Biophysics, Vol. 45, No. 3, 2000, pp. 479–482. Translated from Biofizika, Vol. 45, No. 3, 2000, pp. 491–494.

CELL BIOPHYSICS

Fluorescence Methods of the Photosystem II Activity Biomonitoring in Phytoplankton

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Received May 12, 1999

Abstract—The photosystem II activity regulation by environmental factors was studied in microalgae. Luminescence methods of phytoplankton biomonitoring *in situ* are suggested.

Key words: chlorophyll fluorescence, photosystem II, phytoplankton, biomonitoring

INTRODUCTION

The primary organic matter is formed in aquatic ecosystems as a result of photosynthetic activity. Various natural and anthropogenic ecological factors affect the photosynthetic activity of algal cells, thereby reducing their productivity. The primary light-induced reactions of photosynthesis play the central role in the photosynthetic activity of phytoplankton. These reactions are based on the highly efficient processes of solar energy transformation into the energy of chemical bonds implemented in photosynthetic reaction centers.

In recent years there has been substantial progress in studies of structural and molecular organization of photosystem (PS) II. It should be noted that PS II activity is associated with such biologically important processes as water oxidation and oxygen evolution. Obviously, these processes exerted a significant impact on the evolution of life on the Earth [1]. The reaction center (RC) of PS II was shown to consist of two polypeptides, one of them (D1) being bound to the main electron-transport cofactors of RC [2, 3]. Recent progress in elucidation of the structural organization of PS II provides methodological approaches to studies of the mechanisms of functional-genetic regulation of PS II activity in intact cells of microalgae exposed to various environmental conditions. Elucidation of specific effects of ecological factors on the photosynthetic apparatus and cell membranes of microalgae is of considerable theoretical and practical importance for the development of methods of prognosis of the biological productivity of aquatic ecosystems and biomonitoring of the aquatic environment.

The goal of this work was to consider the processes of regulation of PS II activity in microalgae exposed to various environmental factors. Elucidation of the possibility of luminescence biomonitoring of phytoplankton was an additional goal of this work.

EXPERIMENTAL

Methods based on chlorophyll luminescence detection were used to study photosynthetic processes in algal cells. Because of high sensitivity, these methods can be used for studying natural phytoplankton *in situ* (i.e., without preliminarily concentrating the experimental samples) [5, 6]. Activity of PS II was determined from variable fluorescence parameters (F_v/F_m = $= (F_m - F_0)/F_m$ [5]). The two-beam submerged fluorimeter with pulse excitation developed by us and described elsewhere [7] was used in experiments with natural phytoplankton. This device allows simultaneous measurements of fluorescence parameters

Abbreviations: PS II, photosystem II; RC, reaction center; LPO, lipid peroxidation; TL, thermoluminescence

(F_v/F_m and F_m), water temperature, and underwater illuminance.

RESULTS AND DISCUSSION

The sophisticated photosynthetic system of intact algal cells adapts to environmental factors by changing the structural and functional organization of photosynthetic membranes [8, 9]. The adaptation processes may include changes in the composition of pigment–protein complexes, concentration and activity of photosynthetic reaction centers, regulation of excitation energy distribution between photosystems, change in the lipid composition of cell membranes, and efficiency of energy storage processes [8–12].

Our own results and data obtained by other authors [8, 13, 14] show that changes in the PS II activity play a decisive role in the regulation of photosynthetic activity in microalgae exposed to variable environmental conditions. The PS II working conditions are rather harsh: at extremely high redox potential required to oxidize water and the presence of oxygen radicals produced during water oxidation and oxygen evolution [2]. Therefore, there should be a system of regulation of PS II activity. It was found that the quantum efficiency of PS II in cultures of microalgae declines under intense illumination (photoinhibition) [2, 5, 14], short supply of mineral nutrients [8, 13, 15], low temperature [9, 11], and exposure to anthropogenic pollution [16, 17]. Inhibition of PS II activity is manifested as a decrease in the maximum quantum yield of PS II (F_v/F_m) and the amplitude of the millisecond components of delayed fluorescence (DF). On the other hand, PS I is significantly more resistant to detrimental factors than PS II, because PS I is connected to cyclic electron transport and NADP reduction. It was shown that PS I is damaged only at the late stages of development of a pathological process [2, 11].

Rate constants of photoinhibition (K_i) and reactivation (K_r) of PS II in phytoplankton cells under standard conditions and after adaptation to various growth conditions

Standard conditions			
Temperature,°C	Illuminance, W/m^2	K_i, h^{-1}	K_r, h^{-1}
15	25	7.8 ± 0.6	0.05 ± 0.01
20	25	6.8 ± 0.6	0.10 ± 0.02
35	25	5.0 ± 0.4	0.40 ± 0.04

Adaptation to different growth conditions				
Time, h	Temperature,°C	Illuminance, W/m^2	K_i, h^{-1}	K_r, h^{-1}
7	15	25	3.8 ± 0.4	0.12 ± 0.03
48	15	25	5.5 ± 0.5	0.37 ± 0.02
7	20	25	5.6 ± 0.3	0.26 ± 0.03
48	20	25	5.5 ± 0.4	0.52 ± 0.06
7	35	170	2.9 ± 0.2	1.49 ± 0.11
9*	20	170	2.8 ± 0.2	1.37 ± 0.12
7*	35	170	3.5 ± 0.2	0.22 ± 0.03

* Before illumination conditions were changed, the algae had been grown for 48 h at 20°C and 25 W/m^2.

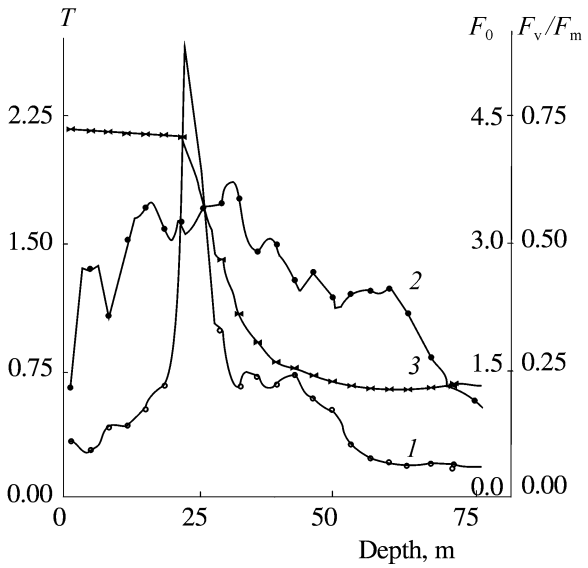
** Illumination conditions were changed simultaneously with nitrogen removal from the culture medium.

It is well known that illumination increases the cell sensitivity to environmental factors [3, 12]. In the dark, the resistance of algal cells to unfavorable temperature, short supply of mineral nutrients, anthropogenic pollution, and some other detrimental factors is sufficiently high. We studied the effect of various detrimental factors on the rate constants of photoinhibition and the slow component of postphotoinhibition recovery of the RC PS II activity (F_v/F_m) associated with synthesis of the D1 protein and sensitive to protein biosynthesis inhibitors (table). It was found that exposure to such factors as unfavorable temperature, short supply of mineral nutrients, and anthropogenic pollution cause a decrease in the rate of the recovery process associated with resynthesis of the D1 protein in microalgal cells. Adaptation of microalgal cells to low temperature or bright light is accompanied by an increase in the rate of PS II activity recovery. These data show that, in addition to such processes as nonphotochemical quenching and excitation energy transfer between photosystems [10], processes of biosynthesis of chloroplast proteins incorporated in the RC of PS II play an important role in regulation of the quantum efficiency of photosynthesis. These proteins are characterized by a high rate of light-induced renewal [3]. Changes in the ratio of rate constants of photodegradation and reactivation of PS II RC induced by environmental factors can inhibit the PS II activity even under physiological levels of illuminance. This can explain an abrupt increase in the

photosensitivity of microalgae grown under unfavorable environmental conditions or in the presence of pollutants [15, 17]. The adaptation capacity of algae under unfavorable environmental conditions is also determined by the activity of biosynthetic reactions. The mechanism of PS II activity regulation by various environmental factors includes optimization of coupling between light and dark reactions. In chloroplasts these reactions have different rates, thereby maintaining the cell metabolism at a required level. Photoinhibition is an example of implementation of such regulation associated with rapid replacement of damaged components of the photosynthetic apparatus [3, 12].

The processes of PS II regulation provide for plant adaptation to variable environmental conditions. These processes prevent generation of excessive amounts of reduced products of the light stages of photosynthesis. This is very important, because if these reduced products are not utilized in biosynthetic reactions, they interact with air oxygen, giving rise to various oxidative reactions, including lipid peroxidation (LPO). It is well known that these oxidative reactions provide a basis of the universal mechanism of membrane degradation [18]. The LPO processes in algal cells were studied in this work using the method of measurement of chlorophyll thermoluminescence (TL) curves at 120°C. Thermoluminescence is emitted as a result of thermal degradation of lipid peroxides, formation of excited products of degradation, and further migration of the excitation energy to chlorophyll, a native chemiluminescence emitter [20, 21]. A comparative kinetic study of PS II inactivation, accumulation of LPO products, and survival rate of algae exposed to extreme environmental factors revealed that reversible inactivation of RC PS II is not caused by LPO processes. Products of LPO are accumulated only after RC inactivation, and their accumulation correlates with cell death. Therefore, RC reactivation is impossible in the presence of LPO products [21]. Because of high sensitivity, the method of high-temperature TL was used in our experiments for studying the LPO rate in phytoplankton cells in natural aquatic environment [22].

Studies of natural phytoplankton *in situ* were performed using a home-made submerged fluorimeter. It was found that the processes of PS II activity regulation described above are also typical of natural phytoplankton communities. In the majority of natural oligotrophic waters (open regions of the Ocean, Lake Baikal, etc.) the phytoplankton growth conditions are generally unfavorable. This conclusion is confirmed by a significant decrease in the quantum efficiency of PS II and low values of F_v/F_m. Short supply of mineral nutrients is the main limiting factor of phytoplankton growth. Intense solar radiation is also a limiting factor of phytoplankton growth in the surface water layers, because against the background of unfavorable environmental conditions of algal growth in various waters, excessive illumination may cause a decrease in the content of active PS II RC (figure). Photoinhibition of algae is determined not only by the intensity and duration of illumination but also by the efficiency of protective and repair reactions associated with biosynthesis of PS II RC proteins. Upon exposure to the dark, photoinhibited phytoplankton cells restore their photosynthetic activity within several hours. Reactivation of PS II in photoinhibited phytoplankton cells was suppressed by a temperature decrease or addition of chloramphenicol, a protein biosynthesis inhibitor.



Results of measurements with a submerged fluorimeter: (*1*) distribution of the amount of natural phytoplankton over depth (F_0, rel. units); (*2*) distribution of the photosynthetic activity of natural phytoplankton over depth (F_v/F_m); (*3*) distribution of water temperature over depth (T°C). Measurements were performed at the Black Sea near the town of Gelendzhik, September 1998; 1.00 p.m. local time.

Phytoplankton cells from oligotrophic waters with a low content of biogenic substances were also characterized by a low rate of post-photoinhibition

recovery. The F_v/F_m value in surface water layers in this case was also low. The functional activity of PS II is increased under favorable growth conditions in upwelling zones enriched in biogenic substances. This is accompanied by enhanced phytoplankton productivity [7]. A sufficiently high correlation is also observed between the primary productivity, as calculated from the fluorescence data including F_0 (phytoplankton concentration) and F_v/F_m (photosynthetic activity) and underwater illuminance at the given horizon, and primary productivity determined *in situ* by the conventional radiocarbon method [7, 23]. Therefore, the photosynthetic productivity of natural phytoplankton is determined by the efficiency of the RC of PS II. This dependence provides a theoretical basis for the use of biophysical luminescence methods in hydrobiology for the purpose of monitoring the state and productivity of phytoplankton communities.

ACKNOWLEDGMENTS

This study was supported by the Ministry of Education of Russian Foundation (project no. 4-20) and The World Ocean Program (project no. 2.1).

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