Photoinduced Cyclic Electron Transfer in *Rhodocyclus tenuis* Cells: Participation of HiPIP or Cyt c_8 Depending on the Ambient Redox Potential

Laure Menin,[‡] Barbara Schoepp,[§] Pierre Parot,[‡] and André Verméglio*[,‡]

C. E. A., DEVM-LBC, C. E. A. Cadarache, 13108 Saint-Paul-lez-Durance Cedex, France, and Laboratoire d'Ingénierie des Protéines, Centre de Génétique Moléculaire, CNRS, 91198 Gif-sur-Yvette Cedex, France

Received May 16, 1997; Revised Manuscript Received July 23, 1997[⊗]

ABSTRACT: We demonstrate the participation of a cytochrome c_8 and a high-potential iron-sulfur protein (HiPIP) in the photoinduced electron transfer in whole cells of *Rhodocyclus tenuis* depending on the redox state or background continuous illumination. At high redox potentials (above 350 mV) or under a strong background illumination (5 W m^{-2}), the cytochrome c_8 acts as the physiological electron donor to the photo-oxidized high-potential hemes of the tetraheme cytochrome bound to the reaction center. For redox potentials ranging from 200 to 310 mV or under weak background illumination (1.25 W m^{-2}), the electron carrier is the HiPIP. The electron transfer between cyt c_8 and HiPIP and the tetraheme cytochrome has half-times of 300 and 480 μs, respectively. A slow electrogenic phase of the membrane potential is linked to their rereduction. This phase is sensitive to a specific inhibitor of the cyt bc_1 complex, indicating involvement of cyt c_8 and HiPIP in the photoinduced cyclic electron transfer at these two redox conditions.

In purple bacteria, the photoinduced cyclic electron transfer is coupled to proton translocation across the cytoplasmic membrane, creating a proton-motive force which drives ATP synthesis and NAD^+ reduction. To perform light-induced cyclic electron transfer, bacteria possess two integral transmembrane complexes, the reaction center (RC)[1] and the cytochrome (cyt) bc_1 complex. These complexes are connected via membrane-diffusive quinone molecules and hydrosoluble periplasmic electron carriers. Excitation of the RC by a photon results in an electron transfer from the primary donor (P) to the primary (Q_A) and secondary (Q_B) quinone acceptors. The primary donor in the photo-oxidized state (P^+) must be rapidly rereduced by a secondary electron donor to prevent wasteful back electron transfer from the semiquinone state of Q_A and Q_B. For some species, such as *Rhodobacter (Rb.) sphaeroides* and *Rb. capsulatus*, this secondary electron donor is a soluble cyt c_2 which in turn is reduced by the cyt bc_1 complex. For most species, however, the reduction of the photo-oxidized primary donor is performed by a RC bound tetraheme cytochrome (Bartsch, 1991). The tetraheme cytochromes have been characterized for many different species in terms of spectral properties, redox potentials, and orientation of the hemes [for a review, see Nitschke and Dracheva (1995)]. In general, they have two hemes with rather high mid-point potentials (HP hemes) and two other hemes with low E_m (LP hemes). In the presence of a tetraheme RC bound cyt, electron transfer between this tetraheme and the cyt bc_1 complex is mediated by an additional electron carrier. This electron donor should be present in the periplasmic space and is expected to have its mid-point potential around 300−400 mV, i.e., between those of cyt c_1 and of the HP hemes of the RC bound tetraheme. In some species, such as *Rhodopseudomonas (Rp.) viridis* (Knaff et al., 1991; Garcia et al., 1993; Meyer et al., 1993) or *Rp. acidophila* (Matsuura & Shimada, 1986), this periplasmic electron carrier is cyt c_2. However, many genera, such as *Rhodocyclus (Rc.), Chromatium (C.), Chloroflexus*, and *Ectothiorhodospira* (*E.*), lack cyt c_2. In the periplasm of *Rubrivivax (Ru.) gelatinosus*, *Rhodospirillum salinarum*, and *E. halophila* cells, the only high-potential electron carrier present is a high potential iron-sulfur protein (HiPIP) containing a cubane $[Fe_4S_4]^{3+/2+}$ redox couple (Tedro et al., 1985b; Bartsch, 1991). Recently, participation of the HiPIP in the photoinduced cyclic electron transfer in intact cells of *Ru. gelatinosus* has been reported (Schoepp et al., 1995). Other species like *R. globiformis* (Ambler et al., 1987; Batsch, 1991), *Rp. marina* (Meyer et al., 1990), *Rhodomicrobium vannielli*, *C. vinosum*, *C. purpuratum* (Kerfeld et al., 1996), *Rhodoferax (Rf.) fermentans*, (Hochkoeppler et al., 1996a) or *Rc. tenuis* (Bartsch, 1991) contain a large amount of both HiPIP and a high-potential cytochrome cyt c_2 or cyt c_8 (formerly designated *c*-551; Ambler, 1991). Cyts c_8 are of smaller size than cyts c_2 and present a less assymetric charge distribution on their surface. In reconstitution experiments using *Rhodoferax (Rf.) fermentans* membranes, Hochkoeppler et al. (1995a,b,c, 1996a,b) have shown that HiPIP is able to donate electron to the photo-oxidized RC bound tetraheme and to mediate electron transfer between the cyt bc_1 complex and the cytochrome oxidase. More recently, the same group found that a high-potential cyt c_8 ($E_m = +287$ mV) is able to reduce heme *c*-560 of the RC bound cyt on a millisecond time scale (Hochkoeppler et al., 1996a).

* Corresponding author. Phone: 33 442254630. Fax: 33 442254701. E-mail: Vermeglio@DRA.CAD.CEA.FR.

‡ CEA Cadarache.

§ Centre de Génétique Moléculaire.

⊗ Abstract published in *Advance ACS Abstracts,* September 15, 1997.

[1] Abbreviations: cyt, cytochrome; E_h, ambiant redox potential; E_m, mid-point potential; EPR, electron paramagnetic resonance; HiPIP, high-potential iron-sulfur protein; HP, high potential; LP, low potential; RC, reaction center; TNBT, tri-(*N*-butyl)tin; *C.*, *Chromatium*; *E.*, *Ectothiorhodospira*; *R.*, *Rhodopila*; *Rb.*, *Rhodobacter*; *Rp.*, *Rhodopseudomonas*; *Rc.*, *Rhodocyclus; Rf.*, *Rhodoferax; Ru.*, *Rubrivivax*.

S0006-2960(97)01163-X CCC: $14.00

In the case of *Rc. tenuis*, a purple bacterium of the β-subdivision, the two electron carriers HiPIP and cyt c_8 have been characterized in terms of amino acid sequence (Tedro et al., 1979, 1985a), Nuclear magnetic resonance spectral properties (Krishnamoorthi et al., 1989; Moor, 1992), mid-point potentials (Przysiecki et al., 1985), and structure (Holden et al., 1986; Rayment et al., 1992). The HiPIP exhibits an E_m of +304 mV, whereas the E_m of the cyt c_8 is +405 mV. These two periplasmic electron carriers are putative electron carriers to the two HP hemes of the RC bound tetraheme cyt whose mid-point potentials have been determined to be equal to +420 mV (Menin et al., 1997). In the present paper, we have studied the role of the two electron carriers HiPIP and cyt c_8 in the *in vivo* photoinduced electron transfer in cells of *Rc. tenuis*.

MATERIALS AND METHODS

Cells Growth. Cells of *Rc. tenuis* were grown in Hutner medium at 30 °C in anaerobic conditions under continuous illumination as described in Menin et al. (1997). To decrease the large light-induced absorption changes due to the carotenoid electrochromic response, blue-green cells of *Rc. tenuis* have been obtained by growth in the presence of diphenylamine (final concentration = 10 μg/mL), a specific inhibitor of carotenoids synthesis (Malhotra et al., 1969; Davies & Than, 1974). The resulting culture presents only small light-induced absorbance changes linked to the carotenoid band shift and have been used for measurements of light-induced absorption changes in the α-band region of cytochromes.

Light-Induced Absorption Changes. The photoinduced absorption changes were measured as described in Menin et al. (1997). For the study of electron transfer in whole cells at poised redox potentials, bacteria were treated with 100 μM *p*-benzoquinone. After centrifugation, they were resuspended in 10 mM Tris-HCl (pH 7) with the following mediators, each added at 10 μM concentration: diaminodurene (E_m = +260 mV), 1,2-naphtoquinone-4-sulfonic acid (+215 mV), 1,2-naphtoquinone (+145 mV), phenazine methosulfate (+80 mV), duroquinone (+5 mV), and 2-OH-1,4-naphtoquinone (−140 mV). The ambiant redox potential was adjusted by addition of small aliquots of potassium ferricyanide (10 mM) and sodium ascorbate (10 mM) and measured with a combined Ag/AgCl reference system electrode (INGOLD). *p*-Benzoquinone was purchased from Merck and repurified by sublimation.

RESULTS

Light-Induced Absorption Changes Following Laser Excitation in a Suspension of Whole Cells. Figure 1 compares the light-induced redox changes of the field-indicating carotenoid band-shift measured as the difference 510−490 nm (part A) to those of cyt recorded at 422 nm (part B), following a series of five saturating flashes in intact cells of *Rc. tenuis*. Cells were placed under anaerobic conditions in the presence of 10 μM TNBT in order to block the ATPase activity responsible for the rapid decrease of the membrane potential. Photo-oxidation of the LP hemes is observed following the first two flashes, as shown by the wavelength position of the light-induced changes centered at 551 nm in the α-band region (Figure 1C). The rereduction of the LP hemes is very slow ($t_{1/2}$ > 5 s). The first two flashes induce

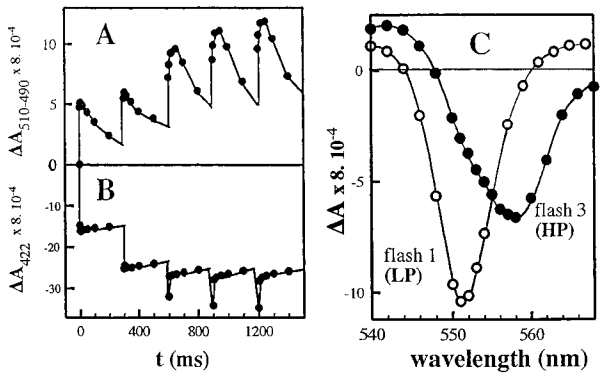

FIGURE 1: Light-induced absorption changes in whole red cells ([RC] = 150 nM) of *Rc. tenuis* placed under anaerobic conditions in the presence of 10 μM TNBT, induced by a series of five saturating flashes spaced by 300 ms. The absorption changes were sampled at discrete times (50 μs, 5 ms, 30 ms, ...) after each actinic flash. (A) Absorbance changes of carotenoid band shift measured as the difference 510−490 nm. (B) Kinetics of cyt photo-oxidation measured at 422 nm. (C) Light-induced difference spectra recorded at 50 μs after actinic flashes number 1 (○) and number 3 (●). Light-induced difference spectra of part C were measured on cells ([RC] = 300 nM) grown in the presence of diphenylamine (10 μg/mL). We have observed no difference on the kinetics recorded at 422 nm for cells grown in the presence or in the absence of this compound.

a fast increase of the membrane potential due to the charge separation between the donor and acceptor sites of the RC, which is not followed by a slower electrogenic phase (Figure 1A). These results are similar to those obtained in *Ru. gelatinosus* cells and show that the two LP hemes are not involved in an efficient cyclic electron transfer. The role of these two LP hemes will not be studied further in this work.

On subsequent flashes, the photo-oxidation of the HP hemes, as shown by the wavelength position of the absorption peak at 557 nm in the α-band region (Figure 1C), is followed by their rapid rereduction. This rapid rereduction of the HP hemes on flashes n = 3−5 is concomitant with a rising phase of the carotenoid band shift, indicating that HP hemes and the cyt bc_1 complex participate in an efficient cyclic electron transfer, as will be further discussed later.

Reduction of the HP Hemes as a Function of the Ambient Redox Potential or Background Continuous Illumination. To study the mechanism of electron transfer between soluble donor(s) and the HP hemes, light-induced absorption changes were studied in intact cells poised at two distinct redox potentials (Figure 2). At around 280 mV, a biphasic reduction of the photo-oxidized cyts is observed in the Soret region (Figure 2A) and in the α-band (Figure 2B). We fitted a double-exponential curve to the data. The fast phase (50%) presents a rate constant of 1.4 ms^{-1} (half-time ≈ 480 μs), and the rate constant of the slow phase is about 0.0125 ms^{-1} (half-time ≈ 55 ms) (Figure 2, curve a of panels A and B). This behavior is very similar to the one observed after the third or fourth flashes for a suspension under anaerobic conditions (Figure 1B). The half-time of HP hemes reduction is of the same order of magnitude as that measured for the electron transfer between the HiPIP and the HP hemes in *Ru. gelatinosus* (Schoepp et al., 1995). At higher redox potentials (400 mV), no fast reduction phase is observed at 422 nm and at (557−540) nm. The disappearance of the absorption change occurs with a half-time of 80 ms (Figure 2, curve b of panels A and B). The different kinetic behavior of HP hemes reduction as a function of redox potential can

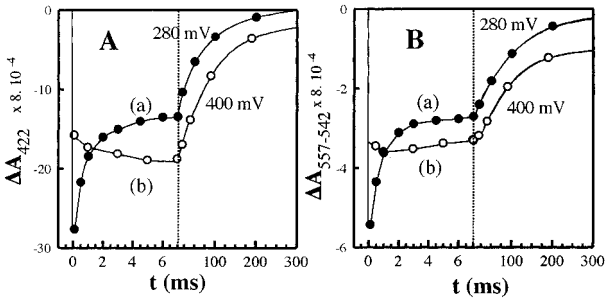

FIGURE 2: Kinetics of HP hemes rereduction (xenon flash excitation) measured (A) at 422 nm and (B) at 557−542 nm on whole cells ([RC] = 230 nM) grown in the presence of diphenylamine (10 μg/mL). Cells were treated with 100 μM *p*-benzoquinone and placed in the presence of mediators at two redox potentials as described in Materials and Methods: (curve a, ●) 280 mV and (curve b, ○) 400 mV. Note the two different time scales.

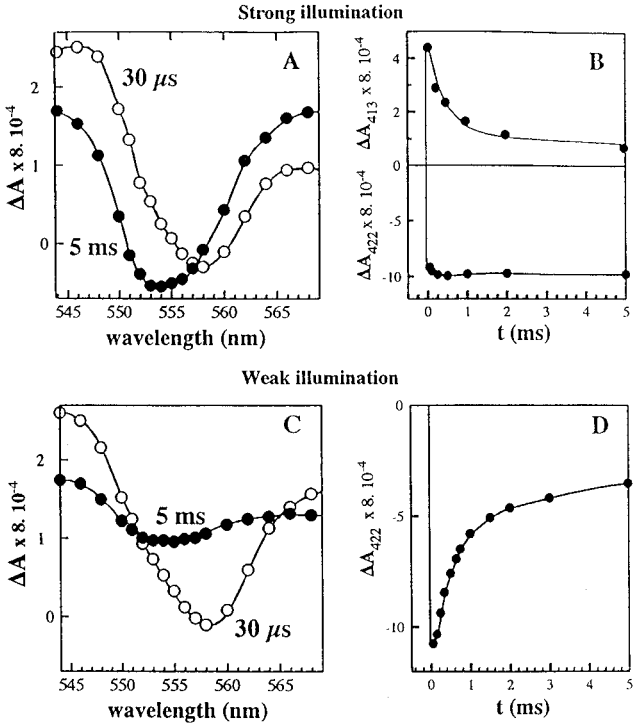

FIGURE 3: Light-induced difference spectra in the α-band recorded at 30 μs (○) and 5 ms (●) after the laser excitation in whole green cells of *Rc. tenuis* ([RC] = 90 nM) under strong continuous light (5 W m^{-2}) (A) or under weak continuous light (1.25 W m^{-2}) (C). Kinetics of electron transfer were recorded at 413 and 422 nm (B) and at 422 nm (D).

be easily reproduced by modulating the intensity of a continuous background illumination of cells under anaerobic conditions. Applying a continuous background light of low intensity (1.25 W m^{-2}) (Figure 3D) does not impair the flash-induced cyt photo-oxidation but, contrary to what is observed for a dark-adapted sample (Figure 1), the HP hemes are photo-oxidized on the first exciting flash as shown by the characteristic fast rereduction kinetics and the wavelength position of the light-induced changes detected at 30 μs (Figure 3 C). The rationale of this experiment is that this weak continuous background illumination causes accumulation of oxidized LP hemes but does not affect the HP hemes. A continuous background illumination of higher intensity (5 W m^{-2}) (Figure 3B) leads to the appearance of the slow kinetics similar to those observed at high redox potential (400 mV, Figure 2). Two hypotheses can explain this result. First, one can suppose that the immediate electron donor to the HP hemes has been photo-oxidized by the strong background continuous illumination. In these conditions, the HP hemes cannot be rapidly rereduced and this leads to stable absorption changes in the 10 ms time range. Alternatively, the HP hemes are rereduced by a cyt which possesses similar spectral contribution as the HP hemes at 422 nm. To discriminate between these two possibilities, flash-induced absorption spectra in the α-band have been recorded under background illumination of 5 W m^{-2}. Figure 3A shows light-induced difference spectra measured at 30 μs and 5 ms after the laser flash, under this 5 W m^{-2} background illumination. At 30 μs, the difference spectrum peaks at 557 nm which is characteristic of the HP hemes. It is clearly shifted to 554 nm when detected 5 ms after the actinic flash. This shift, from 557 to 554 nm, demonstrates the occurrence of an electron transfer between two distinct cytochromes under this background illumination. The difference spectrum calculated between 30 μs and 5 ms shows that the α-band of the soluble donor is centered at 551 nm, consistent with the oxidation of cyt c_8. The HP hemes and the soluble cytochrome present also slightly different absorption bands in the Soret region. At 413 nm, the spectral contribution of the HP hemes is positive, while the spectrum of the soluble donor presents an isosbestic point. Light-induced absorption changes recorded at 413 nm (Figure 3B) allow therefore the determination of a half-time of 300 μs for the fast phase of the electron transfer between the soluble donor and the HP hemes. A similar half-time is also observed for the absorption changes measured at (547−540) nm (data not shown), where the contribution of the cyt c_8 is important compared with that of the HP hemes. At 422 nm, the extinction coefficient of cyt c_8 and of the HP hemes are about equal, which accounts for the stablility of absorption changes in the 10 ms time range at this wavelength (Figure 3B).

Under weak continuous illumination, the amplitude of the absorption changes in the 545−570 nm region diminishes drastically between 30 μs and 5 ms after light excitation (Figure 3C). This indicates that the soluble electron donor involved in such conditions has only a weak spectral contribution in the 540−570 nm region. The spectrum recorded 5 ms after the actinic flash (Figure 3C) shows a band of small amplitude peaking at around 553 nm. This band can be attributed to the cyt c_8 or cyt c_1 oxidation (see next paragraph). Measurement of absorbance change kinetics at 422 nm (Figure 3D) shows that the photo-oxidized HP hemes are rereduced with a half-time of about 480 μs. Such a situation was previously observed in *Ru. gelatinosus*, and was interpreted as due to the participation of HiPIP in the rereduction of the HP hemes. We propose a similar interpretation in the case of *Rc. tenuis* since HiPIP can readily rereduce the tetraheme cyt in reconstitution experiments [see Figure 6 of Menin et al. (1997)]. Moreover, the EPR spectrum recorded at 10 K in intact cells, illuminated at room temperature before rapid freezing, shows typical EPR signals of photo-oxidized HiPIP (data not shown).

To precisely determine the redox range where the HiPIP acts as the electron donor, we plotted the percentage of the fast phase (<1 ms) at 422 nm as a function of the redox potential of the cells suspension. Figure 4 shows that the percent of fast phase is maximal at about 220−260 mV, a potential at which at least 90% of the total amount of HiPIP is reduced; it decreases very sharply at E_h higher than 310

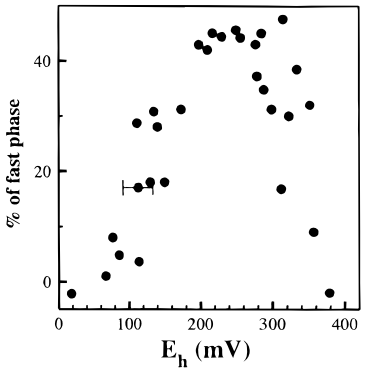

FIGURE 4: Percentage of the fast rereduction phase of the HP hemes calculated between 30 μs and 1 ms after the actinic flash, as a function of the redox potential E_h ($\pm$20 mV) of the cells suspension ([RC] = 150 nM). Cells were treated with 100 μM *p*-benzoquinone and mediators as described in Materials and Methods.

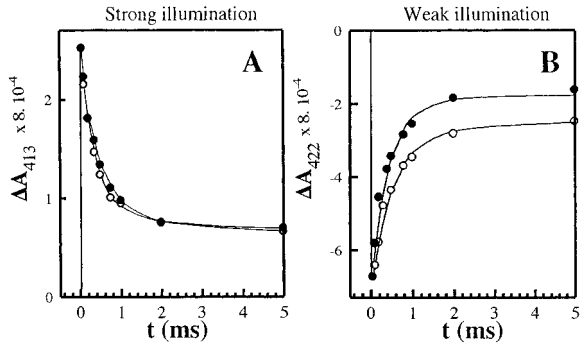

FIGURE 5: Kinetics of laser-induced absorption changes monitored in cells of *Rc. tenuis* ([RC] = 60 nM) under anaerobic conditions. (A) at 413 nm under a strong continuous light of 5 W m^{-2} or (B) at 422 nm under a weak illumination of 1.25 W m^{-2}. The kinetics were recorded after a saturating laser flash (○) or an attenuated actinic flash hitting only 20% of the RC (●). The signal amplitudes were normalized at 50 μs and kinetics were fitted with double-exponential equations.

mV, because cyt c_8 begins to act as electron donor, i.e. the absorption changes are stable during the first milliseconds (see Figure 2A, curve b). Note that the amplitude of the fast phase is already maximum at a potential close to the mid-point potential of the HiPIP (+304 mV) (Przysiecki et al., 1985), which may indicate an excess of the HiPIP in the periplasmic space, compared to the amount of RC. Below 150 mV, the fast phase also collapses and is replaced by the slow phase linked to the LP hemes reduction, as at these potentials, the LP hemes are partly reduced in the dark and can therefore be photo-oxidized by the actinic flash. These results are consistent with the participation of the HiPIP in the HP hemes reduction for redox potentials between 150 and 300 mV and of the cyt c_8 for higher potentials.

To test whether cyt c_8 and/or HiPIP form a complex with the tetraheme cyt, the ratio between the secondary electron donors and the oxidized RC was increased by attenuating the laser intensity. Two laser intensities, which hit 100 and 20% of RCs, respectively, were used. Figure 5 compares, after normalization, the rate of electron transfer between HiPIP or cyt c_8 and the HP hemes for a saturating laser flash or a weak flash. Decreasing the amount of photo-oxidized RC does not affect the half-time of electron transfer between cyt c_8 and the HP hemes ($t_{1/2} \approx 300$ μs), measured at 413 nm under strong illuminating conditions (Figure 5A). Under weak background illumination (Figure 5B), the kinetics at

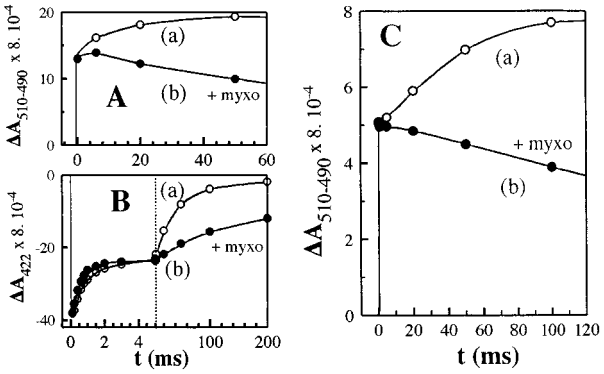

FIGURE 6: Whole cells were placed under anaerobic conditions in the presence of 10 μM TNBT, and kinetics of light-induced absorption changes were recorded at 510−490 nm for the carotenoid band shift (A) and at 422 nm for the HP hemes reduction (B) on the third flash of a train of four saturating flashes spaced by 300 ms, in the absence (curve a, ○) and presence (curve b, ●) of 5 μM myxothiazol ([RC] = 300 nM). (C) Cells ([RC] = 150 nM) were treated with *p*-benzoquinone (100 μM) and the redox potential was poised at 380 mV. Kinetics without (curve a, ○) and with (curve b, ●) 5 μM myxothiazol.

422 nm are biphasic and may be fitted with a sum of two exponentials. The $t_{1/2}$ (420 μs) of the fast phase does not depend on the flash saturation (Figure 5B), whereas the relative amplitude of slow phase decreases from 45% (strong flash) to 33% (weak flash). This decrease is due to the relative increase of the cyt bc_1 complex concentration compared to the low amount of HiPIP photo-oxidized by the attenuated flash. The slight differences in amplitude and half-time of the fast phase measured in the experiments of Figure 5 compared to those obtained in Figure 2 (curves a) are due to some double hits due to excitation with the xenon flash used in this latter case.

Reduction of the HiPIP and the Cyt c_8: Role of the bc_1 Complex. The experiments of Figure 1A and B show that, in conditions where the fast phase at 422 nm linked to the rereduction of the HP hemes by the HiPIP appears (flashes number 3−5), a slow rise of the carotenoid band shift is observed. Figure 6A shows more detailed kinetics of this light-induced carotenoid band shift recorded in the absence (curve a) or presence (curve b) of 5 μM myxothiazol, a specific inhibitor of the cyt bc_1 complex. The half-time of the slow rise is about 10 ms and its amplitude, taking into account the membrane potential decay, is similar to the membrane potential linked to the charge separation at the RC level. Moreover, the addition of myxothiazol strongly abolishes this slow electrogenic phase. Figure 6B shows that myxothiazol does not affect the fast phase of electron transfer between HiPIP and HP hemes, but drastically slows down the second phase of the absorption changes recorded at 422 nm. The rate constant value of this slow phase increases from 50 to 330 ms upon addition of the cyt bc_1 complex inhibitor. Our interpretation is that, in the presence of myxothiazol, the photo-oxidized cyt c_1 and cyt c_8, which contribute to the slow phase measured at 422 nm, are no longer rapidly reduced by the cyt bc_1 complex. The clear effect of addition of myxothiazol on the slow electrogenic phase and on the cyt rereduction indicates that a complete cyclic electron transfer involving HiPIP and a cyt bc_1 complex occurs under these conditions. In order to determine if the cyt c_8 rereduction involves also the cyt bc_1 complex, the carotenoid band shift was studied for cells placed at about

380 mV. Under these conditions, the fast increase of the membrane potential is followed by a slow rise with a half-time of 35 ms (Figure 6C, curve a). The amplitude of the slow rise is in the same order of magnitude as the fast one which is due to charge separation of the RC. This slow rise is completely inhibited by addition of 5 μM myxothiazol (Figure 6C, curve b). The difference in half-time of the slow electrogenic phase, 10 and 35 ms measured in conditions where the HiPIP or the cyt c_8, respectively, are the electron donor to the HP hemes, can be ascribed to a lower concentration of reduced quinone in the pool in the last condition. From these experiments, we conclude that depending on the redox state of the cells, HiPIP or cyt c_8 is involved in the photoinduced cyclic electron transfer between the RC and the bc_1 complex.

DISCUSSION

The present results demonstrate that, in *Rc. tenuis* cells, the cyt c_8 acts as the electron donor and participates to the *in vivo* cyclic electron transfer at high redox potentials (above 350 mV) or under strong background illumination of 5 W m^{-2}. At redox potentials comprised between 300 and 150 mV or under a weak background illumination, our results suggest that HiPIP is the preferential donor to the HP hemes. As light-induced absorption changes linked to the oxidation of HiPIP are too small to be directly detected *in vivo*, our arguments come from the fast reduction phase of the HP hemes in the Soret band without concommitant cyt oxidation, detection by EPR of photo-oxidized HiPIP upon continuous illumination at room temperature and rapid electron transfer between HiPIP and the HP hemes *in vitro* reconstitution experiments (Menin et al., 1997). The difference in the redox mid-point potential of the HiPIP (+304 mV) and of the cyt c_8 (+405 mV) explains that these two electron donors function in different redox conditions. The ratio (R) between the concentration of reduced HiPIP and reduced cyt c_8 can be calculated as a function of the redox potential, taking into account the relative amount of HiPIP and cyt c_8 (HiPIP/cyt c_8 = 2.5:1) present in the periplasm as determined by absorption and EPR experiments (data not shown). Between 320 and 200 mV, R increases from 1 to 2.5. At 350 mV, R is equal to 0.4 while it reaches 0.1 at 400 mV, i.e., the concentration of reduced cyt c_8 is 10 times higher than the one of reduced HiPIP. With these considerations, the preferential participation of cyt c_8 at potentials higher than 320 mV is obvious. *In vivo*, the rate of electron transfer between HiPIP and the HP hemes (480 μs) is of the same order of magnitude as that measured between cyt c_8 and the HP hemes (300 μs). This rate of electron transfer is very similar to that measured between HiPIP and the HP hemes in *Ru. gelatinosus* (Schoepp et al., 1995) and to the reduction rate of the RC bound tetraheme by cyt *c* in some other species (Coremans et al., 1985; Garcia et al., 1993). The net charge of the HiPIP and the cyt c_8 are close (+4 for cyt c_8 and +5 for HiPIP) and this suggests similar binding properties of these electron carriers to the tetraheme RC bound cyt. Since the two HP hemes cannot be distinguished spectroscopically by their α-bands or their redox potentials, it is not possible to determine if the HiPIP or the cyt c_8 donate specifically to one of the two HP hemes, or if each of these carriers can function indiscriminately with both of them. Attenuating the laser intensity did not affect significantly the rate of electron transfer between the HiPIP or cyt c_8 and the HP hemes. These results suggest that both HiPIP and cyt c_8 form a complex with the tetraheme cyt.

The experiments shown in Figure 6 demonstrate that an electron flow through the cyt bc_1 complex is linked to the participation of both HiPIP and cyt c_8. In particular, the slow rise of the membrane potential is abolished by myxothiazol, a classical inhibitor of the cyt bc_1 complex. In *Rc. tenuis* membranes, we estimated, by reduced−oxidized difference absorption spectra (data not shown), that the ratio between bc_1 complex and RC is lower than 0.15. Because of this low concentration of cyt bc_1 complex, HiPIP or cyt c_8 oxidized by the HP hemes have to diffuse to be rereduced by this complex. The complete rereduction of the HiPIP or the cyt c_8 requires several turnovers of the cyt bc_1 complex which explains the high value (10−35 ms) of the half-time of the slow electrogenic phase.

We demonstrated that both HiPIP and cyt c_8 participate *in vivo* to the photoinduced cyclic electron transfer of *Rc. tenuis* cells. Other cases of interchangeability between different electron carriers were previously reported under metal deficiency or deletion. For example, the soluble copper protein, plastocyanin, can be replaced by cytochrome c_6 in cyanobacteria and green algae for transfering electrons between the cyt b_6f and PSI complexes, when these organisms are grown in the absence of copper (Wood, 1978; Lochau, 1981; Ho & Krogmann, 1984; Sandmann, 1986). The interchangeable function of PC and cyt c_6 has been confirmed by spectroscopic, genetic, and physiological experiments. Cyt c_6 appears to be related to *Ectothiorhodospira* cyt c_{551} (Ambler et al., 1993). Another example is the periplasmic electron carrier c_{550} in *Paracoccus denitrificans*, whose expression is strongly increased during growth under denitrifying conditions. The disruption of the gene coding for cyt c_{550} does not change significantly the electron transfer to the terminal reductases of denitrification, but this activity is abolished by a copper chelator (Moir & Ferguson, 1994; Berks et al., 1995). These results suggest that electrons are carried between the cyt bc_1 and the reductases by both cytochrome c_{550} and copper protein(s). Substitution of a *c*-type cytochrome by a copper protein has also been described in methylotrophic bacteria (Auton & Anthony, 1989). In *Rb. sphaeroides*, the deletion of the secondary electron donor cyt c_2 resulted in a photosynthetically incompetent phenotype (Donohue et al., 1988). Spontaneous photosynthetic competent pseudo-revertant of the cyt c_2 deleted mutant overproduces a low abundance soluble *c*-type cytochrome, the iso-cyt c_2 (Fitch et al., 1989; Rott et al., 1992, 1993). In *Rb. capsulatus*, the photo-oxidized primary electron donor P is rapidly rereduced by the soluble cyt c_2 or a membrane-bound cyt c_y. Both of the pathways lead to a rapid and efficient cyclic electron transfer and are present in the wild-type strain (Jenney et al., 1994). However, these two cytochromes have similar E_m values (Jones et al., 1990). To our knowledge, *Rc. tenuis* is the only case where two different electron carriers are involved in the *in vivo* photosynthetic electron transfer depending on the ambient redox potential or background illumination. The physiological significance of this behavior is under study.

ACKNOWLEDGMENT

The authors wish to thank Dr. J. Gaillard for recording EPR spectra, Dr. J. Lavergne for many valuable discussions, and A. LeMouellic for technical assistance.

REFERENCES

Ambler, R. P. (1991) *Biochim. Biophys. Acta 1058*, 42−47.

Ambler, R. P., Meyer, T. E., Cusanovich, M. A., & Kamen, M. D. (1987) *Biochem. J. 246*, 115−120.

Ambler, R. P., Meyer, T. E., & Kamen, M. D. (1993) *Arch. Biochem. Biophys. 306*, 83−93.

Auton, K. A., & Anthony, C. (1989) *J. Gen. Microbiol. 135*, 1923−1931.

Bartsch, R. G. (1991) *Biochim. Biophys. Acta 1058*, 28−30.

Berks, B. C., Ferguson, S. J., Moir, J. W. B., & Richardson, D. J. (1995) *Biochim. Biophys. Acta 1232*, 97−173.

Coremans, J. M. C. C., Van der Wal, H. N., Van Grondelle, R., Amesz, J., & Knaff, D. B. (1985) *Biochim. Biophys. Acta 807*, 134−142.

Davies, B. H., & Than, A. (1974) *Phytochemistry 13*, 209−219.

Donohue, T. J., McEwan, A. G., Van Doren, S., Crofts, A. R., & Kaplan, S. (1988) *Biochemistry 27*, 1918−1925.

Fitch, J., Cannac, V., Meyer, T. E., Cusanovich, M. A., Tollin, G., Van Beeumen, J., Rott, M. A., & Donohue, T. J. (1989) *Arch. Biochem. Biophys. 271*, 502−507.

Garcia, D., Richaud, P., & Verméglio, A. (1993) *Biochim. Biophys. Acta 1144*, 295−301.

Ho, K. K., & Kromann, D. W. (1984) *Biochim. Biophys. Acta 766*, 310−316.

Hochkoeppler, A., Kofod, P., & Zannoni, D. (1995a) *FEBS Lett. 375*, 197−200.

Hochkoeppler, A., Zannoni, D., & Venturoli, G. (1995b) *Biochim. Biophys. Acta 1229*, 81−88.

Hochkoeppler, A., Ciurli, S., Venturoli, G., & Zannoni, D. (1995c) *FEBS Lett. 357*, 70−74.

Hochkoeppler, A., Ciurli, S., Kofod, P., Venturoli, G., & Zannoni, D. (1996a) *PPB*, 10th FESPP Congress, Florence, Italy, 9−13 September, 1996.

Hochkoeppler, A., Zannoni, D., Ciurli, S., Meyer, T. E., Cusanovich, M. A., & Tollin, G. (1996b) *Proc. Natl. Acad. Sci. 93*, 6998−7002.

Holden, H. M., Meyer, T. E., Cusanovich, M. A., & Rayment, I. (1986) *J. Biol. Chem. 261* (9), 4219−4220.

Jenney, F. E., Prince, R. C., & Daldal, F. (1994) *Biochemistry 33*, 2496−2502.

Jones, M. R., McEwan, A. G., & Jackson, J. B. (1990) *Biochim. Biophys. Acta 1019*, 59−66.

Kerfeld, C. A., Chan, C., Hirazawa, M., Kleis-SanFrancisco, S., Yeates, T. O., & Knaff, D. B. (1996) *Biochemistry 35*, 7812−7818.

Knaff, D. B., Willie, A., Long, J. E., Kriauciunas, A., Durham, B., & Millet, F. (1991) *Biochemistry 30*, 1303−1340.

Krishnamoorthi, R., Cusanovich, M. A., Meyer, T., & Przysiecki, C. T. (1989) *Eur. J. Biochem. 181*, 81−85.

Lochau, W. (1981) *Arch. Microbiol. 128*, 336−340.

Malhotra, H. G., Britton, G., & Goodwin, T. W. (1969) *Phytochemistry 8*, 1047−1049.

Matsuura, K., & Shimada, K. (1986) *Biochim. Biophys. Acta 852*, 9−18.

Menin, L., Schoepp, B., Garcia, D., Parot, P., & Verméglio, A., (1997) *Biochemistry 36*, 12175−12182.

Meyer, T. E., Cannac, V., Fitch, J., Bartsch, R. G., Tollin, D., Tollin, G., & Cusanovich, M. A. (1990) *Biochim. Biophys. Acta 1017*, 125−138.

Meyer, T. E., Bartsch, R. G., Cusanovich, M. A., & Tollin, G. (1993) *Biochemistry 32*, 4719−4726.

Moir, J. W. B., & Ferguson, S. J. (1994) *Microbiology 140*, 389−397.

Moor, G. R. (1992) *J. Inorg. Biochem. 28*, 355−362.

Nitschke, W., & Dracheva, S. M. (1995) in *Anoxygenic Photosynthetic Bacteria* (Blankenship, R. E., Madigan, M. T., & Bauer, C. E., Eds.) pp 775−805, Kluwer Academic Publishers, Dordrecht.

Przysiecki, C. T., Meyer, T. E., & Cusanovich, M. A. (1985) *Biochemistry 24*, 2542−2549.

Rayment, I., Wesenberg, G., Meyer, T. E., Cusanovich, M. A., & Holden, H. M. (1992) *J. Mol. Biol. 228*, 672−686.

Rott, M. A., Fitch, J., Meyer, T. E., & Donohue, T. J. (1992) *Arch. Biochem. Biophys. 292*, 576−582.

Rott, M. A., Witthuhn, V. C., Schilke, B. A., Soranno, M., Ali, A., & Donohue, T. J. (1993) *J. Bacteriol. 175*, 358−366.

Sandmann, G. (1986) *Arch. Microbiol. 145*, 76−79.

Schoepp, B., Parot, P., Menin, L., Gaillard, J., Richaud, P., & Verméglio, A. (1995) *Biochemistry 34*, 11736−11742.

Tedro, S. M., Meyer, T. E., & Kamen, M. D. (1979) *J. Biol. Chem. 254*, 1495−1500.

Tedro, S. M., Meyer, T. E., & Kamen, M. D. (1985a) *Arch. Biochem. Biophys. 239*, 94−101.

Tedro, S. M., Meyer, T. E., & Kamen, M. D. (1985b) *Arch. Biochem. Biophys. 241*, 656−664.

Wood, P. M. (1978) *Eur. J. Biochem. 87*, 9−19.

BI971163B