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Effect of Carotenoid Composition on Stability and Light-Induced Oxidative Damage of the LH2 Complexes Isolated from *Ectothiorhodospira Haloalkaliphila*

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Abstract: Earlier, it has been shown that carotenoid-dependent singlet oxygen photogeneration in LH2 of *Ectothiorhodospira haloalkaliphila* leads to damage to pigments and protein. Present work continues this investigation using LH2 complexes with altered carotenoid composition: carotenoid-less LH2, and LH2 complexes with incorporated neurosporene, spheroidene or rhodopin (LH2-Neu, LH2-Sph or LH2-Rho, respectively). It was shown that inhibition of carotenoid synthesis led to a decrease in LH2 thermal stability and reducing the light-induced oxidative damage to bacteriochlorophyll and protein. Re-incorporation of exogenous carotenoids did not return stability of the complexes but reduce tendency of complexes to aggregate, and (in the case of LH2-Rho) reactivated both photooxidation of bacteriochlorophyll and photoproduction of organic hydroperoxides. It was concluded that carotenoids play an important role in the complex stability and are capable of inducing oxidative damage to LH2 components through singlet oxygen photogeneration.

Keywords: purple sulfur bacteria; light-harvesting complex 2; carotenoids; bacteriochlorophyll; reactive oxygen species; organic hydroperoxides

1. Introduction

In purple photosynthetic bacteria, the primary processes of photosynthesis involve several steps: (1) photon absorption by a network of antenna-based light-harvesting (LH) complexes, (2) excitation energy transfer from these complexes to reaction centers (RCs), and (3) a primary charge separation process in the photosynthetic membrane, resulting in the formation of a proton gradient used for ATP synthesis [1–3]. Antenna complexes are able to harvest light from a wider spectral range than RCs, allowing bacteria to increase their efficiency in using solar energy [4–7].

In most purple bacteria, two types of LH complexes are usually distinguished according to their in vivo absorption maxima in the near-IR region [1,7–12]. The first type includes the LH1 complex (B875), which has a main absorption maximum in the near-IR region at ~865-890 nm and forms the so-called 'core' LH1-RC complex with RC. This complex is found in all purple photosynthetic bacteria [2,9]. The second type of LH complex is the LH2 complex (B800-850). It is characterized by two main absorption maxima at ~800 and ~850 nm. The LH2 complex is located more peripherally relative to the LH1 complex and provides energy transfer to the RC via the LH1 complex [5,9]. The above

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complexes are located in invaginations of the inner membrane and are well-ordered structures built from integral membrane proteins that non-covalently bind several photosynthetic pigments (bacteriochlorophyll and carotenoids)[13–16]. For a number of purple bacteria, structures of LH2 complexes have been determined, comprising seven [17], eight [18–20], and nine pairs of α/β-heterodimers [21–23]. For example, the LH2 complex from *Rhodoblastus acidophilus* (formerly *Rhodopseudomonas acidophila*) strain 10050 is a nonamer consisting of nine pairs of α/β-heterodimers forming inner and outer rings with diameters of 36 Å and 68 Å, respectively. The double ring of α-β heterodimers serves as a scaffold for two rings of bacteriochlorophyll *a* molecules (BChl800 and BChl850) and carotenoid molecules. The entire LH2 complex contains 27 bacteriochlorophyll molecules and 9 carotenoid molecules [22,24,25].

Carotenoids in purple bacteria are accessory photosynthetic pigments that perform several important functions [26–30]. They effectively stabilize the structure of LH complexes, collect light in the region of minimal absorption of bacteriochlorophyll (430-570 nm), and protect against potentially dangerous singlet and derivative triplet excited states of bacteriochlorophyll, preventing the formation of reactive oxygen species (ROS). ROS can be represented by a variety of highly reactive and toxic oxygen species that cause damage to proteins, lipids, pigments, carbohydrates and DNA, ultimately leading to cell death [31–34]. In purple bacterial cells, ROS can be generated by light exposure through the formation of excited triplet states of bacteriochlorophyll [29,35,36]. It has also been reported that blue-green light absorbed by carotenoids may participate in the formation of singlet oxygen in LH2 complexes of purple sulfur bacteria [37–42], as well as by individual carotenoids in model systems [43–45]. We have recently shown that blue-green light can also lead to the formation of hydroperoxides of organic molecules and, as a consequence, to a change in the hydrodynamic radius of LH2 complex proteins of the purple sulfur bacterium *Ectothiorhodospira (E.) haloalkaliphila* [46].

This work is devoted to the study of the effect of carotenoid composition on photo-induced (375 > λ > 600 nm) processes in carotenoidless light-harvesting complexes LH2 (LH2-DPA) of the purple sulfur bacterium *E. haloalkaliphila* before and after incorporation of exogenous carotenoids (rhodopin (LH2-Rho), neurosporin (LH2-Neu) and spheroidene (LH2-Sph)). The influence of qualitative carotenoid composition on the photooxidation of bacteriochlorophyll, photoproduction of organic hydroperoxides and stability protein matrix of LH2 complex are discussed.

2. Materials and Methods

2.1. Isolation, Purification and Characterization of LH2-Containing Preparations

Normal or carotenoid-less cells (DPA-cells) of purple sulfur bacterium *E. haloalkaliphila* were grown on Pfenning's medium under illumination provided by 75 W incandescent lamps (2000 lux) at 26±2 °C [47] in the absence or in the presence of 71 μM diphenylamine (DPA), respectively. Cells were collected in the stationary growth phase on the 4th–6th day of cultivation. The obtained biomass was immediately treated with liquid nitrogen and stored at -18°C.

To isolate normal or carotenoid-less pigment-containing membranes, corresponding cells were resuspended in 10 mM Tris-HCl-buffer (pH 8.0) and disrupted on an ultrasonic disintegrator UZDN-1 (Ultrasonics, Russia) [48]. Undestroyed cells and fragments of the cell wall were removed by centrifugation using a K24 centrifuge (Janetzki, Germany) for 10 min at 5000 rpm. The pigment-containing membranes isolated from the experiment were treated with liquid nitrogen and stored at -18°C.

LH2 complexes were isolated by ion-exchange chromatography on a DEAE-TOYOPEARL 650 S column in a linear gradient from 0.06 to 0.22 M NaCl. 2.5% n-Dodecyl β-maltoside (DM) was used for membrane solubilization. The LH2 complexes were eluted at 0.14 M NaCl [49], then desalted and concentrated using Amicon Ultra 50 concentrating tubes (Merck, Darmstadt, Germany) at 2900 rpm on a CM 6M centrifuge (ELMI, Riga, Latvia). Isolated LH2 complexes were suspended (at 1.16 μM) in a medium containing 50 mM Tris-HCl (pH 7.5) and stored at −76 °C.

Incorporation of exogenous carotenoids was performed using the carotenoid-less membranes. Preparations were considered as carotenoid-free samples if the total carotenoid content was less than

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5% in comparison to control samples. Carotenoids used for incorporation into LH2 of DPA-membranes were isolated from membranes of *Allochromatium vinosum*, *E. haloalkaliphila* and *Cereibacter sphaeroides* as described previously [50]. 0.15 ml of Tris-HCl buffer (50 mM, pH 7.5) and 0.05 ml of DM (20%) were added to 0.3 ml DPA-membranes (at density of 35-40 optical units at 850 nm). The resulting concentration of bacteriochlorophyll was ≈0.8 μM. In order to avoid carotenoid aggregation, carotenoid solution was added to the resulting mixture fractionally (100 μl at a time). To remove the acetone and methanol from the mixture, the samples were dialyzed in Tris-HCl buffer (50 mM, pH 7.5) after each addition of carotenoids. Up to 10 portions of carotenoids were added in one procedure.

The bacteriochlorophyll concentration was determined in Tris-HCl solution as described in [42]. PG200N Spectral PAR Meter (UPRtek, Zhunan, Miaoli, Taiwan) was used to estimate light spectra and flux density. The ζ-potential of LH2 preparations was determined using Zetasizer Ultra (Malvern Panalytical, Malvern, UK) at 25 °C. Temperature dependence of viscosity of LH2 preparation in solution was obtained using SmartPave 102 rheometer (Anton Paar GmbH, Germany). The 3D fluorescence spectrum of LH2 preparations was measured using a Jasco FP-8300 spectrofluorimeter (JASCO Applied Sciences, Victoria, BC, Canada) at 25°C. Absorption spectra were recorded on a Cary 50 spectrophotometer (Agilent Technology, USA). Pigment composition was determined with high pressure liquid chromatography (HPLC) using a Shimadzu chromatography system (Shimadzu, Japan) equipped with an Agilent Zorbax SB-C18 column (4.6 × 250 mm) (Agilent Technologies, USA), as described in [50].

2.2. Registration of Organic Hydroperoxides Photoproduced in LH2 Preparations

To determine the photoproduction of organic hydroperoxides in LH2 preparations, the previously described approach was used [51,52]. Spy-LHP (a low-fluorescent compound, which can be oxidized with hydroperoxides to form a high-fluorescent compound) was used to detect them. According to the manufacturer's description, Spy-LHP is highly specific for lipophilic organic hydroperoxides and does not react with hydroxyl radicals, superoxide anion, nitric oxides, peroxynitrite, and alkyl peroxyl radicals, and other species. Two peroxide types (highly lipophilic (LP-OOH) and relatively hydrophilic (HP-OOH)) had different reaction rate with Spy-HP. The LP-OOH peroxide oxidized Spy-LHP in 5 min; the reaction with HP-OOH occurred very slowly and did not end after 3 hours [52]. The quantity of peroxides (LP-OOH and HP-OOH) was determined using a *m*- Chloroperbenzoic acid (MCPBA, a standard of lipophilic peroxide) and *tert*-Butyl hydroperoxide (TBHP, a standard of hydrophilic peroxide) in the presence of LH2 preparations as previously described [46,51,52]..

2.3. Statistical Analysis

Statistically significance of differences between groups were determined by one-way analysis of variance (ANOVA), followed by post hoc comparison using Tukey's test and Student's t-test for independent means. The normality and homoscedasticity requirements were checked using Shapiro–Wilk test and Goldfeld-Quandt test, respectively. The difference was considered statistically significant if $p \leq 0.05$. All measurements were repeated at least 3 times.

3. Results

HPLC analysis (Figure 1, curve 1) and absorption spectrum (Figure 2A, curve 1) of LH2 preparations isolated from untreated cells purple sulfur bacteria *E. haloalkaliphila* (LH2-control preparations) showed that the LH2 complexes used in present investigation contained bacteriochlorophyll, didehydrorhodopine, rhodopin, spirilloxanthin, anhydrorhodovibrin and lycopene. The preparations contained 7.98 ± 0.01 molecules of carotenoid per one LH2 complex.

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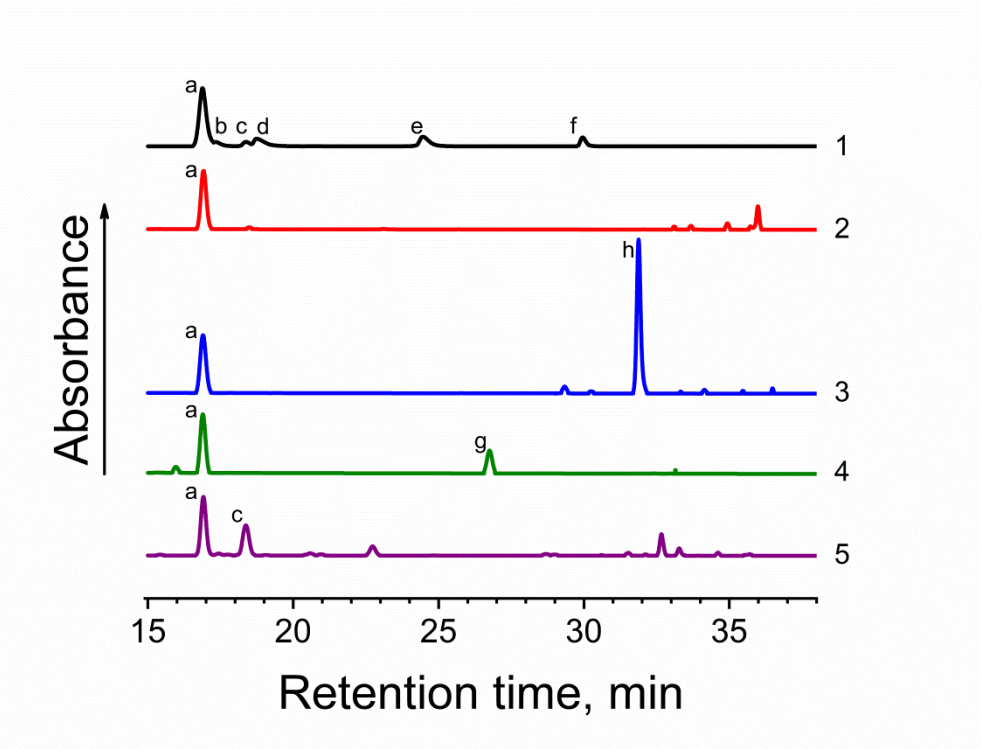

Figure 1. HPLC analysis of pigment composition of the LH2 complexes isolated from cells purple sulfur bacterium *E. haloalkaliphila*; (1) LH2-control preparations, (2) LH2-DPA preparations, (3) LH2-Neu preparations, (4) LH2-Sph preparations or (5) LH2-Rho preparations. Peak identification: (a) bacteriochlorophyll, (b) didehydrorhodopin, (c) rhodopin, (d) spirilloxanthin, (e) anhydrorhodovibrin, (f) lycopene, (g) spheroidene, (h) neurosporene. The chromatograms were normalized according to bacteriochlorophyll peaks. Detection was performed using DAD detector at 190 - 800 nm.

Inhibition of carotenoid synthesis with DPA-treatment of *E. haloalkaliphila* cells led to assembly of LH2-complexes with almost complete loss of colored carotenoids (LH2-DPA preparations; Figure 1, curve 2; Figure 2A, curve 2) which is consistent with the previously published data [53,54]. The carotenoid content in LH2 preparations are presented in the Table 1. LH2-DPA preparations contained trace amounts of neurosporene, ζ—carotene, phytoene and phytofluene. Incubation of LH2-DPA preparations in the presence of purified neurosporene, spheroidene or rhodopin resulted in the incorporation of the carotenoids into the light-harvesting complexes and formation of corresponding LH2-carotenoid complexes (LH2-Neu, LH2-Sph or LH2-Rho, respectively). The Figures 1 and 2 (curves 3-5) confirm the incorporation of the carotenoids into the LH2. It was shown that efficiency of incorporation of neurosporene, spheroidene or rhodopin was 86%, 52% or 72%, respectively. The carotenoid composition of obtained preparations was as follows. LH2-Neu: 0.07, 6.78 and 0.02 molecules of lycopene, neurosporene and ζ—carotene per one LH2 complex, respectively; LH2-Sph: 4.16 molecules of spheroidene per one LH2 complex; LH2-Rho: 0.22, 5.46 and 0.07 molecules of didehydrorhodopine, rhodopin and spirilloxanthin per one LH2 complex, respectively (Table 1).

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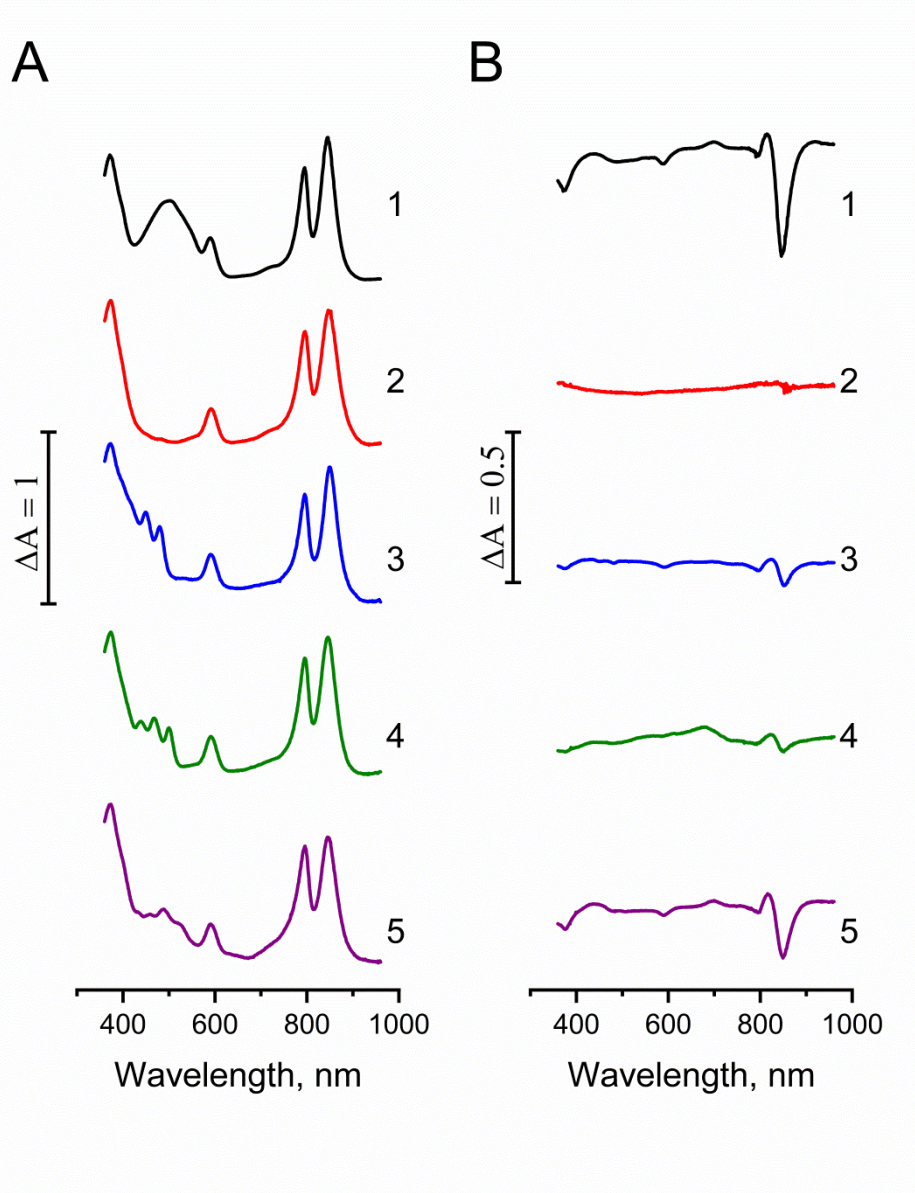

Figure 2. Room-temperature absorption spectra (A) and difference (light minus dark) absorption spectra (B) of LH2 preparations. (1) LH2-control preparations, (2) LH2-DPA preparations, (3) LH2-Neu preparations, (4) LH2-Sph preparations or (5) LH2-Rho preparations. Illumination (650 mmol photons s^{-1} m^{-2}, 375 > λ > 600 nm) or dark incubation of the LH2 preparations was carried out in the medium containing 50 mM Tris-HCl, at LH2 concentration of 16.7 nmol at 25 °C. Measurements of the absorption spectra were done immediately after illumination or dark incubation without dilution of the sample solution.

Illumination (20 min, 375 > λ >600 nm, the carotenoid absorption region) of LH2 preparations led to changes in absorption spectrum. Photobleaching of bacteriochlorophyll band and appearance of 3-acetyl-chlorophyll band in LH2-control preparations were demonstrated (Figure 2B, curve 1). Bacteriochlorophyll photobleaching and appearance of 3-acetyl-chlorophyll band reflect formation of 3-acetyl-chlorophyll as a result of bacteriochlorophyll photooxidation. Absorption spectrum of carotenoidless LH2 preparations does not undergo significant changes after illumination (Figure 2B, curve 2). A slight photobleaching of the bacteriochlorophyll band is observed in LH2-Neu and LH2-Sph preparations (Figure 2B, curves 3 and 4) that may indicate low efficiency of the processes leading to oxidation of bacteriochlorophyll in control samples. Incorporation of rhodopin into the LH2-DPA led to increase of the photosensitivity of the samples. The amplitude of the negative

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bacteriochlorophyll band in the differential spectrum was about 40% in comparison with the amplitude of bacteriochlorophyll band in the LH2-control preparations (Figure 2B, curve 5).

Table 1. Pigment composition of the LH2 complexes isolated from cells purple sulfur bacterium *E. haloalkaliphila*. The measurements were done at least triplicate. The standard deviation did not exceed five percent.

Detected carotenoid	Content of carotenoid in LH2 preparations, molecules per one LH2				
	LH2-control	LH2-DPA	LH2-Neu	LH2-Sph	LH2-Rho
Didehydrorhodopine	0.81	—	—	—	0.22
Rhodopin	0.81	—	—	—	5.46
Spirilloxanthin	2.36	—	—	—	0.07
Anhydrorhodovibrin	2.64	—	—	-	—
Lycopene	1.38	—	0.07	—	—
Spheroidene	—	—	—	4.16	—
Neurosporene	—	<0.01	6.78	—	—
ζ—carotene	—	0.22	0.02	—	—
Phytoene	—	<0.01	—	—	—
Phytofluene	—	0.17	—	—	—

It is known that damage to proteins is usually accompanied by a change in their fluorescent properties. Depending on the target for damage, fluorescence can either increase or decrease [55–60]. The positive changes in protein region of difference "light minus dark" fluorescence spectra of LH2 preparations may reflect damage to protein matrix of the LH2 complexes (Figure 3A). However, light-induced changes in fluorescence intensity of protein component of the LH2-DPA preparations were negligible, as were LH2-Neu and LH2-Sph (Figure 3B-D). LH2-Rho demonstrated small increase in fluorescence intensity of protein component.

A change in the temperature dependence of the sample viscosity before and after illumination is known may reflect a light-induced changes in the stability of the complexes. It was previously shown that illumination of LH2 preparations led to changes in thermal stability of the protein matrix of the samples [46]. The data presented in the Figure 4 repeat this observation (compare curve 1 and curve 2 in the Figure A and B). Thermal stability of the carotenoid-less LH2 complexes is significantly reduced. If in the LH2-control preparations the heat-induced transition started at ≈50 ^{0}C, then in LH2-DPA preparations such a transition was observed already at 31-33 ^{0}C, indicating the instability of the complexes. Surprisingly, incorporation of rhodopin as well as neurosporene, spheroidene did not lead to increase in the temperature of the state transition of the complexes (Figure 4). Preillumination of the LH2-DPA, LH2-Neu, LH2-Sph and LH2-Rho preparations did not result in any additional shift of the transition point to lower temperatures.

Table 2 shows the influence of illumination of LH2 complexes on the ζ-potential, which reflects the tendency of the components of a colloidal solution to aggregate. It was show that illumination of samples did not lead to the changes in ζ-potential (except for the control samples, in which some decrease in the parameter was observed). However, carotenoid composition affects the ζ-potential of LH2 preparations. So, ζ-potential of LH2-control, LH2-Neu and LH2-Sph was significantly higher in comparison with LH2-Rho and LH2-DPA. Present data may indicate that carotenoid composition of LH2 strongly affect the electrical charge of the complex surface and stability of the LH2 complexes in the colloid.

Table 2. Effect of the illumination (650 mmol photons s^{-1} m^{-2}, 375 > λ > 600 nm) of colloidal solutions containing LH2 complexes with different carotenoid composition on the ζ-potential. For details, see caption to Figure 1 and``Materials and Methods'' section. The data are the means of 10 replications with the standard deviation of the mean.

	ζ-potential, mV				
	LH2-control	LH2-DPA	LH2-Neu	LH2-Sph	LH2-Rho
Before illumination	$-28.8^a \pm 1.4$	$-12.7^d \pm 0.8$	$-23.8^b \pm 1.3$	$-23.2^b \pm 1.4$	$-15.8^c \pm 0.9$
After illumination	$-25.7^b \pm 1.6$	$-11.9^d \pm 0.8$	$-24.2^b \pm 0.7$	$-23.3^b \pm 0.8$	$-15.5^c \pm 1.3$

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Letters indicate statistically significant difference between different seed groups ($p \leq 0.05$).

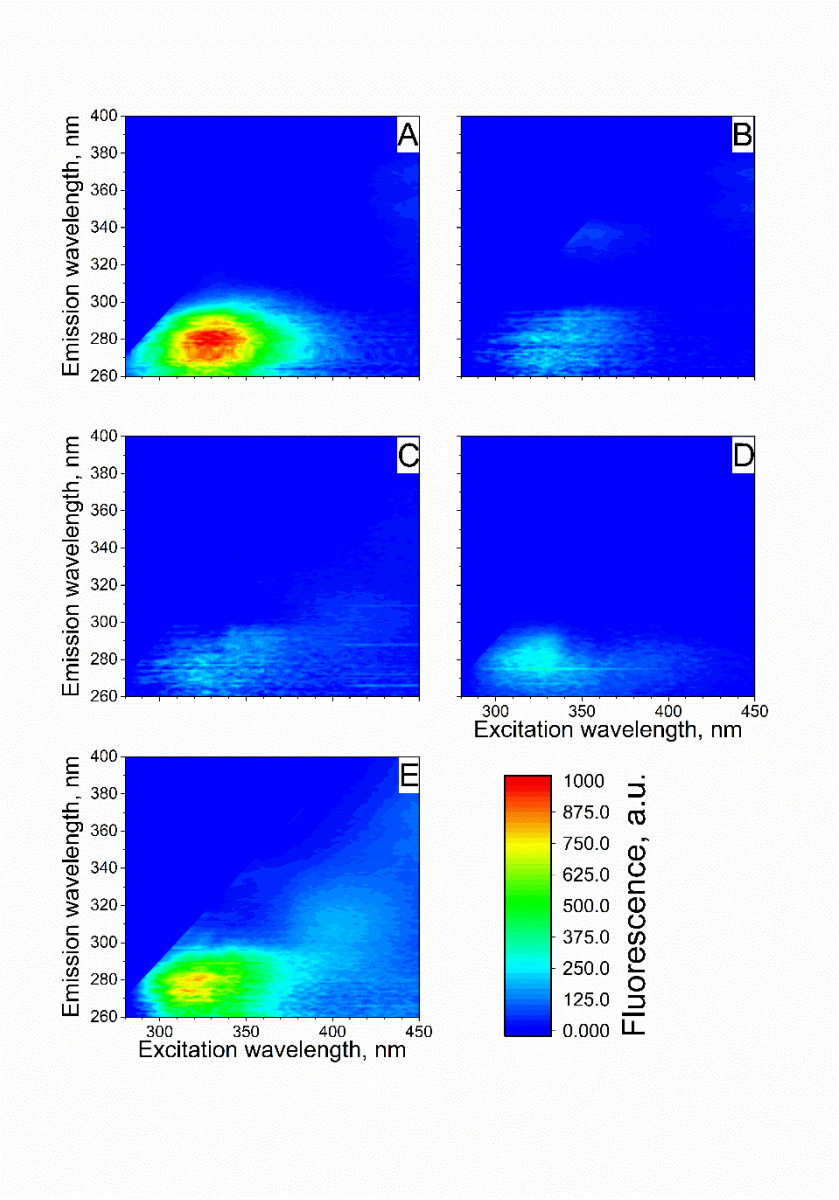

Figure 3. Difference (light minus dark) fluorescence spectra of LH2-control preparations (A), LH2-DPA preparations (B), LH2-Neu preparations (C), LH2-Sph preparations (D) or LH2-Rho preparations (E). Illumination or dark incubation of the LH2 preparations was carried out in the medium containing 50 mM Tris-HCl, at LH2 concentration of 16.7 nmol at 25 °C. Measurements of the fluorescence spectra were done immediately after pre-illumination or dark incubation without dilution of the sample solution. All measurements were done at least three times and the typical spectra are shown.

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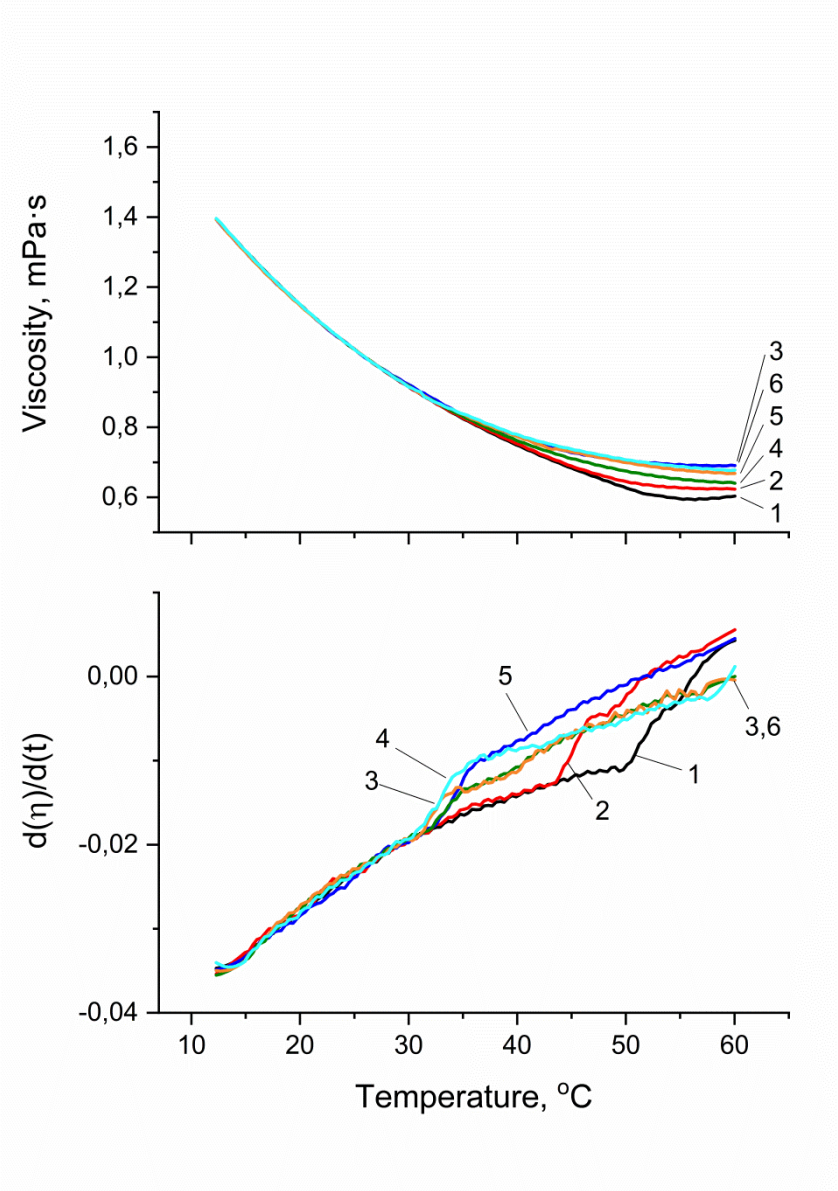

Figure 4. Dependence of viscosity (A) of LH2 solution on temperature and its derivative (B). Measurements were performed before (1, 3, 5) and after (2, 4, 6) illumination (650 mmol photons s^{-1} m^{-2}, 375 > λ > 600 nm) of LH2-control preparations (1, 2), LH2-DPA preparations (3, 4) or LH2-Rho preparations (5, 6) with blue-green light. Illumination or dark incubation of the LH2 preparations was carried out in the medium containing 50 mM Tris-HCl, at LH2 concentration of 16.7 nmol at 25 °C. Measurements were done immediately after pre-illumination or dark incubation with a 3.8-fold dilution of the sample by medium containing 50 mM Tris-HCl. All measurements were done at least three times and the typical kinetics is shown.

Earlier, it has been shown that illumination of LH2 preparations isolated from *E. haloalkaliphila* led to singlet oxygen-dependent formation of organic hydroperoxides (R-OOH) on protein matrix [46]. The results of the present work are consistent with these data. Two types of organic hydroperoxides formed under illumination (20 min, 375 > λ >600 nm) of LH2-control preparations were revealed: highly lipophilic (LP-OOH, 11.4 molecules per one LH2) and relatively hydrophobic (HP-OOH, 70 per one LH2) (Figure 5, curve 1). However, photoformation of R-OOH in LH2-DPA preparations was not detected (curve 2).

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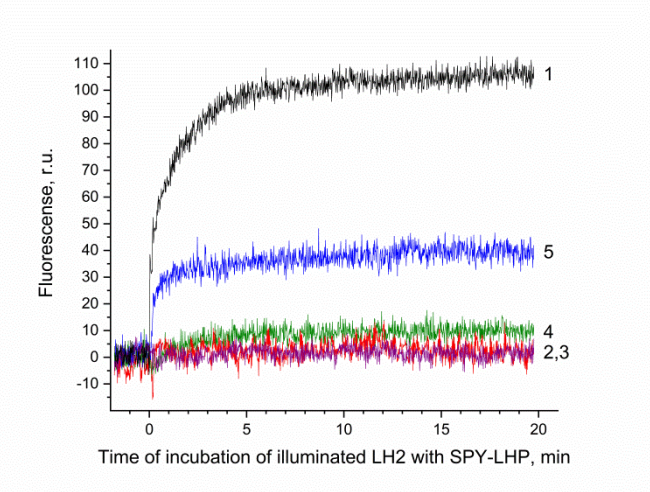

Figure 5. Kinetics of the Spy-LHP fluorescence related to its oxidation by hydroperoxides formed by a 20-min illumination (650 mmol photons s^{-1} m^{-2}, 375 > λ > 600 nm) of LH2-control preparations (1), LH2-DPA preparations (2), LH2-Neu preparations (3), LH2-Sph preparations (4) or LH2-Rho preparations (5). Illumination or dark incubation of the LH2 preparations was carried out in the medium containing 50 mM Tris-HCl, at LH2 concentration of 16.7 nmol at 25 °C. "0" on the timescale indicates the moment of addition of the sample containing LH2 complexes to SPY-LHP solution. Measurements were done immediately after pre-illumination or dark incubation. For other details, see ``Materials and Methods'' section. All measurements were done at least three times and the typical kinetics is shown.

The effect of incorporation of exogenous carotenoids in LH2 on R-OOH photoproduction was dependent on carotenoid species. While, incorporation of neurosporene or spheroidene into LH2 did not lead to R-OOH photoformation (curves 3 and 4), then incorporation of rhodopin resulted in photoformation of both LP-OOH and HP-OOH (curve 5). The quantity of photoproduced LP-OOH and HP-OOH in LH2-Rho reached almost a 30% compared to LH2-control preparations.

Addition of Rose Bengal (RB, singlet oxygen generating photosensitizer) before illumination of the LH2-control samples did not lead to the increase in the photoproduction of R-OOH (Figure 6). RB reduced the photoproduction of LP without affecting HP, which is consistent with previously obtained data. Moreover, RB did not increase R-OOH photoformation in other (LH2-DPA, LH2-Neu, LH2-Sph or LH2-Rho) preparations. On the one hand, this may indicate that hydroperoxide precursor molecules are not formed in LH2 due to the action of DPA. On the other hand, the incorporation of carotenoids into LH2 does not promote their appearance.

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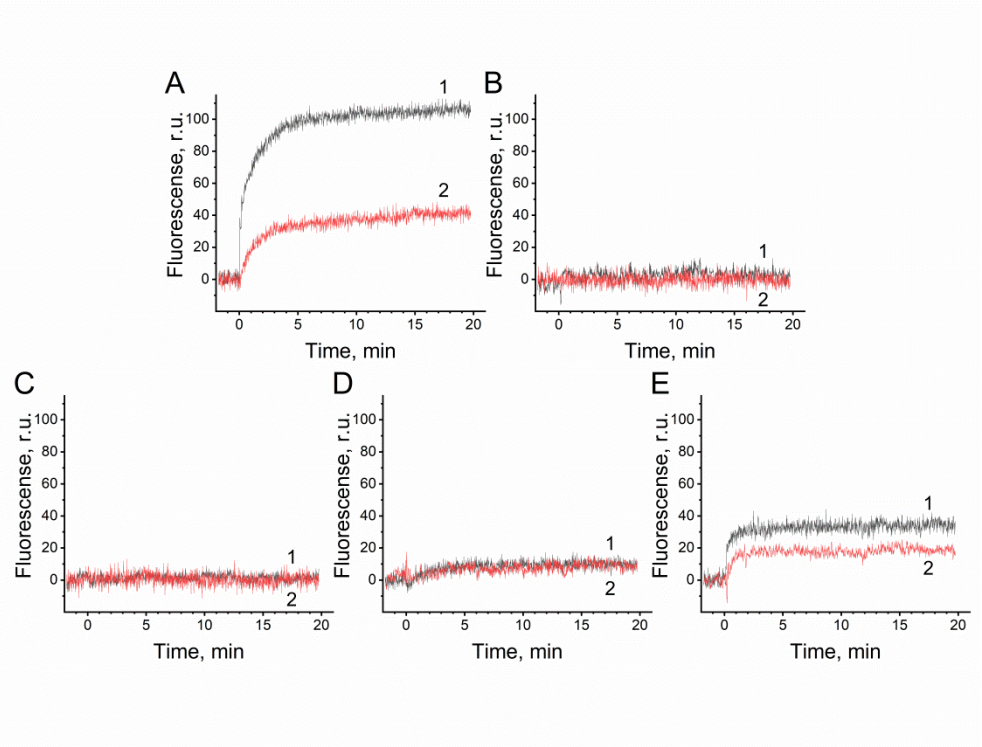

Figure 6. Effect of Rose Bengal on the light-induced (20-min, 650 mmol photons s^{-1} m^{-2}, 375 > λ > 600 nm) hydroperoxides formation in LH2-control preparations (A), LH2-DPA preparations (B), LH2-Neu preparations (C), LH2-Sph preparations (D) or LH2-Rho preparations (E). Illumination or dark incubation of the LH2 preparations was carried out in the absence (1) or in the presence (2) of Rose Bengal. For other details, see caption to Figure 3 and``Materials and Methods'' section. All measurements were done at least three times and the typical kinetics is shown.

Thus, our data suggests that carotenoids both stabilize the LH2 complexes (Figure 4 and Table 2) and promote the light-induced oxidative damage to the LH2 (Figure 2, Figure 5 and Figure 6).

4. Discussion

Previously, the generation of singlet oxygen was shown upon illumination of LH2 preparations with light, which is effectively absorbed by carotenoids but practically does not excite bacteriochlorophyll molecules, which was accompanied by oxidation of bacteriochlorophyll to 3-acetylchlorophyll [37–39,61] and photoformation of R-OOH with damage to protein molecules included in the complexes [46]. Similar conclusions can be drawn from the data presented in this work. Indeed, singlet oxygen is capable of oxidizing bacteriochlorophyll and interacting with protein molecules, damaging them [31,32]. Degradation of plant LHC2 proteins under the influence of singlet oxygen is known [62,63]. Amino acids of the protein (tyrosine, tryptophan, and histidine) are capable of forming hydroperoxides when interacting with singlet oxygen [31,32,64–72]. The photogeneration of singlet oxygen associated with photosynthetic pigments has been known for a long time. For example, in addition to isolated antenna complexes of bacteria, singlet oxygen was detected during illumination of plant antenna complexes [73–76] and in model pigment solutions [77–80]. In this case, photogeneration of singlet oxygen is usually associated with pigments of chlorophyll nature. Nevertheless, the possibility of energy transfer from a carotenoid in the triplet state to molecular oxygen with the formation of singlet oxygen was demonstrated previously [81]. The authors indicate that carotenoids with the number of conjugated double bonds (N) ≥11 are energetically capable of this. The mechanism of formation of the triplet state of carotenoids as a result of singlet-triplet excitation fission ($^1Car^* + Car \rightarrow {}^3Car + {}^3Car$) was proposed earlier [42]. This process is well known in photochemical studies and has been described in the case of light-harvesting complexes of *Alc.*

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vinosum [41,42,82–84]. Moreover, this process is very fast and can have a fairly high quantum yield (up to 0.32) [85]. As shown in Figure 1, all carotenoids of the LH2-control preparation have a sufficient number of conjugated double bonds. In addition, carotenoid-dependent damage to the proteins of the light-harvesting complex and photoformation of R-OOH due to the generation of singlet oxygen were confirmed by inhibitory analysis and the absence of effects upon illumination in the absorption band of bacteriochlorophyll [46]. In this work, we show that illumination of LH2-DPA preparations lacking carotenoids does not result in damage to the proteins of the light-harvesting complex and photoformation of R-OOH, despite the fact that bacteriochlorophyll is retained in their structure. It should be noted that LH2-DPA complexes initially have reduced thermal stability (Figure 5) and a reduced surface charge (Table 2). It has been previously demonstrated that carotenoids play an important role in LH2 complexes, increasing their structural stability [86]. We assumed that the incorporation of carotenoids with different N into LH2 may cause different effects on both the stability of the complexes and the photoproduction of 3-acetyl-chlorophyll and R-OOH. We selected neurosporene (N=9), spheroidene (N=10) and rhodopin (N=11) for incorporation. The choice of carotenoids for incorporation was based on the fact that these carotenoids meet the criteria necessary for incorporation. First, these carotenoids can be obtained in sufficient quantities. Second, these carotenoids can be effectively incorporated into light-harvesting complexes. Most other carotenoids either do not practically incorporate, or their incorporation is very slow and is accompanied by serious destruction of LH2-DPA due to the effect of the solvent. Among the carotenoids selected for incorporation, there are both carotenoids with a relatively short chain of conjugated double bonds and a carotenoid with a chain length of conjugated double bonds sufficient for the generation of singlet oxygen. Indeed, the incorporation of carotenoids with a short chain of conjugated double bonds (neurosporene (N=9) and spheroidene (N=10)) did not result in either activation of bacteriochlorophyll photobleaching (Figure 2) or R-OOH photoformation (Figure 3) or an increase in the stability of the complexes (Figure 5). However, these carotenoids significantly shifted the ζ-potential to a more negative region, bringing its value closer to that measured in the control samples. The incorporation of rhodopin (N=11) also had no effect on the thermal stability of the complexes, but activated the photoformation of R-OOH and photobleaching of bacteriochlorophyll (≈30% and ≈40% compared to the control, respectively). At the same time, the restoration of ζ-potential was not as strong as in the case of neurosporene and spheroidene. It is known that oxidative damage to protein particles (including as a result of the action of reactive oxygen species) is accompanied by a change in ζ-potential [87–91]. The difference in ζ-potential of proteins can reflect the surface charge on proteins. This parameter allows one to estimate the tendency of colloid particles to aggregate or the ability of the colloid to be stable. Table 2 shows that DPA treatment results in a shift of ζ-potential of LH2 colloid from approximately -30 mV to -12 mV. Re-incorporation of carotenoids mitigates this effect. These data indicate a significant effect of carotenoids on the surface charge of the proteins of the complex, which may be of great importance for the functioning of the antenna complex in vivo. Despite this, the incorporation of carotenoids did not lead to the restoration of the thermal stability of the complexes (Figure 5). However, according to other data, carotenoids incorporated into LH2-DPA preparations had a significant effect on the stability of the complexes [86].

There are several possible explanations for why illumination of the LH2-DPA does not result in photoformation of R-OOH, etc. The first possibility is that carotenoids are required for photoformation of R-OOH. The second possibility is that in the presence of DPA, altered complexes without the hydroperoxide precursor molecules are assembled. Addition of an artificial singlet oxygen photosensitizer did not result in an increase in R-OOH production in LH2-DPA preparations. However, given the fact that RB in the control samples surprisingly inhibited R-OOH photoformation, we cannot claim that LH2-DPA preparations do not have sites that singlet oxygen can oxidize. On the contrary, the incorporation of rhodopin showed that incorporation of a carotenoid with the required number of conjugated double bonds is sufficient for reactivation of the studied processes. The question of functional activity of the carotenoids incorporated into LH2-DPA preparations are remains debatable. On the one hand, incomplete restoration of the efficiency of bacteriochlorophyll photobleaching and R-OOH photoformation after rhodopin incorporation may

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indicate that other carotenoids also "work" in the control preparations. On the other hand, the efficiency and physiologicality of exogenous carotenoid incorporation may be incomplete, which leads to a loss of functionality. Third, DPA treatment could lead to the assembly of LH2 complexes with disturbances that are not corrected by carotenoid incorporation.

Thus, our data indicate that carotenoids, on the one hand, stabilized the LH2 complexes and, on the other hand, were involved in the light-induced oxidative damage to the LH2 probably due to redox activity of the singlet oxygen, and rhodopin may be one of the carotenoids that is able to participate in the generation of singlet oxygen in LH2-control preparations.

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