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High-light induced singlet oxygen formation in cytochrome b_6f complex from *Bryopsis corticulans* as detected by EPR spectroscopy

Min Sang [a,e,1], Fei Ma [b,1], Jie Xie [c], Xiao-Bo Chen [d], Ke-Bin Wang [a], Xiao-Chun Qin [a], Wen-Da Wang [a], Jing-Quan Zhao [c], Liang-Bi Li [a,*], Jian-Ping Zhang [b,*], Ting-Yun Kuang [a]

[a] Key Laboratory of Photosynthesis and Environmental Molecular Physiology, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, PR China
[b] Department of Chemistry, Renmin University of China, Beijing 100872, PR China
[c] Key Laboratory of Photochemistry, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, PR China
[d] School of Bioscience and Engineering, Hebei University of Science and Technology, Shijiazhuang, Hebei Province 050018, PR China
[e] Graduate School of Chinese Academy of Sciences, Beijing 100049, PR China

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ABSTRACT

Electron paramagnetic resonance (EPR) spectroscopy was used to detect the light-induced formation of singlet oxygen ($^1O_2^*$) in the intact and the Rieske-depleted cytochrome b_6f complexes (Cyt b_6f) from *Bryopsis corticulans*, as well as in the isolated Rieske Fe–S protein. It is shown that, under white-light illumination and aerobic conditions, chlorophyll *a* (Chl *a*) bound in the intact Cyt b_6f can be bleached by light-induced $^1O_2^*$, and that the $^1O_2^*$ production can be promoted by D_2O or scavenged by extraneous antioxidants such as L-histidine, ascorbate, β-carotene and glutathione. Under similar experimental conditions, $^1O_2^*$ was also detected in the Rieske-depleted Cyt b_6f complex, but not in the isolated Rieske Fe–S protein. The results prove that Chl *a* cofactor, rather than Rieske Fe–S protein, is the specific site of $^1O_2^*$ formation, a conclusion which draws further support from the generation of $^1O_2^*$ with selective excitation of Chl *a* using monocolor red light.

1. Introduction

The cytochrome b_6f complex (Cyt b_6f) together with photosystem II (PSII) and photosystem I (PSI) are key integral membrane protein complexes constituting the photosynthetic electron transport chain in thylakoid membrane. A monomer of the dimeric Cyt b_6f complex (105 kD) contains four major subunits (Cyt *f*, Cyt b_6, Rieske Fe–S protein, subunit-IV) and another four subunits of low molecular weight (Pet G, L, M, N). The first three major subunits bind the redox-active cofactors, a c-type heme to the Cyt *f*, two b-type hemes and a newly discovered 'heme x' to the Cyt b_6, and a [2Fe–2 S] cluster to the Rieske Fe–S protein, while subunit-IV posses the binding site of plastoquinone (PQ) [1–3].

The Cyt b_6f oxidoreductase mediates the electron transfer from PSII to PSI and the Q-cycle around PSI, through which a trans-membrane proton gradient is built up for ATP synthesis [4]. Furthermore, it involves in balancing the excitation energy distribution between the two photosystems, and regulates the gene expression via redox control for maintaining efficient energy convention [4,5]. The Cyt b_6 subunit plays crucial roles in the physiological functions of Cyt b_6f, e. g., the electron transfer activity of the Cyt b_6f complex from spinach can be inhibited by dicyclohexylcarbodiimide (DCCD) bound to this subunit [6]. Each monomer of the Cyt b_6f complex contains a single chlorophyll *a* (Chl *a*) and merely one carotenoid molecule [7,8]. β-Carotene (β-Car) is commonly seen in the Cyt b_6f complexes from various species, but α-Car was identified in the Cyt b_6f complex from *Bryopsis (B.) corticulans*, a marine green alga [9]. Recently, the relative orientation of the two different kinds of pigment cofactors has been resolved by crystallography to 3.1 Å and 3.0 Å, respectively, for the Cyt b_6f complexes from *Mastigocladus laminosus* [7] and *Chlamydomonas reinhardtii* [8], and considerable research efforts have been made to understand the physiological roles of these pigment cofactors [4].

Zhang et al. reported that, under high-light illumination and aerobic conditions, the rate of Chl *a* photo-bleaching in the Cyt b_6f complex correlates inversely to the β-Car content [10]. These authors proposed that Chl *a* photo-bleaching is a consequence of the oxidative reaction with singlet oxygen ($^1O_2^*$), and that the triplet excited state Chl *a* (^{3}Chl *a**) can be deactivated by transferring the excitation energy to β-Car before sensitizing the formation of harmful $^1O_2^*$. However, Suh et al. suggested, on the basis of comparative studies of the intact and the Rieske-depleted Cyt b_6f complexes from spinach, that the Rieske subunit is responsible for $^1O_2^*$ production [11]. In

Abbreviations: Car, carotenoid; Chl *a*, chlorophyll *a*; ^{3}Chl *a**, triplet excited state chlorophyll *a*; Cyt b_6f, cytochrome b_6f complex; β-DM, *n*-dodecyl-β-D-maltoside; EPR, electron paramagnetic resonance; $^1O_2^*$, singlet oxygen; β-OG, *n*-octyl-β-D-glucopyranoside; ROS, reactive oxygen species; SDS, sodium dodecyl sulfate; TEMP, 2,2,6,6-tetramethylpiperidine.

* Corresponding authors.
E-mail addresses: lbli@ibcas.ac.cn (L.-B. Li), jpzhang@chem.ruc.edu.cn (J.-P. Zhang).

[1] Min Sang and Fei Ma contributed equally to this work.

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addition, our previous flash photolysis investigation for the Cyt b_6f complex from *B. corticulans* indicated that the process of direct Chl *a*-to-α-Car triplet excitation energy transfer (EET) may be not efficient enough to deactivate ^{3}Chl *a**, i.e. the direct EET mechanism seems not significant in protecting Chl *a* against photo-bleaching [9]. Thus the specific site and the detailed mechanism of $^1O_2^*$ formation in the Cyt b_6f complex still remain unclear, although the relevant knowledge are indispensible in understanding its photo-protective mechanism.

In the present work, we have attempted to reveal the specific site and the mechanism of photo-induced formation of $^1O_2^*$ in the Cyt b_6f complex from *B. corticulans*, for which three different kinds of Cyt b_6f preparations, i.e., the intact and the Rieske-depleted Cyt b_6f complexes and the isolated Rieske Fe–S protein, have been prepared and investigated comparatively by the use of EPR spectroscopy and photo-bleaching assays. Our results unambiguously show that, for the Cyt b_6f complex from *B. corticulans*, the Chl *a* cofactor rather than the Rieske Fe–S protein is the specific site of $^1O_2^*$ formation, and that the underlying mechanism is triplet sensitization, i.e. Chl *a*-to-O_2 triplet EET.

2. Materials and methods

2.1. Cyt b_6f preparations

All of the chemicals used for the Cyt b_6f preparations and for the subsequent experiments were purchased from Sigma and used as received: 2,2,6,6-Tetramethylpiperidine (TEMP), *n*-octyl-β-D-gluco-pyranoside (β-OG), phenylmethanesulfonyl fluoride (PMSF), sodium cholate hydrate (SC), *n*-dodecyl-β-D-maltoside (β-DM), L-histidine, ascorbate, glutathione, D_2O, hydroxyapatite, sodium lauryl sulfate and acrylamide.

B. corticulans was collected from the intertidal zone at Qingdao, China. Intact Cyt b_6f complex was isolated and purified according to the procedures described previously [12]. The final precipitate formed through ammonium sulfate fractionations, i.e. the purified Cyt b_6f complex, was suspended in 50 mM Tricine–NaOH (pH, 8.0) containing 0.2 mM β-DM, and stored at −80 °C before use.

The Rieske-depleted Cyt b_6f complex and the isolated Rieske Fe–S protein were prepared following the procedures in *ref.* [13]. The intact Cyt b_6f containing 15–20 μM Cyt *f* was loaded onto a hydroxyapatite column (diameter 1.5 cm, length 3 cm) equilibrated with 20 mM potassium phosphate (pH, 6.8) plus 0.5% Triton X-100. Washing the column with the equilibrium buffer resulted in the elution of Rieske Fe–S protein. The Cyt b_6f complex, which is deficient in Rieske Fe–S protein, was eluted with 200 mM potassium phosphate (pH, 6.8) plus 0.05% Triton X-100.

2.2. EPR detection of light-induced $^1O_2^*$

$^1O_2^*$ is a strong electrophile but not a radical, however, it can oxidize TEMP and form the stable N-oxyl radical TEMPO that allows the EPR detection of $^1O_2^*$ [14]. The samples (80 μL) containing Cyt b_6f (1 μM Cyt *f*) and 37.5 mM TEMP in the buffer of 50 mM Tricine–NaOH (pH, 8.0) with 0.2 mM β-DM were saturated with oxygen. The samples were sealed in glass capillaries, and illuminated for 9 min with white light (1.5×10^3 μM m^{-2} s^{-1}) filtered out with a $CuSO_4$ solution of 12 cm optical path from a 150 W halogen lamp, or with monocolor red light (665 ± 1 nm) of comparable photon flux from a continuous wave semiconductor laser. EPR spectra were recorded on a BRUKER E500 spectrometer with the followings parameter settings: Microwave frequency, 9.78 GHz; modulation frequency, 100 kHz; microwave power, 10 mW; modulation amplitude, 1 G; time constant, 40 ms; field sweep, 100 G and center field, 3480 mT. The EPR signal amplitude at a magnetic field of 3479 mT was taken to measure the $^1O_2^*$ production.

The anaerobic condition, when needed, was achieved by adding 5 mM glucose, 0.1 mg mL^{-1} glucose oxidase and 0.05 mg mL^{-1} catalase to the Cyt b_6f preparations [15].

2.3. Photo-bleaching of Chl a in Cyt b_6f complex

A volume of 1 mL Cyt b_6f preparation (1 μM Cyt *f*) in a quartz cuvette of 1-cm optical path length was stirred while irradiated with white light at a photon flux of 1.5×10^3 μM m^{-2} s^{-1}, and the UV–vis absorption spectra were recorded after various irradiation time on a spectrophotometer (UV-2550, Shimadzu) whose spectral resolution was set to 1.2 nm. The difference between the Q_y-band absorbance at 668 nm and 700 nm, $\Delta A = A_{668nm} - A_{700nm}$, was used to evaluate the residual content of Chl *a*. All of the spectroscopic experiments were carried out at room temperature (298°K).

3. Results

3.1. Characterization of the Cyt b_6f preparations

To investigate the site of light-induced $^1O_2^*$ production, we have prepared the isolated Rieske Fe–S protein and the Rieske-depleted Cyt b_6f complex in addition to the intact Cyt b_6f complex. Fig. 1 shows the SDS-PAGEs of the different kinds of Cyt b_6f preparations (1–3) and their corresponding UV–visible absorption spectra. The SDS-PAGE of intact Cyt b_6f clearly displays the protein bands of four major subunits (Fig. 1a, 1). For the Rieske-depleted Cyt b_6f preparation, successful depletion of Rieske Fe–S protein is confirmed by the lack of Rieske protein band (Fig. 1a, 2). For the isolated Rieske Fe–S preparation, only a single band of this protein appears (Fig. 1a, 3).

Comparing the UV–visible absorption spectra of the intact and the Rieske-depleted Cyt b_6f preparations (Fig. 1b), we see that, upon depletion of Rieske Fe–S, the Q_y-band of Chl *a* in the spectral region of 650–700 nm becomes slightly weaker and shifts to the blue for ~3 nm, and that the full width at half maximum of the Q_y-band decreases slightly (556 cm^{-1} to 518 cm^{-1}). Small spectral shift of the Q_y absorption had been reported for the Cyt b_6f complex from *C. reinhardtii* in response to the variation in temperature or in the degree of denature [16], which may originate from the subtle difference in the electrostatic interactions between the chlorine ring of Chl *a* and the surrounding amino acid residues. It is understandable that depletion of Rieske Fe–S may introduce some perturbation to the tertiary structure of the remaining complex and, consequently, may vary the electrostatic interactions between Chl *a* and its surroundings. Note that, after the depletion of Rieske Fe–S, the α-Car absorption in the region of 450–500 nm still remains. Importantly, the isolated Rieske Fe–S protein exhibits no apparent absorption in the Q_y absorption spectral region, indicating that this preparation is free from Chl *a* contamination. Nevertheless, the above results prove that the quality of the target Cyt b_6f preparations is high enough for the subsequent EPR and photo-bleaching experiments.

3.2. Light-induced formation of $^1O_2^*$ in the Cyt b_6f complex

Fig. 2 shows the characteristic EPR signals of TEMPO resulted from the reaction of TEMP with $^1O_2^*$ induced by light illumination. The characteristic hyperfine lines of the N-oxyl radical in spectrum Fig. 2a indicate the light-induced formation of $^1O_2^*$ in the Cyt b_6f complex under aerobic condition. Upon adding 30% D_2O (Fig. 2b), the EPR signal intensity increased by 20% since the lifetime of $^1O_2^*$in D_2O can be prolonged as much as 20 folds compared to that in H_2O [17]. To the contrast, the addition of 50 mM sodium-azide (NaN_3) resulted in 77.5% decrease in the EPR signal intensity (Fig. 2c), because NaN_3 is an efficient $^1O_2^*$ scavenger [18]. Under anaerobic condition, no TEMPO signal is recognized (Fig. 2d), indicating that little $^1O_2^*$ was formed

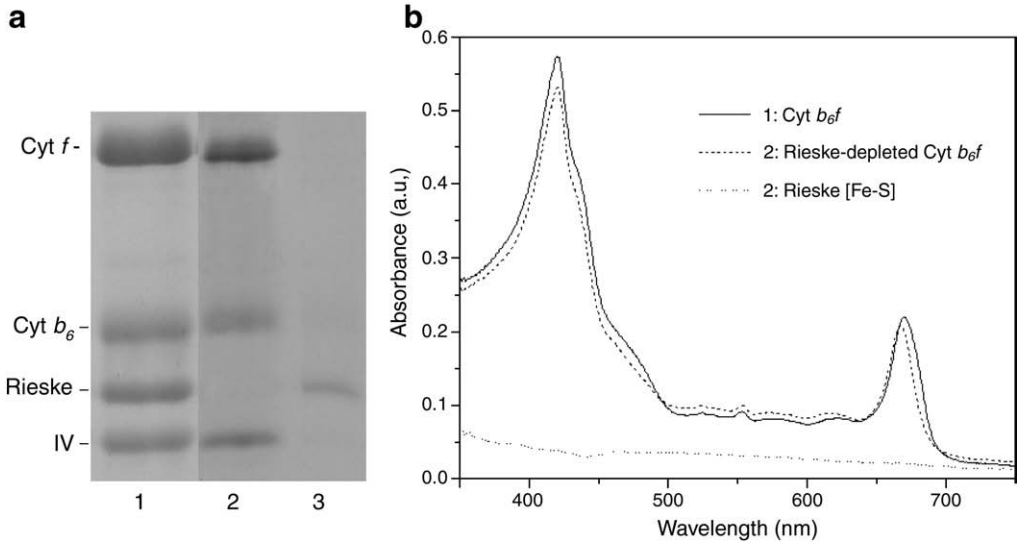

Fig. 1. (a) SDS-PAGEs of 1: intact Cyt b_6f in buffer 50 mM Tricine–NaOH (pH, 8.0) with 0.2 mM β-DM, 2: Rieske-depleted Cyt b_6f in buffer 200 mM potassium phosphate (pH, 6.8) plus 0.05% Triton X-100, and 3: isolated Rieske Fe–S protein in buffer 20 mM potassium phosphate (pH, 6.8) plus 0.5% Triton X-100. (b) UV–visible absorption spectra of 1: Cyt b_6f (1 μM Cyt *f*), 2: Rieske-depleted Cyt b_6f (1 μM Cyt *f*), and 3: isolated Rieske Fe–S protein (0.6 μM).

upon the high-light irradiation, and that O_2 is a prerequisite for the $^1O_2^*$ formation in the Cyt b_6f complex from *B. corticulans*.

3.3. Correlation between photo-bleaching of Chl a and $^1O_2^*$ production in the Cyt b_6f complex

Fig. 3 illustrates the change of $^1O_2^*$ concentration in the intact Cyt b_6f preparation in presence of TEMP and upon different illumination time. The results show the increase of $^1O_2^*$ production in response to the illumination time from 0 min to 15 min (spectra a–f). Obviously, no $^1O_2^*$ can be detected for the Cyt b_6f complex in dark (spectrum a), and the maximal $^1O_2^*$ production is reached at the longest illumination time of 15 min (spectrum f). However, the $^1O_2^*$ production exhibits a tendency of saturation upon further increasing illumination time (*vide infra*, Fig. 4), which can be ascribed to the effect of oxygen consumption (samples were sealed after oxygen saturation).

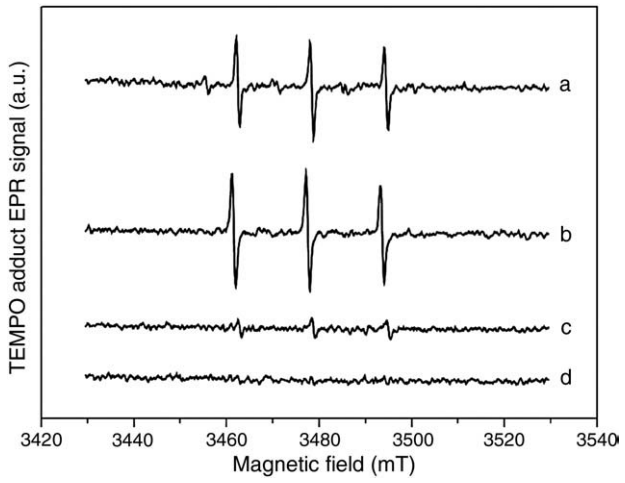

Fig. 2. EPR detection of $^1O_2^*$ for the intact Cyt b_6f preparation from *B. corticulans* (1 μM Cyt *f*; 37.5 mM TEMP; buffer (pH 8.0): 50 mM Tricine with 0.02% β-DM). (a) Aerobic Cyt b_6f preparation without any additive, and those with (b) D_2O (30%) and (c) NaN_3 (50 mM). (d) Intact Cyt b_6f preparation under anaerobic condition and without any additive. TEMP was added before illumination. Spectra were recorded after illumination with white light (1.5×10^3 μM m^{-2} s^{-1}) for 9 min.

Fig. 4 shows the change of Q_y absorption measured by the absorbance difference (ΔA) between 668 nm and 700 nm, which is proportional to the content of residual Chl *a* molecules after photo-bleaching. It is seen that the Chl *a* content gradually decreases as the illumination time increases, indicating that Chl *a* bound in the Cyt b_6f complex is bleached likely owing to the oxidative reaction with $^1O_2^*$. These results are in consistence with a previous report where photo-bleach of Chl *a* in the Cyt b_6f complexes was related to the $^1O_2^*$ formation [10]. On the other hand, the EPR data in Fig. 4 exhibits a prominent increase of $^1O_2^*$ production on increasing the illumination time. These results of photo-bleaching and EPR experiments firmly establish the reverse correlation between remaining Chl *a* content and light-induced $^1O_2^*$ production in the Cyt b_6f complex from *B. corticulans*.

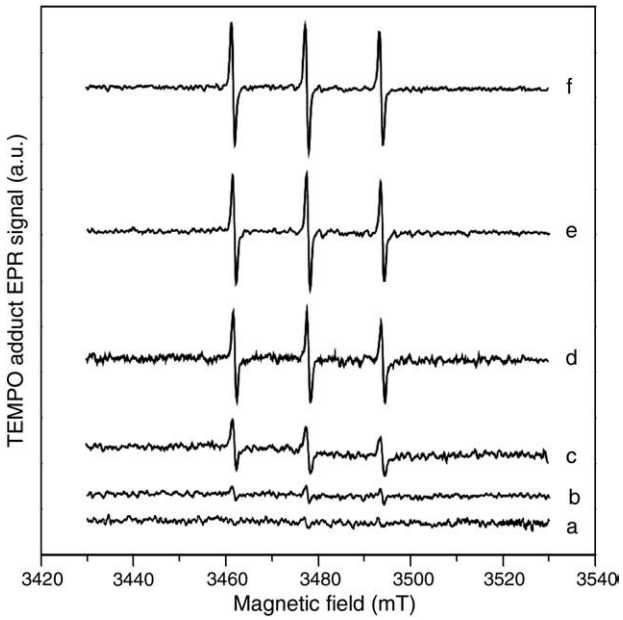

Fig. 3. EPR detection of $^1O_2^*$ for the intact Cyt b_6f complex from *B. corticulans*. The samples were illuminated with white light (1.5×10^3 μM m^{-2} s^{-1}) under aerobic condition for (a) 0 min (in dark), (b) 3 min, (c) 6 min, (d) 9 min, (e) 12 min and (f) 15 min before subjected to EPR measurements. TEMP was added before illumination.

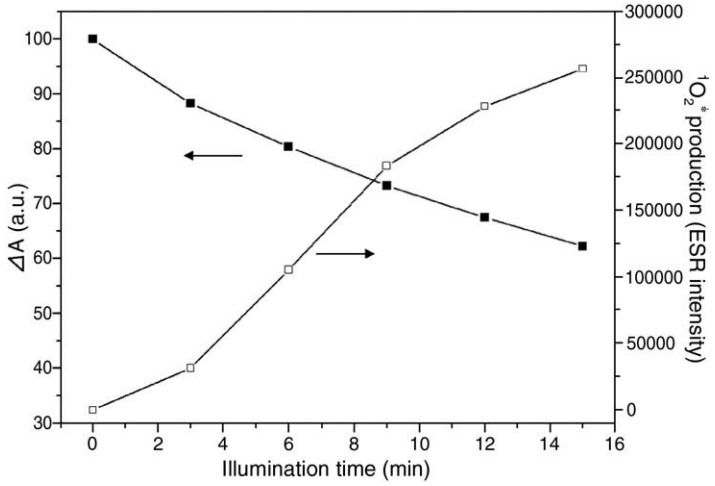

Fig. 4. (■) Time evolving change of the remaining Chl *a* content after photo-bleaching as indicated by the Q_y absorption of Chl *a* (ΔA, see text for details), and (□) change of the $^1O_2^*$ production (amplitude of EPR signal at 3479 mT was used as an indicator) in the Cyt b_6f preparation (1 μM Cyt *f*) from *B. corticulans*. Photon flux of white light was 1.5×10^3 μM m^{-2} s^{-1}. EPR data were extracted from Fig. 3.

3.4. Scavenging of $^1O_2^*$ in the Cyt b_6f complex by extraneous antioxidants

Ascorbate, L-histidine, glutathione and β-Car are important antioxidative substances in plants, and these biomolecules are also known to be effective scavengers of $^1O_2^*$ [19–21]. In photosynthetic organisms, β-Car can prevent the $^1O_2^*$ formation via quenching ^{3}Chl *a**, it can also directly quench or scavenge the $^1O_2^*$ formed inevitably [22]. Fig. 5 illustrates the relative $^1O_2^*$-scavenging activity of these biomolecules added to the intact Cyt b_6f preparations under light illumination. With respect to the controlled case without any additives, the EPR response decreased to zero upon adding L-histidine (spectrum e), glutathione (spectrum d) and ascorbate (spectrum c), but it only dropped by 72.4% when β-Car was added (spectrum b). Note that the EPR hyperfine feature for the case of ascorbate (spectrum c) is the characteristic signal of ascorbate itself rather than the TEMPO adduct. These results further prove the light-induced formation of $^1O_2^*$ in the Cyt b_6f complex from *B. corticulans* and, more importantly, suggest that the photo-induced $^1O_2^*$ can be scavenged by the extraneous antioxidants in thylakoid.

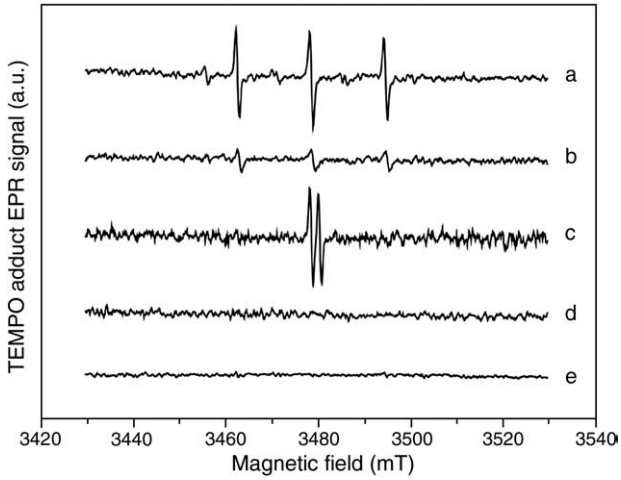

Fig. 5. EPR detection of $^1O_2^*$ for the intact Cyt b_6f preparation from *B. corticulans* as scavenged by extraneous antioxidants: (a) control, (b) β-Car (0.5 mM), (c) ascorbate (25 mM), (d) glutathione (25 mM) and (e) L-histidine (16 mM). Other experimental conditions were similar to those in Fig. 2.

3.5. Light-induced $^1O_2^*$ production in the three different Cyt b_6f preparations

Fig. 6 shows the EPR spectra of the intact and the Rieske-depleted Cyt b_6f complexes, as well as the isolated Rieske Fe–S protein, all of which were recorded after 9 min illumination. Judging from the EPR signal amplitudes, the $^1O_2^*$ yield for the Rieske-depleted Cyt b_6f complex (spectrum b) is 2.7 folds of that for the intact Cyt b_6f complex (spectrum a), which is understandable in view of the perturbation in the Chl *a* surroundings caused by the depletion of the Rieske Fe–S subunit (*vide supra*). In this case, the quenching mechanisms of the Chl *a* singlet and/or triplet excitations may become less efficient, resulting in higher $^1O_2^*$ yield. The effect of free Chl *a* was also examined, because free Chl produces more $^1O_2^*$ than bound one does

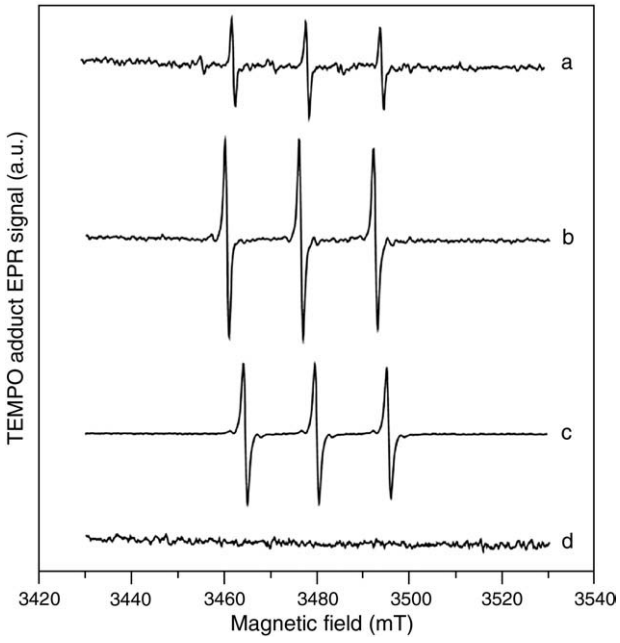

Fig. 6. EPR detection of $^1O_2^*$ for different Cyt b_6f preparations from *B. corticulans*. (a) Intact Cyt b_6f, (b) Rieske-depleted Cyt b_6f, (c) free Chl *a* (2-butyl alcohol solution, OD_{678nm} was kept the same as (a) and (b)), and (d) isolated Rieske Fe–S protein. For clearness signal intensity of spectrum c is scaled by multiplying 0.2. Other experimental conditions were similar to those in Fig. 2.

upon light illumination [23], which is indeed confirmed by comparing the spectra a and c in Fig. 6. Most importantly, no $^1O_2^*$ was detected in the case of isolated Rieske Fe–S preparation (spectrum d). These results prove that light-induced formation of $^1O_2^*$ in the Cyt b_6f complex correlates tightly to Chl *a*, indicating that the specific site of $^1O_2^*$ generation is the Chl *a* cofactor rather than the Rieske Fe–S protein.

To exclude the possible influence from any other types of photosensitizers besides Chl *a*, the intact Cyt b_6f preparations were illuminated with monocolor red light (665 ± 1 nm) to ensure the specific excitation of Chl *a*. It is seen from Fig. 7 that the amplitude of EPR signal under white-light illumination (spectrum a) is comparable to that under red-light illumination (spectrum b), which further proves that the Chl *a* cofactor is the active site of $^1O_2^*$ formation in the Cyt b_6f complex from *B. corticulans*.

4. Discussions

It is well known that excess amount of light irradiation may damage the photosynthetic apparatus or decrease the efficiency of photosynthesis. Such photoinhibition phenomena correlate closely to the production of light-induced reactive oxygen species (ROS) [24–26]. In this context, Chl *a* in thylakoid can be a major photo-sensitizer of $^1O_2^*$ that is extremely cytotoxic to the Chl *a*-containing pigment–protein assemblies [27–29].

To date, different views exist regarding the specific site of light-induced $^1O_2^*$ formation in the Cyt b_6f complex. Zhang et al. proposed that the formation of $^1O_2^*$ in Cyt b_6f originates from the reaction of ^{3}Chl *a** with O_2 [10]. ^{3}Chl *a** lives for ~600 μs under anaerobic condition in the Cyt b_6f complex from *B. corticulans* [30], and the time scale for Chl *a*-to-O_2 triplet EET is ~0.1 μs [31–33], therefore, the unwanted sensitization process can be very efficient. The present work unambiguously shows that the Chl *a* cofactor is the specific site of $^1O_2^*$ formation in the Cyt b_6f complex from *B. corticulans*. This conclusion is based on the correlation between the photo-bleaching of Chl *a* and the $^1O_2^*$ production (Fig. 4), the effects of extraneous $^1O_2^*$ scavengers (Figs. 2 and 5) and the detection of $^1O_2^*$ in the Rieske-depleted Cyt b_6f complex (Fig. 6). In addition, the fact that in the isolated Rieske Fe–S protein $^1O_2^*$ could not be detected serves as a strong supporting evidence. Furthermore, all of the experimental evidences show that the Rieske Fe–S protein is not responsible for the light-induced $^1O_2^*$ production.

To the contrary, by means of EPR spectroscopy, Suh et al. could detect $^1O_2^*$ in the intact Cyt b_6f but not in the Rieske-depleted Cyt b_6f complex from spinach under high-light illumination and aerobic conditions [11]. These authors then concluded that the Rieske protein is responsible for the sensitization of $^1O_2^*$. The apparent discrepancy with our results may originate from the difference between the Rieske-depleted Cyt b_6f preparations: Obviously, Suh et al.'s Rieske-depleted preparation seemed lacking of Chl *a* judging from the UV–visible absorption spectrum (see Fig. 1 in ref. [11]), whereas in the present work the Rieske-depleted Cyt b_6f complex binds the Chl *a* cofactor whose protein surroundings varies only slightly compared to its intact counterpart.

In the membrane proteins of thylakoid, a photo-sensitizer may either transfer its triplet excitation to molecular oxygen to from $^1O_2^*$, or interact with other substrates via electron or hydrogen transfer to yield free radicals [34]. Since $^1O_2^*$ is not a subject of enzymatic processing, carotenoids and other antioxidants intrinsic to chloroplast can be the main scavengers of triplet excited state Chls or $^1O_2^*$ [35]. Normally, the formation of $^1O_2^*$ in photosynthetic organisms can be alleviated by the photo-protective function of Car via efficient Chl-to-Car triplet EET [36]. For the Cyt b_6f complex from *B. corticulans*, however, our previous spectroscopic study could not identify this particular mechanism [9]. In addition to the mechanism of direct quenching ^{3}Chl *a**, photosynthetic carotenoids may be involved in scavenging $^1O_2^*$ via reactions $^1O_2^* + \mathrm{Car} \rightarrow {}^3\mathrm{Car}^* + O_2$ and $^3\mathrm{Car}^* \rightarrow \mathrm{Car} +$ heat [37], i.e., α-Car in the Cyt b_6f from *B. corticulans* can be a potent $^1O_2^*$ scavenger. It had been reported that tocopherols, ascorbate and glutathione etc. are efficient $^1O_2^*$ scavengers in chloroplasts [38–40], the present work clearly shows that L-histidine, glutathione and β-Car *etc.* are also efficient quenchers or scavengers of light-induced $^1O_2^*$ from the Cyt b_6f complex. To the best of our knowledge, this is the first direct evidence of scavenging photo-induced $^1O_2^*$ produced in the Cyt b_6f complex by extraneous antioxidants. We propose that the photoprotection of the Cyt b_6f complex may be partially realized by the antioxidants that are extraneous to the Cyt b_6f complex but endogenous to thylakoid.

Finally, it is worth noting that we have also detected $O_2^{\cdot -}$ by the use of EPR spectroscopy in the Cyt b_6f complex from *B. corticulans* under high-light illumination (data not shown). Since ROS such as superoxide radical ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH) may be derived from $^1O_2^*$ [41], it is interesting to study further the detailed formation mechanisms and the enzymatic scavenging mechanisms of these ROS.

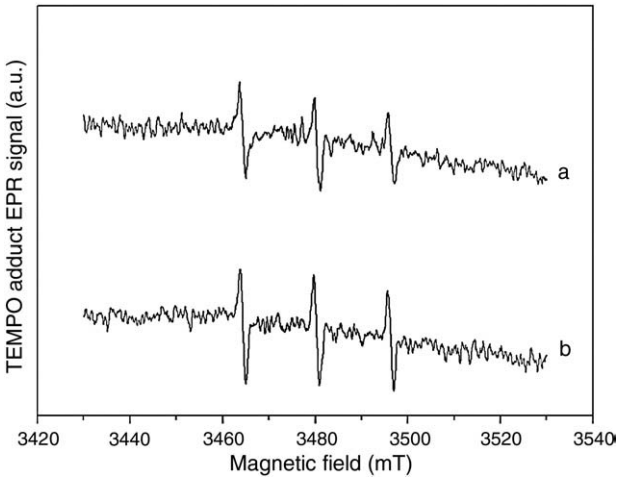

Fig. 7. EPR detection of $^1O_2^*$ in intact Cyt b_6f complex from *B. corticulans* under (a) white light and (b) monocolor red light (665±1 nm) illumination with comparable photon flux (1.5×10^3 μM m^{-2} s^{-1}). Other experimental conditions were similar to those in Fig. 2.

Acknowledgements

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