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Protective effect of histidine against pigment photobleaching in Photosystem I particles♣

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Photosystem I (PSI) is a supercomplex of a reaction centre and light-harvesting complexes. Photosystem I particles isolated from spinach leaves were studied by means of absorbance, 77 K fluorescence, and resonance Raman spectroscopy. During prolonged exposure to high-light intensities, various pigments in Photosystem I exhibited different susceptibilities to photodestruction. This work presents preliminary investigations on the effect of histidine on the photobleaching of pigments in isolated particles of photosystem I. Resonance Raman spectroscopy allowed us to obtain direct information about the effect of histidine on the photobleaching of the lutein molecules, upon excitation with a 514.5 nm laser line. Our results showed that histidine reduces the photobleaching of antenna pigments and especially luteins and the most long-wavelength absorbing chlorophylls located in the PSI antenna complex.

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1. Introduction

The plant photosystem I (PSI) supercomplex is the most efficient nano-photochemical machine in nature. It is remarkable that PSI exhibits a quantum yield of nearly 1, and almost every captured photon is eventually trapped and results in electron translocation [1]. The PSI supramolecular complex is located preferentially in the non-appressed regions of thylakoid membranes and consists of chlorophyll (Chl) *a*, a binding core complex (PSI core) and a Chl *a/b* binding peripheral light-harvesting complex (LHCI), located on one side of the core [1-3]. During prolonged exposure of leaves or choroplasts to high light intensity, two processes were observed - photoinhibition and photobleaching of the pigments. The photobleaching of photosynthetic pigments under high-light illumination involves a loss of bulk photosynthetic pigments [4,5], and it is supposed that these damages are caused by reactive oxygen species and especially by singlet oxygen, a particularly destructive radical. The production of singlet oxygen occurs via interaction between the ground state electrons of triplet oxygen with the triplet state of chlorophyll [6] or by participation of Fe–S centers in thylakoid membranes [7]. A potent artificial scavenger of singlet oxygen is histidine. The effect of histidine in high light-treated isolated thylakoid membranes and submembrane particles of PSII has been studied in detail, and it has been shown that the damaging effect of singlet oxygen in the presence of histidine is reduced and sometimes abolished for the reaction centre proteins [8,9]. Although PSI was believed to be tolerant to strong illumination, photobleaching of photosynthetic pigments was also observed in PSI particles, being different for various pigments [5,9,10]. Recently, using resonance Raman (RR) spectroscopy, we revealed that in addition to bleaching of Chl *a*, long-wavelength lutein molecules were nearly fully photobleached after 120 min illumination [10]. Our results have shown that the photobleached antenna pigments and especially luteins, and the most long-wavelength absorbing chlorophylls, are involved in the photoprotection of the PSI core complex.

In the present report, the effect of histidine on the photo-bleaching of pigments in isolated particles of PSI is investigated.

2. Experimental

2.1. Isolation of Photosystem I particles

The PSI particles were isolated from spinach chloroplasts by mild digitonin treatment [11]. The submembrane particles obtained by this method originated

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mainly from stroma exposed regions of thylakoids and consisted of a PSI core complex and LHCI, the so called PSI-200 complexes. The final pellet was re-suspended in a medium containing 20 mM Tricine-KOH (pH 7.8), 10 mM NaCl, 10 mM NaCl, and 5 mM $MgCl_2$. The pigment concentration was determined using the method of Lichtenthaler [12].

2.2. High-light treatment

The illumination of isolated PSI particles was carried out in a temperature-controlled vessel under continuous stirring at room temperature (22°C). The Chl concentration during illumination was 500 μg/ml. Samples were placed in a flat vessel to ensure equal illumination of all sample layers. High intensity illumination was provided by a 1000 W halogen projector lamp, giving an intensity of 1800 μE m^{-2} s^{-1} on the vessel surface. The light was passed through a heat filter. Samples for analysis were taken at different time intervals (0, 60, and 120 min) during illumination, and where it is indicated 25 mM histidine was added.

2.3. 77 K Chl fluorescence measurements

Samples taken from the temperature-controlled vessel at different time intervals of illumination were transferred into a translucent Dewar and immediately frozen in liquid nitrogen. The 77 K chlorophyll fluorescence emission spectra excited at 436 nm were measured using a JobinYvon JY3 spectrofluorimeter. Actinic light with a low enough intensity was provided by a Xenon lamp "Suprasil"- 150W, the width of the slits being 4 nm. The experimental spectra were corrected for the spectral sensitivity of the detection system. The Chl content of the samples was adjusted to 10 μg/ml.

2.4. Resonance Raman spectra measurements

For the RR spectra measurements, the Chl concentration was 500 μg/ml. RR spectra at 77K were obtained in a translucent Dewar using a 0.85 m double spectrophotometer Spex (model 1403; Spex Industries, Inc., Edison, NJ, USA), equipped with a cooled photomultiplier tube (model R943, Hamamatsu Photonics, K. K., Shizuoka, Japan). The excitation was provided by the line at λ = 514.5 nm of an Ar+ laser (Innova 307, Coherent). Five to ten successive Raman spectra were averaged for each experiment. To compare the absolute RR amplitudes, exactly the same amount of Chl in each sample was used and the signal arising from the buffer at the 814 cm^{-1} was used for normalization of the measured spectra.

3. Results and discussion

3.1. Changes in pigment content

The pigment content of the treated samples was determined during illumination in 80% aqueous acetone, by the method of Lichtenthaler [12]. After 60 min of light treatment of control (without histidine), the PSI particles Chl *a* content decreased by about 30%. After 120 min it was by reduced about two times. For samples containing histidine, during illumination this decrease was by about 8-9% for the entire period of illumination. This protective effect of histidine is also observed for the content of total carotenoids. After 120 min of strong light treatment, this decreased to 38% of the initial level in the absence of histidine, while in presence of histidine, the carotenoids content decreased by about 15-17%. It could be concluded that histidine, scavenging singlet oxygen, probably prevents carotenoids from photobleaching, and thus preserves their own protective function. The Chl *b* content was less affected.

3.2. Changes in the 77K fluorescence spectra

The 77 K steady-state emission spectra of PSI particles illuminated in the absence and in the presence of histidine are compared in Fig. 1a. The steady-state emission properties of PSI-200 complexes at low temperatures (below 80K) were determined by the so called long-wavelength absorbing pigments (LWP) – pigments that absorb at wavelengths longer than the corresponding reaction centres [3]. Two LWP pools, emitting mainly at 720 and 735 nm, named F720 and F735, according to their fluorescence maxima, are generally found. For untreated spinach PSI particles, the maximum of the emission spectrum is located near to 734 nm [3].

In control samples, 60 min high light treatment resulted in a considerable decrease of the emission intensity, and the fluorescence was almost abolished after 120 min of illumination (Fig. 1(a)). The presence of histidine reduced the inhibiting effect of strong light, the effect being more expressed in the last hour of illumination, and after 2 hours of illumination, almost 30 % of the fluorescent intensity remained (Fig. 1b).

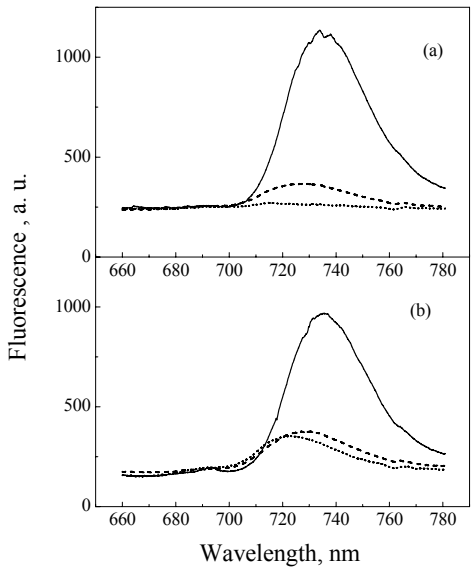

Fig. 1. 77 K fluorescence emission spectra of PSI particles under high light treatment: illuminated for 0 min (solid line), 60 min (dashed line) and 120 min (short dashed line) in: (a) control PSI particles; (b) histidine-containing PSI particles.

The high-light treatment resulted in a remarkable blue-shift of the main fluorescence maximum, indicating that the fluorescence from the LWP pool emitting at 735 nm, located in LHCI-730, was considerably quenched.

3.3. Changes in the resonance Raman spectra

In order to obtain direct information about the effect of histidine on the photobleaching of the lutein molecules, we employed RR spectroscopy. Based on the selectivity of RR and the established strong dependence of the position of the most intense band ν_1 in carotenoid RR spectra [13], we characterized the absorption transitions for lutein, violaxanthin, and 9-*cis* neoxanthin in spinach PSI particles in [14]. The laser line at 514.5 nm, which coincides with the absorption maxima of long-wavelength lutein [10,13,14], excites its RR spectrum selectively. It contains four main bands (ν_1 to ν_4) characteristic of carotenoids [10,14] and the band at 814 cm^{-1} arising from the buffer used. The main carotenoid bands have been assigned [13] as follows: ν_1 – to C=C bonds in phase stretching vibrations; ν_2 – to C_{14}-C_{15} stretches coupled to C_{15}-H in plane bending; ν_3 – to methyl CH_3 in plane rocking vibrations; ν_4 – to C-H out of plane bending modes coupled with C_7=C_8 torsion. The absolute RR band amplitudes are normalized as explained in section 2.4. The RR spectra in the ν_1 region, as a function of the time of illumination, are compared in Figs. 2a and b for lutein molecules in control and histidine-containing PSI particles.

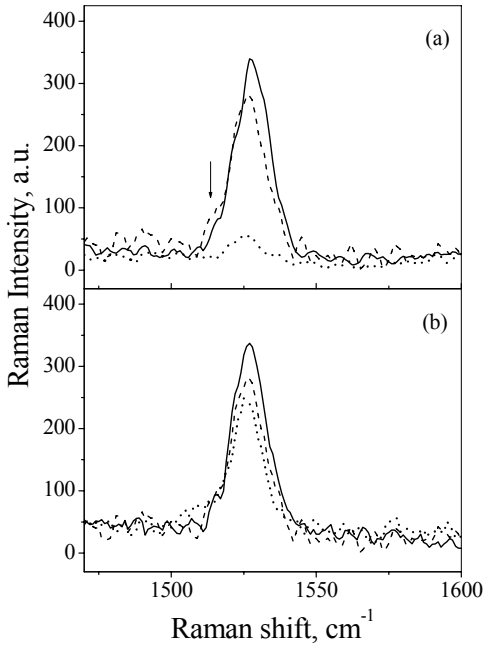

Fig. 2. Effect of light treatment with strong white light (1800 μE $m^{-2}s^{-1}$): illuminated for 0 min (solid line), 60 min (dashed line) and 120 min (short dashed line) on the band ν_1 in the lutein RR spectra: (a) in control PSI particles; (b) in histidine - containing PSI particles.

The most intense band ν_1 in the lutein RR spectrum is located at 1526.5±1 cm^{-1}. The discernible shoulder at smaller wavenumbers, and its weakly increasing intensity, suggest that it originates from zeaxanthin, which is known [13] to exhibit a band at 1524 cm^{-1} and accumulates under high-light illumination. The ν_1 intensity dependencies for lutein molecules in control and histidine-containing PSI particles on the time of illumination are compared in Fig. 3. The comparison reveals that during high-light treatment the intensity of the long-wavelength absorbing lutein ν_1 band decreases drastically, by nearly 10 times in control samples, while in histidine-containing samples the intensity decreases by only 1.4 times.

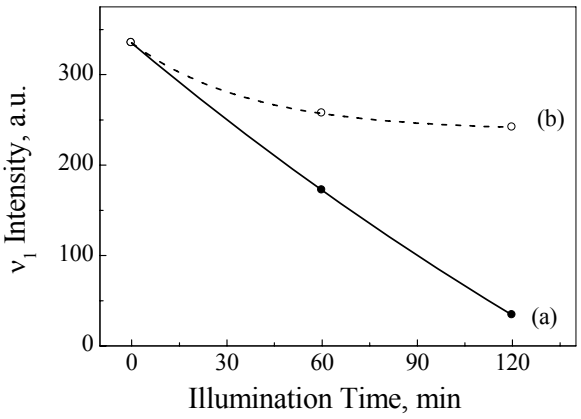

Fig. 3. Dependence of ν_1 band intensity on the time of illumination of lutein molecules: (a) in control PSI particles; (b) in histidine - containing PSI particles.

4. Conclusions

In this study, we examined the effect of singlet oxygen scavenger histidine on the pigment content, 77K fluorescence and RR spectra of Photosystem I particles under high light treatment. The results revealed nearly full photobleaching of lutein and the most long-wavelength absorbing chlorophylls in control samples, while in histidine-containing samples the bleaching was markedly retarded. Histidine had a protective effect against the photodestruction of Chl *a* and carotenids, thus indicating that the photobleaching process in photosystem I particles was mediated by singlet oxygen. Our data provide insights into the photo-protective effect and its mechanism.

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