myopentapeptide	Sequence	Position in myosin[a]
A	Met-Asn-Pro-Pro-Lys	79-83
B	Ile-Thr-Thr-Asn-Pro	306-310

[a] Position of respective peptides in the sequence of the porcine skeletal muscle myosin heavy chain.

Myopentapeptides A and B, and six tripeptides, which have parts of the sequences of the myopentapeptides, were synthesized. The ACE inhibitory activities and antihypertensive effects of eight synthetic peptides are shown in Table 2. Of eight synthetic peptides tested, four peptides demonstrated antihypertensive activities significantly.

To the best of our knowledge, this is the first report of the ACE inhibitory and antihypertensive peptides derived from muscle proteins of domestic animals. Since the sequences of myopentapeptides A and B were not only found in the primary structure of the porcine myosin heavy chain but also in those of myosin of the rat, chicken and human, these sequences are thought to be presence in the myosin of various species, including meat animals.

The results of this study along with previous observation suggest that antihypertensive peptides are easily generated from muscle proteins. Thus, in meat products, such as fermented meat products, antihypertensive peptides could be generated. In fact, we have detected ACE inhibitory activity in several fermented meat products. On the other hand, digestive enzymes in gastrointestinal tracts generated the activity from muscle proteins (Arihara et al., 1999). Therefore, it is thought that antihypertensive activity could be generated in the gastrointestinal tract by ingestion of meat.

CONCLUSIONS

This study demonstrated that antihypertensive peptides can be generated from muscle proteins by enzymatic hydrolysis. The results from this study suggest that antihypertensive peptides derived from muscle proteins would be utilized to develop physiologically functional foods. Although bioactive peptides, such as ACE inhibitors, have not yet been utilized in the meat industry, meat products with such activity could open up a new market in the near future. It is expected that increasing interest will be shown in basic research and potential applications of bioactive peptides for meat products.

Table 2
ACE inhibitory activity and antihypertensive activity of synthetic peptides.

Sequence	IC_{50} (μM)[a]	Decreased SBP[b] (mm Hg)
Met-Asn-Pro-Pro-Lys	945.5	-23.4 ± 3.0*
Met-Asn-Pro	66.6	-19.6 ± 3.5*
Asn-Pro-Pro	290.5	-17.6 ± 6.7
Pro-Pro-Lys	>1000	-24.7 ± 2.9*
Ile-Thr-Thr-Asn-Pro	549.0	-21.0 ± 3.1*
Ile-Thr-Thr	678.5	- 6.8 ± 6.0
Thr-Thr-Asn	672.7	-11.4 ± 3.4
Thr-Asn-Pro	207.4	-11.1 ± 2.8

[a] The concentration of peptide needed to inhibit 50% of the ACE activity.

[b] The number showed the mean value ± SE (n=5) of the decreasing systolic blood pressure (SBP) in SHR. Changes in SBP at 6 h after gastric intubation are shown.

* Different from control ($P<0.01$).

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