

The photosynthetic apparatus and photoinduced electron transfer in the aerobic phototrophic bacteria *Roseicyclus mahoneyensis* and *Porphyrobacter meromictius*

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Abstract Photosynthetic electron transfer has been examined in whole cells, isolated membranes and in partially purified reaction centers (RCs) of *Roseicyclus mahoneyensis*, strain ML6 and *Porphyrobacter meromictius*, strain ML31, two species of obligate aerobic anoxygenic phototrophic bacteria. Photochemical activity in strain ML31 was observed aerobically, but the photosynthetic apparatus was not functional under anaerobic conditions. In strain ML6 low levels of photochemistry were measured anaerobically, possibly due to incomplete reduction of the primary electron acceptor (Q_A) prior to light excitation, however, electron transfer occurred optimally under low oxygen conditions. Photoinduced electron transfer involves a soluble cytochrome *c* in both strains, and an additional reaction center (RC)-bound cytochrome *c* in ML6. The redox properties of the primary electron donor (P) and Q_A of ML31 are similar to those previously determined for other aerobic phototrophs, with midpoint redox potentials of +463 mV and –25 mV, respectively. Strain ML6 showed a very narrow range of ambient redox potentials appropriate for photosynthesis, with midpoint redox potentials of +415 mV for P and +94 mV for Q_A . Cytoplasm soluble and photosynthetic complex bound

cytochromes were characterized in terms of apparent molecular mass. Fluorescence excitation spectra revealed that abundant carotenoids not intimately associated with the RC are not involved in photosynthetic energy conservation.

Keywords *Roseicyclus mahoneyensis* · *Porphyrobacter meromictius* · Aerobic anoxygenic phototroph · Photosynthetic apparatus · Electron transport

Introduction

Obligate aerobic phototrophic bacteria (APB) synthesize bacteriochlorophyll (BChl) *a* incorporated into photosynthetic reaction center (RC) and light harvesting (LH) complexes, however, unlike those found in typical anoxygenic phototrophic bacteria, these pigment protein complexes are incapable of light-induced electron (e^-) transfer under anaerobic conditions (Yurkov and Beatty 1998). In earlier studies, it was found that e^- transfer in APB could be distinguished into two separate types based on the mode of the photooxidized primary e^- donor (P^+) re-reduction. The first one involved a cytochrome (cyt) *c* intimately bound to the RC, allowing for very fast re-reduction of the P^+ . Such e^- transfer has been observed in *Erythromonas ursincola*, *Sandaracinobacter sibiricus*, *Roseococcus thiosulfatophilus* and *Roseobacter denitrificans* (Garcia et al. 1994; Yurkov et al. 1998b; Yurkov and Beatty 1998). In the second type, found in all tested species of *Erythromicrobium* and in *Erythrobacter longus*, no RC-bound cyt was present and P^+ was re-reduced through transfer of an e^- directly from a soluble cyt *c* (Yurkov et al. 1998b). In the case of *Erythrobacter litoralis*, the oxidation of the soluble cyt *c* was relatively slow, further differentiating its photochemistry from other APB (Yurkov et al. 1998b). In each of these cases the path of e^-

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transfer closely resembles those discovered in anaerobic phototrophic bacteria (Yurkov and Beatty 1998), involving the participation of a cyt *bc*₁ complex to complete a light-induced cyclic electron transfer. However, the photosynthetic e^- transport chain in APB was catalytically active only in the presence of oxygen, i.e. under relatively oxidized conditions (Yurkov and Beatty 1998; Rathgeber et al. 2004). A number of speculations have been proposed to explain this difference. (1) The relatively high redox potential of the RC primary e^- acceptor (Q_A) observed in APB (*Erb. litoralis*, *Erm. ursincola*, *S. sibiricus*, *Rsc. thiosulfatophilus*, and *Rba. denitrificans*) (Yurkov et al. 1998a; Rathgeber et al. 2004), results in fully reduced Q_A under anaerobic conditions, leaving it incapable of accepting an e^- and participating in e^- transport (Rathgeber et al. 2004). (2) The redox state of cyt in the e^- transport chain may be responsible for the lack of photochemistry anaerobically (Garcia et al. 1994). Under reduced conditions the low potential hemes of the *Rba. denitrificans* RC-bound tetraheme cyt were capable of re-reducing P^+ , but the hemes may not be re-reduced by the soluble cyt *c* (Schwarze et al. 2000; Rathgeber et al. 2004). Absence of e^- transfer from the soluble cyt to the low potential hemes of the RC-bound cyt results in an incomplete e^- transfer chain. However, this speculation could not be applied to all APB and immediately raised the question: What about species that do not produce a tightly bound tetraheme cyt in the RC? Does the redox potential of a soluble cyt restrict e^- transfer anaerobically? Or is there an alternative low potential soluble cyt that is capable of re-reducing P^+ , but cannot itself be re-reduced by the cyt *bc*₁ complex? Therefore, the major scientific question in regards to the name-sake feature of APB (the inability to grow anaerobically by photosynthesis) remains without a confirmed answer.

Roseicyclus mahoneyensis, ML6 and *Porphyrobacter meromictius*, ML31 are interesting examples of APB isolated from the meromictic Mahoney Lake in British Columbia, Canada (Yurkova et al. 2002). *R. mahoneyensis*, phylogenetically related to the α -3-*Proteobacteria*, is unusual in its tendency to form vibrioid cells, and to produce a rare type of LH II, previously known only in the aerobic *Roseobacter* and *Rubrimonas* (Rathgeber et al. 2005) and the anaerobic purple non-sulfur bacterium *Rhodospseudomonas palustris* (Hartigan et al. 2002). The monomodal LH II (LL B800) of *Rps. palustris* is controlled by a bacteriophytochrome and produced only under low light conditions (Evans et al. 2005), however, this has not been shown for *R. mahoneyensis*, in which BChl *a* production is strongly inhibited by light (Rathgeber et al. 2005). *P. meromictius* is a halotolerant prosthecate bacterium, branching within the α -4-*Proteobacteria*, which produces a relatively large quantity of BChl as compared to other APB (Yurkova et al. 2002; Rathgeber et al. 2007). In the current study, we examine the organization and

function of the photosynthetic apparatus of these species to better understand the aspects of photosynthesis in APB.

Materials and methods

Growth conditions

Batch cultures of *R. mahoneyensis* were grown at 30°C in a dark shaking incubator (170 rpm) in medium N1 (Yurkova et al. 2002). *P. meromictius* was grown under the same conditions using a modification of medium N1 containing in $g\ l^{-1}$: $MgSO_4$, 2.0; KH_2PO_4 , 0.3; NH_4Cl , 0.3; KCl , 0.3; $CaCl_2$, 0.05; $NaSO_4$, 50.0; sodium acetate, 1.0; adjusted to pH 7.8–8.0 and supplemented with 2 ml each of vitamin and trace element solutions (Yurkova et al. 2002).

Isolation of soluble cyt, membranes, and pigment protein complexes

Cells were harvested at the end of the exponential growth phase, washed with 10 mM Tris–HCl buffer, pH 7.8 and concentrated by centrifugation. To 100 ml of a thick cell suspension were added: 1 ml of 10% EDTA in Tris–HCl, pH 7.8; 1 ml of 1 mM PMSF in 95% ethanol; 0.05 g of DNAase (Fisher Scientific). Cells were then disrupted by three passes through a French Press at 20K PSI. Unbroken cells and debris were removed by centrifugation at 12K×g for 20 min. The supernatant was centrifuged again at 150K×g for 12 h (strain ML31) or 15 h (strain ML6) to separate soluble from membrane fractions. The supernatant was reserved and soluble cyt were collected by passing the soluble fraction through a 100 kDa YM ultrafiltration membrane under a stream of nitrogen.

Cell membranes were homogenized in 10 mM Tris–HCl, pH 7.8 and purified by sucrose density gradient centrifugation (0.9–1.8 M sucrose in 10 mM Tris–HCl, pH 7.8) at 38K rpm for 15–16 h. Individual bands were removed from centrifuge tubes using Pasteur pipettes and absorbance spectra were measured to identify photosynthetic membranes (PSM) (presence of BChl peaks at 800 and 870 nm), which were collected, centrifuged at 150K×g to sediment and re-suspended in fresh buffer.

Pigment protein complexes were isolated from PSM of ML31 by treatment with 0.6% LDAO at room temperature for 20 min in the dark followed by sucrose density gradient centrifugation (0.2–0.9 M sucrose in 10 mM Tris–HCl, pH 7.8 containing 0.05% LDAO) at 38K rpm for 15–16 h. Colored bands were removed and absorbance spectra measured to identify fractions containing LH–RC complexes. Sucrose and LDAO were removed by three 8-h dialyses in Tris–HCl, pH 7.8 and concentrated by packing dialysis tubing in Polyethylene glycol-6000 for 2 h. The LH–RC

complexes of ML31 were relatively unstable when purified and partially degraded into RC and free BChl after dialysis (see “[Results and discussion](#)” section for details). These RCs were further purified by anion exchange chromatography on a DEAE cellulose column equilibrated with Tris–HCl, pH 7.8 (0.05% LDAO added). Fractions containing RC were collected and dialyzed to wash and concentrate, as above.

Membranes of strain ML6 were treated with 8% Triton X-100 at room temperature for 30 min to release LH–RC complexes followed by sucrose density gradient centrifugation (0.3–1.0 M sucrose in 10 mM Tris–HCl, pH 7.8; 0.05% Triton X-100) and processed as described for ML31.

Analytical methods

The presence of cyt and their molecular weights were determined by SDS-PAGE on a 15% acrylamide/bis-acrylamide gel or on an 11–17% linear gradient acrylamide/bis-acrylamide gel, stained using 3,3',5,5'-tetramethylbenzidine (TMBZ) in the presence of hydrogen peroxide as described (Thomas et al. 1976).

Spectrophotometric procedures

Absorbance spectra were recorded at room temperature using a Hitachi U-2010 spectrophotometer. Light-induced absorbance spectra were measured in whole cells resuspended in 10 mM Tris–HCl pH 7.8 to which was added bovine serum albumin (BSA) (Fisher Scientific) at a final concentration of 30%. PSM and pigment protein complexes using equipment similar to that described by Joliot et al. (1980), with excitation provided by xenon flash bulbs (Yurkov et al. 1998a). Redox titrations of (P/P^+) and (Q_A/Q_A^-) were performed using whole PSM suspended in 10 mM Tris–HCl, pH 7.8. Redox mediators used included ferrocenecarboxylic acid; 1,1'-dimethylferrocene; tetrachloro-1,2-benzoquinone; 1,2-naphthoquinone; trimethylhydroquinone; and 2-methyl-1,4-naphthoquinone (menadione) all dissolved in ethanol; and potassium ferricyanide(III); hexaamineruthenium(II) chloride; 2-hydroxy-1,4-naphthoquinone; and anthraquinone-2-sulfonic acid all in water. The solutions were prepared at a concentration of 10–20 μ M with 100 mM KCl added for conductivity. Fluorescence excitation spectra were calculated by measuring fluorescence between 830 and 890 nm produced by excitation over a series of wavelengths from 350 to 650 nm.

Results and discussion

Isolation of membranes and purification of pigment protein complexes

Membranes were separated from cells of both strains, ML31 and ML6. PSM of ML31 appeared as a dark-red

band migrating to approximately 1.7 M sucrose, whereas membranes of ML6 appeared dark-purple and migrated to about 0.5 M sucrose. Both strains produced only membranes containing pigment protein complexes. Other membranes, not containing BChl, were not detected. Considering the APB characteristic lack of an intracytoplasmic membrane (ICM) system (Yurkov and Beatty 1998; Rathgeber et al. 2005, 2007), this confirms that the photosynthetic apparatus is indeed located directly in the cytoplasmic membrane. This differs from results found for *Erythromicrobium ramosum* and *Rsc. thiosulfatophilus*, which produced two distinct membrane fractions (Yurkov et al. 1994). The LH–RC complexes were located mainly (*Rsc. thiosulfatophilus*), or wholly (*E. ramosum*) in one fraction indicating a discontinuous organization of the membrane despite the lack of obvious ICM.

The LH–RC complexes of ML31 were separated by treatment of dense membrane suspensions with 0.6% LDAO at room temperature, resulting in liberated LH–RC that migrated to 0.7 M sucrose during density gradient centrifugation. The LH–RC fraction was collected and dialyzed to remove sucrose and detergent (Fig. 1a). After dialysis, most LH–RC complexes had degraded leaving RC intact and free BChl of the decomposed LH I. This is unusual as a second, stronger detergent treatment is typically required to release RC from the LH (Yurkov et al. 1998a), and indicates that the LH antennae of ML31 is very fragile, unstable in solution and may be less tightly associated with the RC than seen in other APB. The liberated and still intact RC component was further purified by anion exchange chromatography on a DEAE cellulose column equilibrated to pH 7.5. The RC collected after chromatography were stable in a 10 mM Tris–HCl solution at pH 7.8, containing 0.05% LDAO (to solubilize complexes) and could be stored at -20°C (Fig. 1b).

Membranes of strain ML6 proved extremely resilient to detergent, remaining intact after treatment with concentrations up to 0.6% LDAO. Treatment with 1% LDAO solubilized membranes but resulted in the destruction of pigment protein complexes, leaving only small amounts of intact RC–LH I contaminated with large amounts of free BChl. However, solubilization of membranes using 8% Triton X-100 incubated for 30 min at room temperature was sufficient to solubilize membranes while leaving RC–LH I complexes intact. The unusual monomodal LH II (806 nm) of ML6 (Fig. 1d) (Rathgeber et al. 2005) was very sensitive to both detergents tested, and degraded to free BChl. Such instability of *Roseicyclus*' monomodal LH II was unexpected because spectrophotometrically similar monomodal LH II of *Roseobacter* resisted purification procedures (Shimada et al. 1985). The difference may arise from variations in amino acid sequence, alignment factors or from specific interacting configurations of the LH I

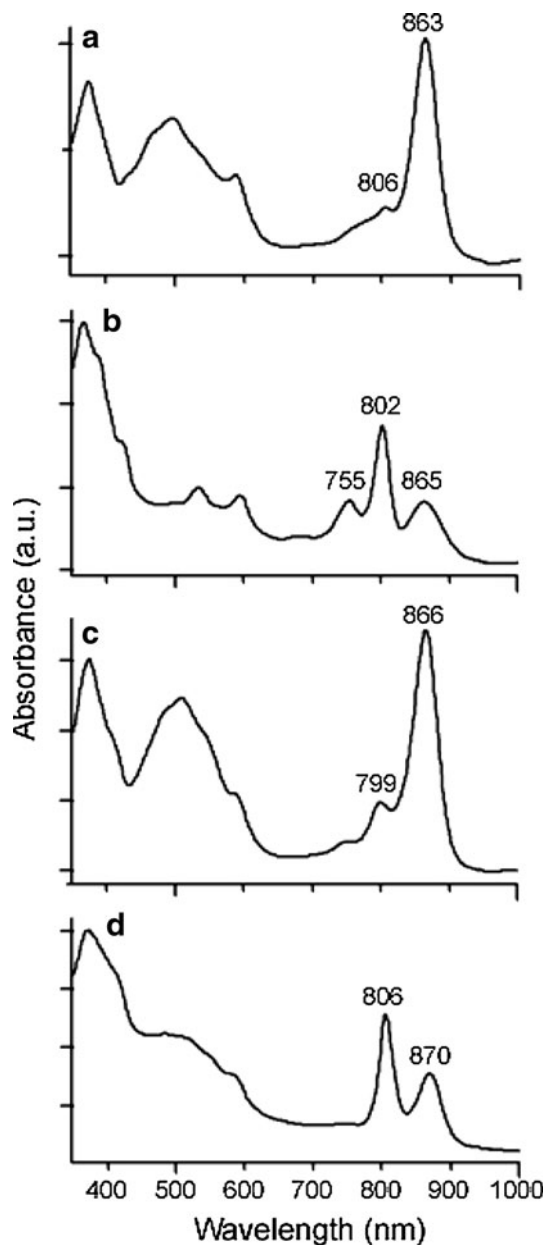


Fig. 1 Absorbance spectra of ML31. **a** Partially purified reaction center-light harvesting complexes, **b** reaction centers, and **c** ML6 reaction center-light harvesting I complexes, suspended in 10 mM Tris-HCl pH 7.8 containing 0.05% LDAO. **d** Intact cells of ML6 show light harvesting complex I (870 nm) and the monomodal light harvesting complex II (806 nm)

components. The detergent resistant RC-LH I complexes were further purified through sucrose density gradient centrifugation (migrated to 0.3 M sucrose) followed by dialysis to remove sucrose and detergent. Final purification was achieved by DEAE cellulose column chromatography as described above, using 0.05% LDAO to maintain solubility of the proteins. The RC-LH I collected after chromatography remained stable in 10 mM Tris-HCl solution

at pH 7.8 containing 0.05% LDAO and could be stored at -20°C (Fig. 1c).

Reaction center-bound tetraheme cytochrome

Reduction of P^+ in most anoxygenic phototrophs does not proceed directly through a soluble cyt (Meyer and Cusanovich 2003). Instead a cyt *c* intimately associated with the RC is responsible for donating e^- to P^+ , and is in turn re-reduced by the soluble cyt. The bound cyt is generally tetrahemic, containing two high, and two low potential hemes, making the molecule fairly large. In APB the size ranges from 37.0 kDa (*S. sibiricus*) to 44.0 kDa (*Rsc. thiosulfatophilus*) (Yurkov and Beatty 1998). To detect RC-bound cyt in our strains, we utilized TMBZ staining of RC polypeptides.

Reduced minus oxidized difference spectra showed the absence of a bound cyt in the RC of ML31, as was also found in light-induced absorbance spectra. As expected, TMBZ staining of gels did not detect the presence of a heme-containing cyt. As discussed below, light-induced absorbance spectra indicated the RC-bound cyt in ML6, which was also revealed by redox difference spectra and conclusively determined by TMBZ (Fig. 2). The cyt had a MW of about 48 kDa, which was in the range of MW reported for known RC-bound cyt (Yurkov et al. 1998b).

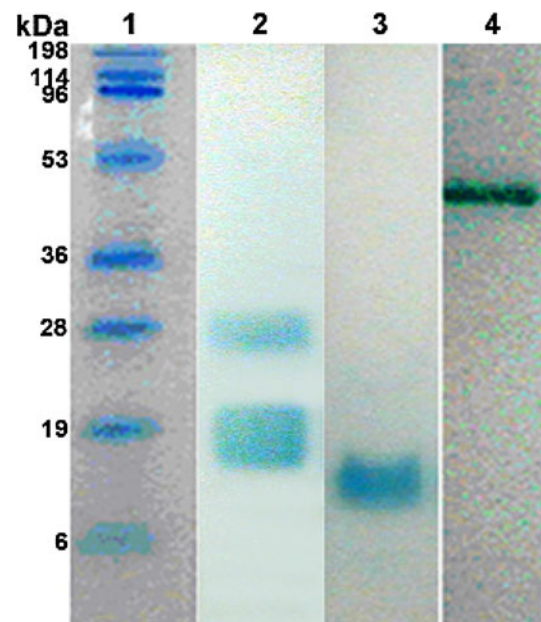


Fig. 2 Polyacrylamide gel electrophoresis of the soluble fractions of ML31 (lane 2) and ML6 (lane 3), and light harvesting I-reaction center complexes of ML6 (lane 4) stained with TMBZ heme stain. Standard ladder is in lane 1

Light-induced e^- transfer

We measured photoinduced absorbance difference spectra in whole cells, PSM and isolated pigment protein complexes of both strains to evaluate their potential for e^- transfer after excitation, and to determine the effect of oxygen on the ability to carry out photosynthesis. Under aerobic conditions the photooxidation of the P^+ in ML31 was observed 50 μ s after an actinic flash confirmed by absorbance changes at 435 and 603 nm (Fig. 3a). In spectra measured 1 and 20 ms after excitation, these changes had been replaced by troughs at 422 and 552 nm, attributed to the oxidation of a c type cyt. The relatively slow re-reduction of the P^+ indicated that e^- were transferred directly from cyt c to P^+ as has been demonstrated for APB of the genera *Erythromicrobium* and *Erythrobaacter* (Yurkov et al. 1998b) and in agreement of the absence of RC-bound cyt observed for the isolated RC.

To calculate the rate of e^- transfer, a kinetic of cyt oxidation was recorded, which showed the half-time of e^-

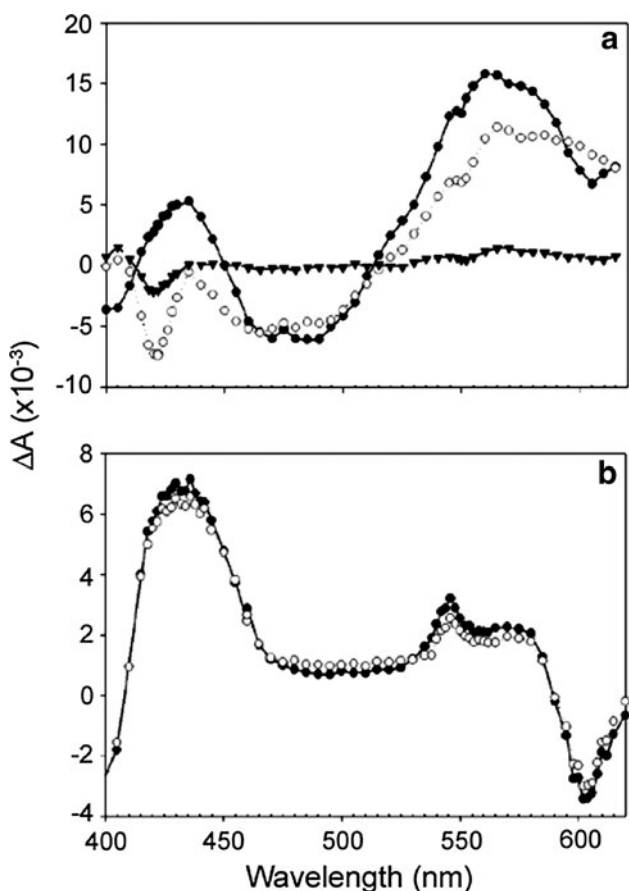


Fig. 3 Light-induced absorbance difference spectra recorded in ML31. **a** Whole cells under aerobic conditions taken at: filled circle 50 μ s, open circle 1 ms, and triangle 20 ms after excitation; and **b** membranes in the presence of 5 mM sodium ascorbate: filled circle 50 μ s and open circle 500 ms after excitation

transfer between the soluble cyt and P^+ to be 170 μ s and the half-time of cyt re-reduction, 10 ms (Fig. 4a). The re-reduction rate was comparable to that reported in whole cells of the genus *Erythromicrobium* (Yurkov et al. 1998b) and *Erb. longus* (Okamura et al. 1984) and indicative of a cyclic electron transfer involving a cyt bc_1 complex.

Light-induced absorbance spectra of ML31 PSM under ambient (oxidized) conditions revealed a strong P^+ signal at 430 and 603 nm, 50 μ s after excitation. This signal remained stable 500 ms after excitation, indicating absence of re-reduction and confirming that re-reduction of the P^+ should be mediated by a soluble cyt that had been washed out during membrane preparation and not by a tetrahemic RC-bound cyt. When a similar experiment was performed under reduced conditions (addition of 5 mM sodium ascorbate), results were the same as seen under ambient conditions, supporting that the absence of P^+ re-reduction was due to the absence of cyt (Fig. 3b). The absence of a RC-bound cyt was further revealed in the absorbance spectra of membranes, and light-induced difference spectra of isolated RC taken under both oxidized and reduced conditions. In absorbance spectra there was no change in characteristics due to the reduction of a RC-bound cyt upon addition of either sodium ascorbate or dithionite.

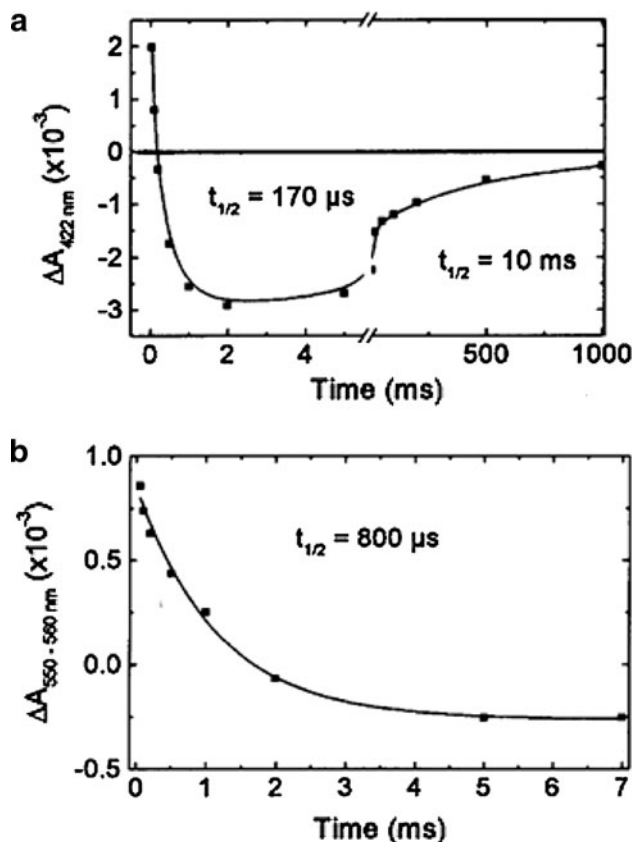


Fig. 4 Kinetic of soluble cytochrome oxidation in whole cells of **a** ML31 and **b** ML6 after excitation (time = 0)

In light-induced difference spectra a signal corresponding to oxidation of P^+ at 50 ms slowly faded over 200 ms, and no signal of cyt oxidation was detected (Figure not shown).

Under anaerobic conditions, no light-induced absorbance changes could be detected, consistent with the strain's inability to photosynthesize under anaerobic conditions and is similar to behavior of other APB (Yurkov and Beatty 1998; Rathgeber et al. 2004).

In whole cells of ML6, light-induced difference spectra recorded under fully aerobic conditions revealed the presence of photooxidized cyt (difference at 422 and 556 nm) superimposed on a signal due to a photooxidized P^+ (603 nm) (Fig. 5a). The P^+ signal (603 nm) remained stable 20 ms after excitation. It appears that for some of the

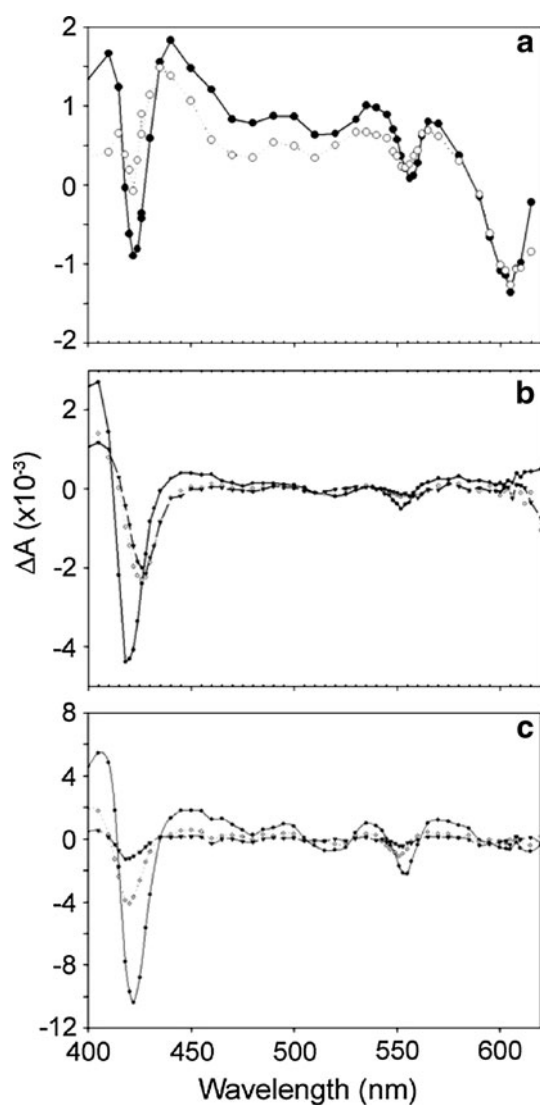


Fig. 5 Light-induced absorbance difference spectra in ML6 whole cells recorded under **a** aerobic, **b** anaerobic, and **c** partially aerobic conditions: *filled circle* 50 μ s, *open circle* 20 ms and *triangle* 50 ms after excitation (**b**, **c** only)

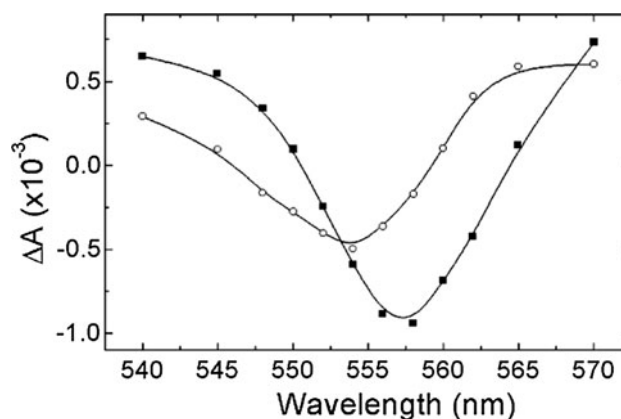


Fig. 6 Light-induced absorbance spectra recorded in ML6 whole cells under partially aerobic conditions: *square* 50 μ s, *circle* 5 ms after excitation

RCs, the e^- transfer was ineffective, possibly caused by partial oxidation of cyt before excitation. For the other RCs, the P^+ was re-reduced in less than 50 μ s by a cyt absorbing around 557 nm which was in turn re-reduced by a cyt absorbing around 553 nm (Fig. 6). This suggests that the RC contains a RC-bound cyt. The P^+ is re-reduced in less than 50 μ s by the high mid-point potential hemes of the tetrahemic cyt which is, in turn, re-reduced by a soluble cyt. When difference spectra were taken under anaerobic conditions, cells were capable of photosynthetic e^- transfer involving a very fast re-reduction of P^+ , giving rise to a difference signal at 422 and 552 nm due to the oxidation of cyt 50 ms after excitation (Fig. 5b). This probably reflects the photooxidation of one of the low potential hemes of the RC-bound cytochrome. The signal, however, was weak indicating that the Q_A may already be partially reduced because of the anaerobic conditions before the excitation flash. Moreover, the re-reduction of the photooxidized cyt was slow.

To better understand the effect of oxygen on photosynthetic e^- transfer in ML6, we analyzed the degree of e^- transfer efficiency as a growing culture respired (and thus removed) available O_2 . Flash-induced absorbance changes were measured in intact cells within a closed cuvette. The active cells consumed O_2 from the medium resulting in its gradual decrease over time. When O_2 levels had been lowered below a certain threshold, the efficiency of photoinduced e^- transfer increased, reaching a maximal activity after 56 min of incubation. With longer incubation, and consistently lower O_2 concentrations, e^- transfer was gradually inhibited (Fig. 7). The pattern of changing efficiency of cyt photooxidation over the time of incubation was attributed to a narrow range of O_2 concentration that supported maximal photosynthetic e^- transfer in living cells, which presumably resulted in optimal redox poise of the e^- carriers. These data correlate with the proposal that

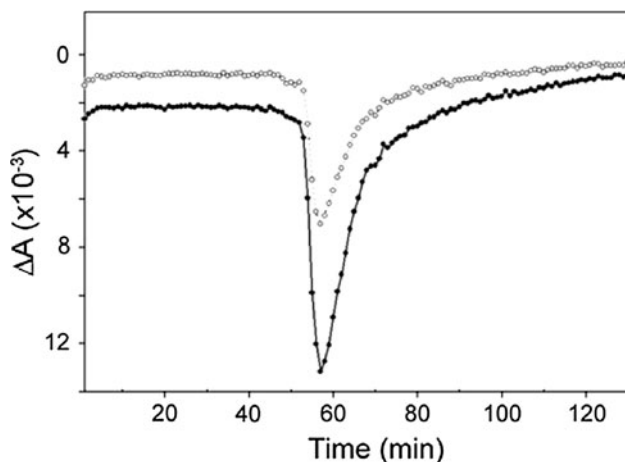


Fig. 7 Flash-induced absorbance differences measured at 422 nm, recorded: *filled circle* 50 μ s and *open circle* 20 ms after excitation every 1 min as cells respired oxygen from the cuvette

the photosynthetic apparatus of ML6 is fully functional only under semi-aerobic conditions.

Flash-induced experiments run on partially (instead of fully) aerobic cells yielded significantly different results. Maximum photochemical activity was obtained under partial aerobiosis by waiting a period of time (between 50 and 60 min) for active cells to respire some of the O_2 to create microaerophilic conditions. A simulated partial aerobiosis could also be achieved by inhibiting respiration of the cells using a low concentration (2 mM) of KCN. In the partially aerobic state, light-induced e^- transfer was maximal with clear differences due to cyt oxidation (422 and 556 nm) 50 μ s after the flash (Fig. 5c). At this time point, P^+ signal was absent. The very fast rate of P^+ re-reduction, indicated the presence of a cyt bound tightly to the RC. A shift from 557 to 553 nm occurs in less than 5 ms (Fig. 6) These results are consistent with an e^- transfer between the high potential hemes of the cytochrome followed by an e^- transfer to a soluble cyt. The signal then faded as the soluble cyt was re-reduced, presumably by the cyt bc_1 complex. The rate of electron transfer between the RC-bound cyt and the soluble cyt occurs with a half-time of about 800 μ s (Fig. 4b), somewhat slower than the rates measured for *Rhodospseudomonas viridis* (Garcia et al. 1993) or *Rubrivivax gelatinosus* (Schoepp et al. 1995).

The above results were supported by measuring light-induced difference spectra in ML6 membranes. Under ambient (oxidized) conditions e^- transfer reactions were absent. Cyt were oxidized before the flash resulting in a P^+ signal at 50 μ s that stayed stable through 500 ms and no cyt oxidation peaks were noticed (Fig. 8a). Under reduced conditions (addition of 5 mM Na-ascorbate), e^- transfer reactions were partially functional, confirming the presence

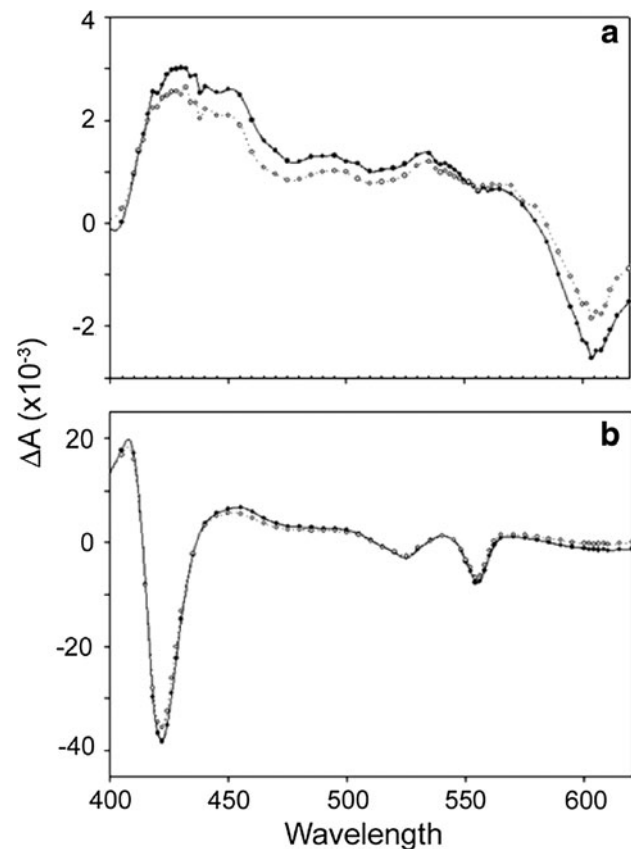


Fig. 8 Flash-induced absorbance spectra of ML6 membranes under **a** ambient conditions and **b** reduced conditions recorded: *filled circle* 50 μ s and *open circle* 500 ms after excitation

of a RC-bound cyt (troughs at 422 and 555 nm) (Fig. 8b). Absence of a difference shift to 552 nm after 20 ms agreed with the shift seen in whole cells due to the RC-bound cyt re-reduction by a soluble cyt washed out in membrane preparations.

Redox potentials of the P^+ and Q_A

To better understand the effect of oxygen on photoinduced e^- transfer, the redox potentials of the P^+ and Q_A were titrated in membranes suspended in 10 mM Tris-HCl pH 7.8. Flash-induced spectral differences were recorded at 605 nm (to detect P^+) and measurements were taken 50 μ s after excitation for ML31, and 100 ns and 50 μ s for ML6, over a range of ambient redox potentials from about -300 to $+490$ mV versus a standard hydrogen electrode at pH 7.8. ML31 midpoint potentials for the Q_A and P^+ were -25 and $+463$ mV, respectively (Fig. 9a). The redox potential of Q_A is somewhat lower than found in other APB, which ranges from $+5$ to $+150$ mV (Yurkov et al. 1998a; Yurkov and Beatty 1998). However, values of -44 and -50 mV have been obtained by Takamiya et al. (1987) and by Schwarze et al. (2000), respectively, for the typical

APB species *Rba. denitrificans*. These values are not different from those found for *Rhodobacter sphaeroides* or *Rhodobacter capsulatus*, two typical anaerobic photosynthetic bacteria (Prince and Dutton 1978).

In the case of ML6, measurements taken 100 ns after excitation showed the midpoint potential of Q_A/Q_A^- to be +94 mV and of the P/P^+ , +415 mV. The midpoint redox potential of the RC-bound cyt was +277 mV as there was no P^+ signal detected 50 μ s after excitation at redox potentials lower than +277 mV (Fig. 9b). Apparently, at redox potentials above +94 mV, the Q_A was oxidized before the flash and capable of accepting e^- from the P^+ , but above +277 mV the RC-bound cyt was oxidized before the flash and could not re-reduce P^+ . Therefore, ML6 was capable of light-induced e^- transfer reactions only under a very narrow range of ambient redox potentials (between +94 and +277 mV). Such peculiar limitation likely accounted for ML6's ability to photosynthesize only semi-aerobically.

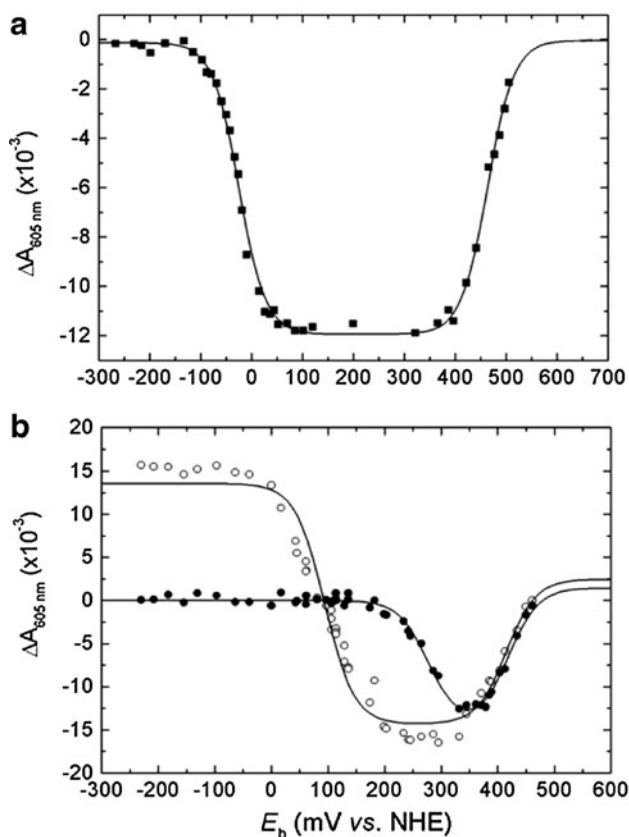


Fig. 9 Light-induced absorbance changes measured at 605 nm **a** 50 μ s (square) after an excitation flash in membranes of ML31, and **b** 100 ns (open circle) and 50 ms (filled circle) after excitation in membranes of ML6, as a function of ambient redox potential (E_h). Fully reduced conditions were achieved by addition of 10 mM $Na_2O_4S_2$, and the solutions were oxidized in increments by addition of small aliquots of 10 mM $FeK_3(CN)_6$, resulting in maximal E_h of 490 mV. The data were fitted using $n = 1$ Nernst curves

According to published reports, the values of the midpoint potentials for the Q_A of other APB species lie between -50 and $+150$ mV. Some are not very different from those obtained from typical anaerobic photosynthetic bacteria. It therefore seems unlikely that the inability of APB to grow under anaerobic conditions is uniquely linked to the redox properties of Q_A . This could, in part, be due to the lack of Rubisco or other NADH-driven major CO_2 -fixation pathway, which could result in the inability to dispose of excess reductant by reverse electron flow from the Q pool to reduce NAD to NADH (no way to dispose of NADH under anaerobic conditions) (Csotonyi and Yurkov 2009). One of us has observed (unpublished results) that a large portion of Q_A is reduced under anaerobic conditions in the typical anaerobic photosynthetic bacterium *Rhb. sphaeroides*. Full photochemical activity could be restored by a short continuous illumination. The re-oxidation of Q_A is linked to a reverse e^- flow from the quinone pool to NAD^+ at complex I. This reverse e^- flow is produced by the light-induced membrane potential driven by the remaining photoactive RCs. In the case of ML6 and ML31, a similar restoration of the photochemical activity by continuous light under anaerobic conditions has not been observed. This could be due to the low amount of RC possessed by APB or the incapacity of complex I to establish a reverse e^- flow.

Photosynthetic function of carotenoids

Carotenoid pigments are found in photosynthetic and non-photosynthetic prokaryotes and eukaryotes and determine the color of organisms. In ML31 and ML6 these pigments contribute to the vibrant colors, red and purple, respectively. Carotenoids can play three major roles in photosynthesis: (1) Contribute structurally to antenna complexes. (2) Act as accessory LH pigments, allowing the use of light in the blue-green and yellow regions of the spectrum (Fraser et al. 2001) that is not absorbed by BChl. (3) Confer protection of the photosynthetic apparatus from triplet oxygen formed under illuminated aerobic conditions when excited BChl reacts with O_2 . Carotenoids have been shown to quench O_2 radicals and the excited BChl triplet itself (Fraser et al. 2001).

The APB are known to produce large quantities of carotenoids relative to BChl, and in significant excess of that normally described in typical anaerobic anoxygenic phototrophs (Yurkov and Beatty 1998). The role of these abundant carotenoids is poorly understood. To act in photoprotection they can be either in direct contact with BChl to quench chlorophyll triplets effectively (Fraser et al. 2001), or involved in singlet oxygen scavenging when distributed throughout the cell and not associated with the photosynthetic apparatus (Yurkov and Beatty 1998;

Yurkov and Csotonyi 2009). To investigate the potential for transfer of light excitation energy from carotenoids to the RC (photosynthetic function), fluorescence excitation measurements on whole cells, membranes and purified RC of ML31, and on whole cells, membranes and LH I-RC complexes of ML6, were performed.

Cells and membranes illuminated by light of 350–650 nm fluoresce, in part, because of the excitation energy transfer from carotenoids to BChl. Light energy that cannot be used for photosynthesis is then emitted by the BChl, between 830 and 890 nm. By comparing Fig. 1d (ML6 whole cells absorbance spectrum) to Fig. 1c (ML6 RC-LH I spectrum) it can be observed that there is a much higher amount of carotenoids in the 400 nm range in whole cells than the amount of carotenoids in the same range associated with the photosynthetic apparatus. This allows us to conclude that there is a significant portion of pigments that are not associated with the photosynthetic complexes. Looking at Fig. 10b (fluorescence excitation spectrum of ML6), particularly the solid line which represents whole cells, the spectrum is almost identical to that of Fig. 1c indicating that only the carotenoids associated with the photosynthetic complexes are involved in photochemical activity. Therefore, the extra carotenoids we see in the absorbance spectrum of whole cells (we know these pigments are not associated with the photosynthetic complexes) are not involved in photosynthesis. The same holds true for ML31 which also has a high amount of carotenoids seen in whole cell absorbance which are not present in RC-LH spectra. Our results correlated with those found previously for other APB. Quantum yields of singlet energy transfer showed the majority of carotenoids did not add to light harvesting in *E. ramosum* and *Rsc. thiosulfatophilus* (Yurkov et al. 1994). Likewise, in *Erb. longus* more than 70% of total carotenoids did not participate in photosynthesis (Noguchi et al. 1992). As most carotenoids are disengaged from energy transduction and evenly distributed throughout the cell, their function is uncertain. Possibly they serve as antioxidants, removing O₂ radicals of non-photosynthetic origin, or they act to filter high intensities of blue light, helping to minimize photodamage during periods of exposure to intense solar radiation (Yurkov and Beatty 1998). Many APB are especially well endowed with polar carotenoids (Yurkov and Csotonyi 2003). Acquisition of the latter, which are particularly antioxidative (Krinsky 1979) is a logical adaptation to illuminated oxic environments.

Electron carriers of the soluble fraction

In all APB tested thus far the periplasmic soluble e^- carrier involved in the connection between the RC and the *cyt bc*₁ was a soluble *cyt c*₂ (Yurkov et al. 1998b), however, in

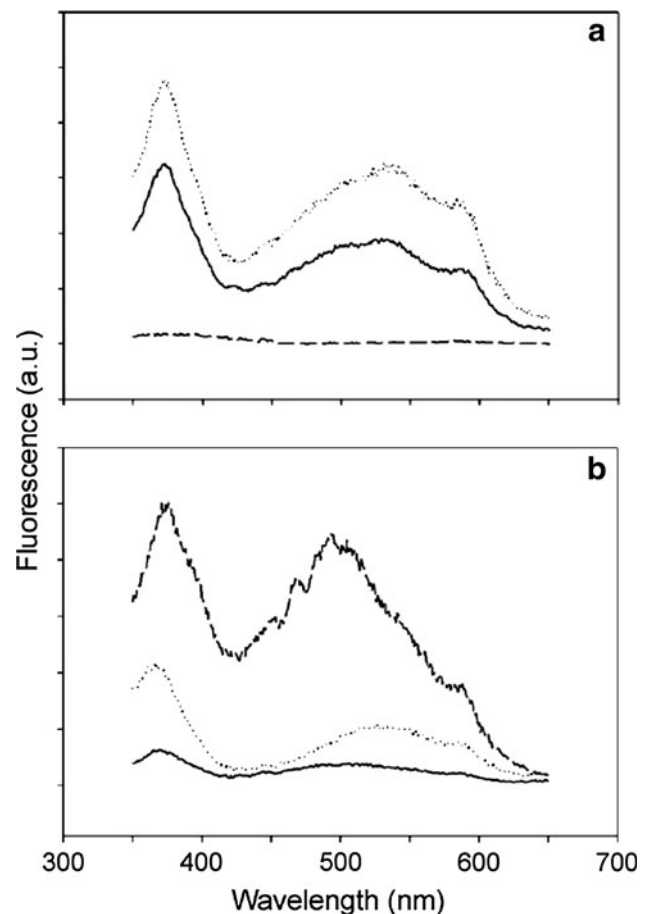


Fig. 10 Fluorescence excitation spectra of: **a** ML31 whole cells (solid line), membrane fragments (dotted line), and purified reaction centers (dashed line) and **b** ML6 whole cells (solid line), membrane fragments (dotted line), and purified light harvesting I-reaction center complexes (dashed line). Fluorescence was measured between 830 and 890 nm produced after excitation between 350 and 650 nm

anaerobic phototrophs alternative e^- carriers such as high potential iron sulfur proteins and *cyt c*₈ can also fulfill this function (Meyer and Cusanovich 2003). *Cyt c*₂ might serve as the immediate e^- donor to P⁺, as in the genera *Erythrobacter* and *Erythromicrobium*, or more frequently it serves to re-reduce a tetrahemic *cyt* bound to the RC.

Soluble *cyts c* have been characterized in several species. *Erythromicrobium hydrolyticum* and *S. sibiricus*, each have only one, and it is believed that this *cyt* functions in both respiration and photosynthesis (Yurkov and Beatty 1998). Some other APB were shown to have 2–4 soluble *cyt*, and in few cases more than one have redox potentials sufficient to participate in both the respiratory and photosynthetic pathways. Unusually small *cyt c* were isolated from *Rsc. thiosulfatophilus* (6.5 and 4.0 kDa) and *Erm. ursincola* (6.5 kDa), but their functions have not yet been clarified. The majority of *cyt* isolated from APB are between 8.0 and 26.0 kDa in size. The species diversity of *cyt* found in phototrophs stimulated us to question if other

interesting e^- carriers could be discovered in APB. Continued study of e^- transport carriers in novel strains of APB will help us to understand diversity of energetic pathways and evolution of the photosynthetic apparatus. Additionally, differences in organization of the photosynthetic apparatus have been used as important criteria in systematics of APB (Yurkov and Beatty 1998), and their continued investigation should help to clarify the taxonomy of this phylogenetically heterogeneous group.

For this purpose we recorded reduced minus oxidized absorbance spectra and performed SDS-PAGE followed by TMBZ heme staining to calculate the molecular weights of each of the cyt present.

Reduced minus oxidized absorbance spectra recorded for the soluble fraction of ML31 revealed two soluble e^- carriers (as determined by difference peaks at 420 and 430 nm) (Figure not shown). One was a c type cyt, while the other appeared to be a cyt c' . The molecular weights were determined to be 17 and 28 kDa (Fig. 2).

Redox difference spectra recorded for the ML6 soluble fraction had a high potential heme-containing cyt reduced by Na-ascorbate, as well as a low potential cyt reduced only by Na-dithionite. However, TMBZ staining detected only one broad cyt band (Fig. 2). Due to the broad appearance of the band between around 11 and 14 kDa, it was likely that two cyts possessed nearly identical molecular weights. Based on redox properties, only the high potential heme-containing cyt could perform a light-induced cyclic electron transfer and therefore play a role in photosynthesis.

Concluding remarks

We have succeeded in isolating pigment protein complexes from two new species of APB isolated from the meromictic Mahoney Lake, and determined that the mild detergent Triton X-100 is suitable for releasing particularly delicate complexes from membranes, such as seen in ML6.

Photosynthesis in *P. meromictius* functions only under aerobic conditions, and the photosynthetic e^- transport involves re-reduction of the P^+ by a soluble cyt. It is interesting to note that of all APB studied thus far, RCs lacking an intimately bound cyt belong exclusively to members of the so-called *Erythrobacter–Porphyrobacter–Erythromicrobium* cluster within the α -4-Proteobacteria.

This finding tends to disagree with suggestions that photosynthesis in APB may have come about by the action of relatively recent lateral gene flow, in which entire operons have been transferred from phototrophic species to non-phototrophs. Had lateral transfer been the case, one would not expect to see homogeneity in RC organization within a phylogenetically coherent group.

Photosynthesis in *R. mahoneyensis* shows unique trends with regards to the effect of oxygen. The e^- transfer reactions were only partially functional under both fully aerobic and anaerobic conditions, and only appeared to occur under conditions of reduced oxygenation. Indeed e^- transfer was only possible over a relatively narrow range of ambient redox potentials, from +270 mV (dictated by redox potential of the RC-bound cyt) and to +415 mV (P/P^+). If ML6 is truly capable of phototrophy only under microaerophilic conditions, than this strain may represent intermediary physiology between characteristic anoxygenic phototrophs that grow robustly under anaerobic conditions, and typical APB, that are photosynthetically active only under full aerobiosis, supporting the idea that photosynthesis in APB has evolved to fit specific ecological niches and requirements. The peculiar trait is evidence that ML6 uses photosynthesis as an auxiliary energy source during periods of O_2 deprivation that may develop when other rapidly respiring heterotrophs within the community consume available O_2 . Because Mahoney Lake is a relatively eutrophic habitat, such a situation can be encountered.

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