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SHORT-TERM HEATING CAUSES THYLAKOID RESTRUCTURING IN PEA CHLOROPLASTS AND MODIFIES SPECTRAL PROPERTIES OF PIGMENT-PROTEIN COMPLEXES

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The effect of short-term heating on ultrastructure of the pea chloroplasts and the spectral characteristics of the subchloroplast fragments has been investigated. The size of chloroplasts decreased at heating for 5 min in the darkness at either 25, 35 or 45 °C. The phenomenon was caused mainly by a decrease of the length of long axis of chloroplast. The extent of the reduction was less after the heating at 45 °C in the presence of light. Detailed analysis has been conducted for heating at 45 °C because more prominent changes in circular dichroism (CD) spectra have been observed indicating the greater effect of heating. A decrease in ψ-bands of the spectrum has indicated a disturbance in long range ordering in arrangement of membrane macrodomains. The quantity of grana with an increased number of thylakoids (more than 6) became greater after the heating of isolated chloroplasts. Low temperature fluorescence spectra of chloroplasts showed a decrease in intensity of both bands of the spectrum. The spectra of subchloroplast fragments corresponding to the grana particles revealed an increase in intensity of both bands. An increase of the short-wavelength band belonging to emission of PSII, indicates an increase of quantity of PSII in grana particles originated from heated chloroplasts. An increase of the long-wavelength band belonging to PSI emission, was caused by an increasing of the size of antenna, that was detected by the rise of intensity in excitation spectrum of fluorescence detected at 735 nm. A broadening of short-wavelength band which was greater after the heating in the presence of the illumination was evoked by a loosening of the PSII complex antenna. An analysis of the spectral characteristics of the fragments of the granal thylakoids margin region provided additional data about the changes in the grana organization caused by heating. The obtained data are in line with the hypothesis that links changes in the organization of the thylakoid membranes to the interaction between PSII and the «mobile antenna» represented by light-harvesting complex II (LHCII). It has also been demonstrated that illumination mitigates the effect of short-term heating.

Key words: *Pisum sativum* L., short-term heating, chloroplasts ultrastructure.

The progress in our understanding of the chloroplast response to the short-term heating relies on the accumulation of the data on the heat-induced

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changes in various characteristics of photosynthetic apparatus, and subsequent generation of the hypotheses regarding the mechanisms behind these changes [1, 2, 3]. Several important experimental reviews have been published by now. It was reported partial destacking of grana thylakoids in bean chloroplasts. Changes in the spatial orientation of grana and in their number per chloroplast have been described for winter wheat plants [4, 5]. Number of thylakoids per grana can also be modified upon heat treatment. The chiral LHCII macrodomains in intact thylakoid membranes have been shown to gradually disassemble after heating at temperatures above 40 °C [6]. Short-term heating also led to alterations in the antenna complexes of both photosystem I (PSI) and photosystem II (PSII) [7, 8]. Interestingly, illumination during heat treatment has been shown to have protective effect on the functional activity of PSII and PSI [9, 10, 11].

In this study, we provide additional experimental evidence of the modifications of the pea chloroplasts ultrastructure after short-term heating and test the hypothesis that designates the light-harvesting complex II (LHCII) as a key player controlling restructuring the chloroplast membranes during the heat stress. Several techniques (electron microscopy, circular dichroism spectra, and low-temperature spectroscopy) were applied to obtain the information related to the different hierarchical organization levels of the organelles performing photosynthetic function in higher plants.

Material and methods

Plant material. The pea (*Pisum sativum* L.) plants were grown outdoors or in greenhouse for two weeks. The leaves of two upper layers were used. The chloroplasts with partially broken sheath were isolated as described earlier [12]. The isolated chloroplasts were suspended in the medium containing 10 mM Tricine buffer (pH 7.5), 0.4 M sucrose, 10 mM NaCl, and 5 mM $MgCl_2$. Subchloroplast fragments were obtained by solubilization of the chloroplasts with 0.3 % digitonin, and the subsequent differential centrifugation according to the previously published procedure [13]. The particles sedimented at 1000 g and 40000 g after 20000 g were used for the measurements. Chlorophyll concentration (Chl) was estimated spectrophotometrically in 80 % acetone extracts [13] and the yield of the fractions was calculated as the ratio of the total Chl in the particles to the total Chl in chloroplast suspension taken for fragmentation. Chl *a/b* ratio was calculated as described [13].

Temperature treatment. The whole leaves were placed into a transparent flexible polyethylene bag and submerged in the water warmed up to a designated temperature. While being under water, the leaves were in the darkness or under illumination with the white light (260 μmol/($m^2 \cdot s$)). The suspension of isolated chloroplasts was let to settle down in a flat-bottomed flask to form a thin layer. Then the flask was placed in the water at certain temperature (25, 35 or 45 °C) and incubated either in the darkness or under the white light (260 μmol/($m^2 \cdot s$)) for 5 min. The control sample was kept at 5 °C in the darkness. Chlorophyll content in the chloroplast suspension used for measurements of low temperature fluorescence spectra and CD spectra was 30 mg/ml and 10 μg/ml, respectively.

Electron microscopy. The preparation of the samples for the electron microscopy was as following: the control and heated leaves were placed into the agarose blocks and fixed with 3 % glutaraldehyde solution in cacodylate buffer. An additional fixation was performed in 1 % osmium tetroxide solution prepared in the same buffer. Then agarose blocks were embedded into Epon–Araldite mixtures and polymerized. Ultrathin sections of fixed material were obtained with an UMTP-6M microtome (Slmi, RF) and stained with lead citrate. Digital images were acquired with a JEM 1230 electron microscope (JEOL, Japan). About 30 images of chloroplasts were selected in a random manner from the pictures of the leaves ultrathin sections for further measurements of the chloroplasts spatial characteristics. The measurements were performed using MapInfo software modified for the analysis of the microscopic images. To obtain the frequency diagram of the thylakoids number per grana, about 80 grana images from the ultrathin sections of the chloroplasts have been processed.

Spectral measurements. Circular dichroism spectra were measured with Jobin Yvon CD6 dichrograph. The measuring cuvette was placed into a thermostated sample holder. The length of optical path within the cuvette was 1 cm. The distance between the sample and a photomultiplier was 5 cm. CD spectra were recorded with a sampling step of 1 nm, the integration time of 0.3 s, and the band-pass length of 2 nm.

Low-temperature fluorescence spectra were measured using air-dried thin films of chloroplasts deposited on a glass disc. The films were prepared as described earlier [14] and placed into an optical cryostat filled with vapours of liquid nitrogen. The fluorescence spectra were measured as described earlier [13] using modified lab-made setup. The films were highly transparent and their low temperature fluorescence spectra proved to be identical to those of corresponding to frozen buffer-glycerol suspension [13]. Light absorption by the samples in a peak of the red band did not exceed 15 %.

Fluorescence was excited with LED light at 450 nm and was recorded at wavelengths from 650 to 800 nm with a sampling step of 0.5 nm. Band-pass of monochromator was 1 nm. Intensity of the actinic light which passed through the sample was monitored using an additional optical channel at the time of recording the fluorescence spectrum of the sample. These data were used for the normalization of the fluorescence spectra to the spectrum of the control sample, to remove small variations in the light absorbance of various samples.

Excitation spectra of fluorescence were obtained at the detection wavelength of 735 nm for the same samples as for the measurement of the fluorescence spectra as described earlier [13]. The samples were illuminated with the monochromatic light in the interval 630–720 nm using an excitation monochromator with the band-pass of 2 nm and a sampling step of 0.5 nm. The fluorescence at 735 nm was detected with band-pass equal to 4 nm using an emission monochromator.

The means were calculated from five replicates. The tables and the figures show the arithmetic mean and standard error of the mean. The obtained data were processed by generally accepted methods of variation statistics.

Results and discussion

The analysis of the electron microscopy images implies that the heat-induced changes in the lengths of the long and short axes of pea chloroplasts varied depending on the mode of treatment. The cross section of the chloroplasts decreased after heating in the darkness with the increase of the temperature maintained during the treatment (Fig. 1). After the short-term heat treatment, the length of the long axis decreased whereas the length of the short axis increased. The higher was the temperature the greater were these changes. The effect of the heating at 45 °C was more pronounced for the leaves kept in the darkness in comparison to the illuminated ones.

The observed alterations in lengths of the axes mainly resulted from changes in the grana organization. The most prominent change was in the number of the thylakoids per stack. The typical image of the heat-treated grana is shown on Fig. 2, *b*. The percentage of the grana consisting of the large quantity of the thylakoids was noticeably higher in case of heating in the darkness (Fig. 3) when compared to samples heated under illumination. The majority of the grana from the control samples had small number of thylakoids (7 or less). The relative amount of the small grana decreased after heating whereas the percentage of the large grana (8 or more thylakoids per stack) increased. Quite a few grana containing 17—23 thylakoids were observed in the heated samples but none was detected in control ones.

CD signals originate from molecular systems of different complexity, such as granal thylakoid membranes or lamellar aggregates of LHCII. PSII and LHCII in the granum forms chirally organized macrodomains (e.g., the so-called psi-type (ψ) aggregates), large arrays with diameters of several hundred nanometers and long-range chiral order. These chiral macrodomains have also been shown to be capable of undergoing termo-induced reversible structural changes. In chirally organized macroarrays, with sizes

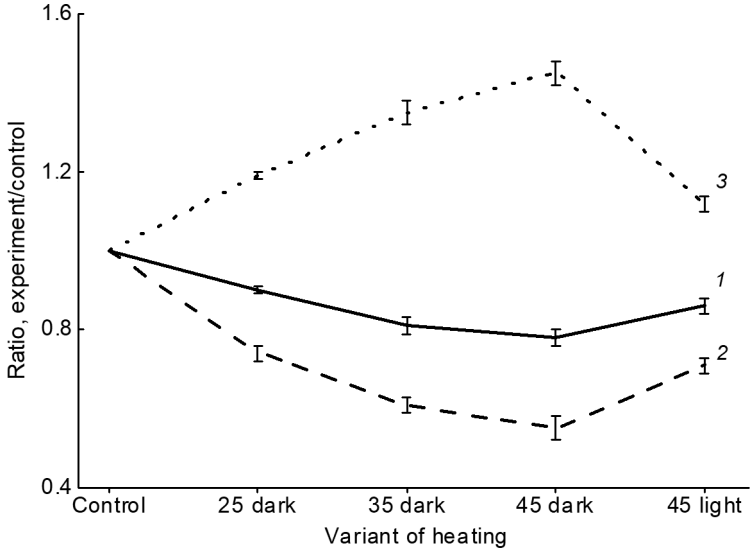

Fig. 1. Changes (experiment/control) of cross section of chloroplast (*1*), lengths of the long (*2*), and the short (*3*) axes

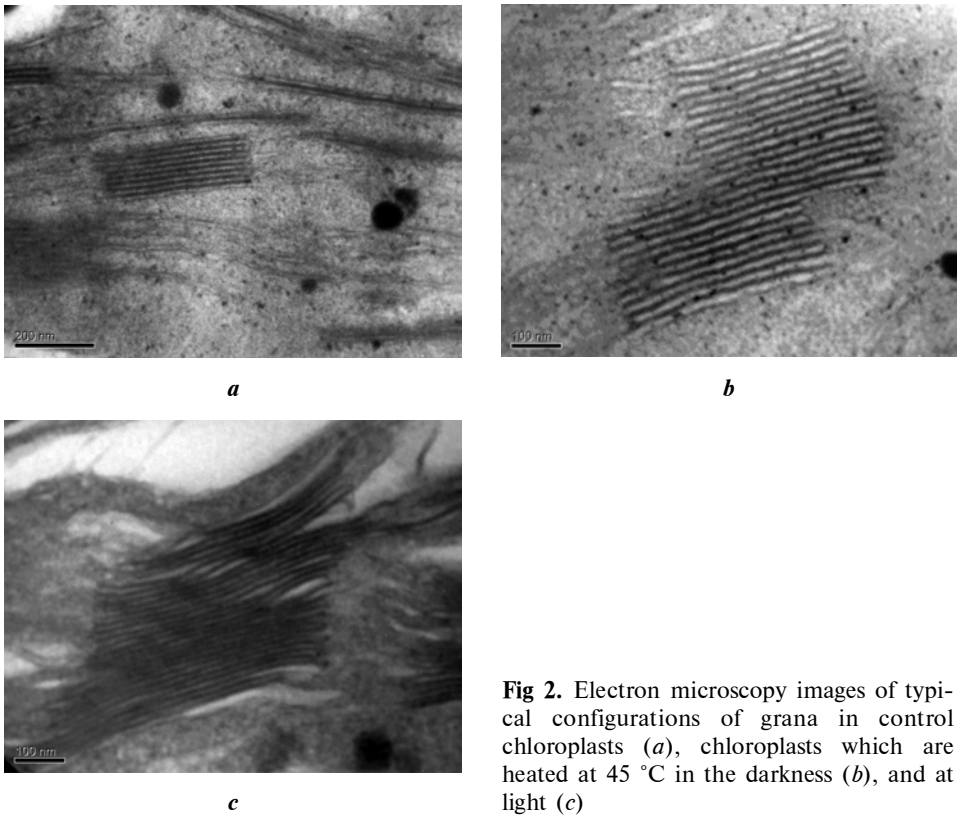

Fig 2. Electron microscopy images of typical configurations of grana in control chloroplasts (*a*), chloroplasts which are heated at 45 °C in the darkness (*b*), and at light (*c*)

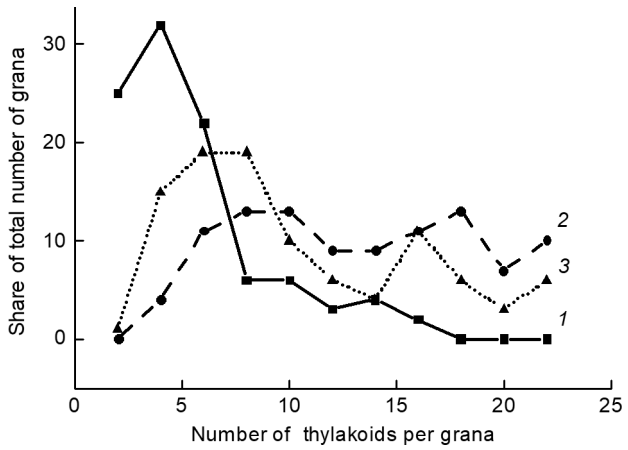

Fig. 3. Changes in a distribution of granas containing various number of thylakoids in the control sample (*1*), in the heated ones in the darkness (*2*), and in the heated ones at light (*3*)

commensurate with the wavelength of the visible light, very intense and «anomalously shaped» so-called ψ-type CD bands are given rise; ψ-type bands are exhibited by intact granal thylakoid membranes, in particular by LHCII-containing macrodomains, as well as by lamellar aggregates of LHCII.

Short-term heat treatment induced changes in CD spectra of the pea chloroplasts manifested mainly in the decreased intensity of both ψ-bands (Fig. 4, Table 1). The most prominent changes occurred after heating at

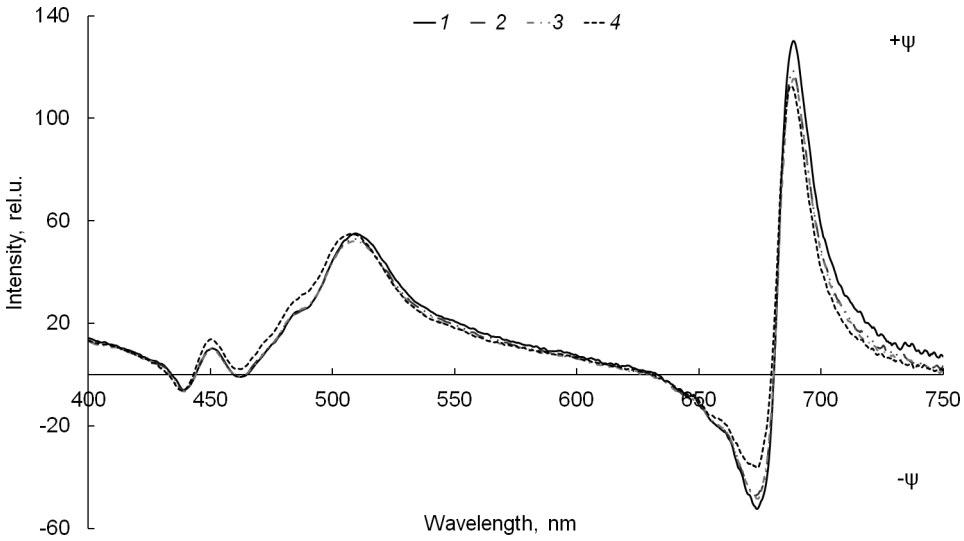

Fig. 4. CD spectra of chloroplasts of control sample (*1*), and of heated in the darkness at 25, 35, 45 °C (*2*, *3*, *4*, respectively)

TABLE 1. Changes in ψ-bands of CD spectra of chloroplasts, heated at various modes of treatment

Treatment	Changes in amplitudes, exp/cont		
	−ψ	+ψ	Sum of amplitudes
Control	1.0	1.0	1.0
Heating 25 °C, dark	0.93±0.03	1.01±0.07	0.98±0.05
Heating 35 °C, dark	0.89±0.03	0.96±0.05	0.94±0.04
Heating 45 °C, dark	0.63±0.05	0.84±0.04	0.79±0.05
Heating 45 °C, light	0.75±0.02	0.82±0.02	0.80±0.02

45 °C. Therefore, this temperature was chosen for further studies performed with subchloroplast particles isolated from chloroplasts subjected to heating in the presence or in the absence of the illumination.

Fragmentation of the chloroplasts and the subsequent stepwise centrifugation at the pre-determined rotation speed allows to obtain subchloroplast particles originated from different regions of the thylakoid membrane system. As shown in our previous publications [13, 15], the subchloroplast particles that sediment at a relatively low speed of 1000 g represent nearly intact grana, and the fraction of fragments obtained after centrifugation at 40000 g contains the membranes from the marginal areas of grana.

The fragment yield was calculated as the ratio (%) of the chlorophyll content in the fraction to the total chlorophyll content in the initial chloroplasts. Table 2 shows that yield increased for the particles isolated from the heated samples whereas Chl *a/b* ratio decreased, when compared to the values obtained for control samples. This effect was similar in the presence or in the absence of the illumination during heating.

Analysis of the chloroplasts low-temperature fluorescence spectra revealed moderate decrease in the intensity of the fluorescence signal

TABLE 2. Changes in characteristics of subchloroplast fragments, induced by short-term heating

Treatment	Changes in the characteristics, exp/cont	
	Yield	Chl *a/b*
	1000 g	
Darkness	1.26±0.01	0.90±0.01
Light	1.23±0.01	0.91±0.01
	40000 g	
Darkness	1.23±0.01	0.94±0.01
Light	1.15±0.01	0.83±0.01

across the spectral range used for the detection (Fig. 5, Table 3). The most prominent changes occurred in the short-wavelength (SW) band (below 700 nm). The ratio between the fluorescence intensity at 685 nm to the intensity at 693 nm was also decreased as the result of the short-term heat treatment. The heat-induced decrease in the intensity of the fluorescence was proportional across the long-wavelength (LW) part of the spectrum (above 700 nm).

Comparison of the low-temperature fluorescence spectra acquired for the subchloroplast particles implies that the fragmentation of the heated chloroplasts closely resembles the fragmentation of the control ones. Fig. 6 shows that the overall shape of the fluorescence spectra of the subchloroplast fragments did not differ substantially between heated and control samples. Minor differences could be associated with altered composition of the thylakoid membranes and/or with variations in their organization in the specific regions.

Various modes of heating also bring about some difference in a shape of the fluorescence spectra (Fig. 6). Low-temperature fluorescence spectra obtained for the particles from heavy (1000 g) and light (40000 g) fractions are depicted in Fig. 6, *a, d*. The spectra of the heavy particles undergo the following changes upon heating: an increase in the area under SW and LW bands, a decrease in I_{685}/I_{693} ratio, and an increase in the value of ΔH parameter, which is represented in Table 4. When compared to the heating in the darkness, illumination during heating resulted in the greater changes, especially for SW band. Fig. 7 shows that the broadening of SW band was associated with the increase in the half-width of both the short-wavelength

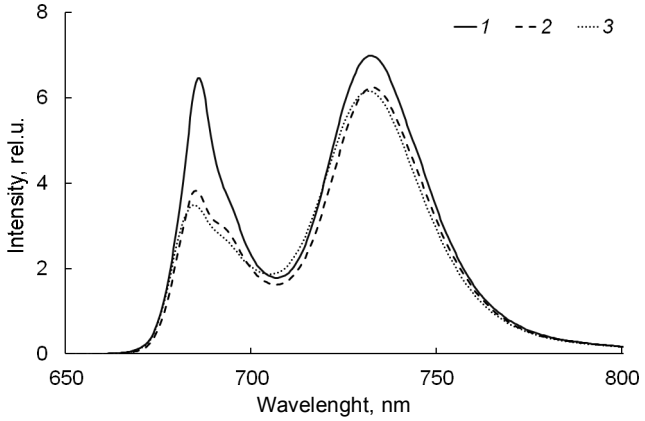

Fig. 5. Low temperature fluorescence spectra of the control chloroplasts (*1*), and of the heated ones in the darkness (*2*), and at light (*3*) (the spectra are normalized to absorption of the control sample)

TABLE 3. Changes of characteristics in low temperature fluorescence spectra of chloroplasts induced by short-term heating at various modes

Treatment	I_{SW}* exp/cont	λ_{max}, nm	ΔH, nm	I_{685}/I_{693}**	I_{LW}* exp/cont	λ_{max}, nm	ΔH, nm
Control	1.00±0.01	686±1	15±1	1.61±0.05	1.00±0.01	731±1	31±1
Heating in darkness	0.72±0.02	685±1	21±1	1.29±0.08	0.91±0.03	732±1	31±1
Heating in light	0.72±0.02	684±1	29±1	1.28±0.08	0.96±0.03	731±1	32±1

N o t e s . * an area under SW or LW band, ** the ratio of the amplitudes.

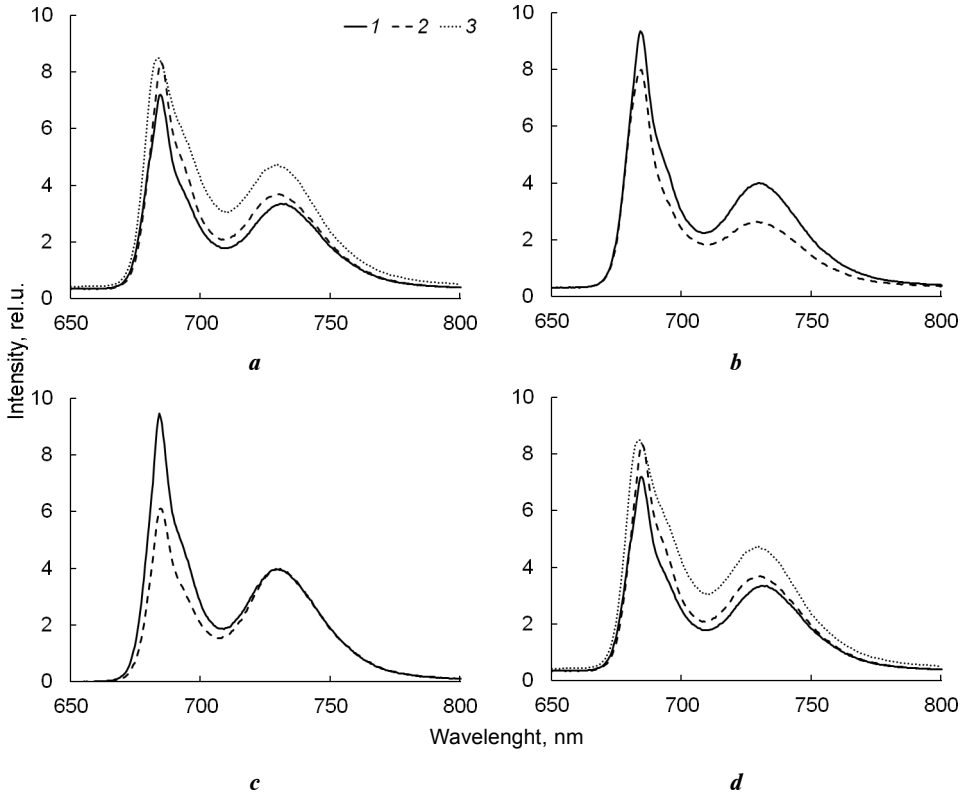

Fig. 6. Low temperature fluorescence spectra of subchloroplast fragments, sediments at 1000, 1300, 20000 and 40000 g (*a*, *b*, *c*, *d*, respectively), isolated from control chloroplasts (*1*), from those heated in the darkness (*2*), and from those heated at low light (*3*) (the spectra are normalized to absorption of the control sample)

and long-wavelength parts of this band. Such changes were due to both the increase in the intensity of the short-wavelength slope of 685 nm band and the increase of relative intensity of 693 nm component. The intensity of LW band increased in the spectra of the heated samples, and to the greater extent for illuminated samples. The half-width and maximum position of the LW band were practically unchanged.

Heating led to an increase in the SW band intensity in the fluorescence spectra of the illuminated particles (Table 4), whereas the intensity of LW band remained unchanged, when heating was performed in the darkness, or decreased if the samples were illuminated during heat treat-

TABLE 4. Changes in characteristics of low temperature fluorescence spectra of subchloroplast fragments, induced by various modes of heating

Treatment	I_{SW}* exp/cont	λ_{max}, nm	ΔH, nm	I_{685}/I_{693}**	I_{LW}* exp/cont	λ_{max}, nm	ΔH, nm
			1000 g				
Control	1.00±0.01	685±1	16±1	1.56±0.08	1.00±0.01	730±1	43±1
Darkness	1.25±0.03	685±1	17±1	1.31±0.07	1.13±0.02	730±1	42±1
Light	1.42±0.02	684±1	22±1	1.32±0.07	1.39±0.03	730±1	42±1
			40000 g				
Control	1.00±0.01	684±1	22±1	1.41±0.07	1.00±0.01	731±1	31±1
Darkness	1.32±0.03	684±1	20±1	1.40±0.07	1.01±0.01	730±1	30±1
Light	1.59±0.02	684±1	23±1	1.25±0.07	0.95±0.01	729±1	31±1

N o t e s . * area under SW or LW band, ** the ratio of the amplitudes.

ment (Fig. 6, *d*). In addition, heating under illumination led to a higher relative intensity of 693 nm component in the SW band in comparison to heating in the darkness (Fig. 7). Overall, the changes in the SW band of the fluorescence spectra of subchloroplast particles indicate heat-induced alterations in the composition or/and the state of the supramolecular complexes of PSII.

The fluorescence excitation spectra detected at 735 nm provided the information about PSI complexes. A rise in the intensity of these spectra and short-wavelength shift was observed for the heated samples (Fig. 8, Table 5). The shift was due to the increase in the intensity of the short-wavelength slope of the spectra (Fig. 8, *b*). The relative intensity at 650 nm (I_{650}/I_{max} ratio in Table 5) was elevated significantly when heating was performed under the light. It indicates higher contribution of the absorption of the cholophyll short-wavelength forms, including Chl *b,* to the low-wavelength part of the spectra.

The fluorescence excitation spectra of the light subchloroplast fragments are shown in Fig. 9, and their characteristics are presented in Table 5.

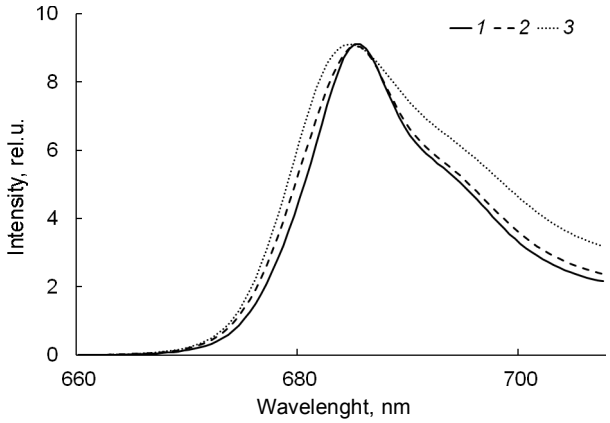

Fig. 7. SW band in low temperature fluorescence spectra of subchloroplast heavy particles (sediment at 1000 g), isolated from control chloroplasts (*1*), from those heated in the darkness (*2*), and from those heated at low light (*3*) (the bands are normalized to intensity at maximum of the band in the spectrum of the control sample)

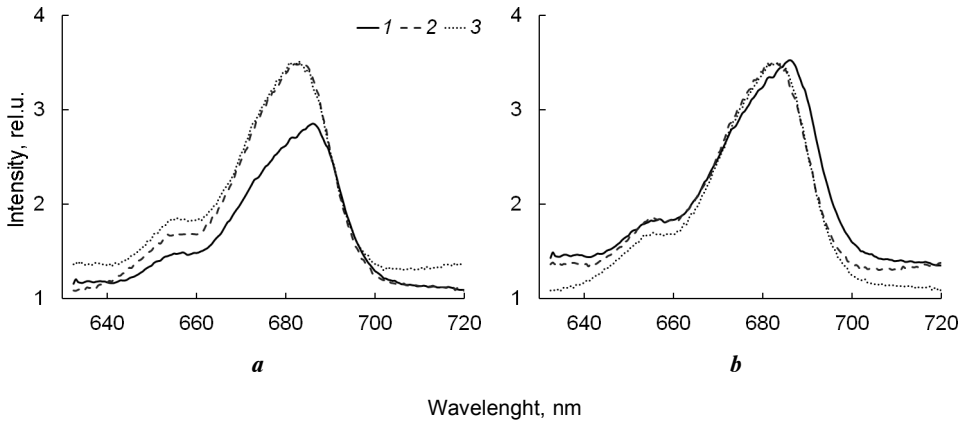

Fig. 8. Excitation spectra of fluorescence detected at 735 nm for heavy subchloroplast fragments from control sample (*1*), the samples, heated in the darkness (*2*) and the samples, heated at light (*3*): *a* — the spectra normalized to absorption of the control sample; *b* — the spectra normalized to intensity in maximum of the control sample

TABLE 5. Changes in characteristics of excitation spectra of fluorescence detected at 735 nm for subchloroplast fragments, induced by various modes of heating

Treatment	Intensity exp/cont	λ_{max}, nm	ΔH, nm	I_{650}/I_{max}
		1000 g		
Control	1.00±0.01	685±1	32±1	0.44±0.03
Darkness	1.33±0.02	683±1	28±1	0.42±0.03
Light	1.38±0.02	682±1	29±1	0.46±0.03
		40000 g		
Control	1.00±0.01	683±1	28±1	0.34±0.03
Darkness	0.92±0.02	683±1	26±1	0.33±0.03
Light	0.97±0.02	683±1	30±1	0.41±0.03

The overall signal intensity decreased slightly after the heating. The half-width of the spectrum was decreased after the heating in the darkness. However, it increased after the heating under illumination. Contribution of the Chl *b* absorption was stronger if heating was performed in the darkness. It appears that more prominent changes occurred due to heating in the presence of the light. They may be associated with the enhanced contribution of the short-wavelength Chl forms including Chl *b*. Most plausible explanation of these changes is an increase in the light-harvesting antenna of PSI. However, the energy transfer from *de novo* associated ingredients seems to be less efficient considering the decrease of the integral intensity of the excitation spectrum.

The analysis of the obtained data reveals changes in various components of the chloroplast ultrastructure. Below, we will summarize and discuss the important distinctions between such changes occurring after short-term heating in the darkness *versus* those occurring when the heating was performed under the light.

Heating in the darkness. Heating at 45 °C in the darkness results in loosening of the packing in the supramolecular PSII complexes located in the grana core region. This conclusion is based on the observed increase in the intensity of the short-wavelength slope of the SW band in the fluorescence spectra of chloroplasts and subchloroplast particles originated from the grana (Tables 3, 4, Fig. 7). Such an increase is explained by the

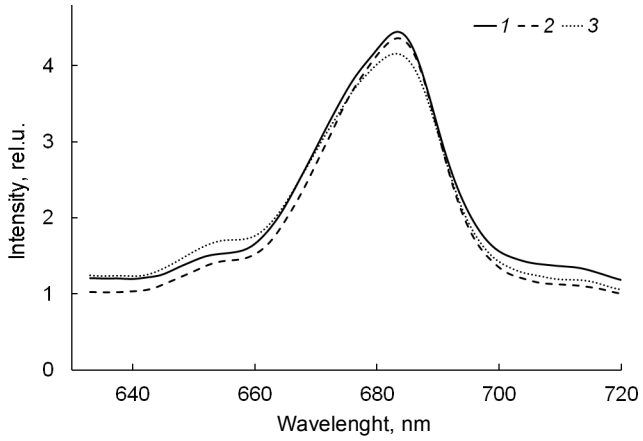

Fig. 9. Excitation spectra of fluorescence detected at 735 nm for light subchloroplast fragments from control sample (*1*), from the samples, heated in the darkness (*2*), and from the samples, heated at light (*3*) (the spectra normalized by absorption of the control sample)

enhanced emission of short-wavelength components of SW band which are linked to LHCII [7]. At the same time heating caused dramatic decrease in the fluorescence at 685 nm. It indicates the impaired energy transfer from LHCII to the inner light-harvesting antenna of PSII, namely, CP43 complex that has the emission band maximum at about 685 nm. Furthermore, a heat-induced decrease in the amplitude of ψ-band in CD spectrum of chloroplasts reveals partial disarrangement in the long-range order of LHCII macrodomains in the grana thylakoids (Fig. 4, Table 1). The heat treatment in the darkness also loosens the stacks of thylakoids in grana as inferred from the increased yield of subchloroplasts fragments originated from the grana margins.

The heating also brings about the alterations at the level of the entire grana. The percentage of the grana with the greater number of thylakoids increases (Fig. 3) and the shape of the pea chloroplasts changes. The length of the long axis is reduced possibly due to a decrease in a length of the grana thylakoids as previously shown [12]. Note that the grana thylakoids are usually aligned along the long axis in pea chloroplasts. At the same time, the length of the short axis is increased likely as the result of the increase in the number of thylakoids per grana.

Based on the obtained data, the following sequence of events initiated by the short-term heating may be assumed. One of the primary targets of the heat action may be the supramolecular pigment-protein complexes in the internal membrane system of the chloroplasts. For instance, the quasi-ordered structure, an inherent property of LHCII-PSII megacomplexes [16], makes it susceptible to the partial disarrangement. Changes in the state of these complexes can affect the entire grana due to their role in the stacking of the grana thylakoids [16, 17], and in the formation of the quasicrystal system in the thylakoid membranes [6, 18, 19, 20]. Modifications in the grana structure eventually lead to the changes in the shape of the entire chloroplasts.

Heating under illumination. This mode of heating induces changes similar to those observed for the heating in the darkness regardless of the

structural organization level those changes were studied. However, the extent of the effects was different. The loosening in the antenna complex of PSII was greater but the extent of the changes in the lateral arrangement of LHCI-PSII complexes, in the stacking of thylakoids, and in the length of both axes of chloroplasts was lesser in comparison to the changes observed after heating in the darkness. Thus, the main feature of the combined action of the heating and light, is less pronounced changes in the grana and chloroplast structure, and stronger effect on the antenna of the PSII complex.

Previously published data suggest that the ability of the electrons to leave the locus of the PSII reaction center is impaired [21], meaning that the phosphorylation of LHCII, which requires the linear electron transport [22], is inhibited. Perhaps, other modifications of LHCII-PSII complex under illumination play role in the protective action of the light under heat stress. The illumination may also modulate the response of the photosynthetic apparatus to heat through the cyclic electron flow around PSI as well as by counteracting the heat-induced increase in the proton conductance of the thylakoid membranes [23], which may affect not only the charge distribution at the surface of the thylakoid membranes but also the luminal pH and the regulatory thermal dissipation in the pigment-protein complexes.

While being in line with the previously reported protective action of the illumination during the heat treatment on the functional activity of the chloroplasts [9, 10, 11], our data provide additional information on the structural changes and lay the groundwork for better understanding of the chloroplast response mechanisms to the short-term heating.

REFERENCES

1. Hu, S., Ding, Y. & Zhu, C. (2020). Sensitivity and Responses of Chloroplasts to Heat Stress. Plants Front. Plant Sci. https://doi.org/10.3389/fpls.2020.00375
2. Wang, Q.-L., Chen, J.-H., He, N.-Y. & Guo, F.-Q. (2018). Metabolic reprogramming in chloroplasts under heat stress in plants. Int. J. Mol. Sci., 19, No. 4, p. 849. https://doi.org/10.3390/ijms19030849
3. Mathur, S., Agrawal, D., & Jajoo, A. (2014). Photosynthesis: response to high temperature stress. J. Photochem. Photobiol. B: Biol., 137, pp. 116-126. https://doi.org/10.1016/j.jphotobiol.2014.01.010
4. Sharkova, V.E. & Buobolo, L.S. (1996). Effect of Heat Shock on the Arrangement of Thylakoid Membranes in the Chloroplasts of Mature Wheat Leaves. Rus. J. Plant Physiol., 43, pp. 358-365.
5. Kisliuk, I.M., Buobolo, L.S., & Vaskovskii, M.D. (1997). Heat Shock-induced Increase in the Length and Number of Thylakoids in Wheat Leaf Chloroplasts. Rus. J. Plant Physiol., 44, pp. 30-35.
6. Cseh, Z., Rajagopal, S., Tsonev, T., Busheva, M., Papp, E. & Garab, G. (2000). Thermooptic effect in chloroplast thylakoid membranes. Thermal and light stability of pigment arrays with different levels of structural complexity. Biochemistry, 39, pp. 15250-15257.
7. Kochubey, S.M. (2010). Changes in antenna of photosystem II induced by short-term heating. Photosinth. Res., 106, pp. 239-246.
8. Kochubey, S.M., Shevchenko, V.V. & Kazantsev, T.A. (2013). Changes of the antenna of Photosystem I induced by short-term heating. Biochemistry (Moscow) Suppl. seria A: Membr. Cell Biol., **7**, pp. 67-77.
9. Janka, E., Korner, O., Rosenqvist, E. & Ottosen, C.-O. (2013). High temperature stress monitoring and detection using chlorophyll a fluorescence and infrared thermography in

chrysanthemum (dendranthema grandiflora). Plant Physiol. Biochem., 67, pp. 87-94. https://doi.org/10.1016/j.plaphy.2013.02.025
10. Kalituho, L.N., Pshybytko, N.L., Kabashnikova, L.F. & Jahns, P.P. (2003). Photosynthetic apparatus and high temperature: role of light. Bulg. J. Plant Physiol. Special iss, pp. 281-289.
11. Kisliuk, I.M., Buobolo, L.S., Bykov, O.D., Kamentseva, I.E. & Sherstniova, O.A. (2008). Protective and Injuring Action of Visible Light on Photosynthetic Apparatus in Wheat Plants during Hyperthermia Treatment. Rus. J. Plant Physiol., 55, pp. 613-620.
12. Bondarenko, O.Yu. (2010). Changes of pea leaves chloroplasts size induced by short-term heating. Physiology and Biochemistry of Cultivated Plants (in Russian), 42, pp. 79-83.
13. Kochubey, S.M., Bondarenko, O.Yu. & Shevchenko, V.V. (2007). A new type of subchloroplast fragments isolated from pea chloroplasts in the presence of digitonin. Biochemistry (Moscow), 72, pp. 1021-1026.
14. Kochubey, S.M. & Samokhval, E.G. (2000). Long-wavelength chlorophyll forms in Photosystem I from pea thylakoids. Photosynth. Res., 63, pp. 281-290.
15. Kochubey, S.M. (2001). Organization of the marginal regions of the pea granal thylakoids. Rus. J. Plant Physiol., 46, pp. 333-339.
16. Dekker, J.P. & Boekema, E.J. (2005). Supramolecular organization of thylakoid membrane proteins in green plants. Biochim. Biophys. Acta., 1706, pp. 12-39.
17. Ford, R.C., Stoylova, S.S. & Holzenburg, A. (2002). An alternative model for photosystem II/light harvesting complex II in grana membranes based on cryo-electron microscopy studies. Eur. J. Biochem., 269, pp. 326-336.
18. Garab, G., Faludi-Daniel, A., Sutherland, J.C. & Hind, G. (1988). Macroorganization of chlorophyll a/b light-harvesting complex in thylakoids and aggregates: information from circular differential scattering. Biochemistry, 27**,** pp. 2425-2430.
19. Barzda, V., Mustardy, L. & Garab, G. (1994). Size Dependency of Circular Dichroism in Macroaggregates of Photosynthetic Pigment-Protein Complexes. Biochemistry, 33, pp. 10837-10841.
20. Garab, G. (1996). Biophysical Techniques in Photosynthesis. In: Amesz, J., Hoff, A. (eds.). Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 11-40.
21. Shevchenko, V.V. (2011). The light of low intensity protects Photosystem II from inhibition at short-term heating. Physiology and Biochemistry of Cultivated Plants (on Russian), 43, pp. 527-532.
22. Baginsky, S. (2016). Protein phosphorylation in chloroplasts — a survey of phosphorylation targets. Journal of Experimental Botany, 67, pp. 3873-3882. https://doi.org/10.1093/jxb/erw098
23. Zhang, R., Cruz, J.A., Kramer, D.M., Magallanes-Lundback, M.E., Dellapenna, D. & Sharkey, T.D. (2009). Moderate heat stress reduces the pH component of the transthylakoid proton motive force in light-adapted, intact tobacco leaves. Plant. Cell and Environment, 32, pp. 1538-1547. https://doi.org/10.1111/j.1365-3040.2009.02018.x

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КОРОТКОТРИВАЛИЙ ПРОГРІВ СПРИЧИНЮЄ РЕСТРУКТУРУВАННЯ ТИЛАКОЇДІВ У ХЛОРОПЛАСТАХ ГОРОХУ І МОДИФІКУЄ СПЕКТРАЛЬНІ ВЛАСТИВОСТІ ПІГМЕНТ-БІЛКОВИХ КОМПЛЕКСІВ

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Досліджено вплив короткочасного прогріву на ультраструктуру хлоропластів гороху та спектральні характеристики субхлоропластних фрагментів. Розмір хлоропластів зменшувався при нагріванні протягом 5 хв у темряві за 25, 35 або 45 °С. Явище було спричинене переважно зменшенням довжини довгої осі хлоропласта. Ступінь змен-

шення знижувався після нагрівання за 45 °C на світлі. Детальний аналіз був проведений для нагрівання за 45 °C, оскільки спостерігались помітніші зміни в спектрах кругового дихроїзму (CD), що свідчить про більший ефект нагрівання. Зменшення ψ-смуг спектра свідчить про порушення великомасштабного впорядкування в розміщенні мембранних макродоменів. Кількість гран зі збільшеною кількістю тилакоїдів (понад 6) зросла після нагрівання ізольованих хлоропластів. Низькотемпературні спектри флуоресценції хлоропластів показали зменшення інтенсивності обох смуг спектра. Спектри субхлоропластних фрагментів, що відповідають частинкам грани, виявили збільшення інтенсивності обох смуг. Збільшення інтенсивності короткохвильової смуги, що належить до випромінювання PSII, свідчить про зростання кількості PSII у частинках грани, отриманих із прогрітих хлоропластів. Підвищення інтенсивності довгохвильової смуги, що належить до випромінювання PSI, спричинене збільшенням розміру антени, яке було встановлено за зміною інтенсивності спектра збудження флуоресценції, детектованого за 735 нм. Після нагрівання при наявності світла спостерігалось розширення короткохвильової смуги, яке було викликане зменшенням антени комплексу PSII. Аналіз спектральних характеристик фрагментів крайової ділянки гранальних тилакоїдів дав змогу отримати додаткові дані про зміни в організації грани, спричинені нагріванням. Отримані дані узгоджуються з гіпотезою, яка пов'язує зміни в організації тилакоїдних мембран із взаємодією між PSII та «мобільною антеною», представленою світлозбиральним комплексом II (LHCII). Також було продемонстровано, що освітлення пом'якшує ефект короткочасного прогріву.

Ключові слова: Pisum sativum L., короткочасний прогрів, ультраструктура хлоропластів.