ALLERGENIC PROTEINS IN FISH

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ALERGENSKI PROTEINI U RIBI

Apstrakt

Riba predstavlja znatan deo ishrane ljudi u svetu. Pre svega, riba je značajan izvor proteina (15-24%) visoke biološke vrednosti, bogata je mineralima, vitaminima, a posebno esencijalnim masnim kiselinama za koje je dokazano da pogoduju u prevenciji mnogobrojnih oboljenja. Zbog velikog značaja polinezasićenih masnih kiselina n-3 klase, u Evropi su date i preporuke o optimalnom dnevnom unosu. Međutim, pored hranljivih svojstava koje ima, riba može biti i izvor različitih bioloških i hemijskih opasnosti. Od bioloških opasnosti posebno su značajni paraziti (*Trematodae, Nematodae, Cestodae*), bakterije (*Salmonella spp, E. coli, Vibrio parahemolyticus, Vibrio vulnificus, Listeria monocytogenes, Clostridium botulinum, Staphyloccocus aureus*), virusi (Norwalk virus, Entero virusi, Hepatitis A, Rotavirus) i biotoksini. Najznačajnije hemijske opasnosti su policiklična aromatična jedinjenja, histamin i teški metali (živa, olovo, kadmijum, arsen, gvožđe). Alergije usled konzumiranja pojedinih vrsta namirnica su u porastu poslednjih godina. Veliki pokret oko pravilnog načina ishrane je doveo do toga da ljudi sve češće konzumiraju ribu, proizvode od ribe kao i različite plodove mora. Pored različitih opasnosti koje mogu poticati iz ribe, posebni značaj poslednjih godina se daje ribi kao potencijalnom alergenu. Naime, veliki broj alergija koje se javljaju u svetu pripisuju se alergenima koji potiču iz mesa ribe, pre svega proteinima mesa ribe. Učestalost alergija koje se povezuju za unosom mesa ribe varira u Evropskim zemljama (Norveška 1,5%; 2,3% Turska, 2,3% Grčka; Švedska 1.2-3.2 %). Kao najznačajniji proteinski alergen iz mesa ribe navodi se parvalbumin (ß tip), koji je izolovan kod velikog broja vrsta. Smatra se da su šaran i bakalar najčešći izvori parvalbumina koji se dovodi u vezu sa različitim vidovima alergijskih reakcija. Potencijalni alergeni su takođe kolagen i želatin koji su izolovani iz kože i pojedinih organa riba. Takođe, značajan alergen iz plodova voda je i tropomiozin, arginin kinaza, aldolaza. Pored ovih alergena, značajni alergeni mogu da potiču iz ikre, pojedih vrsta kavijara, a opisani su slučajevi gde su alergijske reakcije povezane sa kolagenom koji se nalazi u ekstracelularnom matriksu

proteina. Alergeni koji dovode do različitih alergijskih reakcija, pored proteina mesa ribe, mogu poticati i od gotovih proizvoda od ribe. Tu spadaju različiti panirani proizvodi od ribe koji sadrže celer, gluten i druge dodatke koji mogu biti potencijalni alergeni. Zbog značaja koji imaju na zdravlje ljude, tehnologija je omogućila različite metode za detekciju ovih alergena. Kao neke od njih navode se ELISA (Enzyme-linked immunosorbent assay), RAST (Radioallergosorbent test) i RIE (Rocket Immuno-electrophoresis). Koja će se metoda detekcije primeniti, prvenstveno zavisi od dostupnosti alergena i praga njegove detekcije. Industija mesa je razvojem tehnologije uvela pojedine tehnološke prosece koji imaju mogućnost inaktivacije pojedinih alergena, pre svega proteina mesa ribe. Visoke temperature koje se primenjuju u obradi mesa ribe mogu uticati na ove alergene, tako što će smanjiti alergeni potencijal, dok neki tehnološki postipci nemaju tu mogućnost.

Ključne reči: riba, alergeni, protein, identifikacija alergena
Key words: fish, allergens, protein, identification of allergen

INTRODUCTION

It is common knowledge that fish are a nutritious component of a human diet, as they constitute a valuable and desired source of protein and polyunsaturated fatty acids (Baltić i Teodorović, 1997). However, they are likely to pose a risk to consumer health. The hazards of fish are associated with biological and chemical contaminations (Baltić et. al., 2013a). The biological contaminations include pathogenic bacteria such as *Listeria monocytogenes*, *Pseudomonas spp*., *Aeromonas spp*., *Clostridium botulinum* and parasites (Baltić et. al., 2013b). Chemical contamination concerns mainly polycyclic aromatic hydrocarbons, heavy metals such as cadmium, mercury, lead, iron (Ivanović et al., 2014) and histamine (Baltić et al., 2009).

The prevalence of food allergy in Europe is uncertain. Using food challenges as a criterion for diagnoses, the prevalence of food allergy in Europe has been estimated to be between 3 and 4%, both in children and adults. About 75% of allergic reactions among children in Europe are due to eggs, peanut, cows‘milk, fish and various nuts (EFSA, 2014).

Food allergy is increasing at a faster rate than any other allergic disorder. In the last few decades, a large movement toward healthier eating makes seafood one of the major foods consumed worldwide (Wild and Lehrer, 2005). Exposure to seafood can cause a variety of health problems, including gastrointestinal disorders, urticaria, immunoglobulin E (IgE)-mediated asthma and anaphylaxis. A true allergy is known as type-one hypersensitivity that activates the human mast cells, a type of white blood cells, producing an IgE and other inflammatory mediators such as cytokines.

Allergic reactions are directed to two major groups: fish and shellfish. The prevalence of “food allergy” as perceived by the general population is several times larger than the prevalence that can be verified by standard diagnostic procedures. Prevalence of self-reported allergy to fish in children was lower in other Northern European countries with high fish consumption, like Iceland (1.5-2.2%), Norway (1.5%) or Sweden (1.2-3.2%). Fish was one of six foods found in DBPCFC (Double-blind, placebo-controlled food challenge) to be the most common allergens (Bock and Atkins, 1990).

FISH ALLERGENS

The Atlantic cod (*Gadus morhua*) was the first model for studying fish allergens, *Gad c1* (12 kDa). This glycoprotein is identified later as parvalbumin, which buffers calcium during muscle relaxation. In the case of fish, more than 20 proteins, mainly parvalbumins, have been classified as the major allergens (Barros and Cosme, 2013). Parvalbumin represents the major clinical cross-reactive fish allergen with sequence homology ranging from 60-80%. This feature was comprehensively applied to exploit the closeness between fish allergens and their human homologs. The allergenicity of the parvalbumin was studied in purified forms from different types of fish along with two other high molecular weight allergens: 29 and 54 kDa. In addition, other fish allergens are characterized such as collagen and gelatin isolated from skin and muscle tissues (Taylor, 2008), fish hormones like vitellogenin in caviar and many other allergens.

The literature reports that there is no cross-reactivity between fish allergens and shellfish (Lopata and Lehrer, 2009). Codfish allergens were the first food allergens to be purified and characterized. Codfish contains one major allergen contained in the sarcoplasmic proteins of fish muscle, *Gad c 1*. *Gad c 1* is a parvalbumin. Parvalbumin allergen in Atlantic cod, *Gad m 1*, encoded by a gene distinct from that of *Gad c 1*, has been identified. Parvalbumins from fish represent extremely abundant and stable allergens. They are considered by some authors to be the major and sole fish allergens for 95% of patients suffering from IgE-mediated fish allergy. Parvalbumins are small (12 kDa; 108-109 amino acid residues) calcium-binding muscle proteins, and are present in high amounts in the white muscles of lower vertebrates and in lower amounts in fast twitch muscles of higher vertebrates, and have a function in calcium buffering and possibly in muscle relaxation.

Parvalbumin has been found to be a major allergen in various other fish species (Table 1). Hamada et al. (2003) demonstrate that parvalbumin is a major allergen in three species of mackerels (*Scomber japonicus*, *S. australasicus* and *S. scombrus*) said to be the fish most frequently involved in IgE-mediated food allergy in Japan.

Table 1. Fish allergens (EFSA, 2014)

Biochemical name	Allergen	Common name	Scientific name	Source	Molecular weight[a]
β-Parvalbumin	Clu h 1	Atlantic herring	*Clupea harengus*	Fish meat	12[1]
	Cyp c 1	Carp	*Cyprinus carpio*		
	Gad c 1	Codfish	*Gadus callarias*		
	Gad m 1	Atlantic cod	*Gadus morhua*		
	Lat c 1	Barramundi	*Lates calcarifer*		
	Lep w 1	Whiff	*Lepidorhombus whiffiagonis*		
	Onc m 1	Rainbow trout	*Oncorhynchus mykiss*		
	Sal s 1	Atlantic salmon	*Salmo salar*		
	Sar s 1	Pacific pilchard	*Sardinops sagax*		
	Seb m 1	Ocean perch	*Sebastes marinus*		
	Thu a 1	Yellowfin tuna	*Thunnus albacares*		
	Xip g 1	Swordfish	*Xiphias gladius*		
Tropomyosin	Ore m 4	Mozambique tilapia	*Oreochromis mossambicus*	Fish meat	33[b]
β-Enolase	Gad m 2	Atlantic cod	*Gadus morhua*	Fish meat	47.3[b]
	Sal s 2	Atlantic salmon	*Salmo salar*		47.3[b]
	Thu a 2	Yellowfin tuna	*Thunnus albacares*		50
Aldolase A	Gad m 3	Atlantic cod	*Gadus morhua*	Fish meat	40
	Sal s 3	Atlantic salmon	*Salmo salar*		40
	Thu a 3	Yellowfin tuna	*Thunnus albacares*		40
Vitellogenin (β’ component)	Onc k 5	Chum salmon	*Oncorhynchus keta*	Fish roe	18[b]

[1] Approximate – slight variation exists between species; [a] MW (SDS-PAGE); [b] kDa

Collagen has been recently proposed as an important fish allergen (Hamada et al., 2003), although further verification is needed. A high-molecular weight allergen from tuna (*Thunnus albacares*) muscle was found to bind specific IgE from 5 out of 8 allergic patient sera, but none of the normal control sera. The authors concluded that the allergen was collagen, probably type 1 collagen, which is the representative collagen in fish muscle. Mammalian and fish gelatins have some similarities, and the possibility of allergic cross-reactivity must be considered. Hamada et al. (2003) concluded that collagen is commonly allergenic and cross-reactive regardless of fish species. Thus, there appears to be little or no cross-reactivity between mammalian and fish collagens, whereas fish collagens from different species appear to be broadly cross-reactive. It seems reasonable to treat fish collagens from different species as one entity. No data have been found regarding cross-reactivity between collagens from different organs (e.g. skin and muscle) from the same species of fish (EFSA, 2014). A report on repeated anaphylactic reactions after intake of Russian Beluga caviar has recently been published (Untersmayr et al., 2002). The patient had no clinical allergy to fish, and was skin test and specific IgE negative to fish (test fish species not specified). Serum contained specific IgE to several proteins in Beluga caviar (derived from *Hiso huso*) and Sevruga caviar (derived from *Acipenser stellatus*) and also to a lesser degree to proteins in “false” caviar (collected from lumpsucker- *Cyclopterus lumpus*, or salmon and trout), but there was skin test positivity only to Beluga and Sevruga caviars. Considering the near absence of other reports on allergy to fish roe, this allergy appears to be rare (EFSA, 2014). In addition to fish and seafood, potential allergens can be fish products, which contain gluten, celery and other allergens. This is especially true of breaded fish products (Janjić et al., 2015).

SHELLFISH ALLERGENS

In shellfish, crustaceans and mollusks, the protein tropomyosin (TM) seems to be the major allergen responsible for ingestion-related allergic reactions. Tropomyosin belongs to the family of actin filament-binding proteins with different isoforms that can be expressed in muscle, and non-muscle tissues. Complex of TM and troponin regulates the calcium sensitive interaction of actin and myosin. In addition, the allergenicity of TM was confirmed in six species of crustaceans: black tiger prawn (*Penaeus monodon*), kuruma prawn (*Penaeus japonicas*), pink shrimp (*Metapenaeus Monocerus*), king crab (*Lopholithodes Mandtii*), snow crab (*Chionoecetes opilio*), and horsehair crab (*Limulus polyphemus*) by immunoblotting and the overall sequence identity showed more than 90% homology (Motoyama et al., 2007).

Many other allergens have been identified in crustaceans. Yu et al. (2003) identified arginine kinase (AK) (40 kDa) as a novel shrimp allergen. The amino acid sequence of this protein showed 60% similarity to AK of the crustacean, kuruma prawn (*Penaeus japonicas*) (Yu et al., 2003). AK was recently reported as an allergen in different crustacean species which was identified in white shrimp (*Litopenaeus vannamei*), gulf shrimp (*Penaeus aztecus*), chinese shrimp (*Fenneropenaeus chinensis*), black tiger prawn (*Penaeus monodon*) and other shrimp species using a proteomics approach. Moreover, AK has been identified in other crab species: mud crab (*Scylla serrata*), and by group in snow crab (*Chionoecetes opilio*) (Rahman et al., 2011), where 49% of the participant patient's sera have a reactivity with AK. Arginine kinase also has been reported is allergen in some other invertebrates, such as the house dust mite (*Dermatophagoides farinae*), Indian-meal moth (*Plodia interpunctella*), and silkworm larvae (*Bombyx mori*). Recent studies have reported other novel crustacean allergens. Shiomi et al. (2008) identified the immunoreactive band (20 kDa) as sarcoplasmic reticulum Ca-binding protein, which was consequently extracted from black tiger shrimp (*Penaeus monodon*). Recently, this allergen was also identified in white shrimp (*Litopenaeus vannamei*), and in snow crab (*Chionoecetes opilio*). Sarcoplasmic calcium binding protein (SCP) is an invertebrate EF-hand calcium buffering protein that fulfills a similar function in muscle relaxation as vertebrate major allergen parvalbumin. Myosin light chain was identified as an allergen in white shrimp (*Litopenaeus vannamei*) and also identified in black tiger prawn (*Penaeus monodon*). Since TM is a common allergen in both crustaceans and mollusks other potent allergens such as myosin heavy chain, troponin, actine, hemocyanin, and amylase are reported also in molluscan shellfish (Rahman et al., 2012).

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