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Isolation of ACE Inhibiting Peptide from Thermolysin Hydrolysate of Manila clam, *Ruditapes philippinarum* Proteins

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Introduction

The angiotensin converting enzyme (ACE) generates the powerful vasoconstrictor angiotensin II by removing the C-terminal dipeptide from the precursor decapeptide angiotensin I (1). The enzyme also inactivates the vasodilator bradykinin (2). There have been many studies on ACE inhibitory substances as functional in food, and ACE inhibitory peptides were isolated (3-5). During a search for the physiologically functional food material of marine products, we found ACE inhibitory activity in thermolysin hydrolysate of manila clam (*Ruditapes philippinarum*) proteins. In this study, we present the isolation and identification of an active component.

Materials and Methods

Materials : ACE, Hip-His-Leu, and thermolysin were purchased from Sigma Chemicals Co (St. Louis, MO). Leu-Leu-Pro, Leu-Leu-Pro-Pro, and Leu-Leu-Pro-Asn are custom-made peptides which were purchased from the Korea Basic Science Institute (Seoul, Korea). Manila clam was harvested from the southwestern coast of Korea.

In vitro assay for the ACE inhibitory activity : Assays were performed HPLC (Hewlett Packard Co., HP 1100, USA), using 5 mM Hip-His-Leu as a substrate by a modification of the method of Horiuchi et al. (6). The ACE (60 mU/mL) used in this experiment was purified enzyme, which had been extracted from rabbit lung.

Purification and analysis of ACE inhibitor from a thermolysin hydrolysate of manila clam proteitns : One hundred grams of the fresh raw manila clam was boiled for 10 min in 300 mL of distilled water, then minced and homogenized. Thirty mL of the homogenized sample was added to 50 mL of Tris-HCl buffer (100 mM, pH 8.2, containing 10 mM $CaCl_2$), and then 32 mg of thermolysin was added. After 4.5 h of digestion at 37℃, the reaction was terminated by boiling for 10 min at 100℃. The precipitate was removed by filtration with Toyo fiter paper (Toyo Roshi Co., Ltd.), and the filtrate was then ultra-filtrated with PM-10 membrane (Amicon Co.). The crude peptides were applied to a Sephadex LH-20 column (Pharmacia Fine Chemicals, 26× 900 mm) and eluted with 30% methanol at a flow rate of 20.8 mL/h. The active fraction was collected and concentrated, and then applied to a SP-Toyopearl 650S

column (Tosoh Co., Ltd., 16×650 mm) equilibrated with distilled water and was eluted with a linear gradient of NaCl concentration (0 to 1 M) at a flow rate 30 mL/h. The active fraction was collected and concentrated, and then applied to a SuperQ-Toyopearl 650S column (Tosoh Co., Ltd., 16×650 mm) equilibrated with distilled water and was eluted with a linear gradient of NaCl concentration (0 to 1 M) at a flow rate 30 mL/h. The active fraction was purified on a Lichrosphere RP-18 column (Hewlett Packard Co., 4.6×250 mm), which was eluted with a linear gradient of acetonitrile (0~63%) in 0.1% TFA at a flow rate of 0.8 mL/min. The active peak from column was further purified on a μBondasphere C_{18} column (Water Inc., 3.9×150 mm), which was eluted with a linear gradient of acetonitrile (0~63%) in 0.1% TFA at a flow rate of 1.0 mL/min. Each chromatography was monitored by the absorbance at 210 nm. The amino acid sequence of the purified peptide was analyzed with a protein sequencer (Procise 491, Applied Biosystems, USA).

Results and Discussion

The peptide inhibiting ACE was isolated from the hydrolysate of manila clam proteins prepared with thermolysin. The amino acid sequence of the peptide was determined as Leu-Leu-Pro. Chemically synthesized Leu-Leu-Pro showed the IC_{50} value of 158 μM.

Peptides related to the manila clam-derived peptide were synthesized to study the structure-activity relationships. The tetrapeptide, Leu-Leu-Pro-Pro, showed very weak effect on the enzyme. However, Leu-Leu-Pro-Asn had no inhibitory activity.

References

1. Skeggs, L.T., W.H. Marsh, J.R. Kahn, N.P. Shumway. 1954. The existence of two forms of hypertensin. J. Exp. Med., 99, 275~282.
2. Yang, H.Y.T., E.G. Erdös, Y. Levin. 1970. A dipeptidyl carboxypeptidase that convert angiotensin I and inactivates bradykinin. Biochim. Biophys. Acta., 214, 374~376.
3. Maruyama, S., K. Nakagomi, N. Tomizuka and H. Suzuki. 1985. Angiotensin -converting enzyme inhibitor derived from an enzymatic hydrolysate of casein. II. Isolation and bradykinin-potentiating activity on the uterus and the ileum of rats. Agric. Biol. Chem., 49, 1405~1409.
4. Kohama, Y., S. Matsumoto, H. Oka, T. Teramoto, M. Okabe, T. Mimura. 1988. Isolation of angiotensin-converting enzyme inhibitor from tuna muscle. Biochem. Biophys. Res. Commun., 155, 332~337.
5. Wako, Y., S. Ishikawa and K. Muramoto. 1996. Angiotensin I -converting enzyme inhibitors in autolysates of squid liver and mantle muscle. Biosci. Biotech. Biochem., 60, 1353~1355.
6. Horiuchi, M., K.I. Fujimura, T. Terashima and T. Iso. 1982. Method for determination of angiotensin-converting enzyme activity in blood and tissue by high-performance liquid chromatography. J. Chromatogr., 233, 123~130.