The Influence of Habitat Water Temperature on the Kinetic Properties of Ca^{2+} - and Mg^{2+} -ATPase of Myofibrils from Marine Fishes

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The Michaelis constants (K_m), maximum velocity (V_{max}), and temperature dependence of myofibrillar Ca^{2+} - and Mg^{2+} -ATPase were examined using fishes in the sea around Sakishima Islands inhabiting at 28°C and in the Yellow Sea inhabiting at 9°C. The K_m values of myofibrillar Ca^{2+} -ATPase were lower with fishes from Yellow Sea than with fishes from Sakishima Islands, while K_m values of myofibrillar Mg^{2+} -ATPase did not differ between fishes from Yellow Sea and Sakishima Islands. The V_{max} levels of myofibrillar Ca^{2+} -ATPase did not differ between fishes from Yellow Sea and Sakishima Islands, but those of myofibrillar Mg^{2+} -ATPase were higher in fishes from Yellow Sea than in fishes from Sakishima Islands at reaction temperatures of 10°C and 30°C. Temperature dependency of myofibrillar Ca^{2+} -ATPase activity in a saturated substrate concentration was not different between fishes from Yellow Sea and Sakishima Islands, but that of myofibrillar Mg^{2+} -ATPase activity was smaller with fishes from Yellow Sea than with fishes from Sakishima Islands. The differences in Mg^{2+} -ATPase with varying habitat temperature indicate the existence of an efficient mechanism for locomotory activity in fishes living in lower habitat temperatures.

The body temperature of fish differs almost exactly according to habitat water temperature. Fishes living in low water temperatures such as 0°C, however, can be just as active in swimming, feeding and growing as fishes living in higher water temperatures. The mechanism of how fishes living in low water temperatures produce this energy has been a subject of great interest among researchers.

In a previous paper,¹⁾ we reported the effects of rearing water temperature on Ca^{2+} - and Mg^{2+} -ATPase activity of myofibrils from carp reared at 10°C and 30°C, which can adapt to a wide range of environmental temperatures. In that study, Michaelis constant (K_m), maximum velocity (V_{max}), and temperature dependence of both myofibrillar Ca^{2+} - and Mg^{2+} -ATPase were different between a group reared at 10°C and a group reared at 30°C, although the degree of difference varied with each experimental item. We considered that the findings on myofibrillar Mg^{2+} -ATPase, in particular, indicated the existence of an efficient mechanism for the production of locomotive energy in carp living at low water

temperatures. Unlike freshwater carp, most marine fishes can adapt to only a very narrow range of water temperatures and therefore live only in a limited area. In other words, fish have a variety of species which are characteristic to their respective environmental temperatures. Intensive studies have been carried out with regard to how myofibrillar Ca^{2+} - and Mg^{2+} -ATPase of fishes varied depending on their habitat temperatures.²⁻⁸⁾ However, these relationships have not been completely elucidated. We tried to clarify them using fishes from two locations at different water temperatures.

Experimental Methods

Fish Samples

We used fishes collected from the sea around Sakishima Islands (28°C) and from the Yellow Sea (9°C) shown in Fig. 1. The details are shown in Table 1. The water temperature in both waters was measured with a STD (salinity, temperature, and depth recorder: T. S. MICOM model). The fishes were held aboard ships in

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sea-water tanks at habitat temperature, and then were brought to the laboratory. The fishes were killed by cutting the hindbrain in laboratory, and then white muscle adjacent to the dorsal fin was taken to prepare myofibrils.

Preparation of Myofibrils

We followed the method of Kato *et al.*¹⁰⁾ in preparation of myofibrils as described in the previous paper.¹⁾ The prepared myofibrils were stored on ice, and all ATPase assays were finished within 8h after the preparation.

ATPase Activity

Km and Vmax of myofibrillar Ca^{2+} - and Mg^{2+} -ATPase in experiment 1 and temperature dependence of the activities in experiment 2 were measured.

We followed the method of Kato *et al.*¹⁰⁾ for myofibrillar Ca^{2+} - and Mg^{2+} -ATPase assay as described in the previous paper.¹⁾ In experiment 1, 0.5 ml of myofibrils adjusted to approximately 0.9 mg/ml, were pipetted into test tubes, and then 4 ml of 0.6 M KCl (for myofibrillar Ca^{2+} -ATPase) or 0.1 M KCl (for myofibrillar Mg^{2+} -ATPase), 25 mM Tris-maleate buffer (pH 7.0), and 5 mM CaCl_2 (for myofibrillar Ca^{2+} -ATPase) or 1 mM

Fig. 1. A chart showing the fishing grounds where the sample fishes were collected. Bottom water temperatures are also given.

Table 1. A list of fishes collected from the sea around Sakishima Islands at 28°C and the Yellow Sea at 9°C, and numbers used for Km and Vmax of myofibrillar Ca^{2+} - and Mg^{2+} -ATPases in experiment 1 and temperature dependency of the activities in experiment 2

Area	Code no.	Japanese Common name	Common name (Scientific name)	Numbers used		
				Exper. 1	Exper. 2	Total
Sakishima Islands	1	Nokogiridai	Gold-lined sea-bream <i>Gnathodentex aurolineatus</i>	2	1	3
	2	Hooakakuchibi	Pierrot emperor <i>Lethrinus rubrioperculatus</i>	1	—	1
	3	Amifuefuki	Half-belted emperor <i>Lethrinus semicinctus</i>	2	4	6
	4	Momoitoyori	Person's butterfly bream <i>Nemipterus peronii</i>	1	—	1
	5	Akahata	Black-tipped grouper <i>Epinephelus fasciatus</i>	1	—	1
	5			7	5	12
Yellow Sea	6	Ainame	Greeling <i>Hexagrammos otakii</i>	1	3	4
	7	Kurosoi	Rockfish <i>Sebastes schlegeli</i>	1	2	3
	8	Magarei	Brown sole <i>Limanda herzensteini</i>	1	3	4
	3			3	8	11
Total	8			10	13	23

MgCl₂ (for myofibrillar Mg²⁺-ATPase) were added. The reaction was initiated by the addition of 0.5 ml of ATP at designed concentrations. The reaction was terminated using 1 ml of 40% PCA. Precipitated protein was removed by centrifugation, and ATP and ADP was extracted.¹⁰⁾ The extracts were analyzed by our method¹¹⁾ and ATPase activity was determined in experiment 1. ATPase activity was determined by the amount of Pi released in experiment 2.¹²⁾ In experiment 1, Km and Vmax were determined from the activity in low ATP concentration (0.02, 0.03, 0.04, 0.05, 0.1, and 0.2 mM) by myofibrillar Ca²⁺- and Mg²⁺-ATPase. The reaction temperatures were 30°C and 10°C. For myofibrillar Ca²⁺-ATPase, the reaction periods were 0, 0.5, 1.0, 1.5, 2.0, and 2.5 min at 30°C, and 0, 1.0, 1.5, 2.0, and 3.0 min at 10°C. For myofibrillar Mg²⁺-ATPase, the reaction periods were 0, 0.5, 1.0, 1.5, 2.0, and 2.5 min at 30°C, and 0, 1.0, 2.0, 3.0, 4.0, and 5.0 min at 10°C. The increase of the product was in a zero-order reaction with myofibrillar Ca²⁺-ATPase and in a first-order reaction with myofibrillar Mg²⁺-ATPase. The initial velocity was calculated as described in the previous paper.^{1,13)} ATPase activity was expressed as the amount of ATP dephosphorylated in a unit time ($\mu\text{mol/min}$) for 1 mg of myofibrillar protein. Km and Vmax were determined from the activities in low ATP concentrations using Lineweaver-Burk plot. In experiment 2, specific activity and its temperature dependance were measured in the same medium as experiment 1 except that 1 mM ATP was used. The reaction temperatures were 5, 10, 15, 20, 25, and 30°C.

Results

Michaelis Constants (Km) and Vmax of Myofibrillar Ca²⁺-ATPase

In Table 2 are the Km and Vmax of myofibrillar Ca²⁺-ATPase at 30°C and 10°C in fishes collected from the sea around Sakishima Islands and from the Yellow Sea.

Comparison of the mean values of Km at 30°C showed that the value for fishes from the Yellow Sea was under one half that for Sakishima Islands, although the difference was not significant statistically. When the fish species were compared, the values of both greeling and brown sole from Yellow Sea were lower than those of all fish species from Sakishima Islands. Rock fish gave the highest value among fishes from Yellow Sea and was close to the lowest value among fishes from Sakishima Islands. At 10°C, the mean Km value of fishes from Yellow Sea was about one half that from Sakishima Islands and the difference between them was significant ($p < 0.05$). (Moreover, Km values of three fish species from Yellow Sea were lower than any other fish species from Sakishima Islands) These results suggest that the enzyme-substrate affinity is much stronger for myofibrils from fish living in low water temperatures than those from fish living in high water temperatures.

However the differences in the mean values of Vmax were not significant between fish groups from Sakishima Islands and Yellow Sea at a reaction temperature of either 30°C or 10°C, though the level in the fish species were different.

The relationship between Km and presumption Vmax for myofibrillar Ca²⁺-ATPase from each

Table 2. Km and Vmax of myofibrillar Ca²⁺-ATPase at reaction temperatures of 30°C and 10°C with fishes in the sea around the Sakishima Islands and the Yellow Sea

Area	Code no.	Common name	30°C		10°C	
			Km (mM)	Vmax ($\mu\text{mol Pi/min/mg}$)	Km (mM)	Vmax ($\mu\text{mol Pi/min/mg}$)
Sakishima Islands	1	Gold-lined sea-bream	0.1068	0.449	0.1623	0.387
	2	Pierrot emperor	0.1531	0.445	0.0888	0.195
	3	Half-belted emperor	0.0897	0.398	0.1545	0.344
	4	Person's butterfly bream	0.1852	0.660	0.1407	0.336
	5	Black-tipped grouper	0.0579	0.266	0.0779	0.188
Mean			0.1185	0.444	0.1248	0.290
$\pm$ S.D.			± 0.0507	± 0.142	± 0.0388	± 0.092
Yellow Sea	6	Greeling	0.0470	0.383	0.0705	0.248
	7	Rockfish	0.0658	0.398	0.0620	0.194
	8	Brown sole	0.0322	0.275	0.0683	0.214
Mean			0.0483	0.352	0.0669	0.219
$\pm$ S.D.			± 0.0168	± 0.067	± 0.0044	± 0.027

Fig. 2. The relationship between K_m and V_{max} of myofibrillar Ca^{2+} -ATPase for fishes from the sea around Sakishima Islands at 28°C and the Yellow Sea at 9°C at reaction temperatures of 30°C and 10°C. The open and closed symbols show fishes from the sea around the Sakishima Islands and the Yellow Sea, respectively, and the inserted numerals on each symbol represent the code number of fish species shown in Tables 1 and 2. The solid line represents the regression line calculated from the data for five species from the sea around Sakishima Islands.

fish species is shown in Fig. 2. With regard to fish species in Sakishima Islands, a significant positive correlation ($p < 0.05$ at 30°C, $p < 0.001$ at 10°C) were shown. Fishes from Sakishima Islands having a high K_m value gave a high V_{max} value, and the correlation between these two values was very high. Fishes from Yellow Sea failed to fit with the regression line for fishes from Sakishima Islands at 30°C or 10°C. This was because K_m values for a high group from the Yellow Sea were lower than those from the Sakishima Islands, while V_{max} value did not differ significantly from each other between both groups as shown in Table 2. These results indicate that the obvious difference of K_m values between Yellow Sea and Sakishima Islands is not caused only by the difference of fish species between the two waters.

Temperature Dependence of Myofibrillar Ca^{2+} -ATPase

Myofibrillar Ca^{2+} -ATPase activities at various reaction temperatures are shown as Arrhenius plots in Fig. 3. The activity decreased with decreasing reaction temperature in all fish species. A logarithmic plot of the activity against reaction temperature did not result in a straight line as the slope turned slow when the reaction temperature was low. In any case, the temperature dependence of myofibrillar Ca^{2+} -ATPase activity does not differ significantly between fishes from Yellow Sea and Sakishima Islands.

Fig. 3. The Arrhenius plot of myofibrillar Ca^{2+} -ATPase activity with the reaction temperature. The solid and dotted lines with the mean values for the activity represent fishes in the sea around the Sakishima Islands at 28°C and the Yellow Sea at 9°C, respectively. The symbols and numerals are the same as shown in Fig. 1 and Table 2.

Michaelis Constants (K_m) and V_{max} of Myofibrillar Mg^{2+} -ATPase

The K_m and V_{max} of myofibrillar Mg^{2+} -ATPase at 30°C and 10°C with fishes from Yellow Sea and Sakishima Islands are shown in Table 3. The differences in mean values of K_m were not significant between fishes from Yellow Sea and Sakishima Islands at either temperatures of 30°C

Table 3. K_m and V_{max} of myofibrillar Mg^{2+} -ATPase at reaction temperatures of 30°C and 10°C with fishes in the sea around the Sakishima Islands and the Yellow Sea

Area	Code no.	Common name	30°C		10°C	
			Km (mm)	Vmax (μmol Pi/min/mg)	Km (mm)	Vmax (μmol Pi/min/mg)
Sakishima Islands	1	Gold-lined sea-bream	0.0335	0.330	0.0504	0.130
	2	Pierrot emperor	0.0546	0.362	—	—
	3	Half-belted emperor	0.0626	0.368	0.0370	0.225
	4	Person's butterfly bream	0.0981	0.623	0.0976	0.254
	5	Black-tipped grouper	0.0390	0.504	0.0354	0.147
		Mean	0.0576	0.437	0.0551	0.189
		±S.D.	±0.0255	±0.123	±0.0291	±0.060
Yellow Sea	6	Greeling	0.0501	0.696	0.0251	0.253
	7	Rockfish	0.0453	0.584	0.1323	0.468
	8	Brown sole	0.0450	0.820	0.0486	0.344
		Mean	0.0468	0.700	0.0687	0.355
		±S.D.	±0.0029	±0.118	±0.0563	±0.108

Fig. 4. The relationship between K_m and V_{max} of myofibrillar Mg^{2+} -ATPase for fishes from the sea around Sakishima Islands at 28°C and the Yellow Sea at 9°C at reaction temperatures of 30°C and 10°C. The open and closed symbols show fishes from the sea around the Sakishima Islands and the Yellow Sea, respectively. The inserted numerals on each symbol represent the code number of fish species as shown in Tables 1 and 2.

or 10°C, and the K_m level of fish species also tended not to be different between Yellow Sea and Sakishima Islands.

The mean values of V_{max} with Yellow Sea fishes were about two times higher than those of Sakishima Islands fishes at 30°C and 10°C, and the difference was significant ($p < 0.05$) at both reaction temperatures. V_{max} levels of fish species from Yellow Sea were all higher than those from Sakishima Islands.

The relationship between K_m and V_{max} of myofibrillar Mg^{2+} -ATPase for each fish species is shown in Fig. 4. No correlation was seen between two values at either 30°C or 10°C. This

was different from the case with myofibrillar Ca^{2+} -ATPase (Fig. 2).

The V_{max} level at reaction temperature near the habitat temperature was slightly lower with fishes from Yellow Sea (10°C, 0.355 μ mol/min/mg) than with fishes from Sakishima Islands (30°C, 0.437 μ mol/min/mg). The difference, however, was not significant between both fish groups. These result were clearly different from those for myofibrillar Ca^{2+} -ATPase (Table 2).

Temperature Dependence of Myofibrillar Mg^{2+} -ATPase

Myofibrillar Mg^{2+} -ATPase activities at various

Fig. 5. The Arrhenius plot of myofibrillar Mg^{2+} -ATPase activity with the reaction temperature. The solid and dotted lines with the mean values for the activity represent fishes in the sea around the Sakishima Islands and the Yellow Sea, respectively. The symbols and numerals are the same as shown in Fig. 2 and Table 2.

reaction temperatures are shown as Arrhenius plot in Fig. 5. The plot of the common logarithm of the activity against $10^3/T$ gave rise to two phases changing the slope near $10^\circ C$ in all fish species. The slope in the temperature range from $10^\circ C$ to $30^\circ C$ were smaller than those less than $10^\circ C$.

The slope of the regression line between $10^\circ C$ and $30^\circ C$ is shown in Fig. 6. Most slopes for fishes from the Yellow Sea were significantly

smaller than those from the Sakishima Islands ($p < 0.05$) except for an insignificant relationship between rockfish in the Yellow Sea and gold-lined sea-bream in the Sakishima Islands. Comparison within fishes from Yellow Sea gave rise to no significant differences in the slope of the regression line. The difference in the mean values of the slope was also significant between fishes from Sakishima Islands and Yellow Sea ($p < 0.05$).

Discussion

With regard to Ca^{2+} -ATPase activity of myofibril in marine fishes living at very different habitat temperatures, K_m values of fish species in Yellow Sea were much lower than those in Sakishima Islands. In other words, the enzyme-substrate affinities for fishes from Yellow Sea were higher than those from Sakishima Islands. This finding might suggest that myofibrillar ATPase of fish species in Yellow Sea have a more flexible structure than those in Sakishima Islands. Therefore, this finding might also related to the previous finding that the thermostability of myofibrillar Ca^{2+} -ATPase is smaller for fishes living in lower habitat temperatures.²⁻⁴⁾ Both the V_{max} level and the temperature dependency of the activity in a saturated concentration of the substrate were virtually the same between fishes from the two waters. We reported that the K_m value of myofibrillar Ca^{2+} -ATPase was lower in carp

Area	Sakishima Islands		Yellow sea		
Species	Gold-lined sea-bream	Half-belted emperor	Greeling	Rockfish	Brown sole
Specimen number	1	4	3	2	3
Mean value of slope	-0.206	-0.153	-0.072	-0.074	-0.100
± S. D.	-	-0.011	-0.011	-0.011	-0.014
	18.270 p<0.05	154.074 p<0.001	0.039 N. S.	4.656 N. S.	
	111.914 p<0.01	132.509 p<0.001	7.404 N. S.		
		90.750 N. S.	46.302 p<0.01		
			42.704 p<0.05		
± S. D.	-0.038	-0.016			
Mean	-0.180	-0.082			
Number of species	2	3			
Area	Sakishima Islands	Yellow Sea			

Fig. 6. The mean value with standard deviation (S.D.) of the slopes in Arrhenius plot between the reaction temperature ($10^3/T$) and myofibrillar Mg^{2+} -ATPase activity (log specific activity) for fishes from the sea around the Sakishima Islands and the Yellow Sea. The figures show F_0 values and significant levels by F -test on the difference in the mean values for each combination of two fishes.

reared at 10°C than in carp reared at 30°C,¹³ which is consistent with the results of the present study in marine fishes, and that Vmax level and temperature dependence of myofibrillar Ca²⁺-ATPase activity in carp changed with rearing temperature, which differs from the results of the present study in marine fishes. However, myofibrillar Ca²⁺-ATPase activity does not correspond to energy production in the muscle.

Km values of myofibrillar Mg²⁺-ATPase were virtually the same between fishes from Yellow Sea and Sakishima Islands. Hochachka *et al.*¹⁴⁻¹⁷ and Somero *et al.*¹⁸ reported that Km values of acetylcholine esterase, isocitrate dehydrogenase, and pyruvate kinase in fishes were different by the habitat water temperature. They considered that the change of Km is important for adaptation to habitat water temperature. The result of Km in myofibrillar Mg²⁺-ATPase was different from results in their reports. With regard to this difference, it is supposed that the change of Km for adaptation is not important in myofibrillar Mg²⁺-ATPase since ATP concentration in muscle of substrate on myofibrillar Mg²⁺-ATPase is very high level. However, the level of Vmax was higher and the temperature dependence of the activity in a saturated substrate concentration was smaller for fishes from Yellow Sea. Therefore, Vmax levels at reaction temperatures each corresponding to habitat temperature were nearly the same between fishes from Yellow Sea and Sakishima Islands. In a previous paper¹³ we reported that the Km value of myofibrillar Mg²⁺-ATPase was slightly lower with carp reared at 10°C than that with carp reared at 30°C, and that the Vmax level was higher and the temperature dependence of myofibrillar Mg²⁺-ATPase activity was smaller with carp reared at 10°C than with carp reared at 30°C. These comparisons between marine fishes and carp suggest that fishes have an efficient mechanism to maintain high activity in locomotion in lower environmental temperatures since myofibrillar Mg²⁺-ATPase activity is coupled with crossbridge cycling between actin-containing thin filaments and myosin-containing thick filaments.

Myofibrillar Ca²⁺- and Mg²⁺-ATPase activities changed adapting to the habitat temperature for both marine fishes and carp, although the changing patterns were slightly different between two groups. These differences in adaptability may come from the fact that marine fishes live in a narrower temperature range. In the present study, myofibrillar Ca²⁺- and Mg²⁺-ATPase ac-

tivities were different not only between fish species from the Yellow Sea and from the Sakishima Islands but also with different habitat temperatures.

Johnston *et al.*^{8,19} reported that changes in actomyosin ATPase activity by thermal acclimation of goldfish, one of the eurythermal species, was controlled by the calcium regulatory proteins, and that, in contrast, the calcium regulatory proteins of stenothermal species were not directly involved in the temperature adaptation of actomyosins. The authors supposed that stenothermal species had selective modifications in the structure of both the heavy and light chains of myosin. There may be different molecular mechanisms of temperature adaptation between marine fishes and carp. Further study to examine this speculation is necessary.

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