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Oxygen uncouples light absorption by the chlorosome antenna and photosynthetic electron transfer in the green sulfur bacterium *Chlorobium tepidum*

Niels-Ulrik Frigaard *, Katsumi Matsuura

Department of Biology, Tokyo Metropolitan University, Minami-ohsawa 1-1, Hachioji, 192-0397 Tokyo, Japan

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Abstract

In photosynthetic green sulfur bacteria excitation energy is transferred from large bacteriochlorophyll (BChl) *c* chlorosome antennas via small BChl *a* antennas to the reaction centers which then transfer electrons from cytochrome *c* to low-potential iron-sulfur proteins. Under oxidizing conditions a reversible mechanism is activated in the chlorosomes which quenches excited BChl *c*. We used flash-induced cytochrome *c* oxidation to investigate the effect of this quenching on photosynthetic electron transfer in whole cells of *Chlorobium tepidum*. The extent of cytochrome *c* photooxidation under aerobic conditions decreased to approx. 3% of that under anaerobic conditions when BChl *c* was excited under light-limiting conditions. Photooxidation obtained by excitation of BChl *a* was similar under aerobic and anaerobic conditions. We interpret this drastic decrease in energy transfer from BChl *c* to the reaction center as a consequence of the quenching mechanism which is activated by O_2. This reversible uncoupling of the chlorosome antenna might prevent formation of toxic reactive oxygen species from photosynthetically produced reductants under aerobic conditions. The green filamentous bacterium *Chloroflexus aurantiacus* also contains chlorosomes but energy transfer from the BChl *c* and BChl *a* antennas to the reaction center in this species was not affected by O_2.

Keywords: Green bacterium; Chlorosome; Excitation energy transfer; Fluorescence quenching; Cytochrome *c* photooxidation; Reactive oxygen species

1. Introduction

Green sulfur bacteria inhabit anoxic and sulfide-rich waters, muds, sediments, and microbial mats [1,2]. They are obligate photoautotrophs and their growth depends on photooxidation of reduced sulfur compounds and complete absence of O_2. One reason for their anaerobic nature may be related to their photosynthetic reaction centers which produce low-potential reductants including reduced ferredoxin [3,4]. The primary electron donor to the reaction center is a bound cytochrome *c* subunit [3,4] and electron transfer activities in the reaction center can conveniently be monitored by measuring absorption changes in this cytochrome [5–7]. In the present study we have used flash-induced cytochrome *c* oxi-

Abbreviations: BChl, bacteriochlorophyll; EDTA, ethylenediaminetetraacetic acid; E_h, redox potential; FMO protein, Fenna-Matthews-Olson protein; MOPS, 3-(*N*-morpholino)propanesulfonic acid

* Corresponding author. Fax: +81 (426) 772559;
E-mail: nuf@comp.metro-u.ac.jp;
URL: http://www.comp.metro-u.ac.jp/~nuf/

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dation to measure how efficiently excitation energy in the antennas is used for photosynthetic electron transfer.

Green sulfur bacteria use a rather large and efficient light-harvesting antenna known as the chlorosome, which is an oblong structure attached on the cytoplasmic side of the cellular membrane [8,9]. Most of the chlorosome is composed of large aggregates of bacteriochlorophyll (BChl) *c* which are thought to be enveloped in a lipid monolayer and organized essentially without participation of proteins. Light energy absorbed by the BChl *c* antenna is transferred to a very small pool of BChl *a* which probably is located in a thin layer on the surface of the chlorosome called the baseplate. Excitation energy from this chlorosomal BChl *a* is transferred to the reaction center in the cytoplasmic membrane, probably via the BChl *a*-containing Fenna-Matthews-Olson (FMO) protein which is thought to be sandwiched between the chlorosome and the cytoplasmic membrane. Most of the BChl *a* in the cell is located in this FMO protein, the rest is distributed between the chlorosome and the reaction center core [8]. Light-induced oxidation of cytochrome *c* by specific excitation of the chlorosome and FMO protein was first demonstrated by Olson and Sybesma [5].

Fluorescence from BChl *c* in green sulfur bacteria is markedly higher under reducing conditions than under oxidizing conditions [10,11]. BChl *c* fluorescence is quenched in whole cells by less than a few micromolar of O_2 but can be restored by cellular reductants under anaerobic conditions [11,12]. Redox-dependent quenching is also observed in isolated chlorosomes and appears to involve a redox-active component which quenches excited BChl *c* in the oxidized state and is inactive in the reduced state [11,13,14]. We recently showed that chlorosomes from *Chlorobium tepidum* contain isoprenoid quinones which probably are involved in the quenching mechanism [15,16].

Although green sulfur bacteria are obligate anaerobes, they may encounter oxic conditions in their natural environment. This could occur due to vertical mixing of the oxic and anoxic zones across the chemocline in stratified lakes. Or it could happen due to changes in microbial activity, such as oxygenic photosynthesis, in layers above the green sulfur bacteria in stratified lakes and sediments which could allow O_2 to penetrate downwards.

The basis of O_2 toxicity in all living organisms is formation of reactive oxygen species which cause damage to a wide range of biological molecules [17,18]. Such reactive oxygen species can be formed by reaction between O_2 and cellular reductants such as thiols and reduced iron-sulfur proteins such as ferredoxin. Many organisms have therefore developed enzymes, such as superoxide dismutase, and non-enzymatic antioxidants as protection against reactive oxygen species. This apparently includes green sulfur bacteria since superoxide dismutase has been found in *Chlorobium limicola* [19,20].

Another means of protection against reactive oxygen species is to decrease the rate of production of cellular reductants. It has been proposed that green sulfur bacteria use this approach based on the redox-dependent quenching of excited BChl mentioned above [8,11,13,14]. In agreement with this idea we show in the present study that the green sulfur bacterium *Cb. tepidum* greatly decreases energy transfer from the chlorosomes to the reaction centers under aerobic conditions and thereby avoid photosynthetic electron transfer in the presence of O_2.

The photosynthetic green filamentous bacterium *Chloroflexus aurantiacus* also contains chlorosomes and cytochrome *c* as donor to the reaction center but not a quenching mechanism as found in green sulfur bacteria [8,9,11,21]. For comparison we also measured flash-induced cytochrome *c* photooxidation in this species and we show that energy transfer from the antennas to the reaction center is not affected by O_2.

2. Materials and methods

2.1. Preparation of cells and membranes

Cb. tepidum ATCC 49652 was grown in 200 ml flasks at 45°C in medium previously described [22]. Illumination was provided by eight high-infrared 20 W fluorescent light tubes (FL20S, Toshiba, Japan) which practically caused light-saturated growth. A 5% inoculum of stationary-phase cells was used and the cultures were used for experiments 2–3 days later when they had reached the late exponential growth

phase. *Cfx. aurantiacus* J-10-fl was grown heterotrophically in 30 ml screw-cap tubes at 55°C in medium previously described [23] and illuminated by a 60 W incandescent light bulb with an intensity of approx. 10 W m^{-2}.

For preparation of cytoplasmic membranes from *Cb. tepidum*, cells were harvested at 4°C and resuspended in ice-cooled 40 mM MOPS-NaOH buffer pH 7.0 containing 1 mM $MgCl_2$ and approx. 0.1 mg ml^{-1} DNase. This suspension was passed through a precooled French press three times. Unbroken cells and cell debris were removed from the resulting suspension by centrifugation at $15\,000 \times g$ for 15 min at 4°C. The supernatant was then centrifuged at $200\,000 \times g$ for 1 h at 4°C to pellet the cytoplasmic membrane fraction. The pellet was resuspended in a few ml 40 mM MOPS-NaOH pH 7.0 and stored at −80°C until use. Membranes prepared in this manner consisted of cytoplasmic membranes with attached FMO protein and chlorosomes as judged from the absorption spectrum.

The concentration of cells or membranes used in the experiments was estimated from the BChl *c* Q_y absorption band around 745 nm. In the case of cell suspensions, this value was corrected for light scattering effects by subtracting $(A_{600}+A_{900})/2$ from the measured value of A_{745}. An A_{745} value of 1 corresponds to approx. 14 μM BChl *c*. Concentrations of BChl *c* and BChl *a* were determined as previously described [15].

All spectroscopic measurements were carried out at room temperature.

2.2. Flash-induced cytochrome c oxidation

A 4 ml glass cuvette containing 40 mM MOPS-NaOH buffer pH 7.0 was used in the flash experiments involving whole cells. For flash experiments involving cells under anaerobic conditions, the MOPS buffer was bubbled with N_2 and supplemented with 0.5 mM Na_2S before use. A volume of cell culture was then removed from the culture flask and injected directly into the almost completely filled cuvette. The head space was flushed with N_2, the cuvette sealed with a rubber stopper and the suspension incubated for 30 min in the dark. For flash experiments involving cells under aerobic conditions, a volume of cell culture was injected into a few ml air-saturated MOPS buffer and incubated for 30 min in the dark. Before measurements on aerobic cell suspensions of *Cb. tepidum*, $Na_2S_2O_3$ was added to a final concentration of 2 mM.

Flash-induced cytochrome *c* oxidation was measured with a single-beam spectrophotometer assembled in the laboratory as previously described [7,24]. The samples were excited with a 5 μs actinic flash provided by a xenon flash and the resulting transient absorption changes recorded at different wavelengths. Photooxidation of cytochrome *c* in *Cb. tepidum* was measured by subtracting the absorption change at 540 nm from the absorption change at 553 nm [7] and in *Cfx. aurantiacus* by subtracting the absorption change at 542 nm from the absorption change at 554 nm [21]. The following filters were placed in front of the flash to obtain selective excitation: 690 nm cutoff glass filter (R-69, Toshiba, Japan), 710 nm cutoff Wratten gelatin filter (89B, Kodak, USA), 750 nm interference filter, 700–760 nm broad band interference filter, and 810–880 nm broad band interference filter (all interference filters from Nihon Shinku Kogaku, Japan). The transmission spectrum of some of the filters are shown in Fig. 1. Decreased flash intensity was obtained by placing neutral density filters made of photographic film in front of the flash.

Flash experiments with membranes were preformed in the same manner as with cells except that the redox potential of the membrane suspension was controlled as described below.

2.3. Redox titration of membranes

Suspensions of *Cb. tepidum* membranes were redox titrated in a special-designed cuvette continuously flushed with argon as previously described [13,25]. The membranes were suspended in 40 mM MOPS-NaOH buffer pH 7.0 containing 20 μM each of methyl viologen, benzyl viologen, anthraquinone-2-sulfonate, 2-hydroxy-1,4-naphthoquinone, 2,3,5,6-tetramethyl-*p*-phenylenediamine and 50 μM of Fe-EDTA (added from an aqueous solution of 5 mM $FeSO_4$ and 0.15 M trisodium EDTA). Stigmatellin and antimycin A were added to a concentration of 10 μM each to slow down the rereduction of cyto-

chrome *c* after photooxidation [26]. Various redox potentials were obtained by adding small amounts of sodium dithionite or potassium ferricyanide.

3. Results

3.1. Absorption and fluorescence spectra of Cb. tepidum

The absorption spectrum of whole cells showed a maximum at 745 nm due to aggregated BChl *c* in the chlorosomes and a shoulder around 810 nm due to BChl *a* (Fig. 1). Chlorosomal BChl *a* absorbs around 795 nm [8] but was not discernible in our spectrum. The 810 nm peak was due to extrachlorosomal BChl *a* mainly in the FMO protein [8]. The membranes had a similar absorption spectrum except that the BChl *c* maximum was blue-shifted a few nanometers. The cells had a molar ratio of approx. 40 BChl *c* per BChl *a*.

Steady state fluorescence spectra of cells and membranes under reducing and oxidizing conditions are shown in Fig. 2a and b, respectively. Oxidizing conditions were obtained by suspending the samples in air-saturated buffer and reducing conditions were obtained by adding crystalline sodium dithionite to a final concentration of roughly 10 mM. When sulfide was added to an anaerobic cell suspension as described in Section 2, the resulting fluorescence spectrum was similar to that obtained with dithionite.

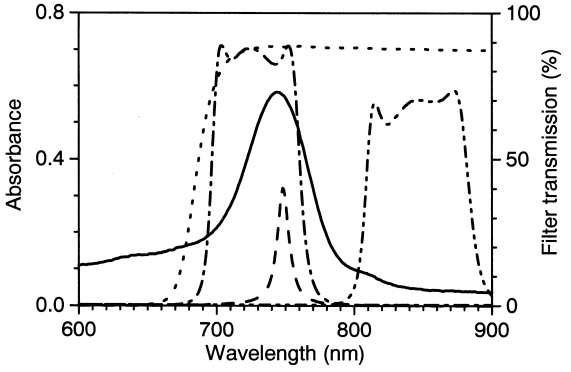

Fig. 1. Absorption spectrum of a suspension of *Cb. tepidum* cells (solid line) and transmission spectrum of filters used for flash excitation: >690 nm (dotted line), 700–760 nm (dash-dot line), 810–880 nm (dash-dot-dot line), and 750 nm (dashed line). The absorption spectrum of the cell suspension was recorded using opal glass to reduce light scattering effects [27].

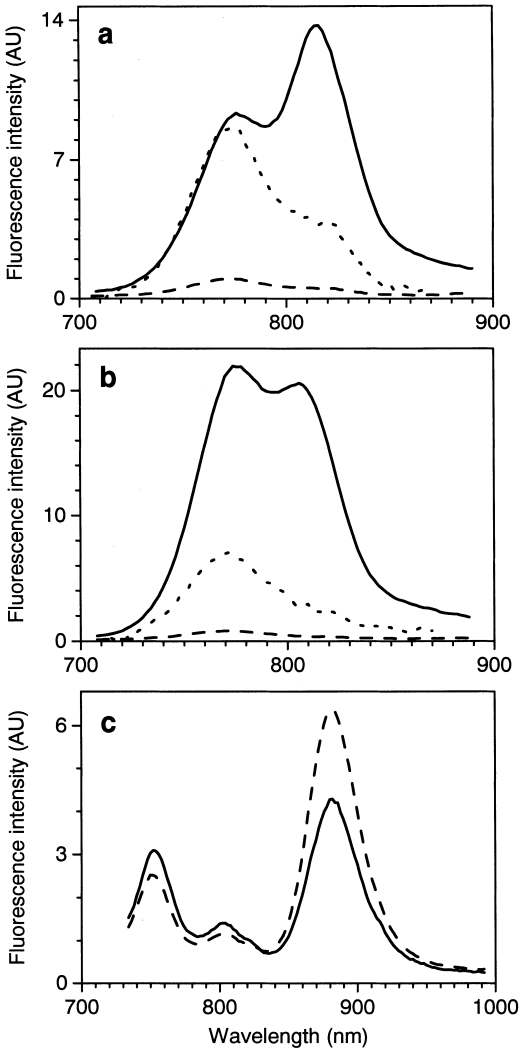

Fig. 2. Fluorescence spectra of suspensions of (a) *Cb. tepidum* cells, (b) *Cb. tepidum* membranes, and (c) *Cfx. aurantiacus* cells under reducing conditions (solid lines) or oxidizing conditions (dashed lines). *Cb. tepidum* was excited at 460 nm and *Cfx. aurantiacus* at 720 nm. The spectra under oxidizing conditions in a and b are also shown as a 10 times enlargement (dotted lines). The BChl *c* concentration was $A_{745} = 0.2$. AU, arbitrary unit.

Two bands were discernible in the fluorescence spectra: BChl *c* fluorescence peaked around 770–780 nm and BChl *a* fluorescence peaked around 800–820 nm. The fluorescence at 775 nm was about 10 times higher in cells and about 30 times higher in membranes under reducing conditions when compared to oxidizing conditions. This shows, in agreement with previous reports, that a quencher of BChl *c* excitation was inactivated under reducing conditions [10–16]. In addition, the ratio of BChl *a* fluo-

rescence to BChl *c* fluorescence also increased dramatically under reducing conditions in both cells and membranes as previously observed [16,28]. This shows that much more excitation energy was transferred from BChl *c* to BChl *a* under reducing conditions than under oxidizing conditions.

By deconvolution of the fluorescence spectra under reducing conditions into Gaussian curves we found that BChl *c* fluorescence peaked at 776 nm in cells and at 774 nm in membranes. This small difference is similar to the small blue-shift in BChl *c* absorption mentioned above. The BChl *a* fluorescence, on the other hand, peaked at 817 nm with high intensity in cells and at 809 nm with low intensity in membranes, even though the major BChl *a* absorption was similar and peaked at 810 nm in both cells and membranes. A likely explanation for this difference is that the BChl *a* fluorescence at 809 nm in membranes mainly originated from chlorosomal BChl *a* (which absorbs at 795 nm), and that the BChl *a* fluorescence at 817 nm in cells mainly originated from extrachlorosomal BChl *a* (most of which absorbs at 810 nm). This interpretation is consistent with the optical properties of isolated FMO protein which absorbs at 809 nm and fluoresces at 818 nm [8] and with the observation that the fluorescence spectra of membranes and isolated chlorosomes were very similar (data not shown). If this interpretation is correct, it suggests that transfer of excitation energy from chlorosomal BChl *c* to extrachlorosomal BChl *a* was disrupted in our membrane preparation. Such a disruption could occur during preparation of the membranes.

3.2. Absorption and fluorescence spectra of Cfx. aurantiacus

The absorption spectrum of whole cells showed a chlorosomal BChl *c* maximum at 743 nm and BChl *a* maxima at around 800 nm due to the chlorosome baseplate and at 867 nm due to the B808-866 antenna complex in the cytoplasmic membrane (data not shown) [8]. These spectral species peaked at 753, 804 and 882 nm respectively in the fluorescence spectrum (Fig. 2c). The fluorescence intensity showed much less dependence on redox potential when compared to *Cb. tepidum* cells (Fig. 2a). BChl *c* fluorescence was only slightly higher in dithionite-reduced *Cfx. aurantiacus* cells than under aerobic conditions (Fig. 2c). This confirms previous observations that an excitation quencher as found in *Cb. tepidum* is not present in *Cfx. aurantiacus* [11,13,16]. The *Cfx. aurantiacus* cells had a molar ratio of approx. seven BChl *c* per BChl *a*.

3.3. Redox titration of fluorescence quenching in Cb. tepidum

The quenching of BChl *c* fluorescence at 775 nm in membranes was redox titrated and the midpoint potential determined as approx. −100 mV at pH 7 (data not shown). This midpoint potential is similar to previously reported values for chlorosomes isolated from *Chlorobium vibrioforme* [13,14] and *Cb. tepidum* [15,16]. A similar redox titration of whole *Cb. tepidum* cells was not possible since we could not obtain stable fluorescence or redox potentials. Therefore, we used aerobic/anaerobic conditions to

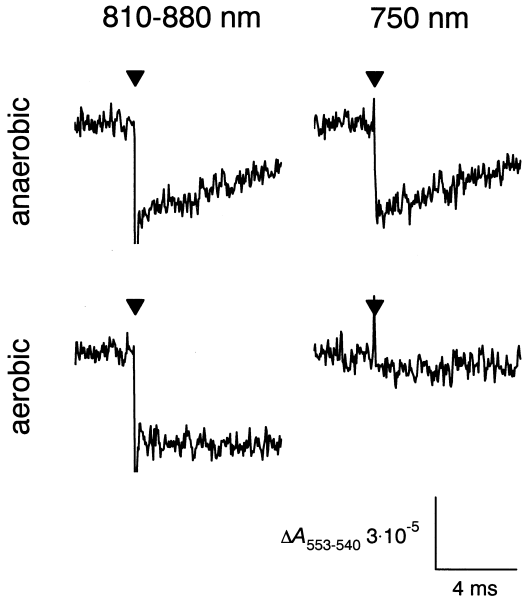

Fig. 3. Flash-induced cytochrome *c* oxidation ($\Delta A_{553-540}$) in *Cb. tepidum* cells in anaerobic or aerobic suspension. At the time indicated by a triangle, the cells were excited by a single flash with either a 810–880 nm filter (for BChl *a* excitation) or a 750 nm filter (for BChl *c* excitation). Each trace represents an average of 1600 traces per wavelength recorded on a 10 ms full time scale and separated by 6 s under anaerobic conditions and by 12 s under aerobic conditions. The BChl *c* concentration was $A_{745} = 0.5$.

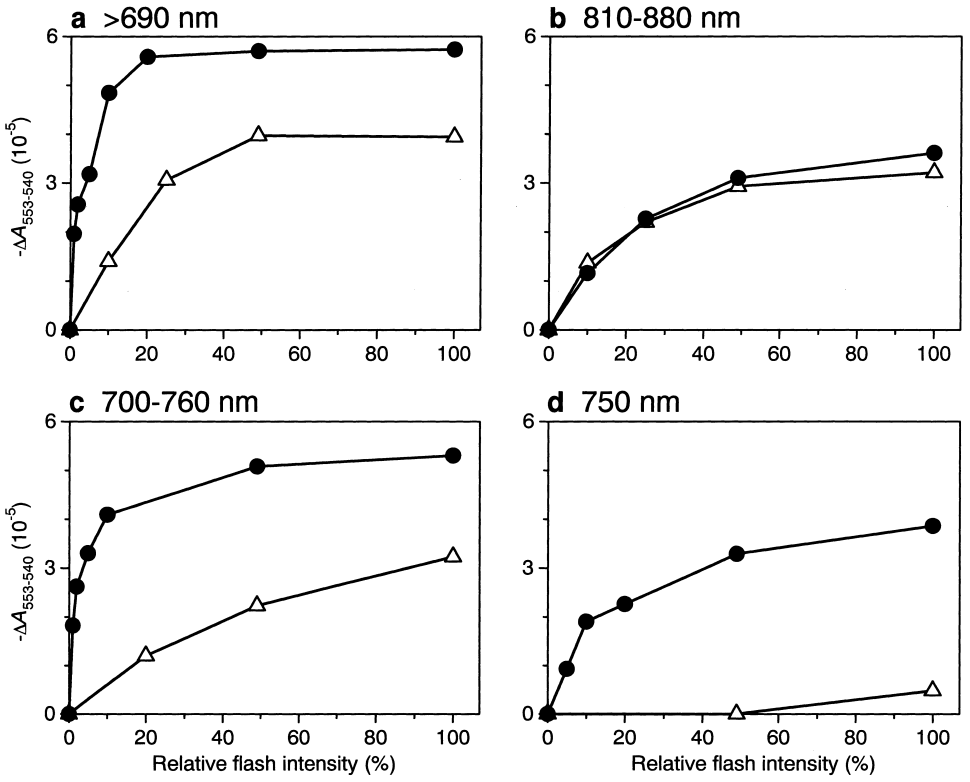

Fig. 4. Extent of flash-induced cytochrome *c* oxidation ($-\Delta A_{553-540}$) in *Cb. tepidum* cells in anaerobic suspension (solid circles) or aerobic suspension (open triangles). The cells were excited using the filter indicated on each graph. Other experimental conditions were as described in Fig. 3, except that 400 traces were averaged for each data point.

investigate the effect of redox potential on flash-induced cytochrome *c* oxidation in whole cells.

3.4. Flash-induced cytochrome c oxidation in Cb. tepidum cells

Fig. 3 shows the transient changes in absorption due to cytochrome *c* oxidation following excitation of whole cells by a single flash. Reaction center-bound cytochrome *c* donates electrons to the photooxidized reaction center BChl dimer ($P840^+$) in less than 0.1 ms and is rereduced by cellular reductants on a millisecond time scale [3,4]. Other membrane-bound or soluble cytochromes *c* may reduce the reaction center-bound cytochrome *c*, so more than one cytochrome *c* may contribute to the measured absorption changes. However, this has little significance for the validity of the current assay since the photoactivity of the reaction center can be evaluated by the total extent of cytochrome *c* oxidation following a single flash.

We used a 810–880 nm broad band interference filter to specifically excite extrachlorosomal BChl *a* and a 750 nm interference filter to specifically excite chlorosomal BChl *c* (Fig. 1). Fig. 3 shows that the extent of flash-induced cytochrome *c* oxidation under anaerobic and aerobic conditions was similar when BChl *a* was excited. A similar photooxidation was obtained by exciting BChl *c* under anaerobic conditions. However, excitation of BChl *c* under aerobic conditions caused only little photooxidation. Thus, energy transfer from BChl *c* to the reaction center was highly inhibited under aerobic conditions. This inhibition was reversible: when a cell suspension which had been aerobically incubated for 6 h in the dark was transferred back to anaerobic conditions and supplemented with sulfide, the fluorescence increased and excitation of BChl *c* resulted in photooxidation as in Fig. 3 (data not shown).

We measured the extent of photooxidation when various filters and various flash intensities were used (Fig. 4). When BChl *a* was specifically excited, there

was hardly any difference between anaerobic and aerobic conditions, even under flash-limiting conditions (Fig. 4b). However, when BChl *c* was specifically excited, the half-saturation intensity was roughly 30 times higher under aerobic conditions than under anaerobic conditions (Fig. 4c,d). To excite both BChl *c* and BChl *a* we used a 690 nm cutoff filter and under these conditions, the half-saturation intensity was roughly 10 times higher under aerobic conditions than under anaerobic conditions (Fig. 4a). The results in Fig. 4 may suggest two different saturation levels where the lowest level is associated with excitation of BChl *a* (Fig. 4b) and the highest level is associated with excitation of BChl *c* under anaerobic conditions (Fig. 4a,c), but this possibility was not further investigated.

From calculations based on the spectra of flash emission, filter transmission, and cell absorption, we estimated that roughly twice as much energy was absorbed by extrachlorosomal BChl *a* in the experiment in Fig. 4a compared to the experiment in Fig. 4b (data not shown). This probably means that most of the photooxidation observed under aerobic conditions in Fig. 4a was due to extrachlorosomal BChl *a*.

3.5. Flash-induced cytochrome c oxidation in Cb. tepidum membranes

Fig. 5 shows the extent of cytochrome *c* photooxidation in isolated membranes when both BChl *c* and BChl *a* were excited. The results shown in the figure were obtained at two different redox potentials, one in which the BChl *c* fluorescence was high (reducing conditions, $E_h = -220$ mV) and one in which the BChl *c* fluorescence was low (oxidizing conditions, $E_h = +45$ mV). The results showed that there was no difference in the extent of photooxidation at these redox potentials. If only BChl *c* was excited using the 750 nm interference filter, photooxidation was not observed (data not shown). We therefore conclude that the chlorosomal BChl *c* antenna was completely and irreversibly uncoupled from the reaction center under both reducing and oxidizing conditions and that the photooxidation observed in Fig. 5 solely was due to BChl *a*. This interpretation agrees well with the interpretation of the fluorescence spectra (Section 3.1).

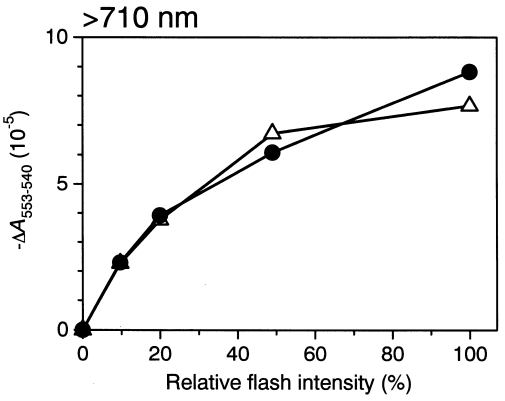

Fig. 5. Extent of flash-induced cytochrome *c* oxidation ($-\Delta A_{553-540}$) in a suspension of chlorosome-containing cytoplasmic membranes from *Cb. tepidum* under reducing conditions ($E_h = -220$ mV, solid circles) or oxidizing conditions ($E_h = +45$ mV, open triangles). The suspension was excited using the filter indicated on the graph. Other experimental conditions were as described in Fig. 4, except that the absorption changes were measured on a 2 ms full time scale and $A_{745} = 1$.

3.6. Flash-induced cytochrome c oxidation in Cfx. aurantiacus cells

Fig. 6 shows the extent of cytochrome *c* photooxidation in whole cells when BChl *c* (Fig. 6a) or BChl *a* (Fig. 6b) was specifically excited. The figure shows that the resulting photooxidation was similar under anaerobic and aerobic conditions even under flash limiting conditions. This means that neither the BChl *c* nor the BChl *a* antennas were affected by O_2.

4. Discussion

Our experiments with flash-induced cytochrome *c* oxidation in whole cells of *Cb. tepidum* showed that energy transfer from the main light-harvesting antenna system was highly inhibited under aerobic conditions. When BChl *c* was selectively excited, the half-saturation flash intensity of cytochrome *c* photooxidation increased roughly 30 times under aerobic conditions when compared to anaerobic conditions (Fig. 4c,d). In other words, when only the BChl *c* antenna was excited the extent of light-limited cytochrome *c* photooxidation decreased approx. 97% under aerobic conditions. This observation agrees well with previous experiments with "*Chloropseudomonas ethylica*" in which cell suspensions were continuously illu-

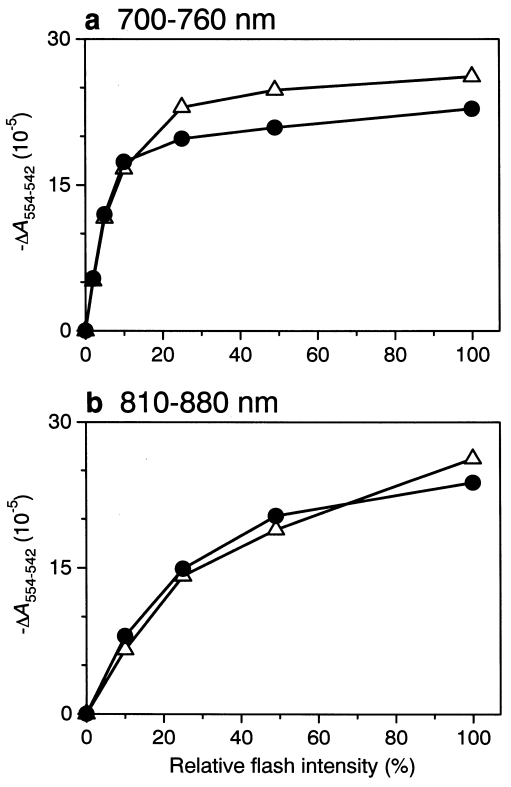

Fig. 6. Extent of flash-induced cytochrome *c* oxidation ($-\Delta A_{554-542}$) in *Cfx. aurantiacus* cells in anaerobic suspension (solid circles) or aerobic suspension (open triangles). The cells were excited using the filter indicated on each graph. Other experimental conditions were as described in Fig. 3, except that 40 traces separated by 20 s were averaged for each data point.

minated with actinic light of 740 nm [29]. The author concluded that under such conditions menaquinone and cytochrome are photooxidized under anaerobic conditions but that this photooxidation is reversibly inhibited under aerobic conditions.

A reasonable explanation for this inhibition is the redox-dependent quenching of excited BChl *c*. Our fluorescence spectra showed that both excited BChl *c* and transfer of excitation energy from BChl *c* to BChl *a* were efficiently quenched under aerobic conditions (Fig. 2a,b). This quenching can therefore explain the decreased photooxidation when BChl *c* is excited under aerobic conditions since less excitation energy is expected to reach the reaction center. The model in Fig. 7 illustrates this idea. No such quenching in the antennas was observed in *Cfx. aurantiacus* cells (Fig. 2c) and cytochrome *c* photooxidation was similar under anaerobic and aerobic conditions (Fig. 6a).

When extrachlorosomal BChl *a* was excited in whole cells of *Cb. tepidum* (Fig. 4b) or *Cfx. aurantiacus* (Fig. 6b), cytochrome *c* photooxidation was similar under anaerobic and aerobic conditions. Thus, the extrachlorosomal BChl *a* antennas in both of these species were apparently not subjected to redox-dependent quenching.

The identity of the quenching species in chlorosomes from green sulfur bacteria is not clear. However, we recently found menaquinone-7 and chlorobiumquinone (1′-oxomenaquinone-7) in the interior of chlorosomes from *Cb. tepidum* in a molar content of approx. 0.02 and 0.1, respectively, per BChl *c* [15,16]. And we suggested that the redox-dependent quenching in *Cb. tepidum* chlorosomes mostly is due to chlorobiumquinone based on this quinone's chemical properties and since *Cfx. aurantiacus* chlorosomes contain menaquinone-10 but not chlorobiumquinone [15,16].

The FMO protein causes most of the absorption by BChl *a* in *Cb. tepidum*. In its isolated form, this

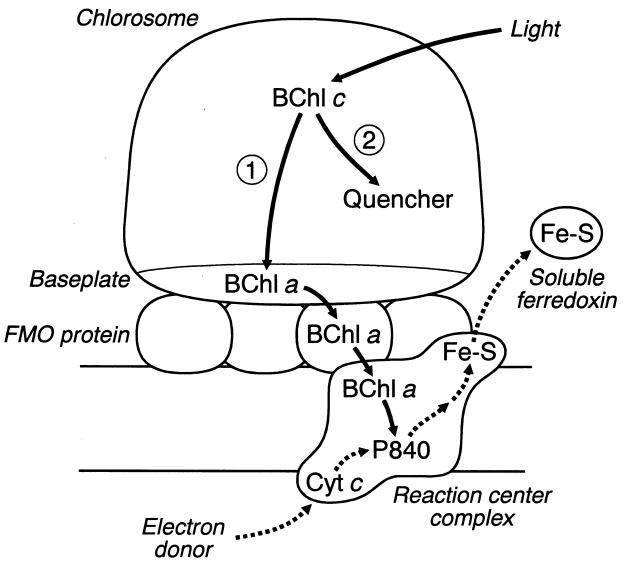

Fig. 7. Simplified model showing the arrangement of the light-harvesting and electron transferring components of the photosynthetic apparatus in *Cb. tepidum*. Excitation transfer is shown by solid lines, electron transfer by dotted lines. Under reducing conditions excitation energy is transferred via pathway 1. Under oxidizing conditions, a quencher is activated which absorbs most of the excitation energy via pathway 2 and thereby inhibits its photosynthetic electron transfer. Cyt *c*, cytochrome *c*; Fe-S, iron-sulfur cluster; P840, primary electron donor.

protein exhibits a redox-dependent quenching of BChl fluorescence, although the mechanism seems to be different from that in chlorosomes [30,31]. It is therefore interesting to note that cytochrome *c* oxidation in whole cells was similar under anaerobic and aerobic conditions when BChl *a* was excited (Fig. 4b). This could mean that quenching in the FMO protein in whole cells was not activated under the aerobic conditions used in this study or that, if the quenching was activated, had no influence on the photooxidation. A rather low transfer of excitation energy from the FMO protein to the reaction center has previously been reported in subcellular preparations from *Cb. tepidum* [32,33]. However, we think this might be explained by a disruption of the energy transfer pathway similar to what apparently occurred with the chlorosome antenna in our membrane preparation from *Cb. tepidum*.

In plants, the primary targets of reactive oxygen species seem to include the iron-sulfur clusters of photosystem 1 and thiol groups of soluble enzymes [18]. The iron-sulfur clusters in the green sulfur bacterial reaction center are also very sensitive to O_2 [34,35] and bubbling a culture of *Cb. vibrioforme* for about 0.5 h with air inactivates the F_A and F_B clusters (I.R. Vassiliev, J.H. Golbeck, personal communication). However, we found that flash-induced cytochrome *c* oxidation in a suspension of *Cb. tepidum* cells was unaffected even after 12 h of flashing under aerobic conditions (using the 810–880 nm filter; data not shown). This means that even though O_2-sensitive components in green sulfur bacteria may easily be damaged under aerobic conditions, the ability of the reaction center to oxidize cytochrome *c* is apparently much more stable.

The low potential reductants produced by the reaction center in green sulfur bacteria react readily with O_2 to form superoxide and other reactive oxygen species [18,36]. By reversibly uncoupling the chlorosomal BChl *c* antenna from photosynthetic electron transfer under oxidizing conditions, the bacteria are apparently able to avoid production of such reductants and might therefore enhance their chance of survival under aerobic conditions. The reduced menaquinone produced in the reaction center in *Cfx. aurantiacus* probably do not readily form reactive oxygen species and green filamentous bacteria are in general rather tolerant towards O_2. This could explain why these bacteria do not possess the same quenching mechanism as green sulfur bacteria.

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References

[1] R.W. Castenholz, J. Bauld, B.B. Jørgensen, Anoxygenic microbial mats of hot springs: thermophilic *Chlorobium* sp., FEMS Microbiol. Ecol. 74 (1990) 325–336.
[2] H.G. Trüper, N. Pfennig, The family Chlorobiaceae, in: A. Balows, H.G. Trüper, M. Dworkin, W. Harder, K.-H. Schleifer (Eds.), The Prokaryotes, 2nd edn., Springer-Verlag, New York, 1992, pp. 3583–3592.
[3] U. Feiler, G. Hauska, The reaction center from green sulfur bacteria, in: R.E. Blankenship, M.T. Madigan, C.E. Bauer (Eds.), Anoxygenic Photosynthetic Bacteria, Kluwer Academic Publishers, Dordrecht, 1995, pp. 665–685.
[4] H. Sakurai, N. Kusumoto, K. Inoue, Function of the reaction center of green sulfur bacteria, Photochem. Photobiol. 64 (1996) 5–13.
[5] J.M. Olson, C. Sybesma, Energy transfer and cytochrome oxidation in green bacteria, in: H. Gest, A. San Pietro, L.P. Vernon (Eds.), Bacterial Photosynthesis, Antioch Press, Yellow Springs, 1963, pp. 413–422.
[6] R.C. Prince, J.M. Olson, Some thermodynamic and kinetic properties of the primary photochemical reactants in a complex from a green photosynthetic bacterium, Biochim. Biophys. Acta 423 (1976) 357–362.
[7] N. Okumura, K. Shimada, K. Matsuura, Photo-oxidation of membrane-bound and soluble cytochrome *c* in the green sulfur bacterium *Chlorobium tepidum*, Photosynth. Res. 41 (1994) 125–134.
[8] R.E. Blankenship, J.M. Olson, M. Miller, Antenna complexes from green photosynthetic bacteria, in: R.E. Blankenship, M.T. Madigan, C.E. Bauer (Eds.), Anoxygenic Photosynthetic Bacteria, Kluwer Academic Publishers, Dordrecht, 1995, pp. 399–435.
[9] J.M. Olson, Chlorophyll organization and function in green photosynthetic bacteria, Photochem. Photobiol. 67 (1998) 61–75.
[10] R.J. van Dorssen, P.D. Gerola, J.M. Olson, J. Amesz, Optical and structural properties of chlorosomes of the photo-

synthetic green sulfur bacterium *Chlorobium limicola*, Biochim. Biophys. Acta 848 (1986) 77–82.
[11] J. Wang, D.C. Brune, R.E. Blankenship, Effects of oxidants and reductants on the efficiency of excitation transfer in green photosynthetic bacteria, Biochim. Biophys. Acta 1015 (1990) 457–463.
[12] N.-U. Frigaard, Light-harvesting Structures in Green Sulfur Bacteria, Ph.D. Thesis, Odense University, Odense, 1997.
[13] R.E. Blankenship, J. Wang, T.P. Causgrove, D.C. Brune, Efficiency and kinetics of energy transfer in chlorosome antennas from green photosynthetic bacteria, in: M. Baltscheffsky (Ed.), Current Research in Photosynthesis, vol. II, Kluwer Academic Publishers, Dordrecht, 1990, pp. 17–24.
[14] R.E. Blankenship, P. Cheng, T.P. Causgrove, D.C. Brune, S.H.-H. Wang, J.-U. Choh, J. Wang, Redox regulation of energy transfer efficiency in antennas of green sulfur bacteria, Photochem. Photobiol. 57 (1993) 103–107.
[15] N.-U. Frigaard, S. Takaichi, M. Hirota, K. Shimada, K. Matsuura, Quinones in chlorosomes of green sulfur bacteria and their role in the redox-dependent fluorescence studied in chlorosome-like bacteriochlorophyll *c* aggregates, Arch. Microbiol. 167 (1997) 343–349.
[16] N.-U. Frigaard, K. Matsuura, M. Hirota, M. Miller, R.P. Cox, Studies of the location and function of isoprenoid quinones in chlorosomes from green sulfur bacteria, Photosynth. Res. 58 (1998) 81–90.
[17] B.P. Yu, Cellular defenses against damage from reactive oxygen species, Physiol. Rev. 74 (1994) 139–162.
[18] K. Asada, Radical production and scavenging in the chloroplasts, in: N.R. Baker (Ed.), Photosynthesis and the Environment, Kluwer Academic Publishers, Dordrecht, 1996, pp. 123–150.
[19] J. Hewitt, J.G. Morris, Superoxide dismutase in some obligately anaerobic bacteria, FEBS Lett. 50 (1975) 315–318.
[20] S. Kanematsu, K. Asada, Crystalline ferric superoxide dismutase from an anaerobic green sulfur bacterium, *Chlorobium thiosulfatophilum*, FEBS Lett. 91 (1978) 94–98.
[21] B.D. Bruce, R.C. Fuller, R.E. Blankenship, Primary photochemistry in the facultatively aerobic green photosynthetic bacterium *Chloroflexus aurantiacus*, Proc. Natl. Acad. Sci. USA 79 (1982) 6532–6536.
[22] T.M. Wahlund, C.R. Woese, R.W. Castenholz, M.T. Madigan, A thermophilic green sulfur bacterium from New Zealand hot springs, *Chlorobium tepidum* sp. nov., Arch. Microbiol. 156 (1991) 81–90.
[23] S. Hanada, A. Hiraishi, K. Shimada, K. Matsuura, *Chloroflexus aggregans* sp. nov., a filamentous phototrophic bacterium which forms dense cell aggregates by active gliding movement, Int. J. Syst. Bacteriol. 45 (1995) 676–681.
[24] K. Matsuura, K. Shimada, Cytochromes functionally associated to photochemical reaction centers in *Rhodopseudomonas palustris* and *Rhodopseudomonas acidophila*, Biochim. Biophys. Acta 852 (1986) 9–18.
[25] P.L. Dutton, Redox potentiometry: determination of midpoint potentials of oxidation-reduction components of biological electron-transfer systems, Methods Enzymol. 54 (1978) 411–434.
[26] H. Oh-oka, M. Iwaki, S. Itoh, Membrane-bound cytochrome c_z couples quinol oxidoreductase to the P840 reaction center complex in isolated membranes of the green sulfur bacterium *Chlorobium tepidum*, Biochemistry 37 (1998) 12293–12300.
[27] K. Shibata, Spectrophotometry of intact biological materials. Absolute and relative measurements of their transmission, reflection and absorption spectra, J. Biochem. 45 (1958) 599–623.
[28] S.C.M. Otte, J.C. van der Heiden, N. Pfennig, J. Amesz, A comparative study of the optical characteristics of intact cells of photosynthetic green sulfur bacteria containing bacteriochlorophyll *c*, *d* or *e*, Photosynth. Res. 28 (1991) 77–87.
[29] K.-I. Takamiya, The light-induced oxidation-reduction reactions of menaquinone in intact cells of a green photosynthetic bacterium, *Chloropseudomonas ethylica*, Biochim. Biophys. Acta 234 (1971) 390–398.
[30] N.V. Karapetyan, T. Swarthoff, C.P. Rijgersberg, J. Amesz, Fluorescence emission spectra of cells and subcellular preparations of a green photosynthetic bacterium. Effects of dithionite on the intensity of the emission bands, Biochim. Biophys. Acta 593 (1980) 254–260.
[31] W. Zhou, R. LoBrutto, S. Lin, R.E. Blankenship, Redox effects on the bacteriochlorophyll *a*-containing Fenna-Matthews-Olson protein from *Chlorobium tepidum*, Photosynth. Res. 41 (1994) 89–96.
[32] C. Francke, S.C.M. Otte, M. Miller, J. Amesz, J.M. Olson, Energy transfer from carotenoid and FMO-protein in subcellular preparations from green sulfur bacteria. Spectroscopic characterization of an FMO-reaction center core complex at low temperature, Photosynth. Res. 50 (1996) 71–77.
[33] H. Oh-oka, S. Kamei, H. Matsubara, S. Lin, P.I. van Noort, R.E. Blankenship, Transient absorption spectroscopy of energy-transfer and trapping processes in the reaction center complex of *Chlorobium tepidum*, J. Phys. Chem. B 102 (1998) 8190–8195.
[34] B. Kjær, Y.-S. Jung, L. Yu, J.H. Golbeck, H.V. Scheller, Iron-sulfur centers in the photosynthetic reaction center complex from *Chlorobium vibrioforme*. Differences from and similarities to the iron-sulfur centers in photosystem I, Photosynth. Res. 41 (1994) 105–114.
[35] M.P. Scott, B. Kjær, H.V. Scheller, J.H. Golbeck, Redox titration of two [4Fe-4S] clusters in the photosynthetic reaction center from the anaerobic green sulfur bacterium *Chlorobium vibrioforme*, Eur. J. Biochem. 244 (1997) 454–461.
[36] D.A. Shill, P.M. Wood, Light-driven reduction of oxygen as a method for studying electron transport in the green photosynthetic bacterium *Chlorobium limicola*, Arch. Microbiol. 143 (1985) 82–87.