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CELL BIOPHYSICS

The Effect of Inhibitors of Cellular Respiration on Self-Oscillating Motility in Plasmodium *Physarum polycephalum*

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Abstract—This work presents the results of spectral analysis of the time dependences, $V(t)$, of endoplasmic shuttle motility in an isolated strand of plasmodium *Physarum polycephalum* that were obtained by laser Doppler microscopy after exposure to inhibitors of cellular respiration, viz., potassium cyanide and salicylhydroxamic acid, which lead to the complete cessation of endoplasmic motion. The results confirm the presence of only two harmonic components of $V(t)$ dependences, with frequencies that differ by a factor of 2, $\omega_2/\omega_1 = 1.972 \pm 0.028$, in different conditions: under normal conditions, without the addition of inhibitors; in a strand that was partially treated with inhibitors; and in the phase of restoring the oscillatory activity after the complete cessation of endoplasmic motion.

Keywords: amoeboid motion, *Physarum polycephalum* plasmodium, inhibitors of cellular respiration, self-oscillating motility, Doppler anemometry

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Autowave processes in living organisms are often studied using the myxomycete *Physarum polycephalum*, whose vegetative form is a unicellular multinuclear plasmodium with an amoeboid type of motility [1]. The plasmodium cell is a network of protoplasmic strands (tubules), in each of which shuttle endoplasmic motions are observed [2]. Motor activity in *P. polycephalum* strands is generated by the process of construction and destruction of actin–myosin filamentous complexes and depends on the free Ca^{2+} concentration [3–5]. Plasmodium is capable of moving along a chemoattractant gradient and favorable humidity and light conditions [6]. Since contraction is generated by a calcium-sensitive actomyosin system whose amino-acid composition is similar to that of contractile muscle proteins, *P. polycephalum* is often used as a model object to study muscular contractions [7, 8].

Local compressions (contractions) and subsequent relaxations that occur in different areas of plasmodium cause a complex motion of the endoplasm. In a general case, such behavior is regarded as a self-oscillating system, i.e., the contractile system of the plasmodium exhibits oscillatory activity. Three types of contractions can be distinguished, which collectively lead to the oscillatory behavior of the plasmodium endoplasm: longitudinal contractions, radial contractions, and cytoplasmic flow as such. In the 1970s it was shown that these oscillations are in phase and are set by one oscillator [9]. The analysis of the contractile rhythm in 30 different areas of the same plasmodium revealed a high synchronicity of contractions and led to the conclusion that plasmodium *P. polycephalum* represents a single synchronized contractile system [10–12]. Plasmodium has a longitudinal system of actomyosin fibrils that are located along the outer part of the strands and a circular system that surrounds the endoplasmic channel. Compression of the middle part of a single strand causes desynchronization of self-oscillations in both parts until the endoplasmic flow is restored. Connecting the protoplasmic mass of two separate strands also leads to synchronization of contractions. This indicates that synchronization is provided by the endoplasmic flow [13]; however, the nature of this phenomenon is not understood completely.

According to [14], the oscillations in the contractile activity in the plasmodium are accompanied by synchronous oscillations of the membrane potential. When studying the correlations of oscillations, radial deformations, endoplasmic flow strength, and membrane potential, it was established that the membrane potential and radial deformation in any portion of the strand oscillate synchronously and in phase.

The motility of plasmodium depends on oxygen consumption: ATP molecules that are synthesized during respiration are required to generate contractions of actomyosin molecules. Thus, by acting on respiration, it is possible to affect the motor activity of the plasmodium endoplasm.

Abbreviations: SHAM, salicylhydroxamic acid.

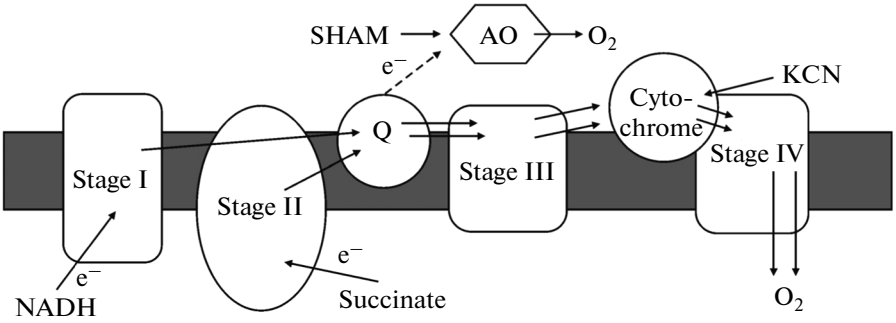

Fig. 1. The scheme of the electron-transport chain of plant mitochondria. Designations: I, II, III, IV, complexes of the electron-transport chain with the coupling sites; KCN, cytochrome *c* oxidase inhibitor; SHAM, alternative oxidase inhibitor [19].

Potassium cyanide (KCN) is often used in studies of the respiratory activity of plasmodium [15]. It is known that KCN inhibits the activity of the enzyme cytochrome c oxidase in complex IV of the respiratory electron transport chain in the inner mitochondrial membrane. The mechanism of action of this inhibitor is based on the ability of the cyanide anion to bind to the Fe–Cu center in cytochrome *c* oxidase due to chemical affinity thereby preventing oxygen reduction by electrons [16]. As a result, cells lose their ability to consume oxygen and the aerobic ATP synthesis stops.

Under exposure to adverse factors (including treatment with KCN), many simple organisms become capable of activating alternative pathways of cellular respiration, which are separated from the main respiratory chain at the level of ubiquinone [17]. This is due to an alternative oxidase, a special enzyme that is located in the inner mitochondrial membrane that is not sensitive to cyanide [18]. The cyanide-resistant alternative oxidase catalyzes electron transfer from reduced ubiquinone (coenzyme Q) to oxygen, bypassing the main cytochrome respiratory chain (Fig. 1). This pathway of electron transport has a smaller yield of ATP compared to the cytochrome electron-transfer pathway. The product of oxygen reduction by cyanide-resistant oxidase is water instead of the superoxide radical or H_2O_2.

Observations of plasmodium contractions in the presence of respiration inhibitors using tensometric sensors showed that in the presence of ketoglutarate (a key product of the citric-acid cycle), the contractile activity of plasmodium does not stop [20]. When glycolysis and the cytochrome pathway are inhibited, ketoglutarate maintains oscillations in the strands as the only source of ATP, which is not observed in the presence of succinate. However, oscillations stop immediately after exposure to salicylhydroxamic acid (SHAM) [21]. Thus, ketoglutarate is used in the cyanide-resistant respiration pathway and the amount of ATP that is produced in the first phosphorylation site is sufficient to maintain normal contractile activity. When the strand was treated with a solution containing only SHAM (without KCN), no change in oscillations was detected.

The cyanide-resistant respiration of *P. polycephalum* has also been studied in the presence of cyanide in dependence on the duration of preliminary oxygen starvation [22]. In this case, the effect of KCN and SHAM directly on the respiration process was assessed. It was shown that the inhibition of plasmodium respiration with KCN changed the baseline rate of O_2 uptake from 30 to 70% depending on the duration of preliminary oxygen starvation. The kinetics of the development of the cyanide-resistant respiration pathway and its sensitivity to SHAM indicated that the cyanide-resistant respiration reflects the activity of the alternative electron-transport pathway. However, evidence that the alternative respiration pathway is active in the absence of cyanide during oxygen starvation was not obtained. KCN completely inhibited oxygen uptake but had no effect on the respiration process as such. The inhibitor acted almost instantly (in milliseconds), which indicated its ability to rapidly penetrate into the cell. Subsequent addition of SHAM always caused an abrupt respiratory arrest. No exceptions to this pattern have been observed, regardless of the sequence of addition of inhibitors.

When added in the absence of cyanide, SHAM has no effect on the oxygen consumption rate in the first several minutes. Nevertheless, the addition of cyanide completely blocks the respiratory activity. The inhibitory effect of SHAM is delayed, which may indicate an indirect correlation with inhibition of the alternative oxidase. The kinetics of the cyanide-resistant respiration pathway under exposure to KCN is ~30% in the first minutes and reaches a maximum of ~70% of the initial oxygen uptake rate. In the case of SHAM, its level remains relatively constant (~80%) throughout the experiment.

Using a tensometric sensor, we studied plasmodium oscillations under the partial influence of inhibitors, when part of the strand was gradually immersed in a solution containing KCN and SHAM [23]. Oscillations continued even when the length of the submerged part reached 90% of the total length of the

strand. Complete immersion led to the blockage of oscillations.

The study of the formation of the endoplasmic flow between the treated and untreated portions of the strand by tensometric methods [13] showed that treatment with inhibitors of one part of the strand and an artificial block of the endoplasmic flow between the parts of the strand terminated force oscillations simultaneously with contraction and increase in the intracellular pressure. The same pattern was observed when the strand was completely immersed in a solution with inhibitors.

After restoration of the endoplasmic flow from the treated half of the strand to the untreated one, a decrease in the intracellular pressure along with a monotonic increase in the isometric strength was observed [13]. Thus, an increase in the intracellular pressure caused by respiration inhibitors is not a necessary condition for stabile contractile activity.

The constant influx of the endoplasm to the treated part of the strand was accompanied by an increase in the generated isometric strength without oscillations. The restoration of the endoplasmic flow was accompanied by the appearance of a contraction–relaxation cycle, despite the presence of respiration inhibitors. Oscillations were not induced during the endoplasmic flow from the untreated part to the treated one; therefore, there are no factors that could be transferred by the endoplasm to create oscillations. The treated part, despite the absence of oscillations, remained as an active system. Thus, an assumption was made that the factors that lead to the synchronization of mechanical and chemical oscillations in plasmodium are of a mechanical origin. It was assumed that the synchronization of oscillations between the treated and untreated parts is due to the mechanical forces of periodic air pressure [13].

In [24] it was shown that the termination of the contractile activity of the *P. polycephalum* plasmodium, which was observed when respiration is completely blocked, was associated with an increase in the level of cytoplasmic nicotinamide adenine dinucleotide. A change in the ratio between the oxidized and reduced NAD forms (NAD^+/NADH) can affect the contractile activity in two ways, viz., either through adenosine diphosphate ribose, which is a strong Ca^{2+}-releasing agent, or through NAD^+-dependent changes in the rate of ATP production in glycolysis.

Previously, by recording the dependences of the rate of the self-oscillatory endoplasmic motions in an isolated plasmodium strand using laser Doppler microscopy, two harmonic components were found in the recorded dependences of the endoplasm flow rate [25]. In addition, it was shown that the frequencies of these harmonics differ exactly by a factor of 2 with a good accuracy and are characteristic of an isolated plasmodium strand under normal conditions, as well as in the case of the restoration of motor activity after a complete stop of endoplasmic motion caused by treatment with the cellular respiration inhibitors KCN and SHAM.

This paper analyzes the shuttle endoplasmic motility after the addition of cellular respiration inhibitors and at the stage of the restoration of complete activity in the buffer solution after the removal (washing off) of these inhibitors.

MATERIALS AND METHODS

The method for recording the rate of the endoplasmic motion activity in a plasmodium strand using a sign-sensitive laser Doppler microscope on the basis of an LUMAM-P1 fluorescent microscope is based on measuring the Doppler shift of the carrier frequency of the signal that is generated by a moving particle in the endoplasm, which depends on the direction and velocity of signal-scattering particles.

He–Ne laser (PH-79-1) radiation beams (λ = 638.2 nm, power ≈1–5 mW) cross in the measured object and form a 5-mm^3 interference volume, which depends on the radiation wavelength, the beam intersection angle, and the geometry of the recording optics. Moving particles, while traversing the interference fringes of the measured volume in the perpendicular direction, reflect and scatter light, forming a Doppler shift with respect to the carrier frequency of the signal, which depends on the direction and velocity of its motion. Thus, the sign-sensitive recording [27] and subsequent computer processing of Doppler spectra allow one to obtain the time dependences of velocities in real time. The instrument records the time dependence of the endoplasmic motion in a plasmodium strand with an accuracy of ±0.5 μm/s.

The plasmodium was cultured by the standard procedure on a nutrient substrate (2% agarose gel with oat flakes) in a Petri dish at a temperature of 22 ± 2°C in the dark [28]. When the plasmodium reached the size of the Petri dish (approximately 10 cm in diameter), a strand 20 mm long and approximately 0.2 mm in diameter was excised from the whole myxomycete together with the nutrient medium, which was placed in a measuring cell (20 × 10 mm at the base) made of thin glass, which was filled with a buffered saline solution (1 mM $CaCl_2$, 2 mM NaCl, 2 mM KCl, and 3 mM $MgCl_2$, pH 7.2 ± 0.2) to maintain favorable conditions for the plasmodium activity [13]. Oscillations in the strand segment were restored within 15 min after immersion in the buffer solution. Experiments were performed using a thermostatic console that provided a constant temperature of 25 ± 1°C.

The time dependences of the endoplasm velocity $V(t)$ were measured in the center of a horizontally oriented strand for an average of 8 s (Doppler spectra were averaged over two to ten measurements, the accumulation time of each spectrum was 0.8–1.6 s). The measurement were recorded at 4 s intervals for

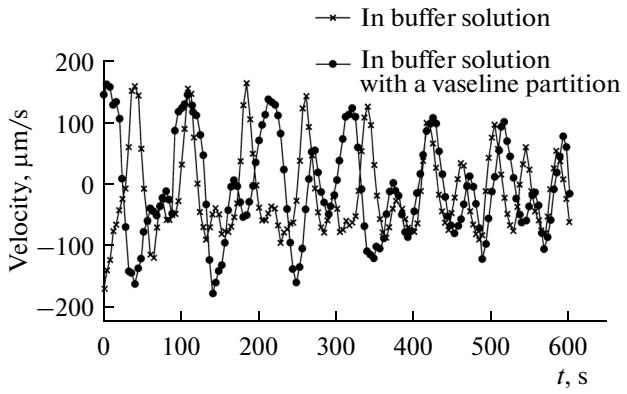

Fig. 2. The experimental time dependences of the endoplasmic velocity ($V(t)$) in buffered saline and in the same solution with a vaseline partition.

20 min. The interference fringes moved along the flow; the distance between them was $d = \lambda/2\sin(\alpha/2) = 1.08$ μm and the beam intersection angle was $\alpha = 38°$C.

The procedure of recording the locomotor activity of the plasmodium included several stages. The first experiment consisted in recording $V(t)$ under favorable conditions for 20 min in the absence of inhibitors in the buffer solution after placing the plasmodium segment in it. The inhibitors (5 mM KCN and 7 mM SHAM) were then added into the cell, which caused a complete cessation of motion of the plasmodium, for 5 min. Thereafter, the isolated strand was washed of the solution containing inhibitors and placed again in the buffered saline and the recovery of the endoplasmic motility was recorded for 20 min [25].

In this experiment, the cell was divided into two parts with a thin (0.5 mm) vaseline partition so that one half of the strand remained in one part of the cell and the other remained in the other part. The partition was required to prevent the flow and mixing of these solutions when different parts of the plasmodium strand were treated with solutions. One part of the cell was filled the buffer solution and the other was filled with the same solution containing the KCN and SHAM inhibitors, after which the $V(t)$ dependence recording was immediately started. It was confirmed experimentally (Fig. 2) that the presence of the partition had no effect on the plasmodium activity. The velocity was recorded in the center of the strand at the boundary between the treated and untreated parts.

RESULTS AND DISCUSSION

Spectral analysis of the experimentally obtained time dependences $V(t)$ was performed using the windowed Fourier transform. Figure 3a shows the time dependence of the endoplasmic velocity measured at the center of the strand, one part of which was placed in a buffer solution and the other was placed in a solution containing inhibitors. The dependence that is shown in Fig. 3b was obtained 30 min after the start of the experiment and reflects the complete recovery of the self-oscillating activity of the plasmodium after the removal of inhibitors.

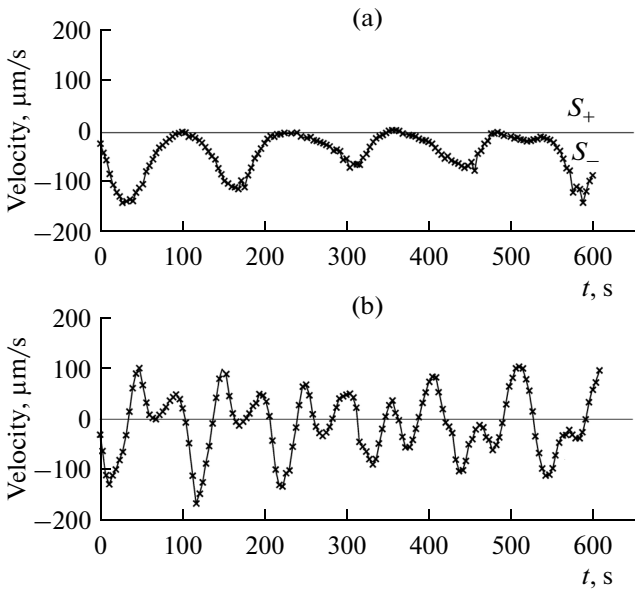

Fig. 3. The experimental time dependences of the endoplasmic velocity $V(t)$ (a) in a plasmodial strand, one part of which was placed in the buffer while the other was placed in the buffer containing KCN and SHAM; (b) 30 min later in the recovery phase of plasmodium oscillations after the removal of inhibitors.

The recorded dependences were fundamentally different; however, the dependence that was obtained at the stage of the complete recovery of activity was similar to the activity for the strand that was recorded earlier under normal conditions in the buffer solution. This fact confirms the return of the system to normal functioning, similar to that observed before the treatment with inhibitors.

The recording of the alternating velocity also makes it possible to quantitatively assess the direction of migration of the plasmodium as a whole organism [27]. The comparison of the integrand areas above (S_+) and below (S_-) the time axis allows one to make a conclusion on the directional migration to one side or the other; when $S_+ = S_-$ migration does not occur. $V(t)$ obtained in the first minutes after the addition of respiration inhibitors (Fig. 3a) shows the migration of the endoplasmic from the treated part of the strand; i.e., the measured velocity is negative. The ratio of the integrand areas under the curve for the first dependence S_-/S_+ was 6.6; i.e., the area below was far superior to the area above the x axis. At the stage of activity recovery (Fig. 3b), the ratio of these areas S_-/S_+ was 1.4. It was found that 15 min after the start of recording, the protoplasm began to return to the treated part, which was accompanied by the restoration of the

oscillatory activity in the entire strand. This was probably due to an unknown factor that was generated in the untreated part of the plasmodium in response to the partial influence of respiration inhibitors.

To test the above hypothesis, we performed an additional experiment in which two identical strands (20 mm long) were placed in the same initial conditions (buffer solution); however, one of them was immediately treated with a solution containing KCN and SHAM, whereas the second was treated successively, at a 15-min interval (first one half of the strand and then the other). Thus, we equalized the conditions in which the two strands were placed. As a result, the two plasmodium strand segments were under identical conditions (solution with inhibitors). However, in the first segment, which was immediately treated with the inhibitor solution, the endoplasmic motion was not recorded with an accuracy of ±0.5 μm/s, whereas in the second, which was treated sequentially, harmonic oscillations of the endoplasm were maintained at a frequency close to $\omega = 0.01$ Hz and an amplitude on the order of ±100 μm/s. In the plasmodium strand segment that was immediately treated with the solution of inhibitors, no directed motion was recorded. However, the change in the interference pattern of speckles in the measuring volume was similar to that observed when the protoplasm motion was stopped under normal conditions. After washing the isolated strands (removal of the solution containing KCN and SHAM and replacing it with the standard buffered saline), the common motion in the strands was resumed. A different sequence of the addition of respiration inhibitors to the cell with plasmodium, with the same initial and final conditions, led to a significantly different behavior of the endoplasm.

Thus, it can be assumed that when only one part of the strand is treated with inhibitors, the untreated part of the strand functions as a stimulant to maintain oscillations in the treated part. According to [23], the endoplasm of plasmodium does not contain factors that are carried by diffusion to create oscillations in other parts of the plasmodial cell.

The described behavior of the velocity time dependences indicates that the endoplasmic motion in the presence of inhibitors in only one part of the plasmodium triggers a new ATP phosphorylation pathway or glycolysis.

The time-dependence period for the strand that was placed in the buffer without inhibitors was, on average, $T_1 = 5$ s. After the treatment with inhibitors and their subsequent removal in the first minutes of the activity recovery, $T_2 = 50$ s (harmonic motion, the prevalence of the first harmonic in the spectrum); endoplasmic motion were not detected. For the dependence that was obtained for the strand for which one half was placed in the buffer solution and the other was placed in the solution with inhibitors, the oscillation period significantly increased to $T_2 = 120$ s, which

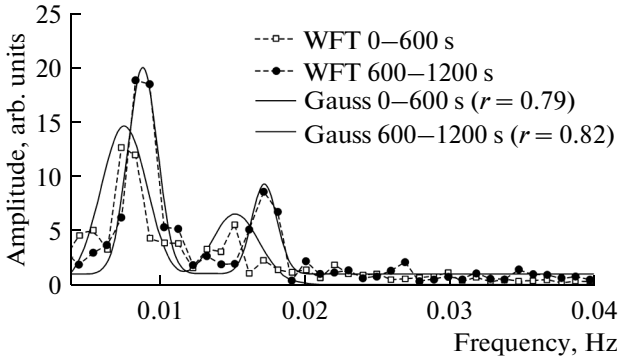

Fig. 4. The power spectra of the time dependence of the endoplasmic velocity in a plasmodial strand, one part of which was placed in the buffer solution while the other was placed in the solution containing KCN and SHAM (see. Fig. 3a). The symbols mark the frequencies of the 10-min intervals (min 0 to 10) that were obtained by the windowed Fourier transform (WFT). The solid lines show the theoretical fitting using Gaussian distributions (sum of Gaussians) with the respective Pearson's correlation coefficients *r*.

is a deceleration of motion. At the stage of motion recovery in the buffer, the period again began to decrease ($T_4 = 100$ s) and returned to the initial value. The comparison of the characteristics of the time dependence recorded after the addition of KCN and SHAM and under the normal conditions showed an increase in the oscillation period, which corresponds to the results that were obtained earlier [25], when an amplitude increase and deceleration of oscillations was detected. However, according to [10], the oscillation frequency does not change under conditions of an oxygen limitation. It was assumed that frequency variations can be significant, but they do not depend on the conditions (aerobic or anaerobic).

Spectral analysis (the windowed Fourier transform) of the time dependencies $V(t)$ was performed by sequential analysis of sections 600 s long (windows) with a 100 s shift. Thus, for a 20-min dependence, seven windows were analyzed, on the basis of which the changes can easily be traced. The use of spectral analysis for velocity time dependences, compared to those obtained previously [25, 26], showed that despite the substantial differences in the sequence of treatment with respiration inhibitors they were also characterized by the presence of two distinct frequency peaks in $V(t)$ spectra (Figs. 4, 5).

The frequencies that correspond to two maximum peaks in the frequency spectrum of the time dependence of the strand for which one half was placed in the solution with inhibitors (Fig. 3a) were $\omega_1 \pm \sigma = 0.0082 \pm 0.004$ and $\omega_2 \pm \sigma = 0.0161 \pm 0.0009$ Hz, respectively, where σ is the corresponding standard deviation. The ratio of frequencies within the measurement error (coefficient of variation $V = 2\%$) was approximately 2 ($\omega_2/\omega_1 = 1.957$). The frequencies of

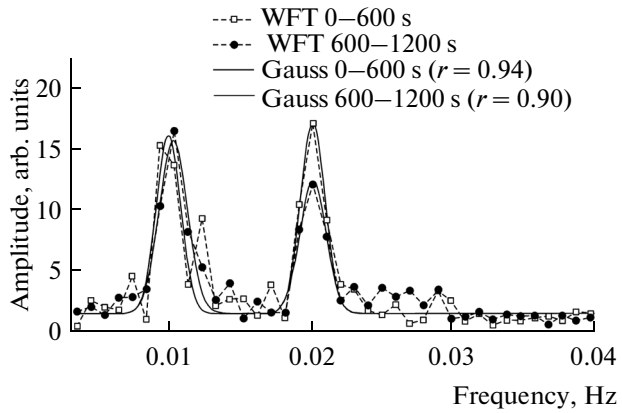

Fig. 5. The power spectra of the time dependence of the endoplasmic velocity in the buffer solution in the recovery phase of activity after washing (see Fig. 3b). Designations: WFT, windowed Fourier transform, the frequencies for 10-min intervals (min 0 to 10). The solid lines show the theoretical fitting using Gaussian distribution with the respective Pearson's correlation coefficients *r*.

the signal during the complete recovery of plasmodium activity were close to the frequencies that were obtained in the buffer before the strand treatment ($\omega_1 \pm \sigma$ and $\omega_2 \pm \sigma$ Hz, respectively). A similar ratio of frequencies was also characteristic of this signal ($\omega_2/\omega_1 = 2.008$ with a coefficient of variation $V = 1.7\%$). It should be noted that the frequencies under the same conditions without treatment in the buffer solution (the first phase dependence and the recovery period) had similar values, which means that the self-oscillating activity can return to the initial state, completely restoring the migratory activity. The frequencies of the harmonics of the time dependences (Figs. 3a, 3b) are summarized in Table 1.

These results indicate the presence of two harmonic oscillators in each of the recorded signals, despite the different conditions of recording the velocity time dependences. In addition, the constant ratio of the frequencies of harmonic oscillations present in $V(t)$ dependences was retained in all cases, which for all four cases (averaged values) was ($\omega_2/\omega_1 = 1.972 \pm 0.025$.

The quantitative comparison of the amplitudes of the obtained harmonics (A_i) showed their energetic relationship. The maximum amplitude values were determined by the corresponding maxima of the peaks for each window and then averaged over the entire spectrum. As can be seen from the frequency spectrum that corresponds to the dependence of the endoplasmic velocity in solutions, the addition of inhibitors affected both frequency peaks in the first minute of measurement ($A_1 = 12.3 \pm 4.2$ arb. units and $A_2 = 5.6 \pm 1.6$ arb. units). This pattern changed significantly 10 min later: the amplitude of each peak increased to $A_1 = 19.5 \pm 5.1$ and $A_2 = 8.1 \pm 1.5$ arb. units. Table 2 shows the values of amplitudes of the first (A_1) and second (A_2) harmonics of the frequency spectrum for all dependences at the beginning of measurement (0 min) and 10 min after the beginning of measurements (10 min). There is a correlation between the redistribution of the intensities of corresponding peaks in the obtained spectra, viz., when the amplitude of the first peak decreased, the amplitude of the second peak increased. This fact indicates the energy interdependence of the sources that set the harmonic oscillations (internal oscillators).

Summing the values of the integrand peak areas for the entire frequency spectrum (Table 3) showed that the total spectrum power in the 600-s window with a 100-s shift remained relatively constant in each of the spectra. Regardless of the effect on the plasmodium, only the ratio of the amplitudes of the harmonic components of the spectrum changed.

The theoretical time dependences of the endoplasmic motion velocities were modeled on the basis of the calculated frequencies [25]. The theoretical dependence is the sum of the harmonic components distinguished in the experimental dependence with the corresponding frequencies, amplitudes, and phases (Figs. 6a, 6b).

$$V(t) = A_1(t)e^{i(\nu_1 t + \varphi_1)} + A_2(t)e^{i(\nu_2 t + \varphi_2)}, \qquad (1)$$

Table 1. The harmonic frequencies of the dependences of the rate that were obtained for a strand, one half of which was placed in the buffer solution, while the other was placed in the solution with inhibitors (see Fig. 3a and Fig. 4)

	t, s		0	100	200	300	400	500	600
$V_1(t)$	Harmonic 1	ω_1	0.0077	0.0077	0.0078	0.0085	0.0085	0.0084	0.0085
	Harmonic 2	ω_2	0.0152	0.0151	0.0151	0.0161	0.0163	0.0169	0.0173
	$\omega_2/\omega_1 \pm 2\%$		1.9612	1.9683	1.938	1.8846	1.9100	2.0094	2.0291
$V_2(t)$	Harmonic 1	ω_1	0.0097	0.0097	0.0099	0.0101	0.0101	0.0101	0.0102
	Harmonic 2	ω_2	0.0200	0.0199	0.0200	0.0201	0.0201	0.0199	0.0201
	$\omega_2/\omega_1 \pm 1.7\%$		2.0606	2.0535	2.0272	1.9856	1.998	1.9607	1.9696

$V_1(t)$, the first several minutes after the addition of inhibitors, and the dependences of the velocity for the strand in the recovery phase ($V_2(t)$).

Table 2. The amplitudes of peaks with frequencies ω_1 and ω_2 for the following dependences: in the buffer solution before the treatment with KCN + SHAM ($V_1(t)$); 10 min after the treatment with KCN + SHAM ($V_2(t)$); one part of the plasmodium was in the buffer solution, while the other was in the solution containing KCN + SHAM ($V_3(t)$); plasmodium in the buffer solution and the solution containing KCN + SHAM 30 min later ($V_4(t)$. See Fig. 3b and Fig. 5

Amplitude		$V_1(t)$	$V_2(t)$	$V_3(t)$	$V_4(t)$
min 0	A_1	17.1 ± 5.3	25.9 ± 7.4	12.3 ± 4.2	14.4 ± 5.1
	A_2	12.4 ± 3.6	3.6 ± 1.1	5.6 ± 1.6	17.1 ± 4.7
min 10	A_1	19.6 ± 6.9	14.8 ± 3.8	19.5 ± 5.1	16.1 ± 4.9
	A_2	11.8 ± 3.7	7.3 ± 2.2	8.1 ± 1.5	12.3 ± 3.6

Table 3. The total peak power of the frequency spectra for all time dependences $V(t)$

Dependence	0	100	200	300	400	500	600	Mean value of the total peak area, arb. units
$V_1(t)$	149.2	126.7	128.2	131.9	128.4	130.6	133.9	132.7 ± 7.7
$V_2(t)$	157.3	159.8	161.3	159.8	164.9	162.1	159.6	160.7 ± 2.9
$V_3(t)$	64.7	60.6	74.5	85.6	89.5	90.6	90.8	79.5 ± 12.8
$V_4(t)$	109.2	110.1	105.9	98	99.3	100.6	98.5	103.1 ± 5.2

where $A_{1,2}$ are the harmonic amplitudes, $\nu_{1,2}$ are respective frequencies, and $\varphi_{1,2}$ are the phases of the harmonics.

Fitting the model signal of velocity dependences (2) taking the amplitudes, frequencies, and phases into account gave a good agreement with the measured velocity values ($V(t)$) in both cases.

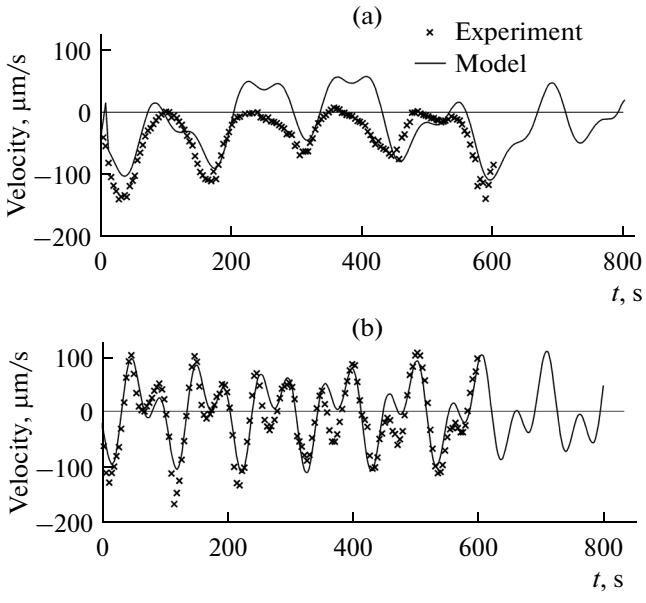

Fig. 6. Comparison of the model (solid bold line) with the experimental values (crosses) of the $V(t)$ dependences: (a) in the center of a strand, one part of which was placed in the buffer while the other was placed in the buffer containing KCN and SHAM; (b) 30 min later in the recovery phase of plasmodium oscillations.

The calculated value of Pearson coefficients was $\chi^2 = 11.4$ at the level of significance $\alpha = 0.05$, indicating the agreement between theoretical and empirical data. Thus, the main contribution to the generation of oscillations of the plasmodium is made by only two harmonic components. A more accurate description may require taking additional factors into account.

CONCLUSIONS

The time dependences $V(t)$ and their spectral analysis, which were performed using the windowed Fourier transform, showed that after the treatment of the isolated strand of plasmodium with the KCN and SHAM cellular respiration inhibitors, the oscillating system is capable of complete restoration of motor activity, provided that the inhibitors are removed and the plasmodium is placed in favorable conditions. Spectral analysis of the time dependences of the endoplasmic velocity confirmed the presence of only two harmonic components, regardless of the conditions in which the isolated plasmodium strand was placed. The frequencies of the harmonics were different, but their ratio was equal to 2 ($\omega_2/\omega_1 = 1.972 \pm 0.028$) with a good accuracy and remained constant in all measurements, regardless of the conditions in which the plasmodium was placed (see [25]). This indicates the presence of one internal oscillator [9], whose frequency is somehow doubled and shifted in phase, or two types of oscillators whose frequencies differ by exactly a fact of 2.

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