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THE REACTION CENTER AND ASSOCIATED CYTOCHROMES OF *THIOCAPSA PFENNIGII*: THEIR THERMODYNAMIC AND SPECTROSCOPIC PROPERTIES, AND THEIR POSSIBLE LOCATION WITHIN THE PHOTOSYNTHETIC MEMBRANE

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Summary

The majority of photosynthetic bacteria possess bacteriochlorophyll *a*, but two species, *Rhodopseudomonas viridis* and *Thiocapsa pfennigii*, possess bacteriochlorophyll *b*, which absorbs at significantly longer wavelengths. The reaction center of *Rps. viridis* has been extensively studied; this paper presents studies on the reaction center of *T. pfennigii*, with the following results.

(i) The "primary donor" bacteriochlorophyll dimer of *T. pfennigii* has a midpoint redox potential of +490 mV at pH 7, and the oxidized form has a gaussian EPR signal centered close to $g = 2.0025$ with a peak-to-peak width of 13 G. The zero field splitting characteristics of the light-induced triplet state of the bacteriochlorophyll dimer, seen when the "primary acceptor" is reduced before illumination, are $D = 158 \cdot 10^{-4}$ cm^{-1}, $E = 39 \cdot 10^{-4}$ cm^{-1}. These EPR properties are very similar to those of the bacteriochlorophyll dimer of *Rps. viridis*, and are quite distinct from those of bacteriochlorophyll *a*-containing species.

(ii) The intermediary carrier, I, which is probably a bacteriopheophytin *b* molecule in close association with the "primary acceptor", can be trapped in its reduced form by the illumination of appropriately poised samples at 200 K. The EPR signals associated with I^- are very similar to those of *Chromatium vinosum*, with the majority of the spins being observed in the form of a broad signal centered close to $g = 2.003$, and split by 68 G; the magnitude of the splitting of this signal is only half that seen in *Rps. viridis*. We have been unable to measure the E_m of the I/I^- couple in *T. pfennigii*.

(iii) The "primary acceptor" of *T. pfennigii* is very similar to that of the

Abbreviations: BChl *a*, bacteriochlorophyll *a*; BChl *b*, bacteriochlorophyll *b*; $(BChl)_2$, bacteriochlorophyll dimer.

majority of other purple bacterial species, being characterized by an EPR signal at $g = 1.82$ and $g = 1.62$. The E_m of the redox couple is pH-dependent below pH 6.5, and the E_m of the unprotonated couple, which is believed to function in electron flow, is −130 mV.

(iv) Four cytochrome hemes can apparently donate electrons to the photooxidized bacteriochlorophyll dimer free radical cation, two of cytochrome *c*-555 (E_{m7} = 340 mV) and two of cytochrome *c*-550 (E_{m7} = 0). If all are reduced prior to illumination, the latter are preferentially oxidized.

(v) The carotenoid bandshift of *T. pfennigii* can be interpreted as indicating that the bacteriochlorophyll dimer is located near the middle of the membrane dielectric, with the high potential cytochrome *c*-555 closer to the inside, and quinone · iron closer to the outside of the chromatophore membrane. The low potential cytochrome *c*-550 seems to be nearer the outside of the membrane than the bacteriochlorophyll dimer.

Introduction

The early steps of purple bacterial photosynthesis occur within the photochemical reaction center, where an actinic photon causes the oxidation of a bacteriochlorophyll dimer, $(BChl)_2$, and the reduction of an intermediary electron carrier, I, which probably involves a bacteriopheophytin. The light reaction is then effectively rendered irreversible as the electron on I^- reduces the "primary acceptor", a quinone · iron complex. Recent reviews on these early events, and on the components involved, can be found in ref. 1—3.

The majority of photosynthetic bacteria possess bacteriochlorophyll *a* (BChl *a*) but two apparently unrelated species have been isolated which contain bacteriochlorophyll *b* (BChl *b*). These are *Rhodopseudomonas viridis*, originally known as *Rhodopseudomonas* sp. [4], and *Thiocapsa pfennigii*, originally known as *Thiococcus* sp. [5]. In both these species, the absorption maximum of the light-harvesting pigments is beyond 1000 nm, whilst species containing BChl *a* have their absorption maxima below 900 nm. This difference is also seen in the reaction center components; BChl *a*-containing reaction centers have absorption maxima near 870 nm, while the BChl *b*-containing reaction center of *Rps. viridis* has its absorption maximum at 985 nm [6]. In a previous paper [7] we discussed how this difference in wavelength maxima might be reflected in different thermodynamic properties of the primary reactants, and measured these parameters in *Rps. viridis*. Several differences were apparent, but it was not clear whether these reflected fundamental differences between BChl *b* and BChl *a* containing reaction centers, or rather differences peculiar to *Rps. viridis*. In order to distinguish between these two possibilities, this paper presents the thermodynamic properties of the reaction center of another BChl *b*-containing organism, *Thiocapsa pfennigii*.

Materials and Methods

Thiocapsa pfennigii 9111 was grown in the laboratory of Dr. John Olson at Brookhaven National Laboratory as previously described [8], and the cells

were stored at −20°C until required. Chromatophores were prepared using a French pressure cell [9]. Optical and electron paramagnetic resonance (EPR) spectroscopy were performed with the rapidly responding dual wavelength spectrophotometer [9] and Varian E-4 and E-9 spectrometers equipped with flowing helium cryostats (e.g., ref. 7) previously described. Redox potentiometry followed the method of Dutton [10].

The actinic light for the optical experiments was provided by a xenon flash lamp (full pulse width at half height = 6 μs) shielded by a Wratten 88A filter. In some experiments a Q-switched ruby laser (Apollo Laser Inc., Calif., Model 22HD) capable of producing either a single 20-ns pulse, or two such pulses separated by 450 μs, was used.

Results

The primary donor (BChl)$_2$

Fig. 1 shows a flash-induced spectrum of the reaction center absorption changes in the 600 nm region, together with a redox titration of the change. The maximal absorption change in the 600 nm region was at 610 nm, and the oxidation-reduction midpoint potential (E_m) at pH 7 was +490 mV.

Fig. 2 shows the EPR spectra of the primary donor of *T. pfennigii*. On the left is the light induced signal seen when quinone · iron was in the oxidized state prior to illumination, corresponding to the $(BChl)_2$ cation radical, $(BChl)_2^+$. The signal is gaussian in line shape, and is centered close to $g = 2.0025$. The peak-to-peak linewidth is approx. 13 G.

On the right of Fig. 2 is the light induced spin polarized triplet or "biradical" signal of the $(BChl)_2$, seen when the primary acceptor was reduced prior to illumination. The zero field splitting parameters are $D = 158 \cdot 10^{-4}$ cm^{-1} and $E = 39 \cdot 10^{-4}$ cm^{-1}.

c-Type cytochromes associated with the reaction center

T. pfennigii possesses several *c*-type cytochromes [11], but as suggested by

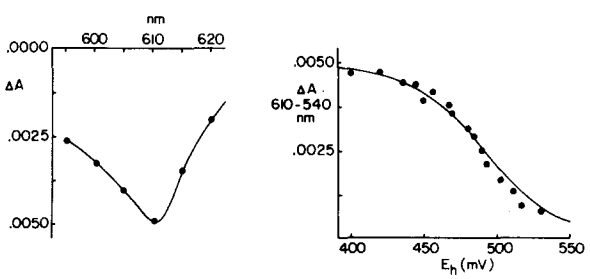

Fig. 1. Flash induced spectrum and redox titration of the reaction center of *T. pfennigii*. Chromatophores (26 μM BChl) were suspended in 20 mM *N*-morpholino-propane sulfonate, 100 mM KCl with 100 μM potassium ferri/ferro cyanide. The total flash activatable $(BChl)_2$ was measured 10 ms after four near-saturating flashes separated by 25 ms. 2 μM valinomycin was present to collapse the carotenoid bandshift (Fig. 7).

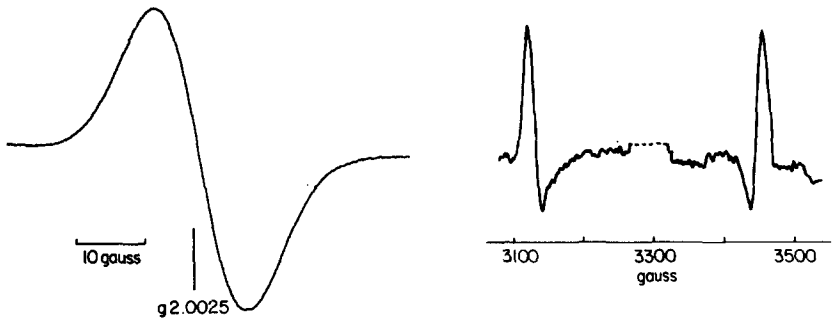

Fig. 2. EPR properties of the $(BChl)_2$ of *T. pfennigii*. Chromatophores (1.1 mM BChl) were suspended in the buffer used in the experiment of Fig. 1 in the presence of a few crystals of ascorbate (left) or dithionite (right) to reduce the $(BChl)_2$ and QFe respectively. Both spectra are light-minus-dark difference spectra, and were recorded at 6 K. EPR spectrometer settings: microwave power, 1 mW; modulation amplitude, 5 G for the spectrum on the left, 12.5 G for the spectrum on the right.

Olson et al. [8] only two are associated with the reaction center. Fig. 3 shows the α-band region of their absorbance spectra; at high potentials, a cytochrome with an α-band maximum at 555 nm was oxidized by a single-turnover flash of light, but at lower potentials this was replaced by another cytochrome with an α-band maximum at 550 nm. Both cytochromes were oxidized within the resolution time of the spectrophotometer (100 μs in these experiments), and took hundreds of milliseconds to go re-reduced. This rate of re-reduction was unaffected by uncoupling agents or by antimycin, suggesting that the cyclic photosynthetic electron flow system seen in whole cells [8] had been disrupted during the preparation of the chromatophores. A single 20-ns ruby laser pulse oxidized approximately half of the total complement of either cytochrome, depending on the ambient redox potential, while two such pulses, separated by 450 μs, oxidized more than 85% of the total cytochrome. This suggests that there are two hemes of each of the two cytochromes per reaction center.

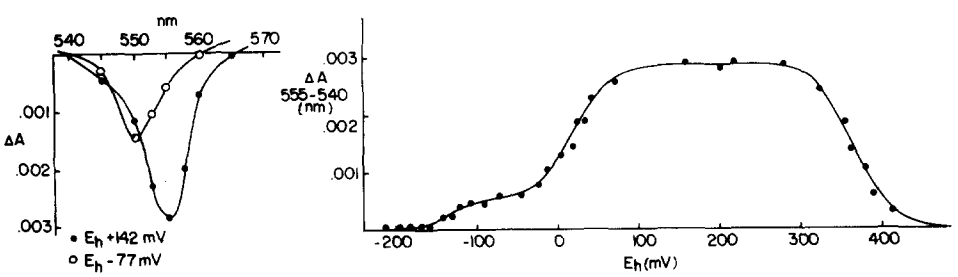

Fig. 3. Flash activated spectra and redox titration of the *c*-type cytochrome associated with the reaction center of *T. pfennigii*. Chromatophores (20 μM BChl) were suspended in the buffer used in the experiment of Fig. 1, together with 10 μM each of 2,3,5,6-tetramethyl phenylenediamine, *N*-methyl phenazonium methosulfate, *N*-ethyl phenazonium ethosulfate, 2-hydroxy-1,4-naphthaquinone and pyocyanine as redox mediators. 2 μM valinomycin was present to collapse the carotenoid bandshift (Fig. 7). On the left of the figure are the changes measured 10 ms after a single turnover flash, at ambient potentials of +142 (±5) mV or −77 (±5) mV. These give the spectra of the high potential and low potential cytochromes, respectively. On the right of the figure is a redox titration of the absorbance changes measured at 555—540 nm.

On the right of Fig. 3 is a redox titration of the cytochrome oxidation after a single turnover flash: the line drawn through the points represents the theoretical curve for a reaction center which possesses two identical high potential (E_{m7} = 340 mV) cytochrome *c*-555 hemes and two identical low potential (E_{m7} = 0) cytochrome *c*-550 hemes, where the low potential cytochrome is preferentially oxidized if all the cytochromes are reduced prior to activation. All cytochrome oxidation ceases at low potential as the "primary acceptor" QFe is reduced prior to activation.

The primary acceptor QFe

Fig. 4 shows the results of experiments similar to that of Fig. 3, where the E_m of the primary acceptor was measured as a function of pH. The data can be separated into a region where the E_m varies with pH by −60 mV/pH unit, and a region where the E_m is pH-independent, the junction between the two regions being at pH 6.5. As will be discussed later, this indicates a p*K* on the reduced form of the primary acceptor. In all cases the titration indicated that the redox reactions of the primary acceptor involve only a single electron (n = 1).

The EPR spectrum of the "primary acceptor" is shown in the top right of Fig. 6; like that of *Rps. sphaeroides* [12,13] *Chromatium vinosum* [14] and *Rps. viridis* [7] it is characterized by a prominent signal at g = 1.82.

The intermediary electron carrier I

In those species which possess cytochromes capable of donating electrons to the reaction center at low temperatures, I can be trapped in a reduced state by the illumination of appropriately poised samples at 200 K [15,17]. With QFe chemically reduced prior to illumination, photochemistry within the reaction center is limited to the transient oxidation of $(BChl)_2$ and reduction of I, which promptly relaxes (see ref. 1). However, the low potential *c*-type cytochrome hemes are capable of irreversible reduction of $(BChl)_2^+$ at low temperatures, and although this is very slow compared to the re-reduction of $(BChl)_2^+$ by I^-, the irreversible nature of the cytochrome oxidation eventually results in the photochemical trapping of I^-, solely at the expense of cytochrome oxidation [15–18]. In both *C. vinosum* and *Rps. viridis* this reaction has a halftime of about

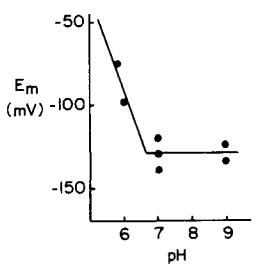

Fig. 4. Thermodynamic properties of the "primary acceptor" of *T. pfennigii*. The figure presents data obtained from experiments similar to those of Fig. 3, where the E_m of the "primary acceptor" was determined from the attenuation of cytochrome oxidation or the carotenoid bandshift (Fig. 8) at low ambient potentials.

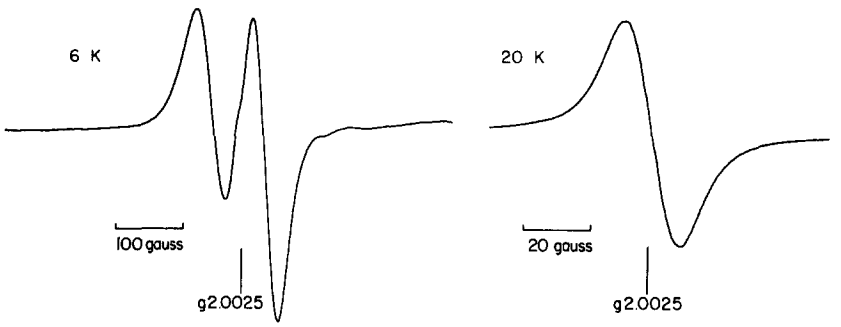

Fig. 5. EPR properties of I^- in *T. pfennigii*. Chromatophores (1.1 mM BChl) were suspended in the buffer of the experiment of Fig. 1, with a few crystals of dithionite to reduce QFe. The sample was cooled to 200 K, illuminated for 5 min (see refs. 15—18) and then cooled to 77 K until measurements were made in the spectrometer. The spectra were taken at the temperatures indicated at a microwave power of 10 mW and a modulation amplitude of 5 G.

30 s at 200 K, and about 60 min at 77 K, although both these rates are limited by the actinic light intensity [15—18].

Fig. 5 shows the EPR spectra associated with I^- in *T. pfennigii* trapped by 5 min of illumination at 200 K; they are very similar to those seen in *Rps. viridis* [17] and *C. vinosum* [15,16] although they are more similar to the latter. At low temperatures (<15 K), I^- is seen as a signal which is split by about 68 G around $g \approx 2.003$. This signal is very difficult to saturate with microwave power, but is hardly detectable at temperatures above 17 K. There is also a small amount of unsplit radical in I^-, which is readily saturated at low temperatures.

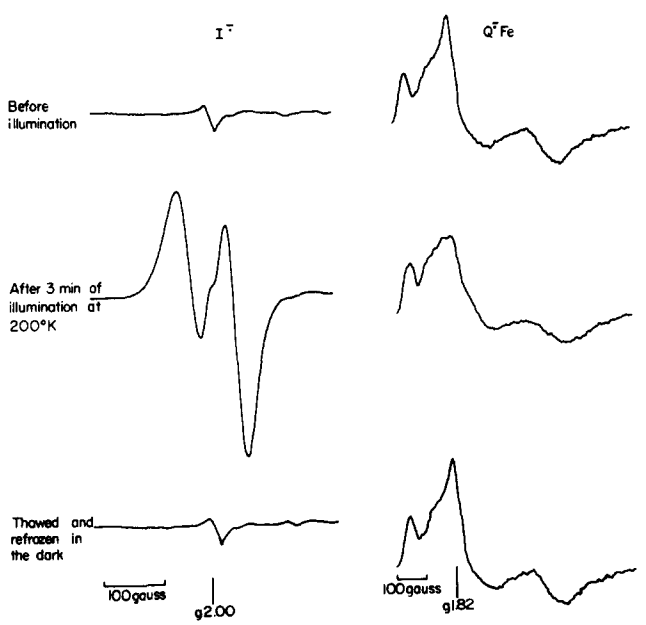

Fig. 6. The reversible reduction of I^-, and its effect on Q^-Fe. The sample was that used in the experiment of Figure 5, and similar instrument settings were used. The temperature was 6 K.

This signal is also centered close to $g = 2.003$, with a peak to peak linewidth of about 12 G. However, it appears to account for less than 10% of the total electron spins in I^-.

At higher temperatures (above 15 K) the broad split signal is replaced by a narrower, unsplit signal which is still centered close to $g = 2.003$, and which is 16 G wide at 20 K. This signal has a line shape intermediate between gaussian and lorentzian, and is difficult to saturate with microwave power.

The photochemical trapping of I^- is readily reversed (Fig. 6) by thawing the sample, because the warmer temperatures allow the oxidation of I^- and the reduction of the ferricytochrome in response to the ambient redox potential. Fig. 6 also shows the effect of trapping I^- on the $g = 1.82$ Q^-Fe signal; it diminishes the total magnitude of the Q^-Fe signal, but in particular it apparently decreases the sharp tip of the band at $g = 1.82$. This change in Q^-Fe is reversed when I^- is allowed to reoxidize at higher temperatures. As we have found in *Rps. viridis* [7,17,18] and *C. vinosum* [15,16], the reduction of I prevents the generation of the spin polarized triplet of $(BChl)_2$, while the reoxidation of I^- again allows the full generation of the triplet.

The carotenoid bandshift of T. phennigii

T. pfennigii possesses 3,4,3′,4′-tetrahydrospirilloxanthin as its major carotenoid [5] and this has peaks at 465, 493 and 530 nm in vivo [8]. As noted by Olson et al. [8], the carotenoids shift their absorption maxima to the red during illumination, and Fig. 7 shows a spectrum of this change after a single turnover flash. In addition to the carotenoid bandshift, Fig. 7 also includes contributions from the oxidation of cytochrome *c*-555. The carotenoid bandshift is formed within the rise time of the instrument (100 μs) and has a decay halftime of hundreds of milliseconds. The decay halftime is related to the "coupling status" of the membrane; in the presence of uncoupler, or valinomycin in the presence of potassium ions, the decay can be rendered complete within 1 ms of the flash.

Fig. 8 shows a redox titration of the carotenoid bandshift, and underneath

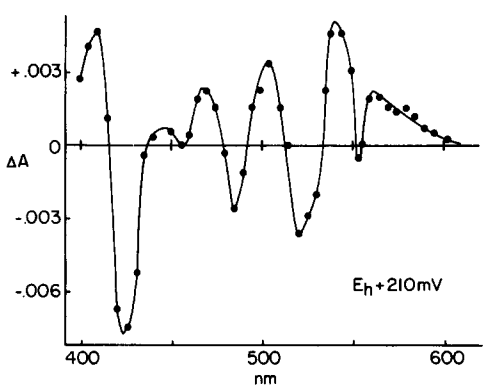

Fig. 7. The carotenoid bandshift of *T. pfennigii*. Chromatophores (20 μM BChl) were suspended as in the experiment of Fig. 3 in the absence of valinomycin. The spectra represents the change 10 ms after a single turnover flash of light and includes the carotenoid bandshift, together with contributions in the 400, 530 and 555 nm regions due to the oxidation of cytochrome *c*-555.

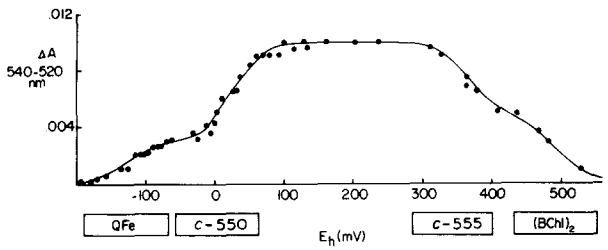

Fig. 8. A redox titration of the carotenoid bandshift of *T. pfennigii*. The experiment used the sample used in the experiment of Fig. 7, and assayed the carotenoid bandshift as the change seen at 540–520 nm.

are shown the thermodynamic properties of the components identified in Figs. 1, 3 and 4, where each box extends over the Nernstian range of ambient redox potential which causes a component to change from 90% oxidized to 90% reduced.

The maximal extent of carotenoid bandshift per single turnover flash occurs at redox potentials near 200 mV, where $(BChl)_2$ and cytochrome *C*-555 are reduced, but QFe and cytochrome *c*-550 are oxidized prior to activation. At an ambient redox potential of 400 mV, where only $(BChl)_2$ is reduced prior to illumination, only half of the maximal carotenoid bandshift was formed per flash, while even less, about one-third, was generated at potentials (e.g., E_h −20 mV) where cytochrome *c*-550 promptly re-reduced $(BChl)_2^+$; no carotenoid shift was generated if QFe were reduced, or $(BChl)_2$ were oxidized prior to activation. The line drawn through the data of Fig. 8 is the amalgam of Nernst curves which describe a situation where two identical cytochrome *c*-555 hemes and two identical cytochrome *c*-550 hemes serve each $(BChl)_2$.

Discussion

The primary donor $(BChl)_2$

It is currently accepted that the primary donor of BChl *a*-containing species is a bacteriochlorophyll dimer, or "special pair", as suggested by the designation $(BChl)_2$. Evidence in support of this has come from two principal sources: (i) the line width of the EPR spectrum of the oxidized reaction center BChl *a* shows a $\sqrt{2}$ narrowing compared to that of monomeric BChl a^+ [19,20] suggesting that the unpaired electron is shared over a rather symmetrical pair of BChl *a* molecules; and (ii) the zero field splitting parameters of the light induced triplet state of the reaction center BChl *a* are consistent with expectations for a biradical state involving two BChl *a* molecules [21,22].

The EPR properties of the reaction center BChl *b* of *T. pfennigii* (Fig. 2) and *Rps. viridis* [7,20] are somewhat different, particularly in the linewidth of the oxidized reaction center BChl *b*. In both cases, this has a linewidth of 12–13 G (Fig. 2 and refs. 2, 7, 20), which is only marginally narrower than the 14 G measured in vitro for monomeric BChl b^+ [2]. Nevertheless the zero field splitting parameters of the reaction center triplets (Fig. 2 and ref. 7), although distinctly different from those of a variety of BChl *a* containing species [7,22],

still suggest that the reaction center primary donors of the BChl *b*-species contain more than a single molecule of BChl *b*. Taken together, the EPR data suggest that the primary donors of both *Rps. viridis* and *T. pfennigii* are dimers of BChl *b*, but that they are perhaps less symmetrically arranged than their counterparts in BChl *a*-containing species. As such they have been referred to as $(BChl)_2$ throughout this work. We may thus conclude that the unusual EPR properties of the $(BChl)_2$ of *Rps. viridis* [2,7,20] and of *T. pfennigii*, probably reflect properties imposed by the presence of BChl *b*.

c-Type cytochromes

Fig. 3 suggests that two kinetically and thermodynamically identical cytochrome *c*-555 hemes, together with two similarly identical cytochrome *c*-550 hemes, share the same reaction center in *T. pfennigii*, and further than when all are reduced prior to light activation, it is the low-potential cytochrome *c*-550 hemes which are preferentially oxidized by the $(BChl)_2^+$. Similar cytochrome complements, with a similar priority for oxidation, are found in other species; there are two high potential cytochrome *c*-555 [23] and two low potential cytochrome *c*-553 hemes [16] per $(BChl)_2$ in *C. vinosum*, and two high potential cytochrome *c*-558 hemes, together with two low potential cytochrome *c*-553 hemes in *Rps. viridis* [7]. Even in those species which lack a low potential *c* type cytochrome capable of donating electrons to $(BChl)_2^+$, such as *Rps. sphaeroides* [9,24], *Rps. capsulata* [24] and *Chlorobium limicola* f. *thiosulfatophilum* [25], there are still two kinetically and thermodynamically identical high potential *c*-type cytochromes per reaction center.

The "primary acceptor" QFe

The classical rationale for defining the "primary acceptor" of photosynthetic bacteria has relied on the attenuation of readily measurable flash-induced changes associated with the reaction center, such as $(BChl)_2$ or *c*-type cytochrome oxidation, or carotenoid bandshifts, as QFe is reduced prior to activation (see refs. 22, 26). It is now recognized that even when QFe is reduced prior to activation, $(BChl)_2$ can undergo transient oxidation, with the concomitant reduction of I [1,28], although this transient state, originally called P^F by Parson et al. [29], decays very rapidly (in less than 10 ns) and was not recognized in earlier work. Nevertheless, although the classically defined "primary acceptor" is not in fact the first acceptor of the electron from $(BChl)_2$, it does play a very important role in the economy of the cell (see ref. 1): it is the reduction of QFe by I^- which effectively renders the light reaction irreversible because the life-time of the $[(BChl)_2^+ I]Q^-Fe$ state is suitably in excess (hundreds of milliseconds) of the halftime of *c*-type cytochrome oxidation ($t_{1/2} < 100$ μs in *T. pfennigii*).

It is now accepted that the primary acceptor of purple bacteria is a quinone iron (QFe) complex, where the electron from $(BChl)_2$ (via I/I^-) resides mainly on the quinone [30]. In *Rps. sphaeroides* [31] the quinone is ubiquinone, while menaquinone apparently fulfils this role in *C. vinosum* [32] and *Rps. viridis* [17,33]. The EPR spectrum of Q^-Fe in all these species has a prominent band close to $g = 1.82$, with another band which seems to vary in different species and preparations from $g = 1.60$ to $g = 1.73$ [7,13,34]. The signal in *T.*

pfennigii is characterized by bands close to $g = 1.82$ and $g = 1.62$, and as such is very similar to that of *C. vinosum* [14,33]. It is quite different from the $g = 1.87$ signal recently seen in reaction centers from *Rhodospirillum rubrum* [35].

The thermodynamic properties of the primary acceptor QFe in *T. pfennigii* are very similar (Fig. 4) to those of QFe in other species [7,26,27]. Near neutral pH the E_m varies with pH by −60 mV/pH unit, while beyond a pK at pH 6.5 it becomes pH-independent. As we have discussed at length elsewhere [26, 27], this indicates a pK on the reduced form ($Q^{\cdot}HFe/Q^{\cdot-}Fe$) at pH 6.5. Evidence from a variety of species (see ref. 27) suggests that the operating redox couple involves the unprotonated reduced species, where the E_m is that measured beyond the pK. In *T. pfennigii* this E_m is −130 mV, which compares with −150 mV in *Rps. viridis* [7] and −160, −180 and −200 mV in *C. vinosum*, *Rps. sphaeroides* and *R. rubrum*, respectively [26]. The redox span (ΔE_m) between (BChl b)$_2$ and QFe in *T. pfennigii* (620 mV) and *Rps. viridis* (650 mV, ref. 7) is thus not significantly different from that between (BChl a)$_2$ and QFe in the three BChl a containing species (630 mV in *Rps. sphaeroides*, 650 mV in *C. vinosum* and *R. rubrum*, see ref. 27), despite the difference in energy of actinic photons of 860 or 985 nm light.

The intermediary carrier I

Since the first indications of the existence of the intermediary carrier, I, in purple bacterial reaction centers [36], considerable progress has been made towards elucidating its chemical identity (see refs. 1—3). The current consensus is that I is probably a single bacteriopheophytin molecule [2] in close association with the iron of the QFe primary acceptor [15—17,37]. This close association between I and QFe appears to be reflected in the broad split EPR signal attributable to $I^{\cdot-}$ [15—17], which suggests that the two centers interact magnetically mainly via exchange coupling. Further evidence in favor of this suggestion comes from the parallel, but rather unusual, magnetic relaxation properties of the $g = 1.82$ signal of $Q^{\cdot-}Fe$ and the broad split signal of $I^{\cdot-}$; both are seen only at very low temperatures (<20 K), and are very difficult to saturate with microwave power [37].

The EPR signals associated with $I^{\cdot-}$ in *T. pfennigii* are very similar to those seen in *C. vinosum* [15,16] and *Rps. viridis* [17], and have similar magnetic relaxation properties. At low temperatures (<15 K), $I^{\cdot-}$ is seen mainly as a signal which is split around close to $g = 2.003$ by about 68 G. This is very similar to the splitting seen in *C. vinosum* [15—17], although it is substantially less than the approximately 130 G splitting seen under identical conditions in chromatophores of *Rps. viridis* [17]. Under ideal conditions, all of the $I^{\cdot-}$ population in *Rps. viridis* chromatophores is seen as the broad, split signal [17], while in *C. vinosum*, some 30—50% of the total $I^{\cdot-}$ population is present as a narrow, unsplit free-radical signal, even in whole cells [15,16]. The chromatophores of *T. pfennigii* used in this work seem to fall between these two extremes, with less than 10% of the total $I^{\cdot-}$ population being present as the unsplit free-radical signal. The magnetic relaxation properties of the unsplit $I^{\cdot-}$ signal seen at low temperatures suggest that it arises from $I^{\cdot-}$ moieties which are not magnetically coupled to $Q^{\cdot-}Fe$, but whether this is a physiological situation, or merely reflects some damage incurred during the low temperature trapping of $I^{\cdot-}$, remains to

be determined. At higher temperatures, I^- in *Rps. viridis* is seen as a broad lorentzian line centered close to $g = 2.003$ [17], which is what might be expected if I^- were exchanged coupled to a rapidly relaxing paramagnetic system, such as the metal containing Q^-Fe. A similar explanation, but with a less intense interaction, could explain the signal seen under these conditions in *T. pfennigii* (Fig. 5). Further evidence in favor of an interaction between Q^-Fe and I^- in *Rps. viridis* comes from the observation that the Q^-Fe signal is apparently split and broadened by its interaction with I^- so that the $g = 1.82$ signal apparently disappears [17]. A similar, although again less intense, interaction would explain the changes in Q^-Fe associated with I reduction seen in Fig. 6.

In *Rps. viridis* we were able to obtain an equilibrium midpoint potential (E_m) for the I/I^- couple by both redox potentiometry [7,18] and by a method which involved comparing the E_m of I/I^- with that of the hydrogen ($H^+/½H_2$) electrode at various values of pH [17]. Both methods gave a value of close to −400 mV, $n = 1$ for the I/I^- couple in chromatophores. Despite several attempts, neither method has yet yielded an E_m value for I/I^- in *T. pfennigii* or *C. vinosum*. The reasons for this failure are unknown, although it is noteworthy that the EPR properties of I^- in these latter two organisms are very similar, while those of I^- in *Rps. viridis* are rather different. It thus seems that the properties of I^- in *Rps. viridis* [7,17] which are different from those of *C. vinosum* [15, 16] and *T. pfennigii* are probably specific properties of *Rps. viridis*, rather than properties imposed by the presence of BChl *b*.

The carotenoid bandshifts of T. pfennigii

Light-induced red shifts of the absorbance spectra of carotenoids occur in a variety of photosynthetic bacteria and, although the mechanism of these shifts is obscure [38], empirical observations suggest that they are in response to membrane potentials across all or part of the chromatophore membrane [9, 10,39,40]. In *Rps. sphaeroides*, three kinetically and thermodynamically distinct phases of the carotenoid bandshift can be readily resolved [40]. The fastest of these, Phase I, is associated with electron flow from $(BChl)_2$ via I to QFe, while Phase II is associated with the re-reduction of $(BChl)_2^+$ by ferrocytochrome c_2. In fact, Phase II of the carotenoid bandshift is itself biphasic, with kinetics apparently identical to the two phases of cytochrome c_2 oxidation [9]. In addition there is a third phase of the carotenoid bandshift which is equal in extent to the sum of Phases I plus II, and which is apparently linked to reactions accompanying the re-reduction of ferricytochrome c_2 [41]. All three phases are additive. Jackson and Dutton [40] interpreted these results as indicating that electron flow from cytochrome c_2, via $(BChl)_2$ and I, to QFe effectively spanned the membrane, with $(BChl)_2$ located within the membrane dielectric. As has been discussed elsewhere [41,42] the antimycin sensitive Phase III of the bandshift seems to represent a separate transmembrane electrogenic event associated, probably indirectly, with ferricytochrome c_2 re-reduction.

Although both *C. vinosum* [43] and *T. pfennigii* (ref. 8 and Fig. 7) show light-induced carotenoid bandshifts, neither seems to have an antimycin sensitive phase. However there are thermodynamically resolvable phases which are clearly associated with electron flow in the reaction center from $(BChl)_2$, via

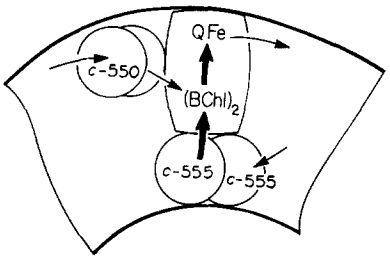

Fig. 9. A model for the arrangement of the reaction center and its associated cytochromes in *T. pfennigii*. The model is based on the carotenoid bandshift data in Fig. 8, as discussed in the text. I is omitted since the carotenoid bandshift provides no hint as to its location. The high potential cytochrome (*c*-555) is assumed to be a part of the cyclic electron transport system which is coupled to ATP synthesis in vivo, while the low potential cytochrome (*c*-550) is assumed to be part of a non-coupled, non-cyclic, probably substrate-linked pathway for replenishing the cyclic system. Rather similar models for other bacteria are discussed in ref. 42.

I to QFe, and with the subsequent re-reduction of $(BChl)_2^+$ by *c*-type cytochromes (ref. 43 and Fig. 8). In *T. pfennigii* there is a carotenoid bandshift associated with electron flow from $(BChl)_2$, via I, to QFe, which can be seen alone at ambient potentials where only $(BChl)_2$ is reduced prior to flash-activation. When cytochrome *c*-555 is also reduced prior to illumination, the carotenoid bandshift is approximately doubled in extent, but when cytochrome *c*-550 is also reduced prior to activation, the total bandshift is actually less than that which occurs when there is no cytochrome oxidation.

In terms of using the carotenoid bandshift to assign intramembrane locations to the reaction center and its associated cytochromes (see, for example, refs. 40--42, 44) Fig. 8 may be interpreted as shown in Fig. 9. Thus electron flow from $(BChl)_2$ via I to QFe is electrogenic, and generates a carotenoid bandshift, which is doubled as the charge separation is increased by the oxidation of cytochrome *c*-555. However, if the low potential cytochrome *c*-550 is reduced prior to activation, and it reduces $(BChl)_2^+$, the amplitude of the carotenoid bandshift decreases, presumably because the ferri-cytochrome *c*-550 heme is closer to the outside of the membrane than $(BChl)_2^+$. Fig. 9 also assumes that the cytochrome *c*-555 complement is near the inside of the chromatophore membrane, by analogy with other species (see ref. 42). Although there is no direct evidence to support the model of Fig. 9, it is worth noting that independent methods of assigning intramembrane locations of electron flow components in a variety of other species have consistently given results in agreement with interpretations of carotenoid bandshifts (see ref. 42).

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