

The Comparative Study of Myofibrillar Proteins of Skeletal Muscles of Some Deep-Sea Fish Species

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Received: 18.01.2017 / Accepted: 10.02.2017 / Published online: 16.02.2017

Abstract:

Comparative characteristics, common and distinctive features of qualitative and quantitative composition of myofibril proteins of skeletal muscles of some deep-sea fish species (*Podonema longipes*, *Coryphaenoides cinereus*, *Coryphaenoides pectoralis*) have been determined. Myofibrillar proteins were extracted from the skeletal muscle of three species deep-sea fish and their relative molecular mass was estimated by SDS/polyacrylamide gel electrophoresis. Subunit and quantitative compositions of deep-sea fish proteins were determined. The molecular weight of predominant contractile proteins, myosin (with heavy chains and two light chains), actin, troponin and tropomyosin was about 492, 47, 38 and 35 kDa, respectively for all fish species. The myosin/actin ratios were determined to be 2.85, 2.76 and 2.56 respectively for *C. cinereus*, *P. longipes*, and *C. pectoralis*. The Ca²⁺-ATPase activity of *C. cinereus* and *P. longipes* actomyosins was significantly higher at low ionic strengths (0.317 and 0.324 $\mu\text{M P}_i \text{ mg}^{-1}\text{min}^{-1}$ accordingly) than at high ionic strengths (0.257 and 0.221 $\mu\text{M P}_i \text{ mg}^{-1}\text{min}^{-1}$ accordingly). At the same time the Mg²⁺-ATPase activity value remained almost constant at both high and low ionic strengths (0.169–0.178 $\mu\text{M P}_i \text{ mg}^{-1}\text{min}^{-1}$). The Ca²⁺-ATPase activity of *C. cinereus*, *P. longipes* and *C. pectoralis* myosins was 0.534, 0.641 and 0.376 $\mu\text{M P}_i \text{ mg}^{-1}\text{min}^{-1}$ respectively.

Keywords: Actomyosin; ATPase; Deep-sea fishes; Myosin; Skeletal muscle tissue

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Introduction

Deep-sea fish have not generally been used for food, but its abundance suggests it could be an important source of high-quality protein. Deep-sea fish have much different significance for industrial fishing. Three species of deep-sea fish *Coryphaenoides pectoralis*, *Coryphaenoides cinereus* and *Podonema longipes* are the most accessible for effective fishing in the Far-Eastern Seas of Russia. These three species were chosen for investigations in the present paper.

For processing of fish meat, the knowledge of the properties of fish muscle proteins is necessary. The main aim of the present paper was to determine the composition of basic myofibrillar proteins and its ratio in skeletal muscle tissue.

Investigations of the biochemistry of muscle tissue of deep-sea fish have only incidental character and systematic study of this has not been carried out. In most cases the studies of researchers have been limited to mesopelagic species. So, there are information about thermostability of deep-sea fishes skeletal muscle actomyosin (Arai, K et al., 1973), myofibrillar proteins as a whole (Uchiyama, H et al., 1978) and water soluble proteins (Taguchi, T et al., 1981). But for all that, the information about the basic and regulatory protein's composition, of the skeletal muscles of deep-sea fish species, is perfectly absent. Biochemical properties of these proteins practically have not been studied. At the same time, great amounts of information about these species habits (Dayton, P.K and Hessler, R.R., 1972) and feeding patterns (Drazen, J.C et al., 2001), along with their biological and biochemical mechanisms of adaptation to low temperatures and high pressures (Wakai, N et al., 2014; Morita, T., 2010) have been accumulated in scientific literature at the present time.

The usual depth of the adults of these species range from several hundred meters to upwards of 2,500 m. The tissue composition of these deep-sea fish also suggests a midwater lifestyle. The majority of deep-sea fish species are characterized by high moisture contents in the muscle tissue (Somero, G.N., 1992; Siebenaller, J.F et al., 1982). So, the white muscle tissue of *Coryphaenoides pectoralis* is composed of approximately 92% water. This high value of muscle water content is likely an adaptation to increase buoyancy without the metabolic costs of buoyant lipids or a gas bladder (Drazen, J.C et al., 2001). The compositions of the skeletal muscle myofibrillar proteins of these species are unknown. Therefore, determining the composition of these muscle proteins seems very important for understanding the contribution of muscle myofibrillar proteins to meat texture.

Materials and Methods

Raw materials

White muscles (*musculus lateralis magnus*) of three species of deep-sea fish *Coryphaenoides pectoralis*, *Coryphaenoides cinereus* and *Podonema longipes* were used for research. All fishes were immediately filleted and dorsal white muscles were coarsely minced, mixed with an equal volume of glycerol, and the mixture was frozen at -40°C and was kept frozen at -24°C until use, not longer than one month.

Preparation of proteins

Preparation of myofibrils isolation was carried out by method of Kato (Kato, N., Uchiyama, H., Tsukamoto, S., Arai, K., 1977; Kato, S., Konno, K., 1993). Actomyosins of deep-sea fish skeletal muscles were isolated by the extraction at high ionic strength, pH 6.4. Actomyosins were purified twice by precipitating at ionic strength 0.1 and recovered as solution in 0.6 M KCl. For additional purification of actomyosins and removing the regulatory proteins the method of fractional precipitation with ammonium sulfate was used. So, pure actomyosins were isolated at ammonium sulfate concentration below 35% of degree of saturation.

Myosins were isolated by the method of Bruggmann and Jenny (Bruggmann, S and Jenny, E., 1975) with some modifications. The solution of 0.3 M KCl, 10 mM $\text{Na}_4\text{P}_2\text{O}_7$, 1 mM MgCl_2 in 0.15 KH_2PO_4 (pH 6.4) was used as basic one for extraction and after dilution with 10 volumes of water for "batch" process on DEAE-Sephrose 4B (Pharmacia Biotech AB). So, myosins with highest possible ATPase activity were prepared.

Tropomyosins were isolated by the method of Bailey (Bailey, K., 1948). The isolation procedure of protein was carried out in high ionic strength solutions (0.5-0.6 M) with using ammonium sulfate as a salting-out agent. Troponins were isolated by the method of Ebashi (Ebashi, S et al., 1968). Actin and actinin were isolated by the method of Ishikawa (Ishikawa, H., 1983).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

The qualitative composition of myofibrillar proteins was investigated by the means of disk-SDS-electrophoresis (PAGE mode) (Weber, K and Osborn, M., 1969). The correlation of molecular weight with relative mobility was obtained with the following standard proteins (ICN Biochemicals Inc.): catalase (molecular weight, kDa, 232), bovine serum albumin dimer (132), bovine serum albumin (66), ovalbumin (45), carbonic anhydrase (29), β -Lactoglobulin (18.4) and α -lactalbumin (14.5).

Polyacrylamide gels with acrylamide concentration 3.5% of concentrating layer were used. Concentrating process was carried out at 15 mA and 210 V. Gels with constant concentration of acrylamide 10, 12.5 or 15% and gradient concentration of acrylamide 10-15% were used as separating ones. The separating process was carried out at 5 mA and 100 V. Disk gel was stained with 0.15% coomassie brilliant blue R-250 in 50% methanol-10% acetic acid at 25°C for 5 min and was detained with 50% methanol-5% acetic acid at 25°C overnight.

Densitometry

Densitometry was carried out on the stained disk gel using a chromatoscanner UV-260 (LKB, Sweden) at wavelength of 640 and 700 nm.

Fluorescence intensity measurement

Ultraviolet fluorescence spectrums were recorded by RF-5000 (Shimadzu, Japan) spectrofluorimeter at extinction wavelength (λ^{Ex}) 280 nm. Ratio of fluorescence intensities at 320 and 360 nm

(A_{320}/A_{360}) was calculated for characterization of proteins state (Turoverov, K.K et al., 2002; Turoverov, K.K et al., 1999).

Determination of protein concentration

Protein concentration was determined by the method of Lowry (Lowry, O.H., Rosebroughm N.J., 1951), UV-spectrums were recorded by UV-1800 Shimadzy spectrophotometer.

ATPase assay

The ATPase activity (EC 3.6.1.3) assay was carried out for 10 min at 25°C in the reaction mixture containing either 0.15 M or 0.6 M KCl, 0.1 mg / ml of protein, 2.5 mM ATP (Kato, N et al., 1977; Seki, N and Narita, N., 1980; Okagaki, T. et al., 2005). The pH value of the mixture was adjusted 7.5 for ionic strength 0.6 and 7.0 for ionic strength 0.15 by using 30 mM Tris-maleate buffer (Mori, Y et al., 1980). The reaction was stopped by adding trichloroacetic acid solution.

ATP- sensitivity was determined by the method of Weber (Weber, H.H and Portzehl, H., 1952). The relative viscosity of 2-3 mg/ml protein solutions was determined before (η_{rel}) and after ($\eta_{rel.ATP}$) addition of 1 mM ATP at 25°C. The ATP- sensitivity was calculated with using formula:

$$ATP_{sens} = (\lg \eta_{rel} - \lg \eta_{rel.ATP}) \cdot 100 / \eta_{rel.ATP}$$

Superprecipitation was induced at 20°C by adding 1 mM ATP to the reaction mixture containing 60 mM KCl, 2 mM $MgCl_2$, 0.1 mM $CaCl_2$, 20 mM Tris-maleate, (pH 6.8), 0.1 mg/ml of actomyosin. Absorbance at 540 nm was recorded by UV-1800 Shimadzy spectrophotometer. Extent of superprecipitation was expressed as $\Delta O.D = A_{max} - A_0$, where A_{max} is the maximum absorbance upon addition of ATP and A_0 is the absorbance in absence of ATP. The rate of super precipitation was expressed as $t_{1/2}$ – time corresponding half-cycle of process when $(A - A_0) = (A_{max} - A_0) / 2$.

Statistical analysis

The results were presented as arithmetic means and their standard deviations. The applied methods were evaluated statistically with the use of STATISTICA 8.0 software.

Results and Discussion

Structure and characterization of actomyosin of deep-sea fish skeletal muscles

Disk-gel electrophoretic patterns of myofibrillar proteins of three species of deep-sea fish obviously were not species-specific (Figure 1). It is significant that the myosin/actin ratio for actomyosins of deep-sea fish skeletal muscles is lower in comparison with traditional vertebrates. The ratios were determined to be 2.85, 2.76 and 2.56 respectively for *C. cinereus*, *P. longipes*, and *C. pectoralis*. How actomyosins of deep-sea fishes skeletal muscle hydrolyzed ATP and ATPase activity depended on their Ca^{2+} and Mg^{2+} concentration. The Ca^{2+} -ATPase activity of *C. cinereus* and *P. longipes* actomyosins was significantly higher at low ionic strengths (0.317 and 0.324 $\mu M P_i \cdot mg^{-1} \cdot min^{-1}$ accordingly) than at high ionic strengths (0.257 and 0.221 $\mu M P_i \cdot mg^{-1} \cdot min^{-1}$

accordingly). At the same time the Mg^{2+} -ATPase activity value remained almost constant at both high and low ionic strengths (0.169-0.178 $\mu M P_i \cdot mg^{-1} \cdot min^{-1}$). There was not dependence on actomyosin of *C. pectoralis* ATPase activity both effectors and ionic strength. Actomyosins of all objects showed highest ATPase activity at 5 mM Ca^{2+} . The lowest values of ATPase activity were shown when both effectors were used in equal concentration (2.5 mM). The results of cross effect studies on the added Ca^{2+} and Mg^{2+} ions showed that the effect of Mg^{2+} overcomes that of Ca^{2+} in the testing conditions. In the presence of ATP, actomyosins of the skeletal muscles dissociated in high ionic strength solutions. It was illustrated in the changes of certain attributes such as viscosity (Figure 2) and parameters of ultraviolet fluorescence (UVF).

As shown (Figure 3), the intensity of actomyosin UVF decreased with the increasing ATP concentration. At that the half-width of UVF-signal increased (Table 1).

As shown in Table 1, increases in the half-width of UVF-signals occurred as a result of protein denaturation. However, the wavelength of emissions remained stable during reaction with

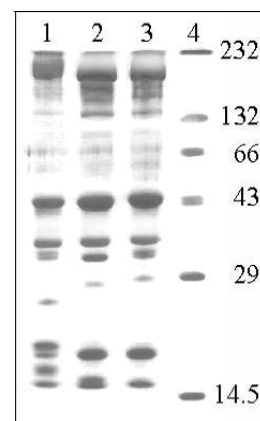


Figure 1: Composition and electrophoretic mobility of myofibrillar proteins of *P. longipes* -1, *C. cinereus* - 2, *C. pectoralis* - 3, and standard proteins - 4. SDS-polyacrylamide slab gel electrophoresis was carried out using a gradient concentration of polyacrylamide from 10 to 15% and 0.1% SDS.

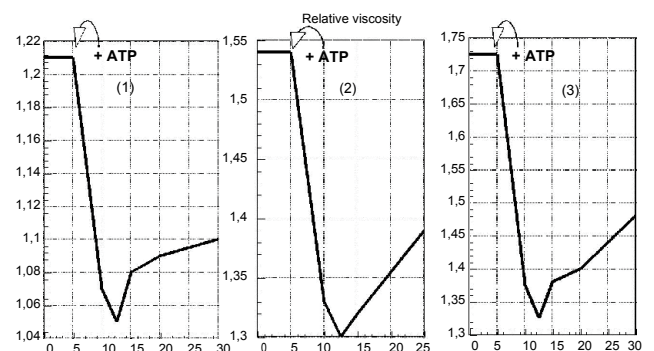


Figure 2: Changing of relative viscosity of deep-sea fishes skeletal muscles actomyosins during reaction with ATP in high ionic strength solutions. Protein concentration 9.0 ± 0.2 mg/ml: (1) *Coryphaenoides cinereus*; (2) *Podonema longipes*; (3) *Coryphaenoides pectoralis*.

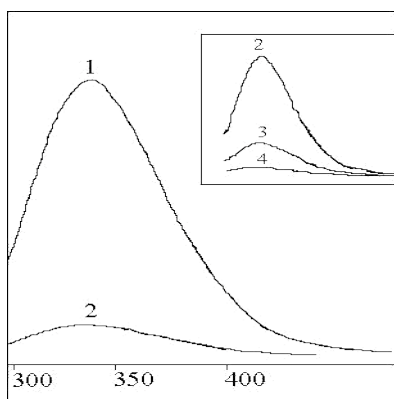


Figure 3: Changing of Ultra Violet Fluorescence (UVF) spectrums of *Podonema longipes* actomyosin of skeletal muscles during reaction with ATP in high ionic strength solutions. ATP concentration, mM: 1-0; 2-0.5; 3-1.0; 4-2.0.

Table 1: Characteristics of Ultra Violet Fluorescence spectrum of *Podonema longipes* actomyosin at the different ATP concentrations.

Spectrum №	C _{ATP} , mM	λ_{Em} (max), nm	A _{Max} , Relative Units	A ₃₂₀ /A ₃₆₀	W _{1/2h} , nm
1	0	332.0	30.27	1.536	59.8
2	0,5	332.0	3.50	1.675	62.6
3	1	332.0	1.04	1.593	67.2
4	2	332.0	-	-	-
Denatured protein*		346.4 ± 1.2	7.2	0.367	81.2

*Denaturation in 10 M urea solution at 80°C, 5 min. C_{ATP} -ATP concentration; λ_{Em} - emission wavelength; A_{Max} - emission maximum; A₃₂₀ -emission at 320 nm; A₃₆₀ - emission at 360 nm; W_{1/2h} - half- width of emission signal Protein concentration – 0.12 mg/ml.

ATP, whereas the shift to the long-wavelength field of spectrum occurred in cases denatured proteins. It is important to note that changes of the UVF-spectrums characteristics were irreversible ones during reaction with ATP, and were only restored after the reprecipitation of proteins.

For cases where actomyosins dissociated at low ionic strengths (Maruyama, K and Gergely, J., 1962), they were accompanied by super precipitation during reactions with ATP (Figure 4). The superprecipitation values correlate with Mg²⁺-ATPase activity in low ionic strength solutions, while superprecipitation rates depend neither on ATPase activity values nor on super precipitation values (Table 2).

We have observed actomyosins aggregation under the influence of heat treatments. The aggregation rates increased with increasing temperatures, and denaturation changes became apparent at temperatures of more than 35°C. By means of UVF (Turoverov, K.K et al., 2002; Turoverov, K.K et al., 1999) we ascertained that the degree of denaturation (as a partition of denatured protein) had a logarithmic dependence on heating time. At that, the denaturation rate constant had an exponential dependence on

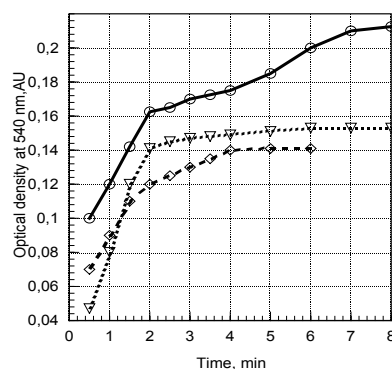


Figure 4: Deep-sea fishes skeletal muscles actomyosins superprecipitation during interaction with ATP in low ionic strength solutions: (◇) *Coryphaenoides pectoralis*; (○) *Podonema longipes*; (▽) *Coryphaenoides cinereus*.

Table 2: Superprecipitation of actomyosins of skeletal muscle of deep-sea fishes.

Source of Actomyosin	A540 (max)	Superprecipitation Value, ΔO.D.	Superprecipitation Rate, t _{1/2} , min
<i>Coryphaenoides pectoralis</i>	0.140	0.070	1.40
<i>Podonema longipes</i>	0.210	0.113	1.80
<i>Coryphaenoides cinereus</i>	0.155	0.106	1.13

temperature at a range of 35-55°C (Figure 5).

Structure and characterization of myosin of deep-sea fish skeletal muscles

The main problem of myosin extraction was the quantitative and selective isolation of this protein from myofibrils. The 0.3 M KCl, 5 mM EDTA, 1 mM dithiothreitol solution, pH 6.4, was used for the selective myosin extraction (Figure 6, Track 1). 10 mM sodium pyrophosphate when added to this solution increased neither selectivity nor effectiveness in myosin extraction (Figure 6, Track 2). At the same time the addition of only 1 mM ATP and 2 mM MgCl₂ to the extraction mixture allowed a remarkable increase in effectiveness and a slight increase in selectivity (Figure 6, Track 3). And vice versa, a pretreatment of the concentrate myofibrils with a low ionic strength solution, (Figure 6, Track 4) or in the aggregate with an extraction using Mg-ATP (Figure 6, Track 5), both allowed to maximum selectivity and poor effectiveness of the extraction of myosin. Extraction was carried out at a ratio of muscle tissue: extractive solvent, 1:5 for all variants.

The most satisfactory results of myosin's isolation were achieved by using the Bruggmann-Jenny method (Bruggmann, S., Jenny, E., 1975). The yields of myosin preparations were around 10-14 mg from every 100 g of muscle tissue. Myosin, prepared in this method, showed all the typical properties of myosin already shown in well-investigated cases: ATP hydrolysis activity, low ATP-sensitivity, negative superprecipitation reaction, and low

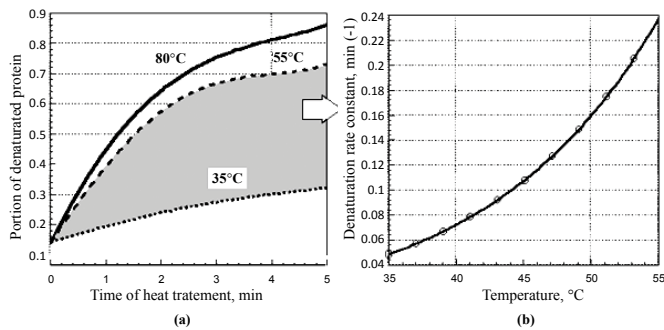


Figure 5: Deep-sea fishes skeletal muscles actomyosins heat denaturation (a) The dependence on the denaturation rate constant and temperature (b) Example of *Podonema longipes*.

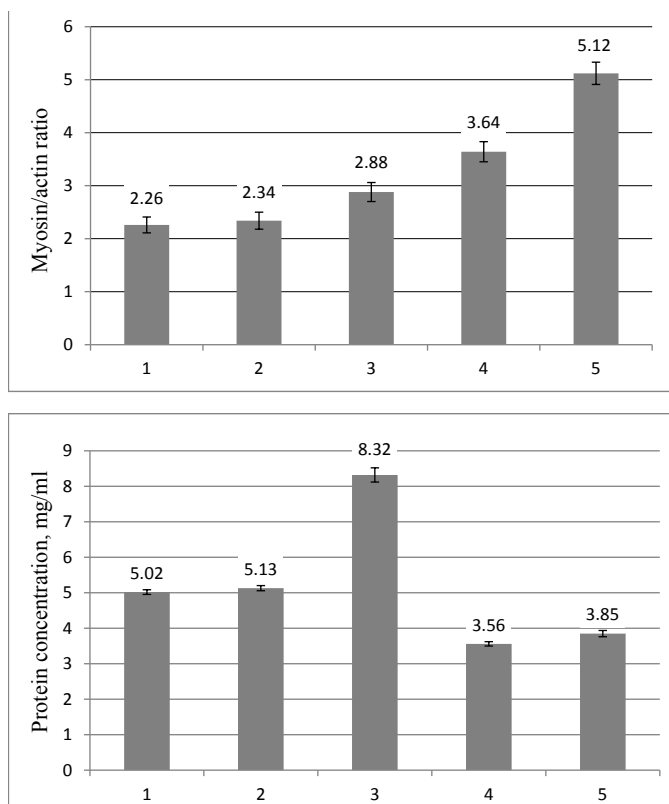


Figure 6: Selectivity and effectiveness of myosin extraction from myofibrils of deep-sea fish. Extraction was carried out at a ratio of muscle tissue: extractive solvent, 1:5 for all variants. An example for muscle tissues of *Coryphaenoides cinereus* is shown. Track 1 - 0.3 M KCl, 5 mM EDTA, 1 mM dithiothreitol. Track 2 - 0.3 M KCl, 5 mM EDTA, 1 mM dithiothreitol, 10 mM Na₄P₂O₇. Track 3 - 0.3 M KCl, 5 mM EDTA, 1 mM dithiothreitol 1 mM ATP, 2 mM MgCl₂; Track 4 - preliminary extraction by 20 mM TRIS-HCl, pH 7; extraction by the means of 1. Track 5 - preliminary extraction by 20 mM TRIS-HCl, pH 7; extraction by the means of 3.

viscosity compared to those of actomyosin. The characteristics of the myosins of deep-sea fish's skeletal muscles are shown in Table 3.

Subunit and quantitative compositions of deep-sea fishes

myosins were determined by mean of total and partial dissociation (Table 4). The full separation of all light chains of myosins occurred in low-concentrated urea solutions. It is necessary to note that different concentrations of urea solutions were used to examine the myosins treatment. So, the necessary and sufficient urea concentration for the separation of *C. cinereus* myosin light chains was 2.0 M, for *P. longipes* - 2.8 M and for *C. pectoralis* - 3.2 M. These values were determined by means of UV-fluorescence (Figure 7). The point where the increase of half-width of UVF-spectrum signals began, during titration of myosin or actomyosin solutions by urea, served as the criterion for the determination of urea concentration. At that, the values of the A_{320}/A_{360} ratio remained more than 1. It signifies the absence of deep delocalized changes in the myosin structure during such treatments.

The most effective results of total myosin light chains separation were realized during brief (3-4 min) intensive heat treatments (53°C) of protein solution. Most of the proteins (about 60%) were aggregated in these conditions (Figure 8, Track 2). Actomyosin may be used as a substitute in this experiment, instead of myosin. A partial light chain separation of myosin was carried out as demonstrated, in Table 4 conditions.

Heat treatments at 40°C and lasting less than 60 min allowed us to selectively remove light chains LC₁ and LC₃ from myosin (Figure 8, Track 3). The light chain LC₃ was removed from myosin by the prolonged incubation of the myosin in a concentrated ammonium chloride solution (4.5-4.7 M) (Figure 8, Track 4). The treatment of myosin with 5, 5'-dithio-bis-2-nitrobenzoic acid solution led to the removal of light chain LC₂ only (Figure 8, Track 5).

Structure and characterization of actin of deep-sea fish skeletal muscles

Actin of skeletal muscle tissues of deep-sea fish has been easily extracted by using low ionic strength solutions. However, most regulatory proteins have been extracted in this manner as well. So, the method, based on depolymerization of fibrillar actin and on higher thermostability of actin globular form, was used (Dominguez, R and Holmes, K.S., 2011). Extraction time was limited to 5 minutes. Because upon the increase of extraction time, the fair quantity that passed into extract impurity proteins exceeded actinin quantity by more than 5 times. The actin and

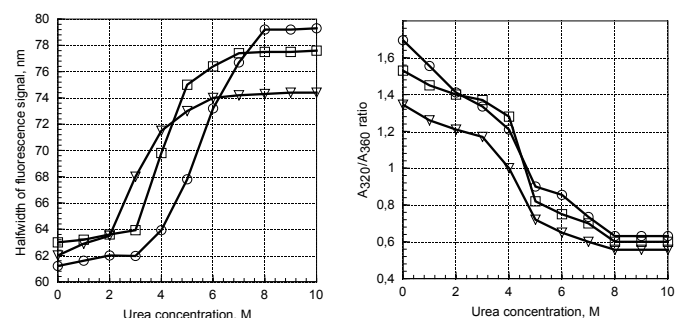


Figure 7: The dependence on some characteristics of Ultraviolet fluorescence spectra of myosins (∇ - *C. cinereus*, \square - *P. longipes*, \circ - *C. pectoralis*) and urea concentration.

Table 3: Characteristics of myosins of deep-sea fish's skeletal muscles.

Myosin Source	AU _{280/260} ^{*)}	Ca ²⁺ -ATPase, μM P _i mg ⁻¹ min ⁻¹	ATP sensitivity, %	A ₃₂₀ / A ₃₆₀ ^{**)}
<i>Podonema longipes</i>	1.78	0.641 ± 0.008	2.32 ± 0.02	1.349
<i>Coryphaenoides cinereus</i>	1.82	0.534 ± 0.004	2.61 ± 0.03	1.423
<i>Coryphaenoides pectoralis</i>	1.76	0.376 ± 0.009	1.21 ± 0.05	1.638

* –Ultraviolet spectrum; ** –Ultraviolet fluorescence spectrum.

Table 4: Myosin subunits dissociation. ^{*)} – 2.0; 2.8 and 3.2 M for myosin of *Coryphaenoides cinereus*, *Podonema longipes* and *Coryphaenoides pectoralis* accordingly.

Processing Environment	Removed Component			Chain
	Molecular weights of chains, kDa			
	<i>C. cinereus</i>	<i>P. longipes</i>	<i>C. pectoralis</i>	
urea ^{*)} or 53°C, 4 min	16.5-17.3-27.2	16.8-17.5-27.0	16.8-18.0-28.5	LC1-3
40°C, 60 min	16.5-27.2	16.8-27.0	16.8-28.5	LC ₁ ; LC ₃
4.7 M NH ₄ Cl	16.5	16.8	16.8	LC ₃
5,5'-dithio-bis-2-nitrobenzoic acid	17.3	17.5	18	LC ₂

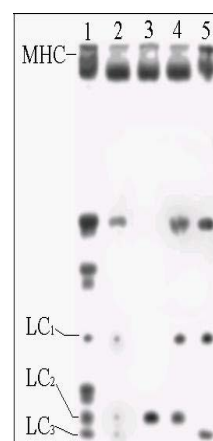
actinin were separated by the means of fractional precipitation. The purity of the actin preparations was calculated, from the area of the band in the densitogram pattern for SDS-PAGE, to be 90-94%. Their yields were 54-70% based on the quantity in the muscle tissue.

Structure and characterization of tropomyosin and troponin of deep-sea fish skeletal muscles

Tropomyosins of deep-sea skeletal muscles were isolated and purified by the Bailey method (Bailey, K., 1948). Tropomyosin is known to be a protein without tryptophane in its structure and its UV-spectrum looks like a spectrum of tyrosine (Staprans, I., Watanabe, S., 1970). Proteins with identical spectral characteristics were isolated by us, from deep-sea fishes myofibrils: UV-spectrum maximum 277.4-278.8 nm, and AU₂₇₈ / AU₂₆₀ ratio-1.98-2.03. However, different compositions of subunits were established (Tables 5 and 6). Only in the case of *C. pectoralis* tropomyosin the α:β ratio was normal according to the value which is known for an overwhelming majority of vertebrates (Shiraishi, F., 1993).

skeletal muscles of *C. cinereus*, *P. longipes* and *C. pectoralis* are almost equal to each other (Table 6).

Troponins of deep-sea fishes muscle tissue were differed in their subunits ratio too. In contrast to traditional objects (Shiraishi, F., 1993; Yates, L.D and Greaser, M.L., 1983; Perry, S.V., 1998) troponins of deep-sea fishes skeletal muscles aren't equimolar

**Figure 8:** Myosin dissociation in the different conditions. SDS-PAGE was performed on a 10-15% gradient acrylamide gel (*Podonema longipes* example is shown).

1. Myofibrillar proteins of *Podonema longipes* skeletal muscles.
2. Dissociation under the influence of 2.8 M urea and during minute heating of up to 53°C.
3. Dissociation during 60-minutes heating of up to 40°C.
4. Dissociation in 4.7 M ammonium chloride solution.
5. Dissociation under the influence of 5, 5'-dithio-bis-2-nitrobenzoic acid.

Table 5: Myosin subunits ratio and molecular weights of myosins.

Myosin source	Molecular Weight	Quantity	Molecular Weight
<i>Podonema longipes</i>	~207	2	492
	16.8	1.1	
	17.5	2	
	27	0.9	
<i>Coryphaenoides cinereus</i>	~207	2	490
	16.5	1.2	
	17.3	2	
	27.2	0.8	
<i>Coryphaenoides pectoralis</i>	~207	2	494
	16.8	1.13	
	18	2	
	28.5	0.87	

The α:β ratio for *C. cinereus* and *P. longipes* tropomyosins was below typical values. Molecular weights of tropomyosins of

complexes. Prevalence of the heaviest component was typical for all examined objects. The distribution of light components was different though, but, on the whole, molecular weights of troponins were close to each other (Table 7).

The non-equimolar composition of troponin was earlier known (Islam, A., 2006) to have been observed. The different rates of proteolytic degradation of troponin components are considered to be the reason for the non-equimolar composition of troponin. The processes of proteolysis of troponin components occurred both during isolation of proteins from muscle tissue and during freeze storage of intact muscle (Tanokura, M et al., 1983; Wei, B and Jin, J.P., 2011). In our case, the troponin components ratio was practically constant or slightly changed during either treatment or storage of muscle tissue. The isolation of all basic protein has allowed us to carry out precise references of signals of the electrophoretic pattern to the relevant proteins (Figure 9) and to calculate its abundance (Table 8).

These results indicated that the composition of proteins of skeletal muscle tissue of examined deep-sea fish species differs in contents of myosin, whereas other main myofibrillar proteins are contained in comparable quantities. Consequently, the different values in the myosin/actin ratio were observed.

Table 6: Composition of deep-sea fishes muscle tropomyosin. MW, MW α , MW β – molecular weight of tropomyosin and α , β -tropomyosin subunits accordingly.

Source of Myofibrils	MW α , kDa	MW β , kDa	a:b ratio	MW, kDa
<i>Coryphaenoides cinereus</i>	33	34	1.1 : 1	67
<i>Podonema longipes</i>	34.2	36.6	1.2 : 1	70.6
<i>Coryphaenoides pectoralis</i>	34.3	36	1.35 : 1	70

Table 7: Molecular weight and quantitative composition of deep-sea fishes' muscle troponins. MW -Molecular weight.

Source of Myofibrils	Troponin Complex's	Relative Quantity	MW of Troponin
<i>Coryphaenoides pectoralis</i>	38	1	
	21	0.9	68.2
	18.5	0.61	
<i>Podonema longipes</i>	39	1	
	20.4	0.66	67.1
	17.7	0.83	
<i>Coryphaenoides cinereus</i>	37.3	1	
	20.5	0.64	67.4
	20.1	0.85	

Table 8: Quantitative composition of deep-sea fish myofibrillar proteins. MW -Molecular weight of protein or its subunits. MW -Molecular weight of protein or its subunits.

Protein	Myofibrillar concentrate sample					
	<i>C. cinereus</i>		<i>P. longipes</i>		<i>C. pectoralis</i>	
	MW, kDa	%	MW, kDa	%	MW, kDa	%
Myosin heavy chains	207		207		207	
Myosin light chains	16.5		16.8		16.8	
	17.3	60.6	17.5	59.8	18	56.8
	27.2		27		28.5	
Actin	47	21.3	47	21.7	47	21.9
Troponin	37.3		39		38	
	20.5	6.2	20.4	6.2	21	5.4
	20.1		17.7		18.5	
Tropomyosin	34	4.8	36.6	4.6	36	4.7
	33		34.2		34.3	

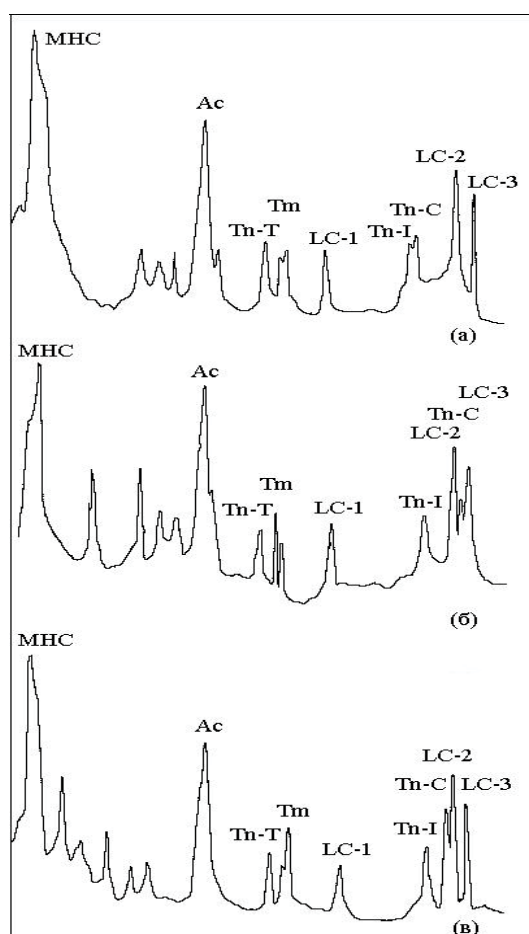


Figure 9: Densitograms of SDS-gel electrophoretic pattern of deep-sea fishes myofibrillar proteins: a) *Coryphaenoides cinereus* b) *Podonema longipes* c) *Coryphaenoides pectoralis*. Abbreviations: MHC-myosin heavy chain; Ac-Actin; Tn-Troponin; Tm -Tropomyosin; LC₁₋₃-myosin light chain.

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