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Isolation of Angiotensin I Converting Enzyme (ACE) inhibitor from fermented oyster, *Crassostrea gigas*

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Introduction

Angiotensin I converting enzyme (ACE) inhibitor was purified from *Crassostrea gigas*. The ACE belongs to the class of metalloprotease. This enzyme plays an important physiological role in regulating blood pressure of the rennin-angiotensin system by converting from angiotensin I to octapeptide angiotensin II, a potent vasoconstrictor and by inactivating bradykinin, which has depressor action.

Recently, many ACE inhibitors have been discovered and isolated from various hydrolysates such as casein, soy sauce, gelatin, and soybean.

The purpose of this study was to isolate from a fermented oyster (*Crassostrea gigas*) with respect to inhibitory effects on ACE.

Materials and Methods

Assay for ACE inhibitory activity The activity of ACE inhibition was assayed by a modification of the method of Cushman and Cheung (1971). The reaction mixture containing 50mM sodium borate buffer (pH 8.3), 0.5M NaCl, 25mU ACE from lung acetone powder, and an appropriate amount of the inhibito solution was preincubated for 10 min at 37℃. The reaction was initiated in incubator for 30 min at 37℃ by adding 4.15 mM of Hip-His-Leu, and terminated by adding 250 ㎕ of 1.0 N HCl. The hippuric acid was extracted with 500 ㎕ of ethyl acetate, after centrifugation. 200 ㎕ the extract was evaporated at room temperature for 2 h in vacuum and the hippuric acid wa dissolved in 1.0 ㎖ of distilled water. The absorbance was measured at 228 nm to evalute the degree of inhibition of ACE activity.

Purification of ACE inhibitor The fermented oyster was fractionated using a

SP-Sephadex C-25 column (Ø 4.0 × 40 ㎝) equilibrated with 20 mM sodium acetate-acetic acid buffer solution (pH 4.0) and eluted with a linear gradient of NaCl in the buffer at a flow rate of 1.0 ㎖/min. The active fractions were collected and lyophilized immediately. Further purification of the ACE inhibitor was carried out by using gel-filtration on a Sephadex G-50 column (Ø 2.5 × 90 ㎝) equilibrated and eluted with distilled water at a flow rate of 1.0 ㎖/min. The active fractions were collected by ultrafiltration, lyophilized, and then separated by reverse-phase high performance liquid chromatography (RPHPLC) using an ODS column.

Results and Discussion

ACE inhibitor from the fermented oyster was purified to the third-step by using sequential chromatographic methods. The first, it was separated three fractions by using cation exchange chromatography on a SP-Sephadex C-25. The second, the active fraction was purified two fractions by using gel-filtration on a Sephadex G-50. In the third, further purification was carried out by using HPLC on C18 column.

References

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