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EXPERIMENTAL ARTICLES

Effect of Methylmercury on the Light Dependence Fluorescence Parameters in a Green Alga *Chlamydomonas moewusii*

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Abstract—The effect of a dangerous toxic substance, methylmercury, on light dependence curves of chlorophyll fluorescence in *Chlamydomonas moewusii* was studied. We found low concentration of methylmercury (10^{-7} M) to cause a decrease in the relative rate of the non-cyclic electron transport activity of PS2, a decline in the maximum utilization of light energy (α), and a decline in the saturation light intensity (E_s). Nonphotochemical fluorescence quenching increased after short-term exposure and decreased in the course of prolonged incubation. These parameters were more sensitive to the action of the toxic substance than the widely used parameter F_V/F_M, which reflects the maximum quantum yield of PS2. We propose the use of the method of fast measurement of light dependence curves of fluorescence to detect the changes in algal cells at the early stages of exposure to mercury salts.

Keywords: Chlamydomonas moewusii, methylmercury, chlorophyll fluorescence, photosystem, bioassay, ecology

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Due to their high toxicity, combined with ability to accumulate in organisms and to be transferred along the trophic chain, heavy metals are important contaminants of the environment (Moore and Ramamoorthy, 1984). In water bodies, heavy metals have a toxic effect on phytoplankton, which is the primary component in the system of food interactions of aquatic organisms determining the state of an aquatic ecosystem as a whole. Mercury compounds are among the most environmentally hazardous (Bertrand and Poirier, 2005). Organic mercury compounds, including extremely toxic methylmercury, which is formed by mercuration of organic compounds, are especially dangerous (Lu et al., 2000; Janeau et al., 2001). Photosynthesis is among the metabolic processes of plant cells sensitive to heavy metals. Mercury compounds are known to inhibit photosynthetic light reactions (Janeau et al., 2001; Antal et al., 2003; Graevskaya et al., 2003). The major mechanism of action of these compounds is inhibition of the membrane processes due to interaction with SH-containing compounds and disulfide groups of proteins, as well as coenzyme replacement (Stohs and Bagchi, 1995).

Requirement for the systems of on-line control of natural water quality and waste toxicity resulted recently in widespread application of biotesting (Zayadan and Matorin, 2015; Filenko, 1988; Perminova et al., 2001). Microalgae, which are the major producers in aquatic environments and act as targets for the metal salts arriving into aquatic ecosystems, are the most important on-line biotest organisms (Filenko, 1988; Vavilin et al., 1995; Zayadan and Matorin, 2015).

The methods based on measuring chlorophyll concentrations are promising for the monitoring of algal photosynthesis in the presence of heavy metal salts (Vavilin et al., 1995; Matorin et al., 2007; Matorin et al., 2014; Black and Frank, 1998). Fluorescent methods are based on the fact that chlorophyll of the photosynthetic membranes acts as a natural indicator of the state of algal cells and their photosynthetic apparatus. Efficiency of photochemical energy transformation in photosystem 2 (subsequently PS2 photochemical activity) and the coefficients of photochemical and nonphotochemical fluorescence quenching are the most important characteristics of photosynthetic primary light reactions, which are determined using PAM (pulse-amplitude-modulation). Methods for rapid measurement of light dependencies (light curves) of various fluorescence parameters, which reflect the development of photochemical and nonphotochemical quenching in the light, are presently actively used for the work with plant leaves and algal cultures. They make it possible to register early changes in the operation of the photosynthetic apparatus as the effect of environmental factors (White and Critchley, 1999; Serodio et al., 2005; Ralph and Gademann, 2005; Herlory et al., 2007). We have previously used this method to investigate the state of environ-

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mental phytoplankton (Matorin et al., 2004; Zayadan and Matorin, 2015).

Fluorescent methods are characterized by rapidity and high sensitivity, providing for real-time characterization of the state of microalgal cells subject to toxicants immediately in their native environment (Matorin et al., 2004). Rapidity of the measurements of the fluorescent parameters is especially important for early detection of pollutants in the environment.

In the present work, changes in the light dependencies of the parameters of chlorophyll fluorescence in the cells of *Chlamydomonas moewusii* in the presence of various concentrations of methylmercury were studied. These dependencies were shown to be applicable for the registration of the changes in energy-storing photosynthetic processes at very low methylmercury concentrations.

MATERIALS AND METHODS

Algal cultures and toxicant treatment. Algologically pure culture of a green alga *C. moewusii Gerloff, Lewin 1002, CALU 228* (flagellaless mutant), which was the subject of research, was obtained from the collection of the Department of Microbiology, St. Petersburg State University, Russia. Unlike the wild type, the cells of this strain do not possess flagella at any stage of growth in the media used. The culture was grown under phototrophic conditions in Tris–acetate–phosphate medium (pH 7.0) at 25°C under illumination by fluorescent lamps (30 μE/(m^2 s) with the duration of light and dark periods 14 and 10 h, respectively. The stationary-phase culture was aseptically dispensed (100 mL) into 250-mL flasks and supplemented with the studied methylmercury salt (MeHg chloride, Aldrich Chemical Co., United States). The algae were incubated with different methylmercury concentrations for several hours to two days under the conditions used for cultivation. Cell concentration prior to methylmercury addition was 250×10^3 cells/mL.

The algae were enumerated by cell count in a Goryaev chamber ($V = 0.0001$ mL) in three repeats.

The fluorescence parameters were measured on a WaterPAM pulse fluorometer (Walz, Germany). Dark-adapted algal samples were used to determine the constant (F_o) and maximal fluorescence (F_M), as well as the relative yield of variable fluorescence (F_V/F_M), which is a measure of the maximal PS2 potential quantum efficiency. Short-time light dependencies of the fluorescence parameters were measured at light intensity increasing stepwise from 0 to 400 μE/(m^2 s) (White and Critchley, 1999). Illumination time was 50 s. Rapid adaptive changes in algal photosynthesis at increased light intensity were shown to occur during this period (Serodio et al., 2005). At the end of each illumination session, a saturating flash (0.8 s, 3000 μE/(m^2 s)) was used to register the F_M' parameters and fluorescence yield in the light $F(t)$. These parameters were used to calculate nonphotochemical fluorescence quenching NPQ = $(F_M - F'_M)/F'_M$, quantum yield of photochemical conversion of absorbed light energy in photosystem 2 as the ratio $Y = (F'_M - F_t)/F'_M$, and the relative rate of noncyclic electron transport as a given light intensity (ETR). The rate of electron transport was determined using the equation $ETR = Y \times E_i \times 0.5$, where E_i is illumination in μE/(m^2 s) (Lippemeier et al., 1999). The light curves (*P/A* curves) thus obtained were used to assess the following photosynthetic parameters: coefficient of maximal utilization of light energy (slope of the *P/A* curve, α), maximal relative electron rate in the electron transport chain (ETR_{max}), and saturating light intensity (E_s). The αvalue was calculated as the coefficient of linear regression built for the points on the light-limited part of the *P/E* curve. ETR_{max} was calculated as the average ETR value for the light-saturating part (Jassby and Platt, 1976). E_s was calculated as $E_s = ETR_{max}/\alpha$ (Platt et al., 1977; MacInture et al., 2002). The designations and determinations of photosynthetic parameters are presented according to accepted nomenclature (Platt et al., 1977).

RESULTS AND DISCUSSION

The concentrations of *C. moewusii* cells during growth under the control conditions and in the presence of methylmercury are presented on Fig. 1a. As can be seen, in the control after the lag phase (3 h) a rapid increase in cell numbers occurs. At low methylmercury concentrations (10^{-7} M), cell number was somewhat higher than in the control after the first few hours, although growth was arrested afterwards. At 5×10^{-7} M methylmercury, no initial increase in cell number occurred, and prolonged incubation (over 24 h) resulted in a significant decrease of cell numbers.

Similar dependencies were observed for the level of constant fluorescence F_o (not shown), which corresponds with a high correlation coefficient to the total pigment content in the algal photosynthetic apparatus responsible for light harvesting. This parameter is considered an indirect indicator of the concentration of algal light-absorbing pigments (Matorin et al., 2004).

Microalgal photosynthetic apparatus is a sensitive target for mercury salts (Janeau et al., 2001; Antal et al., 2003). The F_V/F_M value reflects the PS2 maximal quantum yield (Schreiber et al., 1994). It is associated with the processes of water decomposition and oxygen emission. High F_V/F_M (0.7) was retained in the control throughout the extensive cultivation period (Fig. 1b). Addition of methylmercury (5×10^{-7} M) resulted in complete PS2 inactivation after 24 h, which is in agreement with the data on high toxicity of this compound (Janeau et al., 2001; Graevskaya et al., 2003). At 10^{-7} M methylmercury caused an F_V/F_M decrease from 0.7 to 0.55. The F_V/F_M changed mainly

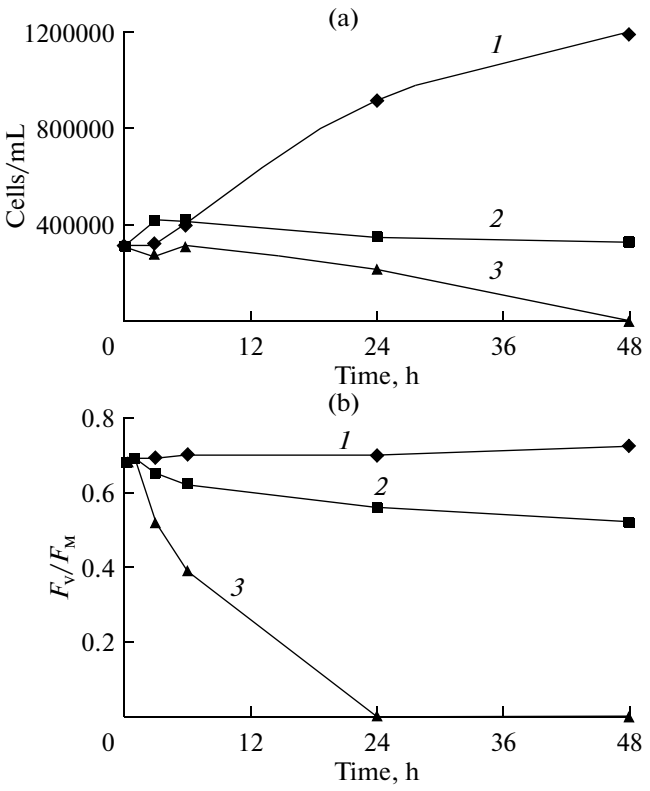

Fig. 1. Cell numbers (a) and the F_V/F_M fluorescence parameter (b) in *C. moewusii* suspensions depending on incubation time with methylmercury. Control (*1*), 10^{-7} and 5×10^{-7} M methylmercury (*2* and *3*, respectively).

due to a decrease in the amplitude of maximal fluorescence, F_M. It should be noted that F_o and therefore the cell number did not change significantly in the case of short-term (several hours) incubation with methylmercury. During short incubation times, absorption spectra of algal suspensions did not change considerably (data not shown), indicating the absence of effect on the pigment apparatus. Thus, monitoring the changes in photosynthetic activity by F_V/F_M makes it possible to detect the effect at early stages of the toxic impact.

The most drastic differences in the algae treated with mercury salts were revealed by measurement and analysis of the fluorescence parameters under different illumination intensities, i.e., under conditions of increasing light load (see Materials and Methods).

Light dependencies of the relative rate of acyclic electron transport (ETR) in the algae exposed to methylmercury calculated as described above are presented on Fig. 2. It can be seen that the rates of electron transport at all levels of illumination decreased considerably in the presence of methylmercury. The maximal rate of electrons in the electron transport chain (ETR_{max}) was the highest in the control (Fig. 2). After addition of methylmercury, lower ETR_{max} values were observed. After 24-h incubation with 5×10^{-7} M methylmercury, ETR was extremely low, which was in agreement with low dark F_V/F_M values under the same conditions.

The parameters describing these dependencies between photosynthetic activity and illumination were determined: the coefficient of maximal utilization of light energy (slope at the linear part of the light curve) (α) and saturating light intensity (E_s). The highest coefficient of maximal utilization of light energy (α) was observed for the control cells (Fig. 3). After addition of methylmercury, the value of this parameter decreased. The highest saturating light intensity (E_s) was observed for the control (204 $\mu E/(m^2\ s)$. After addition of methylmercury the saturating light intensity decreased drastically. Importantly, changes in the coefficient of maximal utilization of light energy (α) and saturating light intensity (E_s) were detected earlier than the F_V/F_M changes (Fig. 1b, *3*).

Decreased PS2 quantum yield (Y) at elevated illumination of the algae results from heat dissipation of excessive light energy, which cannot be utilized in light reactions. This process is reflected in the changes in nonphotochemical quenching of fluorescence under active light, which is calculated as NPQ = $(F_M/F'_M) - 1$ (Schreiber et al., 1994). Mercury-containing compounds affecting the primary processes of energy utilization in PS2 may result in increased energy dissipation as heat in the PS2 antennal complexes. The data on light-induced nonphotochemical fluorescence quenching (NPQ) in *C. moewusii* cells at several time points after addition of methylmercury are shown on Fig. 2. At low methylmercury concentration, especially at low light intensities, NPQ was higher than in the control, while at a high concentration (5×10^{-7} M) and incubation time exceeding 3 h the amplitude decreased. We have previously reported this effect for the action of methylmercury on marine algae (Antal et al., 2003; Graevskaya et al., 2003; Wu and Wang, 2013). Enhanced nonphotochemical fluorescence quenching at low methylmercury concentrations probably resulted from increased contribution of the energization component of the intrathylakoid pH due to impaired phosphorylation processes in the presence of mercury. Higher concentrations cause considerable disruption of the electron transport and the native state of photosynthetic membranes, where the proton electrochemical gradient is developed, which results in impaired nonphotochemical discharge of excessive light energy. In both the control and the methylmercury-containing sample, nonphotochemical fluorescence quenching was completely suppressed by methylamine, an uncoupler of phosphorylation in thylakoid membranes (data not shown). Importantly, the curve of NPQ amplitude depending on light intensity was nonmonotonic, both in the control and especially in methylmercury-treated samples. In the samples treated with 10^{-7} M methylmercury, NPQ initially increased and then

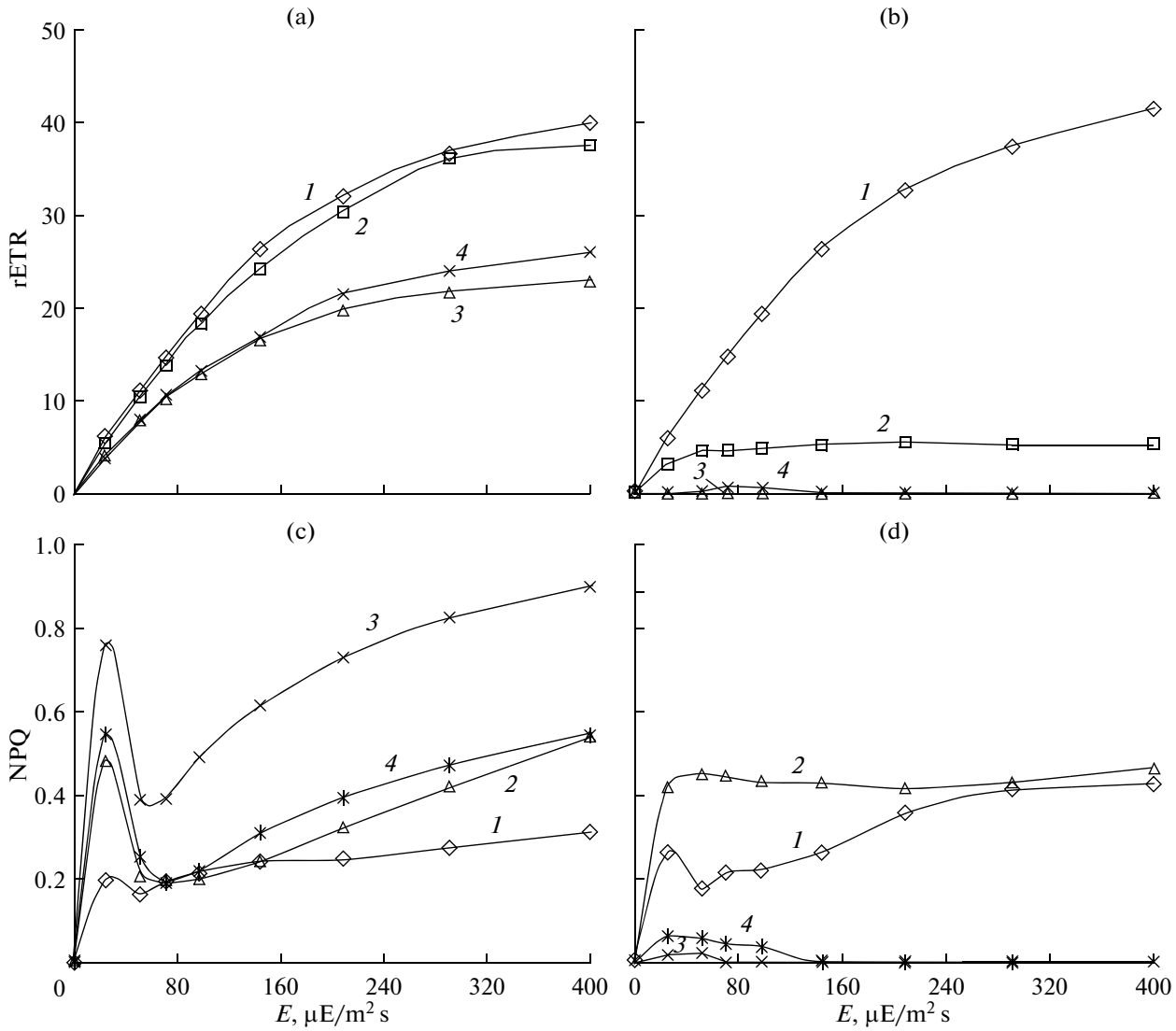

Fig. 2. Fluorescent parameters depending on light intensity in *C. moewusii* cell suspensions treated with 10^{-7} (a, c) and 5×10^{-7} M methylmercury (b, d) at different incubation times: relative rate of electron transport ETR (a, b) and nonphotochemical quenching (c, d). Control (*1*), 3, 24, and 48 h of incubation with methylmercury (*2, 3*, and *4*, respectively).

decreased slightly at 60 μE/(m^2 s) with a subsequent increase. This was probably due to nonlinear nature of the processes during registration of rapid light dependencies.

Salts of heavy metals are known to affect various processes in plant cells. According to the literature data, the ions of heavy metals act via binding to organic acids or phosphate anions, blocking the highly important groups (such as SH groups), or by replacing other metal ions in proteins (Stohs and Bagchi, 1995; Rauser, 1999). These processes result in lipid peroxidation, impaired ion transport and homeostasis, increased ATP concentration, inhibition of the enzymatic systems (antioxidant system enzymes, ATPases), and DNA damage (Luo et al., 1996; Navari-Izzo and Quartacci, 2001). These changes cause growth arrest of algal cell populations. The biotesting techniques used for assessment of water contamination by various agents and establishment of the standards of permissible load in aquatic ecosystems are based on analysis of algal growth rate in the presence of toxicants. Our experiments with methylmercury revealed, however, that short-term (several hours) incubation even with low concentrations of the toxicant resulted in the changes in photosynthetic light reactions, which could be observed as changes in light dependencies of the fluorescence parameters. Quantum yield of photochemical conversion of absorbed light energy in PS2 and relative rate of acyclic electron transport decreased. The coefficient of maximal utilization of light energy (α) and saturating light intensity (E_s) also changed. Moreover, in the presence of methylmercury, thermal energy dissipa-

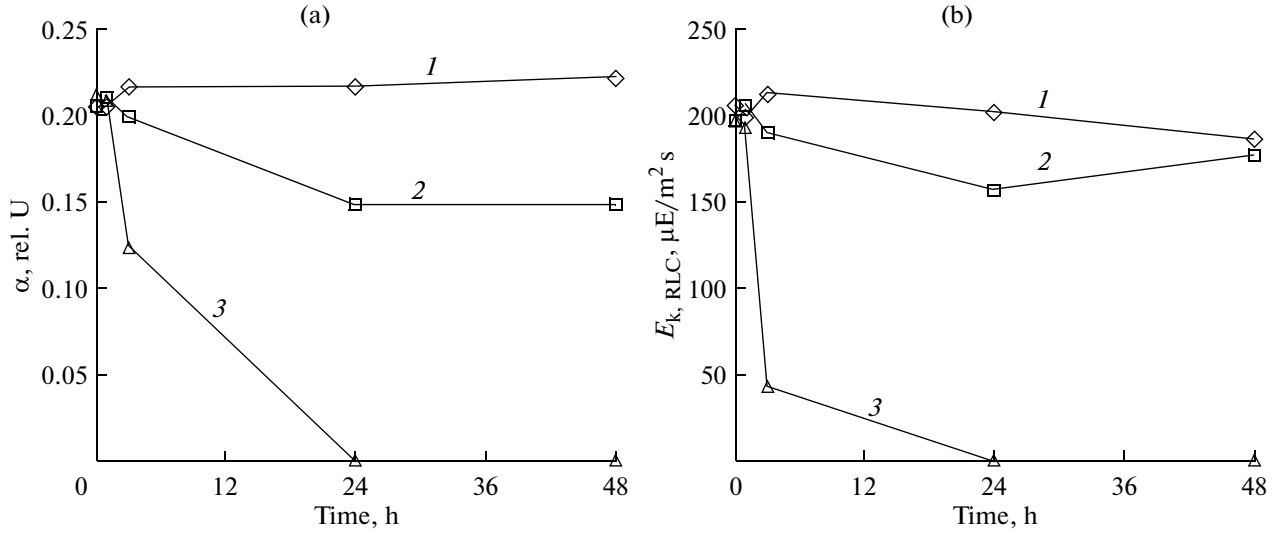

Fig. 3. Parameters of light dependence of the relative electron transport rate ETR in *C. moewusii* cell suspensions depending on incubation time: control (*1*), 10^{-7} and $5 \times 10_{-7}$ M methylmercury (*2* and *3*, respectively). Coefficient of maximal light energy utilization α (a) and saturating light intensity E_s (b).

tion in the PS2 antennal complexes increased, which is, according to preliminary data, associated with impaired phosphorylation in the thylakoids.

Since short incubation times are insufficient for significant differences in cell numbers, control of toxicological effect by growth rates is difficult. Measurement of the changes in photosynthetic activity registered using the light curves of electron transport and NPQ makes it possible to reveal the toxicological effect at early stages and therefore to take the relevant measures for environmental protection at an earlier stage.

Increased toxic effect of methylmercury on PS2 at enhanced illumination is probably due to inhibited reparation of the photosystem. A similar effect has been previously reported for copper salts (Vavilin et al., 1995). It may play a significant role in a decrease of the photosynthetic activity of the cells at low concentrations of these metals under conditions of photooxidative stress and may be used for detection of the action of low concentrations of mercury salts on algal communities.

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