

## Recombinant fish parvalbumins: candidates for diagnosis and treatment of fish allergy

Fish and fish products represent one of the most important causes of IgE-mediated food hypersensitivity. In sensitized individuals contact with and consumption of fish can lead to severe health problems, ranging from urticaria and dermatitis to angioedema, diarrhoea, asthma and, at worst, systemic anaphylactic reactions and death. Parvalbumin, a small calcium-binding protein present in the muscles of vertebrates, was identified as the major fish allergen. We describe the isolation and characterization of cDNA clones coding for carp parvalbumin by IgE immunoscreening of a carp muscle expression library. These clones will be the basis for the production of recombinant carp parvalbumin, a useful tool for *in vitro* and *in vivo* diagnosis of fish allergy.

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Fish and fish products play an important role in human nutrition. Fish is a valuable source of highly assimilated proteins and it contains large amounts of polyunsaturated fatty acids and fat-soluble vitamins. However, it also represents one of the most important causes of IgE-mediated food hypersensitivity (1, 2), especially in countries where the majority of the population lives on fishing and where fish is a mainstay of the diet (3). In allergic individuals, fish ingestion can provoke diarrhoea, angioedema, or severe anaphylactic reactions, inhalation of vapours generated during the cooking of fish may cause asthma, and skin contact can lead to urticaria and dermatitis (2, 4).

Although the term 'fish' refers to a wide variety of aquatic vertebrates and describes rather a life form than a well-defined taxonomic group, the most commonly consumed fish species belong to only a few closely related orders. The taxonomic relationship is reflected in the presence of cross-reactive allergenic components in these fish species. For many years this cross-allergenicity and its clinical significance have been a major point of interest for several research groups. Based on skin tests, passive transfer experiments and double-blind, placebo-controlled food challenge tests high degrees of cross-reactivity were described among

various fish species (2, 5, 6). However, in the cases of some patients, species-specific reactions were observed, which indicated also the presence of additional species-specific allergen epitopes (7, 8).

Several groups analysed allergenic components of fish extracts by immunoblotting and isoelectric focusing and the presence of serum IgE antibodies that cross-reacted with different fish species was proven by ELISA and RAST-inhibition studies (1, 2, 6). IgE-immunoblot inhibition experiments led to characterization of the cross-reactive allergens and identified a small protein of about 12 kDa as the major cross-reactive fish allergen (2, 9). This protein was first described in cod (10), designated Gad c 1, and found to belong to parvalbumins, a group of EF-hand  $\text{Ca}^{2+}$ -binding proteins (11), which are present in high amounts in the white muscles of lower vertebrates (12) and in lesser amounts in fast twitch skeletal muscles of higher vertebrates (13). They are heat stable, acidic proteins, resistant to denaturation and proteolytic digestion (10, 14).

Carp is among the most commonly consumed fish species in our environment. Therefore, we focused our research on the characterization of allergenic proteins of this species. We recently identified sarcoplasmic parvalbumin as the major carp allergen and demon-

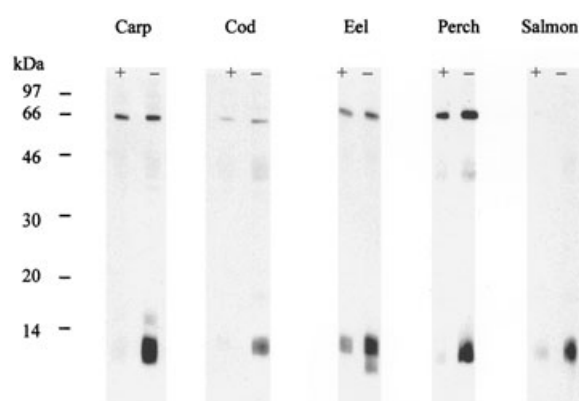


Figure 1. Inhibition of IgE-binding to parvalbumins from several fish species by preadsorption with carp parvalbumin. Nitrocellulose-blotted fish extracts (carp, cod, eel, perch, salmon) were incubated with the serum of a fish allergic patient that had been preadsorbed either with (+ lanes) or without (– lanes) carp parvalbumin.

strated its importance as a cross-reactive fish allergen (15, 16). Carp parvalbumin inhibited IgE binding to parvalbumins of several other fish species and thus contains most of the fish-specific IgE epitopes (Fig. 1). Our next aim was to isolate a cDNA coding for carp parvalbumin and subsequently to synthesize the corresponding recombinant protein.

During the last few years an increasing number of biologically active recombinant allergens were produced by cDNA cloning and expression in heterologous hosts (17). Recombinant allergens were shown to represent valuable tools for investigation of T-cell and B-cell recognition of allergens and for studying the mechanisms of specific IgE regulation (18). Immunologic equivalence of many recombinant allergens with their natural counterparts was demonstrated and it was shown that recombinant allergens are well suited for *in vitro* and *in vivo* allergy diagnosis as well as for patient-tailored specific allergy therapy (18).

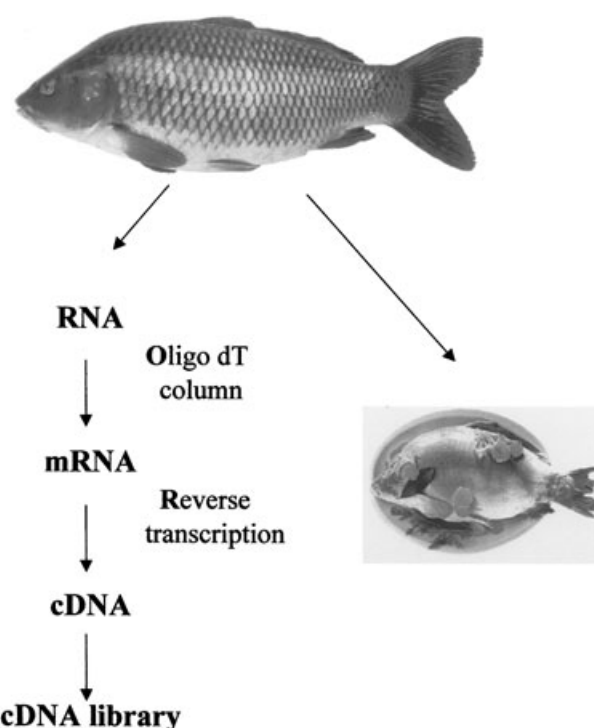


Figure 2. Construction of a carp muscle cDNA library. mRNA is isolated from carp muscles by oligo dT cellulose chromatography. cDNA is synthesized and a cDNA expression library is constructed in  $\lambda$ gt11.

IgE immunoscreening of cDNA expression libraries prepared from the allergen source yields recombinant allergens which most closely mimic their natural counterparts in terms of antibody binding capacity and biologic activity. Therefore, we constructed an expression library from carp muscles in phage  $\lambda$ gt11 (9). For this purpose, mRNA was isolated from muscle tissue and reversely transcribed into cDNA (Fig. 2), which was ligated into the phage DNA. After *in vitro* packaging, the recombinant phages were used to infect

Table 1. Amino acid sequence identities (in %) of parvalbumins – accession numbers are shown in parentheses

|                 | Carp<br>(AJ29211) | Silver<br>hake<br>(P56503) | Toadfish<br>(P05941) | Whiting<br>(P02621) | Chub<br>(P05939) | Atlantic<br>salmon<br>(Q91483) | Pike<br>(P02619) | Hake<br>(P02620) | Cod<br>(P02622) | Leopard<br>shark<br>(P30563) | Boa<br>constrictor<br>(P02615) | Frog<br>(P05940) | Chicken<br>(P19753) | Mouse<br>(P32848) | Human<br>(P20472) |
|-----------------|-------------------|----------------------------|----------------------|---------------------|------------------|--------------------------------|------------------|------------------|-----------------|------------------------------|--------------------------------|------------------|---------------------|-------------------|-------------------|
| Carp            | 100               | 85                         | 82                   | 80                  | 79               | 78                             | 76               | 75               | 68              | 53                           | 69                             | 76               | 71                  | 56                | 56                |
| Silver hake     |                   | 100                        | 74                   | 88                  | 78               | 79                             | 77               | 73               | 67              | 50                           | 65                             | 71               | 63                  | 51                | 53                |
| Toadfish        |                   |                            | 100                  | 71                  | 74               | 73                             | 76               | 72               | 65              | 48                           | 67                             | 68               | 67                  | 56                | 52                |
| Whiting         |                   |                            |                      | 100                 | 74               | 72                             | 73               | 65               | 47              | 66                           | 71                             | 62               | 52                  | 53                |                   |
| Chub            |                   |                            |                      |                     | 100              | 74                             | 72               | 66               | 54              | 61                           | 72                             | 64               | 55                  | 56                |                   |
| Atlantic salmon |                   |                            |                      |                     |                  | 100                            | 93               | 68               | 61              | 50                           | 64                             | 71               | 64                  | 54                | 56                |
| Pike            |                   |                            |                      |                     |                  |                                | 100              | 71               | 61              | 50                           | 64                             | 69               | 64                  | 56                | 59                |
| Hake            |                   |                            |                      |                     |                  |                                |                  | 100              | 65              | 52                           | 64                             | 68               | 59                  | 53                | 52                |
| Cod             |                   |                            |                      |                     |                  |                                |                  |                  | 100             | 49                           | 56                             | 63               | 54                  | 53                | 52                |
| Leopard shark   |                   |                            |                      |                     |                  |                                |                  |                  |                 | 100                          | 41                             | 53               | 68                  | 61                | 58                |
| Boa constrictor |                   |                            |                      |                     |                  |                                |                  |                  |                 |                              | 100                            | 67               | 62                  | 52                | 50                |
| Frog            |                   |                            |                      |                     |                  |                                |                  |                  |                 |                              |                                | 100              | 75                  | 57                | 55                |
| Chicken         |                   |                            |                      |                     |                  |                                |                  |                  |                 |                              |                                |                  | 100                 | 64                | 57                |
| Mouse           |                   |                            |                      |                     |                  |                                |                  |                  |                 |                              |                                |                  |                     | 100               | 87                |
| Human           |                   |                            |                      |                     |                  |                                |                  |                  |                 |                              |                                |                  |                     |                   | 100               |

*Escherichia coli* cells. Expression of the inserted cDNAs as  $\beta$ -galactosidase fusion proteins was induced and phage clones expressing carp allergens were identified with serum IgE antibodies from a fish allergic patient. Sequencing of more than 30 isolated IgE-reactive cDNA clones revealed that they all coded for parvalbumin and demonstrated the presence of two distinct highly homologous carp parvalbumin isoforms.

The deduced amino acid sequence of one of the carp clones was compared to parvalbumins from different fish species and from other groups of vertebrates (Table 1). Significant homologies, between 68% (cod) to 82% (silver hake and toadfish), were observed with parvalbumins of other bony fishes (class Osteichthyes). This was a further support for the notion that parvalbumins represent cross-reactive fish allergens (15). Much lower was the homology to a parvalbumin from leopard shark, a cartilaginous fish of the class Chondrichthyes, which showed a percentage identity of only 53% to carp parvalbumin. Leopard shark parval-

bumin had low similarity indices with parvalbumin sequences from all bony fishes (ranging from 47% to 54%), which clearly separated parvalbumins of bony fishes from those of cartilaginous fishes. Surprisingly, bony fish parvalbumins showed a very high degree of sequence similarity to some of the parvalbumins from amphibia (frog: ranging from 63% to 76%), reptiles (boa constrictor: 56% to 69%) and birds (chicken: 54% to 71%), and even the difference to mammalian parvalbumins was relatively low (51% to 59% identity).

This work will be the basis for the production of recombinant carp parvalbumin and hypoallergenic derivatives for diagnosis and therapy of fish allergy.

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