Zinc in Oysters (Crassostrea gigas): Chemical Characteristics and Action during In Vitro Digestion

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Summary To obtain information on the luminal absorption of oyster zinc, the zinc action during an in vitro protease digestion of oysters was examined. More than 90% of the zinc rendered solute at pH 1.3 or 3.0 irrespective of the pepsin digestion. The solute zinc was partially re-precipitated by neutralization, and trypsin digestion did not render the re-precipitated zinc solute. When the pepsin digestion was performed at pH 5.0, the ratio of soluble zinc in trypsin digest decreased. When the trypsin digest was fractionated by Sephadex G-25, the zinc was eluted later than the peptide fragments. These results indicate that the pH of the stomach juice rather than peptides released by the digestion of oyster protein highly contributes to the oyster zinc in the small intestine becoming solute.

Key Words zinc, oyster, bioavailability, luminal absorption, in vitro digestion

Zinc is an essential trace element in human nutrition. The latest National Nutritional Survey has indicated that there is a suboptimal zinc status in the Japanese population (1). To improve zinc nutrition, effective utilization of several food sources with high zinc concentration is necessary. According to the Standard Tables of Food Composition in Japan, oysters contain zinc at a particularly high level (2), and so their use is expected in the zinc-enrichment of foods or the production of the zinc supplements.

It has been established that zinc in foods shows varying absorption rates due to various dietary factors including chemical characteristics (3). Even though oysters contain zinc at a high level, their contribution to zinc nutrition will remain unclear unless information on the absorption of oyster zinc is obtained.

Many approaches have been used to investigate zinc absorption in foods. The in vitro approach to zinc absorption has been performed by several researchers (4–6). In these reports, the solubility and chemical characteristics of zinc in an in vitro digest were used for an index of absorption. In the present study, to obtain information on the luminal absorption of oyster zinc, the chemical characteristics of oyster zinc and its action during an in vitro protease digestion of oysters were examined.

MATERIALS AND METHODS

Samples. Oysters (Crassostrea gigas) with a shell length of 10 to 15 cm were collected in the northern part of Hiroshima Bay in April 2002. Among them, 4 samples (zinc content: 123±19 μg/g) were selected randomly and homogenized with a homogenizer with a stainless steel blade (Cell Master CM-100, As-one Co., Osaka, Japan) individually.

Fractionation of oyster homogenate. Five grams of the whole oyster homogenate was diluted with 20 mL of Tris-HCl buffer (50 mM, pH 7.4) and centrifuged at 6,000×g for 30 min. After filtration with a membrane filter (0.20 μm), 100 μL of the soluble fraction obtained was analyzed using high-performance liquid chromatography–inductively coupled plasma mass spectrometry (HPLC-ICPMS). The HPLC system consisted of a CCPM-II multi-pump (Tosoh, Tokyo, Japan), an SD-8022 on-line degasser (Tosoh) and a column (4.6 mm i.d. ×250 mm) packed with a molecular exclusive resin (TSK-GEL 2000SWxL, Tosoh). The mobile phase was Tris-HCl buffer or 0.1M NaCl. Elution was performed isocratically at 1.0 mL/min. The eluate was successively monitored by a UV-8020 spectrophotometer (Tosoh) at an absorbance of 280 nm and by ICPMS (ICPM-8500, Shimadzu, Kyoto, Japan) at an ion intensity of m/z 66 derived from Zn66. Another 5 mL of the soluble fraction was fractionated using a column (3.0 cm i.d. ×40 cm) packed with another molecular exclusive resin, Sephadex G-25. The samples were eluted with the Tris-HCl buffer or 0.1 M NaCl at a flow rate of 1.0 mL/min. Eluates were collected every 5 mL, the absorbance was measured at 280 nm and zinc content was determined.

In vitro protease digestion. One gram of the whole oyster homogenate was mixed with 50 mL of 0.1 M HCl containing 100 mg of a dried powder of porcine pepsin (1:10,000, Wako Pure Chemical Industries, Ltd., Osaka, Japan) and incubated and shaken at 37°C for 3 h. The pH of this reaction mixture was 1.3. In another pepsin digestion test under a different pH condition, each homogenate was mixed with deionized water and adjusted to pH 3.0 or 5.0 by dropwise addition of 1 M...
HCl. In the case of the pepsin digestion at pH 1.3, the pH of the reaction mixture was adjusted to 7.4 by dropwise addition of 1 M NaOH after the digestion. After neutralization, 10 mg of a crystalline porcine trypsin (5, 600 USP trypsin unit/mg, Wako) was added to the mixture and incubated and shaken at 37 °C for a further 16 h. At each step of the in vitro protease digestion, a portion of the reaction mixture was centrifuged at 6,000 × g for 30 min. Zinc and nitrogen levels in the soluble fraction obtained were determined and their solubility was estimated as a percent of the contents in the soluble fraction to those in the whole mixture. In addition, the soluble fraction of the pepsin or pepsin-trypsin digest was fractionated by the Sephadex G-25 column (3.0 cm i.d. × 40 cm) with 0.1 M NaCl as elution solvent at a flow rate of 1.0 mL/min.

Analyses. One gram of the homogenate of the whole shelled oyster or each tissue was mixed with 10 mL of nitric acid (metal-free grade, Wako) and heated in a boiling water bath until the insoluble component disappeared. The protease digests were also rendered solute with nitric acid in a similar manner. Then, the acid digests were diluted with deionized water. The zinc level in the diluted acid digests and the eluates of the Sephadex G-25 chromatography was determined with an atomic absorption flame emission spectrophotometer (AA-6200, Shimadzu). The zinc analysis was verified using standard reference materials (RM 8414, bovine muscle powder, National Institute of Standard & Technology, USA). In addition, we confirmed that the zinc values in the oyster homogenate digested with nitric acid were coincident with those digested with nitric acid and perchloric acid. Nitrogen was determined by the Kjeldahl method.

Statistics. When necessary, analytical results were assessed by analysis of variance (ANOVA) followed by Fisher's PLSD test for multiple comparisons using a personal computer (iMac OS 8.6, Apple Computer, Cupertino, CA, USA) with the statistical analysis software package StatView ver. 5.0 (Abacus Concepts, Berkeley, CA, USA).

RESULTS

Fractionation of whole homogenate

The solubility of zinc in the 20% oyster whole homogenate was 22.5%. Figure 1 shows the results of fractionation of the soluble fraction under 4 different gel chromatography systems. When the chromatography was performed using HPLC systems (Fig. 1a and b), zinc was co-eluted with high-molecular weight protein at a retention time that corresponded to a void volume irrespective of the kind of elution solvent. On the other hand, zinc was eluted into two peaks and the elution pattern of zinc was similar to that of the absorbance at 280 nm in the fractionation by Sephadex G-25 with Tris-HCl buffer (Fig. 1c). However, when 0.1 M NaCl was used as the elution solvent in the Sephadex G-25 column chromatography, zinc was eluted with a broad range and the elution pattern of zinc was not coincident with that of the absorbance at 280 nm (Fig. 1d).

Action of zinc during protease digestion

The solubility of zinc in each step of the protease digestion is summarized in Table 1. In the untreated oyster homogenate, more than 70% of the zinc was insoluble at pH 7.4. Addition of 0.1 M HCl to the homogenate made more than 90% of the zinc solute. Neutralization of the acidic pepsin digest caused partial insolubility of the zinc. However, digestion with protease did not affect the solubility of zinc. Even at the end of the in vitro protease digestion, about 30% of the zinc remained insoluble. On the other hand, the digestion with protease increased the soluble nitrogen. Nonethe-
Table 1. Effect of in vitro protease digestion on solubilities of zinc and nitrogen in oysters.

<table>
<thead>
<tr>
<th>Digestion step</th>
<th>Solubility (%)</th>
<th>Nitrogen (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2% homogenate in Tris-HCl buffer (pH 7.4)</td>
<td>26.5±5.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.7±5.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>2% homogenate in 0.1 M HCl (pH 1.3)</td>
<td>93.8±2.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.6±4.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pepsin digest (pH 1.3)</td>
<td>94.9±0.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25.4±3.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Neutralization of pepsin digest (pH 7.4)</td>
<td>68.1±1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.5±4.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Trypsin digest (pH 7.4)</td>
<td>68.6±4.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.9±4.2&lt;sup&gt;c&lt;/sup&gt;</td>
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Samples were collected in the northern part of Hiroshima Bay in April 2002. The weight of the shelled oyster was 19.7±3.9 g (means±SD, n=4). Values (means±SD, n=4) in the same column not sharing a common superscript letter differ significantly (p<0.05).

Table 2. Effect of pH condition during pepsin digestion on the solubility of zinc in oysters.

<table>
<thead>
<tr>
<th>Digestion step</th>
<th>Solubility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pepsin digest</td>
<td>93.7±3.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Trypsin digest (pH 7.4)</td>
<td>70.2±5.7&lt;sup&gt;b&lt;/sup&gt;</td>
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Oyster samples were the same as those in Table 1. Values (means±SD, n=4) in the same row not sharing a common superscript letter differ significantly (p<0.05).

DISCUSSION

As described in Table 1, more than 70% of the zinc was insoluble in the untreated oyster homogenate at pH 7.4. In the fractionation of the soluble fraction by HPLC with a molecular exclusive resin, zinc was co-eluted with the protein fraction (Fig. 1a and b). Thus, it is likely that the soluble zinc is mostly bound to protein. However, the affinity of zinc to protein may be mild because the elution pattern of zinc varied depending on the kind of resin or solvent used in the chromatography (Fig. 1c and d). Several metallothionein-like proteins containing zinc have been found in oysters (7, 8). However, Fig. 1 shows the absence of such a specific zinc-containing component. Further examination is necessary in the search for a specific zinc-containing protein in oysters.

When the oyster homogenate was mixed with 0.1 M HCl, more than 90% of the zinc was rendered solute irrespective of the pepsin digestion. The solute zinc was partially re-precipitated by neutralization of the pepsin digest, but nearly 70% remained solute in the neutralized digest. Probably, most oyster zinc is present as a form tightly bound to oyster component(s), and is insoluble and biologically inactive. Diluted HCl, a main component in stomach juice, releases zinc from the component(s) as a bivalent zinc ion (Zn<sup>2+</sup>) which is
highly soluble at a low pH. Because of the lower solubility of zinc hydroxides at pH 7 to 10 (9), a part of Zn (II) released by HCl could be re-precipitated by neutralization, but several parts remained soluble due to the formation of a complex with a protein or a peptide contained in the pepsin digest.

It has been believed that amino acids or peptides accelerate the luminal absorption of zinc due to the formation of a soluble complex with zinc (3). In addition, it has been reported that solubility of zinc in beef was accelerated by protease digestion (10). Therefore, it was expected that the release of peptides or amino acids by the protease from the oyster protein would increase the ratio of soluble zinc. However, as described in Table 1, pepsin or trypsin digestion did not increase the ratio of soluble zinc. When the trypsin digest was fractionated by Sephadex G-25, zinc was eluted later than the peptide fragments. Probably, the resin acted as a ligand for Zn (II). This implies that the affinity of Zn (II) with the peptide fragments released by the trypsin digestion is lower than that with the untreated protein.

When the pepsin digestion was performed at pH 5.0, the ratio of soluble zinc in the trypsin digest decreased (Table 2). These results indicate that the release of zinc by stomach juice highly contributes to the solubility of oyster zinc even in the small intestine; the pH of stomach juice rather than peptides released by the digestion of oyster protein is an important factor for zinc absorption.

REFERENCES