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# Review

# Temperature plasticity of contractile proteins in fish muscle

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**Summary**

**Three myosin heavy chain isoforms with different actin-activated $Mg^{2+}$-ATPase activities were found in the fast skeletal muscle from carp (*Cyprinus carpio*) acclimated to 10 and 30 °C. The composition of three types of myosin heavy chain was dependent on acclimation temperature, demonstrating the presence of temperature-specific myosin isoforms in carp. Subsequently, the temperature-dependence of the sliding velocity of fluorescent F-actin in myosins isolated from 10 °C- and 30 °C-acclimated carp was measured. At 8 °C, the filament velocity was three times higher for myosin from 10 °C- than from 30 °C-acclimated fish. Activation energies ($E_a$) for the sliding velocity of F-actin were 63 and 111 kJ mol$^{-1}$ for myosins from 10 °C- and 30 °C-acclimated fish, respectively. Activation energy for actin-activated $Mg^{2+}$-ATPase activity was 0.46 kJ mol$^{-1}$ in myosin from 10 °C-acclimated fish and 0.54 kJ mol$^{-1}$ in myosin from 30 °C-acclimated fish. The inactivation rate constant ($K_D$) of $Ca^{2+}$-ATPase was $7.5\times10^{-4}$ s$^{-1}$ at 30 °C for myosin from 10 °C-acclimated fish, which was approximately twice that for myosin from 30 °C-acclimated fish. It is suggested that these differences in thermostability reflect a more flexible structure of the myosin molecule in cold-acclimated carp, which results in a reduced activation enthalpy for contraction and, hence, a higher sliding velocity at low temperatures. Structural analysis of cDNAs encoding the carp myosin heavy chain demonstrated striking differences in two surface loops of myosin subfragment-1 (S1), loops 1 and 2, between the 10 °C and 30 °C types, which were predominantly expressed in carp acclimated to 10 °C and 30 °C, respectively. Chimeric myosins composed of *Dictyostelium discoideum* myosin backbones with loop sequences of carp S1 heavy chain isoforms demonstrated that the diversity of the loop 2 sequence of carp S1 affected the $V_{max}$ of actin-activated $Mg^{2+}$-ATPase activity.**

Key words: actin-activated $Mg^{2+}$-ATPase activity, activation energy, carp, *Cyprinus carpio*, chimeric myosin, *Dictyostelium discoideum*, inactivation rate constant, myosin, myosin heavy chain isoform, sliding velocity, subfragment-1, thermal acclimation, thermostability.

## Introduction

The body temperature of most fish species closely parallels that of their environment. While some species live at a relatively constant temperature, for example those in the deep sea or polar oceans, many experience marked changes in temperature sufficient to produce major changes in their physiological and biochemical rate processes as well as in their behaviour. 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 the level of genetic diversity (Hazel and Prosser, 1974). The changes in phenotype induced by seasonal temperature change have been extensively studied in eurythermal temperate species such as carp *Cyprinus carpio* and goldfish *Carassius auratus*.

One of the best examples of a trait that changes in an acclimation-temperature-dependent manner at the level of the whole animal is the maximum cruising speed. Goldfish acclimated to different experimental temperatures show unique temperature/performance curves (Fry and Hart, 1948). 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. The mechanisms underlying such changes in swimming performance have been shown to involve adaptations in the activity and thermostability of myofibrillar ATPase (Johnston et al., 1975) as well as alterations in force production and maximum contraction speed of isolated muscle fibres (Johnston et al., 1985). It has been reported that changes in myofibrillar ATPase activity following temperature transfer are apparent for carp after 1 or 2 weeks (Heap et al., 1985). However, a steady state is achieved after 4 or 5 weeks, but not in starved individuals, suggesting that protein synthesis or the turnover of myofibrillar component(s) is involved in the response.

Table 1. *Kinetic parameters of actin-activated $Mg^{2+}$-ATPase for carp myosin isoforms and their chimera*

| $V_{max}$ ($s^{-1}$) | | | | $K_m$ ($\mu mol\,l^{-1}$) | | | |
|---|---|---|---|---|---|---|---|
| Myosin | | Chimera | | Myosin | | Chimera | |
| 10 °C-acclimated carp | 1.8 | Loop 1-10 | 0.80 | 10 °C-acclimated carp | 2.7 | Loop 1-10 | 1.1 |
| | | Loop 2-10 | 0.95 | | | Loop 2-10 | 0.7 |
| 30 °C-acclimated carp | 1.1 | Loop 1-30 | 0.80 | 30 °C-acclimated carp | 2.9 | Loop 1-30 | 0.8 |
| | | Loop 2-30 | 0.68 | | | Loop 2-30 | 0.6 |
| | | Wild type | 0.71 | | | Wild type | 0.9 |

$Mg^{2+}$-ATPase activity was measured at 20 °C.

Myosins were prepared from fast skeletal muscle of carp acclimated to 10 and 30 °C (Chaen et al., 1996).

Chimeric myosins were prepared from *Dictyostelium discoideum* backbone with loop 1 sequences from carp 10 °C- (loop 1-10) and 30 °C-type S1 (loop 1-30) and with loop 2 sequences from carp 10 °C- (loop 2-10) and 30 °C-type S1 (loop 2-30).

$V_{max}$, maximum initial velocity; $K_m$, myosin affinity to actin.

This article shows that one of the myofibrillar proteins responsible for changes in muscle plasticity in association with temperature acclimation of carp is myosin, a major protein in the contractile apparatus. Furthermore, the structure/function relationship of a temperature-specific myosin isoform expressed in carp is described.

## Carp myosin isoforms are responsible for the temperature plasticity of fast skeletal muscle

Myosin is the most abundant protein in the contractile apparatus, is essential for the contractile process and has been studied intensively. It consists of two heavy chains of approximately 200 kDa and four light chains of approximately 20 kDa (Harrington and Rodgers, 1984). The N-terminal half of each heavy chain folds into a globular head domain, termed subfragment-1 (S1), with two light chains. The C-terminal halves of the two heavy chains, termed the rod, fold into an α-helical coiled-coil structure and have the ability to form thick filaments. The rod can be further divided into two proteolytic fragments, subfragment-2 and light meromyosin, in order from the N terminus of this domain. Myosin is organized *in situ* into thick filaments in which the heads of the myosin molecules protrude from the surface of the thick filament and form cross-bridges with actin-containing thin filaments. Each cross-bridge is thought to be composed of two myosin heads, or S1 units, and each of these heads contains a site for ATP hydrolysis and a site for interaction with actin.

Myosin was isolated from fast skeletal muscle of carp acclimated to 10 and 30 °C for a minimum of 5 weeks and examined for its ATPase activities (Hwang et al., 1990). $Ca^{2+}$-ATPase activity differed between myosins from cold- and warm-acclimated carp, especially at KCl concentrations ranging from 0.1 to 0.2 $mol\,l^{-1}$, when measured at pH 7.0. The highest activity at a measuring temperature of 20 °C was 0.32 $\mu mol\,P_i\,min^{-1}\,mg^{-1}$ at 0.1 $mol\,l^{-1}$ KCl for cold-acclimated carp and 0.47 $\mu mol\,P_i\,min^{-1}\,mg^{-1}$ at 0.1 $mol\,l^{-1}$ KCl for warm-acclimated fish. Physiologically functional actin-activated myosin $Mg^{2+}$-ATPase activity differed markedly between cold- and warm-acclimated carp. The maximum initial velocity ($V_{max}$) at 20 °C was 1.8 $s^{-1}$ at pH 7.0 and 0.05 $mol\,l^{-1}$ KCl for cold-acclimated carp, which was 1.6 times that for warm-acclimated carp (Table 1). These differences were in good agreement with those obtained with myofibrillar $Mg^{2+}$-ATPase activity for carp acclimated to both temperatures. No differences were, however, observed in myosin affinity to actin ($K_m$). Differences in myosin properties between cold- and warm-acclimated carp were further demonstrated by its thermal stability. The inactivation rate constant ($K_D$) of myosin $Ca^{2+}$-ATPase was $7.5\times10^{-4}\,s^{-1}$ at 30 °C and pH 7.0 for carp acclimated to 10 °C, which was approximately twice that for carp acclimated to 30 °C (Fig. 1A), suggesting that myosin from carp acclimated to 10 °C is much more thermally unstable than myosin from carp acclimated to 30 °C.

## Confirmation of changes in the myosin cross-bridge head, S1, in association with temperature acclimation of carp

Myosin S1 was prepared by α-chymotrypsin cleavage from the myosin of carp acclimated to 10 and 30 °C. The objective of these studies was to extend the study of thermally acclimated changes in the myosin molecule (Hwang et al., 1991) to focus on the myosin cross-bridge head. The $Ca^{2+}$-ATPase activity of S1 was higher in the 30 °C- than in the 10 °C-acclimated carp, while the difference in $K^+$(EDTA)-ATPase activity was not significant between the two S1s. The actin-activated S1 $Mg^{2+}$-ATPase activity at 20 °C of the 10 °C-acclimated carp was approximately 1.8 times that of the 30 °C-acclimated fish. The maximum initial velocity of this activity at pH 7.0 in 0.05 $mol\,l^{-1}$ KCl was 9.3 $s^{-1}$ for the 10 °C-acclimated carp S1, approximately 3.7 times that for the 30 °C-acclimated carp S1. However, no significant difference was observed in the apparent affinity of S1 for actin, as was the case for myosin. The inactivation rate constant ($K_D$) of S1 $Ca^{2+}$-ATPase from the 10 °C-accclimated carp was $32.1\times10^{-4}\,s^{-1}$, compared with $13.2\times10^{-4}\,s^{-1}$ for the 30 °C-acclimated carp, demonstrating that the S1 of 10 °C-acclimated carp is 2.4 times less thermostable than that of the 30 °C-

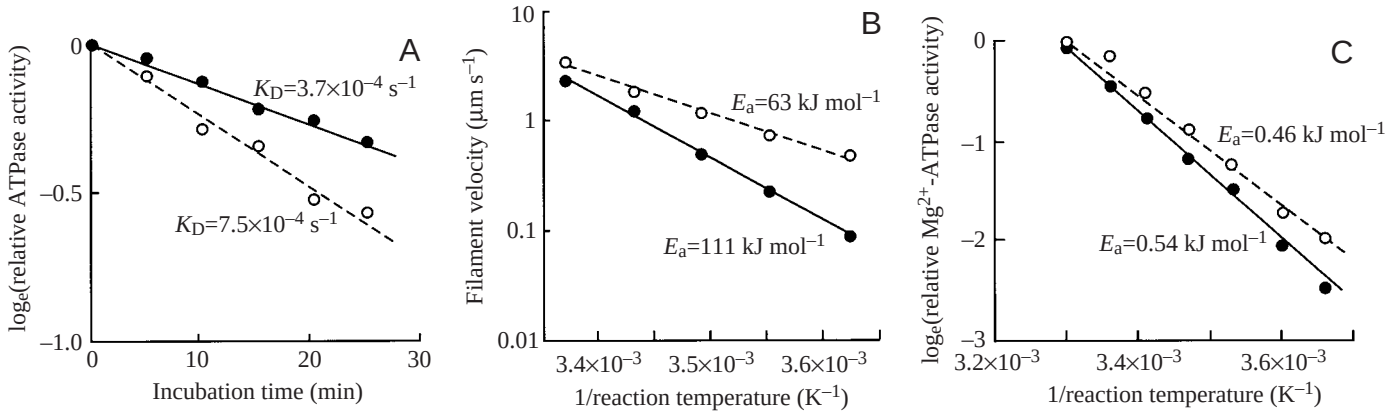


Fig. 1. Structural flexibility and functional differences of myosin isoforms from thermally acclimated carp. (A) A plot of relative $Ca^{2+}$-ATPase activity *versus* incubation time (modified from Watabe et al., 1992) from which the inactivation rate constant ($K_D$) can be derived. (B) Arrhenius plot of the sliding velocity of F-actin on myosin (modified from Chaen et al., 1996) from which the activation energy ($E_a$) for sliding velocity can be derived. (C) Arrhenius plot of actin-activated $Mg^{2+}$-ATPase activity (modified from Watabe et al., 1992) from which the $E_a$ for actin-activated $Mg^{2+}$-ATPase can be derived. Myosin isoforms were prepared from carp acclimated to 10 °C (open circles) and 30 °C (filled circles).

acclimated carp. Such differences in the thermostability of S1 isoforms are roughly consistent with those for myosin isoforms described above.

Polyacrylamide gel electrophoresis in the presence of sodium pyrophosphate ($PP_i$–PAGE) showed that carp acclimated to 10 °C contained four isoforms of chymotryptic S1 (Watabe et al., 1994). Peptide mapping revealed that these consisted of two types of S1 heavy chain, H1 and H2, with different primary structures. Four S1 isoforms in total, H1(A1), H1(A2), H2(A1) and H2(A2), were separated by $PP_i$–PAGE with two associated alkali light chains, A1 and A2. Carp acclimated to 30 °C contained another type of S1 heavy chain, H3, and thus included two S1 isoforms, H3(A1) and H3(A2).

DEAE anion-exchange column chromatography separated these isoforms well, revealing that S1 from carp consisted of three heavy chain isoforms with molecular masses of 96 kDa (H1), 94 kDa (H2) and 92 kDa (H3) (Guo et al., 1994). The composition of these three S1 heavy chain isoforms in carp changed in association with temperature acclimation. The H1 heavy chain was dominant in 10 °C-acclimated carp and responsible for high actin-activated S1 $Mg^{2+}$-ATPase activity and low thermostability. In contrast, the H3 heavy chain predominating in 30 °C-acclimated carp showed low activity and high thermostability. The H2 heavy chain was found in both 10 °C- and 20 °C-acclimated carp, but only at very low levels in 30 °C-acclimated carp. The H1 heavy chain made up approximately 55 % of the total amount of the three heavy chain isoforms in 10 °C-acclimated carp, while the H3 heavy chain made up approximately 85 % of that in 30 °C-acclimated carp (Guo et al., 1994). It is well known that limited proteolysis of S1 with trypsin produces 25, 50 and 20 kDa fragments in order from the N terminus (Harrington and Rodgers, 1984). The H3 heavy chain from 30 °C-acclimated carp produced the three fragments described above (Guo et al., 1994). However, the H1 heavy chain from 10 °C-acclimated carp produced a 23 kDa fragment in addition to these three fragments. N-terminal amino acid sequence analysis suggested that the 23 kDa fragment contained an N-terminal peptide normally found in the C-terminal region of the 50 kDa fragment.

### *In vitro* motility assay of carp myosin isoforms

The temperature-dependency of sliding velocity of fluorescent F-actin was also examined on a slide covered with myosins isolated from 10 °C- and 30 °C-acclimated carp (Chaen et al., 1996). Filament sliding velocity for the myosin from 10 °C-acclimated carp was higher at all measuring temperatures between 3 and 23 °C than that for myosin from 30 °C-acclimated carp (Fig. 1B). For example, the velocity at 3 °C for myosin from 10 °C-acclimated carp was approximately 0.49 μm s$^{-1}$, a value corresponding to that at 13 °C for myosin from 30 °C-acclimated carp. In contrast, myosin from 30 °C-acclimated carp hardly moved on F-actin filaments at 3 °C (0.09 μm s$^{-1}$). Arrhenius plots for the sliding velocity of myosins from 10 °C- and 30 °C-acclimated carp gave activation energies of 63 and 111 kJ mol$^{-1}$, respectively. These differences roughly paralleled those in activation energy of actin-activated $Mg^{2+}$-ATPase activity of approximately 0.46 and 0.54 kJ mol$^{-1}$ for myosins from 10 °C- and 30 °C-acclimated carp, respectively (Fig. 1C) (calculated from the data of Watabe et al., 1992).

These results suggest that the less thermostable, more flexible structure of myosin from cold-acclimated carp has a reduced activation energy for the contractile process, which allows the F-actin to slide fast over myosin filaments even at low temperatures. The present data have shown that the energy barrier for the sliding velocity of F-actin on the myosin from 10 °C-acclimated carp is much lower than that on the myosin from 30 °C-acclimated carp (Fig. 1B). This observation is consistent with the fact that the thermal stability of the myosin

from 10 °C-acclimated carp is reduced in comparison with that for the myosin from 30 °C-acclimated carp (Fig. 1A).

There are numerous data suggesting that the dynamics of the structural fluctuation of the protein molecule and its functional activities are closely related (Huber, 1979; Welch et al., 1982; Karplus and McCammon, 1983; Brooks et al., 1988). It has been observed that the less thermostable a protein, the more flexible its structure (Delpierre et al., 1983; Wrba et al., 1990; Varley and Pain, 1991). Varley and Pain (1991) have shown that, at a given temperature, 3-phosphoglycerate kinase from thermophilic bacteria is more stable, and the activation energy for the kinetic rate of acrylamide quenching of tryptophan fluorescence is lower for the enzyme from mesophilic yeast than for that from the thermophilic bacterium. In the myosin of cold-acclimated carp, increased conformational dynamics resulting from lower thermal stability would reflect the lower activation energy for the sliding process. This low energy barrier for the sliding process would make it possible for carp living at low temperatures to swim as the same speed as those living at warmer temperatures.

A

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10 type              VIQYFATI-AMAGP----KKAEAVPGKMQGSLE
I type               VIQYFATV-AMSGP----KKTEAVPGKMQGSLE
30 type              VIQYFATVGAMSGP----KKPEPVPGKMQGSLE
Chicken skeletal     VIQYFATI-AASGE----KKKEEQSGKMQGTLE
Chicken gizzard      VIQYLAVV-ASSHKGKRTPASLKVHLFPYGELE
Chicken non-muscle   VIQYLAHV-ASSHKSKKDQ----------GELE
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B

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10 type              LALLYVAVP-EA--------EAAG-KKGGKKKGGS
I type               LALLYVAVP-E---------EGGG-KKAGKKKGGS
30 type              LAFLYATHGAEA--------EGGGGKK-GKKKGGS
Chicken skeletal     LALLFATYGGEA--------EGGGGKKGGKKKGSS
Chicken gizzard      VADLWKDVDRIVGLDQMAKMTESSLPSASKTKKGM
Chicken non-muscle   VSELWKDVDRIVGLDQVAGMSETALPGAFKTRKGM
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Fig. 2. Amino acid sequences around loops 1 (A) and 2 (B) in the 10 °C, intermediate (I) and 30 °C types of S1 heavy chain from carp (modified from Hirayama et al., 2000) and in S1 heavy chains of chicken myosin from a variety of tissues. The two loops are boxed.

Nakaya et al. (1995) have reported that, in a differential scanning calorimetric study of a rod portion prepared from the myosin of 10 °C-acclimated carp, the myosin isoform was less thermally stable than that from 30 °C-acclimated carp. The differences in thermal stability between the isoforms are therefore considered to span an entire region of the myosin molecule, although the contribution of a given region, for example S1, of the molecule to the changes in thermal stability might vary. The activation energy of the actin-activated $Mg^{2+}$-ATPase was also lower in cold-acclimated carp myosin, although the difference in $E_a$ between the two carp myosin isoforms, 0.08 kJ mol$^{-1}$, was smaller than the difference in $E_a$ for the sliding velocity, 48 kJ mol$^{-1}$. The difference in the activation energy between the sliding process and actin-activated $Mg^{2+}$-ATPase is consistent with the idea that the rate process that limits sliding velocity is different from that limiting ATPase activity (Siemankowski et al., 1985; Dantzig et al., 1991, 1992). Furthermore, this suggests that the conformational dynamics of a restricted region of myosin governs the mechanical process and the conformational dynamics of the rest of the myosin molecule governs the chemical process.

### cDNA cloning of myosin heavy chain isoforms from thermally acclimated carp

Three types of cDNA clone encoding fast skeletal muscle myosin heavy chains have been isolated from thermally acclimated carp (Watabe et al., 1995; Imai et al., 1997). All clones covered at least the full length of light meromyosin, the C-terminal part of the myosin molecule (Imai et al., 1997). These will be described in more detail in the following section. Three cDNA clones encoding the entire myosin heavy chain were also isolated by extending the 5′ region of the cDNAs described above (Hirayama and Watabe, 1997). Two of the three types were termed the 10 and 30 °C types, since they were the predominant form in carp acclimated to 10 and 30 °C, respectively (Imai et al., 1997). Another type, termed the intermediate type, is intermediate in both the DNA nucleotide sequence and the deduced amino acid sequence between the 10 and 30 °C types. It is not clear at present which clone encodes the H1, H2 or H3 S1 heavy chain isoform isolated by ion-exchange column chromatography. However, it is highly possible that these three S1 heavy chain isoforms correspond to the 10 °C, intermediate and 30 °C types, respectively, in view of their isoform composition determined by elution profiles in ion-exchange column chromatography. DNA nucleotide substitutions occurred throughout the isolated clones, suggesting that different genes exist in carp that encode the three types of cDNA clone. In preliminary experiments using Southern blot analysis, different patterns were found with the 10- and 30 °C-specific carp cDNA probes after digestion of genomic DNA with restriction enzymes (Imai et al., 1997).

Rayment et al. (1993) elucidated the three-dimensional structure of chicken pectoralis muscle S1, showing that ATP is inserted into the cleft formed by the 50 kDa tryptic fragment and located on the opposite side to the actin-binding region of the same fragment. Myosin light chains are bound to the C-terminal region of the α-helical 20 kDa fragment. S1 contains two surface loops, loops 1 and 2. Loop 1, connecting the 25 and 50 kDa tryptic fragments, is situated near the ATP-binding sites, whereas loop 2 is situated on the actin-binding site in the 50 and 20 kDa fragments (Sutoh, 1982, 1983; Chaussepied and Morales, 1988; Rayment et al., 1993). It is known that the two surface loops vary in both length and amino acid sequence between different myosin types, including those from skeletal

and smooth muscle (Yanagisawa et al., 1987; Shohet et al., 1989; Maita et al., 1991; Bobkov et al., 1996).

The amino acid sequences of the entire myosin molecules were deduced from DNA nucleotide sequences for the 10 °C, intermediate and 30 °C types. The three isoforms generally resembled each other in primary structure, showing 96.4, 93.8 and 93.6 % identity between the 10 °C and intermediate types, between the 10 and 30 °C types and between the intermediate and 30 °C types, respectively. In contrast, S1 showed 95.0, 91.9 and 90.9 % identity, respectively, in these comparisons. However, isoform-specific differences were clearly observed between the 10 and 30 °C types in the first 60 amino acid residues from the N terminus, where the intermediate type was intermediate between the sequences of the 10 and 30 °C types (Hirayama and Watabe, 1997). Another striking difference was observed in the two surface loops between the 10 and 30 °C types. Five amino acid residues out of 16 were different in loop 1 near the ATP-binding pocket, and six out of 20 were different in loop 2 on the actin-binding site (Fig. 2). Although the three types of carp S1 showed different amino acid sequences in the two surface loops, both loops were of two skeletal muscle types. The P-loop connecting the β-sheets that are known to surround the ATP-binding pocket had a highly conserved primary structure among the three types. Amino acid substitutions between the 10 and 30 °C types are present not only in the above regions but also in a variety of areas in the motor domain.

In northern blot analysis, the accumulated mRNA levels of the 10 °C and intermediate types were significantly higher in carp acclimated to 10 °C than in carp acclimated to 30 °C, whereas the mRNA levels of the 30 °C type were significantly higher in carp acclimated to 30 °C than in carp acclimated to 10 and 20 °C (Hirayama and Watabe, 1997).

## Functional comparison of loops 1 and 2 of myosin S1 using chimeric myosin consisting of carp loops in a *Dictyostelium discoideum* myosin backbone

As described above, it seems reasonable to speculate that structural differences between the two surface loops of carp myosin heavy chain isoforms affect their motor activities. Chimeric myosins consisting of *Dictyostelium discoideum* myosin backbones with loop sequences of carp S1 heavy chain isoforms were expressed (Hirayama et al., 2000). These chimeras were subjected to $Mg^{2+}$-ATPase activity measurements and *in vitro* motility assays to reveal the structure/function relationship of carp myosin isoforms.

Actin-activated $Mg^{2+}$-ATPase activity was measured for chimeric myosins and for the *Dictyostelium discoideum* wild-type myosin at 20 °C in the absence and presence of various concentrations of F-actin. The two loop-1-associated chimeric myosins, loop 1-10 and loop 1-30, showed $V_{max}$ and $K_m$ values similar to those of *Dictyostelium discoideum* wild-type myosin (Table 1). These results were consistent with the data reported by Murphy and Spudich (1998) for *Dictyostelium discoideum* chimeric myosins containing loop 1 of rabbit skeletal or *Acanthamoeba* myosin, which showed that chimeric substitutions of loop 1 did not affect the $V_{max}$ of actin-activated $Mg^{2+}$-ATPase activity. However, the $V_{max}$ of actin-activated $Mg^{2+}$-ATPase of the loop 2-10 myosin was 1.4 times that of the loop 2–30 myosin, although the $K_m$ values for actin were not significantly different (Table 1). In contrast, all chimeric myosins showed similar sliding velocities in *in vitro* motility assay (Table 2). These results were clearly different from previous findings that the loop 1 structure affected the sliding velocity of actin filaments (Murphy and Spudich, 1998; Bobkov et al., 1996; Kelley et al., 1993). As described above, myosin prepared from carp acclimated to 10 °C moved over actin filaments faster than that from carp acclimated to 30 °C. The differences in motility of carp myosin isoforms are therefore probably caused by amino acid substitutions in regions other than loops 1 and 2.

Table 2. In vitro *motility assay for carp myosin isoforms and their chimera*

| Myosin | Motility ($\mu m\ s^{-1}$) | Chimera | Motility ($\mu m\ s^{-1}$) |
|---|---|---|---|
| 10 °C-acclimated carp | 3.32 | Loop 1-10 | 1.30 |
| | | Loop 2-10 | 1.29 |
| 30 °C-acclimated carp | 2.25 | Loop 2-10 | 1.27 |
| | | Loop 2-30 | 1.29 |
| | | Wild type | 1.29 |

*In vitro* motility assay was carried out with actin-containing filaments over glass slides covered with myosin.

Myosins were prepared from fast skeletal muscle of carp acclimated to 10 and 30 °C (Chaen et al., 1996).

Chimeric myosins were prepared from *Dictyostelium discoideum* backbone with loop 1 sequences from carp 10 °C- (loop 1-10) and 30 °C-type S1 (loop 1-30) and with loop 2 sequences from carp 10 °C- (loop 2-10) and 30 °C-type S1 (loop 2-30).

## Concluding remarks

The myosin heavy chain isoforms of carp changed following temperature acclimation. Cold acclimation induced an increase in actin-activated myosin $Mg^{2+}$-ATPase activity and *in vitro* motility of F-actin on myosin sheets, suggesting a correlation with the improved swimming performance at low temperatures after thermal acclimation reported previously. The carp myosin heavy chain isoforms differed from each other at the level of their primary structure, as was the case with the myosin cross-bridge head, S1, which has a motor function. The results obtained with chimeric myosins constructed from *Dictyostelium discoideum* heavy chain backbone with carp loops 1 and 2 inserted into the S1 heavy chain partly explained the differences in actin-activated $Mg^{2+}$-ATPase activity, but not those in *in vitro* motility. It appears that certain amino acid substitutions in the S1 heavy chain, other than those in loops 1 and 2, that occur in the myosin from cold- and warm-acclimated carp are responsible for the changes in myosin motor function, which is closely related to the muscle plasticity

of thermally acclimated carp. However, carp S1 heavy chain isoforms contain many substitutions that may affect their motor function cooperatively. It is difficult at present to understand the contribution of all amino acid substitutions to the functional changes in myosin isoforms using conventional recombinant techniques. The accumulation of knowledge concerning variations in myosin heavy chain from a variety of origins may give new insight into such ambiguities.

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