ISSN 1995-0829, Inland Water Biology, 2018, Vol. 11, No. 1, pp. 92–96. © Pleiades Publishing, Ltd., 2018.
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ECOLOGICAL PHYSIOLOGY AND BIOCHEMISTRY OF HYDROBIONTS

Effect of Temperature on the Morphometrical and Physical Parameters of Erythrocytes and Polymorphonuclear Leucocytes in *Carassius gibelio* (Bloch)

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Received January 11, 2016

Abstract—Dynamics of the morphometric and physical properties of hemocytes of the Prussian carp *Carassius gibelio* (Bloch) under the influence of a temperature factor has been studied with atomic force microscopy in experiments in vitro. It is found that, at a low incubation temperature (5°C), as opposed to room temperature (20°C), morphometric parameters change in erythrocytes; at a high temperature (40°C) they change in polymorphonuclear leucocytes. The low incubation temperature reduces the adhesion and elasticity of polymorphonuclear leucocytes and erythrocytes of *C. gibelio*, whereas a high incubation temperature leads to a decrease in adhesion in polymorphonuclear white blood cells.

Keywords: erythrocytes, leucocytes, adhesion, elasticity, temperature

DOI: 10.1134/S1995082918010030

INTRODUCTION

Temperature is one of the most important abiotic factors of the environment; the adaptation of living organisms to temperature is achieved through the implementation of various physiological and biochemical mechanisms [10, 27]. There are many studies on the effect of temperature on the body of *Teleostei* representatives in whole and on the blood of animals of this hyperorder in particular: the changes in their heat resistance, growth dynamics, and life cycle were studied [3, 11, 18]; physiological and physicochemical properties of blood cells under the influence of high and low incubation temperatures were revealed in experiments in vitro [14, 15]. However, questions related to the key aspects of the effect of the temperature factor on a number of other parameters of hemocytes in representatives of teleost fish have not been studied.

The purpose of this work is to evaluate the dynamics of morphometric and physical parameters of erythrocytes and polymorphonuclear leucocytes of Prussian carp *Carassius gibelio* under the influence of the temperature factor in experiments in vitro.

MATERIALS AND METHODS

Studies were carried out at the beginning of the autumn period. Peripheral blood of Prussian carp *C. gibelio* (32 individuals) at the age of 12 months and weighing 165 ± 15 g was used in the work. The study subjects were erythrocytes and polymorphonuclear leucocytes (PMNL). Prior to taking blood, the fish was kept in an aquatic habitat at room temperature (20°C). Blood sampling from *C. gibelio* was carried out from the tail vein. The resulting blood was centrifuged for 10 min at 400 rpm. The erythrocyte and leucocyte suspensions were diluted with an isotonic solution (0.65% NaCl) [5], then they were incubated for 2 h with use of three temperature conditions in parallel: room (20°C), low (5°C), and high (40°C). At the end of the incubation period of the cells, smears were made. From each series of sample preparation, 20–25 erythrocytes and leucocytes were examined by AFM. The hemocytes were scanned on an INTEGRA Vita atomic force microscope (configuration based on an inverted Olympus IX-71 optical microscope).

Morphometric parameters of the cells were determined in a semicontact scanning mode [12] with a scanning frequency of 0.6–0.8 Hz using a NSG03 series cantilever with a stiffness of 1.1 N/m and a radius of rounding of 10 nm [9]. Area (S, μm^2), perimeter (P, μm), volume (V, μm^3), large (D, μm), and small (d, μm) cell diameters were measured on the scans. These data were used for the curve construction of the profile of scanned cells with use of the Nova software (NT MDT, Zelenograd, 2009). The adhesion (nN) of the cells was estimated from the curves. Elasticity (Young's modulus, kPa) of erythrocytes and

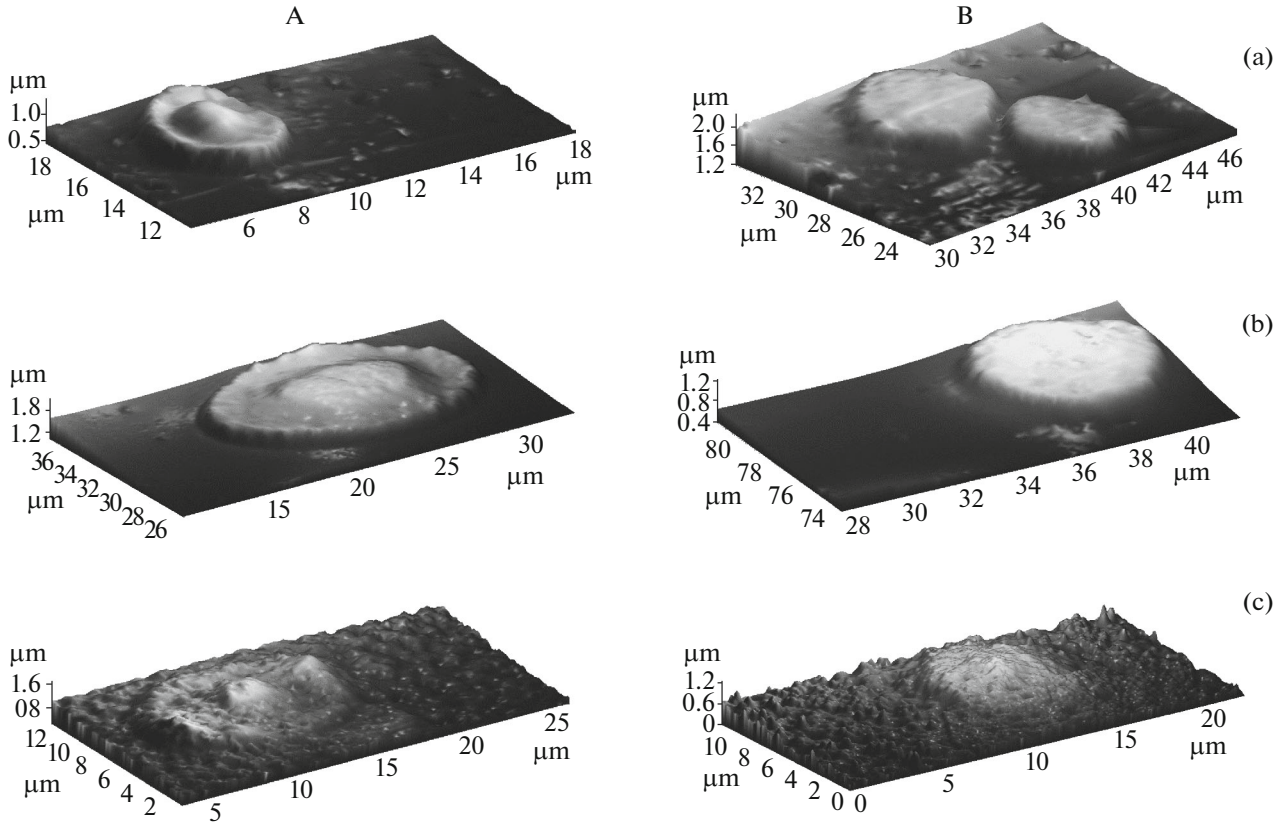

Fig. 1. Erythrocytes (A, μm) and polymorphonuclear leucocytes (B, μm) of the Prussian carp *Carassius gibelio* after incubation at 5 (a), 20 (b), and 40°C (c).

PMNL was measured with use of the Image Analysis 3.5.0.2070 program.

The experimental data were processed by variational statistics methods using special programs on a personal computer. The digital data are represented by the arithmetic mean (M) and the standard deviation ($\pm m$). The reliability of differences in the results was evaluated using the Wilcoxon–Mann–Whitney U-test.

RESULTS

Scans of erythrocytes of *Carassius gibelio* incubated at different temperatures are shown in Fig. 1A. The surface of the plasmalemma of erythrocytes of the Prussian carp under conditions of exposure at 5°C is rough and convex above the nucleus. The mean values of large and small diameters of red blood cells differ insignificantly; the ratio $D : d$ is $11.2 \pm 0.2 : 9.1 \pm 0.4$. After incubation at a temperature of 20°C, erythrocytes have an elliptical shape; they are characterized by the predominance of a large diameter of 15.6 ± 1.7 over a small 9.2 ± 0.4 (and the presence of a roughness on the surface of the cell). At an elevated incubation temperature (40°), the cells are also elliptical, the value of $D : d$ is $13.2 \pm 1.0 : 8.9 \pm 0.2$, and the surface of the erythrocyte plasmalemma acquires a more pronounced rough appearance.

Scans of polymorphonuclear leucocytes of fish incubated at temperatures of 5°C and 20°C, round in shape with close sizes $D : d$ and a rough surface of the plasmic membrane (Fig. 1B (a)–(b)). The value of the $D : d$ indices for PMNL at a low and room temperature incubation is $6.9 \pm 0.6 : 6.8 \pm 0.2$ and $7.2 \pm 0.8 : 6.1 \pm 0.1$, respectively. At an incubation temperature of 40°C, the cell volume significantly increases with the shape maintained; the value of $D : d$ is $11.2 \pm 1.2 : 9.7 \pm 0.7$. It should be observed that the surface of the leucocyte plasmalemma, as well as the erythrocytes, at this incubation temperature acquires a more pronounced rough appearance when compared to the incubation of cells at temperatures of 5°C and 20°C (Fig. 1B (c)).

Morphometric parameters of erythrocytes and leucocytes of *Carassius gibelio*, obtained after incubation at different temperatures, are presented in Table 1.

An increase in the volume of erythrocytes of 54.3% occurred upon the decrease in the incubation temperature from 20 to 5°C during the decreasing area and perimeter of 28.3% and 18.7%; an increase in incubation temperature from 20 to 40°C helps reduce the perimeter of red cells of blood by 17.3%.

In polymorphonuclear leucocytes at a low incubation temperature, only the area changes (decreases by 28.5%); with an increased incubation temperature, all the studied morphometric parameters increase: area (by 112.8%), volume (by 84.7%), and perimeter (by 106.8%).

The indicators characterizing the shifts in the physical properties of erythrocytes and polymorphonuclear leucocytes after incubation at different temperatures are presented in Table 2.

The lower incubation temperature decreases the physical parameters of hemocytes when compared to room temperature: the adhesion of red and white blood cells decreases by 25.6 and 38.1% and elasticity decreases by 63.7 and 61.2%, respectively.

The increased incubation temperature (40°C) causes a decrease in adhesion of PMNL by 19.4% and does not affect other studied physical indices of blood cells.

Table 1. Morphometric characteristics of blood cells of *Carassius gibelio* under different incubation temperatures

Index	5°C	20°C	40°C
Area, μm^2	74.2 ± 3.8** 26.9 ± 5.0*	103.5 ± 19.1 37.6 ± 7.3	95.2 ± 6.5 80.0 ± 15.4*
Volume, μm^3	167.7 ± 46.1* 45.6 ± 14.9	108.7 ± 23.0 36.1 ± 7.4	104.2 ± 34.0 66.6 ± 22.5*
Perimeter, μm	36.6 ± 1.6** 21.3 ± 2.5	45.0 ± 5.1 22.0 ± 1.9	37.2 ± 6.9* 45.6 ± 7.0*

Here and in Table 2, red blood cells are above the lines and polymorphic-nuclear leucocytes are under the line.

*$p \le 0.05$.

**$p \le 0.01$.

Table 2. Change in physical parameters of hemocytes in *Carassius gibelio* under the influence of the temperature factor

Index	5°C	20°C	40°C
Adhesion, nN	18.9 ± 4.1* 15.6 ± 1.9*	25.4 ± 2.6 25.2 ± 2.6	23.8 ± 5.0 20.3 ± 5.0*
Elasticity, kPa	15.8 ± 2.9* 15.7 ± 2.4*	43.5 ± 2.9 40.5 ± 3.1	41.7 ± 6.5 41.8 ± 8.3

* $p \le 0.05$.

DISCUSSION

A comparative evaluation of the results of the study made it possible to detect the peculiarities of the effect of the temperature factor on the morphometric and physical properties of the hemocytes of Prussian carp *Carassius gibelio* in experiments in vitro.

It has been experimentally established that, when the cells are cooled to 5°C and at room temperature (20°C), the surface of the plasmalemma of erythrocytes and polymorphonuclear leucocytes is rough, which is typical for blood cells of lower vertebrates [9, 17]. At an elevated incubation temperature (40°C), the surface of the blood cells of the *Carassius gibelio* acquires a more pronounced rough appearance. The appearance of roughness on the surface of hemocytes is associated with the disorganization of the cytoskeleton elements and the formation of actin-binding domains in the submembrane space that determine the formation of protrusions or protuberances on the plasmalemma [8, 20, 21, 25].

An analysis of the morphometric parameters of erythrocytes of *Carassius gibelio* revealed that these parameters in red blood cells of the Prussian carp change both at elevated and at low incubation temperatures, which is also characteristic of animals of other species, particularly mammals and humans, whose erythrocytes are denuclearized [6, 7].

Unlike erythrocytes, as was already mentioned, at a reduced incubation temperature, only the area decreases in polymorphonuclear leucocytes, whereas, at elevated temperatures, all the studied morphometric parameters are increased. It is known that the incubation of cells under conditions of an elevated temperature (40°C) causes the rapid expression of heat shock proteins (HSP) that perform a number of important protective functions in the cell [4, 16, 22, 23, 26], including interfering with the stress-induced denaturation of other proteins [19, 24]. The expression of HSP is usually triggered a few minutes after the onset of the heat load on cells [27], with some HSP associated with cytoskeleton proteins [28]. This relationship includes the involvement of HSP in the organization of the cytoskeleton in the process and/or after the heat load [1]. Apparently, similar reactions of the recovery of the protein fraction of the cell can lead to an increase in its morphometric parameters a very short period of time after the end of the effect of exogenous overheating.

A reduction of adhesion and elasticity of erythrocytes and polymorphonuclear leucocytes at a low incubation temperature, in comparison with room temperature, indirectly indicates the inclusion of adaptive mechanisms aimed at significantly reducing the functional activity of cells and slowing down metabolic processes as survival mechanisms in conditions unfavorable for the vital functions of hemocytes. Considering that the leading factors causing changes in the physical and physiological properties of blood cells are the properties of the plasmalemma [13, 15], it can be assumed that the decrease in the indices of its adhesion and elasticity after the incubation of erythrocytes of the Prussian carp at a temperature of 5°C for 120 min is due to changes in the microviscosity of the double-lipid layer, the phase distribution of lipids in it, the microenvironment of proteins, protein–lipid interactions, and a number of other features of the structural organization of the membrane [15] (manifested as roughness on the surface).

The stability of elasticity parameters in red and white blood cells, as well as adhesion in erythrocytes at an incubation temperature of 40°C, is consistent with the published data according to which [2] the Prussian carp belongs to the group of the most thermophilic fishes. The upper lethal temperature of these fish is 37–41°C. With the preliminary acclimatization of the Prussian carp to a temperature of >30°C, its upper lethal boundary of vital activity increases to 43.4°C [1]. In addition, under conditions of incubation at an elevated temperature (40°C), deviations in the adhesion and elasticity values of blood cells from their mean values are two times higher than at 20°C, which indicates significant fluctuations in the physical properties of red blood cells and PMNL of the Prussian carp and their high functional activity under conditions optimally favorable for the vital activity of this fish species.

CONCLUSIONS

At a high incubation temperature (40°C), the surface of the plasma membrane of erythrocytes and polymorphonuclear leucocytes of *Carassius gibelio* acquires a more pronounced rough appearance when compared to cells incubated at room temperature (20°C) and low (5°C) temperatures. In the erythrocytes of *Carassius gibelio*, the morphometric parameters change at a reduced incubation temperature (5°C); in polymorphonuclear leucocytes they change at an elevated temperature (40°C) when compared to room temperature (20°C). At an incubation temperature of 5°C, the adhesion and elasticity of polymorphonuclear leucocytes and erythrocytes of *Carassius gibelio* decreases; at 40°C the adhesion rates of polymorphonuclear white blood cells decrease.

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Translated by Z. Litvinenko