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PHOTOPROTECTIIVE STRATEGIES OF UNICELULAR RED ALGA *RHODELLA VIOLACEA* DURING ACCLIMATION TO STRONG LIGHT

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ФОТОПРОТЕКТОРНЫЕ МЕХАНИЗМЫ У ОДНОКЛЕТОЧНОЙ КРАСНОЙ ВОДОРОСЛИ *RHODELLA VIOLACEA* В ПРОЦЕССЕ СВЕТОВОЙ АДАПТАЦИИ

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АННОТАЦИЯ

Красные водоросли содержат в своем фотосинтетическом аппарате водорастворимые антенные комплексы - фикобилисомы (PBS), связанные с тилакоидными мембранами и передающими энергию возбуждения фотосистемам. Сильный свет, поглощаемый PBS, вызывает быструю генерацию транстилакоидного ΔpH, сопровождаемую нефотохимическим тушением флуоресценции хлорофилла (Хл). Генерация ΔpH, очевидно, необходима для фотозащиты фотосинтетического аппарата в отсутствие ксантофиллового цикла, существующего у высших растений. Однако фотозащитные механизмы красных водорослей пока еще подробно не изучены.

Мы представляем здесь исследование по тушению флуоресценции Хл в одноклеточных красных водорослях *Rhodella violacea* и его корреляции с формирующимся градиентом ΔpH. Также была исследована связь этого явления с фотопротекцией фотосистемы 2 (ФС 2) в нормальных и адаптированных к сильному свету клетках *Rhodella*.

Было обнаружено, что в условиях фотоингибирования (белый свет, I= 2000-3000 мкМ квантов / $м^2с$) ΔpH-зависимое тушение флуоресценции Хл замедляет кинетику фотоингибирования ФС 2. Разобщители-ионофоры, такие как нигерицин и NH_4Cl, разрушающие ΔpH-градиент, приводили к диссипации тушения флуоресценции Хл с последующим увеличением скорости фотоингибирования PS 2. Такой же эффект показал дальний красный свет, вызывающий падение транстилакоидного ΔpH. Ингибитор АТФазы, DCCD, не влияющий на ΔpH, не оказывал воздействия на фотоингибирование ФС 2. Это указывает на то, что фотопротекторным действием обладает именно протонный градиент, а не синтез АТФ.

Длительная адаптация клеток *Rhodella* к более высокой освещенности (500–1000 мкМ квантов/ $м^2с$) приводила к частичной потере фикобилисомами периферийных фикоэритрин-содержащих субъединиц. Адаптированные культуры демонстрировали более высокую устойчивость к фотоингибирующему свету, чем контрольные. Это можно объяснить уменьшением транспорта энергии от редуцированных ФБС к ФС 2, а также экранированием света вторичными каротиноидами, которые накапливаются в клетках под действием света.

Полученные данные по низкотемпературной (77 К) флуоресценции Хл проливают свет на молекулярные механизмы супрессии фотоиндуцированной флуоресценции Хл в клетках *Rhodella*, а также ее темнового восстановления.

ABSTRACT

Red algae contain in their photosynthetic machinery water-soluble antenna complexes - phycobilisomes (PBSs) attached to thylakoid membranes to transfer excitation energy to photosystems. Strong light absorbed by the PBSs triggers a fast formation of transthylakoid ΔpH that follows the non-photochemical quenching of chlorophyll (Chl) fluorescence. The ΔpH build-up seems to be essential for photoprotecting the photosynthetic apparatus in the absence of xanthophyll cycle common to higher plants. However, the photoprotective mechanisms of red algae are not studied in details yet.

We present here our research of the Chl fluorescence quenching in unicellular red algae *Rhodella violacea* and its correlation with the ΔpH gradient being formed. The relation of this phenomenon to photoprotection of photosystem 2 (PS 2) in the normal and high light-acclimated *Rhodella* cells is also examined.

Under the photoinhibitory conditions (white light of 2000-3000 μE/m^{2}s), the ΔpH-dependent Chl fluorescence quenching was found to delay the kinetics of PS 2 photoinhibition. The uncouplers like nigericin and NH_4Cl are known to break down ΔpH gradient, lead to the dissipation of Chl fluorescence quenching followed by enhancing the PS 2 photoinhibition rate. The same effect showed far-red (FR) light consuming transthylakoid ΔpH. ATPase inhibitor, DCCD, having no impact on ΔpH didn't influence PS 2 photoinhibition as well this implies the photoprotection to be fulfilled by the proton gradient rather than by ATP synthesis.

Long-term acclimation of *Rhodella* cells to higher irradiances (500-1000 μE/m^{2}s) results in a partial loss of the periphery phycoerythrin-containing subunits by PBSs. The light-acclimated cultures display a higher resistance to the photoinhibitory light than the non-acclimated ones. This could be explained by diminishing the energy transfer from the reduced PBSs to PS 2 as well as light screening by the secondary carotenoids synthesized during light exposure.

Data on low-temperature (77K) fluorescence allow to evaluate the molecular mechanisms of light-induced Chl fluorescence suppression in *Rhodella* cells and its recovery in darkness.

Ключевые слова: *Rhodella violacea*, фикобилисомы, флуоресценция хлорофилла, световая адаптация, фотосистема 2, нефотохимическое тушение флуоресценции

Key words: *Rhodella violacea*, phycobilisomes, chlorophyll fluorescence, light acclimation, photosystem 2, non-photochemical fluorescence quenching

Abbreviations: FR, far red light; LL, low light; HL, high light; XHL, extra-high light; Chl, chlorophyll "a"; PBS, phycobilisome(s); PE, B-phycoerythrin; APC, allophycocianin; PS 1&2, photosystem 1&2; PI, photoinhibtion; ETC, electron transport chain; DCCD, N,N-dicyclohexylcarbodiimide? NPQ, non-photochemical fluorescence quenching; DCMU, (3-(3,4-dichlorophenyl)-1,1-dimethylurea), diuron.

INTRODUCTION

Photosynthetic pigment-protein complexes (CPs) of phototrophic organisms comprise the reaction centers of photosystem 1 (PS 1), those of photosystem 2 (PS 2), and light-harvesting antenna complexes (LHC). Red algae and cyanobacteria differ from higher plants by lacking of large distant antenna complex LHC 2. Instead of it, they have extramembrane antenna complexes, phycobilisomes (PBSs), attached mainly to PS 2 from the stromal part of thylakoids [12, 19].

While photon flow is required for proper functioning of the photosynthetic electron transport chain (ETC), the excessive light can inhibit photosynthesis and inflict irreversible damage to pigments and proteins of thylakoid membranes [1, 3].

Plants developed various photoprotective mechanisms to resist light stress [6, 11, 22, 23]. However, if these mechanisms of excessive energy relaxation are insufficient, the remaining flux of photons leads to the photosensitized formation of triplet chlorophyll and toxic singlet oxygen. The first target of photoinhibition is the PS 2 reaction center, which is associated with a loss of photochemical activity and variable fluorescence Fv [2, 7, 16, 18, 26, 28].

One of the photoprotective mechanisms described for higher plants involves a down-regulation of PS 2 that proceeds simultaneously with the build-up of a proton gradient across the thylakoid membrane (ΔpH) [11]. It was shown to correlat with xanthophyll oxidation in LHC 2 [6, 10, 13, 24, 27].

In *Rhodophyta* peripheral antennae - PBSs transfer excitation to core antennae of PS 2, CP43 and CP47 protein-chlorophyll complexes where xanthophyll cycle is absent [12, 20]. However, a pH-dependent Chl *a* fluorescence quenching can also be formed under strong light [8, 9, 17]. Possible photoprotective role of pH was studied for unicellular red alga *Rhodella violacea* [20, 21]. As a long-term response to light stress, photoacclimation takes place.

Studies on photoacclimation have compared the steady-state exposures to high light (HL) with the low light (LL) exposures. These investigations focused on various aspects of the differences between acclimated and LL-grown cells at the level of their ultrastructure, pigment content, light harvesting antenna size and PS 2 to PS 1 ratios [4, 9, 21, 25].

The aim of the present research was to study different photoprotective mechanisms in red alga *Rhodella violacea* [8, 9, 17]. We characterized light-induced generation of transthylakoid ΔpH in *Rhodella* cells accompanying non-photochemical Chl fluorescence quenching, studied the effects of various irradiances and inhibitors. Under photoinhibitory conditions, the ΔpH-dependent Chl fluorescence quenching exerts a photoprotective role and delays the kinetics of photoinhibition [20, 21]. Here we provide

additional evidence for photoprotective role of ΔpH-mediated non-photochemical Chl fluorescence quenching (NPQ) being formed either in LL-grown *Rhodella* cells or strong light acclimated ones.

Long-term acclimation of *Rhodella* to high light is accompanied by the reduction of antenna size due to the loss of the distal hexamers containing B-phycoerythrin (PE) [4]. We investigated the acclimation and its role in protecting the photosynthetic machinery of *Rhodella* against strong light.

MATERIALS AND METHODS

Cell cultures of *Rhodella violacea* (strain 115-79 from Göttingen University, Germany) were grown photoautotrophically in sterile artificial seawater with the addition of vitamin B_{12} at 25 mg L21 [8, 9]. Cultures of 300 to 700 mL were incubated at 20°C in glass culture flasks continuously flushed with sterile air, and illuminated with fluorescent tubes with a 16-h light/8-h dark photoperiod [20, 21].

Cultures of 3-4 day-old were used for the experiments. Three different light intensities were used for the light acclimation: 40, 500, and 1000 □E/m^{2}s, defined as LL (low light), HL (high light), and XHL (extra high light) conditions, correspondingly. To standardize the culture conditions and minimize self-shading, cells were diluted every 3 days to 700 cells/ml with fresh medium. Full adaptation took 7 days; the cultures were diluted to 700 cells/ml 3 days prior to the photoinhibition experiments.

Photoinhibitory treatment of *Rhodella* suspensions was performed in a thermostatic vessel (20°C) by white light at photon flux densities 2000-4000 □E/m^{2}s. Photoinhibited samples were stored in the dark at 20°C for 15 min prior to fluorescence induction measurements to provide full dissipation of NPQ [4, 20, 21].

Induction kinetics of Chl fluorescence at 20°C were measured in a laboratory-built continuous fluorimeter using green LEDs (□□=□550 nm, I = 40 □E/m^{2}s) to provide continuous illumination of adjustable intensity and duration that served both as actinic and as detecting beam. The fluorescence was detected at 680 nm by a photomultiplier H 5700–50 (Hamamatsu Photonics, Japan). Data were collected in a computer with a 33-□s time resolution. The results were displayed on logarithmic time scale to present all phases of the kinetic curves together [20, 21].

At room temperature, fluorescence emanates mainly from the Chl *a* antenna of PS 2 [15]. Fluorescence yield is dependent on photochemical and non-photochemical processes [25]. Photochemical quenching is dependent on the redox state of the primary acceptor of PS 2, the plastoquinone Qa [15, 19]. When all PS 2 reaction centers (RC) are open (Qa oxidized), they are efficient exciton traps and the fluorescence yield of PS 2 is low. When Qa is reduced, the RCs are unable to trap excitons, the photochemical quenching is suppressed. Chl fluorescence can also be decreased by NPQ ascribed to two main processes occurring under strong light: □pH-dependent quenching and photoinhibition [8, 9, 17, 20, 21, 22, 25]. In the absence of photochemical quenching and NPQ, fluorescence reaches a maximum level Fm. The □pH-dependent quenching can be destroyed by nigericin (an uncoupler functioning as a proton-transporting ionophore) [9, 20, 21].

Low-temperature fluorescence of Chl and other photosynthetic pigments was detected in liquid nitrogen (77K) in Hitachi Spectrofluorimeter (Hitachi, Japan). Fluorescence band ratio PS 2/PS 1 was calculated to characterize either photodamage or changes of photosystems in the result of photoregulatory/photoinhibitory processes. Both excitation and emission spectra of 77K fluorescence were used to estimate energy migration efficiency from PBSs to PS 2 and PS 1.

Absorption spectra of *Rodella* suspension were registered at 20°C in double-beam Aminco spectrophotometer equipped with Shibata plate to diminish light scattering.

RESULTS

Photoprotective role of □□pH-mediated non-photochemical Chl fluorescence quenching.

Similar to other photosynthetic organisms, dark-adapted *Rhodella* cells demonstrate typical OIDPS fluorescence induction curves under continuous irradiation (green light, I = 40 □E/m^{2}s) (Fig. 1). The initial value of Fo corresponds to fluorescence of Chl in photosynthetic antennae when most of PS 2 RCs are open [17]. Once light photons are absorbed, primary and secondary quinone electron acceptors of PS 2 (Qa and Qb, correspondingly) are reduced and fluorescence reaches Fi maximum; this process takes about 20 ms. Since the double-reduced Qb migrate to plastoquinone pool (PQ) with slower rates compared to primary reactions, the Fi peak corresponds to the fully reduced Qb. Fluorescence reaches Fp maximum when all plastoquinones are reduced; usually 500-1000 ms is needed to that. Finally, the long-term monotonous decline of fluorescence is observed. This loss of fluorescence is considered to originate from non-photochemical quenching following the transthylakoid ΔpH build up. After the actinic light was turned on NPQ developed for 80-100 s.

Since PQ pool is always in an unstable equilibrium (strongly dependent on intensity and spectrum of actinic light) between Qb and Cyt b6f-complex consuming electrons from PQ to reduce PS 1, Fp amplitude is lower than the real maximal fluorescence [20, 25]. To obtain the maximal value Fm researchers usually add DCMU, which blocks the electron transport between Qa and Qb so preventing ΔpH formation [17, 20]. When we loaded 10 □M DCMU to *Rhodella* cells prior to light irradiation we observed fast fluorescence rise to Fm in 10 □s (Fig. 1). To elucidate whether ΔpH build up correlates to NPQ we added nigericin, a proton transporting ionophore disrupting transthylakoid ΔpH. The induction curve retains its shape reaching Fm level, but without Fs dip that reflects likely total dissipation of a non-photochemical quenching.

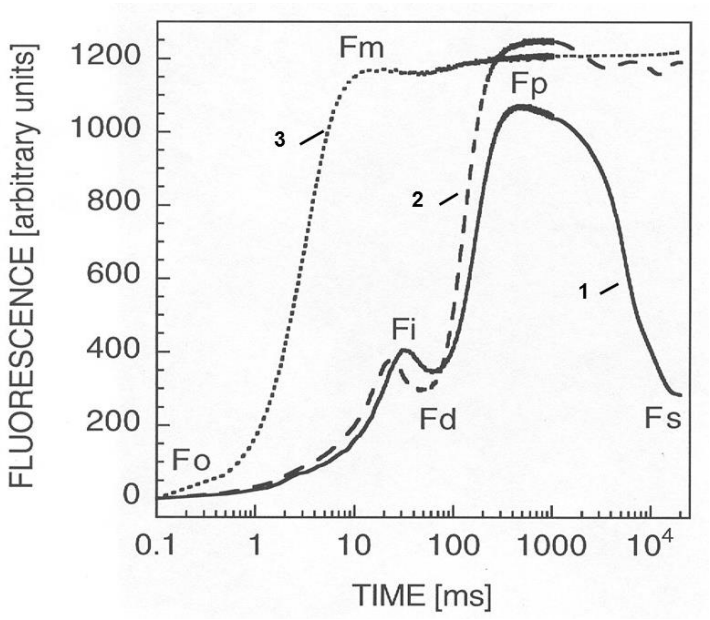

Fig. 1. Fluorescence induction curves of LL-grown Rhodella cultures.
Continuous fluorimeter with green LED excitation. Chl concentration – 5 □g/ml.
(1) - dark adapted cells grown at LL;
(2) - cells preincubated with 80 □M of nigericin
(3) - cells preincubated with 10 □M of DCMU

There are several findings pointing at □pH buil up to be a fast photoprotective mechanism when light induced ΔpH-mediated protein conformational changes in antennae result in enhance of thermal dissipation of excess energy.

According to this suggestion, nigericin added to *Rhodella* suspensions in concentration of 80-100 □M enhanced photoinhibition rates, as it was measured by kinetics of Fv loss (variable Chl fluorescence, Fv = Fm (Fp) – Fo). Adding another uncoupler, NH_4Cl, demonstrated the same effect although the effective concentrations were 10 times exceeding those for nigericin and, according to the fluorescence induction curve, a part of NPQ remains (data not shown).

In full agreement to the above statement, DCCD, inhibitor of chloroplast ATPase which has no effect on□□pH gradient, didn't change kinetics of PS 2 photoinhibition [9].

One more evidence of the photoprotective role of transthylakoid□□pH was found when far red irradiation was applied. Far red light (FR, 710<λ<760 nm) absorbed mainly by PS 1 brings about the dissipation of □pH and restoration of the Chl fluorescence (that is, dissipation of □pH-induced Chl fluorescence quenching). This effect was attributed to activating the chloroplast ATPase via a thioredoxin system [9, 20]. In the extra set of experiments, we examined whether FR influences the photoinhibition rates.

Simultaneous exposure of *Rhodella* suspensions to white light and FR slightly affected kinetics of photoinhibition obtained in the absence of FR. It is reasonable since FR source provided much less light intensity and was unable to dissipate □pH being formed under white light.

However, when we applied short (2 min) FR and white light expositions alternately, that is maintained an average lower level of □pH, we have observed a clear enhancement of photoinhibition rates for all (LL, HL, XHL) types of *Rhodella* cells; for LL-grown culture the difference was about 25% after 20 min of light exposure (data not shown). These results confirm the importance of the fast transthylakoid □pH build up to resist the algal photosynthetic apparatus against strong light.

Acclimation to strong light of LL-adapted *Rhodella* cells.

The low light grown *Rhodella* cultures were acclimated to strong light from 3 to 7 days. LL-grown cultures were transferred to HL or XHL intensities. Such adaptation was previously shown to cause partial loss of PE-containing distal hexamers in PBS antenna and suppression of synthesis of the PE chromophore [21]. This phenomenon can be visualized as a progressive change in the color of the cultures, which turn greenish.

As it follows from Fig. 2, despite of equal Chl content (5 μg Chl/ml) in all three samples (LL, HL, XHL), their absorption spectra differ from each other. Changes in the blue part of absorption spectra of the *Rhodella* suspensions indicate the loss of PE and increased carotenoid bands. The excessive amounts of carotenoids are known to be accumulated in response to light stress; the effect is common for numerous species of microalgae. These "secondary" carotenoids localize in lipid particles outside photosynthetic membranes so that they probably serve as a "screen" to protect CPs from strong light [5, 24].

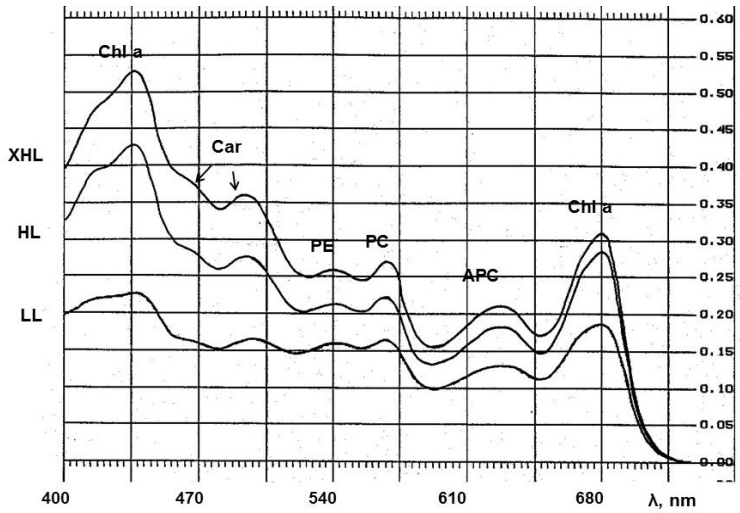

Fig. 2. Absorption spectra of LL-grown and light-adapted Rhodella suspensions at 20°C.
Chl concentration – 5 □g/ml. Optical pathway l = 1 см
LL – low light – 40 μE/м²с
HL – high light – 500 μE/м²с
XHL – extra high light – 1000 μE/м²с
PE – B-phycoerithrin, PC – C-phycocyanin, APC – allophycocyanin.

Because of a more friable thylakoid packing in the light-adapted cultures, the effect was different among LL, HL and XHL samples resulting in the different OD value in the red Chl absorption peak. That is why we measured the total light absorption (1-T) by these suspensions and took these values for final correction of the photoinhibitoin curves.

Light adaptation and photoinhibition.

Since photoinhibition of PS 2 is a complex physiological process induced by oversaturation of electron transport via PS 2 ("acceptor-side photoinhibition"), the influence of photodamage rates on the effective antenna size seems to be natural [2, 3].

We have studied PS 2 photoinhibition in *Rhodella* cultures adapted for strong (HL and XHL) light. Results show that at 2000-4000 μE/m²s photon fluxes the photoinhibition kinetics were as following: LL>HL>XHL (Fig. 3). The difference between HL and XHL was clearly less than one between HL (XHL) to LL probably because of a complete detachment of PE hexamers even under HL light intensity (500 μE/m²s).

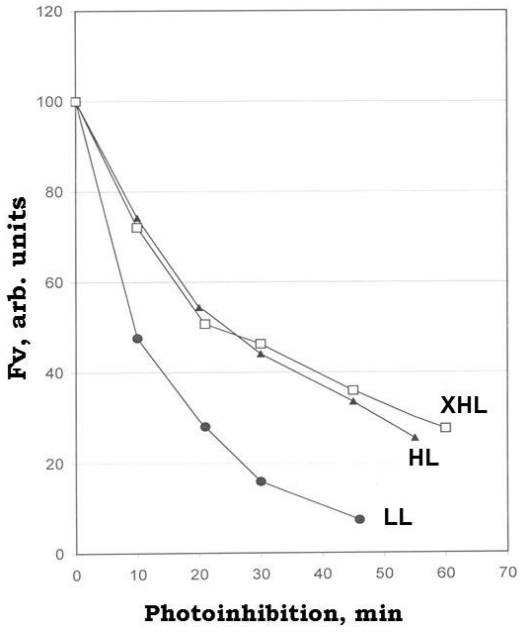

Fig. 3. Kinetics of PS 2 photoinhibition in low light-grown and high light-acclimated Rhodella cells.
Photoinhibition: white light (halogen lamp, I = 4000 μE/м²с)
[Chl] = 5 μг/мл, t° = 20°C
LL – 40 μE/м²с
HL – 500 μE/м²с
XHL – 1000 μE/м²с

The obtained data demonstrate the kinetics of Fv loss to be delayed in HL and XHL-adapted cultures as compared to the LL-grown ones. This is consistent with the fact that PBSs in *Rhodella* are preferentially attached to PS 2 rather than to PS 1, so a smaller antenna size in high light-grown cells prevents PS 2 from excessive energy flow and decreases the photodamage.

Low temperature fluorescence and "state transitions-like" mechanism.

Fluorescence spectra of thylakoid membranes at 77K provide valuable information on both PS 2 and PS 1 since Chl fluorescence at the liquid nitrogen temperature is emitted by both photosystems. Under the excitation at 560 nm (PE absorption maximum in phycobilisome) we detected in *Rhodella* cells fluorescence of PS 2 at 686 and 696 nm and of PS 1 at 720 nm. Additional maximum at about 645 nm was observed and prescribed to APC, the terminal energy acceptor of PBS core. In contrast to PE protein complexes, APC core proteins were stable during the light adaptation to high irradiances, so that this band served as an amplitude marker.

We have shown that the photoinhibited *Rhodella* cells manifest a decrease of both PS 2 and PS 1 fluorescence bands referred to APC emission (Fig. 4), likely, because of the photodamage done to both photosystems with their integrity being impaired. Dark recovery of the photoinhibited cultures demonstrated a partial restoration of PS 2 and PS 1 bands and increase of PS 2 / PS 1 ratio. Faster restoring of PS 2 may be explained by a *de novo* synthesis and a subsequent turnover of D1 protein; the process is light inducible [2, 7].

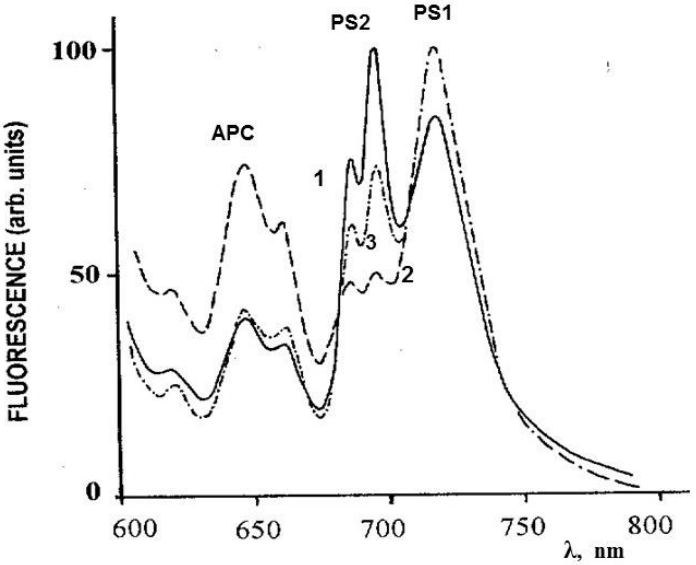

Fig. 4. Low temperature (77K) fluorescence of pigments in the cells of Rhodella violacea
1 – control cells (LL-grown)
2 – Photoinhibition: 25 min
3 – Photoinhibition: 25 min + dark recovery 2 h

Since the 77K fluorescence was excited by green light absorbed mainly by PE, we have calculated the ratio of Chl and PE maxima in fluorescence excitation spectra during high light adaptation of *Rhodella* cultures. We found that LL - HL adaptation during 72 h the ratio of emission bands F686/F648 (PS 2 band to APC band) declined and F718/F648 (PC 1 band to APC band) increased. The ratio of excitation maxima E572/E681 (PE band to Chl band, fluorescence detection at 735 nm) increased supporting the ideas that part of PBSs detaches from PS 2 and move to PS 1 increasing energy migration to PS 1. This process resembles the "state transition", a light adaptation mechanism descried for higher plants; in our experiments it occurred within a longer time scale [8]. Alternatively, the described effect may be explained in terms of light-accelerated *de novo* PBS synthesis where the newly synthesized phycobilisomes associate preferentially with PS 1 [4]. Since the last speculation is not supported experimentally yet, this phenomenon requires further investigation.

CONCLUSION

Photoprotection mechanism in red algae includes the fast light-induced buildup of transthylakoid ΔpH, which is followed by the non-photochemical fluorescence quenching [9, 20]. The important role of ΔpH-gradient in photoprotection was confirmed by the photoinhibition acceleration under FR light known to reoxidize the plastoquinone pool and enhance the dark dissipation of ΔpH.

The presented results reveal that the acclimation of *Rhodella violacea* to strong light (500 or 1000 $\mu E/m^2s$) leads to a better photostability of its photosynthetic machinery. The reasonable explanation may run as follows: when the antenna size is reduced (detachment of PE-containing distal proteins), the energy flow to PS 2 discharging exciton pressure to ETC also goes down.

This subsequently suppresses the photoinhibition rates in HL and XHL acclimated cells. It is worth mentioning that photoinhibition kinetic curves for HL and XHL samples were close to each other pointing at

the possibility that both distal PE hexamers in PBS rods are being detached under HL conditions. Hence, the XHL light intensity doesn't additionally reduce the antenna size.

The "non-photosynthetic" secondary carotenoids, synthesized under high irradiance and accumulated outside the photosynthetic membranes can provide additional barrier against the excess photons.

According to the data on 77K fluorescence, the photoinhibitory light is destructive for both PS 2 and PS 1 though the photosystems are capable to partially recover in darkness. PS 2 recovers faster than PS 1 obviously because fast renewability of the key D1 protein.

Acclimating the *Rhodella* cells to high light for 72 h results in a decrease of energy transfer from PBS to PS 2 and simultaneous increase of PBS-to-PS 1 energy migration. These preliminary results obtained by analyzing as emission as excitation fluorescence spectra point to the "state-transitions-like" process going on the light-acclimating *Rhodella* cells. Although the "state transitions" had been described such as an adaptive mechanism exclusively for higher plants, this process could also exist in red algae. However, this issue has to be further elucidated.

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ИССЛЕДОВАНИЕ ФОТОХИМИЧЕСКИХ СВОЙСТВ И ФИЛОГЕНИИ РАСТИТЕЛЬНЫХ БЕЛКОВ СЕМЕЙСТВА WSCP

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АННОТАЦИЯ

Показана способность хлорофилла в составе белков семейства WSCP (Water-Soluble Chlorophyll-binding Proteins) фотосенсибилизировать окислительные редокс-реакции. Облучение красным светом рекомбинантных хлорофилл-белковых комплексов WSCP подклассов IIa и IIb в присутствии донора электрона (NADH) приводило к окислению донора, т.е., у данных пигмент-белковых комплексов была выявлена фотохимическая активность. При этом не происходило фотодеструкции хлорофилла в составе WSCP, что указывает на фотокаталитический характер обнаруженной редокс-реакции. Кинетические константы фотоокисления NADH были выше для WSCP подкласса IIa (BoWSCP), чем для подкласса IIb (LvWSCP). Для объяснения разности фотосенсибилизирующей активности представителей разных подклассов WSCP был проведен биоинформатический анализ белков класса II данного семейства. С этой целью был проведен поиск членов семейства WSCP в базе данных белковых последовательностей UniProt с алгоритмом поиска BLAST с последующим их множественным выравниванием и построением филогенетического дерева с помощью веб-сервиса EMBL-EBI Clustal Omega и программы MEGA7. Биоинформатический анализ подтвердил филогенетическое разделение семейства белков WSCP класса II на два подкласса, ранее установленное на основании различия их физико-химических свойств. Высказано предположения о связи филогении с фотохимической активностью представителей разных подклассов в семействе WSCP.

ABSTRACT

Here we show the ability of the chlorophyll associated with proteins of the WSCP family (Water-Soluble Chlorophyll-binding Proteins) to photosensitize oxidative redox reactions. Irradiation with red light of the recombinant chlorophyll-protein complexes WSCP subclasses IIa and IIb in the presence of an electron donor (NADH) led to oxidation of the donor, i.e., these pigment-protein complexes showed photochemical activity. Meanwhile there was no photodestruction of chlorophyll associated with WSCP, which indicates the photocatalytic nature of the detected redox reaction. The kinetic constants of NADH photooxidation were higher for WSCP subclass IIa (BoWSCP) than for subclass IIb (LvWSCP). To explain the difference in the photosensitizing activity of representatives of different WSCP subclasses, bioinformatic analysis of class II proteins of this family was carried out. For this purpose, we searched for members of the WSCP family in the UniProt protein sequence database using the BLAST search algorithm, followed by their multiple alignment and construction of a phylogenetic tree using the EMBL-EBI Clustal Omega web service and the MEGA7 program. Bioinformatic analysis has confirmed the phylogenetic division of the WSCP class II protein family into two subclasses, previously established on the basis of the difference in their physicochemical properties. It was suggested that phylogeny is related to the photochemical activity of representatives of different subclasses in the WSCP family.