Proceedings of the 35th European Peptide Symposium
Patrick B. Timmons, Chandralal M. Hewage, Michal Lebl (Editors)
European Peptide Society & PSP, 2018

Novel oligopeptides with angiotensin I-converting enzyme inhibitory activity found in an elastase-treated hydrolysate of porcine aortic elastin

Toshiya Hatakenaka[1], Tamaki Kato[1], Kouji Okamoto[2]

[1]Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology, Japan
[2]Vital Resources Applied Laboratory, Inc., Japan

https://doi.org/10.17952/35EPS.2018.251

Introduction

Hypertension is one of the main risk factors causing cerebro and cardiovascular diseases. Angiotensin I-converting enzyme (ACE) catalyzes the production of a vasoconstrictive peptide, angiotensin II from angiotensin I. Therefore, the inhibition of ACE activity is an essential target for antihypertension. Elastin is an important macromolecular protein which exists widely in elastic tissues such as arteries, ligaments, lung, skin, etc. and exerts its elasticity to such elastic tissues. It was recently shown that elastin is the protein with ACE inhibitory activity. The peptides with ACE inhibitory activity were found in a thermolysin-treated hydrolysate of bovine ligamentum nuchae elastin [1] and an elastase-treated hydrolysate of porcine aortic elastin [2]. However, ACE inhibitory activities of these peptides were relatively weak. Therefore we are particularly interested in novel ACE inhibitory peptides derived from porcine aortic elastin. Here, we investigated to isolate the ACE inhibitory peptides from a pancreatic elastase-treated hydrolysate of porcine aortic elastin.

Methods

Porcine aortic elastin was treated with pancreatic elastase and its hydrolysate was separated into eleven crude fractions (F1~F11) and analyzed by ACE inhibition assay. The mixture of F2 and F3 with ACE inhibitory activity was separated into five refractions (Ref1~Ref5) (Fig. 1) and analyzed by ACE inhibition assay. The separation into crude fractions (F1~F11) or into refractions (Ref1~Ref5) was carried out using Inertsil ODS-3 column (250 mm x 20 mm) by reversed-phase high-performance liquid chromatography (HPLC). The ACE inhibition assay was performed according to the method of Cushman and Cheung [3] as modified by Yamamoto *et al.* [4]. The potent ACE inhibitory refraction, Ref5 was separated and identified by means of LC/MS/MS. Seven peptides identified were synthesized by a conventional solid-phase method using Fmoc strategy, and the molecular weights and purities of these synthetic peptides were confirmed by MALDI-TOF-MS and by HPLC measurement, respectively. ACE inhibitory activity of synthetic peptides was examined by ACE inhibition assay.

Results

The novel ACE inhibitory peptides derived from pancreatic elastase-treated hydrolysate of porcine aortic elastin were oligopeptides in the crosslinked regions as shown in Table 1. The most potent ACE inhibitory oligopeptide was exhibited to be a Leu-Ala-Ala.

Conclusion

The resulting ACE inhibitory peptides may be beneficial as ingredients of functional foods for preventing hypertension.

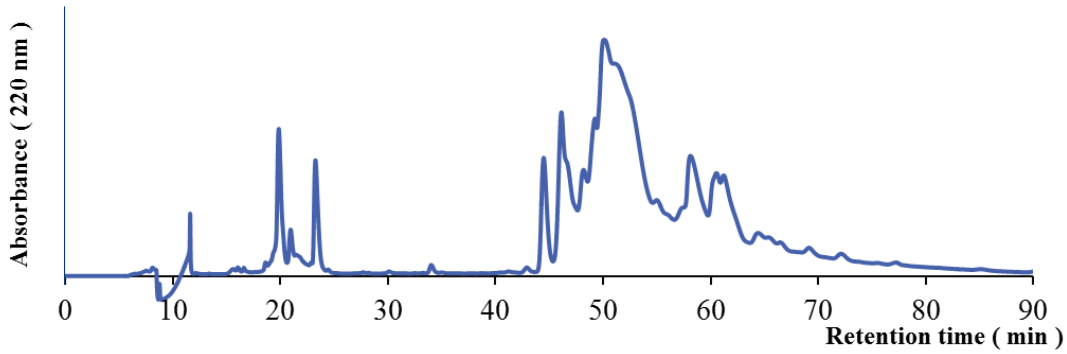

Figure 1: HPLC Chromatogram of Mixture of F2 and F3

The separation was carried out under the following conditions.

Solvent A: CH3CN 5% + 0.1% TFA Solvent B: CH3CN 95% + 0.1% TFA

Flow rate: 10 mL/min

Gradient system for refractionation:

Solvent B 0% (0 - 3 min), 0 - 3.5% (3 - 10 min), 3.5 - 10% (10 - 80 min)

The mixture of F2 and F3 was further fractionated into 5 refractions as follows:

Ref1 (18.3 - 24.0 min), Ref2 (43.5 - 47.5 min), Ref3 (47.5 - 55.3 min),

Ref4 (55.3 - 60.0 min), Ref5 (60.0 - 74.0 min).

*Table 1: IC50 of Oligopeptides Found in Porcine Aortic Elastin * Tropoelastin is a precursor protein of elastin*

Oligopeptides	The number of oligopeptide which is present in tropoelastin*	IC_{50} (μM)
Ala-Ala-Ala	33	214
Leu-Ala-Ala	1	6.1
Ser-Ala-Ala	1	162
Gln-Ala-Ala	3	255
Gly-Ala-Ala	6	258
Pro-Ala-Ala	7	310
Asp-Ala-Ala	1	705

References

[1] Y.Sato,et al., Biosci. Biotechnol. Biochem., 76 (7), 1329–1333(2012).

[2] I.Maeda,et al., Current Enzyme Inhibitation, 14(1), 67-74(2018).

[3] D. W. Cushman and H.S.Cheung, Biochem. Pharmacol., 20, 1637–1648 (1971).

[4] S. Yamamoto,et al., Nihon Kyobu Shikkan Gakkai Zasshi (in Japanese), 18, 297–303 (1980).