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Ig heavy chain genes and their locus in grass carp *Ctenopharyngodon idella*

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ABSTRACT

The cDNA and genomic sequences of IgD and IgZ were characterized in grass carp *Ctenopharyngodon idella* in the present study, and with the identification of a BAC clone covering ζ, μ, and δ genes, the IgH locus containing these Ig genes and other V, D, J genes was also illustrated in this fish. Secretory and membrane-bound IgZ were identified, with two transmembrane exons spliced within the CH4 exon, as reported in IgM of mammals and IgZ in other teleost fish. The first and second constant domains of IgZ shows more than 90% nucleotide identity with respective domains of grass carp IgM. The IgD has a structure of δ1–(δ2–δ3–δ4)$_2$–δ5–δ6–δ7–TM–UTR, with the repeat of δ2–δ3–δ4; but intron was not found between the two repeat, i.e. between the first δ2–δ3–δ4 (δ2.1–δ3.1–δ4.1) and the second δ2–δ3–δ4 (δ2.2–δ3.2–δ4.2), and the intron between δ3.1 and δ4.1 was much shorter than the intron between δ3.2 and δ4.2. The genomic organization of the IgH locus has a pattern of Vn–Dn–Jn–Cζ–Dn–Jn–Cμ–Cδ, as reported in other teleost fish. Thirteen V_H, fourteen D, and twelve J_H genes were observed in this locus, with the similarity of three D segments and four J_H segments being the same in the upstream of Cζ and Cμ. The transcriptional enhancer located at the μ–δ intergenic region was also analyzed and it seems possible that this enhancer is functional as verified in zebrafish and channel catfish.

1. Introduction

Immunoglobulins (Igs), which bind antigens with high specificity, are important molecules in adaptive immune system of jawed vertebrates. The basic structure of Ig is composed of heterodimers of two H and two L chains, and Ig isotypes are defined based on the constant domains of their H chains [1]. In mammals, five isotypes, i.e. IgM, IgD, IgG, IgA and IgE have been reported. Evolutionarily, IgM and IgD are conserved in vertebrates, as they have been reported from piscine to mammal, except in avian [1,2]. Apart from these two Igs, other Ig isotypes reported in different classes of vertebrates may differ at least to some extent.

With the accumulation of genome databases, IgH genes and their loci on chromosomes have been illustrated in either evolutionarily or economically important species or model animals representing different classes of vertebrates. In duck billed platypus (*Ornithorhynchus anatinus*), a much more complicated IgH locus has recently been revealed [3,4], with the finding of two IgA isotypes [5] and a new Ig isotype, IgO [4], which is considered to be a remaining copy of IgY in monotremes [5]. Avian species are reported to have IgM and IgA, and also IgY in duck [6,7], but as IgG in chicken which, from a phylogenetic perspective, is equidistant from mammalian IgG and IgE and which in birds has sometimes been referred to as IgY [8]. Recently, IgM, IgD and IgY genes have been identified in reptile, the green anole lizard (*Anolis carolinensis*) with available genome data [9], and also in an economically important species [10]. An IgA-like immunoglobulin has also been identified in leopard gecko (*Eublepharis macularius*) [11]. Five Ig isotypes, IgM, IgD, IgX, IgY, IgF have been reported from anuran amphibians [12–15], and IgM, IgY and recently IgP identified in urodele amphibians [16].

Fish are the most primitive and diverse group of vertebrates. The Ig identified earlier in cartilaginous and teleost fish is IgM (e.g. [17,18]). Over the last two decades, IgD has been reported continuously in several species of teleost fish [19–21], and in this century several Ig isotypes have been identified in different species of fish, such as IgZ in zebrafish (*Danio rerio*) [22], IgT in rainbow trout (*Oncorhynchus mykiss*) [23] and three-spined sticklebacks (*Gasterosteus aculeatus*) [24], the novel IgH in fugu (*Fugu rubripes*) [25], chimeric IgM–IgZ in common carp (*Cyprinus carpio*) [26]. Ig isotypes in cartilaginous fish have not received much attention as in

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teleost fish; however, several Ig isotypes, such as IgW, IgNAR and IgNARC have been reported in cartilaginous fish. Surprisingly, IgW was recently found also in a teleost fish, the African lungfish (*Protopterus aethiopicus*) [27]. It seems a bit surprising that the number of Ig isotypes reported in fish varied. It has been suggested that some Ig isotypes may be just another name of a formerly reported Ig isotype; for example, IgZ and IgT may be of the same isotype in fish, and the newly identified IgO in platypus [4] has been considered as IgY, as it shares characteristics with IgY [5]. Furthermore, genomic organization of IgH locus has also been identified in several species of teleost fish, such as in zebrafish [22], rainbow trout [23], fugu [25], channel catfish (*Ictalurus punctatus*) [28,29] and three-spined sticklebacks [24]. The genome organization of IgH locus in channel catfish is a translocon arrangement, with three δ genes linked to an μ gene or pseudogene, and the loci in zebrafish and fugu follow the pattern of genomic organization: Vn–Dn–Jn–Cζ/τ/C_H–Dn–Jn–Cμ–Cδ [22,25,28,29]. The organization of the IgH locus in rainbow trout is, in general, similar to that of zebrafish and fugu, except that two V_H genes were found downstream of the Cζ [23]. The IgH locus from three-spined stickleback is very complex, with three regions containing IGHM, IGHD and IGHT and a fourth region having only one IGHT [24]. The illustration of Ig genes and their loci in chromosome may enable the comparison of different Ig isotypes.

Grass carp, *Ctenopharyngodon idella*, is an economically important species in aquaculture industry in China, with the highest production of about 4 million tons each year among any individual fish species cultured [30]. Despite a few reports on some immune-related gene and the IgM (e.g. [31]), any other Ig isotypes have not been reported in this fish. The present study was thus aimed to clone cDNA sequences of other Ig isotypes in grass carp. With the establishment of grass carp BAC library [32], the IgH locus was also illustrated in the present study.

2. Materials and methods

2.1. Fish, RNA isolation and cDNA synthesis

Four grass carp, 90–120 g in body weight, obtained from Guanqiao Experimental Station of the Institute of Hydrobiology, Chinese Academy of Sciences, were reared in a quarantine tank with aerated freshwater at 20 ± 3 °C for 2 weeks before the dissection of organs and/or tissues. The total RNA was extracted using Trizol reagent (Invitrogen, USA) by following the manufacturer's instruction. For rapid amplification of cDNA ends (RACE) PCR, the Smart cDNA was amplified from the RNA extracted from spleen by using SMART PCR cDNA Synthesis Kit (Clontech, USA). For reverse transcription (RT)-PCR, total RNA was isolated from gills, head kidney, intestine, liver, muscle, trunk kidney and spleen from four grass carp, respectively, and reverse-transcribed with PowerScript™ Reverse Transcriptase (Clontech).

2.2. Cloning of IgZ and IgD cDNA sequences

Degenerated primers ZDF/ZDR (Table 1) were designed to obtain the internal region of IgZ. For secretory IgZ (sIgZ), primers UF/UR and ZF1/UPM were used to obtain the 5′ and 3′ untranslated regions (UTR), respectively. For membrane-bound IgZ (mIgZ), specific primer CF1 and adaptor primer UPM were used in 3′-RACE PCR. The large fragment of mIgZ was amplified using gene specific primers CF2 and CR1. Similarly, the internal region of IgD was obtained by PCR using primers DF1/DR1. The 3′ UTR of IgD was amplified by RACE PCR using primers DF2/UPM. The PCR cycling conditions were one cycle of 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 90 s, followed by one cycle of 72 °C for 7 min. All PCR products were cloned into pMD18-T vector (TaKaRa) for sequencing. The cDNA sequences of IgZ and IgD have been deposited in the GenBank database with accession numbers GQ201421, EU243240 and GQ429174.

Table 1
Primers used in the study.

Primer	Sequence (5′–3′)	Application
ZDF	AGACTGA(TC)(GT)CTGAA(GC)CCACC	cloning
ZDR	C(TA)C(CT)TTCCACATGAT(GA)TAGAC	cloning
ZF1	CGATGTCTATATCATGTGGAA	cloning
UF	GGATACTGTAGAGGAGAATCAATCA	cloning
UR	ACAAAAGCATCACACTCACACAGGT	cloning and expression analysis
CF1	TGTCAAAACAAGAGTATGAGGA	cloning
CF2	CTCAACCATCTCCGCCGAAGTC	cloning
CR1	ACTTTATTGATGAGTGTGAACATTGAG	cloning and expression analysis
DF1	GTGCCCACTATGGCTCTGCT	cloning
DF2	AGCGGTCTACCTGTTGGGTCCTTC	cloning
DR1	TGTCTGAGGGTCGGAGGATG	cloning
UPM	CTAATACGACTCACTATAGGC	cloning
ECF1	AGCAGGAAATCCATTGTAATAAAGG	expression analysis
ECR1	CAACAGGGAGCATAAGCATT	expression analysis
EDF	GGTCTACCTGTTGGGTCCTTCC	expression analysis
EDR	GCTTCACTTTGAGCAATGTCACTG	expression analysis
EMF	CGTCTACCTCCAACTCCACCAC	expression analysis
EMR	TACCGCTCTTCCACTCAGAATAAC	expression analysis
ActinF	CCTTCTTGGGTATGGAGTCTT	expression analysis
ActinR	AGAGTATTTACGCTCAGGTGG	expression analysis
BMF	GTCATCGCTGAGGCATCGG	screening BAC library
BMR	ACCGCTCTTCCACTCAGAATAACT	screening BAC library
BCF1	GAGCACAAGACGGGAAAAGAAG	screening BAC library
BCR1	TTGGTGGCTTCAGTGTCAGTGTT	screening BAC library
BDF	ATCCGTGCCACAGCAAGAGT	screening BAC library
BDR	ATGGGAAGAAACCAGTGATGAA	screening BAC library

2.3. Screening of BAC library and annotation of IgH gene locus

To isolate BAC clones containing grass carp μ, and/or δ, ζ chains, a 6.3× grass carp bacterial artificial chromosome (BAC) library [32] was screened by PCR-based method as used by He and Komatsuda [33] with three sets of primers, BMF/BMR for IgM, BCF1/BCR1 for IgZ, BDF/BDR for IgD (Table 1). Twelve BAC clones were found positive, one of which containing the three-chain genes was sequenced. Sequencing of the BAC clone was performed by a shotgun approach as the followings: BAC DNA was sheared with a Hydroshear device (Gene Machines) to generate 1.5–3 kb DNA fragments. These sheared fragments were isolated by gel electrophoresis, and then ligated into a pUC118 plasmid vector, before being transformed into *Escherichia coli* strain DH10B. The shotgun subclones with inserts of 1.5–3 kb were sequenced from both ends on MegaBACE 1000 sequencers (GE Healthcare). The obtained sequences were assembled using Phred (0.020425.c) and Phrap (0.990329) programs, and then examined using Consed (16.0) program. Sequence gaps were closed using PCR with specific primers (Table 1). The BAC clone was sequenced and assembled at 8.78× coverage at Beijing Genomics Institute, Chinese Academy of Sciences.

The ExPASy translate tool (http://ca.expasy.org/tools/dna.html) was used to deduce the amino acid sequence. The software GENSCAN (http://genes.mit.edu/GENSCAN.html), FGENESH (http://linux1.softberry.com/all.htm) and BLASTX (http://blast.ncbi.nlm.nih.gov/Blast.cgi) were employed for the genome annotation. The D and J_H gene segments were identified using FUZZNUC software to search for heptamer–nonamer like motifs. The V gene segments were detected by a BLAST search of the BAC sequence in combination with a procedure developed for the automatic generation of the database VBASE2 [34], and with manual sequence analyses. The positions and exon boundaries of IgH C domains were determined

by aligning the BAC sequences with known grass carp IgH cDNA sequences.

2.4. Expression analysis of IgH isotypes in different organs by RT-PCR

To examine the distribution of IgM, IgD and IgZ in grass carp, RT-PCR was carried out by using specific primers, ECF1/UR for sIgZ, ECF1/CR1 for mIgZ, EMF/EMR for secretory IgM (sIgM), EDF/EDR for membrane-bound IgD (mIgD) and ActinF/ActinR for β-actin as control. The reactions were completed in a thermocycler with the following profile: 94 °C for 4 min, and then 30 s at 94 °C, 30 s at 58 °C and 45 s at 72 °C for 25 cycles (for β-actin) or 30 cycles (for each IgH genes). Upon the completion of PCR, the reaction was incubated at 72 °C for an additional 5 min. Resultant products were examined on 1% agarose gel.

3. Results

3.1. Characteristics of IgZ and IgD cDNA sequences and IgH locus

The secretory IgZ (sIgZ) cDNA sequence of grass carp (GenBank accession no. GQ201421) contains a 3 bp 5′-untranslated region (UTR), a 1716 bp open reading frame (ORF) and a 459 bp 3′-UTR. A putative polyadenylation signal (AATAAA) is located at 20 bp upstream of the polyA tail. The ORF encodes a 571 amino acid protein, which is composed of leader peptide, variable domain, four constant domains and C-terminus. The cDNA sequence of membrane-bound form IgZ, mIgZ (GenBank accession no. EU243240) has two transmembrane exons, TM1 and TM2, which are spliced within the CH4 exon. The CH1, CH2, CH3 and CH4 regions of the grass carp IgZ exhibited 91.9%, 92.0%, 48.1% and 49.7% nucleotide identity with the corresponding regions of grass carp IgM (GenBank accession no. DQ417927).

Amino acid analysis of the grass carp IgZ revealed the presence of conserved cysteine (Cys) residue in CH1 for connecting the H chain to the L chain. Moreover, all the CH domains contained two conserved Cys residues, which were important for the formation of intradomain disulfide bridges [35]. But in CH3 of grass carp IgZ, the first conserved Cys residue was present in a non-canonical position, as in zebrafish IgZ [22], with the second conserved cysteine was at canonical postion. In the grass carp IgZ, there was one additional cysteine in CH3 and four additional cysteine residues in the secretory tail piece. Besides, two conserved tryptophan (W) residues were observed in CH1 and CH2, while only one in the CH3 and CH4. Six putative N-linked glycosylation sites, i.e. one in CH2, two in CH3 and three in CH4, were found in grass carp IgZ.

The cloned grass carp IgD cDNA sequence had an incomplete ORF at the 5′ end (GenBank accession no. GQ429174), with a structure of δ1–(δ2–δ3–δ4)$_2$–δ5–δ6–δ7–TM–UTR. The transmembrane region of grass carp IgD is encoded by only an exon and the last amino acid is the negatively charged glutamic acid (E). Two conserved Cys for the formation of intradomain disulfide bridges were observed in each constant regions of grass carp IgD.

The BAC clone covering immunoglobulin ζ, μ, and δ genes and other V, D, J genes is 136,963 bp long. This BAC clone sequence was annotated except about 7 kb sequence after a gap (Fig. 1; GenBank accession no. GQ480796). The genomic organization of the BAC clone was summarized as: V_{13}–D_5–J_6–Cζ–D_9–J_6–Cμ–Cδ (Fig. 1). The position of grass carp δ gene is immediately downstream of μ gene in the IgH locus. In the IgD genomic sequence, there are 10 δ exons and one TM exon; but intron was not found between the δ2–δ3–δ4 repeats, i.e. between δ4.1 and δ2.2, and the intron between δ3.1 and δ4.1 was much shorter than the intron between δ3.2 and δ4.2.

3.2. V_H, D and J_H genes

There were 13 V_H gene segments, eight of which had an intact structure, each consisted of an octamer-like sequence and a TATA box in the promoter, a leader exon, an exon encoding V gene and a downstream recombination signal sequences (RSS). The RSS is composed of conserved heptamer (CACAGTG) and nonamer (ACAAAAACC) motifs, separated by a spacer of either 12 or 23 base pairs in length. In each of these eight V_H genes, the octomer was either identical to the consensus or differed by only one nucleotide at the most 3′ site (being C rather than T). Besides, the heptamer was almost uniform to the consensus sequence except for one substitution at the 3′ end of the site, and the nonamer was somewhat less conserved, differing by one or two nucleotides. Five other V_H genes appeared to be pseudogenes because of the lack of intact structure or the existence of in-frame stop codons or frame shift mutations. All V_H, D and J_H gene segments were named and numbered based on the IMGT nomenclature convention [36,37].

Analysis of the sequence of the BAC clone revealed five D segments between V_H segments and the J_H segments upstream of Cζ, and nine D segments between Cζ and J_H segments upstream of Cμ, with the similarity of three D segments being the same in the upstream of Cζ and Cμ (Fig. 1). To be specific, the nucleotide sequences of IGHD3S1 are identical to that of IGHD3S3, sequences of IGHD4S1 are identical to that of IGHD4S3, and sequences of IGHD5S1 are identical to IGHD5S2. All D segments had 12-RSS on both sides and most of them were GC-rich motifs.

As illustrated in Fig. 1, six J_H segments were located upstream of Cζ and Cμ, respectively, with four J_H segments being the same in the upstream of Cζ and Cμ. That is, the nucleotide sequences of IGHJ1S1 and IGHJ1S6, IGHJ1S3 and IGHJ1S8, IGHJ1S4 and IGHJ1S9, and IGHJ2S1 and IGHJ2S2 are entirely identical. All J_H segments had heptamer and nonamer sequences separated by a 22–23 nucleotide spacer. When comparing IGHJ1S2 with IGHJ1S7, and IGHJ1S5 with IGHJ1S10, only one or two nucleotide differences were observed in the reading frame, leading to the changes of one amino acid.

3.3. Conserved sequence similar to enhancer in catfish and zebrafish

In the grass carp IgH locus, about 200 bp region between the μTM2 and the Cδ1 exons was homologous to the core of Eμ3′ enhancers in zebrafish and catfish. The sequence alignment of Eμ3′ enhancers from zebrafish and catfish, together with those from grass carp was shown in Fig. 2. The sequence in grass carp exhibited 77.2% and 59.4% nucleotide identity with those in zebrafish and catfish, respectively. Eμ3′ enhancer from catfish and zebrafish contained a unique motif characteristic of an E-box motif and an octamer motif which overlapped with the E-box motif, termed as Oct/E-box [38]. This region in grass carp consisted of two E-box motifs (CATTTG and CAGATG), an Oct/E-box (ATGCAgATG) and a variant octamer motif (ATGtAAAT).

3.4. Constitutive expression of IgH isotypes in organs/tissues

RT-PCR was carried out to assess the expression of IgM, IgD and IgZ in grass carp. The sIgZ, mIgZ and sIgM had similar expression pattern, as they were mainly detected in gill, head kidney, intestine, liver, trunk kidney and spleen. mIgD was expressed mainly in gill, head kidney, trunk kidney and spleen (Fig. 3).

4. Discussion

The IgH locus, which covers immunoglobulin ζ, μ, and δ genes was illustrated in grass carp *C. idella* in the present study. cDNA

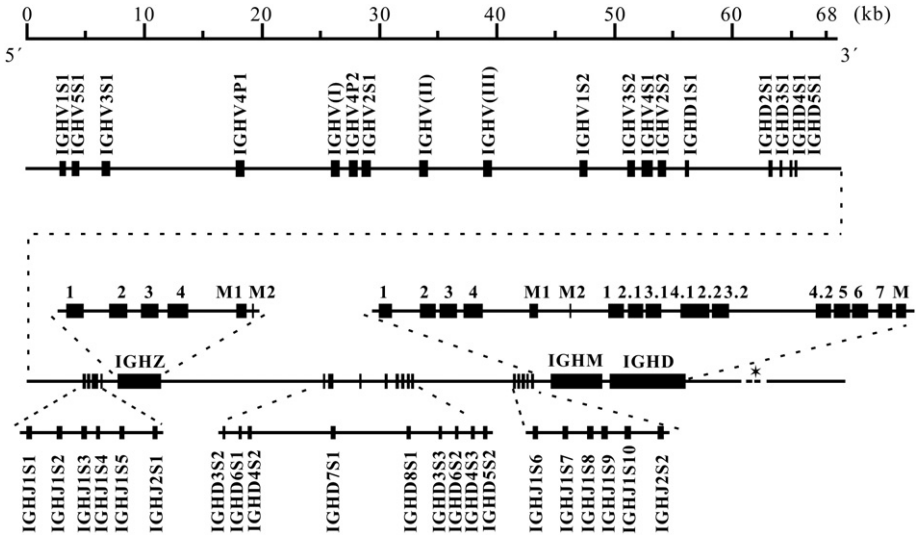

Fig. 1. Organization of IgH locus in grass carp. V_H, D and J_H gene segments were named and numbered based on the IMGT nomenclature convention [36,37]. Open symbols indicate pseudogenes. Black boxes indicate exons. IGHZ, IgZ encoding gene; IGHM, IgM encoding gene; IGHD, IgD encoding gene; M, membrane exon. Constant-encoding exons for each Ig genes are indicated by Arabic numbers. A small sequence gap is indicated with asterisk (*).

sequences encoding heavy chains of membrane IgD, as well as secreted and membrane IgZ of grass carp were also cloned in this study. In consideration of a previous report on IgM cDNA sequence in grass carp [31], the immunoglobulin heavy chain genes and their locus in chromosome have been characterized in grass carp, an important fish species in aquaculture. The grass carp IgH locus organization is similar to the loci reported from zebrafish, rainbow trout and fugu, with the following pattern: Vn–Dn–J–Cζ/Cτ/C_H–Dn–Jn–Cμ–Cδ [22,23,25]. In zebrafish, rainbow trout and fugu, the D and J_H segments upstream of Cζ/Cτ/C_H are completely different from D and J_H segments upstream of Cμ [22,23,25]. However, in grass carp IgH locus, three D segments and four J_H segments upstream of Cζ were found as same as in the upstream of Cμ, indicating that grass carp IgH locus is not completely analogous to the loci in zebrafish, rainbow trout and fugu. In channel catfish, only two immunoglobulin isotypes, μ and δ genes, have been reported so far, and no Cζ or Cτ-like sequences have been reported. The catfish IgH locus is a translocon-type with three δ genes linked to a μ gene or pseudogene [28,29], being thus different from that in zebrafish, rainbow trout, fugu and grass carp. It is suggested that the organization of the IgH locus in different species of teleost fish may vary at least to some extent, as illustrated for the above five species of teleost fish.

However, the complete composition of V_H genes in the IgH loci has been reported only in zebrafish, three-spined stickleback and channel catfish, with 47, 49 and 55 V_H genes identified, respectively [22,24,29]. It seems possible that the number of V_H genes may vary in different species of teleost fish. In grass carp IgH locus, a segment of 53 kb at the 5′ end of the sequenced BAC clone contains 13 V_H genes. When comparing V_H genes among these fish species, it appears likely that the full composition of the V_H gene in the IgH locus of grass carp may need some further research.

The core region of the transcriptional enhancer, Eμ3′ located at the μ–δ intergenic region has been characterized functionally in channel catfish and zebrafish [39,40]. Mutagenesis studies demonstrated that the zebrafish Eμ3′ enhancer in the IgH locus was

```
gr      TTGTTCATTGGGTGACTGAAGCACAACATCTTCTAACTGTGAACATTTGTAA--GCATAA
ze      TTGTTTATTGTGTTACTGAA-CACAGCATTGTCTAGCTCTGCATATTTGTAAT-GAACAA
ca      TTATTTATTGATCTTTTTAGCTAATATATATT-TATTTCTGCATTATTACAAAGGCTTTA
        ** ** ****       * *    *     **   * **  *  ** *    **  **   *     *

gr      ATTTTTCATGCAGCAAAGTATATTAAAACATAAATTGCATAAGAAGGAGAAGCATTTTAT
ze      ATAGCTAATGATGCAAATTATATTAAATCATACATTGCACAACA-GGATAGTTATTATG-
ca      TCATGTCACTGAGCAAAAAGCAA-AAAGCACTCTTTACATAACA-GGCAAAACACTGCAT
              * *     *****     *  ***  **     ** ** ** * **  *    * *

gr      GTAAATGG---------------AAAGGTG----TCTTTCCTGTGCAGATGTGCTCAACC
ze      --AAATTA---------------TAAGATA----TTTTTCCTGTGCAGATGTGCTCAGCC
ca      GTAAATAGTCTAATAATGGATGTAAATGTAGCACTTCTTCCTGTGCAGGTGTGTTTCACT
           ****                      **  *      *   *********** **** *   *

gr      CTGTCTCAGAGATGCAGATGGTTTTCAGTTCTGCTTT
ze      TTGTTTCAGAGATGCAGGTGCTTTTCGGTTCTGCTTT
ca      CCATCTCAGAGATGCAGATGATTTTCACTTCTTCTTT
            * ************ ** *****  **** ****
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Fig. 2. Nucleotide sequence alignment of assumed enhancer region from μ–δ intergenic region in grass carp and Eμ3′ regions from zebrafish and catfish. Asterisks indicate identity, and dashes gaps. The octamer and E-box motifs are underlined and boxed, respectively. The hybrid octamer/E-box motifs are shaded. GenBank accession numbers of sequences used are as the followings: grass carp (GQ201421); zebrafish (AY682723); catfish (X79482). gr, grass carp; ze, zebrafish; ca, catfish.

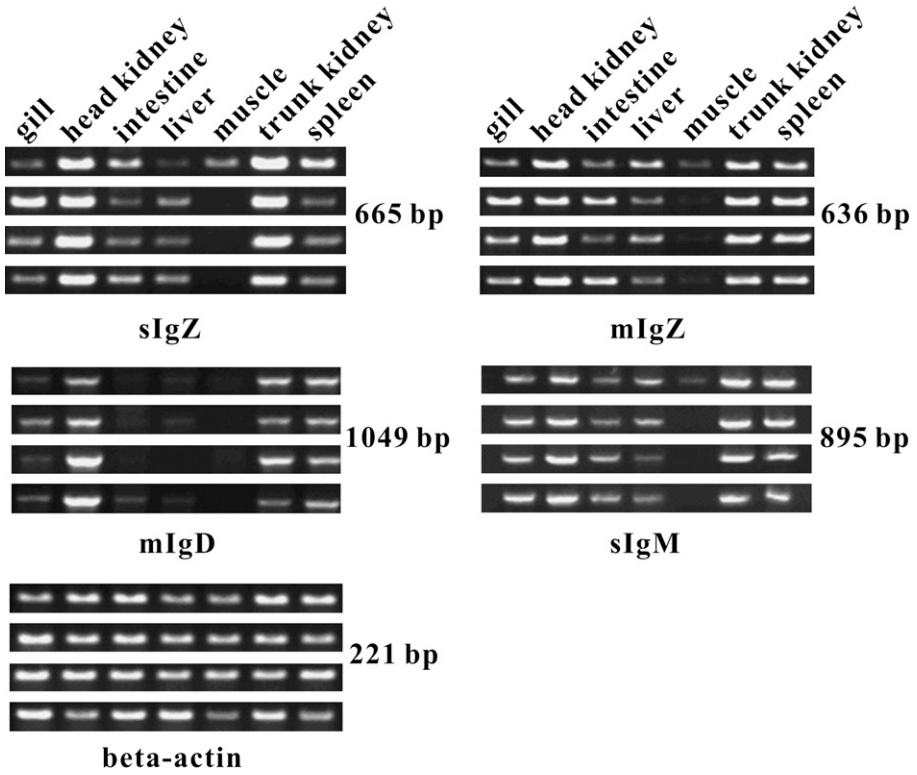

Fig. 3. Expression of IgZ, IgM and IgD in different organs/tissues of grass carp as detected by RT-PCR. β-actin product served as control.

highly dependent on a pair of E-box motifs (CAGATG and CAGGTG) which were found in the enhancer core region [40]. In catfish, the Eμ3′ enhancer relied on two non-canonical octamer motifs (ATGtAAAT) and a μE5 E-box motif (CAGGTG) [38,40]. However, in other species of fish, such as in Atlantic salmon (*Salmo salar*), Atlantic cod (*Gadus morhua*), Japanese flounder (*Paralichthys olivaceus*) and fugu, despite the identification of homologous sequences between the μTM2 and the Cδ1 exons, none of the E-box sites which has been characterized functionally in zebrafish and channel catfish was found. Specifically, in grass carp, there is an E-box site (CAGATG) and a non-canoncial octamer motif (ATGtAAAT) which have been shown to be functionally crucial in the zebrafish and catfish enhancers, respectively. Furthermore, the Oct/E-box (ATGCAgATG), which was identified with binding of the Oct transcription factors and with negative regulatory function by EMSA in channel catfish [38], was also found in the grass carp. The fourth motif in the Eμ3′ enhancer region of grass carp is a new E-box motif (CATTTG), which is not found in any other teleost fish whose enhancers have been revealed, and this motif is a bit far away from other three motifs in the grass carp. Taken together, it is thus at least tentative to speculate that the grass carp enhancer may act through the E-box motif (CAGATG) and the non-canoncial octamer motif (ATGtAAAT).

Grass carp IgZ possesses secretory and membrane forms. Generation of membrane form of the IgZ is the splicing of the TM exons into a cryptic splice site within the CH4 exon, as in IgM of mammals, birds, amphibians and elasmobranches. The same splicing pattern for the expression of membrane form is also reported from IgZ in zebrafish, IgT in rainbow trout and the novel IgH in fugu [22,23,25]. The consensus sequence of the cryptic donor splice site from IgM is AG↓GTAAG [41]. The cryptic donor splice site of IgZ from grass carp is AA↓GTAAA, which deviates from the consensus sequence of IgM. However, membrane version of IgZ which is generated by splicing to the cryptic splice site within the CH4 exon, does exist in grass carp, and identical cryptic donor splice site is also observed in zebrafish IgZ.

The expression of sIgZ, mIgZ in grass carp were observed in immune organs such as in kidney and spleen, and in other organs/tissues such as gills, intestine and liver. Such a similar pattern has been reported for IgM–IgZ in common carp, the novel IgH in fugu and IgT in rainbow trout [23,25,26]. However, Danilova et al. [22] showed that IgZ was almost entirely localized in primary lymphoid organs in zebrafish; but there was also low expression of IgZ in intestine of fish.

In conclusion, the cDNA and genomic sequences of μ, δ and ζ genes have been characterized in grass carp, and especially the IgH locus has been illustrated in grass carp in the present study.

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