Process Biochemistry 49 (2014) 1747–1756

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

Purification, characterization and immunoreactivity of tropomyosin, the allergen in *Octopus fangsiao*

Hai-Wang Shen[a], Yan-Yun Liu[a], Feng Chen[b], Jia-Wei Hu[a], Min-Jie Cao[a], Wen-Jin Su[a], Guang-Ming Liu[a,*]

[a] College of Food and Biological Engineering, Fujian Collaborative Innovation Center for Exploitation and Utilization of Marine Biological Resources, Jimei University, 43 Yindou Road, Xiamen, Fujian, 361021, China
[b] Department of Food, Nutrition, and Packaging Sciences, Clemson University, Clemson, SC 29634, USA

ARTICLE INFO

Article history:
Received 22 April 2014
Received in revised form 27 June 2014
Accepted 10 July 2014
Available online 19 July 2014

Keywords:
Octopus
Allergen
Tropomyosin
Processing
Protease digestion
Immunoreactivity

ABSTRACT

Octopus is one of the foods that can cause food allergies. In this study, tropomyosin (TM) – the primary allergen in octopus – was purified from *Octopus fangsiao* following different food processing treatments. The changes in the allergenicity and digestibility of different TM samples were estimated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and immunological experiments. The results showed that TM was resistant to pepsin but not to trypsin or α-chymotrypsin. Compared with crude TM, the TM treated by ultrasound-heat (UH) and ultraviolet radiation (UVR) showed no significant change in IgG/IgE-binding ability, except high pressure steaming (HP). And the resistance of the treated TM to digestive enzymes was reduced, with HP-treated TM showing the most significant change in stability. It was different that the resistance of TM to the digestive enzymes was increased after treatment with the Maillard reaction (MR); the IgG/IgE-binding activities of the MR products were almost completely eliminated. Thus, all of these results show that the immunoreactivity of octopus TM can be reduced by HP and MR treatment.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

In recent years, the octopus has become a promising candidate for aquaculture [1] and is in great demand in many regions of the world [2]. However, because of allergies, the consumption of octopus is also a serious problem for consumers. Food allergies are representative of type I allergies mediated by IgE antibodies. TM-sensitized individuals can develop urticaria, angioedema, laryngospasm, asthma, and even life-threatening anaphylaxis [3]. Therefore, it is important for octopus food processing methods to be researched and optimized to facilitate the production of hypoallergenic octopus products.

Some common features of "major" food allergens are that they are typically glycoproteins with a molecular weight of 10–70 kDa and are relatively resistant to heat, acid, and proteases [4]. The major allergen in *Octopus vulgaris* is tropomyosin (TM) [5]. TM is also in other species, including golden cuttlefish *Sepia esculenta*, squid [6,7], Japanese abalone *Haliotis diversicolor*, green mussel *Perna viridis*, noble scallop *Chlamys nobilis* [8], turban shell *Turbo cornutus* [9], Japanese oyster *Crassostrea gigas* [10], and crustaceans, such as shrimp [11–13] and crabs [14,15]. TM is a myofibrillar protein composed of two identical subunits with molecular weights of 35–38 kDa, and each subunit is an α-helix coiled-coil [14–16]. The molecular weight of *O. vulgaris* TM is approximately 35 kDa, and it is heat, acid, and alkali stable. Currently, three IgE-binding epitopes of *O. vulgaris* TM have been determined, and the peptides in the epitopes are residues 77–112, 148–160, and 269–281 [5]. However, there have been very few reports on the allergen in *Octopus fangsiao* and any effects of food-processing methods on allergenicity.

It is generally thought that the reason why native or denatured food proteins retain their allergenicity is that key structural and linear epitopes of the allergen survive food-processing treatments and in vivo digestion [17]; thus, the digestibility of the allergen has an important correlation to its immunoreactivity. The immunoreactivity of allergens can be affected if the digestibility of the allergen is reduced by food processing methods [18]. Of the numerous food processing methods available, ultrasound, pulsed ultraviolet light, and high-pressure treatment are the most effective processing methods. The allergenic properties of shrimp are clearly reduced after powerful ultrasound treatment [19]. Likewise, pulsed ultraviolet light reduces IgE binding to peanut extracts and liquid peanut

* Corresponding author at: College of Biological Engineering, Jimei University, Xiamen 361021, China. Tel.: +86 592 6180378; fax: +86 592 6180470.
E-mail addresses: gmliu@jmu.edu.cn, liugmo@sina.com (G.-M. Liu).

http://dx.doi.org/10.1016/j.procbio.2014.07.005
1359-5113/© 2014 Elsevier Ltd. All rights reserved.

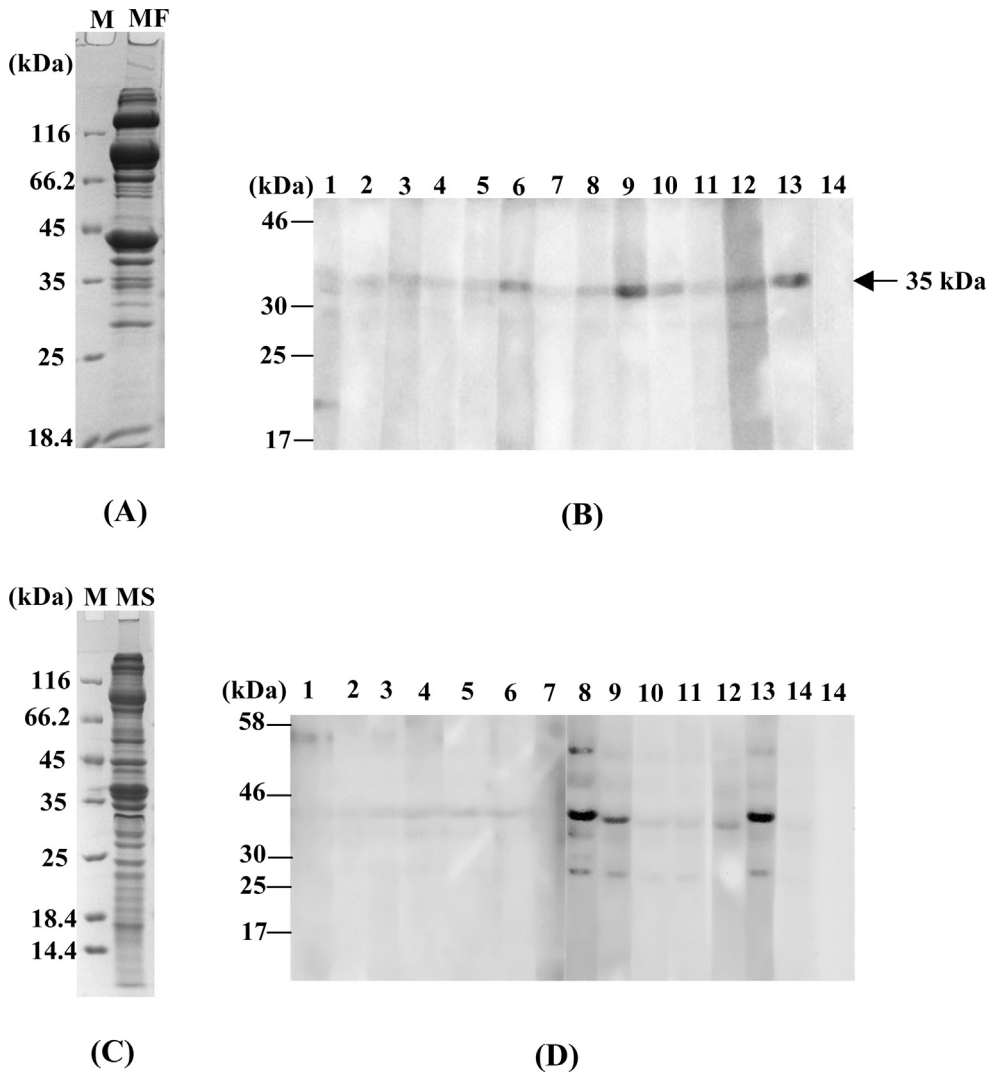

Fig. 1. Allergen detection of octopus. (A) SDS-PAGE analysis of octopus MF. (B) Western-blotting profiles of IgE-binding activity of MF. (C) SDS-PAGE analysis of octopus MS. (D) Western-blotting profiles of IgE-binding activity of MS. Samples were separated on 12% polyacrylamide gels with 5% stacking gels. There were 13 patients' sera (Nos. 1–13); the negative serum pool contained 3 normal persons' sera (No. 14).

butter [20], and high-pressure treatment reduces the antigenicity of rice grains [21] and bovine whey protein hydrolysates [22]. The Maillard reaction (MR) between the allergens and the reducing sugar can affect the immunoreactivity of the allergen [23,24], but it is still unclear if the MR enhances or reduces the immunoreactivity of these allergens. It is important to examine the effects of these different food-processing methods on TM and to establish the methods necessary to reduce the immunoreactivity of octopus.

In this study, the effect of different processing methods (ultrasound-heat, UH; ultraviolet radiation, UVR; high pressure steaming, HP; and the Maillard reaction, MR) on the digestibility of TM was analyzed using simulated gastrointestinal fluid digestion in vitro. The reduction in IgG/IgE binding reactivity toward the processed TM was estimated by Dot-blotting and an inhibition enzyme-linked immunosorbent assay (the iELISA).

2. Materials

2.1. Octopus

Live octopus (*O. fangsiao*) was purchased from Jimei market, Xiamen and either used immediately or stored in a freezer at −80 °C.

2.2. Chemicals

DEAE-Sepharose was purchased from Amersham Biosciences (Uppsala, Sweden). Porcine trypsin and bovine α-chymotrypsin were purchased from Sigma (St. Louis, MO, USA) and Amresco (Cleveland, OH, USA). Porcine pepsin was prepared in our laboratory as previously described [25]. Rabbit anti-crab TM polyclonal antibody (2 mg/mL) was prepared in our laboratory. Horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG antibody was purchased from DAKO (Glostrup, Denmark). Peroxidase-conjugated goat anti-human IgE antibody was obtained from Kirkegaard & Perry Laboratories (Gaithersburg, MD, USA). The enhanced chemiluminescent (ECL) substrate for immunoblotting was obtained from Pierce (Rockford, IL, USA). The 3,3′,5,5′-tetramethylbenzidine (TMB) was purchased from TIANGEN (Beijing, China). All other reagents were of analytical grade.

The proteolytic activity of pepsin was estimated by measuring trichloroacetic acid precipitable bovine hemoglobin after hydrolysis. The proteolytic activity of trypsin was measured using Boc-Phe-Ser-Arg-MCA as a substrate, whereas the activity of α-chymotrypsin was measured using Suc-Leu-Leu-Val-Tyr-MCA as a substrate according to previously described methods [26,27].

Download English Version:

https://daneshyari.com/en/article/34508

Download Persian Version:

https://daneshyari.com/article/34508

Daneshyari.com