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Antibody reactivity to the major fish allergen parvalbumin is determined by isoforms and impact of thermal processing

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ABSTRACT

The EF-hand calcium binding protein, parvalbumin, is a major fish allergen. Detection of this allergen is often difficult due to its structural diversity among various fish species. The aim of this study was to evaluate the cross-reactivity of parvalbumin in a comprehensive range of bony and cartilaginous fish, from the Asia-Pacific region, and conduct a molecular analysis of this highly allergenic protein. Using the monoclonal anti-parvalbumin antibody PARV-19, we demonstrated the presence of monomeric and oligomeric parvalbumin in all fish analysed, except for gummy shark a cartilaginous fish. Heat processing of this allergen greatly affected its antibody reactivity. While heating caused a reduction in antibody reactivity to multimeric forms of parvalbumins for most bony fish, a complete loss of reactivity was observed for cartilaginous fish. Molecular analysis demonstrated that parvalbumin cross-reactivity, among fish species, is due to the molecular phylogenetic association of this major fish allergen.

1. Introduction

Fish is one of the eight prominent foods known to cause allergy (Lopata & Lehrer, 2009). Being an excellent source of proteins and omega fatty acids, the consumption of fish has increased greatly. However, allergy to fish is also increasing, affecting up to 0.2% of the general population. Fish allergy is an important concern in the seafood processing environment. A recent study reported that prevalence of fish allergies can be as high as 8% among fish processing workers (Jeebhay et al., 2008). Along with consumption and handling of fish allergens, patients can also react to aerosolized fish proteins generated during cooking or processing (Sharp & Lopata, 2013; Sicherer & Teuber, 2004). Clinical manifestations of fish allergy may include symptoms ranging from wheezing, tightness of the throat, urticaria, vomiting, diarrhea etc. to the life threatening reaction called anaphylaxis.

The major fish allergen has been identified as parvalbumin, an EF hand calcium binding protein (Beale, Jeebhay, & Lopata, 2009; Bugajska-Schretter et al., 1998). Parvalbumins are globular proteins about 12 kDa in size and are abundant in lower vertebrates such as amphibians and fish (Girija & Rehbein, 1988). These are water soluble and remarkably stable over a broad temperature and pH range (Arif, 2009; Arif, Jabeen, & Hasnain, 2007; Untersmayr et al., 2006). Parvalbumins are abundant in the white muscle tissue of fish, however lower concentrations have also been reported in fish dark muscle tissue (Kobayashi et al., 2006; Wilwert, Madhoun, & Coughlin, 2006). Fish exhibit differences in their environmental habitats and their overall muscle composition. Multiple isoforms of parvalbumin can be expressed in a single fish species during its different developmental stages (Brownridge et al., 2009; Huriaux, Collin, Vandewalle, Philippart, & Focant, 1997; Van Do, Hordvik, Endresen, & Elsayed, 2003). For example, fresh water carp has been reported to express up to eight isoforms of parvalbumin, differing slightly in molecular weight and isoelectric properties (Brownridge et al., 2009).

The detection of fish parvalbumin is challenging compared to other food allergens; this can be attributed to the high biochemical and immunological variability among the different fish species (Gajewski & Hsieh, 2009; Sharp & Lopata, 2013). Fish consumption strongly depends on regional availability. Most studies on characterization of parvalbumins have been conducted on fish commonly consumed in the northern hemisphere. Several fish species such as barramundi, flathead, gummy shark are indigenous to the Asia-Pacific region. However, data on fish allergens in these species is limited. Moreover, not much research has been done on the comparison of the diversity of parvalbumin isoforms across different orders of fish or effect of heat processing on their antibody reactivity.

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The main aim of our study was to compare parvalbumin distribution profiles, specific antibody reactivity and cross-species recognition across 12 different orders of fish and study the impact of heat-processing on the mono and polymeric forms of these parvalbumins. Using bioinformatics tools, we have shown that phylogenetic classification of fish based on the amino acid sequence of parvalbumin, can be linked to the immunological cross reactivity of this allergen. Furthermore, we deduced different antibody binding sites of parvalbumin for bony and cartilaginous fish which can aid in designing specific antibodies for better detection of parvalbumin.

2. Materials and methods

2.1. Fish samples

Nineteen species of fish commonly consumed in the Asian-Pacific region (see Table 1) were analysed. Fresh fillets of each species were purchased from the local fish market and transported on ice to the laboratory. All samples were stored at −80 °C prior to processing.

2.2. Preparation of protein extracts

Fifty grams of fish white muscle was homogenized in 100 ml of phosphate buffered saline (PBS, 10 mM, pH 7.2) using an Ultra Turrax blender (IKA, Staufen, Germany) and extracted overnight with gentle tumbling at 4 °C. The crude extract was centrifuged at 5000*g* for 30 min at 4 °C and filter sterilized using 0.2 μm cellulose acetate filter membranes (Sartorius, Germany) and this is referred to as 'raw extract'. To standardize the heat-processing an aliquot of raw extracts were heated at 95 °C for 15 min in a water bath.

Precipitated proteins were removed by centrifugation at 5000*g* for 15 min and the resulting aliquot referred to as 'heated extract'. The prepared protein extracts were stored at −80 °C until further analysis.

2.3. Protein quantification

Protein concentrations were determined for the raw and heated extracts using the Quick Start™ Bradford assay kit (Bio-Rad, USA). Readymade bovine serum albumin standards (Bio-Rad, USA, 0.125–2.00 mg/ml) were used and the absorbance was determined at 595 nm using a Multiskan Ascent®, (Pathtech, Australia) micro plate reader.

2.4. SDS–PAGE analysis

Protein profiles for raw and heated fish extracts were obtained using sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE). Fish proteins (25 μg) were diluted in a 5× Laemmli sample buffer containing 2-mercaptoethanol, heated for 5 min and loaded on a 12% Tris–Glycine gel. Precision Plus protein standards (Bio-Rad, USA) were used to estimate the molecular weights of individual proteins, using the Mini-PROTEAN® Tetra Cell (Bio-Rad, USA) system at 170 V. Proteins were visualized by Coomassie Brilliant Blue R-250 (Bio-Rad, USA) staining.

2.5. Immunoblotting

The presence of different parvalbumins in raw and heated samples was determined by immunoblotting. Fish protein extracts (5 μg) were separated using SDS–PAGE and then transferred to an activated PVDF membrane (Bio-Rad, USA) using the Semi-dry Trans Blot electrophoretic transfer system (Bio-Rad, USA) for 10 min at 10 V. After blocking with 5% (w/v) skim milk (in TBS) for 1 h at room temperature, membranes were incubated for 1 h with the primary monoclonal anti-parvalbumin antibody (PARV-19; Sigma, USA) diluted 1:3000 in 1% skim milk and TBS. Membranes were subsequently washed in TBS with 0.5% Tween-20 (TBS-T) and incubated with the secondary anti-mouse HRP labelled antibody (Sigma, USA). The protein–antigen interaction was visualised using Enhanced Chemiluminescence substrate (Sigma, USA) followed by exposure to photographic film (GE Healthcare, Australia).

2.6. Inhibition-ELISA

To analyse the cross-species antibody reactivity for the different fish parvalbumins an in-house developed inhibition ELISA was performed. Briefly, 10 μg/ml of heated carp extract was coated on a 96-well high binding plate (Costar, USA) and

Table 1
Biological classification and scientific names of fish species analysed in this study.

Common name	Order	Family	Scientific name
Bony fish			
Snapper	Perciformes	Sparidae	*Pagrus auratus*
Silver bream	Perciformes	Sparidae	*Acanthopagrus australis*
Yellowtail kingfish	Perciformes	Carangidae	*Seriola lalandi*
Barramundi	Perciformes	Latidae	*Lates calcarifer*
Blue fin tuna	Perciformes	Scombridae	*Thunnus maccoyii*
Slimy mackerel	Perciformes	Scombridae	*Scomber australasicus*
Orange roughy	Beryciformes	Trachichthyidae	*Hoplostethus atlanticus*
Tiger flathead	Beryciformes	Platycephalidae	*Neoplatycephalus richardsoni*
Atlantic salmon	Salmoniformes	Salmonidae	*Salmo salar*
Rainbow trout	Salmoniformes	Salmonidae	*Onchorynchus mykiss*
Carp	Cypriniformes	Cyprinidae	*Cyprinus carpio*
Pilchard	Clupeiformes	Clupeidae	*Sardinops neopilchardus*
Rock ling	Ophidiiformes	Ophidiidae	*Genypterus blacodes*
Atlantic cod	Gadiformes	Gadidae	*Gadus morhua*
Cartilaginous fish			
Skate	Rajiformes	Rajidae	*Raja cerva*
Gummy shark	Carchariniformes	Triakidae	*Mustelus antarcticus*
Sparsely spotted stingaree	Myliobatiformes	Urolophidae	*Urolophus paucimaculatus*
Blacktip shark	Carchariniformes	Carcharhinidae	*Carcharias limbatus*
Elephant shark	Chimaeriformes	Callorhinchidae	*Callorhinchus milii*