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STRUCTURAL ORGANIZATION OF *CHIRONOMUS AGILIS* AND *C. MURATENSIS* (DIPTERA, CHIRONOMIDAE) HEMOGLOBINS

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Biochemical studies of two chironomids species show that their hemolymph contains curious hemoglobins. It is unusual that haemoglobin has a low molecular weight, 15.9 kDa for monomeric and 31.4 kDa for dimeric forms, and in a condition of disc-electrophoresis, and they are formed up to 12–16 different fractions of hemoglobins. In hemolymph of *Chironomus agilis* (Shobanov, Djomin 1988) were found octamers, pentamers, tetramers, trimers, dimers, and monomers, which consist of subunits with MW from 9.6 to 15.7 kDa. In *Chironomus muratensis* (Ryser, Scholl, Wülker 1983) hemolymph were found octamers, hexamers, tetramers, trimers, dimers, and monomers, which formed from subunits with MW from 11 to 15 kDa.

Keywords: *Chironomus agilis*, *muratensis*, hemoglobin, Diptera, hemolymph

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INTRODUCTION

The article is a continuation of work on the study of the mechanisms of maintaining homeostasis of the internal environment of the body, which is provided by the multiplicity of variants of monomeric forms of haemoglobin [Shobanov, 2000; Bolshakov, 2013]. Chironomids can inhabit a wide variety of water bodies. They are a very good food for all types of fish and especially for the juvenile stages. Additionally, they are playing a very important role in the self-purification of water. Some species of chironomids live in the river with well-aerated water and in the bottom mud of giant water reservoirs where oxygen is almost absent. Both species of this review refer to sibling-species of plumosus group in *Chironomus* genus. It is the largest and the best genetically and karyologically studied group in *Chironomus* [Golygina et al., 2007], interest in them continues unabated [Bolshakov et al., 2022; Li et al., 2022]. Numerous biochemical reviews have shown that their hemolymph contains hemoglobin [English, 1969; Braunitzer, 1971; Bolshakov, Andreeva, 2012; Bolshakov, Fefilova, 2020]. The typical place of habitat for *Ch. agilis* (Shobanov, Djomin, 1988) and *Ch. muratensis* (Ryser, Scholl et Wülker, 1983) is the bottom of meso- and eutrophic waters with slow water flow and as a consequence of this oxygen deficiency. As we know, oxygen is one of the most essential components of life, and hemoglobin assists chironomids survive under the most difficult low oxygen conditions.

Hemoglobins contents in hemolymph reach 90% of whole protein [Walshe, 1951; English, 1969], and its concentration can reach 8.2 g-%, which can be compared with reptiles [Alyakrinskaya, 2002]. For example, in fish, the concentration of hemoglobin in normal blood of 0.5–2.5 g for 1 kg of weight live organism, and up to 30 g for 1 kg in chironomids [Korzhuev, Nikolskaya, 1951; Korzhuev, Radzinskaya, 1958].

The hemoglobins of chironomids possess are extraordinary high oxygen affinity, some of its molecules can utilize oxygen with 1.3 mm Hg and even with 0.32 mm Hg [Weber, Vinogradov, 2001]. In vertebrates, the same conditions of this characteristic are 26 mm Hg for Seal, 50 mm Hg for a kangaroo and 27 mm Hg for humans [Korzhuev, 1964]. The oxygen capacity of *C. plumosus* (L., 1758) hemoglobin varies from 5.4 to 11.6 cap-%, and depends on hemoglobin concentration [Leitch, 1916]. Larvae can use stored oxygen only for short period from 9 [Walshe, 1950] to 12 minutes [Leitch, 1916], they use this time for a short pause between breathing motions [Walshe, 1947].

Hemoglobin is omnipresent and appears in many organisms at different levels of complexity: from bacteria to vertebrates [Hardison, 1996, 1998; Herhold et al., 2020]. The hemoglobin of vertebrates is intracellular and forms heterotetramers with a molecular weight (MW) about 68 kDa [White et al., 1978]. Nonvertebrate hemoglobins are generally extracellular. It carries out similar functions, but its structure is very diverse, from monomers to multisubunit compositions. Their molecular weights vary widely too, from tens to thousands of kDa's [Lehninger, 1982; Weber, Vinogradov, 2001; Numoto et al., 2005; Herhold et al., 2020; Prothmann et al., 2020]. The molecular weights of the separate subunits in different groups of organisms range from 12–28 kDa, dimers 23–34 kDa, tetramers 50–80 kDa and high molecular weight aggregates up to several thousands kDa [Alyakrinskaya, 1979; Aydemir, Korkmaz, 2023; Braunitzer, 1971; Dangott, 1980; Thompson, Bleecker, English, 1968; Tichy, 1975; Waxman, 1975; Weber, Vinogradov, 2001; White et al.,

1978]. Hemoglobin of chironomids is uncommon; it is extracellular and possesses a low molecular weight, with a MW of 15.9kDa for the monomeric forms [Thompson et al., 1968; Tichy, 1975] and about 31.4 kDa for the dimeric forms [Schmidt-Nielsen, 1979]. The relative content of monomers and dimers in the hemolymph of *Ch. plumosus* depends on pH values. In a solution of hemolymph with the pH from 5.2 to 7.0 dimers predominate, while at pH's from 9.2 to 10 the hemolymph solution is dominated by monomers, and at a pH's of 7.5 to 9 there is a dynamic equilibrium between monomers and dimers [Behlke, Scheler, 1967].

Several electrophoretic studies of chironomids hemoglobins have shown their high heterogeneity [Braunitzer, 1971; Tichy, 1978; Shobanov, 2004]. Different species of chironomids have from 1–2 up to 12 different fractions of hemoglobin, specific for a stage of development and populations [Tichy et al., 1981; Shobanov et al., 1993; Das, Handique, 1996; Bolshakov, Fefilova, 2020]. It has been suggested that fractions with different electrophoretic mobility are functionally diverse: the fractions with low electrophoretic mobility carry out a buffer function, while the middle fractions have the greatest affinity for oxygen and provide for its utilization at low concentrations in the environment, and the fractions with the high electrophoretic mobility may represent the products of degradation of hemoglobin [Shobanov, 2004].

The amino acid sequences of hemoglobins from the laboratory species *Ch. riparius* (Meigen, 1804) and *Ch. dilutus* (Shobanov, Kiknadze et Butler, 1999) have been well studied; however, the reason for the high level of heterogeneity has not yet been defined. The observed heterogeneity cannot be explained only by the presence in the hemolymph of monomers and dimers. Therefore, the objective of our research is to study the structural organization hemoglobins of chironomids *Chironomus agilis* and *C. muratensis* from natural populations.

MATERIALS AND METHODS

Place of sampling. The larvae of *Ch. agilis* (Diptera, Chironomidae) and *Ch. muratensis* (Diptera, Chironomidae) were collected from sediments of the Rybinsk reservoir with Ekman dredge, near Syobla river (N 58º27'00''; E 37º37'00''), from depth 14 m., in October 2010 (Figure 1). The oxygen concentration in near-bottom water ranged from 0.03 to 2 mg/l for all summer period, and in October about 12 mg/l.

Hemolymph extraction and analyses system. Hemolymph was extracted from every single larva in phase 4–9 of the IV-th instar and immediately froze in –24°C or used fresh for electrophoresis. Hemolymph after thaws out was used for only once. To provide the most accurate species identification were used karyological methods with the application of standard chromosomal maps [Keyl, 1962; Kiknadze et al., 2016; Maximova, 1976].

For protein analyses several electrophoretical methods were used. Before electrophoresis hemolymph was mixed with 40% sucrose in 1:1. Native proteins were first separated by discontinuous polyacrylamide gel electrophoresis (disc-PAGE) with 10% acrylamide [Maurer, 1978]. The protein fractions separated by disc-PAGE were then further separated by two-dimensional electrophoresis. The second-dimension separations were by native gradient-PAGE with 5–40% acrylamide [Andreeva, 2008]; denaturing PAGE with 8M urea [Creighton, 1979, 1980]; and denaturing PAGE with SDS [Laemmli, 1970]. All type of electrophoresis was used for every individual of larva, i.e. for 10 larvae's were made 10 lines in disc-PAGE, 10 two-dimensional gradient-PAGE's, 10 — SDS-PAGE's and 10 — 8M urea-PAGE's.

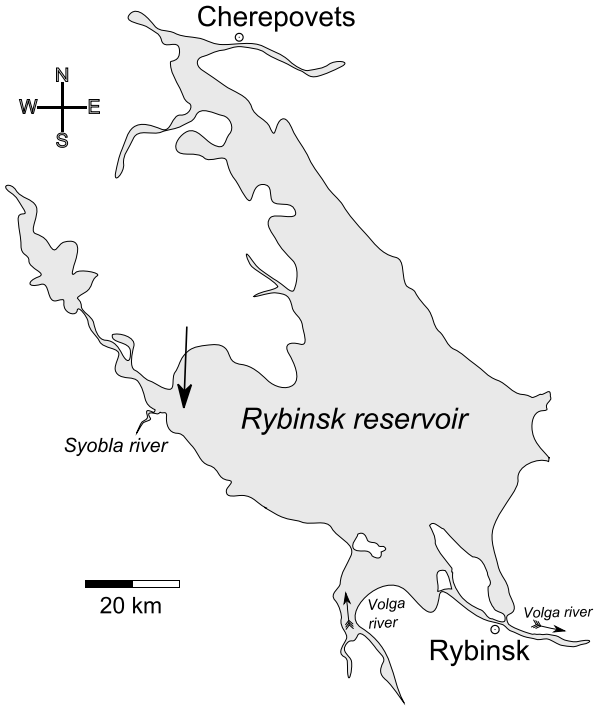

Рис. 1. Схема Рыбинского водохранилища, Россия. Стрелкой показано место отбора проб.

Fig. 1. Scheme of the Rybinsk reservoir, Russia. The arrow shows the sampling place.

Staining and MW markers. Coomassie R-250 (0.1%) was used for protein staining, in mixture: distilled water, ethanol and acetic acid in 30:10:1 [Maurer, 1978]. Native protein gels used HSA (human serum albumin) and OA (ovalbumin) as molecular weight markers, and for denaturated

protein molecular weight markers we used the PageRuler™ Prestained Protein Ladder Plus kit (Fermentas). All results were analyzed with specialized software (OneDScan™).

RESULTS AND DISCUSSION

3.1. Differentiation of proteins in disc-PAGE. Spectral analysis of *Ch. agilis* and *Ch. muratensis* hemoglobins has shown standard absorption bands in 576 nm (alpha), 544 nm (beta) and 414 nm (Soret absorption band) typical for oxyhemoglobins of chironomids [White et al., 1978]. In the native hemolymph pH is 7.45 and agrees with pH 7.45–7.68 in *Chironomus plumosus* [Weber, 1963].

The native disc-PAGE separated hemoglobins from hemolymph of *Ch. agilis* and *Ch. muratensis* into 10 fractions (Figure 2). The observed fractions were designated by numbers from 1 to 10. The observed fractions are distinct from hemoglobin species previously described in laboratory cultures of *Ch. riparius* and *C. tentans* and designated by common symbols such as "CTT I–CTT X" [Osmulski, Leyko, 1986].

Differentiation of native hemoglobin molecules by molecular weight. The fractions defined by native disc-PAGE were further separated by two-dimensional electrophoresis with native gradient-PAGE. The *Ch. agilis* first hemoglobin fraction contained molecules with MW's of about 120 kDa (Figure 3a, Table 1). The second, third and fourth *Ch. agilis* fractions included hemoglobins with MW's of about 68, 56.7 and 45.3 kDa, respectively. In the fifth and sixth fractions gradient PAGE revealed three components with MW's of 44, 35.4 and 24.7 kDa. However, these three components could not be unambiguously assigned to either fraction 5 or fraction 6. In the area of the seventh and eighth fractions, components of MW 33, 26.2 and 18.3 kDa were distinguished, but could not be unambiguously assigned to either fraction 7 or fraction 8. The ninth fraction contained a single component with a MW of 19.4 kDa. In the tenth fraction four components were revealed with MW's of 22.8, 20.9, 18 and 14.2 kDa (Figure 3a, Table 1).

The first fraction of *Ch. muratensis* hemoglobin included one component with a MW of 133 kDa (Figure 3b, Table 2). Fractions 2, 3 and 4 each contained one component with MW's of 95, 63 and 52 kDa, respectively. Fractions 5 and 6 each contained two components with MW's of 50 and 34, and 24 and 20.3 kDa, respectively. Fraction 7 included four components with MW's of 28.3, 24.3 and 15.8 kDa. Fractions 8 and 9 each contained one component with MW's of 19.7 and 16.5 kDa, respectively. Fraction 10 was separated into three components with MW's of 21.9, 17.5 and 14.7 kDa (Figure 3a, b; Table 2).

Separation of hemoglobins of *Ch. agilis* and *Ch. muratensis* in denaturing 8M urea PAGE. In 8 urea PAGE electrophoresis the chironomid hemoglobin fractions formed macromolecular aggregates. Fractions 1, 2, 3 and 4 of *Ch. agilis* formed aggregates with MW's of 142, 152, 114 and 68 kDa, respectively (Figure 4a; Table 1). In the region of fractions 5 and 6 complexes with MW's of 91, 62 and 47 kDa were revealed. The area of fractions 7 and 8 also separated into three units with MW's of 77, 60 and 50 kDa. Fraction 9 contained one component with a MW of 50 kDa. Fraction 10 included three components with MW's of 618, 49 and 38 kDa (Figure 4a; Table 1).

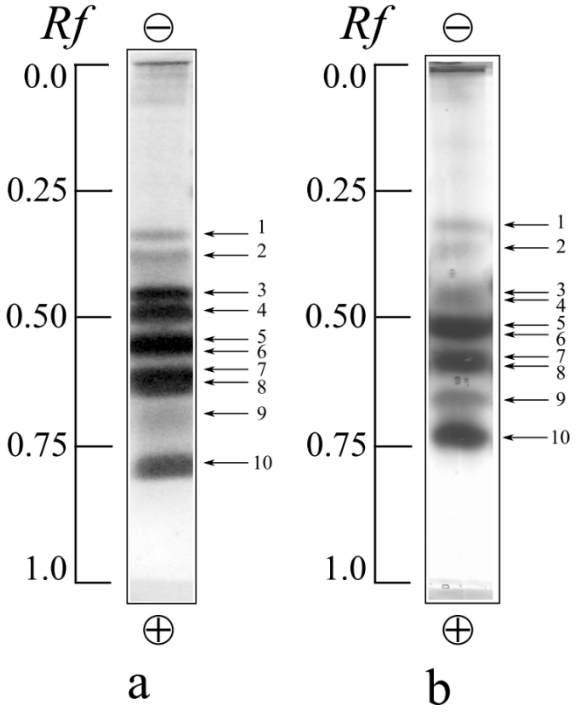

Рис. 2. Распределение гемоглобина в диск-ПААГ, a — *Ch. agilis* и b — *Ch. muratensis*. Анализируемые фракции помечены цифрами от 1 до 10. *Rf* — электрофоретическая подвижность. В обоих случаях для окрашивания использовали Coomassie R-250.

Fig. 2. Representative disc-PAGE separation of hemoglobin proteins from a — *Ch. agilis*, and b — *Ch. muratensis*. The analyzed fractions are marked with numbers from 1 to 10. R_f — retention factor, or relative electrophoretic mobility. In both cases Coomassie R-250 was used for staining.

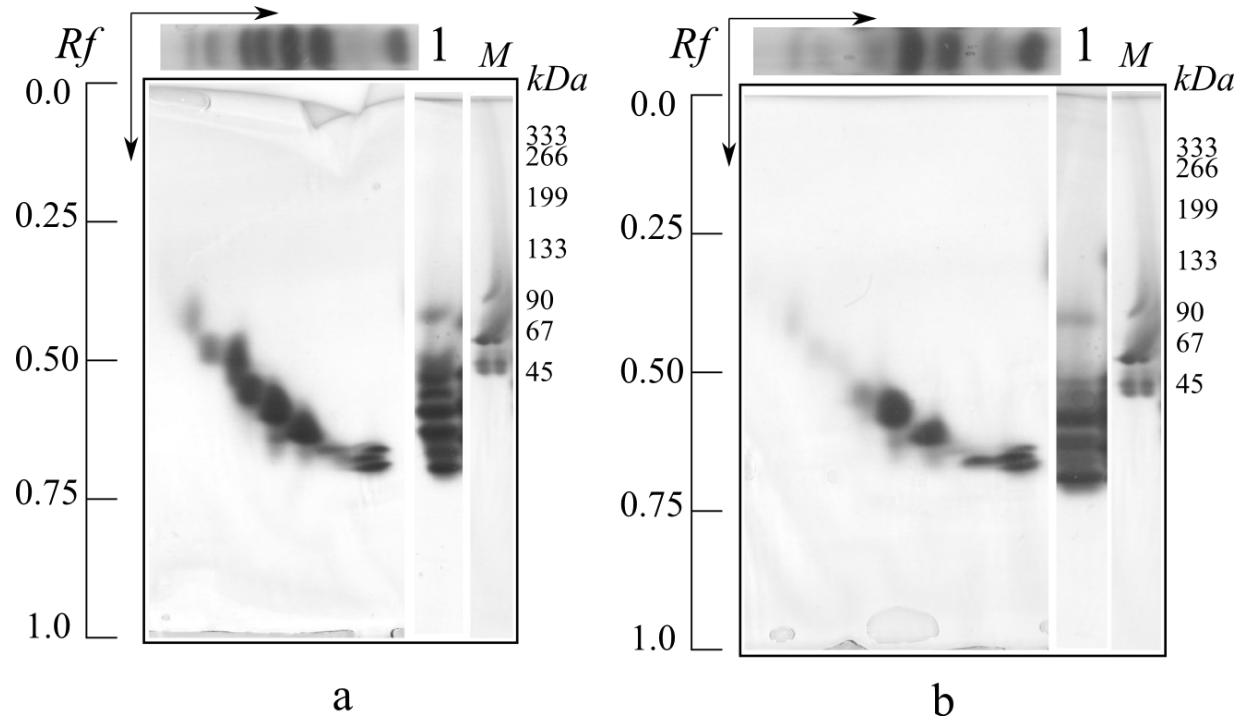

Рис. 3. Двумерный электрофорез нативного гемоглобина личинок хирономид в градиенте концентрации, a — *Ch. agilis*, b — *Ch. muratensis*. Горизонтальная стрелка указывает направление диск-электрофореза, вертикальная стрелка указывает направление электрофореза в градиенте ПААГ. R_f— относительная электрофоретическая подвижность, *M* — маркеры молекулярной массы (ОА и ЧСА), 1 — гемолимфа личинок.

Fig. 3. Two-dimensional electrophoresis of hemoglobin of chironomid larvae in a native gradient-PAGE. a — *Ch. agilis*, b — *Ch. muratensis*. The horizontal arrow indicates the direction of native disc-PAGE and the vertical arrow indicates the direction of native polyacrilamide gradient gel electrophoresis. R_f — retention factor, or relative electrophoretic mobility, *M* — molecular weight markers (OA and HSA), 1 — hemolymph of larvae.

Таблица 1. Значения молекулярных масс компонентов нативного и денатурированного гемоглобина *Ch. agilis*

Table 1. The values of molecular masses of native and denatured hemoglobin components of *Ch. agilis*

Type of Electrophoresis	Hemoglobin fractions number defined by disc-PAGE electrophoresis							
	1	**2**	**3**	**4**	**5–6**	**7–8**	**9**	**10**
Grad.-PAGE	120	68	56.7	45.3	44	33	19.4	22.8
					35.4	26.2		20.9
					24.7	18.3		18
								14.2
8M-urea PAGE	142	152	114	68	91	77	50	618
					62	60		49
					47	50		38
SDS-PAGE	11.4	11.3	10.6		11.2	12.7	11.5	15.7
			10.4		9.6	12.5	10.4	11.6
						11.2		10.2
						10.2		
						9.7		

Fractions 1 and 2 of *Ch. muratensis* complexes with MW's of 130 and 141 kDa respectively (Figure 4b; Table 2). Fraction 3 consisted of two components with MW's of 139 and 103 kDa. Fractions 4, 5, 6, 7 and 8 each contained one component with MW's of 86, 61, 86, 60 and 75 kDa, respectively. Fraction 9 consisted of two components with MW's of 57 and 40 kDa. Finally, fraction 10 yielded three components with MW's of 57, 50 and 41 kDa (Figure 4b; Table 2).

Analysis of the subunit composition of *Ch. agilis* and *Ch. muratensis* hemoglobins by denaturing SDS-PAGE. The fractions defined by native disc-PAGE were dissociated into their component subunit and separated by molecular weight with two-dimensional electrophoresis with denaturing SDS-PAGE. In *C. agilis* fractions 1 and 2 we detected subunits with MW's of 11.4 and 11.3 kDa, respectively. In the region of fractions 3 and 4 two components with MW's of 10.6 and10.4 kDa were found. Fractions 5 and 6 yielded two components

with MW's of 11.2 and 9.6 kDa. In the area of fractions 7 and 8, we identified four components with MW's of 12.7, 12.5, 11.2 and 10.2 kDa. Fraction 9 consisted of two components with MW's of 11.5 and 10.4 kDa. Fraction 10 was resolved into three components with MW's of 15.7, 11.6 and 10.2 kDa (Figure 5a; Table 1).

Таблица 2. Значения молекулярных масс компонентов нативного и денатурированного гемоглобина *Ch. muratensis*

Table 2. The values of molecular masses of native and denatured hemoglobin components of *Ch. muratensis*

Type of Electrophoresis	Hemoglobin fraction number defines by disc-PAGE electrophoresis									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Grad.-PAGE	133	95	63	52	50 34	24 20.3	28.3 24.3 18.9 15.8	19.7	16.5	21.9 17.5 14.7
8M-urea PAGE	130	141	139 103	86	61	86	60	75	57 40	57 50 41
SDS-PAGE	14.3 12.9 12.3	–	–	13 12 11	13.5 12.5 11.5	–	13.6 12.6 11.6	13.8 12.8 11.8	13 12	15 11.6

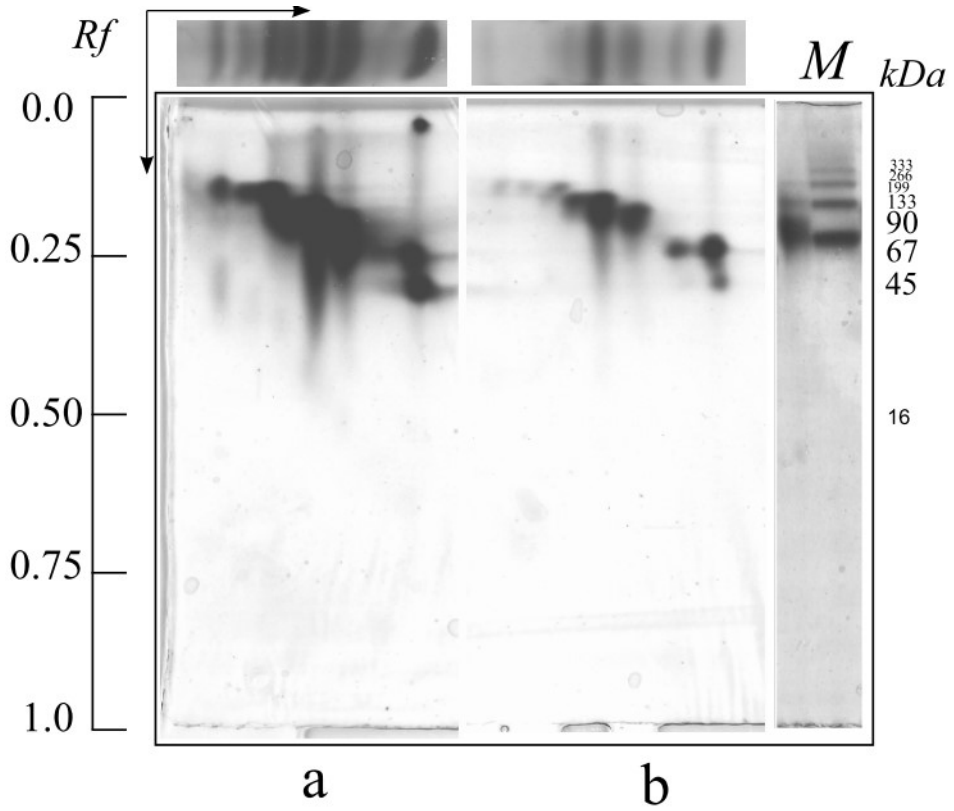

Рис. 4. Двумерный электрофорез гемоглобина личинок хирономид в денатурирующих условиях ПААГ с 8 М мочевиной. a — *Ch. agilis*, b — *Ch. muratensis*. Горизонтальная стрелка указывает направление нативного дискэлектрофореза, вертикальная стрелка — направление денатурирующего электрофореза в ПААГ с 8М мочевиной. R_f — относительная электрофоретическая подвижность, *M* — маркеры молекулярной массы (ОА и ЧСА).

Fig. 4. Two-dimensional electrophoresis of hemoglobin of chironomid larvae in a denaturing PAGE with 8M urea. a — *Ch. agilis*, b — *Ch. muratensis*. The horizontal arrow indicates the direction of native disc-PAGE and the vertical arrow indicates the direction of denaturing PAGE in 8M urea. R_f — retention factor, or relative electrophoretic mobility, *M* — molecular weight markers (OA and HSA).

Analysis of the subunit composition of *Ch. agilis* and *Ch. muratensis* hemoglobins by denaturing SDS-PAGE. The fractions defined by native disc-PAGE were dissociated into their component subunit and separated by molecular weight with two-dimensional electrophoresis with denaturing SDS-PAGE. In *C. agilis* fractions 1 and 2 we detected subunits with MW's of 11.4 and

11.3 kDa, respectively. In the region of fractions 3 and 4 two components with MW's of 10.6 and10.4 kDa were found. Fractions 5 and 6 yielded two components with MW's of 11.2 and 9.6 kDa. In the area of fractions 7 and 8, we identified four components with MW's of 12.7, 12.5, 11.2 and 10.2 kDa. Fraction 9 consisted of two components with MW's of 11.5 and 10.4 kDa. Fraction 10 was resolved into three components with MW's of 15.7, 11.6 and 10.2 kDa (Figure 5a; Table 1).

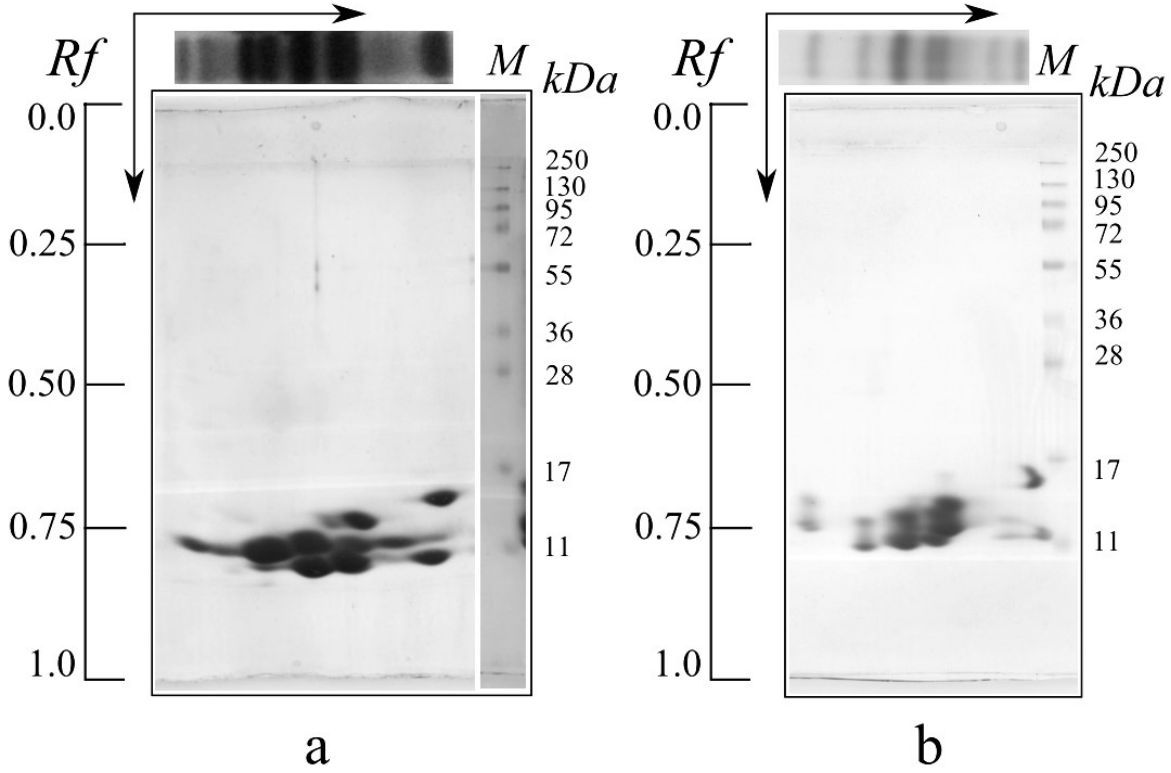

Рис. 5. Двумерный электрофорез гемоглобина личинок хирономид в денатурирующих условиях SDS-ПААГ. a — *Ch. agilis*, b — *Ch. muratensis*. Горизонтальная стрелка указывает направление нативного диск-электрофореза, вертикальная стрелка — направление электрофореза в денатурирующих условиях SDS-ПААГ. R_f — относительная электрофоретическая подвижность. Маркеры *M* — молекулярной массы (набор PageRuler^{TM} Prestained Protein Ladder Plus kit (Fermentas)).

Fig. 5. Two-dimensional electrophoresis of hemoglobin of chironomid larvae in denaturing SDS-PAGE. a — *Ch. agilis*, b — *Ch. muratensis*. The horizontal arrow indicates the direction of native disc-PAGE and the vertical arrow indicates the direction of denaturing SDS electrophoresis. R_f — retention factor, or relative electrophoretic mobility. *M* — molecular weight markers (PageRuler^{TM} Prestained Protein Ladder Plus kit (Fermentas)).

In *Ch. muratensis* fraction 1 revealed three distinct subunits with MW's of 14.3, 12.9 and 12.3 kDa. Fractions 2 and 3 in the investigated specimens were absent as a result of polymorphism. Fraction 4 contained three components with MW's of 13, 12 and 11 kDa, fraction 5 — contained subunits with MW's of 13.5, 12.5 and 11.5 kDa, and fraction 6 was absent. Fraction 7 was composed of subunits with MW's of 13.6, 12.6 and 11.6 kDa, fraction 8 contained subunits with MW's of 13.8, 12.8 and 11.8 kDa, and fractions 9 and 10 each included two subunits with MW's of 13 and 12, and 15 and 11.6 kDa, respectively (Figure 5b; Table 2).

Analysis of the structural organization of the hemoglobin proteins in the larvae of chironomid species studied. In *Ch. agilis* hemolymph we observed a high diversity of hemoglobin proteins (Table 1). Fraction 1 in native conditions involves a protein, probably an octamer, with a MW of 120 kDa, dissociated into subunits of MW 11.4 kDa, and in 8M urea aggregated into complexes with MW 142 kDa. Subunits of the second fraction have a MW of approximately 11.3 kDa, and in native conditions, they form a complex with a MW of 68 kDa, presumably a pentamer, while in 8M urea fraction 2 forms a complex with a MW of 152 kDa. Subunits of fractions 3 and 4 have similar MW values of 10.4 and 10.6 kDa, and were poorly differentiated. Under native conditions they formed a pentamer with a MW of 56.7 kDa (fraction 3) and a tetramer with a MW of 45.3 kDa (fraction 4). Under native conditions fractions 5 and 6 contained a tetramers with a MW 44 kDa, a trimers with a MW 35.4 kDa, and a dimers with a MW of 24.7 kDa. In SDS-PAGE these complexes

dissociated into subunits with MW's of 9.6 and 11.2 kDa and in 8M urea they aggregated into complexes with MW's of 47, 62 and 91 kDa. The components of fraction 7 could not be distinguished from the components of fraction 8, so the two fractions were analyzed together. In total, five types of subunits with MW's 9.7, 10.2, 11.2, 12.5, and 12.7 kDa were isolated. In native conditions they formed a monomer with a MW of 18.3 kDa, a dimer with MW of 26.2 kDa, and a trimer with a MW of 33 kDa. In 8M urea these components aggregated into complexes with MW's of 50, 60 and 77 kDa. Fraction 9 is presumably a monomer with a MW of 19.4 kDa, and contained subunits of 10.4 and 11.5 kDa. Fraction 10 was formed by three monomers with MW's of 14.2, 18, 20.9 kDa, including subunits of MW 10.2 and 11.6 kDa, and a monomer with a MW of 22.8 kDa, including, presumably, subunits of 15.7 kDa.

In the hemolymph of *Ch. muratensis* we also found a high diversity of hemoglobin proteins (Table 2). The fraction 1 hemoglobin defined by native disc-PAGE contained subunits with MW's of 12.3, 12.9 and 14.3 kDa, in native conditions formed an octamer of 133 kDa, and in 8M urea formed an aggregate with a MW of 130 kDa. In the hemolymph of the individual larvae subjected to SDS-PAGE, fractions 2, 3 and 6 were not found. Therefore, predictions of multimeric structure were based on the assumption that the MW's of the hemoglobin subunits were approximately 12–14 kDa. Thus, the fraction 2 hemoglobin with a MW of 95 kDa was predicted to be a hexamer. In 8M urea fraction 2 formed a complex with a MW of 141 kDa. Under native conditions, the subunits of hemoglobin fraction 3 formed aggregates of 139 and 103 kDa. Hemoglobin fraction 4 was predicted to be a tetramer with a MW of 52 kDa under native conditions. Fraction 4 contained subunits with MW's of 11, 12 and 13 kDa, and aggregated in 8M urea to a complex with a MW of 86 kDa. Under native conditions, the subunits of fractions 5 formed trimers and tetramers with MW's of 34 and 50 kDa, respectively, while in 8M urea fraction 5 formed an aggregate of 61 kDa. The subunits of fraction 6 were predicted to form dimers with MW's of 24 and 20.3 kDa, and aggregated in 8M urea to complexes with a MW 86 kDa. In the region of the fraction 7 we found three types of subunits with MW's of 11.6, 12.6 and 13.6 kDa, which in native conditions formed monomers with apparent MW's of 15.8 and 18.9 kDa, dimers with MW's of 24 kDa, and trimers with MW's of 28.3 kDa. Fraction 8 in native conditions consisted of monomers with an apparent MW of 19.7 kDa, formed by three types of subunits with MW's of 11.8, 12.8 and 13.8 kDa. In 8M urea fraction 8 aggregated into a complex with a MW of 75 kDa. Protein fraction 9 consisted of monomers with an apparent MW of about 16.5 kDa, including two types of subunits with MW's of 12 and 13 kDa. In 8M urea fraction 9 aggregated into complexes with MW's of 40 and 57 kDa. In the area of fraction 10 we observed monomers with apparent MW's of 14.7, 17.5 and 21.9 kDa, formed by two subunits with MW's of 11.6 and 15 kDa. In 8M urea fraction 10 aggregated into complexes with MW's of 41, 50 and 57 kDa.

CONCLUSION

This work is a follow up to a thorough study of the mechanisms of adaptation in chironomids at the biochemical and cytogenetic levels. While previous studies have detected only monomeric and dimeric forms of hemoglobin, the present study demonstrates the novel result that oligomeric forms of hemoglobin are simultaneously present in the hemolymph of chironomid larvae. Dr. Shobanov suggested that the high level of chromosomal polymorphism may contribute to an increase in the diversity of haemoglobin forms in different species of chironomids in the genus *Chironomus*. In the studied species *Ch. agilis* and *Ch. muratensis*, which have a high chromosomal polymorphism, we found a high diversity of structural forms of both multimeric haemoglobins and their subunits. This indirectly confirms Dr. Shobanov's theory. In addition, our results reveal a high degree of heterogeneity in the electrophoretic spectra of chironomid haemoglobins, which may be useful in the characterization of species, populations and developmental stages.

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СТРУКТУРНАЯ ОРГАНИЗАЦИЯ ГЕМОГЛОБИНОВ *CHIRONOMUS AGILIS* И *C. MURATENSIS* (DIPTERA, CHIRONOMIDAE)

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Биохимические исследования двух видов хирономид показали, что в их гемолимфе содержатся различные формы гемоглобинов. Они обладают низкой молекулярной массой: мономеры — 15.9 кДа, и димеры — 31.4 кДа. В условиях диск-электрофореза выявлено до 12–16 различных фракций гемоглобинов. В гемолимфе *Chironomus agilis* (Shobanov, Djomin 1988) были обнаружены октамеры, пентамеры, тетрамеры, тримеры, димеры и мономеры, в состав которых входят субъединицы с молекулярной массой от 9.6 до 15.7 кДа. В гемолимфе *Chironomus muratensis* (Ryser, Scholl, Wülker 1983) были обнаружены октамеры, гексамеры, тетрамеры, тримеры, димеры и мономеры, состоящие из субъединиц с молекулярной массой от 11 до 15 кДа.

Ключевые слова: *Chironomus agilis*, *muratensis*, гемоглобин, Diptera, гемолимфа.