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Molecular cloning and sequence analysis of stearoyl-CoA desaturase in milkfish, *Chanos chanos*

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Abstract

Stearoyl-CoA desaturase (EC 1.14.99.5) is a key enzyme in the biosynthesis of polyunsaturated fatty acids and the maintenance of the homeoviscous fluidity of biological membranes. The stearoyl-CoA desaturase cDNA in milkfish (*Chanos chanos*) was cloned by RT-PCR and RACE, and it was compared with the stearoyl-CoA desaturase in cold-tolerant teleosts, common carp and grass carp. Nucleotide sequence analysis revealed that the cDNA clone has a 972-bp open reading frame encoding 323 amino acid residues. Alignments of the deduced amino acid sequence showed that the milkfish stearoyl-CoA desaturase shares 79% and 75% identity with common carp and grass carp, and 63%–64% with other vertebrates such as sheep, hamsters, rats, mice, and humans. Like common carp and grass carp, the deduced amino acid sequence in milkfish well conserves three histidine cluster motifs (one HXXXXH and two HXXHH) that are essential for catalysis of stearoyl-CoA desaturase activity. However, RT-PCR analysis showed that stearoyl-CoA desaturase expression in milkfish is detected in the tissues of liver, muscle, kidney, brain, and gill, and more expression sites were found in milkfish than in common carp and grass carp. Phylogenic relationships among the deduced stearoyl-CoA desaturase amino acid sequence in milkfish and those in other vertebrates showed that the milkfish stearoyl-CoA desaturase amino acid sequence is phylogenetically closer to those of common carp and grass carp than to other higher vertebrates.

Keywords: Stearoyl-CoA desaturase; cDNA; Milkfish; Rapid amplification of cDNA ends (RACE); Expression; Polymerase chain reaction (PCR); Histidine motifs; Phylogenetic relationship

1. Introduction

Fatty acid desaturases play important roles in the physiological functioning of cell membranes and in fatty acid metabolism (Jeffcoat, 1979; Tiku et al., 1996). To date, three types of desaturases have been recorded: Acyl-CoA, Acyl-lipid, and Acyl-ACP desaturases (reviewed in Los and Murata, 1998). Acyl-CoA desaturases are the enzymes involved in the CoA-bound desaturation of fatty acids in animals and yeasts (Thiede et al., 1986; Luo et al., 1997). In plants and cyanobacteria, acyl-lipid desaturases introduce double bonds into the fatty acids, which are bound to glycerolipids (Sperling et al., 1990). In addition, higher plants contain acyl-ACP desaturase, which catalyzes desaturation reactions when fatty acids are bound to the acyl carrier protein (ACP) (Thompson et al., 1991).

Stearoyl-CoA desaturase (EC 1.14.99.5), one of

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the acyl-CoA desaturases representing the first regulatory step in the formation of long-chain unsaturated fatty acids (Enoch et al., 1976), is responsible for introduction of the first double bond between carbons 9 and 10 of palmitoyl (16:0)- and stearoyl (18:0)-CoA to form the mono-unsaturated palmitoleic (16:1) and oleic acids (18:1), respectively (Jeffcoat et al., 1977). These desaturation processes are also catalyzed by microsomal enzyme systems: cytochrome b_5, NADH-dependent cytochrome b_5 reductase and stearoyl-CoA desaturase (Holloway, 1971; Rogers and Strittmatter, 1973; Strittmatter et al., 1974). Cytochrome b_5 can transfer electrons from its internal heme to the iron centers of the stearoyl-CoA desaturase (Itoh et al., 1998).

In thermal acclimation, there are species-specific differences in the types of desaturase that contribute to lipid restructuring. For examples, Δ^9 desaturase plays an important role in the lipid composition for carp in the cold (Macartney et al., 1996; Tiku et al., 1996), and Δ^{12} plays a more significant role in thermal acclimation than Δ^6 and ω3 desaturases for *E. coli* (Panpoom et al., 1998). Living organisms, poikilotherms in particular, respond to a downward shift in temperature by desaturating the fatty acids of their membrane lipids, which exert modulative effects upon the actions of membrane-bound enzymes. This homeoviscous acclimation response reflects the ability to maintain the fluidity of biological membrane over a certain range of temperatures. The ability of cells to modulate the physical characteristics of the membrane lipids is mainly determined by the action of desaturase, which introduces double bonds into fatty acids (Lee and Cossins, 1990; Murata and Wada, 1995). Since more than 30 species of fatty acids may be combined in different pairings on the membrane phospholipids, the compositional differences between cold- and warm-acclimated animals are extremely complex. However, the most common pattern is that cold acclimation leads to an increased proportion of fatty acids containing unsaturated bonds at the expense of saturated homologues (Cossins and Bowler, 1987).

Although stearoyl-CoA desaturase is not the only desaturase involved in modulating membrane lipid restructuring, it has also been recognized as one of the important desaturases involved in the processes of acclimation to stressful environments, including lipid metabolism, obesity, cell-membrane fluidity and signal transduction (Legrand and Hermier, 1992; de Antueno et al., 1993; Györfy et al., 1997). Among these, the maintenance of cell membrane fluidity through changes in the fatty acid composition of the membrane is achieved by the action of stearoyl-CoA desaturase, which therefore plays a significant role in the thermal adaptation of fish in fluctuating environmental conditions (Nakashima et al., 1996).

Stearoyl-CoA desaturase has been isolated from a variety of vertebrates. These include Long Evans rats (Thiede et al., 1986); mice, *Mus musculus* (Ntambi et al., 1988; Kaestner et al., 1989); hamsters, *Mesocricetus auratus* (Ideta et al., 1995); common carp, *Cyprinus carpio* (Tiku et al., 1996); sheep, *Ovis aries* (Ward et al., 1998); humans, *Homo sapiens* (Zhang et al., 1999); and grass carp, *Ctenopharyngodon idella* (Chang et al., 2001).

Milkfish, widely distributed in tropical and subtropical waters, have traditionally been considered a commercially important cultured warm-water species in the Southeast Asian region (Bagarinao, 1994). Heavy mortality by winter kill of this species has often been reported. Since stearoyl-CoA desaturase has only been reported in cold-tolerant teleosts, common carp and grass carp to date, the objectives of this research were to confirm the presence of stearoyl-CoA desaturase in milkfish, and furthermore to pursue the cloning and sequencing of the cDNA encoding the milkfish stearoyl-CoA desaturase by RT-PCR and RACE methods and gene expression in this species.

2. Materials and methods

2.1. Animals

Milkfish (*Chanos chanos*) were collected from the Tainan Station of the Taiwan Fisheries Research Institute. Upon arrival in our laboratory, they were acclimated in freshwater at 25°C and a 12 L/12 D photoperiod regime, and fed with commercially formulated feed. The average weight was 140.03 ± 8.74 g.

2.2. RNA isolation

Total RNA was isolated following the method described by Chomczynski and Sacchi (1989).

Liver was homogenized in a guanidine solution (5 M guanidinium thiocyanate, 50 mM Tris, pH 7.5, 10 mM EDTA, 8% β-mercaptoethanol and 2% sarcosyl). The homogenate was centrifuged at $15\,000 \times g$ for 30 min. The supernatant was applied onto a 5.7 M CsCl cushion and ultracentrifuged at $35\,000 \times g$ for 16 h. The RNA pellet was dissolved in DEPC-treated water and extracted twice with phenol/chloroform (1:1, v/v), followed by chloroform extraction. Total RNA was precipitated in 75% ethanol containing sodium acetate, and was then washed with 75% ethanol. The RNA pellet was dissolved in a small amount of DEPC-treated water, and its concentration was adjusted to 1 μg/μl. Samples were stored at −80°C until use.

2.3. Molecular cloning

Full-length cDNA was obtained by the procedures of reverse transcription polymerase chain reaction (RT-PCR) and rapid amplification of cDNA ends (RACE). For RT-PCR, first-strand DNA was synthesized by MMLV reverse transcriptase (Life Sciences) using poly(A)$^+$ RNA with oligo(dT) as the primer. The reverse transcripts were used as a template for the following PCR with two degenerate primers. The forward primer was 5′-A(A/G)AA(T/C)GA(T/C)AT(T/C/A)TA(T/C)GA(A/G)TGG-3′, and the reverse primer was 5′-GT(A/G)TG(A/G)TG(A/G)TA(A/G)TT(A/G)TG(A/G)AA-3′, corresponding to amino acid sequences QNDIYEW and FHNYHHT, respectively. PCR was performed with the two primers under the following conditions: 25 cycles of 94°C for 1 min, 45°C for 1 min, and then 72°C for 2 min. The last step was extended for 10 min at 72°C. The PCR product was electrophoresed in 1% (w/v) agarose gel. Every 50-μl PCR reaction contained approximately 1 μg total RNA as the template, 200 μM dNTPs (dATP, dCTP, dGTP and dTTP), 200 nM of the desaturase primer set, 1× PCR buffer (containing 1.5 mM $MgCl_2$), and 2.5 units of Taq DNA polymerase.

For 5′-RACE, 3 μg of total RNA was reverse-transcribed with the first nested P1 primer, which is specific to the milkfish desaturase gene. The first-strand cDNA used for 5′-RACE was performed as above, then tailed at the 5′-end by terminal transferase TdT (Boehringer Mannheim) and dGTP. The primer sets consisted of P1 and the anchor primer, PT1, for the first-run PCR, and P2 and PT2 for the second-run PCR (Table 1). The PCR conditions described above were followed for RT-PCR, except that the annealing temperature was elevated to 50°C. The procedure for 5′-RACE was also applied to that for 3′-RACE. Total RNA was reverse-transcribed after annealing with anchor primer PT1. Two successive PCRs were performed with the primer sets Q1 and PT2 for the first run and Q2 and PT2 for the second run (Table 1). Amplification products were fractionated on 1% agarose gels, from which selected DNA bands were purified. They were ligated directly into the vector of PGEM-T Easy (Promega), and the plasmids were used to transform them into *Escherichia coli* XL1 blue cells. Plasmid DNA was purified for sequencing by the QIAperp miniperp kit (Qiagen). Positive clones were further confirmed according to the presence of the expected amino acid sequence.

Table 1
Nucleotide sequence of the primers in milkfish

Name	Nucleotide sequence (5′–3′)	cDNA position
N1	ARAAY GAYAT HTAYG ARTGG	332–351
C2	GTRTG RTGRT ARTTR TGRAA	790–781
MD1	ACACT TTCCT TGGAC AAATT GTGTG	88–100
MD2	AGCCA GGTCG CGTTA AGGAC	680–661
MFQ1	GTCCT TAACG CGACC TGGCT	661–680
MFQ2	TGTGG GGCAT GAGGC CCTAC	701–720
MFP1	ACCAG CAGCC AGCCG ACGTG	452–433
MFP2	CTTGT GGTGA ACACG ATGGT	377–358

The symbols used to donate multiple nucleotides are as follows: R = A + G; Y = C + T; M = A + C; K = G + T; W = A + T; H = A + C + T; B = C + G + T; V = A + C + G; D = A + G + T; and N = A + T + C + G.

2.4. Tissue expression analysis

Tissue expression of stearoyl-CoA desaturase mRNA was demonstrated by RT-PCR. Total RNA at 10 μg from various tissues (liver, muscle, kidney, brain, heart, and gill) was reverse-transcribed with oligo(dT). Two primers (MD1 and MD2) specific to milkfish desaturase were used (Table 1). The reaction conditions included an initial step at 94°C for 2 min, followed by 30 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min. A final extension step was performed at 72°C for 6 min. The PCR reaction without the cDNA template was performed simultaneously.

2.5. Nucleotide DNA sequencing

The nucleotide sequence of the cDNA was determined with a dye terminator-cycle sequencing kit (Perkin-Elmer, Applied Biosystems) on a model 373A DNA sequencer (Perkin Elmer, Applied Biosystems). Clones were the sequence M13 forward and reverse primers.

2.6. Sequence analysis

The BLAST sequence analysis program (http://www.ncbi.nlm.nih.gov/BLAST/) was used for initial sequence comparisons. Multiple alignment of stearoyl-CoA desaturase from human (*Homo sapiens,* GenBank accession no. AF097514), rat (Long Evans, GenBank accession no. J02585), mouse (*Mus musculus*, GenBank accession no. NM009127), sheep (*Ovis aries*, GenBank accession no. AJ001048), hamster (*Mesocricetus auratus*, GenBank accession no. L26956), carp (*Cyprinus carpio,* GenBank accession no. U31864) and grass carp (*Ctenopharyngodon idella,* GenBank accession no. AJ243835) was performed with CLUSTAL W, a Windows interface for the CLUSTAL W multiple sequence alignment program (Thompson et al., 1994). PHYLIP, the Phylogeny Inference Package (version 3.57C) (Felsenstein, 1995) was used to construct the phylogenetic trees. Distances were calculated with 'protdist' of the PAM-Dayhoff matrix, and the trees were constructed using the neighbor-joining method after 1000 bootstrap replicates using programs from the PHYLIP 3.57 package.

3. Results

3.1. Cloning of cDNA encoding stearoyl-CoA desaturase

Two degenerate oligonucleotide primers, N1 and C1, were designed following the sequence characteristics of stearoyl-CoA desaturase presented in Table 1. Using these primers, PCR was carried out with single-stranded cDNA synthesized with poly(A)$^+$ RNA from milkfish liver as a template. A major product of 468 bp was compared with stearoyl-CoA desaturases reported elsewhere. The cDNA sequence of this fragment has a high identity to common carp (81%), grass carp (82%) and rat (85%) stearoyl-CoA desaturases. According to this sequence information, four specific primers were designed, and the 3′- and 5′-ends of the cDNA were obtained using a RACE protocol (Table 1). The nucleotide and deduced amino acid sequences of DNA encoding the putative desaturase of milkfish stearoyl-CoA desaturase are presented in Fig. 1. Using similar approaches, 323 bp of the cDNA fragment was amplified by 5′-RACE, and 135 bp of the 3′-end cDNA was obtained by 3′-RACE. The full-length cDNA comprises 1073 bp, including 36 bp in the 5′-untranslated region, 972 bp in the open reading frame (ORF) that encodes 323 amino acid residues, and 65 bp in the 3′-translated region.

3.2. Comparison of amino acid sequences

The amino acid sequences from eight different vertebrates are compared in Fig. 2. The milkfish stearoyl-CoA desaturase amino acid sequence shows relatively high identity and similarities to deduced desaturase amino acid sequences of other vertebrates: 79 and 87% to common carp; 75 and 84% to grass carp; 64 and 79% to rat; 64 and 80% to human; 63 and 79% to sheep; 63 and 79% to hamster; and 64 and 80% to mouse, respectively. Like most desaturases in other vertebrates, three histidine clusters are well conserved in the sequence in milkfish. One HXXXXH (residues 84–89) and two HXXHH (residues 121–125 and 262–266), as underlined in Fig. 2, are essential for catalysis of stearoyl-CoA desaturase activity (Shanklin et al., 1994). On the contrary, the N-terminal sequences are highly variable between fish and other vertebrates, indicating that this region is probably not as relevant to enzymatic functions. Hydropathy profiles of the deduced amino acid sequence analysis are presented in Fig. 3. Two hydrophobic regions of milkfish stearoyl-CoA desaturase are conserved the same as in vertebrate rat, common carp and grass carp stearoyl-CoA desaturase.

3.3. Polymorphism

In order to clarify the molecular evolutionary relationship between milkfish stearoyl-CoA desaturase and those of other vertebrates, a phylogenetic tree produced by the CLUSTAL W program was attempted (Thompson et al., 1994) (Fig. 4). The milkfish stearoyl-CoA desaturase sequence is phylogenetically closer to those of teleosts, com-

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   ATTGGGGGGCGACGTTCGGCGTGCGGCCGGCCGGACATGCCTGCCGCGGAGATCTCGATT  60
 8                                          M   P   A   A   D   I   S   I
   ATGCGCGCGGCCACGGAGGAGAGGGTGCTTTTGGTGGACAGTCAGAAGGAGAAAGAAGGT 120
28 M   R   A   A   T   E   E   R   V   L   L   V   D   S   Q   K   E   K   E   G
   CCGACACTTCCTGGACAAATTGTGTGGAGAAACGTCGTTTTAATGGTGTTGCTGCATATC 180
48 P   T   L   P   G   Q   I   V   W   R   N   V   V   L   M   V   L   L   H   I
   GGAGCTCTGTAGGGGCTGGTTACTATTCCAGCGGCTTCTGCTCTAACCTTCGTTTGGACG 240
68 G   A   L   Y   G   L   V   T   I   P   A   A   S   A   L   T   L   V   W   T
   GGGGTGTGCTTCATGATAAGTGCCCTGGGAATTACGGCGGGGGCCCATCGGCTGTGGAGC 300
88 G   V   C   F   M   I   S   A   L   G   I   T   A   G   A   H   R   L   W   S
   CACAGGTCCTACAAAGCCTCGATGCCTTTACGAATCTTCTTAGCCGTTGCAAATTCCATG 360
108 H   R   S   Y   K   A   S   M   P   L   R   I   F   L   A   V   A   N   S   M
   GCCTTTCAGAATGATATTTATGAATGGTCCCGGGATCACCGCGTGCATCACAAGTACTCC 420
128 A   F   Q   N   D   I   Y   E   W   S   R   D   H   R   V   H   H   K   Y   S
   GAGACGGACGCCGACCCTCACAACTCAAACCGGGGCTTTTTCTTTTCTCACGTCGGCTGG 480
148 E   T   D   A   D   P   H   N   S   N   R   G   F   F   F   S   H   V   G   W
   CTGCTGGTTCGAAAACACCCGGAAGTCATCGAGAGAGGACGCAAACTAGACCTCACTGAC 540
168 L   L   V   R   K   H   P   E   V   I   E   R   G   R   K   L   E   L   T   D
   CTGAAGGCCGATAAGGTGGTCATGTTTCAGAGGAGATTCTACAAGCTGTCTGTGGTGTTG 600
188 L   K   A   D   K   V   V   M   F   Q   R   R   F   Y   K   L   S   V   V   L
   ATGTGCTTCGTTGTCCCCACGGTTGTGCCCTGCATCATGTGGGGCGACTCTCTCTGGATC 660
208 M   C   F   V   V   P   T   V   V   P   C   Y   M   W   G   E   S   L   W   I
   GCGTATTTCATCCCGACGCTCCTCAGATACGCACTAGTCCTTAACGCGACCTGGCTGGTC 720
228 A   Y   F   I   P   T   L   L   R   Y   A   L   V   L   N   A   T   W   L   V
   AACAGTGCAGCCCACATGTGGGGCATGAGGCCCTACGACCAAAACGTCAACCCAAGAGAA 780
248 N   S   A   A   H   M   W   G   M   R   P   Y   D   Q   N   V   N   P   R   E
   AACAAGTTTGTCGCCTTCAGTGCTATAGGAGAGGGATTTCACAACTACCACCACACCTTC 840
268 N   K   F   V   A   F   S   A   I   G   E   G   F   H   N   Y   H   H   T   F
   CCTTATGACTACGCAACCAGTGAGTTTGGCAGCCGGCTGAACCTGACCAAAGCCTTTATT 900
288 P   Y   D   Y   A   T   S   E   F   G   S   R   L   N   L   T   K   A   F   I
   GATTTCATGTGCTACCTCGGCCAGGCCTGAGACTGCAAGAGAGTGTCTCATGAAACCGTT 960
308 D   F   M   C   Y   L   G   Q   A   S   D   C   K   R   V   S   H   E   T   V
   ATGGCACGAGTTCGCCGCACAGGAGACGGTAGCCACAGGAGTGGCTAAGCGGTTTGATTT1020
333 M   A   R   V   R   R   T   G   D   G   S   H   K   S   G   *
   GTTTTCAAAAGCCCCCCAAGAAAATAAACTGCTGACGCAAAAAAAAAAAAAAA        1073
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Fig. 1. Nucleotide and deduced amino acid sequences of milkfish desaturase. The amino acid sequence is shown below the nucleotide sequence in a single letter code. The translation start codon and termination codon are marked by an M and asterisk (*), respectively. The polyadenylation signal is underlined.

mon carp and grass carp, than to those of different phyla.

3.4. Tissue expression

Tissue expression of stearoyl-CoA desaturase in various tissues of milkfish is shown in Fig. 5. Expression of stearoyl-CoA desaturase mRNA was demonstrated by RT-PCR. In a RT-PCR study, a 592-bp fragment was amplified with the stearoyl-CoA desaturase-specific primers (MD1 and MD2, Table 1) that were derived from the cDNA of all tissues examined, i.e. the liver, brain, kidney, gill and muscle. However, the desaturase was strongly detected only in liver and brain for grass carp (Chang et al., 2001).

4. Discussion

Full-length stearoyl-CoA desaturase cDNA from milkfish (*Chanos chanos*) was successfully cloned using an RT-PCR strategy. Since the temperature tolerance and adaptation mechanisms for fish in different temperature niches differ, variabilities in the subcellular desaturase expression and activity, which are intimately related to thermal adaptation, are expected (Schünke and Wodtke, 1983). In searching for stearoyl-CoA desaturase in milkfish, a 1073-bp cDNA encoding a protein sequence of 323 amino acids by reverse-transcription polymerase chain reaction (RT-PCR) and rapid amplification of cDNA ends (RACE) was obtained. The deduced amino acid

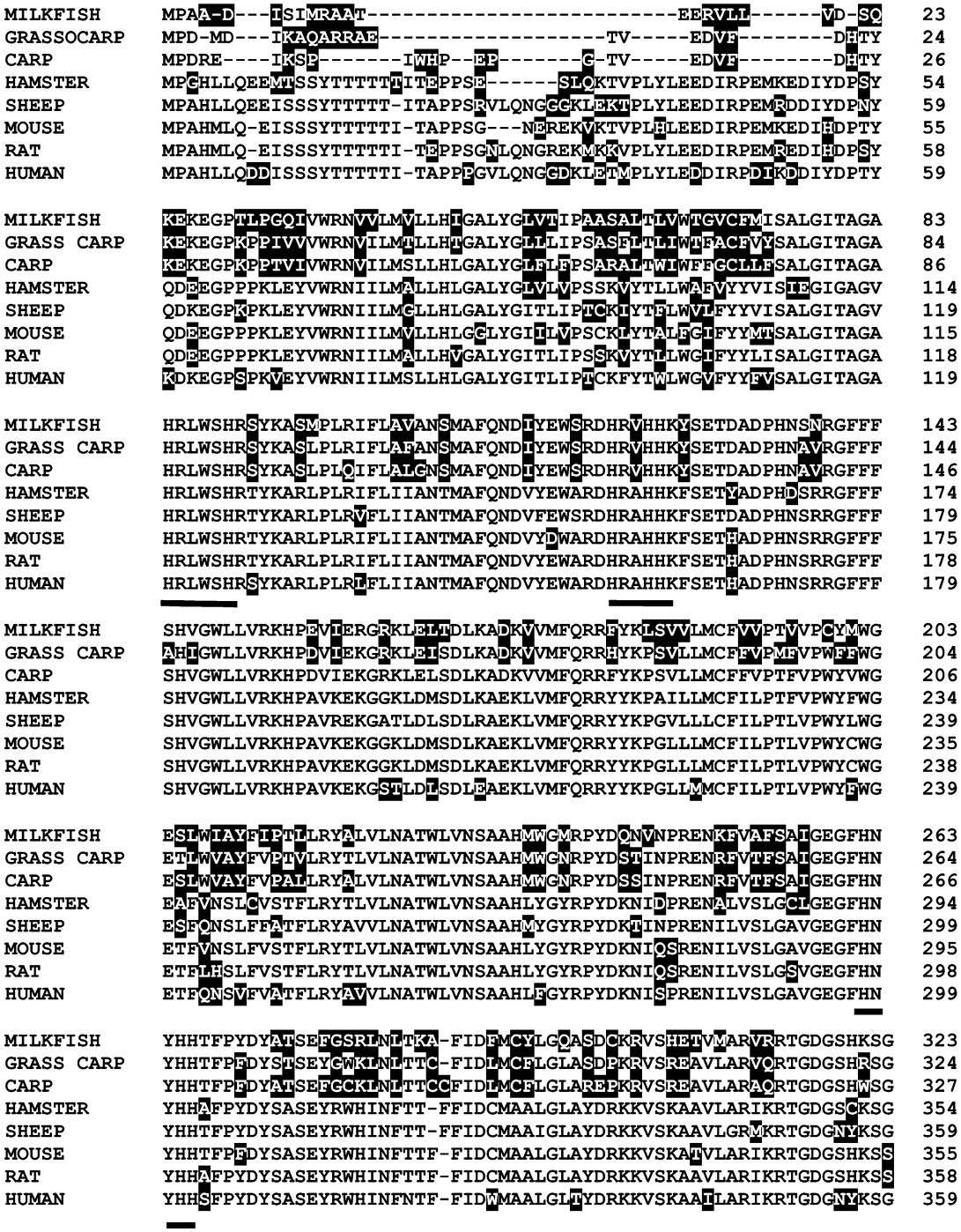

Fig. 2. Multiple alignments of stearoyl-CoA desaturases. Different amino acid residues among species are presented on a black background. Three conserved histidine clusters (one of HXXXXH and two of HXXHH) are underlined. Deduced amino acid sequences were obtained from human desaturase (GenBank/EMBL accession no. AF097514), mouse (NM009127), rat (J02585), sheep (AJ001048), hamster (L26956), carp (U31864), and grass carp (AJ243835).

sequence of stearoyl-CoA desaturase in milkfish shares high homology with those of other cold-tolerant teleosts, 79% to common carp, and 75% to grass carp. Fairly high identities to mammalian desaturase in the range 63–64% were also demonstrated. The phylogenetic analysis suggests

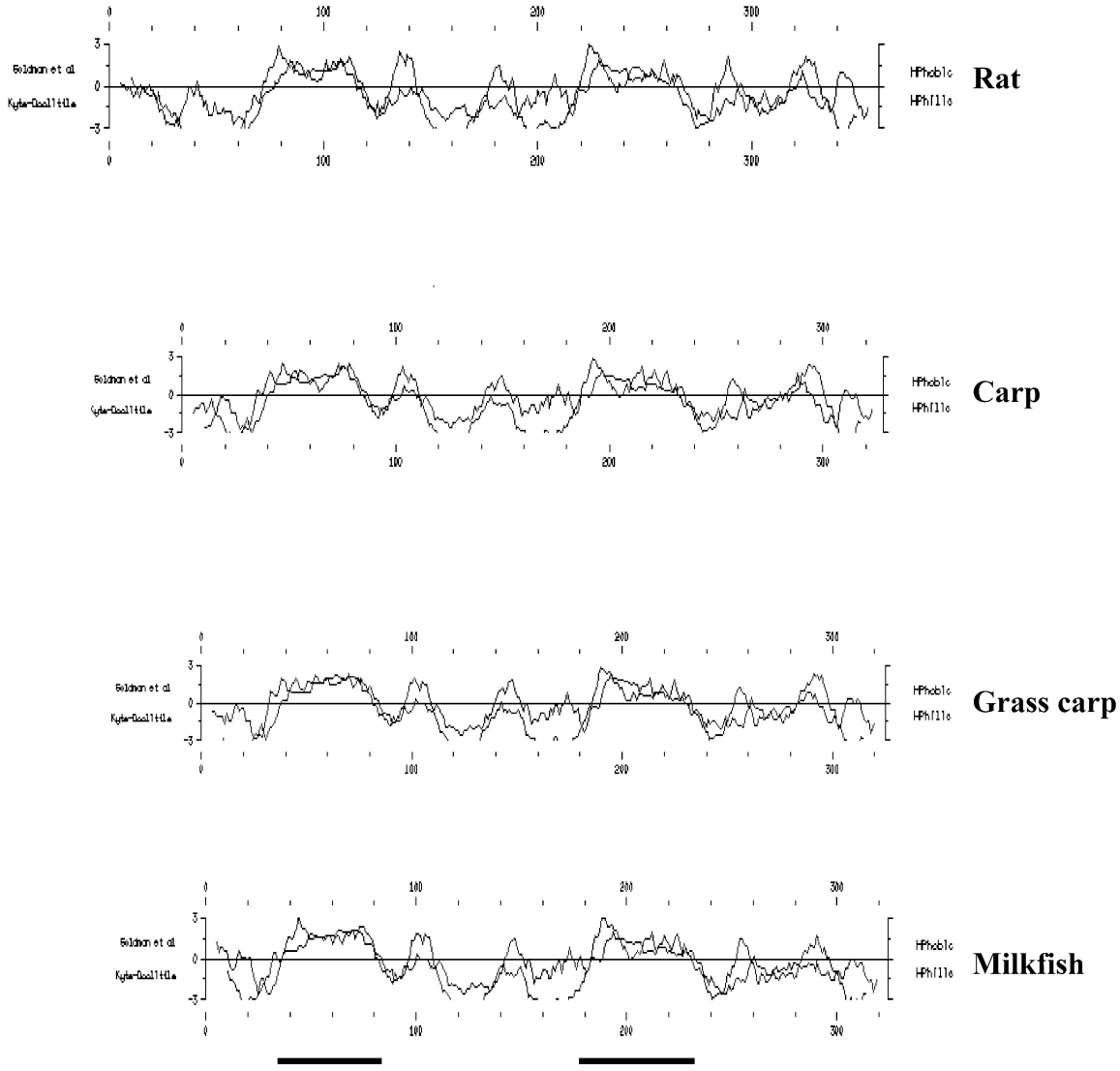

Fig. 3. Kyto–Doolittle hydropathy analysis of rat, carp, grass carp, and milkfish stearoyl-CoA desaturase (Kyto and Doolittle, 1982). The presumptive double membrane-spanning regions are underlined.

that milkfish desaturase is closely related to those of other vertebrates, with 100% bootstrap value to common carp and grass carp. The stearoyl-CoA desaturase sequence in milkfish contained three histidine cluster motifs: one HXXXXH and two HXXHH, which are all conserved in the stearoyl-CoA desaturases of vertebrates reported to date. The role of these histidine residues is likely to act as ligands to iron atoms that are involved in electron transfer, and thus oxidation–redaction reactions for the insertion of double bonds into fatty acids (Strittmatter et al., 1974).

By hydropathy analysis, two long hydrophobic regions were found to be conserved in milkfish stearoyl-CoA desaturase. These regions potentially exist in two membrane-spanning domains that are characteristic of membrane-anchored proteins and generally found in both acyl-CoA and acyl-lipid desaturases (Stukey et al., 1990; Murata and Wada, 1995). This speculation is consistent with the finding that stearoyl-CoA desaturase is tightly bound to microsome membranes.

Influences on desaturase activities and expression have been the subject of controversy. Factors, including nutrient conditions (Ntambi, 1992; Tocher et al., 1996), hormones (Miller et al., 1997; Waters et al., 1997), peroxisomal proliferation (Miller and Ntambi, 1996), developmental

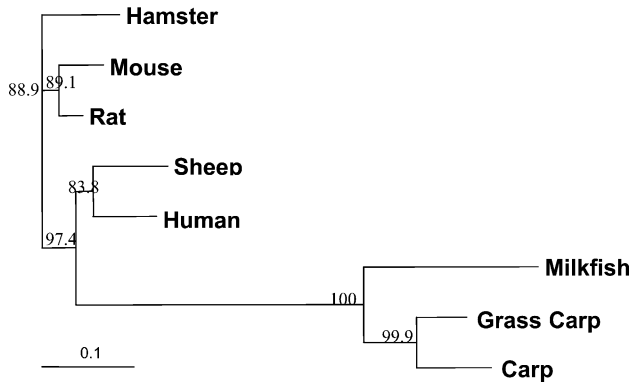

Fig. 4. Phylogenetic tree of eight vertebrate stearoyl-CoA desaturase sequences. A multiple alignment of amino acid sequences was performed using CLUSTAL W (Thompson et al., 1994). PHYLIP (Phylogeny Inference Package) version 3.57c (Felsenstein, 1995) was used to construct phylogenetic trees. Bootstrap values are indicated at the nodes. The scale bar indicates distance in units of fraction of amino acid differing between two sequences.

processes (Casimir and Ntambi, 1996), and environment conditions such as temperature (Tiku et al., 1996), have been investigated in various species. In starved rainbow trout, refeeding produced significant effects on the induction of stearoyl-CoA desaturase expression and activity within 1 week (Tocher et al., 1996). Furthermore, desaturase was notably expressed by cold shock in common carp, and the transcription of stearoyl-CoA desaturase in hepatic tissue increased 40–50-fold (Trueman et al., 2000). Similarly, transcription of stearoyl-CoA desaturase in milkfish liver also increased under cold shock (data not shown).

Although the molecular structure of desaturase is well conserved among various vertebrates, the expression pattern of desaturase appears to differ among them, and species-specific variations exist. Two types of desaturase genes were found in rodents: SCD-1 and SCD-2. SCD1 and SCD2 are different isoforms of stearoyl-CoA desaturase enzyme. Nucleotide sequence analysis of SCD1 and SCD2 genes revealed that the six exons and five introns span approximately 15 kb. Unlike the SCD1 gene, SCD2 lacks a typical 'TATA' box in the 5′-flanking region, but has two 'CCAAT' boxes at the transcription site (Kaestner et al., 1989). SCD-1 is principally expressed in adipose tissue and mildly expressed in the lung, while SCD-2 is predominantly expressed in the brain and less abundantly in the lung, spleen, and kidney (Ntambi et al., 1988; Kaestner et al., 1989; Mihara, 1990). In sheep, a single desaturase mRNA is highly expressed in the liver and mammary gland, whereas a low level of expression is detectable in the brain, muscle, lung, kidney, heart, and adipose tissue (Ward et al., 1998). As for human desaturase, two alternative transcripts are generated from a single gene, and its transcripts are abundant in the brain and liver, and also detectable in the lung, heart, placenta, skeletal, muscle, and kidney (Zhang et al., 1999). Among teleosts, the expression of the stearoyl-CoA desaturase in milkfish is tissue-specific, and

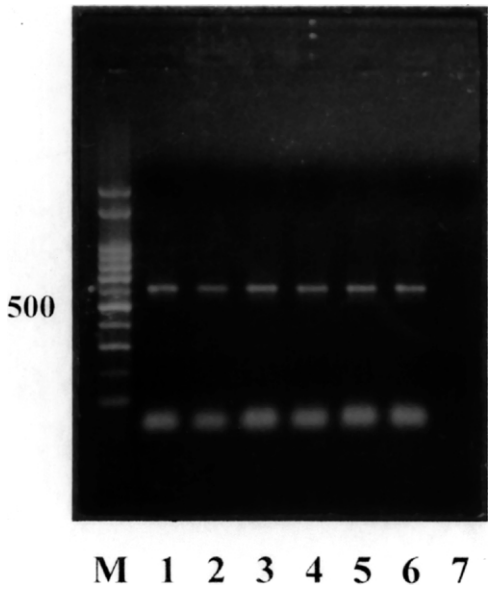

Fig. 5. RT-PCR analysis of stearoyl-CoA desaturase expression in various tissue. Tissues are indicated as follows: lane M, DNA marker; lane 1, liver; lane 2, brain; lane 3, kidney; lane 4, gill; lane 5, heart; and lane 6, muscle. The expression of stearoyl-CoA desaturase was also analyzed by RT-PCR with primers specific to milkfish stearoyl-CoA desaturase. The PCR products were analyzed on 1.2% gels.

transcripts of the gene are expressed in liver, brain, kidney, gill, heart and muscle, as determined by RT-PCR analyses. In grass carp, the distribution of desaturase transcripts appears to be more confined, being highly detectable in the liver and much less in the brain (Chang et al., 2001). In contrast to its expression in grass carp, expression of stearoyl-CoA desaturase in milkfish was detectable in tissues of the liver, brain, kidney, gill, heart and muscle. These observations suggest that gene-regulation mechanisms for this enzyme are present in a variety of tissues, and that desaturase is regulated through multiple tissue-specific regulatory factors.

In comparison with tissue expression patterns in other teleosts, stearoyl-CoA desaturase transcripts in milkfish are widely distributed. Differences in the expression profile of desaturase among vertebrates imply disparate regulation mechanisms and possibly reflect species-specific differences in the physiological essentiality and requirements of this enzyme in physiological adaptation (Chang et al., 2001).

The stearoyl-CoA desaturase sequence of milkfish is as highly conserved as those of common carp (Tiku et al., 1996) and grass carp (Chang et al., 2001), but these species differ greatly in their cold tolerance and adaptability. This may suggest that milkfish are incapable of expressing stearoyl-CoA desaturase in sufficient quantities to cope with cold temperature challenge, even though the expression distribution of stearoyl-CoA desaturase in milkfish is much wider than that in grass carp. An alternate possibility is that stearoyl-CoA desaturase is not the only factor involved in the thermal adaptation of fish (Hazel, 1984; Cossins, 1994), and that other factors, such as energy metabolic enzymes and cold shock proteins, might be simultaneously involved in the process of physiological compensation and adaptation (Jones et al., 1996). This remains to be further clarified.

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