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Isolation and Characterization of Parvalbumin Beta Gene from Channel Catfish (*Ictalurus punctatus*)

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Our previous studies of both microarray analysis in channel catfish muscle gene expression of 2 different ages and channel catfish muscle expressed sequence tag profiles demonstrated parvalbumin beta is one of the highly expressed muscle transcriptome. We have cloned and sequenced complementary DNA encoding the channel catfish parvalbumin which encode 109 amino acids. The deduced amino acid sequences of the catfish parvalbumin are highly conserved with those cloned from other teleosts. The availability of the catfish parvalbumin provides the opportunity of studying fish epitopes.

Keywords: Expression, Gene, Muscle, Parvalbumin

Fish and fish products play an important role in human nutrition. Fish is a valuable source of highly assimilated proteins, polyunsaturated fatty acids, and fat-soluble vitamins. However, it also causes IgE-mediated food hypersensitivity (Aas, 1987; Bernhisel et al., 1992). Patients allergic to fish often have dramatic symptoms and severe asthmatic attacks can be triggered by the smell of fish (De Besche, 1937; Pascual et al., 1992). The frequency of fish allergy varies according to geography and exposure. In Norway, fish allergy is 1/1000; in Sweden, about 39% of the pediatric population is affected; and in Spain the figure ranges between 18-30% (Zinn et al., 1997). A general figure for all populations is approximately 22%.

Parvalbumin has been known as one of the major allergen in fish (Elsayed, 1993). Allergen Gad cI (allergen M) from cod was the first allergen to be isolated as a pure fraction (Elsayed, 1993). It is a high affinity calcium-binding protein belonging to the parvalbumins found only in the white muscles of fish and amphibians (Perchere, 1997) and in lesser amounts in fast twitch skeletal muscles of higher vertebrates (Lehky et al., 1974). They are heat stable, acidic proteins, resistant to denaturation and proteolytic digestion (Aas and Elsayed, 1969; Elsayed and Aas, 1971). Parvalbumin plays an important role as a soluble relaxing factor in fast skeletal muscle fibers by acting as a delayed Ca^{2+} sink (Wahr et al., 1999).

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The general shape of parvalbumin itself can be described as 6 helices named A, B, C, D, E and F. These helices are arranged into two identical protein motifs called E-F hands (Kretsinger et al., 1991). There are two distinct phylogenteic lineages of parvalbumins, named α and β generally recognized in lower and higher vertebrates (Goodman and Pechere, 1977). Most species in muscles contain only one parvalbumin of either α or β whereas both of parvalbumin α and β were found in frog skeletal muscles (Tanokura et al., 1986).

We already published two genes (creatine kinase and alpha actin) of three highly expressed transcriptomes in channel catfish muscles (Kim et al., 2000; Liu et al., 2001). Here we report the full sequence of channel catfish parvalbumin beta cDNA clones highly expressed in channel catfish skeletal muscles.

Muscle tissues were collected from channel catfish. Muscles were used for preparation of total RNA. Muscle tissues were frozen in liquid nitrogen for grinding with a mortar/pestle and then homogenized with a hand-held tissue tearor (Model 985-370, Biospec Products, Inc., WI, USA) in RNA extraction buffer following the guanidium thiocyanate method (Chomczynski and Sacchi, 1987). Poly $(A)^+$ RNA was then purified by using the Oligotex Spin Column Kit from Qiagen according to manufacturers instructions (Qiagen Inc., Chatsworth, CA, USA). The muscle cDNA library used in this study has been reported (Kim et al., 2000). DNA sequences were analyzed using the DNAsis and DNAstar software packages. Homology searches were conducted using the BLASTN pro-

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agtctttgagaatctacagctcaccaccaaaaagacgaaagagctaaagATGGCATTCGC  60
                                                    M  A  F  A   4
TGGTGTCCTCAACGATGCTGACATCACTGCAGCCCTGGATGCATGCAAAGCCGACGGTTC 120
  G  V  L  N  D  A  D  I  T  A  A  L  D  A  C  K  A  D  G  S  24
CTTCAACCACAAGAGCTTCTTCACTAAGGTCGGTCTGACCGGCAAGTCTGCCGATGACGT 180
  F  N  H  K  S  F  F  T  K  V  G  L  T  G  K  S  A  D  D  V  44
CAAGAAGGCTTTCGCCATCATTGATCAGGACAAGAGTGGCTTCATTGAGGAGGATGAACT 240
  K  K  A  F  A  I  I  D  Q  D  K  S  G  F  I  E  E  D  E  L  64
GAAACTGTTCCTGCAGAACTTCAAATCCAGCGCGAGGGCACTCACTGATGCCGAGACCAA 300
  K  L  F  L  Q  N  F  K  S  S  A  R  A  L  T  D  A  E  T  K  84
GACTTTCCTCAAGGCTGGAGACACCGATGGTGATGGCAAGATTGGAGTTGATGAGTTCGC 360
  T  F  L  K  A  G  D  T  D  G  D  G  K  I  G  V  D  E  F  A 104
TAGCCTGGTTAAGGCATAAaatgcttctgaccaacaaacaatacttctcgctctgaagga 420
  S  L  V  K  A  *                                          109
Acaactttgcccgagacctcccatgttcatctgactataaataatttttataccaatgct 480

tatgagaagtttcctcccatgcagcacactgtccaaaatgatgattgtgaatgtagcgcg 540

gctgtgtatcctgtcttaaatatgaattttgcttactgtaaaatctgatgcacttttacg 600

ggaacggactataagaaaag<u>aataaa</u>tattctttgcaaaaaaaaaaaaaaaaaaaaaa 660
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Fig. 1. The nucleotide and deduced amino acid sequences of cDNA encoding channel catfish parvalbumin beta. Nucleotide sequences are numbered on the top, far right, and the amino acid sequence on the bottom, far right. The coding region is in upper case letters and the non-coding region in lower case letters. The stop codon is indicated by an asterisk. The polyadenylation signals (AATAAA) are underlined.

gram of the National Center of Biotechnology Information (NCBI) via the internet.

In an analysis of channel catfish expressed sequence tag profiles, fifteen clones of parvalbumin were found. The parvalbumin cDNAs are small and, therefore, their complete cDNA sequences were obtained by automated sequencing on the LI-COR sequencer using the forward and reverse universal primers. Its nucleotide sequence and the deduced amino acid sequences are shown in Fig. 1. The sequences were submitted to Genebank with Accession number AF227795. The nucleotide sequence analysis of the 15 clones revealed single unique parvalbumin cDNA sequences. The cDNA consisted of a 330 base pairs (bp) of coding region (include the termination codon) and a 49 bp 5' untranslated region (UTR), and a 258 bp 3'-UTR. A typical polyadenylation signal AATAAA was found 17 bp upstream of the poly (A)+ sequences. The coding region encoded a peptide of 109 amino acids. The nucleotide sequences of channel catfish parvalbumin were

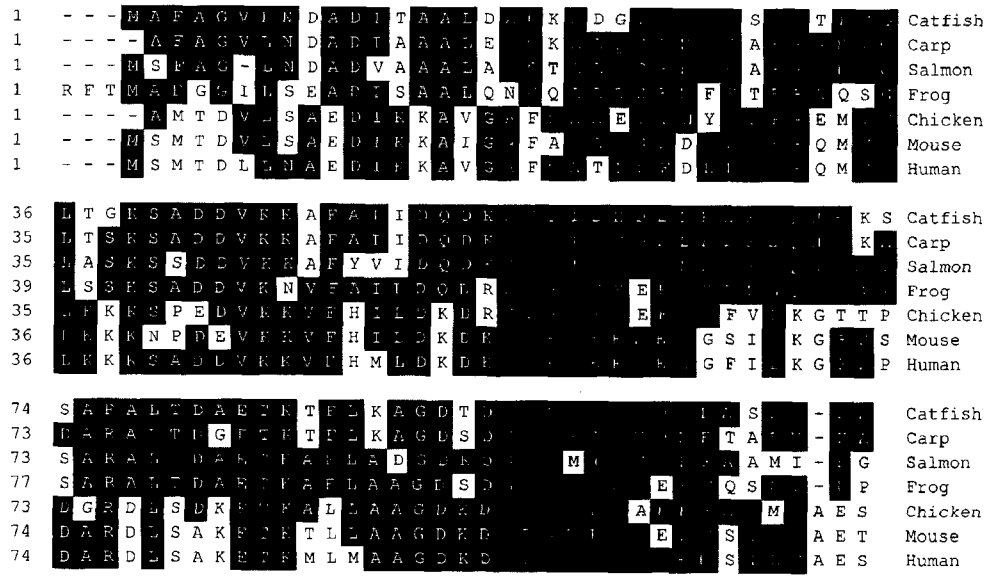

Fig. 2. Alignment of the parvalbumin amino acid sequences from selected species. Amino acid positions are indicated on the left margin. Species name are indicated on the right margin. The sequences used for alignment were obtained from the published studies deposited in the non-redundant Genbank+EMBL+DDBJ+PDB database or Swissprot protein database. The following accession numbers were used in this alignment. Common carp: P02618; chicken: AAB20408; Atlantic salmon: CAA66404; *Xenopus laevis*: AAA49925; mouse: X54613; human: X63578.

compared to parvalbumin from different fish species and from other groups of vertebrates (data not shown). Significant homologies, between 90.0% (common carp) and 77.7% (atlantic salmon), were observed with parvalbumin of other fishes. The alignment of the deduced amino acid sequences of the channel catfish parvalbumin with those from common carp, atlantic salmon, frog, chicken, mouse, and human are shown in Fig. 2. Clearly, the protein primary structures have been well conserved through evolution to have the typical structure of EF-hand, helix-loop-helix, Ca^{2+}-binding proteins (Pauls et al., 1996). This sequence and structure homologies support that catfish parvalbumin like other species represent cross-reactive fish allergens (Bugajska et al., 1998).

In channel catfish muscles, there are 3 dominantly expressed genes, creatine kinase, alpha actin, and parvalbumin which mainly function in muscle contraction and relaxation (Kim et al., 2000; Liu et al., 2001). Parvalbumin is expressed mostly in muscles, but expression in other tissues has been reported such as in testis, nervous tissue, and endocrine glands (Endo et al., 1985; Macmanus et al., 1985; Celio 1986, Berchtold et al., 1987; Kagi et al., 1987). The highly and tissues-specific expressed genes provide valuable information for strength of promoters involved in transcription of these genes although gene copy numbers are not known. Assuming a single copy gene is responsible for the transcription of creatine kinase and alpha actin, its strong promoter may be useful for purposes of driving strong, muscle-specific gene expression in transgenic studies. Similarly, promoters of parvalbumin may also be valuable resources for genetic engineering. And also the availability of channel catfish parvalbumin clones will be the basis for the production of recombinant channel catfish parvalbumin and hypoallergenic derivatives for diagnosis and therapy of fish allergy.

References

Aas, K., 1987. Fish allergy and the cod fish allergen model. (*in*) Brostoff J, Challacombe S. J., eds. Food Allergy and Intolerances. Bailliére Tindall, London. pp. 356–366.

Aas, W. and S. Elsayed, 1969. Characterization of a major allergen (cod). Effect of enzymic hydrolysis on the allergenic activity. J. Allergy, **44**: 333–343.

Berchtold, M. W., M. R. Celio and C. W. Heizmann, 1987. Parvalbumin in non-muscle tissues of the rate. J. Biol. Chem, **259**: 5189–5196.

Bernhisel-Broadbent, J., S. M. Scanlon and H. A. Sampson, 1992. Fish hypersensitivity. J. Allergy Clin. Immunol., **89**: 730–737.

Bugajska-Schretter, A., L. Elfman and T. H. Fuchs, 1998. Parvalbumin, a cross-reactive fish allergen, contains IgE binding epitopes sensitive to periodate treatment and Ca^{2+} depletion. J. Allergy Clin. Immunol., **101**: 67–74.

Celio, M. R., 1986. Parvalbumin in most gamma aminobutyric acid-containing neurons of the rat cerebral cortex. Sci., **231**: 995–997.

Chomczynski, P. and N. Sacchi, 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Analyt. Biochem., **162**: 156–159.

De Besche A., 1937. On asthma bronchiale in man provoked by cat, dog and different other animals. Acta. Med. Scand., **42**: 237–255.

Elsayed, S. and K. Aas, 1971. Characterization of a major allergen (cod). Observations on effect of denaturation on the allergenic activity. J. Allergy, **47**: 283–291.

Elsayed, S., 1993. Allergens. (*in*) Van Regenmortel M. H. V., ed. Structure of Antigens, Vol. II. CRC Press Inc., London. pp. 293-317.

Endo, T., K. Takazawa and T. Onaya, 1985. Parvalbumin exists in the rat endocrine glands. Endocrinol., **117**: 527–531.

Goodman, M. and J. F. Pechére, 1977. The evolution of muscular parvalbumins investigated by the maximum parsimony method. J. Mol. Evol., **9**: 131–58.

Kagi, U., M. W. Berchtold and C. W. Heizmann, 1987. Ca^{2+} binding parvalbumin in rat testis, characterization, localization, and expression during development. J. Biol. Chem., **262**: 7314–7320.

Kim, S., A. Karsi, R. A. Dunham and Z. Liu, 2000. The skeletal muscle alpha-actin gene of channel catfish (*Ictalurus punctatus*) and its association with piscine specific SINE elements. Gene, **252**(1-2): 173–181.

Kretsinger, R. H., D. Tolbert, S. Nakayama and W. Pearson, 1991. Novel Calcium-Binding Proteins. (*in*) Heizmann C. W., ed. Fundamentals and Clinical Implications. Springer, New York. pp. 17–37.

Lehky, P., H. E. Blum, E. A. Stein and E. H. Fisher, 1974. Isolation and characterization of parvalbumins from skeletal muscle from higher vertebrates. J. Biol. Chem., **249**: 4332–4334.

Liu, Z., S. Kim, H. Kucuktas and A. Karsi, 2001. Multiple isoforms and an unusual cathodic isoform of creatine kinase from channel catfish (*Ictalurus punctatus*). Gene, **275**(2): 207–215.

Macmanus, J. P., D. C. Watson and M. Yaguchi, 1985. Rat skin calcium binding protein is parvalbumin. Biochem. J., **229**: 39–45.

Pascual, C., M. E. Martin and J. F. Crespo, 1992. Fish allergy evaluation of the importance of cross-reactivity. J. Pediatr., **121**: 29–34.

Pauls, T. L., J. A. Cox and M. W. Berchtold, 1996. The Ca^{2+} binding proteins parvalbumin and oncomodulin and their genes: new structural and functional findings. Biochim. Biophys. Acta, **1306**: 39–54.

Perchere, J. F., 1997. The significance of parvalbumins among muscular calcium proteins. (*in*) Calcium-Binding Proteins and Calcium, (ed) R. H. Wassermann, R. Corradino, E. Carafoli, Elsevier, Amsterdam. pp. 47–58.

Tanokura, M., H. Aramaki, K. Goto, U. Hashimoto, Y. Toyomori and K. Yamada, 1986. Preparation and characterization of two major isotypes of parvalbumins from skeletal muscle of bullfrog (*Rana catesbeiana*). J. Biochem., Tokyo **99**(4): 1211–1218.

Wahr, P. A., D. E. Michele and J. M. Metzger, 1999. Parvalbumin gene transfer corrects diastolic dysfunction in diseased cardiac myocytes. Proc. Nat. Acad. Sci., **96**: 11982–11985.

Zinn, C., A. Lopata, M. Visser and P. C. Potter, 1997. The spectrum of allergy to South African bony fish (Teleosti). S. Afr. Med. J., **87**: 146–52.

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