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IgE reactivity to type I collagen and its subunits from tilapia (*Tilapia zillii*)

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ABSTRACT

Acid-soluble collagen (ASC) was isolated from the skin of tilapia (*Tilapia zillii*) *via* acetic acid (HAc) extraction and NaCl precipitation. ASC from tilapia consists of α chains (α1 and α2), β chains and γ chains and is classified as type I collagen. A comparison of the properties of tilapia collagen and silver carp parvalbumin showed that tilapia collagen was less stable under heat treatment and more resistant to pepsin digestion. Both tilapia collagen and silver carp parvalbumin were degraded at pH 2.0 but stable at pH 3.0–11.0. Subunits α1 and α2 were further purified from tilapia collagen by carboxymethyl (CM) cellulose column chromatography with linear gradient elution and stepwise elution, respectively. Enzyme-linked immunosorbent assay (ELISA) and immunoblotting results demonstrated the specific IgE activity of different fish-allergenic patients' sera towards the α1 and α2 chains of tilapia collagen. It can be inferred that tilapia collagen and its subunits are all potential allergens.

1. Introduction

Anaphylactic reactions to measles, mumps, and rubella vaccines stabilised by bovine gelatin have been reported repeatedly. Kelso reported that a child who developed anaphylaxis after receiving a combined measles, mumps, and rubella vaccine had IgE antibodies to gelatin (Kelso, Jones, & Yunginger, 1993). Thus, bovine gelatin included in live vaccines is considered to be an allergen (Nakayama, Aizawa, & Kuno-Sakai, 1999). Type I collagen, which is the main component of gelatin, was confirmed as the major allergen of bovine gelatin allergy (Sakaguchi et al., 1999).

Fish is one of the most commonly allergenic foods (Aas, 1966). It has been reported that many fish cause food allergies. Yuki Hamada reported that fish collagen may possibly be a new allergen (Hamada, Nagashima, & Shiomi, 2001; Hamada et al., 2003), in addition to the major allergen of fish, parvalbumin (Elsayed & Bennich, 1975). The skin and meat of fish, but especially the skin, contains abundant type I collagen. A previous study by Japanese researchers has preliminarily investigated the IgE reactivity of human sera against marine fish collagen. Sakaguchi et al. (2000) compared discrepancies between the IgE reactivity towards fish collagen in patients with fish allergy and bovine allergy. They concluded that fish gelatin (type I collagen) may be a potential allergen in subjects with fish allergy.

Currently, collagen is widely used in the film industry and tissue engineering, as well as in sausage casings, food additives, and cosmetics (Zhang, Liu, & Li, 2009). A number of collagen samples from the skin and bones of fish have been isolated and characterised. Recently, collagen from the skin and scales of several freshwater fish has also been isolated and characterised (Duan, Zhang, Du, Yao, & Konno, 2009; Pati, Adhikari, & Dhara, 2010; Singh, Benjakul, Maqsood, & Kishimura, 2011). Freshwater fish is one of the most popular foods in China, where tilapia is widely distributed. The skin and meat of tilapia are known to contain a large quantity of collagen. Nowadays, tilapia is also a major source of collagen for industrial uses (Zhang, Duan, Tian, & Konno, 2009; Zhang, Liu, et al., 2009).

Whether freshwater fish collagen also has the potential ability to cause an anaphylactic reaction in consumers and affect the healthy, especially in light of the extensive applications of tilapia collagen, is currently an important issue. A previous study confirmed an allergenic response can be invoked by the whole collagen molecule. However, little is known about the subunits of fish collagen and the relationships between them. We do know that the structure of type I collagen is a right-handed triple super-helical rod consisting of three polypeptide chains. Although one report indicated that it is the α2 subunit of bovine gelatin that mainly causes an anaphylactic reaction (Sakaguchi et al., 1999), there has not been sufficient research on fish collagen and its subunits to identify the specific source of its allergenicity. On this basis, it is meaningful to study the allergenicity of tilapia collagen and its subunits.

2. Materials and methods

2.1. Materials and pretreatment

Live tilapia (about 1000 g) were purchased from a local market in Jimei, and brought to the laboratory alive in a bucket of water.

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Tilapia skin was prepared manually and immediately washed with chilled distiled water to remove the adhering tissues. All materials were stored at −70 °C until use.

2.2. Human sera

Sera from patients were obtained from the Hospital of Jimei University and the Second Hospital of Xiamen. Sera from 8 tested patients, who had been found to be allergic to fish, were used for ELISA and immunoblotting, and sera from non-allergic individuals were pooled and used as a negative control. All sera samples were stored at −30 °C until use.

2.3. Isolation of acid-soluble tilapia collagen

The methods described by Zhang, Duan, et al. (2009) and Liu, Zhong, et al. (2010), with some slight modifications, were used to isolate and purify tilapia collagen.

All procedures were performed at 4 °C with gentle stirring. Tilapia skin was cut into small pieces using scissors and homogenised with 0.1 M NaOH at a solid/solvent ratio of 1:10 and extracted for 24 h; the solution was changed twice during that time to remove non-collagenous proteins. The residue from the alkaline extraction was then rinsed with cold distiled water repeatedly until the pH became stable or nearly neutral. It was then soaked in 10% *n*-butyl alcohol with a solid/solvent ratio of 1:10 for 24 h, with a change of solution each 12 h, to remove fat. The residue was washed several times and extracted in 0.5 M acetic acid with stirring for 3 days. The supernatant was salted out by adding NaCl to a final concentration of 0.9 M, and the precipitate was collected by centrifugation at 20,000*g* for 30 min. The precipitated collagen was re-dissolved in 0.5 M acetic acid and dialysed against 0.1 M acetic acid for 3 days; the sample was then lyophilised.

2.4. SDS–polyacrylamide gel electrophoresis (SDS–PAGE)

SDS–PAGE was performed according to the method of Laemmli (1970), with slight modification, using the discontinuous Tris–HCl/glycine system with 7.5% resolving gel and 4% stacking gel. Tilapia collagen was mixed with loading buffer and heated at 95 °C for 3 min. A high molecular weight marker containing bands from 10 to 250 kDa was used as a reference. After electrophoresis, the gel was stained with 0.25% Coomassie Brilliant Blue R-250.

2.5. Comparison of the properties of tilapia collagen and silver carp parvalbumin

The properties were compared with simulated gastric fluid (SGF) digestion, heat treatment, and different pH buffer treatment, respectively.

In the SGF digestion, silver carp parvalbumin isolated by our laboratory was used for comparison. Briefly, the silver carp muscle was homogenised with 20 mM Tris–HCl (pH 7.5) containing 5 mM EDTA, the supernatant was collected and fractionated with ammonium sulphate from 60% to 100% saturation. The precipitate was dissolved and fractioned into DEAE−Sepharose; the proteins were eluted by NaCl and applied to Superdex 75, then the column was eluted and fractions were collected for SDS–PAGE (Liu, Wang, et al., 2010). SGF was prepared as described in the *US Pharmacopoeia* (1995) and consisted of porcine pepsin in 25 mM NaCl at pH 1.2. The contents of collagen and parvalbumin were adjusted to be equal and dissolved in SGF. The total volume of the reaction solution was 1 mL. For each sample, SGF was preheated at 37 °C for 15 min before adding the test protein. Digestion was performed at 37 °C with continuous rocking. At each scheduled time point (0, 1, 2, 5, 10, 15, 30, and 60 min), a 100-μL aliquot of the reaction solution was removed, and the reaction was immediately terminated by adding 30 μL 200 mM Na_2CO_3. A protein sample dissolved in SGF with no added pepsin was the control. SDS–PAGE was performed using a linear gradient gel ranging from 6% to 20%.

In the study on thermal stability, tilapia collagen and silver carp parvalbumin were treated for 30 min at temperatures of 0, 20, 30, 40, 50, 70, and 100 °C. After heat treatment, the samples were then mixed with the loading buffer and reacted at 95 °C for 3 min before SDS–PAGE.

To investigate pH stability, the proteins were mixed with buffer at pH 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, and 11.0. The sample and the buffer were mixed at a ratio of 1:10 and maintained at ambient temperature for 1 h. The samples were then mixed with the loading buffer and reacted at 95 °C for 3 min before SDS–PAGE.

2.6. Isolation of the subunits of tilapia collagen

CM (5 mL) column chromatography was used to isolate the α1 and α2 subunits, according to the method of Piez, Eigner, and Lewis (1963) with slight modification. The tilapia collagen sample was dissolved in 10 mL of sodium acetate buffer (20 mM, pH 4.8) at a concentration of 1 mg/mL. Prior to fractionation on the column, the sample was heated at 45 °C for 40 min. To isolate the α1 subunits, elution was achieved using a starting buffer with a linear gradient of 0–1 M NaCl, at a flow rate of 60 mL/h. Fractions were collected automatically every 3 min. To isolate the α2 subunits, a starting buffer containing 0.13 M NaCl, followed by a starting buffer containing 0.17 M NaCl, was used. The absorbance was monitored at 223 nm. The fractions indicated by numbers were examined by SDS–PAGE (7.5% gels).

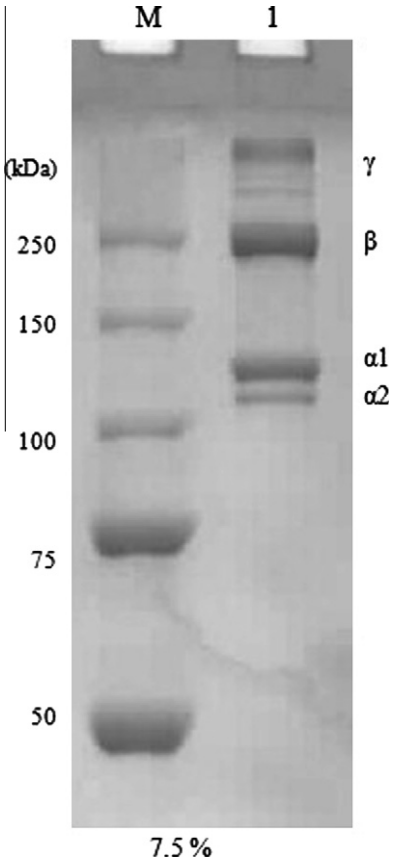

Fig. 1. SDS–PAGE analysis of the purified collagen from tilapia skin using 7.5% gel. M: High molecular protein marker ranging from 10 to 250 kDa. 1: Tilapia skin collagen.