

Molecular and Immunological Characterization of Carp Parvalbumin, a Major Fish Allergen

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Key Words

Type I allergy · Parvalbumin · Cross-reactive allergen ·
Ca²⁺-binding proteins · Fish allergens, recombinant

Contact, inhalation and mostly ingestion of fish can cause mild (diarrhoea) to severe and life-threatening (anaphylactic shock) IgE-mediated reactions in sensitized individuals [1–3]. By immunochemical techniques, parvalbumins have been identified as the major and cross-reactive allergens in various fish species [1, 4, 5]. Parvalbumins represent low molecular weight (~12 kDa), acidic, water-soluble calcium-binding proteins with a remarkable stability regarding thermal and enzymatic degradation [1–5]. Parvalbumins contain three classical EF-hand domains, of which the first, N-terminal domain does not bind calcium but rather functions as a cap [6–8]. Parvalbumins are mainly expressed in muscle of vertebrates where they are considered as a Ca²⁺/Mg²⁺ exchange system and to a lower extent in the central nervous system and some endocrine glands of higher vertebrates [9–11].

Diagnosis of fish allergy is currently performed with crude fish extracts, which, in addition to parvalbumin, contain numerous other undefined components, among them biological mediators. Crude fish extracts are therefore less suited for biological testing (e.g., basophil histamine release tests, skin testing) and therapeutic purposes (allergen-spe-

cific immunotherapy) [12]. Also for this reason, provocation testing (e.g., double-blinded placebo-controlled oral challenge tests) is required in addition to serology, skin testing and anamnesis in order to confirm the diagnosis of fish allergy. In the last years an increasing number of recombinant allergens has been produced by cDNA cloning and expression in heterologous hosts. It has been demonstrated that recombinant allergens are suited for component-resolved diagnosis of type I allergy by *in vitro* as well as *in vivo* testing [reviewed in 13]. Recombinant hypoallergenic variants are currently developed for more safe and patient-tailored forms of immunotherapy.

We have recently characterized carp parvalbumin as a major cross-reactive fish allergen which contains most of the fish-specific IgE epitopes [5, 14]. We also found that depletion of protein-bound calcium leads to a great reduction of the allergen's IgE binding capacity [5, 14]. Here we report the isolation of a cDNA coding for the major fish allergen, carp parvalbumin. We constructed an expression cDNA library from carp muscle tissue in phage λ gt11. Using serum IgE from a fish allergic patient, several IgE-reactive cDNA clones were isolated. Their cDNA and deduced amino acid sequence was highly homologous to parvalbumins from other species and allowed to construct the structural model depicted in figure 1. The model of the parvalbumin's three-dimensional structure contains three EF-hand domains which are each flanked by two alpha helices. The

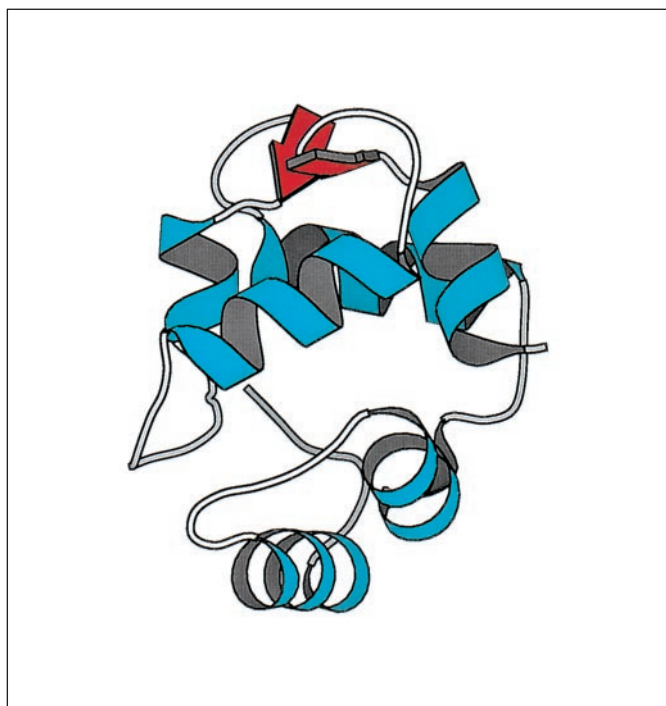


Fig. 1. Molscript representation of the carp parvalbumin structure. The alpha helices flanking the EF-hands are shown in blue and the short beta sheets in red.

N-terminal EF-hand domain reportedly does not bind calcium ions whereas the other two EF-hand domains can bind Ca^{2+} and/or Mg^{2+} ions, a process which is known to dramatically alter the protein's general conformation [15, 16]. This conformational change may be responsible for the different IgE binding capacity of the calcium-bound and calcium-free (apo-)form of parvalbumin.

When expressed as β -galactosidase fusion protein, recombinant clone 7-encoded carp parvalbumin, but not *Escherichia coli*/phage proteins, bound serum IgE from fish-allergic patients (fig. 2). We propose using purified recombinant carp parvalbumin for in vitro as well as in vivo diagnosis of fish allergy. Conversion of carp parvalbumin in a stable apoform will likely yield a hypoallergenic variant that may later on be used for component-resolved and thus patient-tailored specific immunotherapy with reduced anaphylactic side effects.

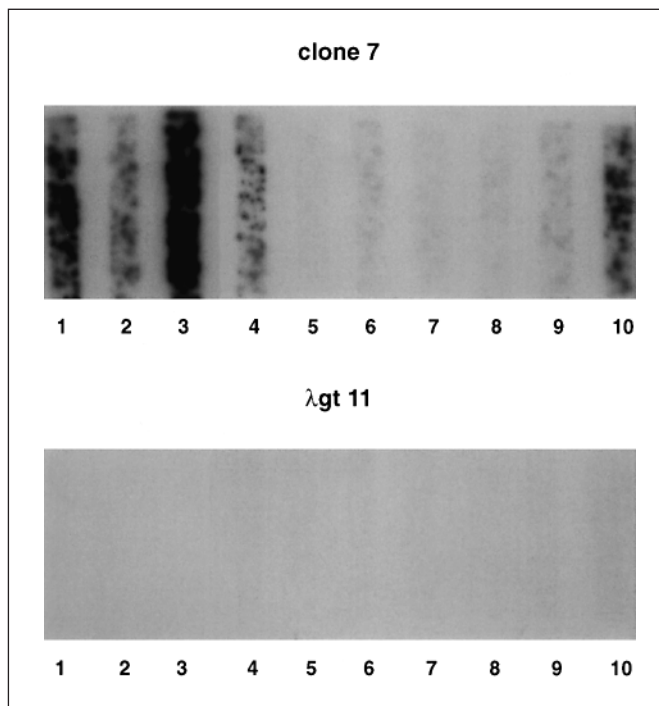


Fig. 2. IgE reactivity of recombinant β -gal-fused carp parvalbumin. Nitrocellulose stripes containing recombinant β -gal-fused carp parvalbumin (clone 7) and, for control purposes, *E. coli*/phage proteins (λ gt11) were incubated with sera from 10 fish-allergic patients (1–10). Bound IgE antibodies were detected with ^{125}I -labeled antihuman IgE.

Acknowledgements

This study was supported by a grant from Pharmacia & Upjohn, Sweden, by a grant from the 'Bürgermeister Fonds', Austria, and by grant P13383-MED of the Austrian Science Found.

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