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Inhibition of Angiotensin 1-Converting Enzyme by Phosphopeptides in Proteolytic Hydrolysates Derived from Oyster, *Crassostrea gigas*.

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ABSTRACT

Hydrolysates which inhibit the angiotensin 1-converting enzyme (ACE) were prepared from oyster with three kinds of proteases.

The inhibitory activity of ACE detected in the hydrolysates by three kinds of proteases of oyster was fractionated into two major phosphopeptides fractions of P-1 and P-2 in peptic hydrolysates, T-1 and T-2 in tryptic hydrolysates, C-1 and C-2 in chymotryptic hydrolysates by gel filtration chromatography on Sephadex G-50, respectively.

The inhibition of ACE of the six kinds of phosphopeptides fractions (P-1, P-2, T-1, T-2, C-1 and C-2) was investigated in vitro.

The IC$_{50}$ values of P-1, P-2, T-1, T-2, C-1, and C-2 of phosphopeptides for ACE were 0.3, 2.9, 2.7, 2.6, 1.5 and 1.4 mg protein/ml, respectively.

The pepsin treated fraction P-1 had most inhibition activity and showed 0.3 mg protein/ml inhibition against ACE at IC$_{50}$ value.

The phosphono-compounds was found in the phosphopeptides fractions with three kinds of proteases.

It has been demonstrated that the P-1, P-2, T-1, T-2, C-1 and C-2 contained about 79.13%, 79.19%, 11.07%, 4.71%, 15.26% and 4.49% as phosphonate-phosphorus of total phosphorus.

The amino acid compositions of the phosphopeptides fractions (P-1, P-2, T-1, T-2, C-1 and C-2) were characterized by relatively high percentage for glutamic acid, aspartic acid, alanine, lysine and threonine.

INTRODUCTION

Protein from natural resources are important for supplying nutrient and energy. Recently, it has become apparent that they also have many functions relating to physio-
logical regulation\(^1,\ 2\). A number of functional peptides derived from milk, soybean and fish have been found, which may serve to promote Ca absorption\(^3\), lowering of blood pressure\(^2,\ 4-9\), regulation of cholesterol in serum\(^10\) and other benefits\(^11\).

The ACE inhibitory abilities of foods have recently been investigated\(^12\). ACE (EC. 3.4.15.1) play an important physiological role in regulating blood pressure, ACE catalyzes the conversion of inactive angiotensin I to a potent vasoconstrictor angiotensin II\(^13,14\).

Our interest has been focused on the preparation and isolation of phosphopeptides from natural resources that are involved in blood pressure regulation. In previous papers\(^15-17\), phosphoproteins and phosphopeptides that inhibited the ACE were isolated from edible shellfish.

Many investigators have tried to prepare and isolate the ACE Inhibitory peptides. In this report, we investigate the effectiveness of three kinds of proteolytic hydrolysates as a physiologically functional food and attempt to isolate the ACE inhibitory phosphopeptides from the three kinds of proteolytic hydrolysates of Oyster.

**MATERIALS AND METHODS**

**Materials:** Edible Oyster, *Crassostrea gigas* was purchased on the local market. Sephadex G-50 was a product of Pharmacia. Proteolytic enzymes (pepsin, trypsin, chymotrypsin) was obtained from Boehringer Co. ACE from rabbit lung acetone powder were obtained from Sigma Chemical Co. (U. S. A.).

Hippuryl-L-histidyl-leucine (HHL) as a substrate was obtained from the Peptide Institute (Osaka, Japan). All other reagents were obtained from Nacalai Tesque (Kyoto, Japan).

**Assay of ACE inhibitory activity:** The activity of ACE inhibition was assayed by the method of Yamamoto et. al\(^18\).

For each assay, 100\(\mu\)l of ACE inhibitor and 50\(\mu\)l of Hip-His-Leu (2.5 mM in a borate buffer containing 200 mM NaCl at pH 8.3) were incubated with 100\(\mu\)l of 2.5 mu/ml of ACE at 37°C for 30 min. The reaction was stopped by adding 250\(\mu\)l of 1N HCl, and 1.5 ml of ethyl acetate was then added to the mixture, and the mixture was shaken for 30 sec. The mixture was centrifuged at 2500 rpm for 10 min., and 1.25 ml from the supernatant was transferred to test tube, and then heated at 120°C for 30 min., 0.5 ml of 1M NaCl was then added to the dried material, and the solution was shaken for 30 sec., and the absorbance of the yielded hippuric acid at 228 nm was measured with a Hitachi 101 spectrophotometer.

The ACE inhibitor concentration required to inhibit 50% of the ACE activity is defined as the IC\(_{50}\) value.
Isolation and preparation of ACE inhibitors from proteolytic hydrolysates:

1. **Hydrolysis by pepsin:**
   The freeze-dried material (26.4 g) of Oyster, *Crassostrea gigas* was extracted with 550 mL of 0.01 M Tris-HCl buffer (pH 8.0) by an efficient magnetic stirrer in a 1000 ml beaker at room temperature for 2 hrs. The extracts were centrifuged at 3000 rpm for 15 min.

   The supernatant was freeze-dried and approximately extract 15 g was obtained. The freeze-dried extract (4.9 g) was dissolved in 100 ml of HCl-KCl buffer (pH 2.0), and then 100 mg of pepsin was added to the mixture, and the mixture incubated at 37°C for 24 hrs. After proteolysis, the solution was then heated for 15 min. in a boiling-water bath.

   After the precipitate had been removed by centrifugation at 3000 rpm for 15 min., the supernatant was then dialyzed for 24 hrs. to remove inorganic salts. Dialyzed solution was centrifuged again, and supernatant was freeze-dried.

2. **Hydrolysis by trypsin and chymotrypsin:**
   The freeze-dried extract (4.9 g) was dissolved in 100 ml of 0.01 M Tris-HCl buffer (pH 8.0), and then 100 mg of trypsin or chymotrypsin was added to mixture, and the mixture incubated at 37°C for 24 hrs. After proteolysis, the solution was then heated for 15 min. in a boiling-water bath.

   After the precipitate had been removed by centrifugation at 3000 rpm for 15 min., the supernatant was then dialyzed for 24 hrs. to remove inorganic salts. Dialyzed solution was centrifuged again, and supernatant was freeze-dried.

**Fractionation of inhibitor by column chromatography:**

The freeze-dried hydrolysate of pepsin, trypsin and chymotrypsin was dissolved in 15 ml of 0.01 M Tris-HCl buffer and then applied to a 2.5 x 40 cm column of Sephadex G-50 (prewashed with 0.01 M Tris-HCl buffer). The column was eluted with 500 ml of 0.01 M Tris-HCl buffer. The eluate was collected in 10 ml fractions, while monitoring the absorbance at 280 nm for peptides and at 820 nm for total phosphorus. (Peptic, tryptic and chymotryptic fractions were denoted as P-1, P-2, T-1, T-2, C-1 and C-2, respectively).

Each fraction was collected, and determined the phosphonate phosphorus, amino acid and ACE inhibitory activity.

**Quantitative analysis:**

Total phosphorus was estimated by the method of Chen et al. Phosphonate-phosphorus was estimated by the method of Tamari et al. Amino acid analyses were carried out with a JTC-200A amino acid analyzer. The sample for amino acid analyses was hydrolyzed in 6 N HCl at 110°C for 24 hrs. The nitrogen and protein were analyzed in the usual way (% protein = % N x 6.25).
RESULTS

Fig. 1 showed the Sephadex G-50 column chromatogram of the peptic hydrolysates of Oyster, *Crassostrea gigas*. The experimental details are described in the text. The entire effluent was collected in tubes on a fraction collector for quantitative analysis of the total phosphorus and for qualitative analysis of the phosphopeptides at 280nm.

![Chromatographic Profile of Phosphopeptides Eluted by Sephadex G-50 Column Chromatography from the Hydrolysates Obtained by Peptic Hydrolysis of Oyster, *Crassostrea gigas*.](image)

The experimental details are described in the text.

- ○○ indicate total phosphorus and ■■ indicate phosphopeptides at 280nm in the collected fractions. P-1 and P-2 represent combined fractions 6~10 and fractions 18~24, respectively.

Two phosphorus-containing peaks and two peptides peaks were eluted from the column in the case of peptic hydrolysates. The first peak and second peak designated as P-1 and P-2, respectively.

The maximum total phosphorus content was observed in fraction No. 7 in the case of P-1, and in fraction No.19 in the case of P-2 as shown in the Fig. 1.

In addition, the maximum absorption at 280nm was observed in fraction No. 7 in the case of P-1, and in fraction No.21 in the case of P-2. Fraction No. 6~10 of the P-1 and fraction No.18~24 of the P-2 were pooled, and analysed for phosphonate-phosphorus(C-P), amino acids and ACE inhibitory activity.

Fig. 2 shows the Sephadex G-50 column chromatogram of the tryptic hydrolysates of
Oyster, *Crassostrea gigas*. The experimental details are described in the text.

The entire effluent was collected in tubes on a fraction collector for quantitative analysis of the total phosphorus and for qualitative analysis of the phosphopeptides at 280nm.

![Graph](image)

**Fig. 2.** Chromatographic Profile of Phosphopeptides Eluted by Sephadex G-50 Column Chromatography from the Hydrolysates Obtained by Tryptic Hydrolysis of Oyster, *Crassostrea gigas*.

The experimental details are described in the text.

- - indicate total-phosphorus and - - indicate phosphopeptides at 280nm in the collected fractions. T-1 and T-2 represent combined fractions 5～10 and fractions 15～26, respectively.

Two phosphorus-containing peaks and two peptides peaks were eluted from the column in the case of tryptic hydrolysates.

The first peak and second peak designated as T-1 and T-2, respectively.

The maximum total-phosphorus content was observed in fraction No. 8 in the case of T-1, and in fraction No.19 in the case of T-2 as shown in the Fig. 2.

In addition, the maximum absorption at 280nm was observed in fraction No. 7 in the case of T-1, and in fraction No.19 in the case of T-2. Fraction No. 5～10 of the T-1 and fraction No.15～26 of the T-2 were pooled, and analysed for phosphonate-phosphorus, amino acids and ACE inhibitory activity.

**Fig. 3** shows the Sephadex G-50 column chromatogram of the chymotryptic hydrolysates of Oyster, *Crassostrea gigas*.

The experimental details are described in the text.

The entire effluent was collected in tubes on a fraction collector for quantitative
Fig. 3. Chromatographic Profile of Phosphopeptides Eluted by Sephadex G-50 Column Chromatography from the Hydrolysates Obtained by Chymotryptic Hydrolysis of Oyster, Crassostrea gigas.

The experimental details are described in the text.

- indicate total phosphorus and ■ indicate phosphopeptides at 280 nm in the collected fractions. C-1 and C-2 represent combined fractions 6–10 and fractions 16–27, respectively.

analysis of the total phosphorus and for qualitative analysis of the phosphopeptides at 280 nm.

Two phosphorus-containing peaks and two peptides peaks were eluted from the column in the case of chymotryptic hydrolysates.

The first peak and second peak designated as C-1 and C-2, respectively. The maximum total-phosphorus contents were observed in fraction No. 7 in the case of C-1, and in fraction No. 20 in the case of C-2 as shown in the Fig. 3.

In addition, the maximum absorption at 280 nm was observed in fraction No. 7 in the case of C-1, and in fraction No. 20 in the case of C-2. Fraction No. 6–10 of the C-1, and fraction No. 16–27 of the C-2 were pooled, and analysed for phosphonate-phosphorus, amino acid and ACE inhibitory activity.

In Table 1 is reported the phosphorus contents of the P-1, P-2, T-1, T-2, C-1 and C-2 from the proteolytic hydrolysates of the Oyster, Crassostrea gigas.

The total-phosphorus content in the P-1, P-2, T-1, T-2, C-1 and C-2 fractions were 5.08, 714.54, 23.13, 390.71, 12.38 and 261.72 µg per ml of the peaks of the Crassostrea gigas, respectively.

The amounts of phosphonate-phosphorus (C-P) in the P-1, P-2, T-1, T-2, C-1 and
Inhibition of Angiotensin 1 Converting Enzyme by Phosphopeptides in Proteolytic Hydrolysates Derived from Oyster, *Crassostrea gigas*.

Table 1. Phosphorus Contents of the P 1, P·2, T·1, T·2, C·1 and C·2 from the Proteolytic Hydrolysates of the Oyster, *Crassostrea gigas*.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>T·P (µg/ml)</th>
<th>C·P (µg/ml)</th>
<th>C·P / T·P (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P·1</td>
<td>5.08</td>
<td>4.02 (0.67)</td>
<td>79.13</td>
</tr>
<tr>
<td>P·2</td>
<td>714.54</td>
<td>565.86 (93.61)</td>
<td>79.19</td>
</tr>
<tr>
<td>T·1</td>
<td>23.13</td>
<td>2.56 (0.24)</td>
<td>11.07</td>
</tr>
<tr>
<td>T·2</td>
<td>90.71</td>
<td>18.41 (3.05)</td>
<td>4.71</td>
</tr>
<tr>
<td>C·1</td>
<td>12.38</td>
<td>1.89 (0.31)</td>
<td>15.26</td>
</tr>
<tr>
<td>C·2</td>
<td>261.72</td>
<td>11.76 (1.95)</td>
<td>4.49</td>
</tr>
</tbody>
</table>

Abbreviations used are:
- C·P, Phosphonate-phosphorus ; T·P, Total phosphorus ;
- ( ), as % of Total C·P

Table 2. Amino Acid Composition in the Acid Hydrolysates of Phosphopeptides in the P·1, P·2, T·1, T·2, C·1 and C·2 Fractions Eluted by Sephadex G·50 Column Chromatography of the Three Different Enzymatic Hydrolysates of Oyster, *Crassostrea gigas*.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>P·1</th>
<th>P·2</th>
<th>T·1</th>
<th>T·2</th>
<th>C·1</th>
<th>C·2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>249.6</td>
<td>433.8</td>
<td>269.3</td>
<td>954.1</td>
<td>136.4</td>
<td>986.4</td>
</tr>
<tr>
<td>Thr</td>
<td>91.8</td>
<td>441.2</td>
<td>218.1</td>
<td>426.2</td>
<td>130.3</td>
<td>487.1</td>
</tr>
<tr>
<td>Ser</td>
<td>102.6</td>
<td>293.5</td>
<td>166.9</td>
<td>459.9</td>
<td>87.1</td>
<td>508.2</td>
</tr>
<tr>
<td>Glu</td>
<td>448.6</td>
<td>442.1</td>
<td>269.1</td>
<td>1729.4</td>
<td>115.1</td>
<td>1573.4</td>
</tr>
<tr>
<td>Gly</td>
<td>160.2</td>
<td>286.9</td>
<td>149.1</td>
<td>731.1</td>
<td>80.0</td>
<td>709.2</td>
</tr>
<tr>
<td>Ala</td>
<td>174.5</td>
<td>293.2</td>
<td>131.4</td>
<td>608.4</td>
<td>86.0</td>
<td>601.2</td>
</tr>
<tr>
<td>Cys</td>
<td>7.3</td>
<td>100.0</td>
<td>32.9</td>
<td>19.7</td>
<td>8.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Val</td>
<td>81.2</td>
<td>163.9</td>
<td>119.1</td>
<td>445.6</td>
<td>79.8</td>
<td>464.0</td>
</tr>
<tr>
<td>Met</td>
<td>38.3</td>
<td>51.5</td>
<td>24.3</td>
<td>21.1</td>
<td>8.8</td>
<td>0.0</td>
</tr>
<tr>
<td>Ileu</td>
<td>68.4</td>
<td>102.1</td>
<td>82.4</td>
<td>378.3</td>
<td>41.7</td>
<td>379.8</td>
</tr>
<tr>
<td>Leu</td>
<td>144.0</td>
<td>129.0</td>
<td>127.1</td>
<td>639.7</td>
<td>68.0</td>
<td>658.3</td>
</tr>
<tr>
<td>Tyr</td>
<td>76.3</td>
<td>446.6</td>
<td>218.1</td>
<td>233.5</td>
<td>123.9</td>
<td>171.9</td>
</tr>
<tr>
<td>Phe</td>
<td>88.0</td>
<td>294.7</td>
<td>150.0</td>
<td>323.9</td>
<td>74.8</td>
<td>364.1</td>
</tr>
<tr>
<td>His</td>
<td>52.2</td>
<td>232.0</td>
<td>122.6</td>
<td>334.1</td>
<td>50.2</td>
<td>381.1</td>
</tr>
<tr>
<td>Lys</td>
<td>137.4</td>
<td>679.3</td>
<td>119.1</td>
<td>977.2</td>
<td>71.9</td>
<td>911.7</td>
</tr>
<tr>
<td>Arg</td>
<td>123.6</td>
<td>200.0</td>
<td>55.0</td>
<td>396.7</td>
<td>32.0</td>
<td>601.9</td>
</tr>
<tr>
<td>Pro</td>
<td>140.0</td>
<td>393.4</td>
<td>143.3</td>
<td>724.3</td>
<td>73.7</td>
<td>812.5</td>
</tr>
<tr>
<td>2·AEP</td>
<td>16.1</td>
<td>2,263.4</td>
<td>10.2</td>
<td>73.6</td>
<td>7.6</td>
<td>47.0</td>
</tr>
</tbody>
</table>

Total: 2,200.0, 7,246.6, 2,407.9, 9,477.1, 1,275.5, 9,657.8

* Values are expressed as µg/mg Nitrogen.
* Abbreviations used are: 2·AEP, 2· aminoethylphosphonic acid.
C-2 were 4.02, 565.86, 2.56, 18.41, 1.89 and 11.76 μg per ml of the peaks.

In addition, about 79, 79, 11, 4, 15 and 4% of the total phosphorus in the P-1, P-2, T-1, T-2, C-1 and C-2 fractions was found to be phosphonate, which was primarily 2-aminoethylphosphonic acid.

The P-2 fraction contained 93.6% of the phosphonate, and the amount of phosphonate in the P-2 fraction was 141, 221, 31, 299 and 48 times higher than those in the P-1, in the T-1, in the T-2, in the C-1 and in the C-2 fractions, respectively.

Table 2 summarises the amino acid composition of the P-1, P-2, T-1, T-2, C-1 and C-2 fractions obtained under three different proteolytic hydrolysis.

Of note in P-1, P-2, T-1, T-2, C-1 and C-2 peaks is the very high acidic amino acids (Asp and Glu), Gly, Ala, Leu, Lys and Pro content, and the presence of unique amino acid, 2-aminoethylphosphonic acid.

In P-2 phosphopeptide, the 2-AEP content was high, when compared to P-2, other peaks (P-1, T-1, T-2, C-1 and C-2) had a low 2-AEP.

Cheung et al. 21) and Matsui et al. 6) have reported that it was essential for strong and competitive ACE inhibition that a peptide had aromatic amino acid residues at the C-termini (e.g., Try, Tyr, Pro.), and hydrophobic or basic ones at the N-terminus.

Table 3. Angiotensin Converting Enzyme Inhibitory Activity of the P-1, P-2, T-1, T-2, C-1 and C-2 Phosphopeptides Eluted by Sephadex G-50 Column Chromatography of the Hydrolysates Obtained by Various Enzymatic Hydrolysis of Oyster, Crassostrea gigas

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein (mg/ml)</th>
<th>IC50 (mg protein/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-1</td>
<td>0.17</td>
<td>0.30</td>
</tr>
<tr>
<td>P-2</td>
<td>1.94</td>
<td>2.90</td>
</tr>
<tr>
<td>T-1</td>
<td>0.66</td>
<td>2.73</td>
</tr>
<tr>
<td>T-2</td>
<td>4.60</td>
<td>2.64</td>
</tr>
<tr>
<td>C-1</td>
<td>0.85</td>
<td>1.53</td>
</tr>
<tr>
<td>C-2</td>
<td>2.30</td>
<td>1.47</td>
</tr>
</tbody>
</table>

Table 3 shows angiotensin converting enzyme inhibitory activity of the P-1, P-2, T-1, T-2, C-1 and C-2 phosphopeptides eluted by Sephadex G-50 column chromatography of the hydrolysates obtained by various enzymatic hydrolysis of oyster, Crassostrea gigas.

The IC50 values for P-1, P-2, T-1, T-2, C-1 and C-2 were 0.3, 2.9, 2.7, 2.6, 1.5 and 1.5 mg protein/ml, respectively.

The pepsin treated fraction P-1 had most inhibition activity and showed 0.3 mg protein/ml inhibition against ACE at IC50 value, and the IC50 value in the P-1 fraction was 9.6, 9.0, 8.7, 5.0 and 4.7 times higher than those in the P-2, in the T-1, in the T-2, in the C-1 and in the C-2 fractions, respectively.
DISCUSSION

Our interest has been focused on the preparation and isolation of phosphopeptides from natural resources that are involved in blood pressure regulation. To date, there is still no report on the ACE inhibition activity of the phosphopeptides and phosphonopeptides in nature. This report describes the isolation of an ACE inhibitory phosphopeptides having antihypertensive effect from Oyster, *Crassostrea gigas*.

To investigate the resistance of phosphoprotein to digestion by gastrointestinal proteases, phosphoprotein was digested by various proteases: pepsin, trypsin, chymotrypsin.

In this study, in the case of peptic hydrolysates of oyster contained two major ACE inhibitors (P-1 and P-2) that were fractionated by gel filtration. The specific activity for ACE inhibition of the P-1 fraction was about 10 times higher than that of the P-2 fraction.

In the case of tryptic hydrolysates contained two major ACE inhibitors (T-1 and T-2) that were fractionated by gel filtration. The specific activity for ACE inhibition of the T-1 fraction was almost the same that of the T-2 fraction.

In the case of chymotryptic hydrolysates contained two major ACE inhibitors (C-1 and C-2) that were fractionated by gel filtration. The specific activity for ACE inhibition of the C-1 fraction was almost the same that of the C-2 fraction. The pepsin treated fraction P-1 had most inhibition activity and showed 0.3mg protein/ml inhibition against ACE at IC50 value.

In previous papers15-17, author et. al. have reported that the inhibitory activity of ACE detected in several shellfishes was fractionated into two major fractions of high molecular weight and low molecular weight by gel filtration chromatography on Sephadex G-50.

On the other hand, Sugiyama et. al.22) have already reported that the ACE inhibitory activity of the alkaline protease hydrolysate from defatted sardine meal was reduced by 40% after a digestion test with gastrointestinal proteases.

Matsuda et.al.23) and Ogihira et.al.24) have reported that di- and tri-peptides would have low susceptibility to hydrolysis by any digestive enzymes.

Matsufuji et. al.7) and Matsui et. al.6) have reported that the ACE inhibitor isolated from alkaline protease hydrolysate of sardine muscle was inhibited ACE competitively.

Kinoshita et. al.11) have reported that the inhibitory activity of ACE detected in soy sauce was fractionated into two major fractions high molecular weight (HW) and low molecular weight (LW) by gel filtration chromatography on Bio-gel P-2; the HW fraction reduced the blood pressure in hypertensive rats after orally administering, while the LW fraction did not.

On the other hand, the search related to the structure and function of phosphonomacromolecules will be one of the most fascinating fields. Aminophosphonic acid are present in various bound forms other than the phosphonolipids and antibiotic
tripeptides. For instant, 2-AEP is found in *Tetrahymena* associated with macromolecular complexes which resist hydrolysis by proteolytic enzymes such as protease and trypsin\(^2\).

Gibson and Dixon\(^26\) isolated from the sea anemone *Metridium senile* three chymotrypsin-like protease, of which two were proved by Stevenson et al.\(^27\) to contain 2-AEP. The two enzymes, protease A and B, have properties similar to the mammalian \(\gamma\)-chymotrypsin with respect to the active-site amino acid sequence and the mode of enzyme action.

Protease A contained 6 2-AEP residues per 243 residues of total amino acids and protease B had 4 2-AEP residues per 239 residues of total amino acids.

The site of attachment of the 2-AEP to three proteases are yet unknown. Quin\(^28\) purified a polypeptide containing 4.4% 2-AEP by pepsin hydrolysis of the insoluble residue from alcohol and chloroform extracts of *M. dianthus* and found that none of the 2-AEP with the amino group free was present.

From the results above, it has been demonstrated that the isolated peptide inhibitors occurs as the phosphonate containing phosphonopeptides and phosphate containing phosphopeptides, but more experiments will be needed to prove the ACE inhibition of the phosphopeptides isolated from oyster.

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Inhibition of Angiotensin 1–Converting Enzyme by Phosphopeptides in Proteolytic Hydrolysates Derived from Oyster, *Crassostrea gigas.*

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