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EFFECT OF LIGHT OF DIFFERENT INTENSITY ON CHLOROPHYLL FLUORESCENCE OF *ULVA PERTUSA* KJELLMAN (*CHLOROPHYTA*)

The effects of high-intensity optical radiation on chlorophyll fluorescence of marine alga *Ulva pertusa* Kjellman (*Chlorophyta*) were investigated. Method of pulse amplitude modulated (PAM) chlorophyll fluorescence was used for studying the photoinhibition of photosynthesis of alga. It was shown that the treatment of the dark-adapted samples with low-intensity (30 μmol·m^{-2}·s^{-1}) white light doesn't provide any significant difference between values of optimal quantum yield Y_{opt} of illuminated samples and those samples which were kept in the darkness. The treatment of the samples with high (1800 μmol·m^{-2}·s^{-1}) intensity of white light provokes the photoinhibition: the value of effective quantum yield Y_{eff} decreases up to 33% during 2 hours in comparison with the samples which were kept in darkness. The damage of the photosynthetic apparatus of alga by high irradiances is reversible – after the end of the photoinhibitory treatment the values of fluorescence parameters reached the initial level during the next 2 hours. The possible mechanisms of quenching of chlorophyll fluorescence are discussed. The fluorescence parameters such as effective and optimal quantum yields, photosynthetic efficiency and capacity makes it possible to estimate the effects of high-intensity irradiation on photosynthetic organisms.

Keywords: Ulva pertusa, photosynthesis, chlorophyll, fluorescence, high irradiance, photoinhibition.

Introduction

Photosystems, PSI and PSII are principal functional units which provide photosynthetic activity of plants and algae. These systems contain about 300 molecules of pigments (Forti, 1996) which can absorb photons, but only one chlorophyll molecule of such photosystems can transform absorbed energy to photochemical reaction. This molecule of chlorophyll is defined as *reaction centre* of photosystem while the others – as *antenna.* The transfer of the light absorbed energy to reaction centres of photosystems is accompanied with excitation of chlorophyll molecule P_{680} and transfer of electrons on upper energy level. Excited molecule P_{680} transfers electrons to acceptor – pheophytin *Ph*, then to primary quinone acceptor, Q_A, secondary quinone acceptor, Q_B, plastoquinine (*PQ*) pool, iron sulfur protein, FeS_R, cytochrome, b_6, cytochrome, *f*, plastocyanin, *PC*, and *PSI* where the light energy transfers the electrons from chlorophyll molecule P_{700} to primary electron acceptor, A_0, A_1, three iron-sulfur centres, ferredoxin, *Fd*, which forms complex with flavoprotein ferredoxin-NADP reductase, *FNR*, for the formation of NADPH.

Besides, absorbed energy can be released as heat or radiation process such as fluorescence. At room temperature the chlorophyll fluorescence originates from PS II;

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the contribution of PSI to fluorescence is significant only in the long-wavelength part of spectrum (740 nm). Variable fluorescence is related mainly to PSII while excitation transfer to PSI can be considered as additional competitive pathway of de-excitation of PSII. Chlorophyll *a* fluorescence emission presents a small (2-5%) part of absorbed energy and depends on the type of chloroplast, physiological state of photosynthetic organism, stress conditions, intensity and wavelength of excitation radiation.

Electron transfer along electron-transport chain is accompanied with decrease (*quenching*) of the chlorophyll fluorescence. The quenching which is related to the oxidation of acceptor is defined as *photochemical quenching*. This process is characterised by coefficient of photochemical quenching *qP*. Simultaneously there are other mechanisms of quenching of nonchemical nature or *nonphotochemical quenching*, which are characterised by coefficient of nonphotochemical quenching *qN*. It is necessary to distinguish energy dependent quenching which is associated with proton-induced gradient across thylakoid membrane, and quenching which can be significant under high intensity irradiation; this type of quenching provokes photoinhibition. In such a way, fluorescence is a complementary process in relation to photochemical and thermal processes: the more energy on photochemical reactions or heat is wasted the less fluorescence is yield.

Photosynthetic activity of plant can be estimated through the measurement of fluorescence induction kinetics of dark-adapted green plant sample or "Kautsky effect" (Kautsky & Hirsch, 1931). The technique of direct recording of fluorescence induction of green sample is characterised with certain shortcomings – dependence of the signal which is recorded on the intensity of radiation, effects of surrounding light. That is why the more promising method – Pulse Amplitude Modulation fluorometry – is used. This method is based on the modulation of chlorophyll fluorescence by saturated light pulses (Shreiber et al., 1986).

Marine algae are exposed to high irradiances during life cycle. The amount of light which is received by alga depends on the Sun position, clouding, and, particularly, the tides. Certainly, light regime affects the photosynthetic activity and, therefore, fluorescence parameters of alga. A number of articles are dedicated to the investigation of the effects of solar high-intensity radiation on algae such as *Ecklonia radiata* (Wood, 1987), *Dictyota dichotoma* (Nultsch et al., 1987; Hanelt & Nultsch, 1991; Hanelt et al., 1995; Flores-Moya et al., 1999), *Fucus serratus* (Huppertz et al., 1990), *Pelvetia canaliculata* (Duval et al., 1992), *Laminaria saccharina* (Benet et al., 1994; Hanelt et al., 1997), *Padina boryana*, *Sargassum polycystum*, *Turbinaria ornata* (Hanelt et al., 1994), *Adenocystis utricularis* (Hanelt, 1996), *Alaria esculenta* (Bishof et al., 1999), *Porphyra perforata* (Herbert, 1990), *P. leucostista* (Figueroa et al., 1997), *Polyneura hilliae* (Nultsch et al., 1990), *Palmaria palmata* (Hanelt & Nultsch, 1995), *Plocamium cartilagineum* (Kain, 1987), *Corralina elongata* (Häder et al., 1997), *Chlamydomanas reinhardtii* (Leverenz et al., 1990), *Anaulus australis* (de Preez et al., 1990), *Ulva rotundata* (Franklin et al., 1992), and *Halimeda tuna* (Häder et al., 1996).

Molecular mechanisms of photoinhibition of algae under high-intensity solar radiation are studied by several authors (Nultsch et al., 1990; Hanelt et al., 1992, 1993; Hanelt & Nultsch, 1994; Baker et al., 1996; Häder et al., 1996; Hanelt, 1996, 1998; Hanelt & Nultsch, 2003).

The main objective of this research work is experimental investigation of the effects of high-intensity radiation on chlorophyll fluorescence of marine alga *Ulva*

pertusa, illumination of possible mechanisms of destructive action of this radiation on photo-synthetic activity of alga and the search of relevant fluorescence parameters which can be used as stress indices.

Material and methods

The thalli of green alga *Ulva pertusa* from the coast of Iwaya, in Awaji Island (Japan) were used in this experiments. The samples were kept in running sea water under 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation (PAR). Experiments were performed with dark-adaptated and light-adaptated samples of *Ulva*.

The portable chlorophyll fluorometer "Diving-PAM-2000" ("Walz", Effeltrich, Germany) was used for the measurement of fluorescence characteristics of alga.

The PAM measuring principle is based on the rapid switching on/off of the measuring light; it is not strong enough to stimulate photosynthesis but does promote a fluorescence signal. The fluorescence signal follows the on/off pattern (i.e. modulated) of the measuring beam and is measured with suitable light filters and electronics in the instrument. The fluorescence value obtained by the measuring beam is termed F_0 in dark-adapted sample and F when the sample is illuminated by actinic light (points A or A', Figure 1).

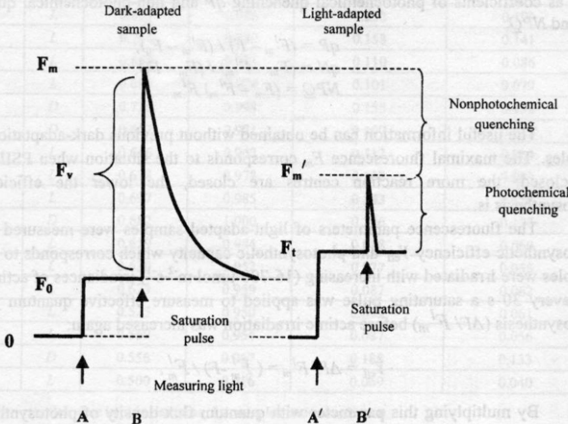

Figure 1. Explanation of the measurement of chlorophyll fluorescence using excitation with pulse-modulated measuring light: the fluorescence value obtained by the measuring beam is termed F_0 in dark-adapted sample and F when the sample is illuminated by actinic light (points A or A'); then the sample is illuminated with intense light pulse which saturates all reaction centres so that all reaction centres become closed; at this point, fluorescence becomes maximal, and the value is noted as F_m in dark-adapted samples or as F'_m in samples under actinic light (points B or B').

Then the sample is illuminated with intense light pulse which saturates all reaction centres so that all reaction centres become closed. Closed reaction centres are reduced and unavailable, temporarily, to do photochemistry. At this point, fluorescence becomes maximal, and the value is noted as F_m in dark-adapted samples or as F'_m in samples under actinic light (points B or B', Figure 1).

With a very strong light pulse the electron transfer chain between PSII and PSI is quickly interrupted, photochemical quenching becomes zero and any remaining quenching must be nonphotochemical.

Photosynthetic activity was estimated by the measurement of optimal quantum yield:

$$Y_{\text{opt}} = F_v / F_m = (F_m - F_o) / F_m,$$

where F_v / F_m is the ratio of variable fluorescence $F_v = F_m - F_o$ to maximal fluorescence F_m of dark-adaptated samples; here F_o is the initial fluorescence when all *PSII* reaction centres are opened, and F_m is maximal fluorescence when PSII centres are closed. Dark adaptation was realised due to use of glass volume which was covered with black paper. The same thallus was divided into two parts – one was kept in darkness, the other – under high irradiation. All the samples were dark-adapted during 15 min before the measurements.

Besides, the fluorescence parameters of dark-adapted samples were measured such as coefficients of photochemical quenching qP and non-photochemical quenching qN and NPQ:

$$qP = (F'_m - F) / (F'_m - F_0);$$
$$qN = (F_m - F'_m) / (F_m - F_0);$$
$$NPQ = (F_m - F'_m) F'_m.$$

The useful information can be obtained without previous dark-adaptation of the samples. The maximal fluorescence F_m corresponds to the situation when PSII centres are closed; the more reaction centres are closed, the lower the efficiency of photosynthesis is.

The fluorescence parameters of light-adapted samples were measured such as photosynthetic efficiency Y_{eff} and photosynthetic capacity which corresponds to $ETR_{\max}$. Samples were irradiated with increasing (16-700 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) irradiances of actinic light and every 30 s a saturating pulse was applied to measure effective quantum yield of photosynthesis ($\Delta F / F'_m$) before actinic irradiation was increased again:

$$Y_{\text{eff}} = \Delta F / F'_m = (F'_m - F) / F'_m.$$

By multiplying this parameter with quantum flux density of photosynthetically active radiation (*PAR*) relative electron transport rates (*ETR*) were calculated (Schreiber et al., 1986):

$$ETR = \Delta F / F'_m PAR,$$

where *PAR* is measured in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Light curves were defined as relative electron transport rate (*ETR*) of photosynthesis versus irradiance (samples should be well adapted to a moderate light intensity, which is close to the light intensity experienced by the alga in its natural environment); the photosynthetic efficiency Y_{eff} was estimated as linear

part of light curve $ETR = f(I)$ (where I is light intensity); photosynthetic capacity was defined as ETR_{max} when the light curve is saturated.

The investigation of light-adapted samples were performed through irradiation of Petri dishes with thalli of *Ulva* by white light of halogen lamp; the irradiance of the lamp was 1800 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. The treatment of the samples by high intensity radiation lasted two hours; then the samples were kept under 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR to study the photorecovery process for several hours.

Diving-PAM fluorometer was equipped also with PIN-photodiode, sensors of irradiance, temperature and water depth; besides, PAM fluorometer was connected with the WinControl software – instrument which can display all data on the monitor screen, store on hard disc kinetic recordings and light response curves, process and analyse data. All the measurements were repeated three times for estimation of mean values and standard deviation.

Table 1. **Effect of low (30 $\mu mol \cdot m^{-2} \cdot s^{-1}$) intensity light on fluorescence parameters of *Ulva pertusa* Kjellman**

N	Regime	*Y*	*pQ*	*qN*	*NPQ*
1	*D*	0.722	0.991	0.148	0.126
2	*L*	0.692	0.983	0.126	0.102
3	*L*	0.758	0.989	0.136	0.120
4	*L*	0.667	0.986	0.222	0.193
5	*L*	0.744	0.989	0.158	0.141
6	*L*	0.688	0.981	0.110	0.086
7	*L*	0.685	0.974	0.101	0.079
8	*D*	0.725	0.994	0.155	0.134
9	*D*	0.667	0.971	0.107	0.082
10	*L*	0.646	0.962	0.112	0.084
11	*L*	0.678	0.978	0.107	0.083
12	*L*	0.697	0.985	0.143	0.118
13	*D*	0.682	1.000	0.206	0.177
14	*L*	0.619	0.984	0.088	0.060
15	*L*	0.630	0.952	0.077	0.055
16	*L*	0.623	0.959	0.087	0.062
17	*L*	0.525	0.950	0.099	0.061
18	*L*	0.569	0.954	0.087	0.056
19	*D*	0.556	0.967	0.188	0.133
20	*L*	0.500	0.916	0.069	0.040

Note. *D* – samples were kept in darkness, *L* – samples under irradiation.

Results

The results of measurements of fluorescence parameters of dark-adapted samples of algae are presented in Table 1 (low-intensity treatment) and Table 2 (high-intensity treatment). The dependence of optimal quantum yield $Y_{opt} = F_v / F_m$ on the duration of illumination of the samples by white light of low (30 $\mu mol \cdot m^{-2} \cdot s^{-1}$) intensity is shown in Figure 2. It is clear that there is not considerable difference between

illuminated samples (light columns) and those samples which were kept in the darkness (dark columns).

Table 2. **Effect of high (1800 μmol·m⁻²·s⁻¹) and low (30 μmol·m⁻²·s⁻¹) intensity light on fluorescence parameters of *Ulva pertusa* Kjellman**

N	Regime	*Y*	*pQ*	*qN*	*NPQ*
1	*D*	0.746	0.988	0.089	0.073
2	*HI*	0.638	0.987	0.153	0.117
3	*HI*	0.320	0.983	0.243	0.105
4	*HI*	0.411	0.965	0.325	0.205
5	*HI*	0.245	0.887	0.317	0.128
6	*D*	0.639	0.971	0.105	0.077
7	*D*	0.700	0.993	0.176	0.151
8	*LI*	0.377	0.894	0.113	0.054
9	*LI*	0.562	0.937	0.121	0.082
10	*D*	0.698	0.974	0.088	0.069
11	*LI*	0.675	0.996	0.143	0.113

Note. *D* – samples were kept in darkness, *HI* – samples under high intensity irradiation, *LI* – samples under low intensity irradiation.

The treatment of the samples with high (1800 μmol·m⁻²·s⁻¹) intensity of white light provokes the photoinhibition (left part of Figure 3). The value of effective quantum yield Y_{eff} decreases up to 33% in 2 hours in comparison with the samples which were kept in darkness (left part of Figure 3). The damage of the photosynthetic apparatus of alga by high irradiances is reversible – after the end of the photoinhibitory treatment the values of F_v/F_m reached the initial level in the next 2 hours (right part of Figure 3). This fast recovery process after the irradiation can be defined as dynamic photoinhibition (Osmond, 1994); chronic photoinhibition corresponds to the recovery process which takes place for several days.

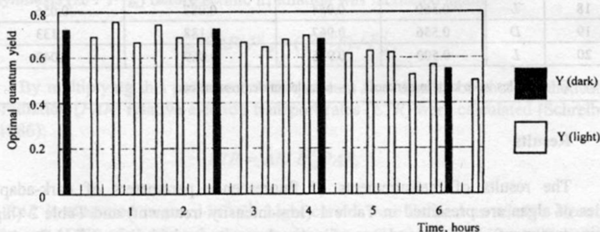

Figure 2. Dependence of the optimal quantum yield on the duration of illumination of the samples of *Ulva pertusa* Kjellman by white light of low (30 μmol·m⁻²·s⁻¹) intensity.

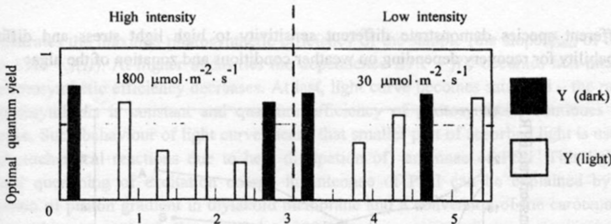

Figure 3. Dependence of the optimal quantum yield on the duration of illumination of the samples of *Ulva pertusa* Kjellman by white light of high (1800 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and low (30 $\mu mol \cdot m^{-2} \cdot s^{-1}$) intensity.

The character of light curves depends strongly on the level of irradiance: increasing the irradiance from 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provokes the corresponding decrease of the photosynthetic efficiency and ETR_{max}; the treatment of the sample with high intensity (500 $\mu mol \cdot m^{-2} \cdot s^{-1}$) irradiance for one hour decreases the level of ETR_{max} (Figure 4). The damage of the light-adapted samples by high irradiances is reversible after the end of the photoinhibitory treatment the values of ETR_{max} approached the initial level (Figure 5).

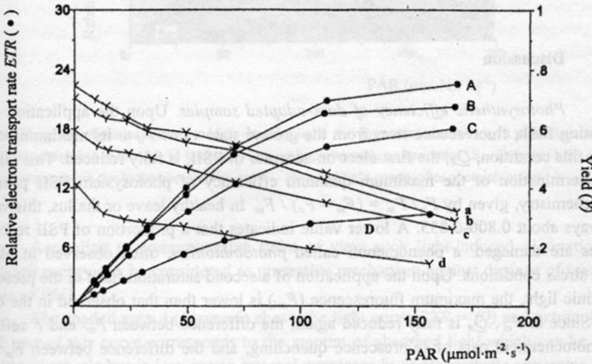

Figure 4. Dependence of the light curves (*A-D*) and effective quantum yield (*a-d*) on the irradiance: *A, a* – 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$; *B, b* – 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$; *C, c* – 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, *D, d* – after 1 hour of the treatment of the sample with high intensity (500 $\mu mol \cdot m^{-2} \cdot s^{-1}$) irradiance.

Changes in effective quantum yield, photosynthetic efficiency, capacity and kinetics of recovery under high light stress were observed in different life-history and developmental stages of *Delesseria sanguinea* (Huds.), *Chondrus crispus* (Stackh.), *Porphyra* spp., *Petalonia fascia* Kuntze (Hanelt et al., 1993), *Laminaria saccharina* (*Phaeophyta*) (Hanelt et al., 1997), *Ecklonia cava* (*Laminariales, Phaeophyceae*).

Different species demonstrate different sensitivity to high light stress and different capability for recovery depending on weather conditions and zonation of the algae.

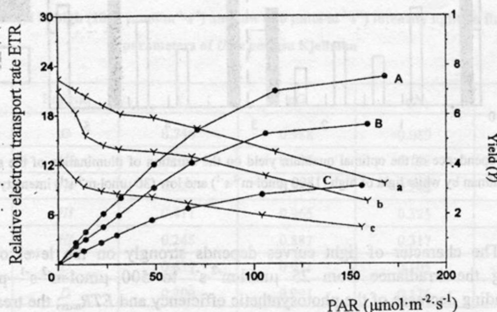

Figure 5. Photoinhibition of photosynthesis of *Ulva pertusa* Kjellman induced by high intensity *A-C* – light curves, *a-d* – effective quantum yield; *A, a* – 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$, *B, b* – 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, *C, c* – 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Discussion

Photosynthetic efficiency of dark-adapted samples. Upon the application of a saturating flash, fluorescence rises from the ground state value F_o to its maximum value F_m. In this condition, Q_A, the first electron acceptor of PSII, is fully reduced. This allows the determination of the maximum quantum efficiency of photosystem PSII primary photochemistry, given by $F_v / F_m = (F_m - F_o) / F_m$. In healthy leave or thallus, this value is always about 0.800-0.835. A lower value indicates that a proportion of PSII reaction centres are damaged: a phenomenon called *photoinhibition*, often observed in plants under stress conditions. Upon the application of a second saturation flash in the presence of actinic light, the maximum fluorescence ($F_{m'}$) is lower than that observed in the dark (F_m). Since at $F_{m'}$, Q_A is fully reduced again, the difference between $F_{m'}$ and F reflects the photochemical part of fluorescence quenching, and the difference between F_m and $F_{m'}$ reflects fluorescence quenching due to heat dissipation (Figure 2). The photochemical quenching *qP* reflects the redox state of the primary electron acceptor of PSII. The non-photochemical quenching *qN* or *NPQ* reflects energy dissipated as heat related to energization of the thylakoid membrane due to lumen acidification. The quantum yield of electron transfer at PSII, calculated as $Y = (F_{m'} - F) / F_{m'}$, is a measure of the overall efficiency of PSII reaction centres in the light.

Photosynthetic efficiency of light-adapted samples. The response of photosynthesis to high-intensity light is characterised by light curves. Let's discuss a light curve which was recorded in our experiments (Figure 6). At the first stage photosynthesis increases linearly with intensity of optical radiation; this part of curve

demonstrates the maximal photosynthetic efficiency of the sample (the slope ϕ_{max} of the curve $ETR = f(I)$). At higher intensities the dependence $ETR = f(I)$ becomes non-linear and photosynthetic efficiency decreases. At last, light curve becomes saturated – the rate of photosynthesis is constant and quantum efficiency of photosynthesis continues to decrease. Such behaviour of light curve means that smaller part of absorbed light is used for photochemical reactions due to heat dissipation of antennae of PSII. This light-induced quenching of excitation energy in antennae of PSII can be explained by a formation of proton gradient in thylakoid membrane and a conversion of the carotenoid to zeaxanthin via xanthophyll cycle (Baker, 1996).

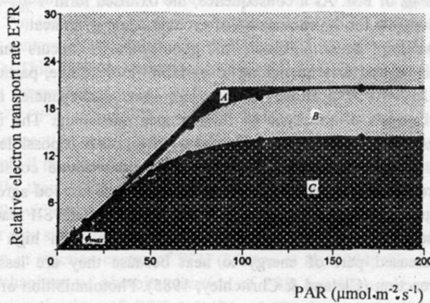

Figure 6. Response of photosynthesis to high-intensity light: *A* – amount of absorbed light which can be used for photosynthesis but is lost due to internal processes in photosynthetic apparatus; *B* – absorbed light energy lost to photosynthesis due to the photoinhibition; *C* – absorbed light energy after photoinhibition.

According to comprehensive point of view such light-induced dissipation of excitation energy can be considered as protective mechanism against damage of reaction centres of PSII.

The shaded area *A* (Figure 6) above the light curve $ETR = f(I)$ and extrapolated linear part of this curve corresponds to the amount of absorbed light which can be used for photosynthesis but is lost due to internal processes in photosynthetic apparatus. The high-intensity light provokes photoinhibition of photosynthesis; the area *B* between the normal and photoinhibited curves corresponds to the absorbed light energy lost during photosynthesis due to the photoinhibition. The area *C* is related to the absorbed light energy after photoinhibition.

Photoinhibition of photosynthesis in wide meaning is defined as the light-induced decrease of CO_2 assimilation; more narrow interpretation of this term means light-induced damage to the PSII reaction centre under the high-intensity light treatment and increasing loss of absorbed energy as heat (Baker, 1996).

qE-quenching. This form of energy-dependent nonphotochemical quenching is significant under excess of irradiance of photosynthetic organisms. The formation of *qE-*

quenching is related to a proton gradient ΔpH across the thylakoid membrane. It was shown that the extent of *qE*-quenching depends linearly on the intrathylakoid proton concentration (Briantais et al., 1979). The main proportion of absorbed photons under optimal irradiation is used for control of electron transport which induces the formation of proton gradient. High irradiances induce regulatory processes which are related to the intensification of energy conversion to heat in controlled manner: an increase in the proton gradient would induce the *qE*-quenching mechanism (Krause & Weis, 1988). In such a way, the process of *qE*-quenching protects PSII from photodamage. The experimental results which were obtained due to the application of picosecond fluorescence spectroscopy (Bruce et al., 1997) can suggest that not antenna of PSII but a reaction centre is responsible for nonphotochemical quenching of chlorophyll fluorescence. De-excitation of PSII provokes the limitation of electron transfer to reaction centre P_{700} of PSI. As a consequence, the oxidised form P_{700}^{+} is accumulated; this form is responsible for the conversion of excitation energy to heat.

qI-quenching. Photoinhibition of photosynthesis occurs under excessive irradiation and is related to a quenching of variable fluorescence, particularly, optimal quantum yield $Y_{opt} = F_v/F_m$. However, there are some discrepancies in views of the origin and mechanisms of this type of fluorescence quenching. The investigation of chlorophyll fluorescence under low temperature (77 K) made it possible to suggest that the mechanism of *qI*-quenching is based on an increased rate constant of thermal deactivation (Krause & Weis, 1988). These conclusions are in good agreement with the hypothesis that photoinhibition is related to a transformation of PSII reaction centres to the peculiar quenchers which can trap excitation energy with high efficiency and dissipate an increased part of energy to heat because they are less capable of a photochemical reaction (Cleland & Chritchley, 1985). Photoinhibition of photosynthesis according to another hypothesis (Kyle, 1988) is a result of primary inactivation and following degradation of the proteins which are bound with the Q_B-acceptor. Some of authors suggest that process of *qI*-quenching has an important negative effect on photosynthesis during a diurnal cycle because of its slow relaxation kinetics (Baker et al., 1994; Ogren, 1994). The results of a number of experiments *in vitro* and *in vivo* (Horton, 1996) offered to consider *qI*-quenching as a more stable form of *qE*-quenching.

The xanthopyll cycle. Photosynthetic organisms have also the accessory pigments which can absorb light and transfer excitation to chlorophyll *a*. The main accessory pigments are carotenoids and phycobilins. The carotenoids can be divided into two classes – carotenes and xanthophylls. The principal role of accessory pigments is protection of photosynthetic organisms from destructive effects of photooxidation under high-intensity illumination; absorption of light by chlorophyll leads to the excitation of the triplet state of pigment which is the source of damage because of the reaction with molecular oxygen and formation of strong reactive species such as singlet oxygen. The carotenoids of antenna quench rapidly any triplet state and regulate the process of excitation conversion into heat under high-intensity illumination. Such control of quantum yield of photosynthesis was defined as the xanthophyll cycle (Demmig-Adams & Adams, 1992). *qE*-quenching can occur either in the PSII antenna, or in the reaction centre, or both (Horton et al., 1994). The first suggestion is supported by the fact that process of *qE*-quenching is associated with the xanthophyll cycle which provides the conversion of violaxanthin to zeaxanthin and antheraxanthin – pigments found only in the peripheral antenna complexes (Demmig-Adams, 1990; Demmig-

quenching is related to a proton gradient ΔpH across the thylakoid membrane. It was shown that the extent of *qE*-quenching depends linearly on the intrathylakoid proton concentration (Briantais et al., 1979). The main proportion of absorbed photons under optimal irradiation is used for control of electron transport which induces the formation of proton gradient. High irradiances induce regulatory processes which are related to the intensification of energy conversion to heat in controlled manner: an increase in the proton gradient would induce the *qE*-quenching mechanism (Krause & Weis, 1988). In such a way, the process of *qE*-quenching protects PSII from photodamage. The experimental results which were obtained due to the application of picosecond fluorescence spectroscopy (Bruce et al., 1997) can suggest that not antenna of PSII but a reaction centre is responsible for nonphotochemical quenching of chlorophyll fluorescence. De-excitation of PSII provokes the limitation of electron transfer to reaction centre P_{700} of PSI. As a consequence, the oxidised form P_{700}^{+} is accumulated; this form is responsible for the conversion of excitation energy to heat.

qI-quenching. Photoinhibition of photosynthesis occurs under excessive irradiation and is related to a quenching of variable fluorescence, particularly, optimal quantum yield $Y_{opt} = F_v/F_m$. However, there are some discrepancies in views of the origin and mechanisms of this type of fluorescence quenching. The investigation of chlorophyll fluorescence under low temperature (77 K) made it possible to suggest that the mechanism of *qI*-quenching is based on an increased rate constant of thermal deactivation (Krause & Weis, 1988). These conclusions are in good agreement with the hypothesis that photoinhibition is related to a transformation of PSII reaction centres to the peculiar quenchers which can trap excitation energy with high efficiency and dissipate an increased part of energy to heat because they are less capable of a photochemical reaction (Cleland & Chritchley, 1985). Photoinhibition of photosynthesis according to another hypothesis (Kyle, 1988) is a result of primary inactivation and following degradation of the proteins which are bound with the Q_B-acceptor. Some of authors suggest that process of *qI*-quenching has an important negative effect on photosynthesis during a diurnal cycle because of its slow relaxation kinetics (Baker et al., 1994; Ogren, 1994). The results of a number of experiments *in vitro* and *in vivo* (Horton, 1996) offered to consider *qI*-quenching as a more stable form of *qE*-quenching.

The xanthopyll cycle. Photosynthetic organisms have also the accessory pigments which can absorb light and transfer excitation to chlorophyll *a*. The main accessory pigments are carotenoids and phycobilins. The carotenoids can be divided into two classes – carotenes and xanthophylls. The principal role of accessory pigments is protection of photosynthetic organisms from destructive effects of photooxidation under high-intensity illumination; absorption of light by chlorophyll leads to the excitation of the triplet state of pigment which is the source of damage because of the reaction with molecular oxygen and formation of strong reactive species such as singlet oxygen. The carotenoids of antenna quench rapidly any triplet state and regulate the process of excitation conversion into heat under high-intensity illumination. Such control of quantum yield of photosynthesis was defined as the xanthophyll cycle (Demmig-Adams & Adams, 1992). *qE*-quenching can occur either in the PSII antenna, or in the reaction centre, or both (Horton et al., 1994). The first suggestion is supported by the fact that process of *qE*-quenching is associated with the xanthophyll cycle which provides the conversion of violaxanthin to zeaxanthin and antheraxanthin – pigments found only in the peripheral antenna complexes (Demmig-Adams, 1990; Demmig-

main attention must be concentrated on the measurements of the following parameters: *effective quantum yield* (samples under light-adaptation), *optimal quantum yield* (samples under dark-adaptation) and *light curves* (samples should be well adapted to a moderate light intensity, which is close to the light intensity experienced by the plant in its natural environment).

Conclusions

Exposure of photosynthetic organisms to higher irradiances which exceed their energy requirement for photosynthetically operated metabolisms provokes a reduction of photosynthetic capacity, called photoinhibition – the process which is related with the degradation of the reaction centre protein (*D1*) of photosystem PSII (Hanelt & Nultsch, 2003). The transfer of excitation energy along electron transport chain is associated with the process of quenching of chlorophyll fluorescence which occurs due to the oxidation of acceptor. The main mechanisms of quenching are energy-dependent *qE*-quenching which is dependent on the presence of proton gradient across the thylakoid membrane, and *qI*-quenching which occurs in excessive irradiation; this type of quenching provokes photoinhibition.

It is possible to suggest the effect of protein structural change in chlorophyll fluorescence quenching. The aggregation of proteins prevents high levels of non-radiative energy dissipation and leads to quenching.

Fluorescence is related to photochemical and heat processes. The measurement of fluorescence parameters such as effective and optimal quantum yields, photosynthetic efficiency and capacity makes it possible to estimate the effects of high-intensity irradiation on photosynthetic organisms.

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ВЛИЯНИЕ СВЕТА РАЗЛИЧНОЙ ИНТЕНСИВНОСТИ НА ФЛУОРЕСЦЕНЦИЮ ХЛОРОФИЛЛА *ULVA PERTUSA* KJELLMAN (*CHLOROPHYTA*)

Исследовано влияние высокоинтенсивного излучения на флуоресценцию хлорофилла морской водоросли *Ulva pertusa* Kjellman (*Chlorophyta*). Для изучения фотоингибирования фотосинтеза

водоросли был использован метод импульсной амплитудно-модулированной флуоресценции хлорофилла. Показано, что воздействие белого света низкой (30 мкмоль·м$^{-2}$·с$^{-1}$) интенсивности на адаптированные к темноте образцы не вызывает особых изменений величины оптимального квантового выхода Y_{opt} у освещенных образцов по сравнению с образцами, которые хранились в темноте. В то же время, воздействие интенсивного (1800 мкмоль·м$^{-2}$·с$^{-1}$) белого света привело к ингибированию фотосинтеза водоросли: величина эффективного квантового выхода Y_{eff} уменьшилась до 33% в течение 2 ч по сравнению с образцами, которые хранились в темноте. Повреждение фотосинтетического аппарата водоросли интенсивным светом было обратимым – параметры флуоресценции после прекращения освещения достигали своей первоначальной величины в течение 2 ч. Обсуждаются возможные механизмы тушения флуоресценции хлорофилла. Измерение таких флуоресцентных параметров, как эффективный и оптимальный квантовые выходы, фотосинтетическая эффективность и фотосинтетическая способность, позволяет оценить влияние высокоинтенсивного излучения на фотосинтетические организмы.

Ключевые слова: *Ulva pertusa,* фотосинтез, хлорофилл, флуоресценция, высокоинтенсивное излучение, фотоингибирование.

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