Note

Effect of Boiling on the Elution of the Major Allergen Tropomyosin from Shrimp and Squid Muscles

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Shrimp and squid often cause food allergy. In order to prevent malnutrition, allergic patients are advised that they can eat foods that contain allergy-inducing ingredients as long as the intake does not cause an allergic reaction. However, there is no hypoallergenic treatment applicable to invertebrate seafood. Thus, in this study, we aimed to develop a hypoallergenic treatment for invertebrate seafood by removing the major allergen tropomyosin (TM) in invertebrate seafood. When muscle samples of shrimp and squid were subjected to boiling for 10 min, only 11.2 and 35.0% of TM remained in the muscles, respectively. Thus, boiling is considered to be an efficient hypoallergenic method, particularly with respect to shrimp.

Keywords: allergy, boiling, muscle, shrimp, squid, tropomyosin

Introduction

Food allergy is an adverse immunologic reaction to proteins after the ingestion of certain ingredients (Johnston et al., 2014). It is well known that shrimp and squid cause food allergy. Thus, shrimp and squid are designated as specified ingredients by the Consumer Affairs Agency in Japan; the former requires labeling, while labeling of the latter is recommended. Tropomyosin (TM) is a pan-allergen of invertebrates including shrimp and squid (Goetz and Whisman, 2000). Shrimp shows higher allergenicity than other allergenic invertebrates such as squid, abalone and scallop. Thus, shrimp TM has been intensively studied; the amino acid sequence (Leung et al., 1994) and epitopes (Ayuso et al., 2002 and 2010; Ravkov et al., 2013) of TM have been determined.

Since rice can be the cause of food allergy, development of hypoallergenic treatment of rice has been intensively studied (Yamada et al., 2006). In contrast, there have been few reports to date of the effective hypoallergenic treatment of shrimp or other invertebrates (Liu et al., 2010). While hypoallergenic treatment of invertebrate TM by high-pressure extraction (Jin et al., 2015) and enzymatic treatment (Gámez et al., 2014) have been reported, neither have been successfully applied to the processing sector. In the laboratory, extraction of TM requires the mincing or homogenization of muscle (Motoyama et al., 2006 and 2007; Ozawa et al., 2011). This process is not applicable as a hypoallergenic treatment because mincing or homogenization would decrease the value of the seafood.

In Japan, in order to prevent malnutrition, allergic patients are advised that they can eat foods that contain allergy-inducing ingredients as long as the intake does not cause an allergic reaction (Ebisawa, 2014). Thus, hypoallergenic treatment would increase the potential edible quantity of allergenic foods. In addition, the hypoallergenic ingredient may prevent the sensitization of healthy subjects. It is important to develop processing methods capable of partially removing allergens, especially for the prevention of infant allergies (Yamada et al., 2006). In addition, a processing method to
completely eliminate allergens could be developed by combining imperfect but simple hypoallergenic treatments. In the present study, we aimed to develop a hypoallergenic processing treatment for shrimp and squid by eluting TM from the muscle.

Materials and Methods

Frozen specimens of whiteleg shrimp (Litopenaeus vannamei) and raw Bleeker’s squid (Heterololigo bleekeri) were purchased from a local supermarket in Kanagawa, Japan. The weight of shrimp tail muscle, without the shell, feet and gut, was 7.98 ± 0.81 g (n = 4). The body weight of squid was 143.7 ± 5.5 g (n = 3) and rings of skinned mantle, around 6 g (around 3 mm in width), were prepared. Each muscle was cut into two pieces along the midline. One piece was used for boiling water treatment, and the other was used as a control. For the boiling water treatment, the muscle was dipped in 10 volumes of distilled water in a 50 mL glass beaker. For the volume calculations for the experiments below, the raw sample weights were used. Then, the glass beaker was placed in a pot containing 600 mL of DW, vigorously boiled using an IH cooking heater IHK-T32 (1000 W, IRIS OYAMA, Miyagi, Japan), for 10 min. The temperature in the beaker reached 100°C at 4 min. After heating, the beaker was immediately put on ice and cooled. Then, to remove excess moisture, the muscle was washed with cold distilled water and wiped with paper towels.

To the control muscle or the muscle after boiling water treatment, 4 volumes of 10 mM phosphate potassium buffer (pH 7.0) containing 150 mM NaCl was added to a 30 mL glass beaker in accordance with Emoto et al. (2009). Then, the sample was cut into pieces with scissors and homogenized with a homogenizer ULTRA-TURRAX T10 basic with S10N-8G-ST (IKA, Osaka, Japan) for 1 min at maximum speed. The homogenized muscle was heated and cooled as described above. Next, the sample was centrifuged (10,000 × g, 10 min, 4°C). The supernatants were prepared for SDS-PAGE (Ozawa et al., 2011) and applied to a 12.5% separating slab gel (4.5×8.5 cm). For the SDS-PAGE, EzAppley, EzGel Ace, EzRun MOPS and EzStain AQua (ATTO CORPORATION, Tokyo, Japan) were used as the sample buffer, gel buffer, electrophoresis buffer and Coomassie Brilliant Blue staining solution, respectively. The extracts were mixed with an equal volume of the sample buffer, and 5-µL samples were loaded to the lanes of the gel. Image J (Abramoff et al., 2004) was used to determine the TM band in the SDS gel after destaining. For quantitative determination of TM, a standard curve was prepared using bovine serum albumin standard (Thermo Fisher Scientific, MA, USA).

Table 1. The contents and remaining ratio of tropomyosin in muscles

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Content a (mg/g)</th>
<th>Ratio b (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shrimp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.48</td>
<td>9.4</td>
</tr>
<tr>
<td>2</td>
<td>2.30</td>
<td>11.1</td>
</tr>
<tr>
<td>3</td>
<td>1.38</td>
<td>14.3</td>
</tr>
<tr>
<td>4</td>
<td>2.13</td>
<td>16.7</td>
</tr>
<tr>
<td>Average</td>
<td>2.06 ± 0.25</td>
<td>11.2 ± 2.0</td>
</tr>
<tr>
<td>Squid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.95</td>
<td>35.4</td>
</tr>
<tr>
<td>2</td>
<td>3.56</td>
<td>35.7</td>
</tr>
<tr>
<td>3</td>
<td>3.95</td>
<td>33.8</td>
</tr>
<tr>
<td>Average</td>
<td>3.49 ± 0.50</td>
<td>35.0 ± 1.0</td>
</tr>
</tbody>
</table>

a Tropomyosin content (mg) in 1 g of muscle.

b Ratio of tropomyosin in muscle after boiling for 10 min against the total tropomyosin.

Results and Discussion

TM was detected in the gel as a band of around 35 kDa (Fig. 1), as reported previously (Ozawa et al., 2011). TM bands were observed in lanes 2 and 5, indicating that TM was eluted from the muscle by boiling. In addition, a smaller amount of TM was observed in lanes 3 and 6 compared to that in lanes 1 and 4, respectively. This demonstrated that boiling water treatment decreased the TM content in muscle. Based on densitometric analysis, 2.07 ± 0.25 and 3.49 ± 0.50 mg of TM was eluted from 1 g of control muscle of shrimp and squid, respectively (Table 1). In the boiling water treatment, only 11.2 ± 2.0 and 35.0 ± 1.0% of TM remained in the muscle of the shrimp and squid, respectively. In a preliminary experiment involving shrimp muscle, treatment at 95°C for 10 min resulted in 72.7 ± 6.4% (n=3) elimination of TM, while boiling treatment for 2 min and cooling treatment at 4°C for 1 hour resulted in 0.3% and 2.9% (n=1 for each) TM elimination, respectively. In boiling treatment, the majority of proteins are...
likely denatured and precipitated, and TM and a few protein bands, which were not identified in previous studies (Motoyama et al., 2006 and 2007; Ozawa et al., 2011), were observed (Fig. 1). Purified TM is quite soluble under relatively mild conditions, for example, in 150 mM NaCl solution (pH 7) (Ozawa et al., 2011). However, in muscle fiber, TM is bound to actin filaments, and is not easily extracted by low ionic strength solutions. In boiling water, however, TM and actin are thought to unfold. Thus, TM was dissociated from actin (Singh and Hitchcock-Degregori, 2009) and eluted into the boiling water in the present experiment. However, the reasons for the differences in the efficiency of TM removal between shrimp and squid remain unclear.

As mentioned above, food allergy patients are advised that they can consume some foods containing allergy-inducing ingredients so long as the intake does not cause an allergic reaction (Ebisawa, 2014). Thus, the boiling treatment of shrimp muscle would increase the allowable intake that would not cause allergy by tenfold. On the other hand, it is likely that taste-active components such as free amino acids and nucleotides, which are important for the consumer’s preference for seafood, would be largely eluted (Abe et al., 2001). However, additional appropriate seasoning would compensate for this loss in taste.

References