Temperature-induced Precocious Transitions of Myosin Heavy Chain Isoforms in the White Muscle of the Arctic Charr, *Salvelinus alpinus* (L.)

Iciar Martinez and Gerd Wibeche Pettersen

Norwegian Institute of Fisheries and Aquaculture, Tromsø, Norway

Abstract

The myosin heavy chain isoforms from white muscles of Arctic charr (*Salvelinus alpinus* (L.), reared in freshwater during two years at either natural temperature (fluctuating between about 0.2°C in winter and 11°C in summer) or at a constant temperature of 10°C, were analyzed by α-chymotryptic peptide and immunopeptide mapping of electrophoretically isolated myosin heavy chain bands. Fish reared at constant 10°C had myosin heavy chains whose peptide maps were different from those of the same age reared under natural temperature, but similar to those of one year older charrs reared under natural temperature. Our results indicate that during the period under study, warmer temperatures seem to precociously induce the sequential transitions in the expression of myosin heavy chains which take place during the normal development of the white muscle in this species and not the expression of an alternative specifically "warm"- or "low"-temperature-adapted type of myosin heavy chain.

Key words: Myosin; temperature; muscle development; fish; charr.

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Myosin is a hexameric protein consisting of two heavy chains and four light chains. Differences in the heavy or in the light chain subunits give rise to different myosin isoforms which may have similar or different ATPase activities [35]. During development of skeletal muscles, different myosin heavy chains are expressed in a sequential fashion in mammals [14, 23, 41], birds [42], amphibians [8, 13] and fish [9, 25]. The expression of the genes encoding for the different myosin heavy chain isoforms has been shown to be regulated by hormonal and neural factors [6, 8, 24, 29, 32, 35, 40].

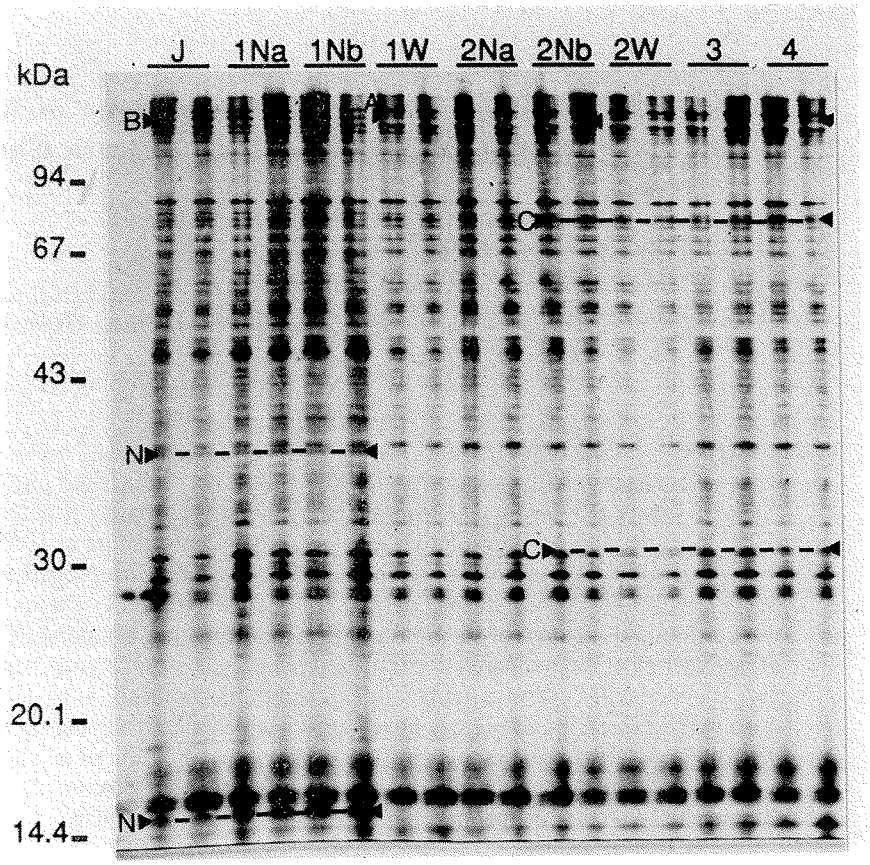
Due to their inability to regulate body temperature, freshwater fish may suffer large seasonal fluctuations and yet remain active throughout the year. One of the biochemical mechanisms involved in temperature adaptation is the switch between isoenzymes [19]. Because of the polymorphic nature of the myosin heavy chain, the possibility of a similar isoenzyme switch depending on the temperature has been examined in the carp (*Cyprinus carpio*, L.) by several authors [11, 18, 21]. However, the results from these studies are controversial, and while some authors found that there seemed to be a change in the type of myosin heavy chains expressed in white muscles depending on the acclimation temperature [18, 21], others did not [11].

Salvelinus alpinus held under constant temperature conditions display maximal growth rate at temperatures between 12-16°C [22, 34]. However, natural temperatures may vary between 0.2°C and 11°C in the Tromsø area. Therefore, we wished to assess the effect of temperature on the expression of myosin heavy chain isoforms during development of the white muscle in this species. The results seem to indicate that at constantly higher temperatures the transitions in the expression of myosin heavy chain isoforms which take place during normal development of the white muscle are precociously induced. On the other hand, we did not find evidence to sustain the presence of specifically "warm"- or "cold"-temperature adapted myosin heavy chain isoforms.

Materials and Methods

Animal samples

Arctic charr, *Salvelinus alpinus* (L.) of the anadromous Hammerfest strain were hatchery-reared under natural temperature until the beginning of the free swimming and exogenous feeding stage (sample J). Then some fish were transferred to tanks were the temperature of the water was kept constant at about 10°C during the rest of the period under study, while others were transferred to tanks receiving water at natural temperature. Table 1 shows the aver-



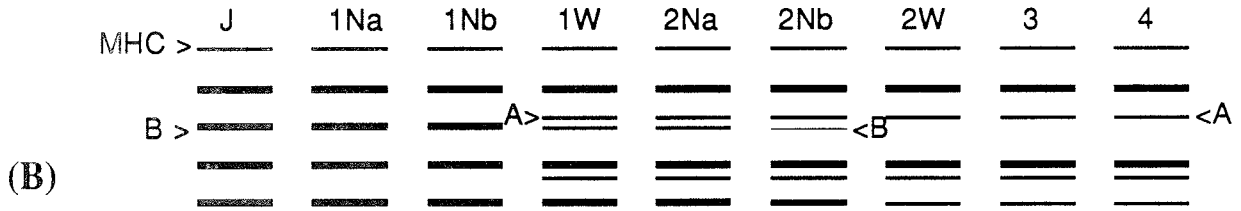


Figure 1. A) Silver stained α-chymotryptic polypeptide mapping (15% polyacrylamide) of electrophoretically isolated myosin heavy chain bands from pooled samples of each group. Arrow indicates the position of the myosin heavy chain. Name of the samples as in table 2. The pattern of the samples reared at 10°C was similar to one year older charrs reared at natural temperature. N, fragments corresponding to the neonatal [25] myosin heavy chain, A, B and C, fragments referred to in the text. kDa, molecular masses of the standards: phosphorylase b (94 kDa), albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa) and A-lactalbumin (14.4 kDa). B) Schematic drawing of the upper part of the gel shown in A).

age temperatures for the years covered by this study and table 2 the data on the Arctic charr used for this work.

Myosin extraction

Myosin from the deep white muscle under the dorsal fin was extracted according to [12]. The protein content in the samples was determined by the Bradford method [4] using bovine serum albumin as the standard.

Myosin heavy chain purification and peptide mapping

The myosin heavy chains contained in 1 µg of total protein were isolated by SDS-5%PAGE on gels containing 37.5% glycerol [7, 9]. Piperazine diacrylamide was used as crosslinker [20]. Gels were stained with Coomassie

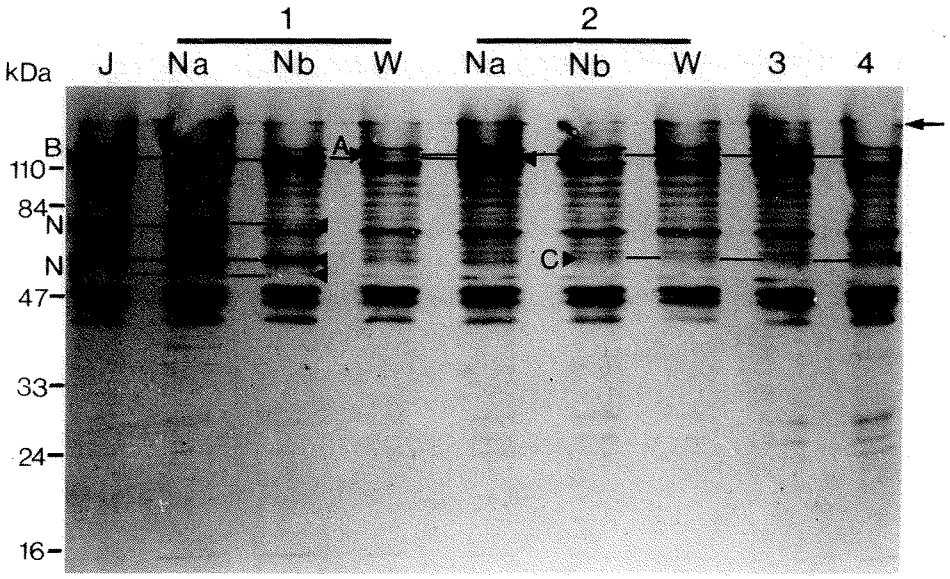


Figure 2. Immunoreactivities of α-chymotrypic myosin heavy chain fragments (resolved in 12.5% polyacrylamide gels) with IgG anti-myosin heavy chain from a pool of 2-year-old Arctic charr white muscles. Symbols and samples as in Figure 1. Some peptides are labelled as in Figure 1 to illustrate the same features, although they may not correspond to the same actual fragment.

brilliant blue R-250 for 2 min and destained in distilled water. Slices of the gel containing the myosin heavy chains were cut and peptide mapping was performed on the isolated myosin heavy chains using 80 ng of α-chymotrypsin from bovine pancreas according to [10] as described in [25], per half slice of myosin heavy chain. Separating gels contained 15% acrylamide and 0.087% piperazine diacrylamide [1, 20]. The gels were silver stained [2].

Antibodies

The myosin heavy chain from a pool of white muscles from 2-year-old charrs was electrophoretically isolated on 36% glycerol, 6% polyacrylamide gels as described above. The slice of gel containing the myosin heavy chain was visualized by immersion in 5% $CuCl_2.2H_2O$, cut, washed and sonicated in phosphate buffered saline, and used to immunize a rabbit. The IgG fraction of the serum was affinity purified on a protein A column (Pharmacia). At the used concentration of 5 µg IgG/ml, these polyclonal antibodies recognize, in immunoblots, whole myosin heavy chains from red and white muscles of several fish species and of fast and slow muscles of the rat, as well as some of their proteolytic fragments.

Immunoblotting

Myosin heavy chain purification and peptide mapping was carried out as above with the following modifications. One slice containing the myosin heavy chain of 1 µg (total protein) of the myosin extract was loaded into the sample wells of slab gels (7 cm x 8 cm) with a 12.5% polyacrylamide separating gel [1, 20]. The myosin heavy chains were incubated with 160 ng of α-chymotrypsin for 20 min. The proteolytic fragments were transferred to nitrocellulose according to [38]. The blots were incubated for 1 h in 5 µg/ml of the above described primary antibodies. Secondary antibody was peroxidase labelled goat anti-rabbit IgG (Sigma) and 3,3'-diaminobenzidine was used as substrate with Co^{2+} enhancement.

Results

Only one myosin heavy chain was detected by SDS-PAGE in the presence of glycerol in white muscles from Arctic charr reared either under natural temperature [25] or at constant 10°C (not shown). Since it has been documented that there may be different myosin heavy chains displaying very similar [30, 36], or even equal [3, 25, 33], electrophoretic mobilities in this system, peptide mapping analysis of the myosin heavy chain bands was performed in order to establish whether the rearing temperature had an effect on the type of myosin heavy chain expressed in the white muscle.

Figures 1 and 2 show the α-chymotryptic peptide and immunopeptide mappings respectively, of the electrophoretically isolated myosin heavy chain bands. For comparison, some peptides have been labelled in both figures with the same letter, although they may not correspond to the same actual peptide. In every case, the peptide pattern of the myosin from fish reared at 10°C was indistinguishable from the pattern of one year older charrs reared under natural temperature (lanes 1W and 2Na and lanes 2W and 3 in Figures 1 and 2).

The most striking difference was the precocious disappearance of the neonatal [25] myosin heavy chain from muscles of 1-year-old charrs reared at 10°C, as shown by the absence of "N" peptides from the sample 1W, which is

Table 1. Average natural temperatures in °C during the years covered by the study

Month	1989	1990	1991	1992
January	0.7	0.6	0.3	0.4
February	0.7	0.5	0.2	
March	1	0.3	0.2	
April	1.5	1.3	0.9	
May	2.5	2.2	3.4	
June	3.8	4.2	6.9	
July	6.1	7.1	10.1	
August	8.2	8.5	10.9	
September	6.3	-	7.5	
October	3.1	2.6	3.3	
November	1.9	0.6	0.9	
December	0.9	0.4	0.6	

-, data not available

best seen in Figure 2. These fragments were clearly detectable in charrs of the same age reared under natural temperature. In addition, fragment A, from the myosin heavy chain type II-2 [25], is already present at 1 year of age when reared at 10°C, but undetectable until 2 years under natural temperature (lanes 1Na, 1Nb, 1W and 2Na, Figures 1 and 2).

Among the 2-year-old charrs reared at natural temperature, one group (2Nb) suffered during the previous summer temperatures of 10-11°C (table 1). In this group, the fragments labelled C, considered to arise from the myosin heavy chain type II-3 [25] were already detectable, as they were in charrs of the same age reared at 10°C and older charrs (lanes 2Nb, 2W and 3, Figures 1 and 2), but not in charrs of the same age reared under natural temperature (lane 2Na, Figures 1 and 2). In addition, peptide B (possibly from the myosin heavy chain type II-1 [25]) was still present at 2 years of age when reared under natural temperature (lane 2Na, Figures 1 and 2) but was undetectable in charrs of the same age reared at 10°C, as well as in older charrs (lanes 2W, 3 and 4, Figures 1 and 2) and in those charrs which suffered a warmer summer (Lane 2Nb, Figures 1 and 2).

However, the peptide patterns of these myosin heavy chains could not be grouped according to the rearing temperature, as could be expected if temperature acclimation was the main determinant of the myosin heavy chain phenotype. Thus, charrs reared under the same temperature conditions did not display the same peptide pattern: samples 1Na, and 2Na were different as well as samples 1Nb and 2Nb, and samples 1W and 2W.

Discussion

The type of myosin heavy chain expressed in the white muscle of fish may be affected by temperature acclimation in several, not mutually exclusive, ways. One possibility is a switch between "cold"- or "warm"-temperature adapted isoforms [19]. We did not find any evidence to sustain this possibility, since the peptide maps of myosin heavy chains from the white muscles of Arctic charr did not show a temperature-dependent pattern (Figs. 1 and 2).

A second possibility may be a fast-to-slow transition induced by low temperature. Rome *et al.* [31] showed that carp compensates the loss of mechanical power at low temperatures by recruiting more and faster fibre types. Increased muscular activity, or chronic electrical stimulation of fast muscles, favours fast-to-slow transitions [28] and induces a rearrangement of the fast myosin isoform pattern [37]. However, it is not known how many different myosin heavy chains are expressed in white muscles of fish, although it has been shown that there is more than one in some species [9, 25, 26, 27]. Therefore, although this type of neural influence is likely to have an effect on the expression of fish myosin heavy chains as well, assessment of its real effect with the available data would be fairly speculative.

A third and novel possible explanation, proposed here, would be a speeding up in muscle development induced by warmer temperatures. During development of the white muscles of fish [9, 25] several myosin heavy chains are sequentially expressed. However, the white muscles of fish [9, 25], as well as the rat diaphragm [23] do not seem to acquire the final adult phenotype immediately after the elimination of the early embryonic and neonatal developmental isoforms and production of the adult types. Rather, activation of some "adult" myosin heavy chain forms may be delayed in both the rat [23] and fish [9, 25].

Thyroid hormones induce precociously the expression of the fast-IIb myosin heavy chain in the rat [32] and seem to induce differentiation of adult fibre types in amphibians [8]. However, perinatal undernutrition delays the neonatal-to-adult fast myosin heavy chain transition and lowers

Table 2. Data on the fish samples. Arctic charr, Salvelinus alpinus *(L.) of the anadromus Hammerfest strain were used. N, natural environmental temperature. Age is given in months after hatching. Lenght and mass expressed as mean or mean ± SD.* n, *number of charrs used.*

Sample	Temperature	Age	Length (cm)	Mass (g)	*n*	Sampling
J*	N	3	1.9	~ 0.06	25	April, 1991
1Na**	N	11	5.40 ± 1.40	1.25 ± 0.86	10	December, 1989
1Nb	N	12	7.38 ± 0.45	3.17 ± 0.65	10	January, 1992
1W	10°C	12	12.05 ± 0.96	17.46 ± 5.01	10	January, 1992
2Na	N	23	13.52 ± 1.93	25.20 ± 10.84	10	December, 1989
2Nb	N	22/24	15.30 ± 0.82	35.65 ± 4.99	10	November, 1991 and January, 1992
2W	10°C	22	20.50 ± 0.28	91.15 ± 3.99	10	November, 1991
3	N	35	19.20 ± 1.46	73.38 ± 16.87	10	December, 1989
4	N	47	36.25 ± 2.49	547.68 ± 127.16	10	December, 1989

*, Juveniles immediately after resorption of the yolksac and beggining exogenous feeding and free swimming.
**, Names of the samples are: the approximate age in years (1 to 4), followed by N or W to indicate that they were reared at warmer temperatures respectively, and *a* or *b* to differentiate the two groups reared under natural temperature.

the levels of serum T_3 in the rat [5]. Food intake in Arctic charr in also reduced at lower temperatures [22]. The number of T_3 receptors in nuclei of skeletal muscle in piglets was found to be reduced by low temperature and low energy intake [15]. Warmer temperatures, on the other hand, increase food intake and growth in Arctic charr [22 and present results: table 1], the levels of thyroid hormone in rainbow trout (*Onchorhynchus mykiss*) [17] and the rate of secretion of growth hormone in fish [39]. It has been shown that growth hormone increases peripheral conversion of thyroxine to T_3 in the eel [16]. It is therefore possible that either the levels of thyroid or growth hormones or the tissue responsiveness to these hormones may be increased by an increase in temperature.

Keeping in mind the differences between species, our results may help to understand some of the discrepancies observed in the carp [11, 18, 21] and add an alternative new explanation. Studies at the mRNA level in the white muscle of small (65-85g) carps revealed an increase in the rate of synthesis of a particular, but unidentified, type of myosin heavy chain in all carps acclimated at 28°C, in some acclimated at 18°C but not in those acclimated at 10°C [18]. However, no study has been undertaken to examine whether or not, and how many, different myosin heavy chains are sequentially expressed during muscle development in this species, which seems to be the case in fast skeletal muscles of all species examined [8, 9, 13, 14, 23, 25, 35, 41, 42]. It is possible, therefore, that the increased mRNA levels corresponded to the "next" type of myosin heavy chain following a normal sequential pattern of expression. Contradictory results regarding the type of myosin heavy chain present in muscles of older (400-700g) carps [11, 21] can be explained because small differences can be obscured either by technical limitations or sampling, mainly since the previous life-history of the fish seems to be of consequence in these type of studies, as illustrated in this work by the groups 2Na and 2Nb.

In summary, the major effect of constant 10°C rearing had on the myosin phenotype in the white muscle of the Arctic charr, during the period under study, was to precociously induce the transitions in myosin heavy chain expression which take place during the normal development of the white muscle. The peptide and immunopeptide maps of the myosin heavy chains were dependant on the age of the fish and at 10°C they were simply similar to those of older charrs reared at natural temperature.

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Address correspondence to:

Dr. I. Martinez, Norwegian Institute of Fisheries and Aquaculture, PO Box 2511, 9002 Tromsø, Norway.

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