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cDNA CLONING OF MYOSIN HEAVY CHAIN ISOFORMS FROM CARP FAST SKELETAL MUSCLE AND THEIR GENE EXPRESSION ASSOCIATED WITH TEMPERATURE ACCLIMATION

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Summary

We have isolated cDNA clones encoding fast skeletal muscle myosin heavy chains of carp acclimated to 10, 20 and 30 °C for over 5 weeks. All clones covered at least the full length of L-meromyosin, the C-terminal part of the myosin molecule. Nucleotide sequence analysis on cDNA clones showed three types of 3′ untranslated sequences, demonstrating that carp expresses at least three myosin heavy chain isoforms in fast skeletal muscle in an acclimation-temperature-dependent manner. cDNAs were identified which were the predominant types expressed in 10 °C- and 30 °C-acclimated fish, as well as an intermediate type present at all acclimation temperatures. Northern blot analysis using probes of three kinds of DNA fragments from the 3′ untranslated region of carp acclimated to 10, 20 and 30 °C further confirmed the presence of acclimation-temperature-specific isoforms. In addition, it was found that mRNA levels of three isoforms were altered in an acclimation-temperature-dependent manner. When the deduced amino acid sequences of three types of carp L-meromyosin were compared with those of homoiotherms, the 30 °C-acclimated type was more similar to those of homoiotherms than was the 10 °C-acclimated type.

Key words: carp, *Cyprinus carpio*, fast skeletal muscle, myosin heavy chain isoforms, L-meromyosin, temperature acclimation, cDNA.

Introduction

Muscle contraction is achieved by interaction between myosin and actin. The N-terminal globular domain of myosin subfragment 1 (S1), is especially important for the interaction with actin (Harrington and Rodgers, 1984; Mornet *et al.* 1989). The three-dimensional structure of skeletal α-actin was first elucidated by X-ray crystallography in the form bound to DNAase I (Kabsch *et al.* 1990). Recently, the three-dimensional structure of S1 has also been determined from a chicken fast skeletal muscle preparation. A unique pocket-like structure has been described which holds ATP, allowing its subsequent hydrolysis in the presence of Mg^{2+} following interaction with actin (Rayment *et al.* 1993*a*,*b*). The myosin molecule from skeletal muscles has a molecular mass of approximately 500 000 Da and contains a rod-like C-terminal tail which forms a coiled-coil α-helix structure, connecting the globular S1 domain to its N-terminal part (Harrington and Rodgers, 1984). The rod portion is responsible for the assembly of myosin to form the functional thick filaments, and the S1 domains protrude from the filament surface to form crossbridges with the actin-containing thin filaments. The myosin rod region is thought to play an important role in the regulation of mechanochemical energy transduction, although less information is available on its role compared with that for the S1 domain. The S1 domain also contains four light chains of approximate molecular mass 20 000 Da which are non-covalently bound.

The body temperature of the majority of fish species closely parallels that of their environment. Whereas some species live at relatively constant temperatures, for example in the deep sea or polar oceans, many experience marked changes in temperature sufficient to produce major changes in behavior and in their physiological and biochemical rate processes. Three major time courses of thermal adaptation have been distinguished: immediate, with a time-scale of minutes or hours; seasonal, requiring several weeks or months; and evolutionary, involving changes at a genetic level (Hazel and Prosser, 1974). The changes in phenotype induced by seasonal temperature change have been extensively studied in eurythermal temperate species such as carp and goldfish. One of the best examples of a trait which changes in an acclimation-temperature-dependent manner at the whole-animal level is maximum cruising speed. Goldfish *Caraussius auratus*

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acclimated to different experimental temperatures show unique temperature-performance curves. In general, swimming speed increases at low temperatures and decreases at high temperatures following cold-acclimation. The opposite responses are observed following acclimation to warm temperatures (Fry and Hart, 1948). The mechanisms underlying changes in swimming performance with temperature acclimation have been shown to involve adaptations in the activity and thermostability of myofibrillar ATPase activity (Johnston *et al.* 1975) as well as alterations in force production and maximum contraction speed of isolated muscle fibres (Johnston *et al.* 1985). Changes in myofibrillar ATPase activity following temperature transfer are apparent after 1 or 2 weeks and a steady state is achieved after 4 or 5 weeks (Heap *et al.* 1985), but such changes are not observed in starved individuals (Heap *et al.* 1986), suggesting that protein synthesis or the turnover of myofibrillar components is involved in the response. We have recently shown that carp express different types of myosin heavy chains in fast myotomal muscle following temperature acclimation (Hwang *et al.* 1990, 1991; Watabe *et al.* 1992, 1994; Guo *et al.* 1994; Nakaya *et al.* 1995). Cold-acclimation induces structural changes in myosin S1 and an increase in actin-activated Mg^{2+}-ATPase activity. Recently, we have shown that temperature acclimation also causes changes in the rod region of the myosin molecule as revealed by differential scanning calorimetry (Nakaya *et al.* 1995). Although carp myosin isoforms are probably expressed by different genes in an acclimation-temperature-dependent manner (Gerlach *et al.* 1990; Watabe *et al.* 1995*a*), no evidence has been provided on expression at the level of transcription.

The objective of the present study was to isolate cDNA clones encoding myosin heavy chain isoforms from fast skeletal muscles of thermally acclimated carp in order to demonstrate whether there was an acclimation-temperature-dependent expression of these proteins. At least three types of cDNA clones were isolated with acclimation-temperature-specific 3′ untranslated regions. Since the three clones covered at least the region encoding the C-terminal part of the myosin molecule, L-meromyosin, we were able to compare the coiled-coil structure of the α-helix between three types of L-meromyosin.

Materials and methods

Fish

Common carp, *Cyprinus carpio* L. (body mass 0.5–0.8 kg), were acclimated to either 10, 20 or 30 °C for a minimum of 5 weeks under a 14 h: 10 h L:D photoperiodic regime. Fast (white) muscle was dissected from the dorsal epaxial myotomes, taking care to avoid other muscle fibre types (red and pink). Hepatopancreas tissues of carp from a local supplier were freshly isolated and used for Southern blot analysis.

cDNA cloning

cDNA clones were isolated from carp muscle λZAPII libraries which were constructed as follows. Total RNA was prepared from the dorsal skeletal muscle of carp acclimated to either 10 or 30 °C by the acid guanidinium thiocyanate-phenol-chloroform extraction method (Chomczynski and Sacchi, 1987). Poly(A^+) mRNA was prepared with an mRNA purification kit including columns of oligo($dT)^+$ coupled to cellulose (Pharmacia). Carp muscle cDNAs were synthesized using a kit (Pharmacia) and poly(A^+) mRNA. First-strand synthesis was performed with oligo($dT)^+$ primers, whereas second-strand cDNA synthesis employed a modification of the procedure described by Gubler and Hoffman (1983). The cDNA products were inserted into λZAPII phage according to the manufacturer's instructions (Stratagene).

cDNA probes were labeled by DIG DNA labeling and detection kits (Boehringer Mannheim). Hybridization was carried out at 68 °C in 5× SSC (saline sodium citrate, 1× concentration = 0.15 mol l^{-1} NaCl, 15 mmol l^{-1} sodium citrate, pH 7.0) containing 1.0 % blocking reagent, 0.1 % *N*-laurylsarcosin and 0.02 % sodium dodecyl sulphate (SDS). A fragment consisting of 231 bp was originally obtained in an unrelated polymerase chain reaction (PCR) cloning effort (Watabe *et al.* 1995*a*). The deduced amino acid sequence was consistent with that of the N-terminal region of L-meromyosin previously reported for chicken fast skeletal muscle myosin heavy chain (Maita *et al.* 1991). This fragment was used to screen cDNA libraries from carp acclimated to 10 and 30 °C. *In vivo* excision of clones from λZAPII libraries was carried out according to the methods of Short *et al.* (1988).

Sequence analysis

cDNA restriction fragments of carp myosin heavy chain isoforms were subcloned into plasmid pBluescript SK^- using *Escherechia coli* strain MV1190 as a host bacterium. Sequencing was performed for both 5′ and 3′ strands of subclones labeled with Dye Deoxy™ terminator cycle sequence kits using a DNA sequencer (model 373A; Perkin Elmer Applied Biosystems). The protein homology search was performed using the Swiss-Prot database coordinated with the Inherit program (Perkin Elmer Applied Biosystems).

Northern and dot blot analyses

The following procedures were performed essentially according to Sambrook *et al.* (1989). Three individuals each of carp acclimated to 10, 20 and 30 °C were killed in the laboratory and fast skeletal muscle tissues were used immediately for RNA extraction. Total RNA was isolated from tissues using an Isogen solution (Nippon Gene). The concentration of RNA was determined spectrophotometrically by reading ε_{260} for each sample before gel electrophoresis. RNA was size-fractionated by electrophoresis on a 1.5 % agarose gel containing formamide, and capillary-transferred from the gel to nylon membranes (Amersham). Gels were stained with ethidium bromide and photographed before transfer to ascertain that an equal amount of intact RNA was loaded. The membranes containing RNA blots were prehybridized in a solution containing 6× SSC, 1× Denhardt's

solution, 0.5 % SDS, 50 % formamide, and 100 mg ml^{-1} of sonicated and heat-denatured calf thymus DNA for 14 h at 42 °C. Blots were then hybridized for 18 h at 42 °C in the same solution containing ^{32}P-labeled cDNA probes which were randomly primed in the presence of α-[^{32}P]dCTP. After washing with 2× SSC plus 0.5 % SDS at room temperature for 20 min and subsequently with 1× SSC plus 0.5 % SDS at 65 °C for 20 min, the blots were exposed to X-ray films for a period of 24–72 h at –80 °C using intensifying screens. The hybridized membranes were scanned using a Fujix Bas 1000 computerized densitometer scanner and quantified using a recommended scanning program.

In dot blot analysis, unlabelled DNA fragments at concentrations of 100, 10 and 1 pg were blotted onto nylon membranes and reacted with the homologous and heterologous probes which were used in Northern blot analysis.

Statistical analysis

Student's *t*-tests were employed to compare Northern blot analysis data among carp acclimated to 10, 20 and 30 °C.

Results

Isolation and characterization of three types of cDNA clones encoding myosin heavy chain isoforms

In order to obtain cDNA clones which encode myosin heavy chains specific to various acclimation temperatures, cDNA libraries from 10 °C- and 30 °C-acclimated carp were screened with a PCR product for carp L-meromyosin DNA reported previously (Watabe *et al.* 1995*a*).

Several clones encoding carp skeletal muscle myosin heavy chain isoforms were isolated from carp cDNA libraries. These were subjected to determination of nucleotide sequences for 5′ and 3′ translated regions and 3′ untranslated regions. There were three types of cDNA clones: the two clones predominantly found in cDNA libraries from the 10 °C- and 30 °C-acclimated carp, respectively, in addition to an intermediate type between the above two clones at the level of the nucleotide sequence (Figs 1 and 2). Of the total 10 cDNA clones screened from the cDNA library of the 10 °C-acclimated carp, eight clones belonged to the 10 °C type and two clones belonged to the intermediate type. However, no clone was found to be the 30 °C type. On the other hand, five out of the total seven clones screened in the cDNA library of the 30 °C-acclimated carp were those of the 30 °C type, whereas the remaining two were of the 10 °C type. The most conspicuous differences among the three types of cDNA clones were with respect to the sequences connecting 3′ translated and 3′ untranslated regions (Fig. 2). Surprisingly, a stop codon of the intermediate-type clone was located three nucleotides upstream from those of the 10 °C- and 30 °C-type clones. The 10 °C- and 30 °C-acclimation types were further confirmed by comparing the respective deduced amino acid sequences with those obtained from N-terminal amino acid sequence analysis for L-meromyosin preparations from 10 °C- and 30 °C-acclimated carp (Watabe *et al.* 1995*a*). We selected and analyzed the longest cDNA clones for the three types: those predominantly expressed at 10 °C (pMHC10-3) and 30 °C (pMHC30-6), as well as the isoform present at all temperatures (pMHC10-25) (see Figs 1 and 4). The nucleotide sequences have been submitted to the GSDB/DDBJ/EMBL/NCBI data bases with accession numbers D50474, D50475 and D50476 for pMHC10-3 (10 °C type), pMHC10-25 (intermediate type) and pMHC30-6 (30 °C type), respectively.

Comparison of the nucleotide sequence in the corresponding region among the three types of cDNA clones produced a 93.8 % homology between the 10 and 30 °C types and a 95.6 % homology between the 10 °C and intermediate types. In the 3′ untranslated region from after the stop codon to just before the poly(A$^+$) tails, however, the 10 °C-type clone showed less homology at the level of the nucleotide sequence compared with the intermediate-type clone (86.5 % including deletions). The homology between the 10 °C- and 30 °C-type clones was much less and in the region of 66.0 %. These results suggest that acclimation-temperature-specific probes for Northern blot analysis can be designed using nucleotide sequences around a 3′ untranslated region (Figs 1 and 2).

Northern and dot blot analyses

Northern blot analysis was carried out to reveal expression of fast skeletal myosin heavy chain isoforms of carp at the transcriptional level. Probes 1, 2 and 3 which encode the

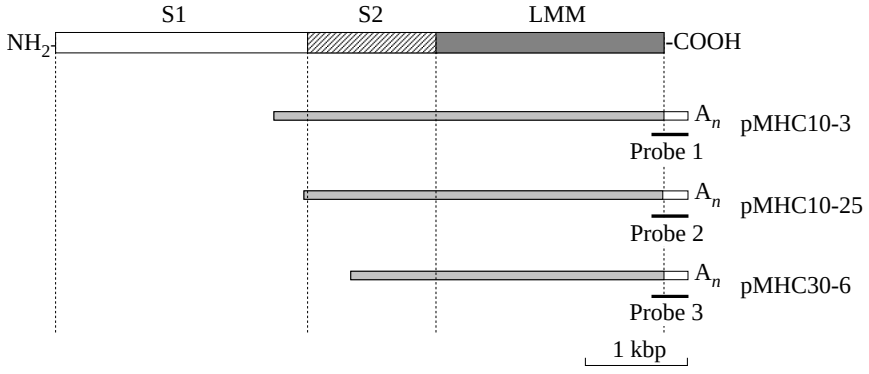

Fig. 1. Schematic representation of three types of cDNA clones encoding the myosin heavy chain of carp fast skeletal muscle. The primary structure of the myosin heavy chain molecule is shown at the top for orientation purposes, those of the three cDNA clones are shown below. cDNA clones of pMHC10-3, pMHC10-25 and pMHC30-6 represent the longest clones for 10°C, intermediate and 30 °C types, respectively. Hatched areas of cDNA clones correspond to translated regions. The bars under the clones indicate the locations of probes 1–3 used in Northern blot analysis. Abbreviations: S1, myosin subfragment 1; S2, myosin subfragment 2; LMM, L-meromyosin; A_n, poly(A$^+$) tail.

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pMHC10-3  AAGAAGAACGTGACTCGCCTGCAGGATCTGGTAGACAACCTGCAGCTGAA  50
pMHC10-25 .......GT........A...................G...........  50
pMHC30-6  ............TC......................G...........  50

pMHC10-3  AGTGAAGGCCTACAAACGCCAAGCTGAAGAAGCTGAGGAGCAGGCCAACA 100
pMHC10-25 ................................................. 100
pMHC30-6  ........T......G.....G...........CG........A...... 100

pMHC10-3  CTCACCTGTCCAGGTACAGGAAGGTCCAGCATGAACTGGAGGAGTCTCAT 150
pMHC10-25 ........................G........G.............. 150
pMHC30-6  ........................G.....C..G.........G....G 150

pMHC10-3  GAGCGCGCTGACATCGCCGAGTCCCAGGTCAACAAGCTGAGAGCCAAGAG 200
pMHC10-25 ..........................................  200
pMHC30-6  .....T......G.T..T.....A...............T...........  200

pMHC10-3  CCGTGAAGCTGGGAAGACTAAAGTTGAAGAATGAAGACAAGAAA--CTAG 248
pMHC10-25 ............A..............T.............---.A.C 248
pMHC30-6  ...C..C.........GC..G.A.......  ...TG..CC.TC.C.C 250

pMHC10-3  -ACAT--ACAAGCAAGCATAT-----GACTGACTTGTGCTGAGTTCTTCT 290
pMHC10-25 ---CT--.....------------------------------------ 290
pMHC30-6  C....CT.............AATAT....T..........T..-...A. 299

pMHC10-3  TTGTCCATTAAATGGCAGAGTTT-----G                    314
pMHC10-25 G............A..-.-...AATT-.                     316
pMHC30-6  A.A.G........A-.-------.CTCAA.GC                  327
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Fig. 2. Aligned nucleotide sequences of 3′ translated and 3′ untranslated regions of three types of cDNA clones encoding the myosin heavy chain of carp fast skeletal muscle. Identical and gapped nucleotides are shown by dots and dashed lines, respectively. The termination codons are boxed, the enclosed area represents the region used for probes in Northern and Southern blot analyses. Refer to the legend of Fig. 1 for identification of the three types of cDNA clones.

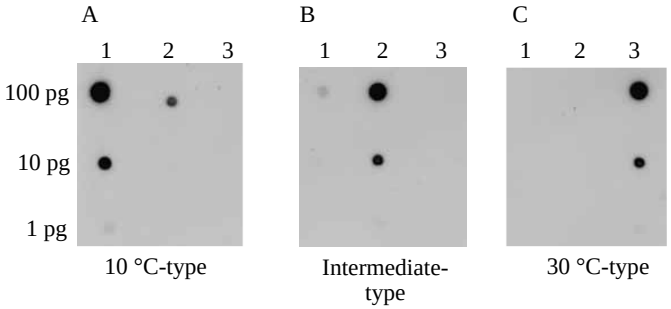

Fig. 3. Specificity of probes for Northern hybridization from three different types of cDNA clones encoding myosin heavy chain isoforms of carp fast skeletal muscle. ^{32}P-labelled DNAs for probing 10 °C- (A), intermediate- (B) and 30 °C-type (C) mRNAs in Northern hybridization were applied to the nylon membrane incorporating the same region of unlabelled DNA fragments from 10 °C (1), intermediate (2) and 30 °C (3) types.

boundary of translated and 3′ untranslated regions (Fig. 1) and are specific to 10 °C-, intermediate- and 30 °C-type cDNA clones, respectively (Fig. 2), were prepared from plasmid DNAs. Specificity was further confirmed by dot blot analysis using three types of cDNA clones (Fig. 3). Fig. 4 shows hybridization patterns in Northern blot analysis and accumulated levels of mRNA for three types of carp myosin heavy chain isoforms. The relative mRNA levels of the three myosin heavy chain types were dramatically changed following temperature acclimation. The level of the 10 °C-type isoform in 10 °C-acclimated carp was sixfold higher than that in the 30 °C-acclimated carp (P<0.05) (Fig. 4D). The level of the intermediate type in the 10 °C-acclimated carp was fivefold higher than that in the 30 °C-acclimated fish (Fig. 4E), whereas that of the 30 °C type was 11-fold higher in the 30 °C-acclimated carp than in the 10 °C-acclimated fish (P<0.01) (Fig. 4F). Slight cross reactions were observed between probes from the 10 °C and intermediate types in DNA-DNA dot blots (Fig. 3). Since DNA-DNA hybrids are less stable than those of DNA-RNA (Sambrook *et al.* 1989), our dot blots do not really counter the argument that on the Northern blot and subsequent quantitations, the signals of the 10 °C and intermediate types could reflect cross-reactions next to perfectly matching hybrids. In spite of such ambiguities, the results demonstrate that the expression of myosin heavy chain isoforms of carp fast skeletal muscle is regulated *via* the availability of their specific mRNAs in an acclimation-temperature-dependent manner.

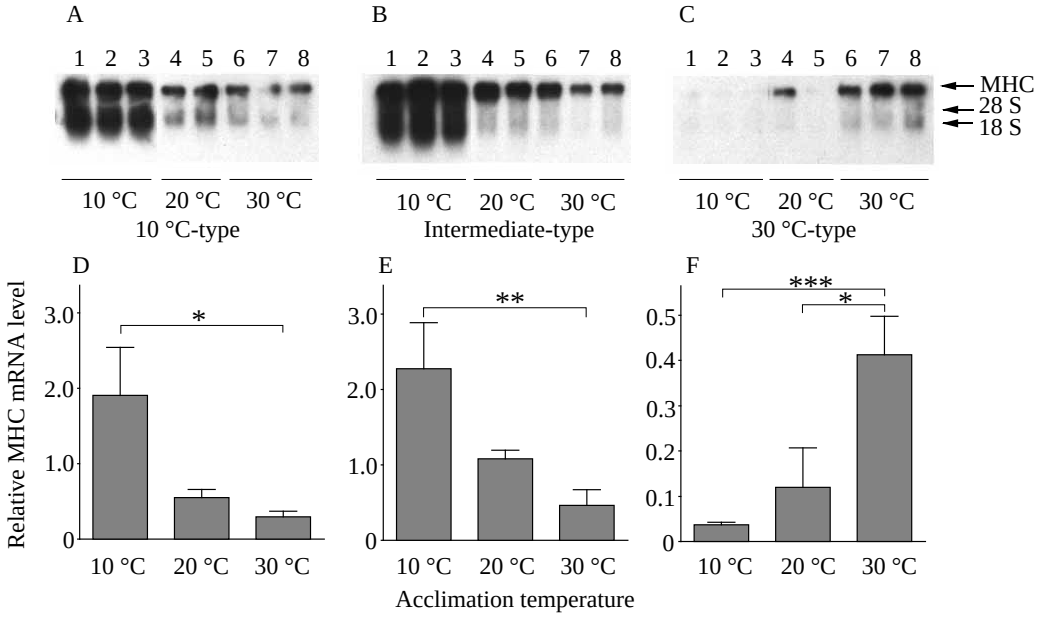

Fig. 4. Northern blot analysis of mRNAs encoding three types of carp myosin heavy chains in thermally acclimated carp. The total RNAs from fast skeletal muscle were prepared from three individuals each of carp acclimated to 10, 20 and 30 °C and hybridized with DNA probes derived from 3′ translated and 3′ untranslated regions of pMHC10-3 (A), pMHC10-25 (B) and pMHC30-6 (C) (see Fig. 1). Data in D–F are means + S.E.M. (N=3 fish) with significant differences at P<0.05 (*), P<0.02 (**) and P<0.01 (***). Refer to the legend of Fig. 1 for identification of the three types of cDNA clones.

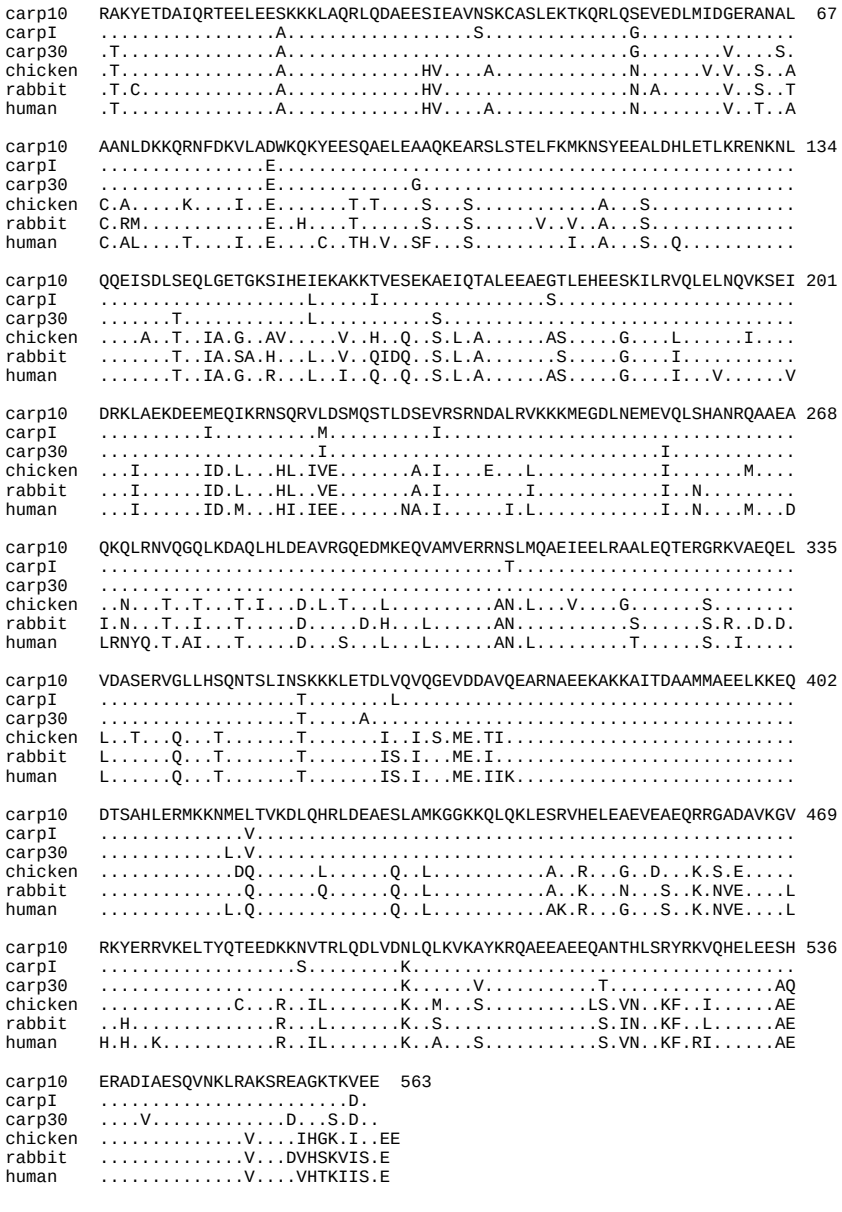

Fig. 5. Comparison of amino acid sequences of fast skeletal muscle L-meromyosin from thermally acclimated carp with those of homoiotherms. Carp10 and carp30 represent the L-meromyosin types predominating in carp acclimated to 10 and 30 °C, respectively, whereas carpI represents L-meromyosin with intermediate structural properties between carp10 and carp30. Sequences of L-meromyosin for comparison are from chicken (Maita *et al.* 1991), rabbit (Maeda *et al.* 1987) and human (Saez and Leslie, 1986) fast skeletal muscles.

Comparison of amino acid sequences of L-meromyosin isoforms from thermally acclimated carp

The amino acid sequence homology of carp fast skeletal muscle L-meromyosin isoforms was 95.6 % between the 10 and 30 °C types and 97.0 % between the 10 °C and intermediate types (Fig. 5). When these were compared with those of three homoiotherms (Saez and Leslie, 1986; Maeda *et al.* 1987; Maita *et al.* 1991), the 30 °C-acclimation-type L-meromyosin was found to be more similar to homoiothermic L-meromyosins than was the 10 °C-acclimation-type L-meromyosin (Table 1), suggesting that the amino acid sequence of L-meromyosin is altered according to the body temperature of the animal.

Table 1. *Comparison of amino acid sequences of fast skeletal muscle L-meromyosin from thermally acclimated carp with those of homoiotherms*

	Carp		
	10 °C acclimation-type	Intermediate-type	30 °C acclimation-type
Chicken	79.3	79.8	80.3
Rabbit	79.8	80.5	80.9
Human	76.2	77.1	78.0

Values are given as percentage identity.

It is well known that the amino acid sequence of L-meromyosin is highly repetitive and shows features typical of an α-helical coiled-coil protein. L-meromyosin, or even the myosin rod containing both the S2 fragment and L-meromyosin, has a characteristic regular pattern consisting of seven amino acid residues, a, b, c, d, e, f and g, where hydrophobic amino acid residues are concentrated at alternate

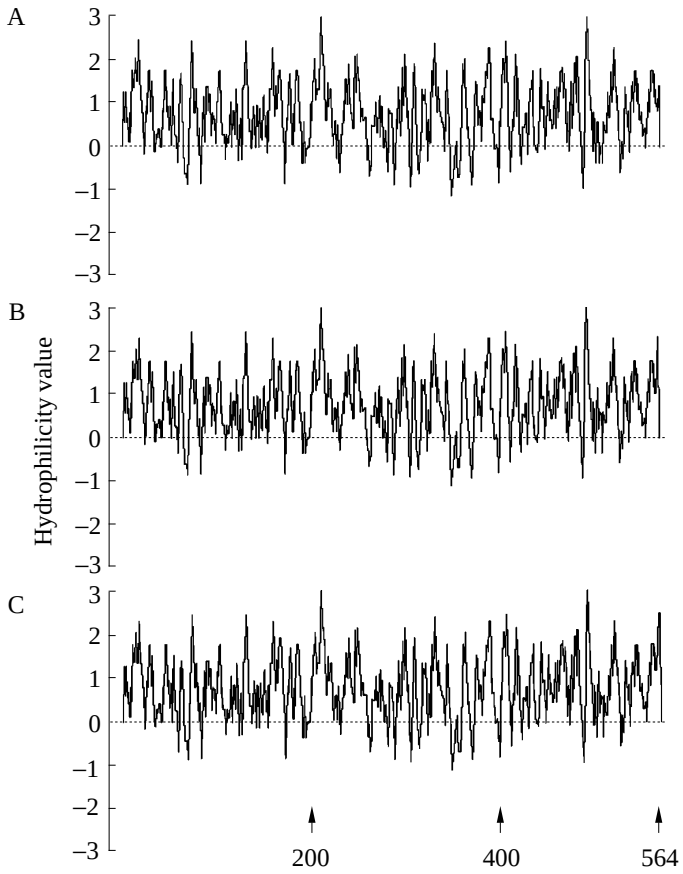

Fig. 6. Comparison of hydrophilicity values between three types of fast skeletal muscle L-meromyosins from thermally acclimated carp. A, B and C represent 10 °C, intermediate and 30 °C types of L-meromyosin. The numbers indicate amino acid residues from the N terminus of L-meromyosin.

intervals of three and four residues along the length of the chain at positions a and d (Parry, 1981; McLachlan and Karn, 1982). The residues at positions a and d form a closely packed hydrophobic interface or 'core' of knobs and holes between the two strands of the coiled-coil structure (Parry, 1981). The surface of the coiled-coil structure is highly charged, with acidic and basic residues clustered mainly in the outer positions b, c and f. There is strong evidence that the myosin rod is formed from a 28-residue unit with a repetitive sequence (McLachlan and Karn, 1982). The three types of carp L-meromyosin, 10 °C, intermediate and 30 °C types described in the present study were also made up of repeating 28 amino acid residue zones (data not shown), as was also found for L-meromyosins from acanthamoeba (Hammer *et al.* 1987; Rimm *et al.* 1989), nematode (McLachlan and Karn, 1982), chicken (Molina *et al.* 1987), rat (Strehler *et al.* 1986) and rabbit (Offer, 1990). However, no marked differences in periodicities of hydrophobic and charged amino acid residues were observed among the 10 °C, intermediate and 30 °C types in the present study even by analysis according to Hopp and Woods (1981), except for an extreme C-terminal region (Fig. 6).

Discussion

We have isolated cDNA clones which encode three types of myosin heavy chain isoforms from fast skeletal muscles of carp acclimated to 10 and 30 °C. Although slight cross reactions were observed between DNA probes from the 10 °C and intermediate types (Fig. 3), Northern hybridization clearly demonstrated that the mRNA levels of the three isoforms were dependent on acclimation temperature (Fig. 4). mRNA levels of α-actin have previously been determined for fast skeletal muscles of carp acclimated to different temperatures, using a specific probe (Watabe *et al.* 1995*b*). Although the levels were very consistent in the 10 °C- and 30 °C-acclimated carp, values were unexpectedly low in the 20 °C-acclimated carp. Since standardization of mRNA levels with those of α-actin has not been validated, we did not refer to these data to argue for different mRNA levels of carp myosin heavy chain isoforms.

Three cDNA clones were isolated from carp fast skeletal muscle encoding at least the C-terminal half of the myosin rod, called L-meromyosin. Deduced amino acid sequences of these clones revealed that two out of the three isoforms correspond to predominant isoforms in 10 °C- and 30 °C-acclimated carp, respectively, compared with data reported previously (Watabe *et al.* 1995*a*). Another isoform showed an intermediate sequence between the above two isoforms. Comparison of the amino acid sequences of these three types of L-meromyosin from carp with those of homoiotherms (see Fig. 5 and Table 1) suggests that certain regions or even just a few amino acid substitutions are responsible for changes in the structural stability of L-meromyosin. Nakaya *et al.* (1995) reported that the α-helical structure of the rod region from 10 °C-acclimated carp melted at lower temperatures than did that from 30 °C-acclimated carp. Furthermore, it seems that structural changes in carp L-meromyosin in response to temperature acclimation are related to the functional properties of this portion of the myosin molecule, which have not yet been elucidated satisfactorily.

Ennion *et al.* (1995) isolated a complete gene of approximately 12.0 kbp which encodes the carp myosin heavy chain. This gene was transcribed only in fast skeletal muscle following an increase in environmental temperature. These results were consistent with those from Northern hybridization which demonstrated that the expression of one RNA isoform for the myosin heavy chain was increased in warm- relative to cold-acclimated carp (Gerlach *et al.* 1990; Goldspink *et al.* 1992). Three types of cDNA clones encoding myosin heavy chains were isolated in the present study for fast skeletal muscle in carp (see Fig. 1). Their expression was apparently controlled at the transcription level in an acclimation-temperature-dependent manner (see Fig. 4). It has been reported that myosin heavy chains are encoded by a multigene family (Emerson and Bernstein, 1987). Although the exact number of genes within the family is unknown and certainly differs between species, the expression of myosin heavy chain isoforms is controlled in a tissue- and developmental-stage-specific manner with each isoform apparently optimized for the specific requirements of the different muscles (Emerson and Bernstein, 1987). Several

myosin heavy chain isoforms have been shown to be expressed by alternative splicing from a single gene (Collier *et al.* 1990; Kelly *et al.* 1993; Nyitray *et al.* 1994). However, DNA nucleotide or deduced amino acid substitutions spread over the whole region of the isolated clones for myosin heavy chains of carp fast skeletal muscle in this study, suggesting the presence of different genes encoding the three types of cDNA clones. In preliminary experiments using Southern blot analysis, different patterns have been found with the 10 °C- and 30 °C-specific carp cDNA probes after digestion of genomic DNA with restriction enzymes (data not shown). Although these results also suggest that different isoforms are probably expressed by different genes, Southern blot patterns were not always reproducible. Therefore, it seems necessary to isolate genomic clones from carp to determine whether they encode the different transcripts described in this study.

The true number of myosin heavy chain isoforms expressed in carp fast skeletal muscle in an acclimation-temperature-dependent manner has yet to be established. The compensation of locomotory performance following temperature acclimation is complex and involves changes in myosin light chain ratio (Crockford and Johnston, 1990), and in the activities of sarcoplasmic reticulum Ca^{2+}-ATPase (Fleming *et al.* 1990; Ushio and Watabe, 1993), in addition to changes in myosin heavy chain expression. Furthermore, the temperature compensation of locomotory activity involves changes in central patterns of muscle fiber recruitment and in the properties of the peripheral nervous system (Johnston *et al.* 1990). Future studies will attempt to determine and compare the amino acid sequences of the S1 region from thermally acclimated carp, since this is functionally and structurally the most characterized part of other myosins.

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