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Photosynthetic Electron Transfer in the Absence of Cytochrome c_2 in *Rhodopseudomonas capsulata*: Cytochrome c_2 Is Not Essential for Electron Flow from the Cytochrome bc_1 Complex to the Photochemical Reaction Center

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ABSTRACT: Photosynthetic electron flow from the cytochrome bc_1 complex to the reaction center has been studied in a strain of *Rhodopseudomonas capsulata* which has had the gene for cytochrome c_2 deleted from its genome. Previously, cytochrome c_2 was thought to be essential for electron flow between these two complexes, but we find this not to be the case in *R. capsulata*. Indeed, in this organism it seems likely that cytochrome c_1 is able rapidly ($t_{1/2} < 100$ μs) to transfer electrons directly to the reaction center. However, this reaction is incomplete; only some 20% of the reaction centers are reduced in this way. In the wild type, a further 20% is rapidly reduced by cytochrome c_2, but the remaining reaction centers are reduced rather more slowly by an as yet unidentified route that may involve cytochrome c_2 shuttling between complexes. The deletion of the cytochrome c_2 gene allows a determination of the oxidation–reduction midpoint potential of cytochrome c_1 in the absence of cytochrome c_2: cytochrome c_1 has an E_{m7} of 345 mV. Furthermore, the finding that a phase of the electrogenic carotenoid bandshift accompanies the oxidation of cytochrome c_1 in the absence of c_2 indicates that the heme of cytochrome c_1 must be near the inner aqueous–membrane interface of the chromatophore.

Photosynthetic electron transfer in *Rhodopseudomonas capsulata* involves two membrane-associated complexes: the photochemical reaction center and the cytochrome bc_1 complex [for recent reviews, see Prince et al. (1982) and Crofts (1985)]. Both contain several distinct redox centers and together are responsible for electrogenic proton translocation. Up until now, there has been little evidence to suggest that they interact directly with each other; rather, it has been assumed that mobile electron carriers "shuttle" between the two. Ubiquinone is thought to fulfill this role in transferring electrons from the reducing end of the reaction center to the cytochrome bc_1 complex, while cytochrome c_2 is thought to transfer electrons from the bc_1 complex to the reaction center's oxidizing site. Furthermore, cytochrome c_2 is also involved in aerobic growth, where it is thought to function in an analogous fashion between the cytochrome bc_1 complex and the terminal oxidase known as cytochrome b_{410} (Baccarini-Melandri et al., 1978).

Using site-directed mutagenesis, Daldal et al. (1986) have deleted the gene coding for cytochrome c_2 from *R. capsulata* and found that the resulting strain is still capable of photosynthetic and aerobic growth. This paper presents an investigation of the effects of the absence of cytochrome c_2 on photosynthetic electron transfer.

Materials and Methods

Rhodopseudomonas capsulata [*Rhodobacter capsulatus* in the proposed taxonomy of Imhoff et al. (1984)] strain MT-G4/S4, which lacks cytochrome c_2, and the parent strain MT1131, which possesses it, were grown aerobically in shake flasks from frozen stocks until they reached early logarithmic phase in 300 mL, and were then transferred to 1.5-L sealed bottles and grown photosynthetically to late log phase as described previously (Daldal et al., 1986). Chromatophores were prepared by using a French pressure cell following standard procedures and were "purified" on a discontinuous sucrose gradient (10–40% sucrose in 10 mM *N*-morpholinopropanesulfonate, pH 7); spheroplasts were prepared by using lysozyme and ethylenediaminetetraacetate (Prince et al., 1975; Takemoto & Bachmann, 1979).

Flash-induced optical absorbance changes were measured with a rapidly responding double-beam spectrometer constructed by the Bio-Instrumentation Group at the University of Pennsylvania, interfaced via a Nicolet 3091 oscilloscope to an IBM personal computer. Actinic flashes were provided by a xenon flash lamp (full width at half-height about 12 μs)

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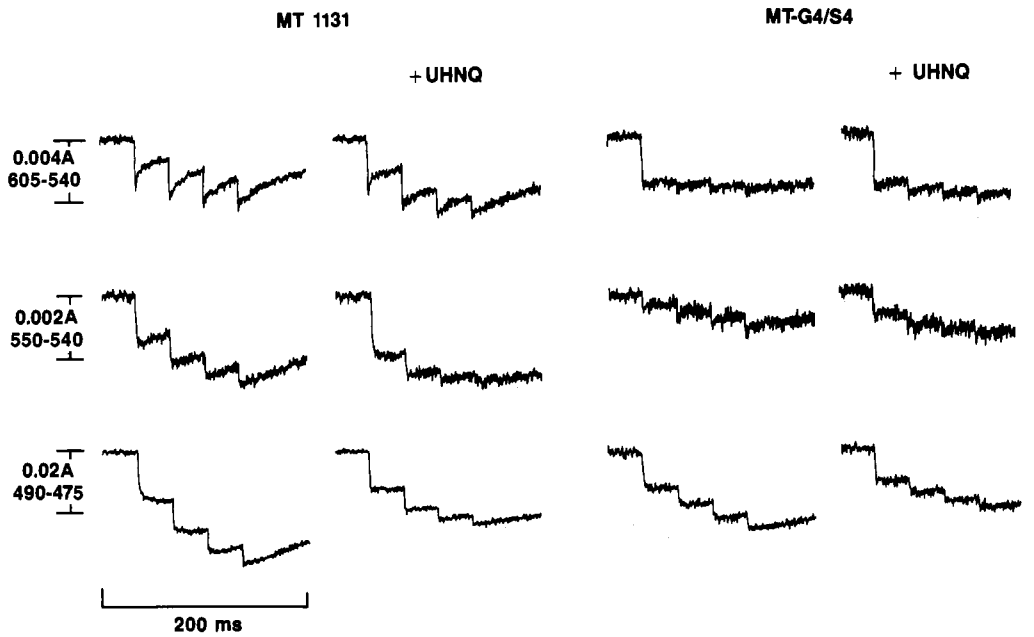

FIGURE 1: Response of the reaction center, *c*-type cytochromes and the carotenoid bandshift to four actinic flashes. Whole cells, diluted so that the bacteriochlorophyll concentration was 20 μM, were suspended in growth media supplemented with 20% sucrose to slow cell settling. 3-Undecyl-2-hydroxy-1,4-naphthoquinone (UHNQ) was added to 10 μM as indicated. The traces represent the average of four flash sequences, delivered 1 min apart.

filtered through a Schott RG780 filter. These flashes were greater than 95% saturating under the conditions used here. Redox titrations of the *c*-type cytochromes used a Hitachi 557 spectrophotometer operating in the dual-wavelength mode. Redox potentiometry followed the method of Dutton (1978).

RESULTS

Initially we studied photosynthetic electron transfer in whole cells, where presumably no soluble components have been lost during the preparation of the sample. The top traces of Figure 1 show the response of the reaction center primary donor, measured at 605–540 nm, to a train of four flashes delivered 32 ms apart. Oxidation of the primary donor results in a bleaching of the 605-nm band, which is seen as a downward deflection in these traces. In fact, not all the photooxidized reaction center is revealed after a single flash, because it is promptly, albeit partially, reduced by what has been thought, until now, to be cytochrome c_2 (Dutton et al., 1975). This in turn has been thought to be rereduced by cytochrome c_1 of the cytochrome bc_1 complex, and because these two *c*-type cytochromes are spectrally very similar, both contribute to the changes measured at 550–540 nm in Figure 1.

In the presence of the inhibitor 2-undecyl-3-hydroxy-1,4-naphthoquinone (UHNQ), the usual reduction of cytochrome c_1 by the Rieske iron sulfur center [see Prince et al. (1982) and Crofts (1985)] is inhibited (Matsuura et al., 1983), and more net cytochrome c_1 and c_2 oxidation is revealed. Furthermore, because the cytochromes are no longer being rereduced between the flashes by cyclic electron flow, the reaction center soon runs out of reduced electron donors, and the full extent of the reaction center oxidation is revealed after the third or fourth flash.

The events discussed above are clearly seen in the wild-type strain MT1131. Qualitatively similar changes are seen in the cytochrome c_2 deficient strain MT-G4/S4. Small changes are seen at 550–540 nm, attributable to cytochrome c_1, even in the absence of UHNQ, and these become much more apparent in the presence of the inhibitor. Similarly, the reaction center changes respond to the inhibitor as described for MT1131, with an approximately 20% increase in observed bleaching in the presence of the inhibitor after the fourth flash.

Both strains used here exhibit the well-known carotenoid bandshift, measured at 490–475 nm. This electrochromic red shift of the carotenoid absorption spectrum is known to respond as a voltmeter to transmembrane potentials and is kinetically and potentiometrically resolvable into several distinct phases following a single turnover flash (Jackson & Dutton, 1973; Bashford et al., 1979; Glaser & Crofts, 1984; Robertson et al., 1985). This is shown in Figure 1, where all phases of the bandshift can be seen in the uninhibited state. The rapid phases accompany processes within the reaction center and its rereduction by cytochrome c_2, while the slower component accompanies electron flow through the cytochrome bc_1 complex (Jackson & Dutton, 1973; Bashford et al., 1979; Glaser & Crofts, 1984; Robertson et al., 1985). The latter is prevented by the addition of UHNQ (Matsuura et al., 1983). There is some UHNQ-sensitive carotenoid bandshift in strain MT-G4/S4, although this is shown more clearly with chromatophores, as shown in Figure 2.

The use of whole cells involves some inherent disadvantages; they scatter light so that the actual light path is not constant, and they have substantial reserves of reductant so that the redox poise of the sample cannot be varied. Figure 2 shows a repeat of the experiment of Figure 1, but using chromatophores. These small vesicles are some 400 Å in diameter, so they do not scatter light significantly, and they are substantially free of endogenous reductant because this is removed during the cell fractionation; they are thus amenable to redox potentiometry. Clearly, Figure 2 is qualitatively similar to Figure 1, but now direct comparisons can be made between the samples because the ambient redox potential has been poised at the optimal potential for cyclic electron transfer and associated photophosphorylation (Prince et al., 1982; Crofts, 1985). The preparations from the two strains have been normalized so that the reaction center concentration is the same in both cases, and it is clear, particularly in the presence of the inhibitor UHNQ, that the change at 550–540 nm in strain MT-G4/S4 is approximately half that seen in MT1131. The spectra of the species responsible for the changes at 550–540 nm are shown in Figure 3; in both strains, the spectra

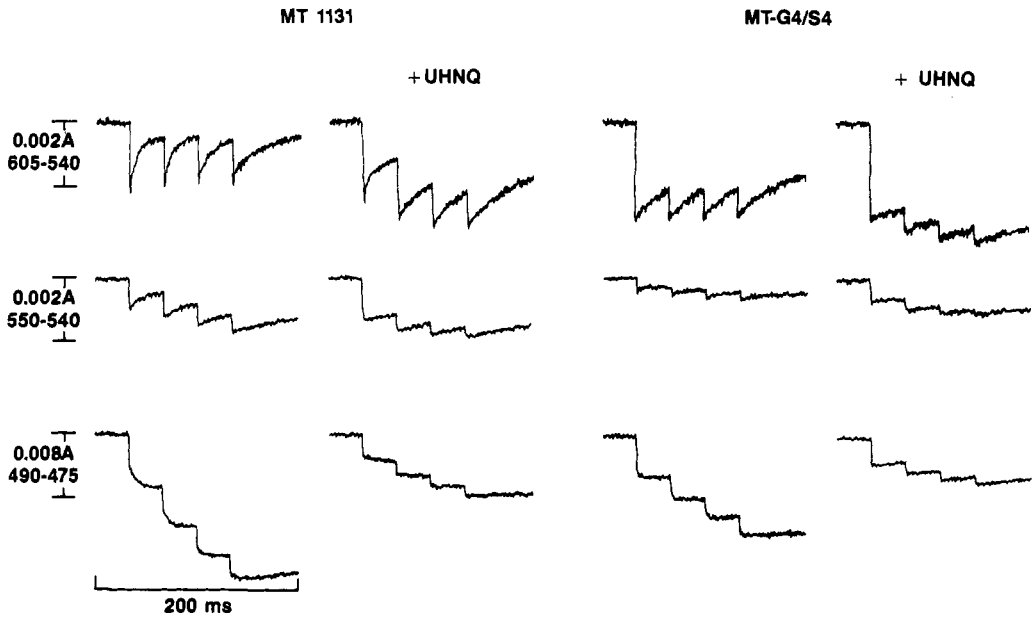

FIGURE 2: Response of the reaction center, *c*-type cytochromes and the carotenoid bandshift to four actinic flashes. Chromatophores were diluted to 25 μM bacteriochlorophyll in 10 mM *N*-morpholinopropanesulfonate with 2 μM *N*-methylphenazonium methosulfate, 2,3,5,6-tetramethylphenylenediamine, and 2-hydroxy-1,4-naphthoquinone, and the E_h was adjusted to 100 mV at pH 7. UHNQ was added as indicated to 5 μM.

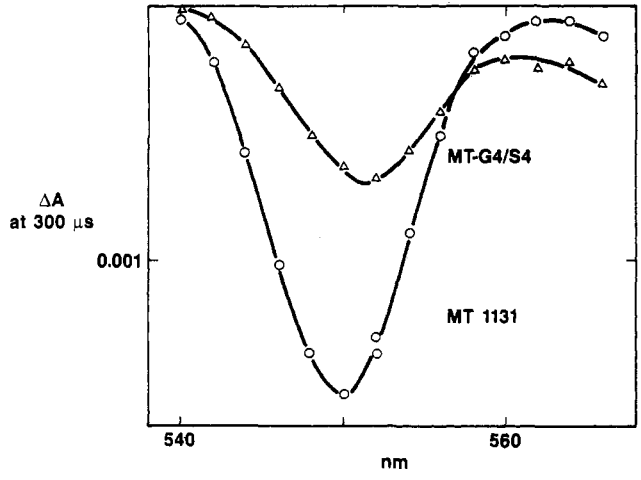

FIGURE 3: Flash-induced absorbance changes attributable to *c*-type cytochromes. Chromatophores were used as in Figure 2, and the absorbance changes, using 540 nm as a reference wavelength, were measured 300 μs after the flash. 5 μM UHNQ was present, and the E_h was 100 mV.

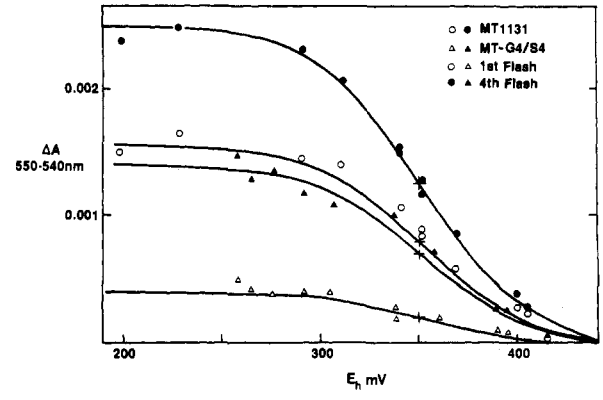

FIGURE 4: Redox titration of *c*-type cytochrome photooxidation. Chromatophores (25 μM bacteriochlorophyll) were suspended in 20 mM *N*-morpholinopropanesulfonate, 100 mM KCl, 2 μM *N*-methylphenazonium methosulfate, 5 μM 2,3,5,6-tetramethylphenylenediamine, and 2-hydroxy-1,4-naphthoquinone with 2 μM antimycin. The extent of the flash-induced change at 550–540 nm measured 5 ms after the first and fourth flash is shown, and theoretical curves for an E_m of 350 mV are shown.

are characteristic of *c*-type cytochromes, and the data suggest that the component in MT-G4/S4 has a slightly longer α-band absorption maximum than the wild type. Redox titrations of the flash-induced changes are shown in Figure 4, after both the first and the fourth flash. These measurements were made in the presence of antimycin instead of UHNQ. Antimycin does not inhibit between the Rieske iron–sulfur cluster and cytochrome c_1 as UHNQ does but rather inhibits cytochrome *b* oxidation (Prince et al., 1982; Crofts, 1985). There is thus little effect on the extent of observed cytochrome *c* oxidation after the first flash, but the *c*-type cytochromes become fully oxidized after four flashes. In both the wild-type strain and the cytochrome c_2 deficient strain, the E_{m7} of the *c*-type cytochrome(s) being oxidized by the reaction center is +350 mV. Similar values are found in equilibrium titrations, where all the *c*-type cytochromes are measured (Figure 5).

The oxidation of the *c*-type cytochromes in MT1131 is biphasic, as shown in Figure 6; this has been widely interpreted as the rapid oxidation of cytochrome c_2, followed by the slower oxidation of cytochrome c_1 by cytochrome c_2 which is itself reoxidized by the reaction center (Prince et al., 1982; Crofts, 1985; Meinhardt & Crofts, 1982). In this model, MT-G4/S4 would have been predicted to have cytochrome *c* oxidation kinetics that, at best, approximated the slower phase seen in the wild type. In fact, MT-G4/S4 exhibits only the fast phase of *c*-type cytochrome oxidation, with a half-time faster than 100 μs (Figure 6).

Taken together, we interpret the data presented above as indicating that cytochrome c_1 of the cytochrome bc_1 complex is rapidly oxidized following a single turnover flash even in the absence of cytochrome c_2. However, is this a direct oxidation of the cytochrome by the reaction center primary donor, or is a small soluble intermediary involved? Figure 7 shows one attempt to answer this question. Cytochrome c_2 is found in the periplasmic space (Prince et al., 1975), so it is washed off during the removal of the cell wall in the preparation of spheroplasts. If the oxidation of cytochrome c_1 in MT-G4/S4 is direct, we would expect this to be unaffected by the removal

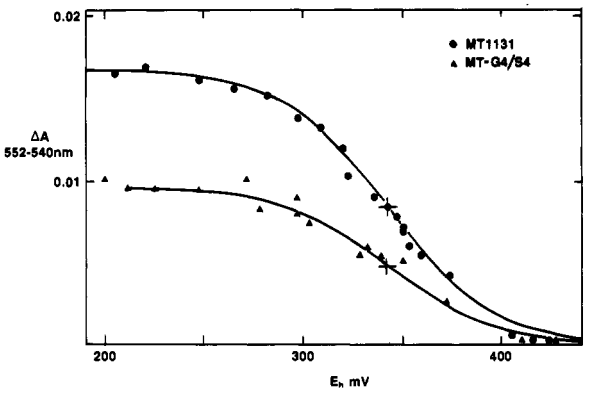

FIGURE 5: Redox titrations of *c*-type cytochromes. Chromatophores (113 μM bacteriochlorophyll) were suspended in the buffer used in Figure 4, with 40 μM 2,3,5,6-tetramethylphenylenediamine and *N*-methylphenazonium methosulfate and 200 μM potassium ferrocyanide.

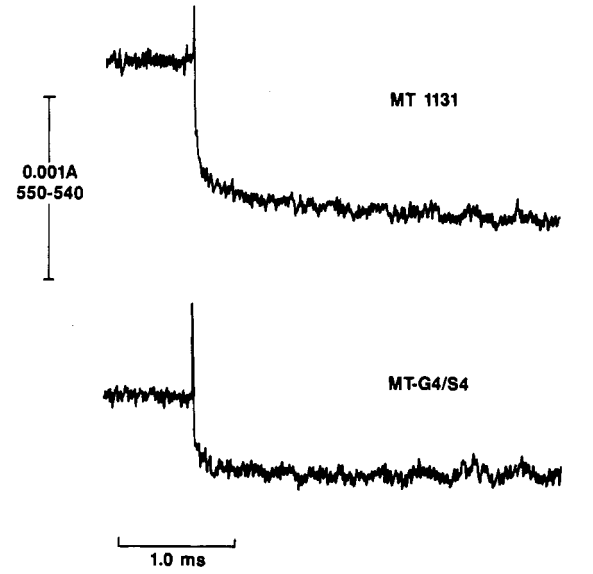

FIGURE 6: Kinetics of *c*-type cytochrome photooxidation. Chromatophores were suspended in the buffer used in Figure 4, with no redox dyes but a few crystals of sodium ascorbate added to bring the E_h near 150 mV. 10 μM UHNQ and 1 μM valinomycin were present, and the time constant for measurement was 3 μs. The traces represent the average of 100 flashes, spaced 1 min apart.

of the cell wall, and for the removal of the cell wall in MT1131 to convert the pattern of oxidation in this strain to one similar to that seen in MT-G4/S4. On the contrary, if cytochrome c_1 oxidation in MT-G4/S4 is via a soluble intermediary, then we would expect this to be washed off during the preparation of spheroplasts and for all *c*-type cytochrome oxidation to cease in both strains. Figure 7 shows that the former expectation is obtained; removing the cell wall has little effect on *c*-type cytochrome oxidation in MT-G4/S4 but converts the pattern seen in MT1131 to one similar to that seen in MT-G4/S4.

An alternative approach to the question of an intermediary electron carrier is seen in Figure 8. The E_{m7} of any donor to the reaction center can be determined by monitoring the extent of observed photooxidation as a function of potential. Although the kinetics of electron transfer are so rapid (Figure 6) that the full extent is not seen after the first flash (Figures 1, 2, and 7), the extent of unresolved electron donation to the reaction center can be obtained by comparing the amount of stably oxidized reaction center after the first and fourth flashes in the presence of UHNQ. As can be seen in the traces in Figure 8, this is the same after the first and fourth flashes at an E_h of +450 mV, where the primary donor is partially oxidized before the flash [E_{m7} = +450 mV; see Evans & Crofts (1974)]. However, at an E_h of +274 mV, the extent after the first flash is some 20% less than that seen after the fourth. A redox titration of such data is shown in Figure 8, fit to the E_{m7} of the donor being +350 mV (Figures 4 and 5), and the agreement of data with expectation is excellent.

Finally, we have assessed whether any electrogenic event accompanies the oxidation of cytochrome c_1 in MT-G4/S4. Phase I of the carotenoid bandshift occurs in response to charge separation between the primary donor and acceptor of the reaction center, while phase II has always been thought to be associated with the rereduction of the primary acceptor by cytochrome c_2 (Jackson & Dutton, 1973). Figure 9 shows that a similar bandshift occurs in MT-G4/S4, despite the lack of cytochrome c_2, and still titrates in at the same E_h, +350 mV. Phase III of the bandshift is associated with electron transfer in the cytochrome bc_1 complex (Jackson & Dutton, 1973; Bashford et al., 1979) and is seen in both MT1131 and MT-G4/S4. The total extent of phase III is reduced in the latter, presumably because the absence of cytochrome c_2 allows fewer cytochrome bc_1 complexes to be involved in reaction center driven electron flow.

DISCUSSION

R. capsulata MT-G4/S4 has the gene for cytochrome c_2 deleted and a cartridge conferring kanamycin resistance in its place (Daldal et al., 1986). The strain produces no cytochrome c_2 and yet is able to grow almost as well as the wild type in both aerobic and photosynthetic modes of growth under laboratory conditions. In this paper, we have investigated the effects of the lack of cytochrome c_2 on electron transfer. The total amount of *c*-type cytochrome in MT-G4/S4 is approximately half that in MT1131 (Daldal et al., 1986; Figures 1–5), which we interpret as indicating that the wild type has essentially equal amounts of cytochromes c_1 and c_2. This implies that both strains have similar amounts of the cytochrome bc_1 complex and they have similar amounts of total *b*-type cytochromes (Daldal et al., 1986) and the Rieske iron–sulfur center (data not shown).

We find that electron transfer is still inhibited by inhibitors of the cytochrome bc_1 complex, such as UHNQ (Figures 1, 2, and 7), antimycin (Figure 4), and myxothiazol (not shown), so we conclude that electron transfer is still cyclic and involves the cytochrome bc_1 complex. The question then is how do electrons get from the cytochrome bc_1 complex to the reaction center. In some genera of blue-green algae, such as *Scenedesmus*, plastocyanin serves as the electron conduit from the cytochrome b_6f complex to photosystem I unless the cells are copper deficient, in which case this role is fulfilled by a soluble *c*-type cytochrome (Crofts & Wood, 1978; Sandmann et al., 1983). We find no evidence that an alternative electron carrier is involved in MT-G4/S4. Indeed, all the evidence suggests that cytochrome c_1 is able to donate electrons directly to the reaction center with rapid kinetics. If an intermediary electron carrier is involved, our data indicate that it must have no prominent absorption bands in the 540–570-nm region of the spectrum that change with oxidation status and be tightly associated with the membrane, have intrinsically very rapid rates of oxidation by the reaction center and reduction by cytochrome c_1, and have an E_{m7} essentially identical with that of cytochrome c_1.

These findings provide a rationalization of our earlier findings with antibodies raised against cytochrome c_2 from *Rhodopseudomonas sphaeroides* and *R. capsulata* (Prince et al., 1975). These antibodies, which did not cross-react between species, had no effect on *c*-type cytochrome oxidation by the

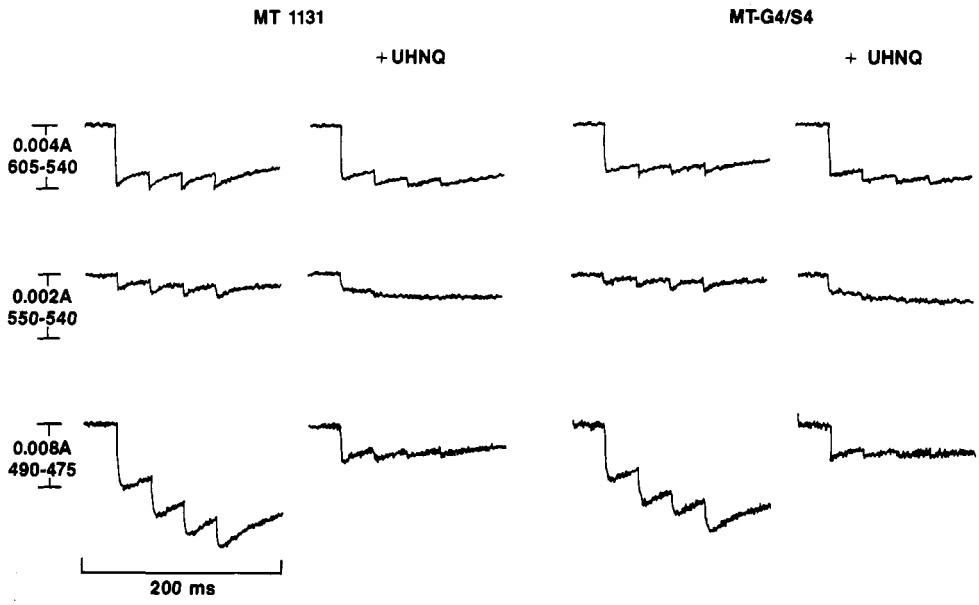

FIGURE 7: Response of the reaction center, *c*-type cytochromes and the carotenoid bandshift to four actinic flashes. Spheroplasts (25 μM bacteriochlorophyll) were suspended in 10 mM *N*-morpholinopropanesulfonate, 100 mM KCl, and 25% sucrose and examined as in Figure 1.

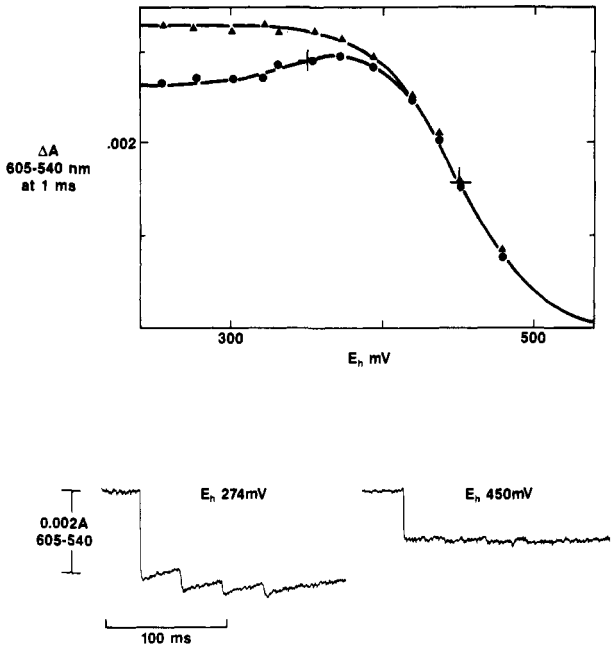

FIGURE 8: Redox titration of the electron donor to the reaction center in MT-G4/S4. Chromatophores (25 μM bacteriochlorophyll) were suspended in the buffer of Figure 4 with 20 μM 2,3,5,6-tetramethylphenylenediamine and 10 μM UHNQ. The bottom part of the figure shows representative traces; the upper part shows a redox titration of the extent of detectable (at 1 ms) photooxidized reaction center after the first (●) and fourth (▲) flashes.

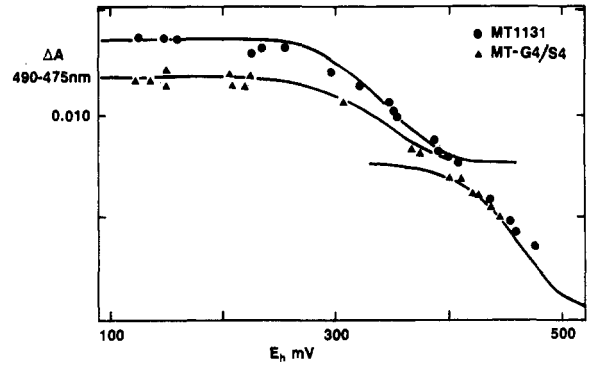

FIGURE 9: Redox titration of the carotenoid bandshift in the presence of an inhibitor of *c*-type cytochrome rereduction. Chromatophores (25 μM bacteriochlorophyll) were suspended in the buffer, and with the redox dyes, of Figure 4, with 2 μM 5-undecyl-6-hydroxy-4,7-dioxobenzothiazole. The latter inhibits at the same site as UHNQ (Bowyer et al., 1980) but has the advantage that it has less of an uncoupling effect. The extent of the carotenoid bandshift was measured 5 ms after the flash, and the lines drawn through the points are theoretical curves expected if the E_m of the reaction center "primary donor" is 460 mV and that of cytochromes c_1 and c_2 is 345 mV.

reaction center in chromatophores unless 1% cholate was added. Upon addition of the detergent, which did not completely solubilize the chromatophores, *c*-type cytochrome photooxidation could be completely inhibited by the anti-c_2 antibody in *R. sphaeroides*, but only approximately 50% inhibited in *R. capsulata*. In light of our present findings, these data can be interpreted as indicating that cytochrome c_1 oxidation by the reaction center obligatorily involves cytochrome c_2 in *R. sphaeroides*, but not in *R. capsulata*. This notion is currently being tested by experiments designed to delete the cytochrome c_2 gene in *R. sphaeroides*.

Our data provide an E_{m7} for cytochrome c_1 in the absence of complications arising from the presence of cytochrome c_2; Evans and Crofts identified only a single thermodynamic population of high-potential *c*-type cytochrome in *R. capsulata*, which at the time was attributed solely to cytochrome c_2 (Evans & Crofts, 1974). With the recognition by Wood (1980) that these organisms also contained a cytochrome c_1, the E_m values of the cytochromes c_1 and c_2 became a matter of debate, because values different by up to 60 mV would be hard to tell apart in the chromatophore membrane, given the experimental uncertainties. By resolving both the spectra and the kinetics of photooxidation, Meinhardt and Crofts (1982) determined the E_{m7} values for cytochromes c_1 and c_2 as 260 and 348 mV in the closely related *R. sphaeroides*, with the latter being oxidized by the reaction center more rapidly than the former. While cytochrome c_1 appears to have the longer α-band wavelength maximum in both species, our thermodynamic and kinetic data for *R. capsulata* are rather different: we find the two cytochromes to have very similar E_{m7} values (Figures 4 and 5) and that cytochrome c_1 is the more rapidly oxidized cytochrome (Figure 6). We attribute the relatively slow phase of cytochrome oxidation, following the initial rapid (<100 μs) phase seen in MT1131 in Figure 6, to the oxidation

of cytochrome c_2 by the reaction center, rather than the oxidation of cytochrome c_1 by c_2.

Our data indicate that "phase II" of the carotenoid bandshift (Jackson & Dutton, 1973) is a response, at least in part, to the oxidation of cytochrome c_1 by the reaction center (Figure 9). Previously, this phase has been attributed to the oxidation of cytochrome c_2 sitting at the membrane aqueous interface (Jackson & Dutton, 1973; Takamiya & Dutton, 1977), but our data indicate that the transmembrane charge separation responsible for the bandshift may instead arise from the oxidation of cytochrome c_1 of the cytochrome bc_1 complex. Thus, the heme of cytochrome c_1 is apparently close to the membrane–aqueous interface, appropriately placed to be near the iron–sulfur cluster of the Rieske iron–sulfur protein (Prince, 1983). Such a location is in concert with the location of the heme of the analogous cytochrome *f* in chloroplasts (Wiley et al., 1984; Cramer et al., 1985) which is oriented with the heme plane some 30° from the membrane plane (Crowder et al., 1982; Bergstrom & Vanngard, 1982).

Surprisingly, even the wild-type strain, MT1131, did not possess a rapid electron donor for every reaction center, even in whole cells grown photosynthetically for many (>10) generations (Figure 1). Similar results were seen in stationary phase cells, so this does not seem to be a function of growth conditions. Only about 20% of the reaction centers of MT-G4/S4 receive an electron rapidly from cytochrome c_1 (Figures 1, 2, 7, and 8), and the data suggest that a similar number are so reduced in MT1131, because there is an approximately equal extent of very fast cytochrome *c* oxidation, which we attribute to cytochrome c_1 (Figure 6). As we discussed above, we attribute the slower phase of cytochrome *c* oxidation seen in MT1131 in Figure 6 to electron donation from cytochrome c_2 to the reaction center, and since this phase is approximately equal in extent to the fast phase seen in MT-G4/S4, we conclude that only some 20% of reaction centers in MT1131 promptly receive electrons from cytochrome c_2, even in whole cells. This is rather less than estimates made earlier [Dutton et al., 1975; Bowyer et al., 1980; see Robertson et al., (1986)] and suggests that the extinction coefficients for the reaction center changes that have been used in the past are significant overestimates. Evaluating data similar to those in Figure 2 implies that the extinction coefficient for the reaction center measured at 605–540 nm is very similar to that for cytochrome c_1 measured at 550–540 nm. In other words, chromatophores contain rather more reaction centers than previously thought, perhaps by as much as 40% if we assume a millimolar extinction coefficient of 20 $mM^{-1}\cdot cm^{-1}$ for cytochrome c_1 at 550 nm. Such a reassessment of the reaction center concentration necessitates a reevaluation of the relative concentrations of cytochrome bc_1 complexes per reaction center. Using the original estimate for the extinction coefficient of the reaction center (29.8 $mM^{-1}\cdot cm^{-1}$; Dutton et al., 1975), we estimated that there was 0.7 cytochrome bc_1 complex per reaction center (van den Berg et al., 1979; Prince et al., 1978; Bashford et al., 1979; Prince, 1983): using an extinction coefficient of 20 $mM^{-1}\cdot cm^{-1}$ changes the ratio to 0.5, in line with predictions made by Crofts et al. (1983).

Furthermore, examination of the pattern of reaction center reduction after an actinic flash (Figures 1, 2, and 7) suggests that earlier attempts to quantitate the electron transfer between *c*-type cytochromes and the reaction center may have been misled by the apparently stable oxidation of the *c*-type cytochromes, following the flash, in the presence of inhibitors of the cytochrome bc_1 complex, for it is clear that the rate of rereduction of the photooxidized reaction center in the period from 2 to 32 ms after the flash is very different in MT1131 from that in MT-G4/S4. Even though the *c*-type cytochromes have reached a "steady-state" level of oxidation in both strains, the rate of rereduction of the reaction center in MT1131, containing cytochrome c_2, is much faster than in MT-G4/S4, which lacks it. This is true both in whole cells, with no added redox mediators (Figure 1), and in chromatophores with added mediators (Figure 2). In the past, we have assumed that this slow rereduction of the reaction center, in the absence of concomitant *c*-type cytochrome oxidation, was due to nonspecific reductants, but from the data presented here, it is clear that the effect is dependent on the presence of cytochrome c_2. In other words, even apparently "fully oxidized" cytochrome c_2 is an efficient mediator of electron flow to the reaction center.

We find that only about 20% of the reaction centers receive an electron from cytochrome c_1 on the first flash and that only about 66% of the total cytochrome c_1 is photooxidized by four flashes (Figures 2, 4, and 5). These ratios do not change when chromatophores are "purified" on a sucrose gradient and seem to indicate that some of the cytochrome c_1 is kinetically isolated from the reaction centers. While we have no explanation for this anomaly, the finding that only a portion of the reaction centers have a rapid reductant, even in whole cells of the wild type, calls into question the assignment of relative contributions to electrogenic events occurring in cyclic photosynthetic electron flow. The pioneering experiments of Jackson and Dutton (1973), subsequently repeated in many laboratories [see Prince et al. (1982), Crofts (1985), Bashford et al. (1979), Glaser & Crofts (1984), and Robertson et al. (1985)], indicated that the reactions within the reaction center gave rise to a phase of the carotenoid bandshift known as phase I. This was followed by phase II that seemed to report electrogenic events associated with the oxidation of *c*-type cytochromes. A subsequent phase, phase III, was associated with the reactions within the cytochrome bc_1 complex. Because phases I plus II thus seemed to report one complete transmembrane electrogenic translocation, and because the extent of phase III was equal in extent to phases I plus II, the cytochrome bc_1 complex was thus interpreted as responsible for a second complete transmembrane electrogenic translocation. Among the many unproven assumptions implicit in this assignment is the notion that all reaction centers are connected to a complete cyclic system. If, as now seems likely, only some reaction centers are connected to a complete cycle, then the number of cytochrome bc_1 complexes functioning to generate phase III of the bandshift is less than stoichiometric to the number of reaction centers. If all the reaction centers are contributing to phase I, the original assignment that the sum of phases I plus II equals phase III implies that a functioning cytochrome bc_1 complex may be generating more than 1 equiv of electrogenically translocated charge. Classical chemiosmotic models have been based on the underlying assumption that the electrochemical gradient of protons, the protonmotive force, is generated by the electrogenic movement of electrons across the membrane, and their electrically neutral return, accompanied by a proton (Mitchell, 1966). If each cytochrome bc_1 complex is contributing more than 1 equiv of charge, per reaction center, one explanation is that there could be electrogenic proton translocation in addition to the neutral translocation discussed above. Just how such electrogenic protons might be transported remains a matter of speculation, but photosynthetic bacteria present an excellent experimental vehicle for further tests of such possibilities.

Registry No. Cytochrome c_2, 9035-43-2.

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An Intermediate Polymer in the Assembly of Clathrin Baskets

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ABSTRACT: Clathrin (8 S) is known to polymerize into two varieties of basket structures (150 S or 300 S) under the normal buffer conditions [100 mM 2-(*N*-morpholino)ethanesulfonic acid (Mes), pH 5.9–6.7] used for the isolation of coated vesicles. However, it is now observed that under very low salt conditions (2 mM Mes, pH 5.9), it forms a homogeneous species with a sedimentation coefficient of 27 S. Increasing the salt concentration to 50 mM Mes completely converts all the 27S species into 150S baskets. Sedimentation equilibrium data show that this 27S species has a molecular weight that is 6 times that of the clathrin protomer and is the result of highly cooperative reversible self-association of the 8S protomer. Light-scattering studies show that the stabilities of 27S species and baskets (150 S or 300 S) are comparable. Fluorescent labeling of sulfhydryl groups with *N*-(1-anilinonaphthalenyl)maleimide indicates that the conformation of clathrin in 27S species and baskets (150 S or 300 S) is similar. Trypsin digestion reveals that in the 27S species clathrin has a conformation differing from that in both the 8S species and baskets.

Clathrin is the structural protein responsible for the polygonal organization of the coat in coated pits and coated vesicles (CVs)[1] (Pearse, 1976). The clathrin protomer (M_r ≈630 000), as isolated from CVs, contains three identical chains of 180 kDa and three light chains of two sizes, i.e., 33 or 36 kDa (Kirchhausen & Harrison, 1981), and has a sed-

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[1] Abbreviations: AN, anilinonaphthalene; ANM, *N*-(1-anilinonaphthalenyl)maleimide; CV(s), coated vesicle(s); kDa, kilodalton; Mes, 2-(*N*-morpholino)ethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane; Trp, tryptophan; SDS, sodium dodecyl sulfate.