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Characterization of Novel Antihypertensive Angiotensin I- Converting Enzyme Inhibitors from Edible Fungi

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This study describe on the characterization of the novel antihypertensive angiotensin I-converting enzyme (ACE) inhibitory peptides from edible mushroom, *Tricholoma gigantum* and *Saccharomyces cerevisiae*. The maximum ACE inhibitory activity (IC_{50} : 0.31 mg) of commerical *Tricholoma gigantum* was obtained when the fruiting body of *T. gigantum* was extracted with distilled water at 30℃ for 3 hours. After the purification of ACE inhibitor with ultrafiltration, Sephadex G-25 column chromatography and reverse phase HPLC, an active fraction with an IC_{50} of 0.04 mg and a yield of 0.3% was obtained. The ACE inhibitory peptide was a novel tripeptide which was sequenced as Gly-Glu-Pro and showed very low similarity to other ACE inhibitory peptide sequences. The purified ACE inhibitor inhibited competitively ACE and it also showed clear antihypertensive effect in spontaneously hypertensive rats(SHR) at dosage of 1 mg/Kg. Cell-free extracts of *Saccharomyces cerevisiae* exhibited the greatest inhibitory activity on ACE and its ACE inhibitory activity was increased about 2 folds by pepsin treatment on the cell-free extracts(42%). After purification of the ACE inhibitor from *S. cerevisiae* with ultrafiltration, Sephadex G-25 column chromatography and reverse phase HPLC, an purified ACE inhibitor with an IC_{50} of 0.07 mg obtained with 3.5% of yield. The inhibitory peptide is a novel decapeptide with 1116 Da of M.W and sequence (Tyr-Asp-Gly-Gly-Val-Phe-Arg-Val-Tyr-Thr, IC_{50} : 8.4 μM) which shows very low similarity to the other ACE inhibitory peptide sequence. The purified ACE inhibitory peptide inhibited competitively ACE.

Angiotensin I-converting enzyme (ACE, dipeptidyl carboxypeptidase I, kininase II, EC. 3.4.15.1) is a multifunctional enzyme which plays a key physiological role in the control of blood pressure by virtue of rennin-angiotensin system. ACE converts the inactive decapeptide, angiotensin I, to the potent vasopressor octapeptide, angiotensin II, and inactivates bradykinin. Many research groups have screened for ACE inhibitors from natural products and microbial sources including *Doratomyces putredinis, Nocardia orientalis, Streptomycetes, Actinomycetes, Actinomadura spiculosopora, Actinomadura* sp.. Food-derived ACE inhibitory peptides have been isolated from food or enzymatic digestion of food proteins including gelatin, casein, fish, fig tree latex and α-zein. Other ACE inhibitors were found from sake and its by-products, Korean traditional rice wines and liquors, cereals and legumes, and microbes such yeasts and *Basidiomycetes*, etc. Since the original discovery of ACE inhibitors in snake venom, captopril

(d-3-mercapto-2-methylpranory-l-proline), enalapril and lisinopril, an effective oral inhibitor, have been developed and are currently used as clinical antihypertensive drugs. However, even though synthetic ACE inhibitors including captopril are remarkably effective as antihypertensive drugs, they have certain side effects such as cough, allergies, taste disturbance, skin rashes, etc. Therefore, research and development on safer, innovative, and economical ACE inhibitors is necessary for the prevention and remedy of hypertension.

This study describes on the characterization of novel ACE inhibitory peptides from the fruiting body of *Tricholoma giganteum* and cell-free extract of *Saccharomyces cerevisiae* which can be used as antihypertensive drugs.

1. Characterization of ACE inhibitor from edible mushrooms, *T. giganteum*

After the purification steps by ultrafiltration, Sephadex G-25 column chromatography and HPLC, the ACE inhibitor with an IC_{50} of 0.040 mg was obtained, and the yield was 0.3%. Molecular mass of the purified ACE inhibitor from *T. giganteum* was estimated to be 301 daltons using LC/MS analysis(Fig. 1). The amino acid composition of the ACE inhibitor was identified Gly-Glu-Pro. ACE inhibition pattern of the purified ACE inhibitor from the water extract of *T. giganteum* was investigated by Lineweave-Burk plot. It was found to be competitive inhibition pattern on ACE. When the ACE inhibitors were treated with pepsin, trypsin and protease N, the ACE inhibitory activities decreased only slightly, though the trypsin - treated ACE inhibitor was inactivated at approximate 11%.

Antihypertension action of the purified ACE inhibitor from *T. giganteum* was investigation (Fig.2) The average blood pressure of the ACE inhibitor group rats showed about 193 mmHg just before the administration. After 2 hour of administrating the ACE inhibitor, 1 mg/kg/rat, blood pressure was decreased into 157 mmHg, and afterward, slightly increased to the average blood pressure. It was similar to that of commercial antihypertensive drug, captopril (190 mmHg → 163 mmHg).

2. Characterization of the ACE Inhibitor from *S. cerevisiae*

The ACE inhibitor was purified from the pepsin-hydrolysates of cell-free extracts of *S. cerevisiae* by ultrafiltration, Sephadex G-25 Chromatography, and HPLC. After the final purification step, the ACE inhibitor with an IC_{50} of 0.07 mg was obtained, and the yield was 3.5%. The molecular mass of the ACE inhibitor was estimated to be 1,178 Daltons by LC-MS analysis (Fig. 1). The amino acid sequence of the ACE inhibitor was found to be Tyr-Asp-Gly-Gly-Val-Phe-Arg-Val-Tyr-Thr by tandem LC- MS analysis. The ACE inhibition pattern of the purified ACE inhibitor was investigated by Lineweave-Burk plot. It was found to be a competitive inhibitor on ACE, suggesting that the ACE inhibitor from *S. cerevisiae* binds competitively with the substrate at the active site of ACE.

Antihypertensive action of the purified ACE inhibitor from *S. cerevisiae* was investigated. Average blood pressure of the ACE inhibitor group rats was found to be roughly 192 mmHg just before the administration.

After 2 h of administration of the inhibitor at, 1 mg/kg rat body weight, blood pressure decreased to 161 mmHg, slightly increasing later to average blood pressure. These results were similar to that of the commercial antihypertensive drug, captopril (192 mmHg → 162 mmHg), suggesting that the purified ACE inhibitor produces a clear antihypertensive effect in SHR at a dosage of 1 mg/kg rat body weight.

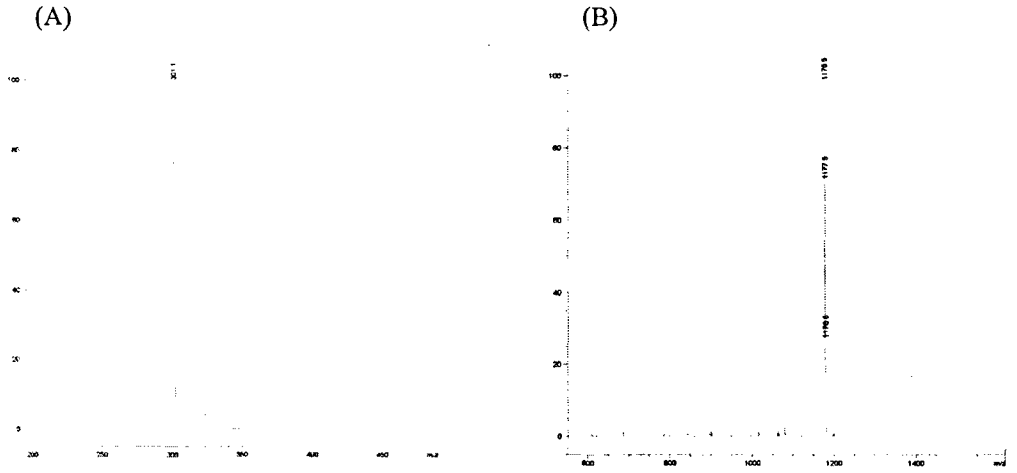

Fig. 1 Mass spectrum of the purified ACE inhibitor from *Tricholoma giganteum*(A) and *S. cerevisiae*(B)

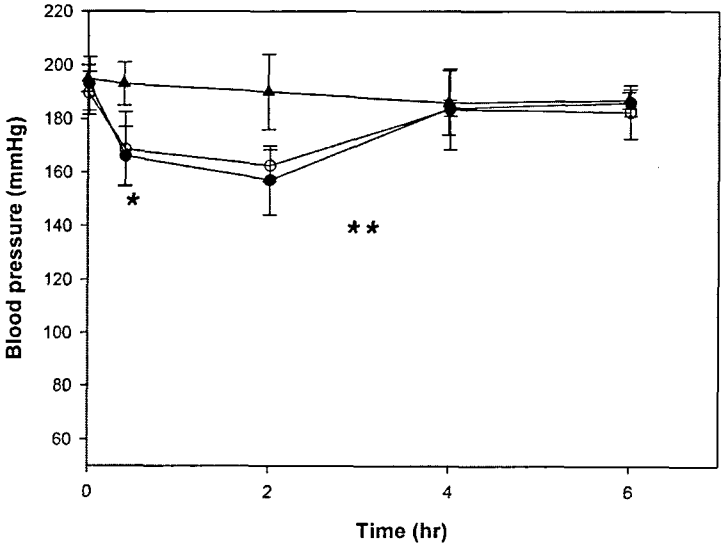

Fig. 2 Effect of orally administered the ACE inhibitor from *T. giganteum* on blood pressure in SHR.
●, ACE inhibitor 1 mg/kg, ○, positive control(captopril) 1 mg/kg, ▲, negative control.
*, ** significantly different from test group at $p<0.05$ by Tukey's test

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