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Adsorption of tropomyosin from pink shrimp (*Pandalus eous*) on stainless steel surface

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ABSTRACT

Cross contamination of allergen to other food products is a serious problem in the food plant where many products shared the same equipment or processing lines. In order to decide a suitable cleaning method, understanding on adsorption of allergen onto the food-contact surface is required. In this study, the adsorption behavior of tropomyosin, a major allergen in shrimp, on stainless steel surface was investigated using shrimp extract as a sample food material. It was found that tropomyosin was adsorbed favorably on stainless steel surface than other proteins in the shrimp extract. Furthermore, the adsorption of proteins in the shrimp extract, including tropomyosin, was hardly removed by water.

1. Introduction

Shrimp has been reported as a cause of food hypersensitive reaction mediated by IgE antibodies, such as urticaria, asthma, and vomiting, in allergic individuals (Lehrer, Helbling, & Daul, 1992). In Thailand, shrimp is the most common causative agent of seafood hypersensitive (Chaidaroon, Visitsunthron, & Vichyanond, 2003). In Japan, a label indicating the use of shrimp as a material is required on package of foods due to law. The major allergen of shrimp is tropomyosin (Daul, Slattery, Reese, & Lehrer, 1994; Shanti, Martin, Nagpal, Metcalfe, & Subba Rao, 1993). Tropomyosin is a heat stable coiled-coil protein found in animal muscle. Tropomyosin is composed of two identical subunits with a molecular mass of 34–38 kDa, depending on species (Daul et al., 1994; Jeoung et al., 1997; Leung et al., 1996; Motoyama, Ishizaki, Nagashima, & Shiomi, 2006; Shanti et al., 1993).

In food plants where many products shared the same equipment or processing lines, cross contamination of allergen to other food products may occur if cleaning is inadequate (Jackson et al., 2008). In order to determine the suitable cleaning conditions, understanding on adsorption of allergen on the food-contact surface is required. Several researches on cleaning of food-contact surface focusing on residual allergen from plants such as peanut, and tree nut have been reported in literature (Schlegel, Yong, & Foo, 2007; Stephan, Weisz, & Vieths, 2004). In contrast, information of adsorption of allergen from shrimp on food-contact surface and their removal is limited although shrimp is a major allergic food materials. Thus, this work aims to study the adsorption behavior of tropomyosin from pink shrimp onto stainless steel surface. Stainless steel is chosen as a target surface in this study because it is widely used in food plant due to its toughness and corrosion resistant. In order to verify the removability of tropomyosin adsorbed on stainless steel, desorption experiment using water was also conducted.

2. Materials and methods

2.1. Shrimp sample

Pink shrimp (*Pandalus eous*) (*Amaebi* in Japanese) was selected as a sample because it is one of the most abundant shrimps caught in Japan. It is commonly served as raw dishes (*Sashimi* and *Sushi*) in Japan and used as a raw material and as an ingredient in food in both Japan and other countries. In this work, fresh pink shrimps were purchased from market and preserved at −85 °C until use.

2.2. Stainless steel particles

Fine stainless steel particles of type 316L (diameter 8–10 μm) were purchased from Yasui Kikai Co. (Osaka, Japan). Use of the fine stainless steel particles provided a large surface area of substrate for adsorption. The stainless steel particles were washed before adsorption experiment by the method of Sakiyama et al. (1999). Briefly, they were washed with 0.1 N NaOH at 60 °C for 2 h at first. After being thoroughly rinsed with distilled water, and then with

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ethanol, they were dried at 50 °C. Based on the result of the nitrogen adsorption experiment, the specific surface area of the particles was estimated to be 0.58 m^2/g.

2.3. Preparation of shrimp extract

Three frozen shrimps were thawed using running tap water. Five grams of edible part of shrimp was mixed with 100 ml distilled water using stomacher (Stomacher 400 circulator, Seward) at 230 rpm for 3 min. Distilled water without any additives such as salts, surfactants, and buffers was used for extraction. This is because such additives are not used frequently in cleaning of shrimp itself in food plant and restaurant. Extract was centrifuged at 4000 rpm for 20 min and supernatant was filtered through a 0.45 μm syringe filter (Sartorius Stedim Biotech GmbH, Goettingen, Germany) to remove insoluble substances. In order to adjust protein concentration, initial protein concentration in the filtrate was measured by BCA method (Pierce, IL, USA). Initial tropomyosin concentration in the filtrate was determined by sandwich ELISA (Crustacean Tropomyosin Residue ELISA, ELISA SYSTEMS Pty Ltd., Queensland, Australia). The tropomyosin concentration in the filtrates were 0.2–0.5% of those of total proteins, varying with the lot of extraction.

2.4. Adsorption experiment

Adsorption experiment was conducted using the depletion method (Itoh et al., 1995) as follows. To a 20-ml bottle containing two grams of stainless steel particles was added 1 ml of shrimp extract. The protein concentration of the extract was varied from 250–2500 μg/ml. The initial tropomyosin concentration in these extracts varied from 0.85 to 7.34 μg/ml irrespectively to the increase of total protein concentration due to the variation in lot of extraction. The bottle was tightly sealed and incubated at 25 °C for 1–2 h with vigorous shaking (120 rpm). From preliminary experiment, there was no significant difference in the amount of adsorbed protein on stainless steel surface within the range of incubation period. The bottle contained 1 ml of shrimp protein extract without stainless steel particles (blank sample) was also incubated as described above. After the incubation, the concentrations of total protein and tropomyosin in supernatant were measured by BCA method and sandwich ELISA, respectively. The amount of protein or tropomyosin adsorbed on stainless steel surface, q (μg/m^2), was calculated using the following equation:

$$q = \frac{(C_i - C_e) \times V}{W \times S_{SS}} \quad (1)$$

where C_i is the concentration in extract before adsorption (μg/ml), C_e the concentration in supernatant after adsorption (μg/ml), V the volume of extract in bottle (ml), W the weight of stainless steel powder (g), and S_{ss} the specific surface area of stainless steel particles, respectively. The procedure mentioned above was conducted at least triplicate for each initial concentration.

2.5. Desorption experiment

Three 20-ml bottles, each containing two grams of stainless steel particles and 1 ml of shrimp extract (1500 μg/ml), were incubated at 25 °C as described above. After the incubation, 400 μl of supernatant from each bottle was taken out to measure concentrations of protein and tropomyosin for the evaluation of their adsorbed amounts on stainless steel surface. The rest of solution and stainless steel particles were poured into filter funnel (20 mm i.d., maximum pore size 5–10 μm). The stainless steel particles left on the filter funnel were washed by pouring 5 ml distilled water more than three times until protein in effluent was not found. Concentration of total protein and tropomyosin in effluents were measured with BCA method and ELISA to evaluate the amounts of protein and tropomyosin still left on stainless steel surface. The procedure mentioned above was conducted triplicate.

3. Results and discussion

3.1. Adsorption isotherm

Fig. 1 shows the adsorption isotherm of total protein on the surface of stainless steel at 25 °C. The adsorbed amount of total protein increased with the increase of equilibrium total protein concentration at a low concentration level and it tended to approach a saturation value ~2500 μg/m^2. This suggested that there was a limit on the amount of adsorbed total protein. Similar limitation behaviors of adsorption on stainless steel surface and amount of saturated adsorbed total protein were reported for other proteins such as gelatin (2080 μg/m^2, pH 7.5, 40 °C) (Sakiyama et al., 1998), and β-lactoglobulin (β-Lg) (2700 μg/m^2, pH 6.85, 25 °C) (Itoh et al., 1995). From the limitation behaviors and the relation between protein molecule size and surface area, they speculated that the protein molecules mentioned above are adsorbed onto the stainless steel surface until a monolayer is formed. Based on their speculation, it is suggested that the adsorption mode of shrimp proteins in the extract may be also same as those of the protein mentioned above.

The amount of adsorbed tropomyosin in the extract is shown in Fig. 2 as a function of the equilibrium tropomyosin concentration. As same as total protein, with the increase of the equilibrium tropomyosin concentration, the amount of tropomyosin adsorbed on stainless steel surface increased (Fig. 2). An amount of adhered tropomyosin ceased to increase at a maximum level when the equilibrium tropomyosin concentration was higher than 0.6 μg/ml. The maximum value was about 7 μg/m^2. This indicates that there is also a limit for tropomyosin to be adsorbed on stainless steel surface.

3.2. Comparison of adsorption ratio of total protein and tropomyosin

Fig. 3 shows percentages of total protein and tropomyosin on stainless steel surface after the incubation at 25 °C relative to the initial amounts. Both of the adsorption percentages decreased with the increase of initial total protein concentration because there

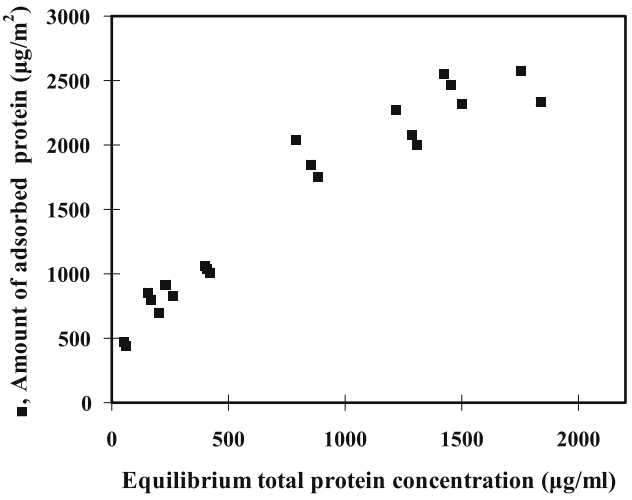

Fig. 1. Adsorption isotherms of total protein on the surface of stainless steel particles at 25 °C plotted against equilibrium total protein concentration.

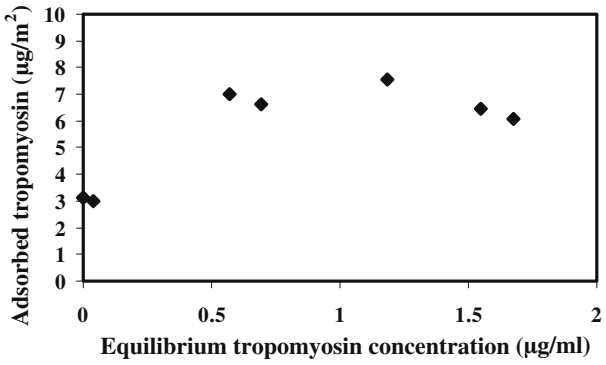

Fig. 2. Adsorption isotherms of tropomyosin in the shrimp protein extract on the surface of stainless steel particles at 25 °C.

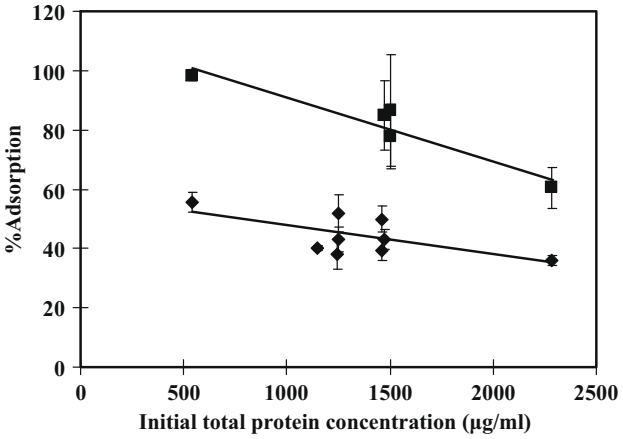

Fig. 3. Percentage of adsorption of total protein and tropomyosin on stainless steel surface incubated at 25 °C. ♦: Total protein, ■: tropomyosin. The initial tropomyosin concentration were 0.85, 4.86, and 4.05 μg/ml for initial total protein concentration 500, 1500, and 2250 μg/ml, respectively.

was the limit of adsorbed amount for both of total protein and tropomyosin as mentioned in the above section (Figs. 1 and 2). At the initial total protein concentration of 500 μg/ml, the percentage of adsorption of tropomyosin may be less-accurate because the concentrations of tropomyosin in supernatant after incubation resulted in less than 0.5 μg/ml; in a strict sense the ELISA kit used here is only effective at more than 0.5 μg/ml due to its accuracy. However, at higher initial protein concentrations, measured tropomyosin concentrations in supernatant after incubation were above 0.5 μg/ml. Therefore, obtained values of adsorption percent of tropomyosin were fairly accurate. Comparison of the adsorption percentages obviously shows that the adsorption percentage of tropomyosin was higher than that of total protein in all initial protein concentrations. It should be noted that the variation in the initial concentration of tropomyosin had no effect on this tendency. Namely, at the same total protein concentration such as 1500 μg/ml, the initial concentration of tropomyosin may be varied from 3.84 to 7.37 μg/ml for the different lot of extraction. However, percentage of adsorption of tropomyosin was obviously higher than that of total protein in all lots. Therefore, tropomyosin tended to be adsorbed on stainless steel surface more favorably than other proteins in shrimp extract. This result is similar to the result reported by Vejborg, Bernbom, Gram, and Klemm (2008) for the adsorption of tropomyosin from cod fish extract on stainless steel surface. They reported that cod tropomyosin was the most protein adsorbed on stainless plate among cod proteins. Cod tropomyosin is a non-allergic substance. In addition, amino acid composition of tropomyosin is significantly different between cod and several shrimp species (Daul et al., 1994; Leung et al., 1994; Miyazawa et al., 1996; Shanti et al., 1993; Vejborg et al., 2008). For example, while composition ratio of leucine in tropomyosin of brown shrimp (*Penaeus aztecus*) is only 1.9% (Daul et al., 1994), that in cod tropomyosin is 11.5% (Vejborg et al., 2008). Amino acid composition of pink shrimp used in this study has not been reported. However, amino acid composition of pink shrimp tropomyosin should be different from cod because of the high amino acid sequence homology in tropomyosin among several difference shrimp species (Daul et al., 1994; Leung et al., 1994; Miyazawa et al., 1996; Shanti et al., 1993). In spite of these expected differences, it is interesting that the favorableness of tropomyosin to be adsorbed on stainless steel surface is common.

The adsorbed amount of protein on solid surface is affected by various factors such as type of protein, properties of solid surface, and environmental conditions (pH, temperature, ionic strength, solvent, etc.) (Nakanishi, Sakiyama, & Imamura, 2001). These factors are keys for determining the main interaction between protein and adsorbent surface. Generally, hydrophobic and electrostatic interactions have been reported to play an important role in protein adsorption on solid surface. Hydrophobic interaction was reported as an important interaction when protein adheres to hydrophobic surface such as silicon and polystyrene (Dewez, Berger, Schneider, & Rouxhet, 1997; Krisdhasima, Mcguire, & Sproull, 1992). In contrast, electrostatic interaction is important when surfaces are charged in aqueous solution like metal oxide (e.g. TiO_2, ZrO_2 and stainless steel) and SiO_2 (Fukuzaki, Urano, & Nagata, 1996). For example, electrostatic interaction was reported as the main interaction for the adsorption of β-Lg, BSA, carbonic anhydrase, lysozyme, and protamine (Imamura, Shimomura, Nagai, Akamatsu, & Nakanishi, 2008; Itoh et al., 1995; Sakiyama et al., 1999). Besides these interactions, other interactions such as van der Waals interaction (Hagiwara, Sakiyama, & Watanabe, 2009; Nassauer & Kessier, 1986), chemisorption (Cabilio, Omanovic, & Roscoe, 2000) may be also involved in adsorption of protein on solid surface. Further study on the factors affecting adsorption of tropomyosin on stainless steel surface is necessary to understand interaction between tropomyosin and stainless steel surface since it is important for both preventing an adsorption and deciding a suitable cleaning method.

3.3. Desorption of protein and tropomyosin

Fig. 4 shows ratio of tropomyosin left on stainless steel surface after desorption with water to that before the desorption. The fact that the ratio was close to one implies that there was no tropomyosin

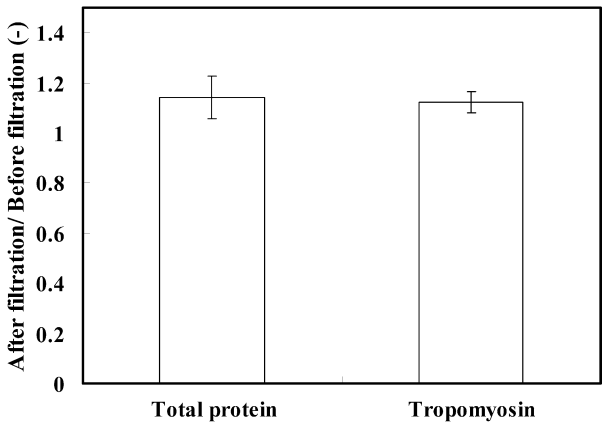

Fig. 4. The ratio of amount of total protein and tropomyosin on stainless steel surface before and after desorption.

removed from stainless steel surface during the desorption. Ratio between the amount of total protein left on stainless steel surface before and after desorption was also shown to be close to one. Therefore, all proteins in the shrimp extract, including tropomyosin, were strongly adsorbed on stainless surface and were hardly removed by water. The strong adhesion on stainless steel surface was reported for several proteins such as fibrinogen (Gattens & Gilbert, 2006), and β-Lg (Sakiyama et al., 1999). However, there is no study that investigated the removability of adsorbed tropomyosin from solid surface.

Even though the tropomyosin was strongly adsorbed on stainless steel surface at the condition used in this work, the adsorbed ability of tropomyosin may be altered when the environment conditions such as pH, ionic strength of the solution above stainless surface is changed due to other kinds of food. This results in the possibility of tropomyosin to be removed from the surface and contaminates in the other kinds of food.

According to the strong adsorption of tropomyosin on stainless steel surface, the extra care of cleaning is needed for preventing the cross contamination of tropomyosin to other foods. Furthermore, since total protein was also strongly adsorbed on stainless steel surface, protein remaining on surface may serve nutrition to microorganisms for growth. Many factors such as type of detergent, mechanical force, and temperature can affect on the cleaning efficiency (Jeurnink & Brinkman, 1994; Nagata et al., 1995; Sakiyama et al., 1998). For efficient removal of adherent shrimp proteins, including tropomyosin, from stainless steel surface, further study on effect of those factors is necessary.

4. Conclusion

In this work, the adsorption of shrimp protein extracted with water on stainless steel surface at room temperature was studied. Tropomyosin (shrimp allergen) was found to be adsorbed favorably on stainless steel surface than other proteins in shrimp extract. Moreover, tropomyosin and other proteins in shrimp extract were hardly removed from stainless steel surface.

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