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Detecting Fish Parvalbumin with Commercial Mouse Monoclonal Anti-frog Parvalbumin IgG

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Parvalbumin is a calcium-binding muscle protein that is highly conserved across fish species and amphibians. It is the major cross-reactive allergen associated with both fish and frog allergy. We used two-dimensional electrophoretic and immunoblotting techniques to investigate the utility of a commercial monoclonal anti-frog parvalbumin IgG for detecting parvalbumin present in some commonly consumed fish species. The 2D electrophoresis and immunoblots revealed species-specific differences in proteins that appear to represent various numbers of isoforms of parvalbumin in carp (5), catfish (3), cod (1) and tilapia (2). No parvalbumin was detected in yellowfin tuna. Based on minor differences in relative intensities of protein staining and immunodetection, parvalbumin isoforms may have slight differences in the epitope region recognized by the anti-frog parvalbumin antibody. These results suggest that the frog anti-parvalbumin antibody can be used as a valuable tool to detect parvalbumins from the fish tested in this study, except yellowfin tuna.

KEYWORDS: Fish; parvalbumin; allergen; 2D electrophoresis; immunoblot; antibody

INTRODUCTION

Fish is one of the most frequent causes of food allergy in many countries of the world (*1*). The prevalence of fish allergy in the United States has been estimated to be 0.4% (*2*). Fish-allergic patients can experience a variety of symptoms including atopic dermatitis, emesis, diarrhea, urticaria, angioedema, and life-threatening anaphylactic shock (*1, 3, 4*).

Gad c 1, a codfish parvalbumin, was the first purified and characterized fish allergen (*5, 6*). Parvalbumins are a family of calcium-binding proteins that play an important role in muscle relaxation (*7, 8*). Parvalbumins are found in all vertebrates, including man, but are present at much higher quantities in the muscles of lower vertebrates, such as fish and amphibians (*9*). Close sequential and structural similarities of parvalbumins are believed to be responsible for the general cross-reactivity of fish and frog muscle in fish-allergic individuals (*10, 11*). Parvalbumin is considered as a pan allergen in fish (*12*) and is known to be the major allergen from cod, salmon, carp, horse mackerel, several species of mackerel, and bigeye tuna (*13–17*). Parvalbumin is also the major allergen of frog (*18*).

Parvalbumins have molecular weights of approximately 10−13 kDa and acidic pI values. They are water soluble and resistant to heat treatment and enzymatic degradation (*19*). Amino acid sequences of parvalbumins from several fish species and amphibians have been determined and show significant sequence homology (*11, 20*). The most conservative amino acid sequences lie in the regions that form the two calcium binding sites, each containing 12 amino acid residues (*20*).

Based on comparison of amino acid sequences, parvalbumins are divided into α- and β-phylogenetic lineages (*21*). The α-parvalbumins have a pI of 5.0 or higher, while the β-parvalbumins contain more acidic amino acids, resulting in a pI value of 4.5 or lower. Members of both lineages have been identified in a number of fish species (*9*). The distribution of the parvalbumins differs according to fish species, stage of development, and muscle type (*22*). Parvalbumins are found mostly in striated skeletal muscle. White muscle generally contains more parvalbumin than red muscle (*23*). In carp, white trunk muscle contains 10 times more parvalbumin than red muscle along the lateral line or supracarinalis (*24*). The swim bladders of fish also contain parvalbumin (*25*).

In an effort to identify and characterize fish allergens, a number of monoclonal anti-parvalbumin antibodies have been developed against different fish species (*26, 27*). Their specificities against parvalbumins from other fish species have not been well-studied. A monoclonal anti-frog parvalbumin of unspecified identity from Sigma-Aldrich Corp. (St. Louis, MO) was previously reported to bind to mackerel parvalbumin (*17*). As Sigma-Aldrich only lists one monoclonal line of anti-frog parvalbumin (PARV-19), which was previously reported to bind to both α- and β-frog parvalbumins in another study (*11*), we were interested in testing this antibody across additional species of fish.

The aim of the present study was to evaluate a commercial anti-frog parvalbumin monoclonal antibody for detecting parvalbumin from the muscle tissue of various fish species.

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MATERIALS AND METHODS

Materials. Frozen samples of carp, catfish, cod, tilapia, yellowfin tuna, and lean beef were purchased from local grocery stores (Lincoln, NE). Fresh skeletal muscle from an albino rat was collected at the University of Nebraska animal facility. All samples were stored at −20 °C prior to extraction. Monoclonal mouse anti-frog parvalbumin IgG (PARV-19) was obtained from Sigma-Aldrich. Preparation of recombinant carp parvalbumin (rCyp c 1.01) was described previously (*20*). Recombinant rat parvalbumin was obtained from Swant (Bellinzona, Switzerland).

Protein Extraction. A 5 g amount of each muscle sample was extracted with 50 mL of phosphate buffered saline (PBS, 0.01 M, pH 7.4), containing 1% (v/v) Triton X-100 and 1 tablet of complete EDTA-free protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN). Samples were blended in extraction buffer for 1 min using a Waring blender. The mixture was then centrifuged at 3000*g* for 30 min at 4 °C. The supernatant solution was collected and stored at −20 °C until analysis.

Protein Assay. The protein concentration of each extract was determined using a Lowry protein assay (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions using heat-shocked fraction V bovine serum albumin (BSA) of greater than 96% purity (catalog no. A9647, Sigma-Aldrich) as a standard.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS−PAGE). All protein extracts were diluted to 2 mg of protein/(mL of PBS) with an equal volume of Laemmli sample buffer (Bio-Rad Labs), containing 2-mercaptoethanol as a reducing agent, and heated at 95 °C for 5 min. A 10 μL aliquot of each fish extract (containing 10 μg of protein) was loaded onto a Novex tris-glycine 10−20% ready gel (Invitrogen, Carlsbad, CA). Precision Plus protein standards of 5 μL each (Bio-Rad Labs) were also used.

Electrophoresis was carried out at a constant 125 V for 1.5 h in a Surelock minigel system (Invitrogen). Following electrophoresis, proteins were transferred to Invitrolon PVDF membranes (Invitrogen) for immunoblotting or gels were fixed and stained with Coomassie brilliant blue G (Sigma-Aldrich), following the manufacturer's instruction. Images of freshly destained gels were captured and analyzed using a Kodak GL440 image station and 1D image analysis software (Eastman Kodak Co., Rochester, NY).

Two-Dimensional Electrophoresis. Isoelectric focusing was performed on 7 cm nonlinear pH 3−10 Zoom strips in a Zoom IPGRunner system (Invitrogen). The strips were rehydrated for 1 h at room temperature in a solution consisting of 7 M urea, 0.5% CHAPS, 100 mM 1,4-dithiothreitol, 1% pH 3−10 ampholytes (Invitrogen), and 35 μg (for protein stain) or 3.5 μg (for immunoblotting) fish protein extract. Focusing conditions were controlled with a multistep program: 200 V for 20 min, 450 V for 15 min, 750 V for 15 min, and 2000 V for 30 min. After focusing and prior to the second dimension, strips were equilibrated in a mixture of 1 mL of NuPage reducing agent (Invitrogen) and 9 mL of 1X NuPage LDS sample buffer (Invitrogen) for 15 min and then alkylated by incubation with 125 mM iodoacetamide in 1X NuPage LDS sample buffer (Invitrogen) for 15 min. The second dimension electrophoresis was conducted in a Novex 4−20% tris-glycine Zoom gel (Invitrogen). Completed gels were used for immunoblotting and protein stain. The pI gradient of the Zoom strips was calibrated by a low-range pI isoelectric focusing calibration kit (Amersham Biosciences Corp, Piscataway, NJ).

Immunoblotting. Proteins separated in 1D or 2D gels were transferred to PVDF membranes using an XCell II blot module (Invitrogen) according to the manufacturer's instruction. Following transfer, membranes were blocked with 5% nonfat dry milk in PBS (PBS−NFDM) for 2 h at room temperature and washed four times with PBS containing 0.5% (v/v) Tween 20 (PBS-T) with vigorous shaking. The membranes were then incubated overnight at 4 °C with anti-frog parvalbumin IgG (PARV-19) diluted 1:5000 (containing 1.54 μg/mL IgG) in 2.5% PBS−NFDM. After four washes in PBS-T, the membranes were incubated for 1 h with goat anti-mouse IgG conjugated with horseradish peroxidase (Pierce Biotechnology, Rockford, IL), diluted 1:5000 (v/v) in 2.5% PBS−NFDM. Membranes were washed four times with PBS-T and wetted in ECL substrate (Amersham Biosciences) to visualize the immunocomplex. The chemiluminescent signal was captured by exposing Kodak BioMax light films for various times (Eastman Kodak Co.). A similar concentration of unconjugated monoclonal IgG1 (M5284, Sigma-Aldrich) was used as a negative isotype control in place of PARV-19 on identical blots.

Sequence Alignment and pI Calculation. Parvalbumin sequences were obtained from the National Center for Biotechnology Information (www.ncbi.nih.gov). The multiple sequence alignment to carp parvalbumin sequence GI no. 179777825 was performed using the Align program (Scientific and Educational Software, Cary, NC), which uses a PAM250 scoring matrix with a mismatch penalty of 2, gap penalty of 4, and gap extension penalty of 1. The pI value for each sequence was calculated for each sequence using Protean protein structure prediction and annotation software (DNASTAR, Inc., Madison, WI).

RESULTS

Detecting Fish Parvalbumin with Anti-frog Monoclonal Antibody by Immunoblotting. The protein profiles of some commonly consumed fish species were revealed by SDS−PAGE, shown in **Figure 1A**. All five species showed similar protein banding patterns above a molecular weight of 25 kDa. Differences in protein bands were observed at lower molecular weights among fish species. Either one or two distinct bands of high concentration were observed in the molecular weight range of 10−13 kDa expected for parvalbumins in carp (2), catfish (2), cod (1), and tilapia (2). No significant bands were noted in the yellowfin tuna sample below 25 kDa.

Rat and beef muscle proteins were also separated by SDS−PAGE (**Figure 1A**), with dramatically different banding patterns than those of fish. Rat (lane 6) had a relatively strong band with a molecular weight of 13 kDa, corresponding to the size of parvalbumin. Beef (lane 7) did not show a band in the 10−13 kDa range.

Immunoblots obtained using the anti-frog parvalbumin IgG are shown in **Figure 1B**. All visible fish protein bands in the molecular weight range of 10−13 kDa were recognized by anti-frog parvalbumin IgG. The antibody did not bind to any bands in yellowfin tuna (**Figure 1B**, lane 5). The molecular weight and relative abundance (determined by densitometry using the Kodak image analysis software) of the antibody-binding fish proteins are summarized in **Table 1**. Lane 9 of **Figure 1B** contains recombinant carp parvalbumin (rCyp c 1.01) with a molecular weight of 11.4 kDa (*20*).

One band in rat protein extract (**Figure 1B**, lane 6) was recognized by the anti-frog parvalbumin IgG, and it possessed the same molecular weight (13 kDa) as recombinant rat parvalbumin (lane 8). No antibody-binding band was observed in beef extract (lane 7).

Two-Dimensional Electrophoresis and Immunoblot of Fish Proteins. Figure 2 shows the 2D electrophoresis maps of carp, catfish, cod, and tilapia extracts in conjunction with immunoblots. Yellowfin tuna was not included because of negative results in 1D SDS−PAGE.

Three protein spots from carp extract were clearly resolved, and two additional spots were smeared (approximately pH 3.5); however, all were apparently bound by the anti-frog parvalbumin antibody (**Figure 2A2**). Three of the five polypeptides had a molecular weight of 10.6 kDa, and the other two were of 11.6 kDa. Their pI values ranged from 3.5 to 4.25, based on protein markers in the low-range pI (Amersham Biosciences); data not shown. Catfish (**Figure 2B2**) had two fairly strong antibody-binding proteins at pI 4.25 and a barely visible spot at pI 3.5. Only one antibody-binding protein was observed in cod (**Figure 2C2**), with a molecular weight of 11.6 kDa and pI 3.6. Anti-frog parvalbumin antibody recognized two bands in tilapia (**Figure 2D2**). Even though these two proteins showed similar intensity in SDS−PAGE (**Figure 2D1**), their antibody-binding capacities were markedly different (**Figure 2D2**). The 11.1 kDa
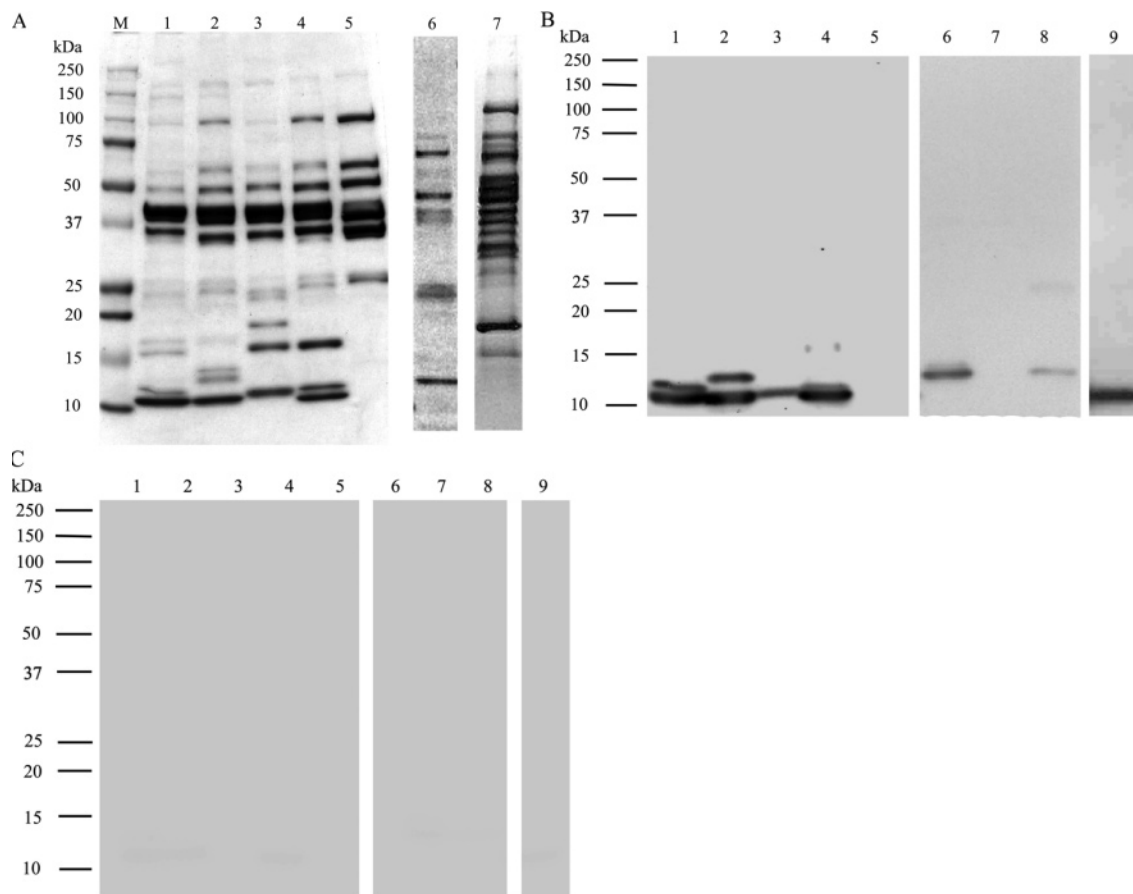
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Figure 1. (**A**) Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE), (**B**) immunoblot using commercial monoclonal antibody, and (**C**) immunoblot using mouse IgG1 isotype control (Sigma-Aldrich) as negative control. Lane 1 = carp; lane 2 = catfish; lane 3 = cod; lane 4 = tilapia; lane 5 = yellowfin tuna; lane 6 = rat; lane 7 = beef; lane 8 = recombinant rat parvalbumin; lane 9 = carp recombinant parvalbumin (rCyp c 1.01).

Table 1. Anti-frog Parvalbumin IgG Binding Fish Proteins Visualized by Immunoblotting[a]

fish	no. of IgG-binding bands[b]	mol wt (kDa)	abundance (%)	total abundance (%)
carp	1	11.6	2.9	17.1
	2	10.6	14.2	
catfish	1	13.1	2.4	16.7
	2	10.9	14.3	
cod	1	11.6	12.5	12.5
tilapia	1	12.1	5.0	14.7
	2	11.1	9.6	

[a] Densitometry analysis was conducted on SDS-PAGE using Kodak 1D image analysis software to calculate the abundance of the antibody-binding protein in the total fish extract. [b] Parvalbumin bands were numbered starting from high molecular weight to low molecular weight.

protein with pI 3.6 exhibited much stronger binding than the 12.1 kDa subunit with pI 4.0.

Comparison of Parvalbumin Sequences. Published sequences of carp (2), cod (3), catfish (1), frog (2), rat, and beef parvalbumin were aligned on the basis of the sequence of the carp parvalbumin (20), shown in **Figure 3**. No sequence information is available for parvalbumin from tilapia. Since we do not know the epitope, or amino acid sequence, of the parvalbumins recognized by the anti-frog monoclonal antibody, we performed an alignment of known parvalbumin sequences to evaluate whether there are specific regions of identities and differences that could be used to identify the likely epitope based on antibody binding results. While the sequences of the fish parvalbumins are highly conserved particularly in the regions

identified previously as the calcium binding sites (20), the frog and mammalian parvalbumin sequences are more divergent except at the calcium binding sites. Furthermore, Hilger et al. (11) previously demonstrated that the PARV-19 antibody binding is dependent on the presence of calcium. We observed that PARV-19 binds to rat parvalbumin, but there was no apparent binding to a beef protein. The beef sequence differed from rat by 12 out of 109 amino acids, although only two changes represent nonconservative substitutions (H at position 8 in beef, P at position 41 in beef), and these are not close to either calcium binding pocket. The conservative substitutions in the calcium binding domains of beef and rat, should not represent differences compared to some of the antibody-bound fish parvalbumins, indicating those substitutions are unlikely to prevent binding to beef parvalbumin. Many nonconservative substitutions are evident comparing parvalbumin sequences from rat, frog, and various fish sequences that are bound by PARV-19, leaving open the question of the probable location of the epitope.

DISCUSSION

The first step in evaluating the potential binding of the PARV-19 monoclonal antibody to various fish parvalbumins demonstrated clear binding to proteins in the correct molecular weight range for carp, catfish, cod, and tilapia, but not yellowfin tuna. However, since there were multiple bands identified by the PARV-19 antibody in carp, catfish, and tilapia, and previous reports of multiple isoforms of parvalbumin in some species of fish (28, 29), we decided to further evaluate the antibody binding to proteins separated by 2D gel electrophoresis.
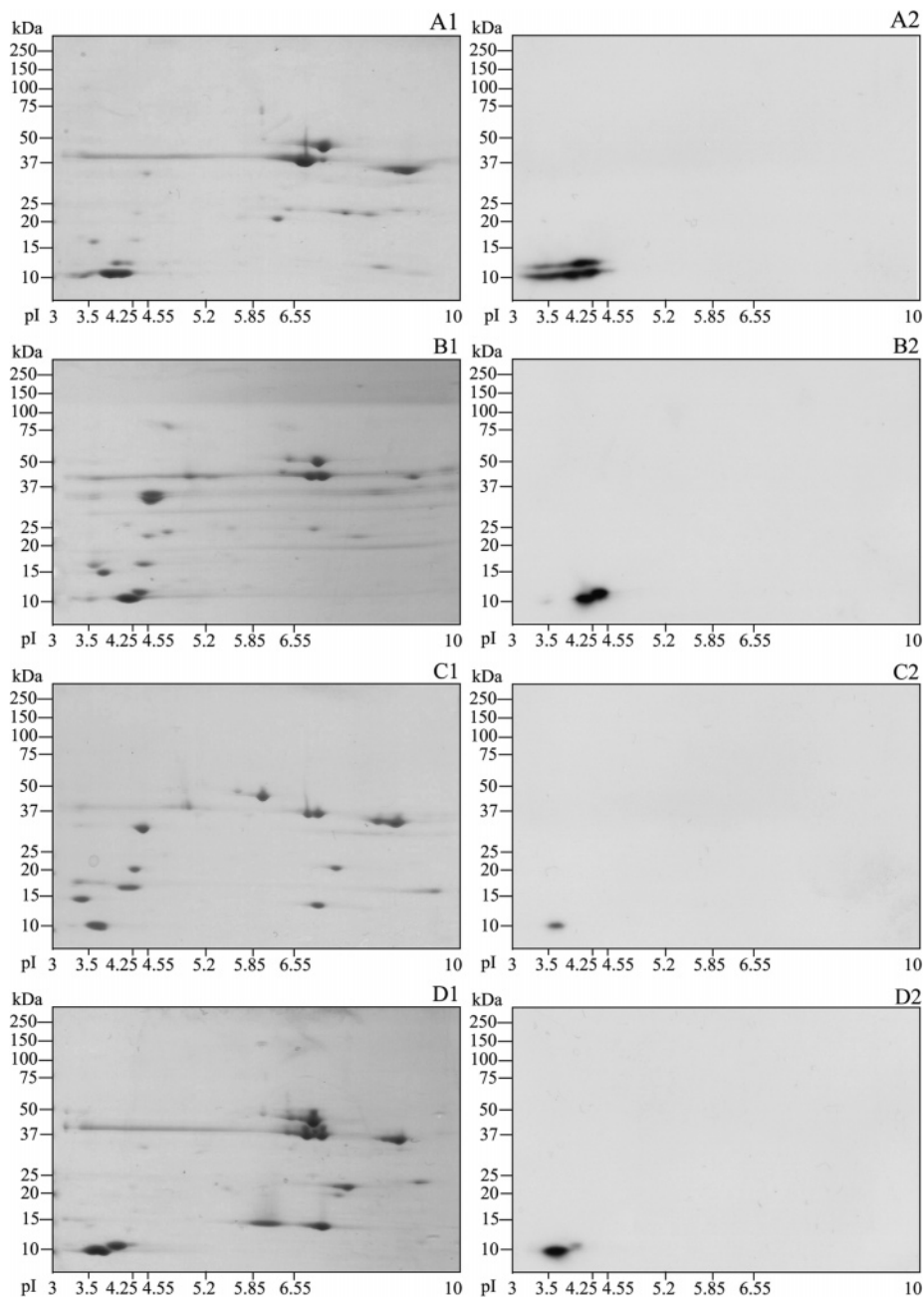
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Figure 2. Two-dimensional electrophoresis maps of carp (**A1**), catfish (**B1**), cod (**C1**), and tilapia (**D1**) extracts and their corresponding immunoblotting profiles: carp (**A2**), catfish (**B2**), cod (**C2**), and tilapia (**D2**).

We found minor differences from our 2D electrophoresis blotting data compared to the number and pI value of apparent isoforms in some of the fish species previously reported by other researchers. We observed five isoforms in carp ranging in pI values from 3.5 to 4.25. Pechere et al. (*29*) reported four isoforms with pI values ranging from 3.95 to 4.47, while Giriga and Rehbein (*28*) reported four isoforms ranging from 3.51 to 4.12. Our cod extract showed only one parvalbumin isoform (pI 3.6), in contrast to Giriga and Rehbein (*28*) who found 2 isoforms (pI 3.75, 3.88) in cod fish by isoelectric focusing. The observed pI values from our 2D gel electrophoresis are lower than calculated values from published sequences of specific parvalbumins (**Figure 3**). The discrepancies may be due to the shift of the protein spots along the pI scale during the second dimension electrophoresis or perhaps nonlinearity of the pI gradient in the Zoom strips used in this experiment. Alternatively, some parvalbumin may be modified posttranslationally, changing the pI values.

It is important to note that there are differences in the number of isoforms across species of fish. The differences in molecular weights and pI values suggest that one might find more variation in parvalbumin sequences and therefore possibly in binding by IgE from fish-allergic individuals to different species of fish parvalbumins than might be expected on the basis of known sequences.

We observed an apparent difference in the spot size and intensity of staining of the two isoforms of parvalbumin in tilapia, relative to the antibody spot size and intensity caused by binding with the PARV-19 (**Figure 2D1,2D2**). It appears that the PARV-19 antibody binds more efficiently to the acidic, smaller molecular weight isoform in tilapia. However, more quantitative work would be needed to confirm this observation.

Differences in antibody binding to parvalbumins might be expected due to differences in the amino acid sequences. It has been well-recognized that the most conserved regions of parvalbumins lie in the two calcium binding motifs. Hilger et

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Organism   GI#              10        20        30        40        50        60
                            *         *         *         *         *         *
Carp       17977825    MAFAGILNDADITAALQGCQAADSFDYKSFFAKVGLSAKTPDDIKKAFAVIDQDKSGFIE
Carp       17977827    .....V..........EA.K.....NH.T.......TS.SA..V.....I..........
Cod        32363376    ................AA.K.EG...H.A..T....A..S.A....V.EI......D.V.
Cod        14531013    .......A...CA..VKA.E..E..S..A....C...G.SA......F...........
Catfish    27883551    .....V..........DA.K.DG..NH....T....TG.SA..V.....I..........
Cod        131112     -..K...SN...K..EAA.FKEG...EDG.Y.....D.FSA.EL..L.KIA.E..E....
Frog       20796733    .PMTDV.AAC..SK.MAAFP..EP.NH.K..ELC..KG.SQ..M..V.HML.K.Q.....
Frog       20797085    .SITD.VSEKN.E...ES VK.EG..E..C..Q....AG.SA..A..V.EIL.R....Y..
Rat        131107     .SMTDL.SAE..KK.IGAFT......H.K..QM...KK.SA..V..V.HIL.K.......
Beef       61830056    .SMTDL.HAE..KK.VGAFT.V....H.K..QM...KK.S.E.V..V.HIL.K.......
                                                                            ( EF 1 )

                            70        80        90        100               %ID   pI
                            *         *         *         *
Carp       17977825    EDELKLFLQNFSAGARALTDAETKAFLKAGDSDGDGKIGVDEFAALV -----K-A  100   4.21
Carp       17977827    ..........K........G...T..................T...  -----.-.   84   4.62
Cod        32363376    .................S.....V..................G.MI  -----.-.   81   4.42
Cod        14531013    .........V.K..........................A....W.. V.-----.-.  82   4.39
Catfish    27883551    ..........KSS......T......T...........S..  -----.-.   80   5.05
Cod        131112     .........IA.A.DL...................G...DKWGA.  -G   68   4.21
Frog       20796733    K...A.I.KG.TPEG.D.S.K..T.L.A...K....... ....VK.. -----SEC  51   4.71
Frog       20797085    K...C......KSS....N....S................E..Q...  -----.-.   64   4.56
Rat        131107     ....GSI.KG..SD..D.SAK...TLMA...K........E..ST..  -----AES   54   4.88
Beef       61830056    .E..GFI.KG..PD..D.SVK...TL.A...K.. .....A...ST.. -----AES   53   5.09
                       ( EF 1)                               ( EF 2 )
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Figure 3. Alignment of parvalbumin sequences from various species. Residues identical to carp recombinant parvalbumin rCyp c 1.01 (GI no. 17977825) are indicated by dots. Gaps are indicated by dashes, and shaded boxes are the calcium binding motifs. Sequence identities to rCyp c 1.01 are expressed in percentage (%ID). The pI value of each sequence was calculated using Protean protein structure prediction and annotation software (DNASTAR, Inc.).

al. (*11*) previously demonstrated that Ca^{2+} is essential for the PARV-19 antibody to bind to frog parvalbumin. This suggests that the anti-frog parvalbumin antibody may bind to a conformational structure associated with the calcium-binding motif and broad cross-reactivity is most likely to occur within the conserved regions. The first calcium binding site has only one conservative amino acid substitution between rat and beef parvalbumins; the second has two. It does not seem likely that these minor differences between beef and rat would oblate antibody binding, but it is possible as none of the fish species have the same amino acid substitutions. It is also possible that the concentration of parvalbumin in beef was simply too low to detect.

We did not detect any PARV-19 antibody binding to tuna extract. Is there little or no parvalbumin in yellowfin tuna? Or is yellowfin tuna parvalbumin not recognized by this antibody? Conflicting reports of IgE binding to tuna parvalbumin have been published from studies using sera from fish-allergic subjects. Bernhisel-Broadbent et al. (*12*) tested the skin prick test reactivity of extracts of 10 species of fish in 11 fish allergic subjects and also performed in vitro IgE binding with extracts from raw and cooked flesh of nine of the fish species using sera from some of the subjects. They also performed double-blind placebo-controlled food challenges with cooked meat from the fish, except that canned tuna (unspecified type) was used in place of cooked tuna. Reactions to the canned tuna were similar to placebo controls. Skin test results were lower for tuna (species not identified) than other fish species. Immunoblots of two "representative" donor sera showed a minor band at about 12 kDa for one subject, and no band for the other. Bugajska-Schretter et al. (*30*), detected IgE binding to an extract of bluefin tuna (*Thunnus thynnus*) protein thought to be parvalbumin by immunoblotting using sera from two of three fish allergic individuals who had demonstrable IgE binding also to cod, salmon, perch, carp, and eel in samples that were denatured and reduced and only denatured. Yamada et al. (*31*) detected IgE-binding to an approximately 12 kDa band in yellowfin (*Thunnus albacares*) and albacore (*Thunnus alalunga*) tuna by immunoblotting with sera from only one out of eight tuna-

allergic subjects. James et al. (*32*) tested IgE binding and skin prick tests and, for those with less than severe clinical histories, also performed double-blind placebo-controlled food challenges of five pediatric and five adult subjects using extracts of catfish, cod, snapper, and tuna (species not specified). The only demonstrable IgE binding to tuna protein was to a 40 kDa band, and no clearly distinct stainable protein band of approximately 12 kDa was observed for the tuna extract. The main, or only protein in catfish, cod, and snapper that was bound by IgE from these subjects was an approximately 12.5 kDa band and that binding was inhibited by preincubation of sera with codfish extract.

It is possible that skeletal muscle from some species of tuna contains little or no parvalbumin relative to the amount of parvalbumin in many other fish species, as indicated by the Coomassie stained gel of fish extracts in the Bernhisel-Broadbent study (*12*). Our results show only two faintly stained proteins below 25 kDa (22 and 14 kDa, larger than expected for parvalbumin) in yellowfin tuna extract (**Figure 1**). A similar SDS–PAGE profile of yellowfin tuna extract was also obtained by Etienne et al. (*33*). Shiomi et al. (*16*) isolated two highly similar proteins from bigeye tuna (*Thunnus obesus*) that were identified as parvalbumins on the basis of size, amino acid composition, and antibody binding to an anti-carp parvalbumin. Lim et al. (*34*) detected parvalbumin in very restricted tissues of white, but not red, muscle of longtail tuna (*Thunnus tonggol*), using the same anti-frog parvalbumin used in the present study. We did not detect parvalbumin from yellowfin tuna (*T. albacares*) with the anti-frog parvalbumin antibody. Interestingly, Swoboda et al. (*20*) reported that recombinant carp parvalbumin was able to partially inhibit IgE binding of fish-allergic subjects to the tuna Pharmacia CAP–FEIA (*T. albacares*). Our data along with previously published data demonstrate uncertainty of the amount of parvalbumin in various species, or tissues of tuna. There appears to be marked differences in IgE binding to parvalbumin of various tuna. It is therefore not clear whether the anti-frog parvalbumin antibody binds effectively to parvalbumin from various tuna species or if the concentration of parvalbumin is just remarkably low in some

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species or some tissues. However, the data suggest that this antibody would not be useful for detecting tuna parvalbumin.

In summary, the commercial PARV-19 anti-frog parvalbumin monoclonal antibody tested in this study could be a useful reagent for detecting fish parvalbumins from a number of commercially important species of fish.

ACKNOWLEDGMENT

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