Biochimica et Biophysica Acta, 440 (1976) 622–636

BBA 47152

THERMODYNAMIC PROPERTIES OF THE REACTION CENTER OF *RHODOPSEUDOMONAS VIRIDIS*

IN VIVO MEASUREMENT OF THE REACTION CENTER BACTERIOCHLOROPHYLL-PRIMARY ACCEPTOR INTERMEDIARY ELECTRON CARRIER

ROGER C. PRINCE, JOHN S. LEIGH, Jr. and P. LESLIE DUTTON

Johnson Research Foundation, Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, Pa., 19174 (U.S.A.)

(Received March 16th, 1976)

SUMMARY

The thermodynamic properties of redox components associated with the reaction center of *Rhodopseudomonas viridis* have been characterized with respect to their midpoint potentials and relationship with protons. In particular a midpoint potential for the intermediary electron carrier acting between the reaction center bacteriochlorophyll and the primary acceptor has been determined. The rationale for this measurement was that the light-induced triplet/biradical EPR signal would not be observed if this intermediate was chemically reduced before activation. The midpoint potential of the intermediary at pH 10.8 is about -400 mV ($n = 1$).

INTRODUCTION

The primary electron transfer reactions of bacterial photosynthesis occur within the reaction center bacteriochlorophyll protein (see ref. 1 for a recent review). Following the absorption of light energy, the reaction center primary donor (a bacteriochlorophyll dimer, termed $(BChl)_2$) becomes oxidized and the primary acceptor (probably a quinone associated with iron, termed X) becomes reduced. Recent work on isolated reaction centers from *Rhodopseudomonas sphaeroides* has measured the kinetics of this oxidation and reduction of the primary reactants [2–4] and it has become clear that while the reduced primary acceptor is the first reduced species which is stable on a ms time scale, at least two primary reactions occur within the bacteriochlorophyll/bacteriopheophytin complement of the reaction center before the reduction of X. ps spectroscopy has revealed that the electron is ejected from the bacteriochlorophyll dimer within 10 ps of a flash of light [4], although it does not arrive at the primary acceptor until some 100–200 ps later [2, 3]. During this time, the electron is presumed to reside on what must be a strongly reducing intermediary electron carrier (designated I), which Fajer et al. [5] have suggested may be bacterio-

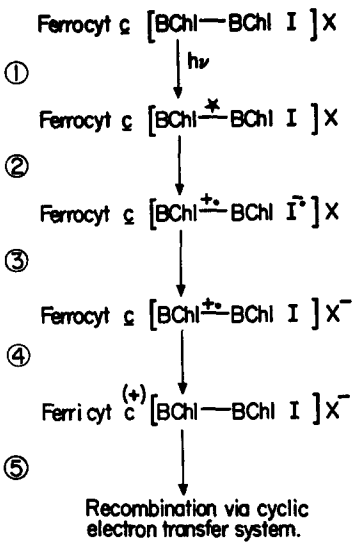

Fig. 1. A working scheme for the early forward steps of photosynthesis.

pheophytin. Current evidence thus supports the scheme shown in Fig. 1 (ref. 4.) Illumination (step 1) excites the reaction center bacteriochlorophyll to an excited singlet state which loses an electron within 10 ps (step 2) to form I^- and [$BChl^+$–BChl]. This transient state of oxidized bacteriochlorophyll dimer, reduced intermediate appears to be the State P^F identified by Parson et al. [6]. If normal photochemistry is to occur, the reduced intermediate reduces (step 3) the primary acceptor in 100–200 ps, but if this is blocked by the prior chemical reduction of X, the lifetime of the intermediary state is longer (10–30 ns, ref. 6) and the electron falls back from I^- to the bacteriochlorophyll dimer. At low temperatures this decay seems to occur solely via a spin polarized triplet or biradical [7–9]. The zero field splitting parameters of this triplet state, and its strong polarization, suggest that it is formed by the recombination of the electron on the reduced intermediate with the unpaired electron remaining on the oxidized bacteriochlorophyll dimer, and as such its formation is analogous to chemically induced dynamic electron spin resonance (CIDEP, see ref. 10). If these considerations are correct, the light-induced triplet should only be observable if X, but not I, is reduced prior to activation, since if I was also in the reduced state prior to illumination, the electron would not be able to leave the excited bacteriochlorophyll dimer, and so could not return to form the spin-polarized triplet. Support for this model can therefore be sought experimentally by establishing reducing conditions in the environment of the reaction center negative enough to abolish the light-induced triplet state. Quantification of such an effect, using redox potentiometry to establish known redox potentials (E_h) of samples to be examined for the light-induced triplet formation, would provide us with the thermodynamic course of oxidation-reduction of the I/I^- couple and hence its midpoint potential (E_m). We have tested this postulated behaviour in the experiments reported here, using *Rps. viridis*.

Rps. viridis differs from other members of the *Rhodospirillaceae* (purple non-

sulfur photosynthetic bacteria) in that it possesses bacteriochlorophyll *b* (BChl *b*) instead of the bacteriochlorophyll *a* (BChl *a*) found in the other species [11–13]. The absorption bands of BChl *b* are at longer wavelengths than those of BChl *a*, so the amount of energy available in the lowest excited singlet of the former may be considerably less than in the latter, being 1.26 eV compared with 1.42 eV. We would thus expect that the redox span generated in the light in *Rps. viridis* would be less than that in *Rps. sphaeroides* or other BChl *a*-containing species, and that this might perhaps be reflected in a more positive midpoint potential of I, bringing the latter within experimental reach. Using the rationale described above we have been able to determine a midpoint potential of the I/I^- couple, and we have also examined some other thermodynamic and kinetic properties of the reaction center of this organism.

MATERIALS AND METHODS

Rps. viridis (strain NHTC 133) was grown, and chromatophores (sometimes known as lamellae) were prepared, essentially as described previously [14] except that the cells were grown in a medium containing 0.3 % yeast extract instead of succinate as the carbon source. Redox potentiometry and the measurement of rapid flash induced spectroscopic changes were performed as before [14, 15], using a xenon flash lamp (full width at half height = 6 μs) as the actinic source. Control experiments using a 20 ns Q-switched ruby laser pulse indicated that the xenon flash was approximately 85 % saturating in intensity, and did not appear to elicit more than a single turnover of the reaction center.

Electron paramagnetic spin resonance measurements were made using a Varian E-4 EPR spectrometer equipped with a flowing helium cryostat and temperature control. Redox potentiometric control of samples and anaerobic transfer to EPR tubes was as described previously [7–9]. Samples were maintained in the near darkness before analysis. To establish very low potentials the pH of the suspending medium had to be appropriately alkaline to avoid problems arising from the hydrogen electrode (e.g., E_m $H^+/\frac{1}{2}H_2$ at pH 7.0 −420 mV; at pH 11 −660 mV). Such high values of pH did not appear to cause any serious damage to the chromatophores as judged by redox titrations of light induced changes associated with the reaction center bacteriochlorophyll or *c*-type cytochromes (see also ref. 15). Light minus dark EPR spectra were obtained using a Nicolet 1074 computer.

RESULTS

The midpoint potential of the reaction center bacteriochlorophyll

Holt and Clayton [13] showed that the reaction center absorption changes in *Rps. viridis* had bleaching maxima at 985 nm and 600 nm. Fig. 2 shows a flash induced spectrum of the latter region, and also shows a redox titration of the change. The oxidation-reduction midpoint potential (E_m) at pH 8 was +500 mV, and other workers have obtained essentially similar results in the neutral pH range [16, 17]. Performing the titrations at widely differing pH values did not markedly change the measured E_m value (E_m = +540 mV at pH 5.3, +475 mV at pH 10.3).

The midpoint potentials of the cytochrome c

Rps. viridis possesses two different *c*-type cytochromes which can donate

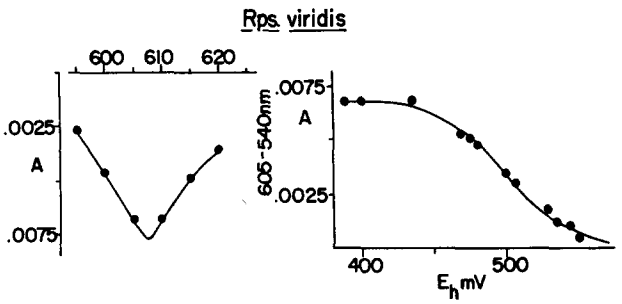

Fig. 2. Flash induced spectrum and redox titration of the reaction center in *Rps. viridis*. Chromatophores (18 μM BChl) were suspended in 20 mM Tris/100 mM KCl pH 8, with 100 μM potassium ferri-ferro cyanide. The total flash activatable $(BChl)_2$ at each redox potential was measured after four flashes spaced 25 ms apart to avoid problems where cytochrome c_{558} was reduced prior to activation, since this cytochrome can donate electrons to $(BChl)_2^+$ faster than the instrumental response time [18]. However, the slow ($\blacktriangleright$ 25 ms) re-reduction of the cytochrome allows no rapid re-reduction problems of $(BChl)_2^+$ after the third flash.

electrons to the reaction center, designated cytochromes c_{553} and c_{558} from their α-band maxima [16, 18]. Their midpoint potentials, determined in dark equilibrium titrations [16] have been reported as -12 mV and $+330$ mV respectively. Multiple flash activation of chromatophores poised at an E_h of $+200$ mV (i.e., c_{558} reduced, c_{553} oxidized) reveals that two turnovers are required to completely oxidize the cytochrome c_{558} complement, suggesting that two cytochrome hemes share each reaction center. Fig. 3 shows a redox titration of the first and second flash induced cytochrome c_{558} oxidations. The lines drawn through the points are the theoretical curves expected if two identical cytochrome c_{558} hemes shared the same reaction center (see refs. 19 and 20); the fit to the data is very good, and gives an E_m at pH 7 of $+346$ mV. This E_m was also essentially independent of pH, being $+310$ mV at pH 10.3.

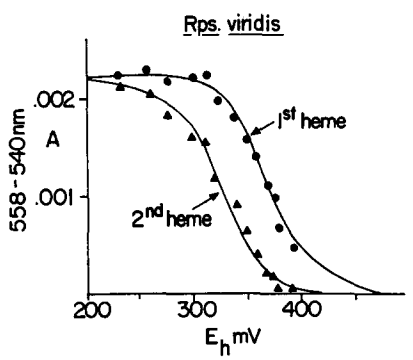

Fig. 3. Flash induced redox titration of cytochrome c_{558}. The conditions were similar to those for fig. 2 except that the experiment was performed at pH 7, and 7 μM each of *N,N,N',N'*- and 2,3,5,6-tetramethyl phenylenediamine was present. The titration of the first cytochrome heme was the extent of first flash induced oxidation, the second heme, the extent of the second flash induced change. The flashes were 25 ms apart, and there was no appreciable re-reduction of the cytochrome in this time.

Cogdell and Crofts have demonstrated that both cytochromes c_{558} and c_{553} donate electrons to the same reaction center, and that if both cytochromes were reduced prior to activation (e.g., chromatophores poised at an E_h of -50 mV), the low potential cytochrome was oxidized preferentially. As the ambient redox potential was made more positive, and this cytochrome was chemically oxidized before the flash, cytochrome c_{558} oxidation occurred after a flash of light. The cross-over between the oxidation of the two cytochromes was reported to be at approximately $+25$ mV, and independent of pH between pH 6 and 9. We have found similar values at pH 7 and pH 10.3 ($+10$ mV and -50 mV respectively) implying that the midpoint potential of cytochrome c_{553} is also independent of pH between pH 7 and pH 10.3.

The midpoint potential of the primary acceptor

Cogdell and Crofts [21] also titrated the E_m of the primary acceptor (X) in *Rps. viridis*, by monitoring the disappearance of flash induced cytochrome oxidation as the ambient redox potential was lowered. At pH 7 the E_m was -100 mV and in their work this appeared to vary with pH by -30 mV/pH unit between pH 6 and 9. Their data are shown in Fig. 4, which also includes our recent studies. Over this wider pH range, the -30 mV/pH unit dependency can be resolved into a region where the E_m varies by -60 mV/pH unit, and a region where the E_m is pH independent, the junction of the two regions being at pH 7.8. As will be discussed later, this indicates a pK on the reduced form of the couple. In all cases the titrations indicated that the redox reaction involved a single electron ($n = 1$).

As in *Rps. sphaeroides* and *Chromatium vinosum* [22], the addition of *o*-phenanthroline (1,10-diazaphenanthrene) shifts the midpoint potential of the primary acceptor, measured at neutral values of pH, to a more positive value [21]. Fig. 4 reveals that this is due to a shift in the pK of the reduced form to a more alkaline

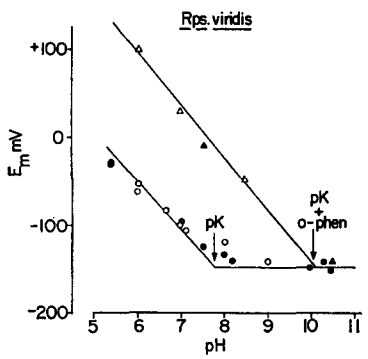

Fig. 4. The E_m of the primary acceptor as a function of pH. The E_m of the primary acceptor was determined as the midpoint of the attenuation of cytochrome *c* oxidation following a single-turnover flash. In our experiments (solid points) the chromatophores (18 μM BChl) were suspended in 20 mM of an appropriate buffer, 100 mM KCl, with 7 μM of the following redox mediators; *N*-methyl phenazonium methosulfate, *N*-ethyl phenazonium ethosulfate, pyocyanine, 2-OH-1,4-naphthaquinone. Open symbols represent the data of Cogdell and Crofts [21]; and triangles represent midpoint potentials determined in the presence of 2 mM *o*-phenanthroline (o-phen).

value, as it was in *C. vinosum* and *Rhodospirillum rubrum* [15]. The E_m above the pK in all three species is independent of *o*-phenanthroline.

EPR measurements of reactions at low temperatures

The primary photochemical events and closely associated electron transport steps can be effectively isolated at the low temperatures used for EPR analysis. Like *C. vinosum* [7], light induced reactions in *Rps. viridis* at temperatures of 77 °K and below are restricted to the reaction center and the low potential cytochrome *c* (in this case cytochrome c_{553}). The electrons do not go beyond X into the cyclic electron transport system because of the high activation energies required; a similar high temperature dependence of the reaction would also explain the incompetence of the high potential cytochrome c_{558} to transfer an electron to $(BChl)_2^+$ at these temperatures.

Thus at ambient redox potentials where cytochrome c_{553} and the primary acceptor are both oxidized and the reaction center bacteriochlorophyll dimer is reduced prior to illumination at low temperatures (Fig. 5A), illumination results in the reversible generation of $(BChl)_2^+$ and X^-; after illumination the electron returns from X^- to $(BChl)_2$ in about 9 ms [23]. The EPR spectrum of the light induced $(BChl)_2^+$ radical is shown in Fig. 6, with that of *Rps. sphaeroides* for comparison. Both signals are centered at approximately $g = 2.0025$, but the peak to peak width

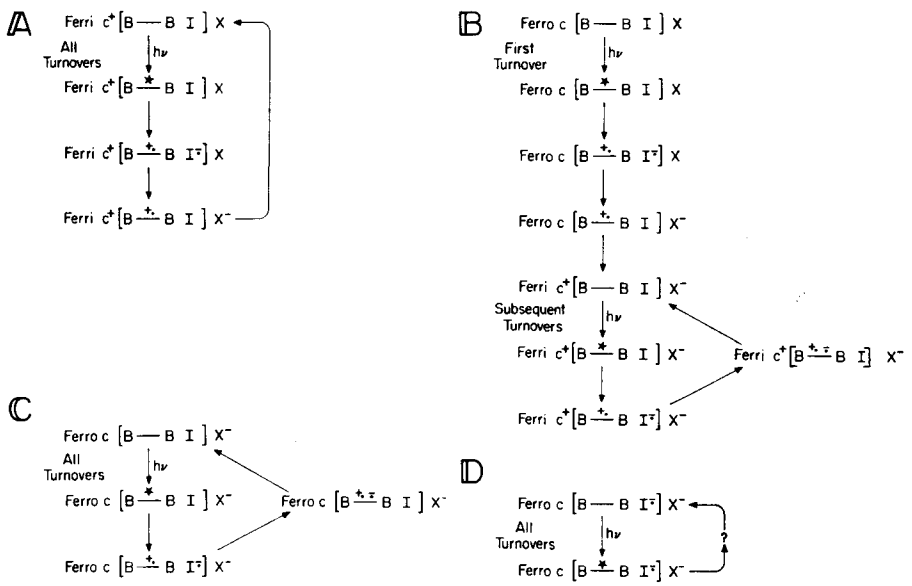

Fig. 5. Light induced reactions at 5 °K in *Rps. viridis*. (A), at an E_h where only the $(BChl)_2$, which is abbreviated here to B-B, is reduced prior to activation (e.g., +200 mV), illumination generates $(BChl)_2^+$ and X^-: (B), at an E_h where cytochrome c_{553} and $(BChl)_2$ are both reduced prior to activation (e.g., −50 mV) or (C), where X is also reduced (e.g., −250 mV), illumination generates the spin polarized triplet signal ($B^+ \cdot\cdot B$). In (D), where I is also reduced (e.g., at an E_h of −600 mV), illumination does not generate a spin polarized triplet, but the decay pathway for the excited $(BChl)_2$ is unknown.

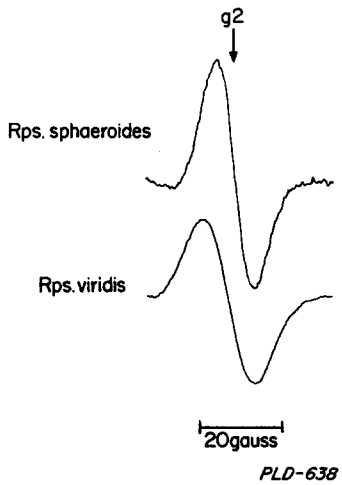

Fig. 6. EPR spectra of the light induced oxidized bacteriochlorophyll radical in *Rps. viridis* and *Rps. sphaeroides*. These are light minus dark spectra, obtained at 6 °K with a modulation amplitude of 2 gauss, and a microwave power of 1 mW.

of the signal in *Rps. viridis* is appreciably wider than in *Rps. sphaeroides*, being 12 gauss compared to 10 gauss. McElroy et al. [23] obtained very similar numbers. The EPR spectrum of X^- is shown in Fig. 7; it is very similar to the signal seen in *C. vinosum* and *Rps. sphaeroides* [24–28] characterized by a prominent signal at $g = 1.82$.

At lower ambient redox potentials, where cytochrome c_{553} is reduced (but X is oxidized) prior to activation, illumination causes an irreversible oxidation of the

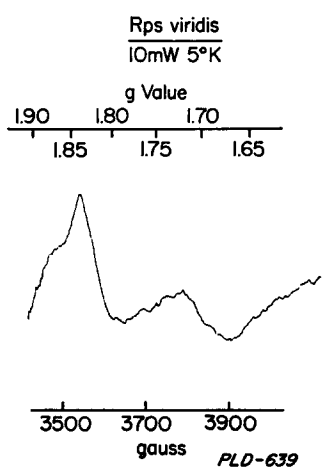

Fig. 7. EPR spectrum of the reduced primary acceptor in *Rps. viridis*. This spectrum was obtained in the dark in a reduced sample, but a similar signal was generated in the light at higher potentials. The spectrum was obtained at 5 °K with a modulation amplitude of 12.5 gauss and a microwave power of 10 mW.

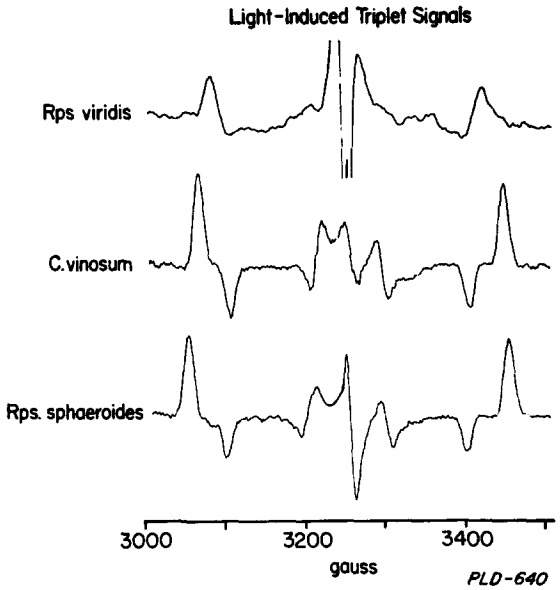

Fig. 8. EPR spectra of the light induced triplet signals in *Rps. viridis*, *C. vinosum*, and *Rps. sphaeroides*. These are light minus dark spectra obtained at 5 °K at a modulation amplitude of 12.5 gauss and a microwave power of 2 mW. In addition to the prominent triplet lines, free radical signals at g 2.0 and iron-sulfur centers at g 2.03 and g 1.94 are also evident.

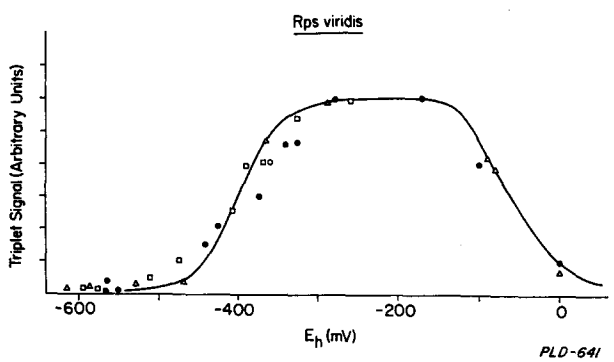

Fig. 9. Redox titration of the light-induced triplet signal in *Rps. viridis*. The size of the triplet signal, measured as the height of the outer wings of the triplet in arbitrary units was measured as a function of redox potential. Chromatophores (1.5 mM BChl) were suspended in 100 mM glycine, 100 mM KCl pH 10.8 with benzyl and methyl viologens and Triquat (1-1′-trimethylene-2-2′-dipyridylium dibromide) present as redox mediators at the following concentrations, ●, 10 μM; □, 20 μM; △, 100 μM. Samples were taken during both oxidative and reductive phases of each titration. The line drawn through the points represents the theoretical line for two identical cytochrome c_{553} hemes per reaction center, with an E_m of −80 mV, and an E_m for I/I^- of −400 mV.

cytochrome on the first turnover, re-reducing $(BChl)_2^+$ to its neutral state and irreversibly reducing X, as depicted in the working scheme of Fig. 5B. The second and subsequent turnovers generate the light induced triplet signal (see refs. 7 and 8) since the criteria for its formation are that $(BChl)_2$ and X are both reduced before activation. This triplet signal (Fig. 8) is therefore also seen if X is reduced prior to activation but obviously without the involvement of cytochrome oxidation (Fig. 5C).

Fig. 9 shows a redox titration of the light induced triplet signal of *Rps. viridis* which is consistent with the working schemes of Fig. 5B and C. The signal induced by steady-state light at 5 °K appears as the redox potential is lowered over the expected range where cytochrome c_{553} would be reduced prior to activation (E_m −70 mV; pH 10.8). However, at potentials substantially below the E_m of X/X^- (−150 mV at pH 10.8) an additional factor apparently affects the generation of the triplet signal. At potentials below −300 mV the size of the signal begins to diminish, and its disappearance approximates a Nernst $n = 1$ curve with a midpoint potential of −400 mV at pH 10.8; if our experimental rationale is correct, this would be the E_m of the I/I^- couple. The titration is reversible, and the E_m is independent of the concentration of redox mediators between 10 and 100 μM. These criteria lend weight to the conclusion that it is a chemical reduction of a redox agent (I) which prevents the formation of the triplet at 8 °K and not some form of quenching via the reduced mediators. This chemical reduction of I and the absence of a light induced triplet signal at low potentials is shown schematically in Fig. 5D. The decay pathway for the excited $(BChl)_2$ is currently unknown (Fig. 5D).

DISCUSSION

The primary donor bacteriochlorophyll

The oxidation-reduction midpoint potential of the reaction center BChl of *Rps. viridis* is +500 mV at pH 8, and the oxidation or reduction involves only a single electron and no proton (Fig. 2). These redox properties are very similar to those of this couple in other purple bacteria (see ref. 1 for a review). The EPR spectra of the oxidized reaction center BChl in BChl *a*-containing species (such as *Rps. sphaeroides*, Fig. 6) show a $\sqrt{2}$ narrowing of the signal compared with that of monomeric BChl a^+ [30, 23], indicating that the unpaired electron of the reaction center BChl is delocalized over two molecules as indicated by the designation $(BChl)_2^+$. The zero field splitting parameters of the triplet signals are also consistent with its delocalization over two BChl molecules [8, 31]. The observation that the oxidized reaction center BChl signal of *Rps. viridis* (ref. 23 and Fig. 6) is 12 gauss wide (cf. 9–10 gauss in BChl *a* species) suggests either that the unpaired electron in *Rps. viridis* is localized on only one BChl molecule, or that the line width of BChl b^+ in solution may be considerably broader than that of BChl a^+. Unfortunately BChl b^+ has not been measured in vitro because it is unstable (Fajer, J., personal communication), but other evidence, although circumstantial, strongly suggests that the primary donor BChl of *Rps. viridis* is a dimer; spectrophotometrically its BChl complement is similar to that of *Rps. sphaeroides*, although shifted to the red [13], including the long wavelength (1315 nm) band of the oxidized state which is apparently not found in the oxidized monomer [32], and the zero field splitting values of the triplet are similar to those of other species (Fig. 8). If the reaction center BChl is indeed a dimer in *Rps.*

viridis, this would suggest that the line width of BChl b^+ in vitro could be as much as 17 gauss if the $\sqrt{2}$ narrowing was to occur in this species.

c-Type cytochromes

The redox titration of Fig. 3 indicates that two kinetically and thermodynamically identical cytochrome c_{558} hemes share the same reaction center in *Rps. viridis*. In addition, the low potential cytochrome c_{553} also shares the same reaction center [21], and it is perhaps noteworthy that the difference in E_m values for cytochrome c_{553} determined by dark equilibrium methods [16] or flash induced (first turnover) kinetic titrations (ref. 21 and this work) suggest that there may be two identical cytochrome c_{553} hemes per reaction center. Similar functional ratios have been found in other organisms; there are two high potential cytochrome c_{555} [19], and probably two low potential cytochrome c_{553} [33], hemes per reaction center in *C. vinosum*, and two cytochrome c_2 molecules and two cytochrome c_{553} hemes per reaction center in *Rps. sphaeroides* [14] and *Chlorobium limicola* f. *thiosulfatophilum* [34] respectively.

The primary acceptor X

The rationale for determining the E_m of X has been based on the attenuation of readily measurable (long-lived) flash induced changes such as reaction center BChl or cytochrome *c* oxidation, or carotenoid bandshifts, as X is chemically reduced at measured redox potentials before activation (see refs. 1 and 15 for reviews). Direct measurements of X by EPR [25–28] show good agreement with these indirect measurements. Although it now seems likely that $(BChl)_2$ undergoes light induced oxidation to the $((BChl)_2^+ I^-)$ state even with X reduced before activation, the lifetime of this state under these conditions is far too short (10–40 ns in *Rps. sphaeroides* [6]) to permit reactions outside the reaction center (e.g., cytochrome *c* oxidation) after a single turnover. The experimental identity of X thus rests on its function to lead to the long-lived formation of $(BChl)_2^+$ required for the slower reactions of the chromatophore.

The measured E_m values for X in a variety of purple photosynthetic bacteria, including *Rps. viridis* (Fig. 4), are pH dependent in the neutral pH range by -60 mV/pH unit, indicating that both a proton and an electron (see ref. 35 for a fuller discussion of this point) are required for the equilibrium reduction of X, i.e.,

$$X + e^- + H^+ \rightleftharpoons XH$$

At higher values of pH, a pK on the reduced form of X is encountered, and under conditions more alkaline than this the E_m of the couple becomes independent of pH, and the equilibrium reduction involves only an electron

$$X + e^- \rightleftharpoons X^-$$

The E_m value for this X/X^- couple in *Rps. viridis* is -150 mV and the pK is at pH 7.8. Studies on *Rps. sphaeroides*, *R. rubrum* and *C. vinosum* [15] have revealed similar properties of their respective primary acceptors, with pK values at pH 9.8, 8.8 and 8.0, and E_m values for the X/X^- couple of -180, -200 and -160 mV respectively.

As we have discussed in an earlier paper [15] there is good evidence that the reduction of the primary acceptor in *Rps. sphaeroides* and *C. vinosum* on the timescale

of light induced electron flow involves only an electron; although chromatophores do take up a proton from the external aqueous phase following single turnover flash activation, this is associated with the reduction of a secondary acceptor, probably ubiquinone [36–38]. Preliminary experiments with *R. rubrum* and *Rps. viridis* (Prince, R. C., Baltscheffsky, M., Petty, K. M. and Dutton, P. L., unpublished observations) suggest that the same is also true in these species. The functional primary acceptor couple is thus the unprotonated couple (X/X^-), and the E_m of this couple has the value of that measured above the pK of the reduced form. It is interesting that this latter value is very similar in the four species of purple bacteria so far examined (E_m of $X/X^- = -175$ mV$\pm$25 mV), and in addition the EPR spectra of the reduced primary acceptors (g_y 1.82) are very similar in *Rps. viridis* (Fig. 7), *C. vinosum* and *Rps. sphaeroides* [24–28]. These may represent common features of the primary acceptors of purple bacteria, although thus far we have not detected the EPR signal in *R. rubrum*.

The intermediary carrier I

The data discussed in the previous section, that the primary acceptor X of *Rps. viridis* has essentially similar redox properties to the primary acceptor in BChl *a*-containing species suggested that the difference in photonic energy available to BChl *a*- or BChl *b*-containing reaction centers might be reflected in the midpoint potential of the intermediate I/I^- component. As discussed in the introduction (and Figs. 1 and 5) we have monitored the light-induced triplet signal as a function of ambient redox potential (Fig. 9), and interpreted the disappearance of the triplet at low potentials as indicating the reduction of the intermediate prior to illumination. The finding that triplet formation can be eliminated by the establishment of a suitably negative redox potential supports the proposed mechanism [4] for the generation of the triplet state on $(BChl)_2$ at low temperatures as depicted in Figs. 5B and C and reveals I as a chemically reducible entity. In *Rps. viridis* the E_m of the I/I^- couple is -400 mV $\pm$25 mV.

Energetic considerations

The energetics of the reaction center of *Rps. viridis* can thus be expressed diagrammatically as in Fig. 10. An incident photon of 985 nm light (1.26 eV) excites the $(BChl)_2$ to its lowest singlet excited state which may be considered as having a reducing potential of -760 mV (subtracting 1.26 eV from the E_m of $(BChl)_2^+/BChl_2$ of $+500$ mV). This then reduces I (E_m of $I/I^- = -400$ mV) forming $[(BChl)_2^+ I^-]$ and apparently releasing some 360 mV of free energy in the process; if this is similar to *Rps. sphaeroides*, this reaction will take < 10 ps. The free energy release between the excited singlet state of the $(BChl)_2$ and I is probably related to the maximal efficiency for the conversion of radiant energy to free energy. It is interesting that three somewhat different approaches to calculating the maximal efficiency of this process predict an approximately 70 % conversion efficiency [39–42]. The difference in midpoint potential (ΔE_m) between the primary donor $[(BChl)_2/(BChl)_2^+]$ and the intermediate (I/I^-) is 900 mV, which is approximately 70 % of the 1.26 eV in the incident photon.

The reduced intermediate then transfers an electron to the primary acceptor X (E_m of $X/X^- = -150$ mV) forming $[(BChl)_2^+ I]X^-$; if this reaction is similar to

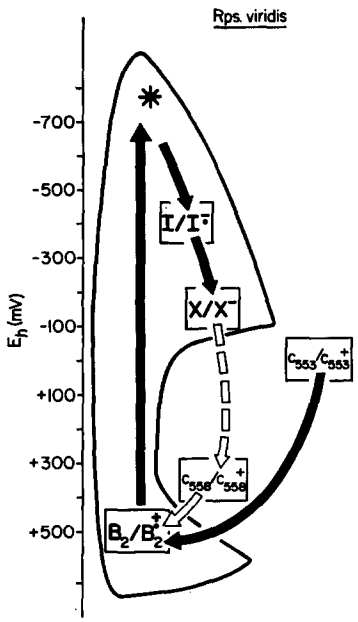

Fig. 10. Diagrammatic representation of the thermodynamic properties of the reaction center of *Rps. viridis*. The shape encloses the primary photochemical reactants and B_2 is used as an abbreviation for $(BChl)_2$. By analogy with other bacteria [18, 27, 33] the high potential cytochrome c_{558} is assumed to be part of the cyclic electron transport system coupled to ATP synthesis, while low potential cytochrome c_{553} is assumed to play some role in the non-cyclic replenishment of the cycle. Solid arrows represent reactions at 5 °K.

that in *Rps. sphaeroides*, it will take 100–200 ps. The reduction of the primary electron acceptor effectively renders the light reaction irreversible, since once the electron has arrived on X, the lifetime of the resulting $[(BChl)_2^+ I]X^-$ state is suitably in excess of the time for the delivery of an electron to $(BChl)_2^+$ by *c*-type cytochrome oxidation. The net result of the reaction center absorbing 1.26 eV of photonic energy may thus be expressed as the ΔE_m between the primary donor and primary acceptor. In this respect it is remarkable that the redox span (ΔE_m) generated between the E_m values of the primary donor $[(BChl)_2^+/(BChl)_2]$ and primary acceptor (X/X^-) is essentially identical in the four species where this has been measured, being approx. 650 mV. This is despite the fact that the energy of an actinic photon in *Rps. viridis* may have only about 90 % of the energy of an incident photon in BChl-*a* containing species.

Apart from this redox free energy of 650 mV, there has also been a charge separation, since an electron has moved from the $(BChl)_2$ to X. It is possible that the apparent free energy available in the I to X redox span ($\Delta E_m = 350$ mV) drives this physical separation of the positive and negative charges involved in the final $[(BChl)_2^+ I]X^-$ state. At the chromatophore membrane level it appears that the reaction center in many species is oriented in the membrane such that X is close enough to the outer surface to donate an electron to secondary ubiquinone, which then takes up a proton from the outer aqueous phase. In *Rps. sphaeroides* this has been measured to be 1.0 H^+ per reaction center per turnover [38], indicating that all electrons move to the

outer surface. On the other hand the cytochrome c_2 which serves to reduce $(BChl)_2^+$, is located on the inner membrane aqueous interface [43], and carotenoid bandshift studies [44] suggest that $(BChl)_2$ occupies a place within the membrane dielectric. Thus, at least in *Rps. sphaeroides*, the reaction center and its immediate electron donors and acceptors spans the chromatophore membrane, and is thus electrogenic. During steady-state illumination, the membrane potential across coupled chromatophore membranes has been reported to be some 200 mV (inside minus outside) [45, 46]. The 350 mV available in the ΔE_m of the I to X span would appear to be sufficient to sustain normal forward operation of the light reaction against such a gradient without loss of quantum efficiency, especially if the primary light reaction only operates across part of the membrane dielectric, and hence against only a part of the membrane potential. Following single turnover activation, when there is little or no pre-existing membrane potential, the light reaction would generate the membrane potential, and as suggested by Crofts et al. [42], it is conceivable that this

TABLE I

ZERO FIELD SPLITTING PARAMETERS OF THE LIGHT INDUCED TRIPLET SIGNAL IN A VARIETY OF BACTERIAL SPECIES

The triplet signals were measured as shown in Fig. 8, and D and E values calculated as before [8]. *C. vinosum* DPA treated cells were grown in the presence of 12 mg/l diphenylamine to repress the synthesis of carotenoids.

		D ($\cdot 10^4$ cm^{-1})	E ($\cdot 10^4$ cm^{-1})
Rps. viridis			
strain NHTC 133	cells	157	37
	chromatophores	158	38
C. vinosum			
strain D	cells	177	34
	DPA treated cells	177	34
	chromatophores	178	33
	Fraction A	177	33
Rps. sphaeroides			
strain 2.4.1	cells	185	31
strain Ga	cells	186	31
	chromatophores	185	31
strain R_{26}	cells	186	31
	chromatophores	186	31
	reaction centers	183	31
Rps. capsulata			
strain St. Louis	cells	183	31
strain SB 25	cells	184	31
strain BY 761	cells	183	31
Rps. gelatinosa			
strain I	cells	186	27
Rps. palustris			
strain 2.1.6	cells	184	34
R. rubrum			
strain S1	cells	186	34
strain G9	cells	185	31

would give the bacterium a substantial extra amount of biochemically usable energy per photon in addition to the 650 mV available in the $(BChl)_2$ to X redox span.

A note about the EPR triplet signals in various photosynthetic bacteria

Fig. 8 compares the light induced triplets seen in *Rps. viridis*, *Rps. sphaeroides* and *C. vinosum*. In each case the zero-field splitting parameters of the triplets are somewhat different, and are given in Table I. Unfortunately the table in our earlier paper [8] contained some errors due to an unsuspected non-linearity in the EPR spectrometer, and the values given here supercede those given earlier. It is noteworthy that the triplets of *Rps. sphaeroides*, *Rps. capsulata*, *Rps. gelatinosa*, *Rps. palustris* and *R. rubrum* have rather similar zero field splitting parameters while *C. vinosum* and *Rps. viridis* are markedly different. In addition it is interesting that different preparations from different strains of a particular species share similar D and E values. Thurnauer et al. [29] obtained very similar D and E values for the triplet of *Rps. sphaeroides*, *Rps. palustris*, *Rps. gelatinosa* and *R. rubrum*, but obtained values of $D = 184 \cdot 10^4$ cm^{-1} and $E = 33 \cdot 10^4$ cm^{-1} for *Rps. viridis*. We are unable to account for this difference.

NOTE ADDED IN PROOF (Received July 13th, 1976)

More recently, Fajer et al. [47] have succeeded in measuring the EPR signal associated with monomeric BChl b^+ in vitro; it has a gaussian line-shape centered at $g = 2.0025 \pm 0.0001$, with a peak to peak width of 14 gauss.

ACKNOWLEDGEMENTS

We would like to thank the following generous providers of the original bacterial cultures; Dr. J. M. Olson for *Rps. viridis*, Dr. T. Kihara for *Rps. gelatinosa* strain I, a wild type, Dr. P. Weaver for *Rps. palustris* 2.1.6, a wild type, and Dr. B. Marrs for *Rps. capsulata* strain SB 25 and BY 761, which are both green mutants. The Triquat was a generous gift from Dr. W. Butler. This work was supported by NSF grant GB 28125, and P.L.D. acknowledges his R.C.D.A. 1-K4-GM-70771, and J.S.L. his R.C.D.A. 1-K4-GM-00142.

REFERENCES

1 Parson, W. W. and Cogdell, R. J. (1975) Biochim. Biophys. Acta 416, 105–149
2 Kaufmann, K. J., Dutton, P. L., Netzel, T. L., Leigh, J. S. and Rentzepis, P. M. (1975) Science 188, 1301–1304
3 Rockley, M. G., Windsor, M. W., Cogdell, R. J. and Parson, W. W. (1975) Proc. Natl. Acad. Sci. U.S. 72, 2251–2255
4 Dutton, P. L., Kaufmann, K. J., Chance, B. and Rentzepis, P. M. (1975) FEBS Lett. 60, 275–280
5 Fajer, J., Brune, D. C., Davis, M. S., Forman, A. and Spaulding, L. D. (1975) Proc. Natl. Acad. Sci. U.S. 72, 4956–4960
6 Parson, W. W., Clayton, R. K. and Cogdell, R. J. (1975) Biochim. Biophys. Acta 387, 265–278
7 Dutton, P. L., Leigh, J. S. and Seibert, M. (1972) Biochem. Biophys. Res. Commun. 46, 406–413
8 Leigh, J. S. and Dutton, P. L. (1974) Biochim. Biophys. Acta 357, 67–77
9 Wraight, C. A., Leigh, J. S., Dutton, P. L. and Clayton, R. K. (1974) Biochim. Biophys. Acta 333, 401–403

10 Pedersen, J. B. (1973) J. Chem. Phys. 59, 2656–2667
11 Eimhjellen, K. E., Aasmundrud, O. and Jensen, A. (1963) Biochem. Biophys. Res. Commun. 10, 232–236
12 Olson, J. M. and Nadler, K. D. (1965) Photochem. Photobiol. 4, 783–791
13 Holt, A. S. and Clayton, R. K. (1965) Photochem. Photobiol. 4, 829–831
14 Dutton, P. L., Petty, K. M., Bonner, H. S. and Morse, S. D. (1975) Biochim. Biophys. Acta 387, 536–556
15 Prince, R. C. and Dutton, P. L. (1976) Arch. Biochem. Biophys. 172, 329–334
16 Thornber, J. P. and Olson, J. M. (1971) Photochem. Photobiol. 14, 329–341
17 Carithers, R. P. and Parson, W. W. (1975) Biochim. Biophys. Acta 387, 194–211
18 Case, G. D., Parson, W. W. and Thornber, J. P. (1970) Biochim. Biophys. Acta 223, 122–128
19 Case, G. D. and Parson, W. W. (1971) Biochim. Biophys. Acta 253, 187–202
20 Jackson, J. B. and Dutton, P. L. (1973) Biochim. Biophys. Acta 325, 102–113
21 Cogdell, R. J. and Crofts, A. R. (1972) FEBS Lett. 27, 176–178
22 Jackson, J. B., Cogdell, R. J. and Crofts, A. R. (1973) Biochim. Biophys. Acta 292, 218–225
23 McElroy, J. D., Feher, G. and Mauzerall, D. C. (1972) Biochim. Biophys. Acta 267, 363–374
24 Leigh, J. S. and Dutton, P. L. (1972) Biochem. Biophys. Res. Commun. 46, 414–421
25 Dutton, P. L. and Leigh, J. S. (1973) Biochim. Biophys. Acta 314, 178–190
26 Evans, M. C. W., Lord, A. V. and Reeves, S. G. (1974) Biochem. J. 138, 177–183
27 Dutton, P. L., Leigh, J. S. and Reed, D. W. (1973) Biochim. Biophys. Acta 292, 654–664
28 Dutton, P. L., Leigh, J. S. and Wraight, C. A. (1973) FEBS Lett. 36, 169–173
29 Thurnauer, M. C., Katz, J. J. and Norris, J. R. (1975) Proc. Natl. Acad. Sci. U.S. 72, 3270–3274
30 Norris, J. R., Uphaus, R. A., Crespi, H. L. and Katz, J. J. (1971) Proc. Natl. Acad. Sci. U.S. 68, 625–628
31 Uphaus, R. A., Norris, J. R. and Katz, J. J. (1974) Biochem. Biophys. Res. Conmmun. 61, 1057–1063
32 Otten, H. A. (1971) Photochem. Photobiol. 14, 589–596
33 Dutton, P. L. (1971) Biochim. Biophys. Acta 226, 63–80
34 Prince, R. C. and Olson, J. M. (1976) Biochim. Biophys. Acta 423, 357–362
35 Dutton, P. L. and Wilson, D. F. (1974) Biochim. Biophys. Acta 346, 165–212
36 Cogdell, R. J., Jackson, J. B. and Crofts, A. R. (1972) J. Bioenergetics 4, 413–429
37 Halsey, Y. D. and Parson, W. W. (1974) Biochim. Biophys. Acta 347, 404–416
38 Petty, K. M. and Dutton, P. L. (1976) Arch. Biochem. Biophys. 172, 335–345
39 Duysens, L. N. M. (1958) Brookhaven Symp. Biol. 11, 10–23
40 Ross, R. T. and Calvin, M. (1967) Biophys. J. 7, 595–614
41 Knox, R. S. (1969) Biophys. J. 9, 1351–1362
42 Crofts, A. R., Wraight, C. A. and Fleischmann, D. E. (1971) FEBS Lett. 15, 89–100
43 Prince, R. C., Baccarini-Melandri, A., Hauska, G. A., Melandri, B. A. and Crofts, A. R. (1975) Biochim. Biophys. Acta 387, 212–227
44 Jackson, J. B. and Dutton, P. L. (1973) Biochim. Biophys. Acta 325, 102–113
45 Jackson, J. B. and Crofts, A. R. (1969) FEBS Lett. 4, 185–189
46 Casadio, R., Baccarini-Melandri, A. and Melandri, B. A. (1974) Eur. J. Biochem. 47, 121–128
47 Fajer, J., Brune, D. C., Davis, M. S., Forman, A. and Spaulding, L. D. (1976) Brookhaven Symp. Biol. 28, in the press