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High-temperature thermoluminescence of chlorophyll as a method to study lipid peroxidation in planktonic algae

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With 7 figures and 3 tables

Abstract: Chlorophyll-containing cells of algae, photosynthetic bacteria, and plants often exhibit strong red luminescence when heated to temperatures above 70 °C. This high-temperature thermoluminescence (HTL) originates mostly from thermally induced degradation of lipid cycloperoxides present in thylakoid membranes. The degrading cycloperoxides can transiently form carbonyl species in excited triplet state, whereas chlorophyll molecules readily accept energy from the triplet carbonyls and emit it as luminescence. The HTL emission was measured in phytoplankton collected from different waters and in laboratory cultures of algae and cyanobacteria. The intensity of HTL generally increased at mid-day in the phytoplankton sampled in oligotrophic waters (Lake Baikal, Kandalakshskaya Bay of the White Sea) but showed little changes throughout the day in waters with high concentration of mineral nutrients (Lake Geneva, Marseille Gulf of the Mediterranean Sea, Kotorska Gulf of the Adriatic Sea). When the culture of *Chlorella pyrenoidosa* was grown under the nitrogen- or phosphorus-limited conditions at moderate light intensity, an increase in the HTL was observed. However, the HTL emission remained low when *C. pyrenoidosa* or other species of algae were grown in nutrient-rich media even if the algae were exposed to a strong light. Consequently, nutrient availability may be of importance for cell resistance to the light-induced peroxidation of membrane lipids. The HTL is an easy and convenient method to access lipid peroxidation in natural phytoplankton assemblages.

Key **words:** Lipid cycloperoxides, nutrient deficiency, *Chlorella pyrenoidosa.*

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Introduction

Light plays a key role in the performance of photosynthetic photochemical reactions in plants and algae. However, photosynthesis can be viewed as a very risky process because a large amount of oxygen is formed in thylakoids in the vicinity of powerful oxidation-reduction systems that readily reduce oxygen to dangerous superoxide and produce harmful singlet oxygen (DALTON 1995). Excess illumination may damage a variety of cellular constituents, especially thylakoid membranes. In addition to inactivation of Photosystem II and Photosystem I reactions in the thylakoids (ARO et al. 1993, SONOIKE 1995), prolonged exposure to a strong light can cause subsequent degradation of thylakoid membrane lipids, a process associated with lipid peroxidation (HEATH & PACKER 1968, MISHRA & SINGHAL 1992). The lipid peroxidation is a universal mechanism of damage to membranes under various stress conditions (FOYER et al. 1994). Hence, a continuous emphasis on exploring the susceptibility of photosynthetic organisms to photooxidative stress is needed in order to understand competitive relationships between photosynthetic species in ecosystems and effects of stress conditions on the overall productivity of water ecosystems (ALSCHER & HESS 1993).

Despite a great variety of literature concerning oxidative stress in plants, algae and cyanobacteria, little is known about peroxidation processes taking place in natural phytoplankton assemblages. The lipid peroxidation is most commonly detected and quantified by measuring by-products of the reaction, such as ethane, conjugated diens, malondialdehyde, and other carbonyl compounds (HEATH & PACKER 1968, PRYOR & CASTLE 1984, CHATTERJEE & AGARWAL 1988, HAVAUX & NIYOGI 1999). Separation of specific products of lipid oxidation by HPLC was also employed in a number of studies (e.g. HAVAUX & NIYOGI 1999). However, these techniques cannot be routinely used for the assessment of lipid oxidation in phytoplankton because the analysis requires high amounts of biological material. The second problem is the difficulty of separating the peroxidative reactions that take place in phytoplankton from the reactions occurring in non-phytoplankton paniculate matter.

When chlorophyll-containing material is gradually heated to temperatures above 70 °C, an enhancement of chlorophyll luminescence emission is often observed. This increase in the luminescence intensity at high temperature was named high-temperature thermoluminescence (HTL) (MATORIN et al. 1989, VENEDIKTOV et al. 1989). HTL peaking at 120-130 °C arises due to a thermally induced breakdown of lipid peroxides (presumably lipid cycloperoxides) that were present in the samples before heating. The high-temperature stimulated breakdown of lipid peroxides leads to the formation of carbonyl species in excited triplet state; chlorophyll molecules readily accept the energy of excited

carbonyls and emit it as luminescence upon transition to the ground state (MATORIN et al. 1989, VAVILIN & DUCRUET 1998).

Recently, we have shown a good correlation between the formation of malondialdehyde and intensity of the HTL emission at 120-130 °C in alga *Chlorella pyrenoidosa* exposed to a variety of conditions that provoked oxidative stress (VAVILIN et al. 1998). Measurements of the HTL have been used to detect lipid peroxidation in leaves of higher plants subjected to low-temperature stress (MATORIN et al. 1991), water stress (MERZLYAK et al. 1992), and strong light (HAVAUX & NIYOGI 1999). In the present paper, we demonstrate that lipid oxidation in natural assemblages of phytoplankton, as well as in laboratory planktonic photosynthetic organisms, can be conveniently assessed by measuring the HTL.

Materials and methods

The following strains of micoalgae were used in experiments: the chlorophyceae *Chlorella pyrenoidosa* CALU-175, *Chlorella vulgaris* SHL 108b and *Selenastrum capricornutum* CCAP 278/4; the diatoms *Navicula accomoda* SHL 107 and *Phaeodactylum tricornutum; Synechocystis* PCC 6803, a cyanobacterium. *C. pyrenoidosa* strain originated from the collection of the Biology Institute (St. Petersburg State University, Russia), *S. capricornutum* was purchased from the Culture Collection of Algae and Protozoa (Ambleside, UK), *C vulgaris* and *N. accomoda* were isolated from Lake Geneva at the Station d'Hydrobiologie Lacustre (Thonon, France), and *P. tricornutum* was taken from the culture collection of the Institute of Marine Fisheries and Oceanography (Moscow, Russia).

C. pyrenoidosa and *Synechocystis* sp. were grown at 30 °C, under continuos light of 50μErrT^{2} s"^{1} provided by a set of fluorescent tubes (see Table 1 for the recipes of growth media). Other algae were grown at 22 °C, under indirect daylight (maximum light intensity of 120μEirr^{2}s~'). For the nitrogen and phosphorus depletion experiments, cells of *Chlorella pyrenoidosa* in mid-logarithmic growth phase were harvested by centrifugation and resuspended in the modified BG-11 medium that was lacking NaNO3 or K_2HPO_4, respectively. In some experiments, 50 uM methylviologen (1,Γ-dimethyl-4,4'-bipiridinum dichloride) or 10 uM diuron (3-(3,4-dichlorophenyl)-l,l-dimethylurea) was added to the growth media to stimulate lipid peroxidation in algae. Methylviologen is a herbicide that promotes generation of superoxide anion radicals in Photosystem I pigment protein complex, whereas diuron is a known inhibitor of electron transfer reactions in Photosystem II.

HTL measurements were done essentially as described by VAVILIN & DUCRUET (1998) using the setup similar to that reported by MIRANDA & DUCRUET (1992). In brief, fixed volumes (up to 50 mL) of cell suspension were filtered through 15 mm polyamide filters. Filters with pore diameter of 1 цт were used for phytoplankton and cyanobacteria and of 5)im for unicellular algae. The final pigment density varied from 50 ng to 2 jig of chlorophyll-a per square cm of the filter area. Immediately after the

Table 1. Culture media recipes.

Medium	Species	Composition
BG-11 (RIPPKA et al. 1979)	*C. pyrenoidosa, Synechocystis* sp.	$NaNO_3$ (1.5 g/L), $MgSO_4$-$7H_2O$ (75mg/L), $CaCl_2 \cdot 2H_2O$ (36mg/L), K_2HPO_4 (31 mg/L), Na_2CO_3 (20 mg/L), citric acid (6 mg/L), ferric ammonium citrate (6 mg/L), H3BO3 (2.86 mg/L), $MnCl_2 \cdot 4H_2O$ (1.81 mg/L), $ZnSO_4 \cdot 7H_2O$ (220μg/L), $Na_2MoO_4 \cdot 2H_2O$ (390μg/L). $CuSO_4 \cdot 5H_2O$ (79μg/L), $Co(NO_3)_2$-$6H_2O$ (49μg/L), NaEDTA (lOmg/L), 5mM TES buffer (pH = 8.2)
A	*C. vulgaris, S. capricornutum*	KNO3 (1.25 g/L), KH_2PO_4 (200 mg/L), $MgSO_4$-$7H_2O$ (1.00g/L), $CaCl_2$-$2H_2O$(100mg/L), $NaHCO_3$ (40mg/L), H_3BO_3 (114mg/mL), $FeSO_4 \cdot 7H_20$ (50mg/L), $ZnSO_4 \cdot 7H_2O$ (88mg/L), $MnCb$-$4H_2O$ (14.5mg/L), $Na_2MoO_4 \cdot 2H_2O$ (12mg/L), $CuSO_4$-$5H_2O$ (16mg/L), $Co(NO_3)_2 \cdot 6H_2O$ (100μg/L), NaEDTA (50 mg/L)
B	*N. accomoda*	Medium A supplemented with Na_2SiO_3 (25 mg/L)
C	*P. tricornutum*	Medium B supplemented with NaCl (27.0 g/L), $MgSO_4$-$7H_2O$ (5.6g/L), $MgCl_2$ (5.5 g/L), $CaCl_2$-$2H_2O$(1.2g/L)
Nitrogen-less	*C pyrenoidosa*	BG-11 medium; $NaNO_3$ was replaced by equimolar amount of $NaHSO_4$
Phosphorus-less	*C. pyrenoidosa*	BG-11 medium; K_2HPO_4 was replaced by KNO_3 (36 mg/L)

end of filtration, the filter was fixed in a pre-cooled aluminum sample holder equipped with a miniature electric heater, incubated for 5 min in darkness and then heated to 150 °C at a constant heating rate of 0.1 °C/s (heating at a faster rate shifted thermoluminescence peaks to higher temperatures). Thermoluminescence detected by a photomultiplier tube was recorded during the heating. A red longpass filter (X>650nm) was placed between the sample and the photomultiplier to increase selectivity of the detection system with respect to chlorophyll luminescence (X_{MAX} - 670-680nm). Temperature was controlled using a thermocouple mounted between the filter and the heating element. In some cases, to decrease the time -period required for recording the thermoluminescence curves, the dark-adapted samples were first rapidly (5-10 s) heated to 75 °C and then the heating was continued at a slower rate (0.1 °C/s). This procedure did not change intensity of the thermoluminescence above 100 °C. It should be emphasized that all the samples were allowed to dry in air during the heating; when evaporation of water was retarded by covering the sample with a glass slide, a strongly modified HTL signal was observed (see DUCRUET & VAVILIN 1999). Simulation of the measured HTL signal by a set of calculated thermoluminescence components was done by a numerical method described by MIRANDA & DUCRUET (1992) and by VAVILIN & DUCRUET (1998).

Table 2. General characteristics of the sampling sites.

Sampling site	Global coordinates	Sampling time	Trophic status
Lake Baikal (near the Biological Station of Irkutsk University, Bolshie Koty); Eastern Russia	52°N 105°E	June, 1987	Oligotrophic (KOZHOVA 1987)
Kandalakshskaya Bay (near the Biological Station of Moscow State University), White Sea	68°N, 37°E	July, 1988	Oligotrophic to mesotrophic (MAKSIMOVA 1978)
Kotorska Gulf, Adriatic Sea	42°40'N, 18°40'E	May, 1988	Highly eutrophic (VILICIC 1989)
Marseille Gulf, North-Western Mediterranean Sea	43°20'N, 4°50'E	March, 1989	Eutrophic (YORO et al. 1997)
Lake Geneva (near Thonon); Western France/Switzerland	46°24'N, 6°28'E	August, 1996	Mesotrophic to eutrophic (PELLETIER 1998)

To measure chlorophyll content, cells were harvested by filtration and the air-dried filters were kept frozen until analyzed. Pigments were extracted with small volume of 100 % methanol (cultures of algae) or with 100 % acetone (phytoplankton samples). Chlorophyll was determined using extinction coefficients referred in PORRA (1991). The sampling sites of phytoplankton are listed in Table 2.

Results and discussion

Thermoluminescence emission in different species of algae and cyanobacteria

A typical thermoluminescence curve recorded from the dark-adapted green alga *Chlorella pyrenoidosa* is characterized by a band peaking around 25 °C and by an unresolved band or a shoulder above 100 °C (Fig. 1 A, thin line). Incubation of *Chlorella* cells in the presence of methylviologen, a herbicide that stimulates oxidation of the membrane lipids, inhibited the 25 °C band and caused a significant rise in the amplitude of the HTL (Fig. 1 A, solid line). Similar enhancement of the luminescence at high temperatures and suppression of the low temperature luminescence (-25 °C) were observed in methylviologen-treated cultures of *Synechocystis* sp. PCC 6803, *Phaeodactylum tricornutum*, and in phytoplankton sampled in a pond near the laboratory at CEN, Saclay (Fig. 1 B-D). In *Synechocystis* and *Phaeodactylum*, the methylviologen treatment also stimulated thermoluminescence at about 60-75 °C (Fig. 1B.C).

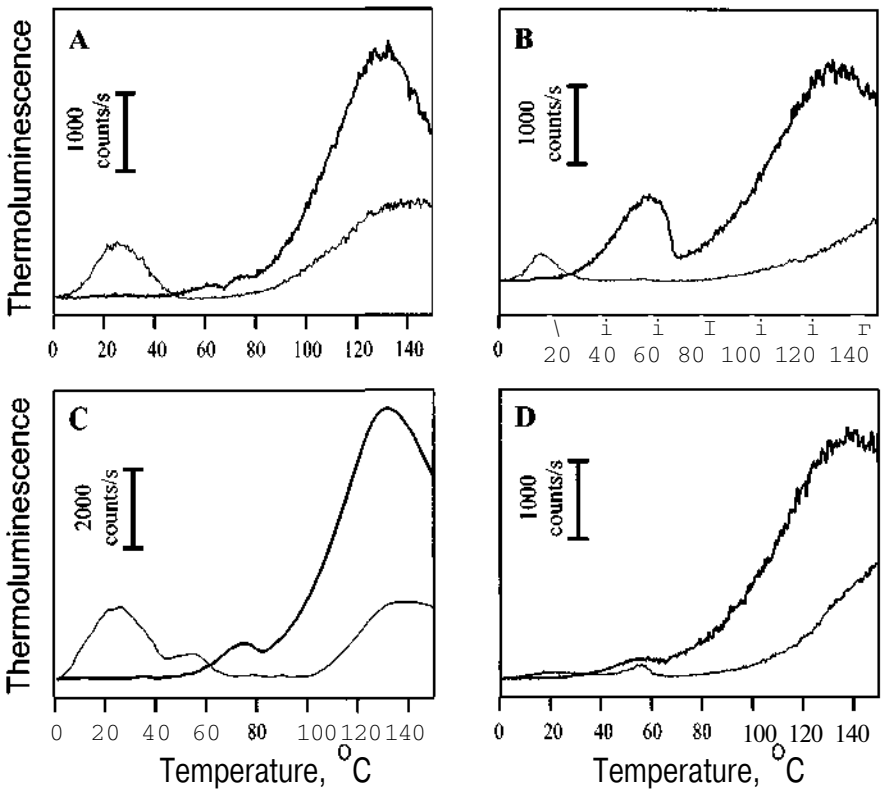

Fig. 1. Thermoluminescence curves measured in *Chlorella pyrenoidosa* (A), *Synechocystis* sp. PCC 6803 (B), *Phaeodactylum tricornutum* (C) cells, and in phytoplankton sampled in a pond on the territory of the Centre d'etudes Nucleaires (Saclay, France) early in the morning on August, 1998 (D). Thermoluminescence was recorded in non-treated cells (thin lines) and in the cells incubated for 5 h in the presence of 50 μM methylviologen at light intensity of 40 μEm^{-2}s^{-1} (thick lines) at 25 °C.

Thermoluminescence emitted by photosynthetic organisms in the temperature range from 0 to 150 °C has a heterogeneous origin. The 25 °C band (photosynthetic thermoluminescence) is produced by charge-recombination reactions in the Photosystem II pigment-protein complex (reviewed by VASS & GOVINDJEE 1996). These reactions were inhibited in algae subjected to methylviologen-induced oxidative stress. The mechanism of thermoluminescence emission intensified at 60-75 °C remains unclear. Some evidence suggests involvement of hydrogen peroxide in the generation of this band (DUCRUET & VAVILIN 1999). The HTL emission at 90-140 °C (peak maximum around 125 °C), which is the focus of this study, arises due to the thermally induced breakdown of lipid cycloperoxides accompanied by the formation of chloro-

phyll molecules in excited state (VENEDIKTOV et al. 1989, VAVILIN & DUCRUET 1998).

Similar to the photosynthetic thermoluminescence (MIRANDA & DUCRUET 1992), the HTL bands can be modeled assuming that formation of a band is driven by two competing factors: (i) rise of temperature causes an increase in the rate of decomposition of the peroxidized specie(s) and therefore an increase in the luminescence intensity; (ii) diminution in the concentration of these species as decomposition proceeds leads to a decrease in the luminescence (VAVILIN & DUCRUET 1998). The first process dominates at relatively low temperatures, whereas the second process is responsible for the decay of thermoluminescence at higher temperatures.

According to the Arrhenius-Eyring rate constant theory, the rate of decomposition of lipid peroxides can be written as

$$dP / d t = P S T \exp (-E_A / k_B T) \quad (1),$$

where P represents the amount of lipid peroxides, which degradation leads to the luminescence emission, S is a frequency factor, E_A is the activation energy, кв is the Bolzmann constant, and t is time. During the time period At, AP lipid peroxide molecules degrade at a given temperature T:

$$ЛP = P (t, T) - P (t + At, T) \quad (2).$$

Consequently, the amount of lipid peroxides in the sample by the end of the At period becomes equal to

$$P (t + At, T) = P (t, T) - P (t, T) S T At \exp (-E_A / k_B T) \quad (3).$$

Starting at $T = T_o$ and P = Po (Po equals the initial amount of lipid peroxides in a non-heated sample, which degradation at higher temperatures leads to the thermoluminescence emission) and increasing the temperature by At V_T (V_T is the heating rate) every time after calculating the corresponding P (t +At, T) point, the values of P (or AP) and T can be obtained at every time point t for the given parameters S and E_A. Considering that thermoluminescence intensity is proportional to the rate of degradation of lipid peroxides AP/At, the plot of AP (or AP / At) versus T represents a theoretical thermoluminescence band, in which P_o corresponds to the area under the thermoluminescence curve. In reality, the measured thermoluminescence curves represent superposition of several thermoluminescence bands; each of these bands can be characterized by a set of the parameters S, E_A, and PQ. By varying these parameters in order to find the best match between the theoretical and experimental thermoluminescence curves, the relative concentration of lipid peroxides in the sample (P o) can be estimated as illustrated in Fig. 2.

Fig. 2 compares two HTL curves measured in *Chlorella* before (control) and after 4 days of incubation in the presence of photosynthesis inhibitor diuron. The inhibition of photosynthesis finally results in the culture death ac-

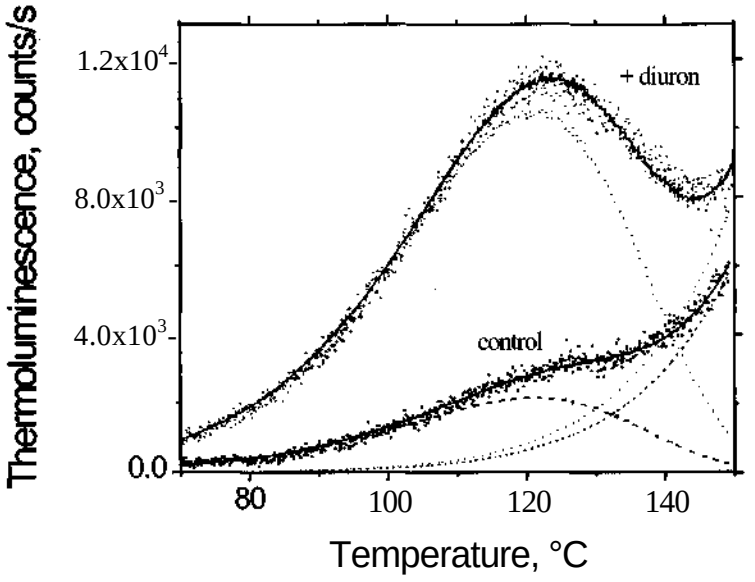

Fig. 2. Mathematical resolution of high-temperature thermoluminescence glow curves measured in *Chlorella pyrenoidosa* cells before (control) and after 4 days of incubation in the presence of 10 μM diuron. Shown are experimental data points (both samples had 1 (ig of chlorophyll-a per square cm of the filter area), individual thermoluminescence bands obtained from the mathematical curve resolution into two components (dotted line, control cells; dashed line, diuron-treated cells) and resulting theoretical thermoluminescence curves (solid lines). Decomposition parameters of the individual thermoluminescence bands are: control - Po (1) =7.6 x 10^4 photon counts, $E_A(1)$ = 16.6kcal/mol, S_o (1) = 2.3x 10 V \ P_o (2) = 2.7x 10^7 photon counts, $E_A(2)$ = 24.2kcal/mol, S_o (2) = 1.4x 10^5 s^{-1}; diuron-treated cells - P_o (1) = 3.5 x 10^5 photon counts, $E_A(1)$ = 16.8 kcal/mol, S_o (1) = 3.0x10^4s-\ P_o (2) = 3.0 x 10^7 photon counts, $E_A(2)$ = 24.0kcal/mol, S_o (2) = 1.2 x 10^5 s^{-1}.

companied by light-stimulated oxidative degradation of lipid membranes. Both HTL curves can be satisfactory modeled by a sum of two bands, peaking at about 125 °C and above 150 °C. As can be seen from comparison of the integral intensities of the individual bands (see the legend to Fig. 2 for P_o values), lipid peroxidation stimulated the 125 °C component, whereas the component peaking at higher temperature exhibited substantially less pronounced changes. According to our previous results (VAVILIN & DUCRUET 1998, DUCRUET & VAVILIN 1999), the band peaking above 150 °C is not related to the preaccumulated lipid peroxides but likely is caused by thermal oxidation of other cellular components. It should be also noted that in certain cases introduction of a third component peaking at 60-75 °C (see for example Fig. 1C) is required to obtain a good agreement between theoretical and experimental thermoluminescence curves in the range of temperatures from 70 to 150 °C.

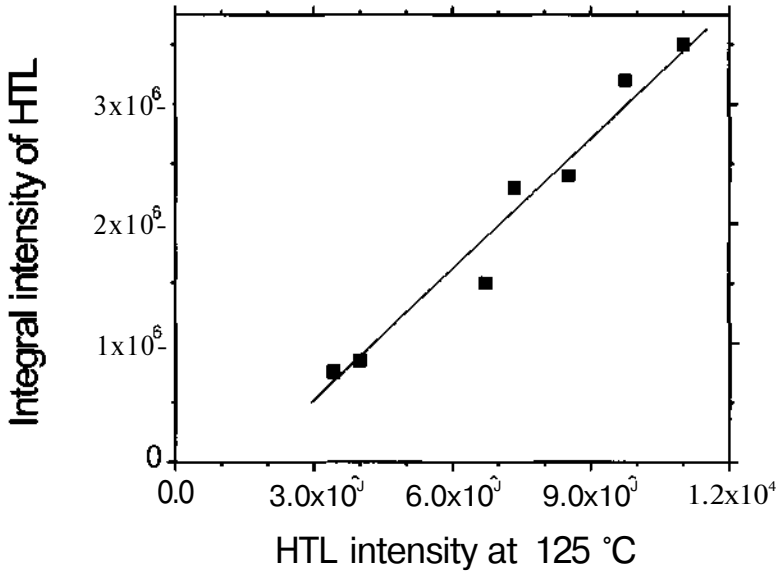

Fig.3. Relationship between the integral intensity of the 125 °C HTL band (Po(l)) and the amplitude of the HTL measured at 125 °C. TL curves were recorded from *Chlorella pyrenoidosa* cells incubated with the diuron for different periods of time (up to 10 days).

The above-described procedure of decomposition of the thermoluminescence curves into separate components, which involves adjustment of the three parameters (P_o, Ед and S) for every band, is rather time-consuming. However, the band peaking above 150 °C generally has a minor contribution to the 125 °C emission, therefore measurements of the amplitude of the HTL at 125 °C can be used as a reasonably good indicator of the lipid peroxides content in the samples (Fig. 3). This simplified approach was employed for the detection of lipid peroxides in phytoplankton sampled in different waters.

The high-temperature thermoluminescence in phytoplankton

Fig. 4 shows typical diurnal changes in the HTL intensity of phytoplankton from Lake Baikal sampled from different depths. The phytoplankton community was dominated by *Chroomonas acuta* (Cryptophyta), *Koliella longiseta* (Chlorophyta), and *Nitzschia acicularis* (Bacillariophyta). The measuring period was preceded by a stormy weather, which caused almost uniform distribution of the phytoplankton biomass throughout the water column with a chlorophyll concentration of about 0.6 ug/L. The HTL signal increased strongly during the first half of the day in the samples collected near the surface of the lake, whereas in deeper water layers the HTL exhibited minor

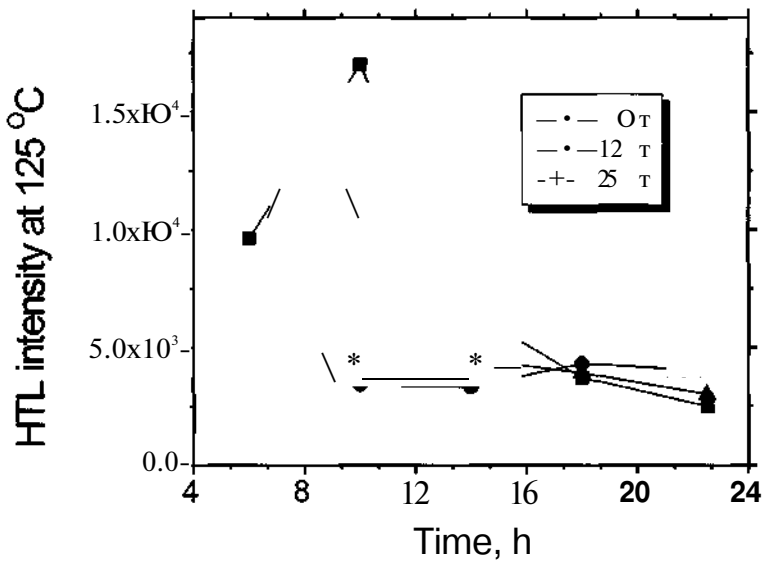

Fig. 4. Diurnal dynamics of the amplitude of HTL measured at 125 °C in phytoplankton sampled in the Lake Baikal at the depth of 0, 12 and 25 m. Samples contained about 50 ng of chlorophyll-a per cm^2 of the filter area. Each data point represents an average of two to three measurements. S.D. was less than 15 % of the average values.

changes. The phytoplankton biomass varied within about 15 % throughout the whole day at different depths. Therefore, the observed changes in the HTL emission are likely to reflect intensification of lipid peroxidation upon exposure of surface phytoplankton to strong daylight. It should be emphasized that strong HTL was also characteristic for the phytoplankton probes collected in mid-day time at other sites of the lake.

Similar effect of light on the diurnal dynamics of the HTL emission was observed in phytoplankton sampled in Kandalakshskaya Bay of the White Sea on the North-East of Russia (Fig. 5). The diatom *Skeletonema costatum* overwhelmingly dominated in the samples; the diatoms *Chaetoceros decipiens, Ch. curvisetus,* and *Ch. diadema* were also abundant. In this experiment, the phytoplankton collected at midnight from approximately 0.5 m depth was placed into two 3-L trace-metal clean glass containers in order to prevent effects of vertical phytoplankton mixing throughout the day. One of the containers was screened from sunlight by black cloth and both containers were left floating on the surface of the water. The HTL signal increased sharply in phytoplankton exposed to direct sunlight, whereas no increase in the HTL emission was observed in the darkened container.

Contrary to the results obtained in Lake Baikal and in the White Sea, no light-stimulated mid-day rise in the intensity of the HTL was detected in

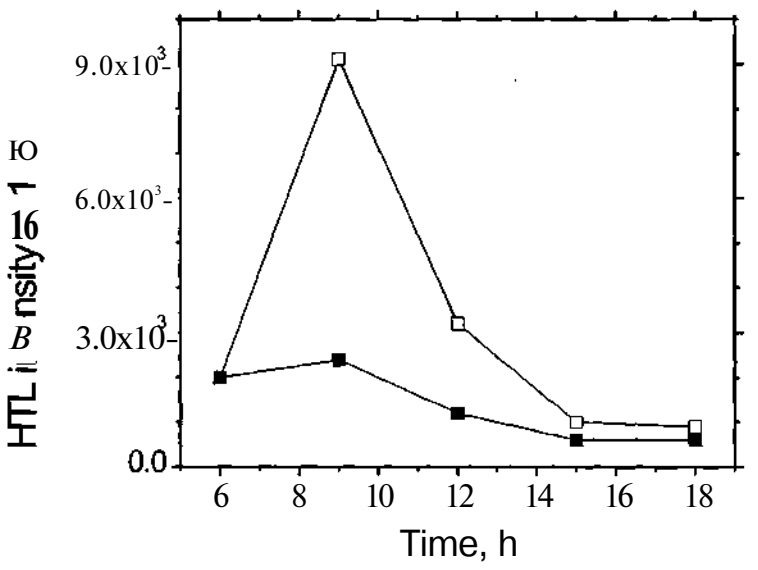

Fig. 5. Diurnal dynamics of the amplitude of thermoluminescence signal measured at 125 °C in phytoplankton incubated under full sunlight (•) and in the dark (•). Phytoplankton was sampled at the Kandalakshskaya Bay of the White Sea. Filters prepared for the HTL measurements contained about 200 ng of chlorophyll-a per cm^2 of the filter area. Each data point represents an average of two to three measurements. SD was less than 11 % of the average values.

phytoplankton sampled in the Kotorska Gulf of the Adriatic Sea, in the Marseille Gulf of the Mediterranean Sea, and in Lake Geneva. For example, intensity of the HTL in the Marseille Gulf samples varied within the range of ± 14 % of an average value during the 40 h observation period that started at 6:00 in the morning (samples from the sea surface were taken every 1.5-4.0 h), with the highest values detected before the sunrise on the second day of the experiment. Average intensity of the HTL from phytoplankton sampled in Lake Geneva between 11:00 and 13:00 was only 1.05 ± 0.15 times higher compared to the intensity of HTL in the samples collected in the morning at 8 :30 or in the evening at 18 :00 (the samples were taken for 6 days and the signal intensity measured in mid-day was normalized to the value obtained in the morning or in the evening at the same day). It should be pointed out that all experiments with phytoplankton were done on cloudless days when maximum subsurface light intensities at noon reached values above lOOQuEm^s"[1]. Therefore, low light intensity due to a cloudy sky, for example, cannot account for the absence of diurnal dynamics of the HTL in the above-mentioned regions. Interestingly, when different species of algae and cyanobacteria grown

Table 3. Effect of strong light on the amplitude of the HTL measured at 125 °C in different cultures of algae and cyanobacteria. The amplitude of the HTL in non-illuminated cells was taken as 100%. Shown are the average values ±SD of 3 to 4 measurements.

Species	Light intensity, µEirT^2 s$^{"1}$	Duration of light treatment, h	HTL intensity
C. pyrenoidosa	1000	4.0	11±12
C. vulgaris	1000	4.0	98±10
S. capricornutum	1000	2.0	97± 6
N. accomoda	1000	3.5	93±10
Synechocystis sp. PCC 6803	1500	2.0	105± 8
		3.5	100±13

in the laboratory under optimum conditions were exposed to strong light for up to 4 hours, the HTL intensity remained low as well (Table 3).

Besides the light intensity, numerous factors or a combination of factors can affect the physiological state of phytoplankton in natural environment and thereby modulate the population response to high light stress. Thus, nutrient deficiency can increase production of potentially toxic reactive oxygen species such as hydrogen peroxide, superoxide anion radicals, or hydroxyl radicals in plants (FOYER et al. 1994). In turn, these highly reactive forms of oxygen can cause peroxidation of the membrane lipids. We propose that combination of strong light and nutrient limitation might be responsible for the mid-day stimulation of lipid peroxidation in phytoplankton. This trend can be clearly seen when trophic status of different phytoplankton sampling sites (see Table 2) is compared to the results of the HTL measurements. Indeed, Lake Baikal is characterized by extremely low concentration of dissolved mineral nutrients (KOZHOVA 1987). Productivity and biomass of phytoplankton in the White Sea are usually low in mid-summer period primarily because of nitrogen limitation (MAKSIMOVA 1978). Accordingly, phytoplankton sampled in these two regions in the middle of the day was characterized by increased HTL (however, we cannot exclude the possibility that bottle artifacts contributed to some extent to the HTL rise observed in the light-exposed bottle in the White Sea). In contrast, waters of Lake Geneva, Kotorska Gulf and the coastal region of the Mediterranean Sea are generally enriched in mineral nutrients and no mid-day enhancement of the HTL emission was detected at these sampling sites.

Effect of macronutrients limitation on the high-temperature thermoluminescence in *Chlorella*

In order to test the effect of mineral deprivation on lipid peroxidation under controlled conditions, the culture of *Chlorella pyrenoidosa* was grown for two

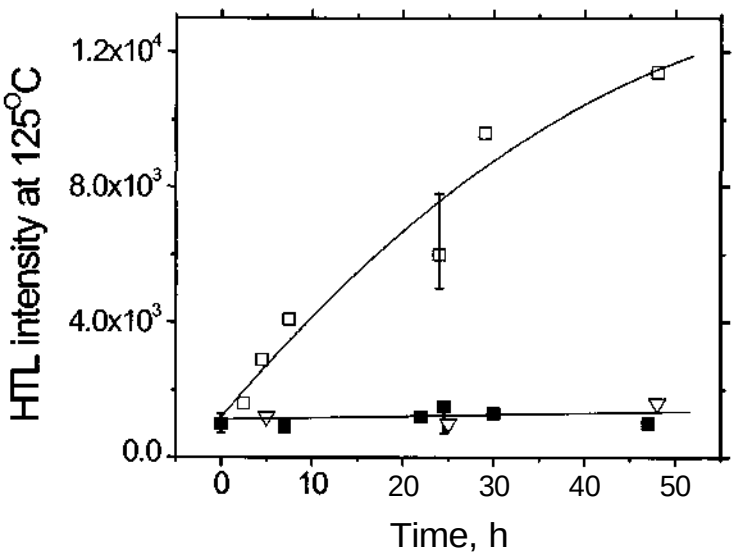

Fig. 6. Amplitude of the HTL signal measured at 125 °C in *Chlorella pyrenoidosa* cells incubated in the nitrogen-deficient (•) and in the nitrogen-rich media (V) at 100 μE $m^{-2} s^{-1}$ and in the nitrogen-deficient medium in darkness (•). Different volumes of the culture were harvested by filtration to achieve equal amounts of chlorophyll-a (1 ng/cm^2) for the HTL measurements. Shown are the results of a single experiment and SD values obtained in three independent experiments in the cultures grown for 24 h under nitrogen-deficient conditions.

days in the absence of mineral nitrogen. In this experiment, initial concentration of NO_3^- ions was less than 0.01 mM, compared to 1.8 mM in the full growth medium. Under these conditions, early symptoms of nitrogen deficiency (e.g. decrease in photosynthetic activity) were observed within about 4 h following the nitrogen removal (VAVILIN et al. 1999). Besides the inhibition of photosynthesis, the nitrogen removal also caused a continuous rise in the intensity of HTL in the culture exposed to light, whereas no **HTL** rise was observed in the dark-incubated culture (Fig. 6). Similar results were obtained with *Chlorella* cells grown at strongly reduced concentration of mineral phosphorus. However, in the latter case the increase in HTL was observed 5 to 7 days after the phosphorus was removed from the medium (Fig. 7).

Concluding remarks

The HTL measurements hold substantial potential to access the importance of peroxidative reactions in natural phytoplankton assemblages. High sensitivity of photomultiplier tubes used to detect the luminescence allows reliable re-

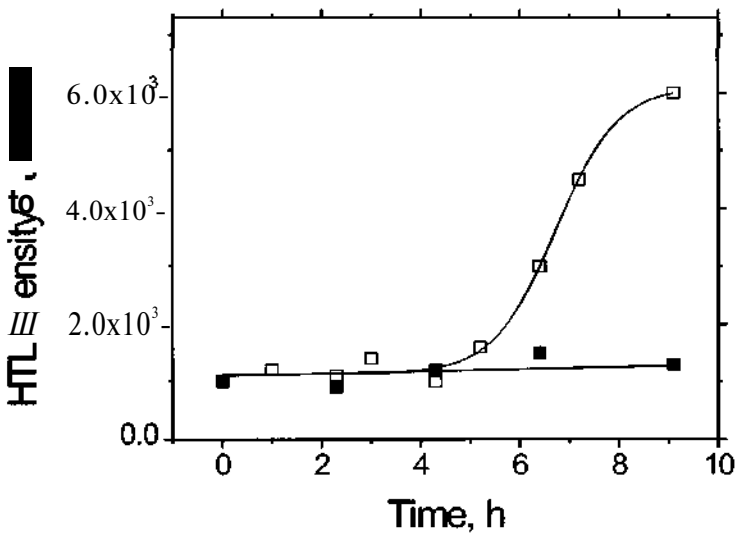

Fig. 7. Amplitude of the thermoluminescence signal measured at 125 °C in *Chlorella pyrenoidosa* cells incubated in the phosphate-deficient medium at 100 µE irT² s"' (Π) and in darkness (•). Different volumes of the culture were harvested by filtration to achieve equal amounts of chlorophyll-a (1 |ig/cm ²) for the HTL measurements. On the 3 day of experiment, the culture was diluted 50 times with fresh BG-11 medium containing no phosphate. Shown are the results of a single experiment. The rate of HTL increase in the phosphate-deficient *Chlorella* culture exposed to light varied to some extent from experiment to experiment. However, in all experiments the amplitude of the HTL increased at least 3 times during the 7-day period of light incubation in the absence of phosphate in the medium.

cording of the **HTL** signals in samples collected from highly oligotrophic waters with chlorophyll concentration of about 0.1µg/l.

We believe that HTL peaking at ~125 °C mostly reflects lipid peroxidation reactions occurring in the phytoplankton material. Really, the **HTL** is emitted by chlorophyll molecules in excited state, which are formed upon temperature-stimulated degradation of the lipid peroxides (VENEDIKTOV et al. 1989, VAVILIN & DUCRUET 1998). Consequently, selective detection of thermoluminescence in the red region of the spectra corresponding to the wavelengths of maximum chlorophyll emission (670-680 nm) provides a good separation of the luminescence originating from chlorophyll-containing photosynthetic organisms from chemiluminescence emitted by chlorophyll-less paniculate matter, which is generally blue-shifted compared to the chlorophyll luminescence. Moreover, results of the modeling of the thermoluminescence curves (see Fig. 2) suggest that only reactions with certain thermodynamic characteristics (i.e. S and E_A values) may lead to the HTL emission at 90-140 °C.

The intensity of lipid peroxidation can be assessed from measurements of the integral intensity of the 125 °C band (Po) or from measurements of the amplitude of HTL around 125 °C. The latter approach is the easiest; however, it should be taken into account that under certain conditions the 125 °C emission can be significantly overestimated because of the contribution of thermoluminescence peaking above 150 °C.

The results of the experiments presented in this paper demonstrate significant variations in the intensity of lipid peroxidation reactions in phytoplankton. These variations were modulated by diurnal changes in light intensity. However, comparison of the diurnal dynamics of the HTL in the phytoplankton sampled at different places, as well as the experiments done with the cultures of algae, show that light intensity is essential but not the only factor responsible for the increase in the lipid peroxides content in the cells. Preliminary results give evidence for the importance of nutrient availability in cell resistance to the light-induced oxidative damage.

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