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Conformational changes following reduction of the bacteriopheophytin electron acceptor in reaction centers of *Rhodopseudomonas viridis* *

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An electron acceptor, designated I, functions between the bacteriochlorophyll dimer (B_2) and the quinone acceptor (Q) in bacterial photosynthetic reaction centers. The acceptor is thought to be a complex composed of a bacteriochlorophyll (BChl) and a bacteriopheophytin (BPheo). We have generated the trapped $B_2I^-Q^-$ redox state at 100 K in reaction centers of *Rhodopseudomonas viridis*, and have examined the optical and EPR properties of this redox state as a function of temperature. Changes in the optical absorption spectra show that the $B_2I^-Q^-$ state converts from a low temperature form, which is frozen in at temperatures below 150 K, to a 'relaxed' form at higher temperatures. The conversion is not reversible. Optical dichroism and EPR spectra show that the conversion of the $B_2I^-Q^-$ state from the low temperature to relaxed form is not the result of a change in the electron distribution between BChl and BPheo. Instead, this conversion appears to affect the optical absorption of the accessory BChl. We propose that nuclear relaxations occur within the accessory BChl and/or surrounding protein following the reduction of I at temperatures above 150 K. Comparison of these optical spectra to those of the transient $B_2^+I^-$ state suggests that these processes may be functional during the ps electron transfer from I^- to Q.

Introduction

Sub-picosecond transient spectroscopy has found that there is only one electron acceptor operating between the bacteriochlorophyll dimer, B_2, and the quinone acceptor, Q, in the purple photosynthetic bacteria [1–4]. The electron transfer from the light-generated excited state of B_2, to the acceptor, I, occurs in 2.8–6 ps [1–4]. Besides the B_2, reaction centers contain two bacteriopheophytins, BPheo, and two additional bacteriochlorophylls, BChl. The optical-absorbance changes which accompany reduction of the acceptor are complicated, and involve changes in both BPheo and BChl bands [5–9]. The X-ray crystal structures of reactions centers from *Rhodopseudomonas viridis* and *Rhodobacter sphaeroides* show that there are two BChl/BPheo pairs located at equivalent positions about the B_2 C2 symmetry axis [10,11]. The two BPheo are

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Abbreviations: BChl, bacteriochlorophyll; BPheo, bacteriopheophytin; Cyt, cytochrome; LDAO, lauryldimethylamine *N*-oxide.

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distinguished by differences seen in the positions of their optical absorption maxima at low temperature [12–14]. Transient picosecond spectroscopy [5,6] and photochemical trapping of the reduced BPheo acceptors [7–9,14,15] have shown that it is mainly the red-shifted of the BPheo's which functions as an electron acceptor at low temperature. Calculations of the exciton couplings between pigments of *Rps. viridis* reaction centers have predicted that the complexity of the optical spectra associated with the reduction of I can be explained in part by the proximity of the accessory BChl and BPheo [16,17].

However, several features of the experimental spectra are not yet explained by these models. For example, the absorbance changes in the BPheo/BChl Q_y region for the transient $B_2^+I^-$ state in *Rb. sphaeroides* are temperature dependent [18]. The kinetics of the absorption changes associated with the decay of the $B_2^+I^-$ state to form $B_2^+Q^-$ are also found to be wavelength and temperature dependent [19]. Shuvalov and Parson have proposed that the temperature dependence of the transient $B_2^+I^-$ spectrum reflects a temperature-dependent redox equilibrium: $B_2^+BChlBPh^- \rightleftarrows B_2^+BChl^-BPh$ [18], while Kirmaier et al. [19] have suggested that changes in the BChl bands reflect nuclear relaxations in the BChl and/or protein rather than a reduction of the BChl itself.

In this paper we have examined the temperature dependence of the optical and EPR spectra of the trapped I^- state in reaction centers of *Rps. viridis*. The low temperature, electron tunneling between the reaction center *c*-cytochromes and B_2^+ allows the acceptor operating between B_2 and Q to be trapped following a single electron reduction [7,9,15]: Cyt $B_2IQ^- + h\nu \rightleftarrows$ Cyt $B_2^+I^-Q^- \rightarrow$ Cyt$^+B_2I^-Q^-$. The advantages of this technique are that (i) the optical changes associated with the reduction of I are not altered by the presence of an oxidized B_2^+ or mixed with absorbance changes induced by changes in the redox state of Q (unlike difference spectra obtained by subtraction of the transient $B_2^+I^-Q$ and $B_2^+IQ^-$ spectra), and (ii) the optical properties of reaction centers in the trapped B_2I^- state can be directly studied as a function of temperature, without initiating further photochemistry. We find that the optical spectrum of the trapped B_2I^- state changes abruptly between 100 and 200 K. This alteration is analogous to that seen in the transient $B_2^+I^-$ spectra in *Rb. sphaeroides* [18], but is not reversible. Furthermore, we report EPR and optical linear dichroism spectra of the B_2I^- state before and after this transition which show that the optical absorbance changes are not brought about by a change in the electron distribution between BChl and BPheo. Instead, these data support the notion that nuclear relaxations occur in the accessory BChl and/or the protein following BPheo reduction at temperatures above 150 K. The conformation changes appear to alter both the absorbance intensity and orientation of the accessory BChl Q_y transition.

Materials and Methods

Reaction centers were prepared from *Rps. viridis* using the detergent lauryl dimethylamine *N*-oxide (LDAO) as previously described [20,21]. The LDAO was replaced with Triton X-100 by chromatography on DEAE Sephadex, using 0.1% Triton/250 mM NaCl/10 mM Tris (pH 8) to elute the reaction centers. The reaction center concentration was adjusted to yield an absorbance of about 0.8 mm^{-1} at 830 nm in 65% glycerol. Reaction centers were also obtained by redissolving crystals. The reaction center crystals were a kind gift from Dr. Peter Gast (University of Chicago), and were prepared as described in Refs. 21 and 22. The reaction center crystals were redissolved in 0.1% LDAO/10 mM Tris (pH 8), and subsequently transferred to the Triton/glycerol buffer as described above.

Photochemical trapping of I^- followed procedures described earlier [7,9,15]. Reaction centers were poised at an E_h of −350 mV in a sealed, argon purged redox vessel using sodium dithionite to reduce the quinone. Samples were then transferred to a sealed, argon purged cuvette. The cuvette was made from a copper block, having quartz windows separated by a 1.5 mm spacer. The cuvette attached to a 'cold finger' cooled by a liquid nitrogen reservoir. The sample temperature was monitored with a thermocouple attached to the copper cuvette on the surface most distant to the cold finger. With a liquid nitrogen reservoir, the cuvette maintained a constant temperature of

100–110 K. With this cryostat we could not regulate the sample temperature, but could monitor the cuvette temperature as it warmed following a boil off of the liquid nitrogen and purging of the reservoir with room temperature nitrogen gas.

The trapped B_2I^- state was generated by illumination for 20 min at 100 K using a 300 W Xe arc lamp with 10 cm water and 590 nm visible cutoff/near infrared transmitting filters. An orientational selection (photoselection) during the photochemical trapping was obtained by exciting the randomly oriented, frozen reaction center solution only within the B_2Q_y band, using linearly polarized light ($\lambda > 900$ nm). For a complete description of the photoselective trapping experiment, see Ref. 25.

Optical spectra were recorded on a Cary 14 spectrophotometer in infrared 1 mode. EPR spectra were measured on a Varian E-9 spectrometer, equipped with an Air Products helium flow cryostat. Linear dichroism ($A_{\parallel} - A_{\perp}$) was measured with an apparatus described previously [25,33]. The spectrophotometer used a photoelastic modulator to rotate the linearly polarized measuring beam between vertical and horizontal directions. The $A_V - A_H$ difference spectrum was detected with synchronized, lock-in detection.

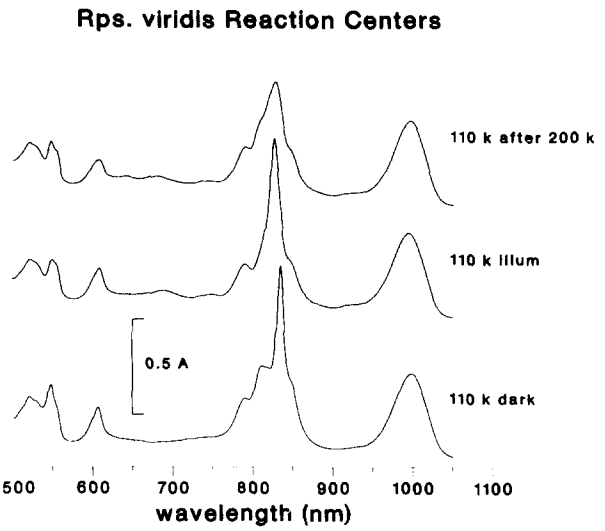

Fig. 1. Optical absorption spectra of *Rps. viridis* reaction centers at 110 K. The spectra were recorded sequentially on the same sample, starting with the bottom spectrum. The lower spectrum was recorded for reaction centers poised at $E_h = -350$ mV, and frozen in the dark. The middle spectrum was taken following 8 min illumination ($\lambda > 590$ nm) at 110 K. The top spectrum was recorded following a heating cycle which warmed the sample just until it reached 200 K, and then it was rapidly cooled. The warming/cooling cycle took about 5 min. All spectra were recorded at 110 K, with a 1.5 mm cuvette. The buffer was 35% 10 mM Tris/0.1% Triton X-100 (pH 7.8)/65% glycerol. Spectra were taken without a reference cuvette, but spectra of just the buffer alone showed that it contributed to less than 5% of the absorbance at 1000 nm.

Results

Optical absorbance spectra

A sequence of optical absorption spectra are shown in Fig. 1 for a reaction center sample poised at $E_h = -350$ mV and cooled to 110 K. The reaction centers were obtained from redissolved crystals (kindly provided by Dr. Peter Gast, University of Chicago). The bottom spectrum was recorded before photochemistry. The peaks resolved at 790 nm and 810 nm can be assigned as principly due to the individual Q_y transitions of the two BPheo's. The absorption peak at 834 nm is probably mainly due to accessory BChl absorption. However, optical absorption in this region, and particularly the shoulder at 850 nm, is likely to contain components due to exciton interactions between the accessory pigments, and components of the B_2 exciton spectrum [16,17,26]. The spectra of reaction centers before crystallization were very similar except that the Q_y peak of the red-shifted BPheo was positioned at 808 nm instead of 810 nm. In neither case was an absorption component seen at 820 nm, which had been tentatively assigned as due one of the reaction center BChl based upon its sensitivity to $NaBH_4$ [23]. We suggest that this component may be a tightly bound, but labile impurity.

The middle spectrum in Fig. 1 shows a spectrum of the reaction centers in the B_2I^- state. The spectrum was recorded following illumination at 110 K. The $I^- - I$ difference spectrum obtained by subtraction of the ground state B_2I spectrum is shown in the lower trace of Fig. 2. The bleaching of the BPheo bands at 808 nm and 545 nm and symmetrical shifts of the BChl bands centered at 830 nm and 609 nm indicate that the B_2I^- state is characterized by the selective reduction of the long-wavelength BPheos, which is accompanied by nearly conservative electrochromic shifts in the

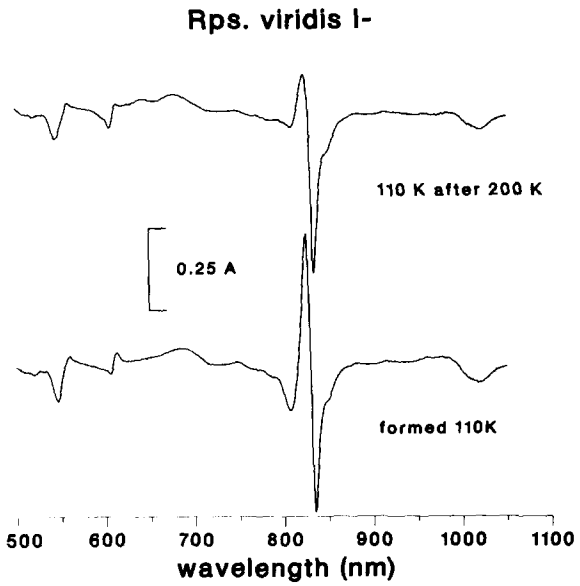

Fig. 2. Optical difference ($I^- - I$) at 110 K. The lower spectrum is the difference associated with the generation of the low temperature $B_2I^-Q^-$ state. It is the difference of the middle spectrum (Fig. 1), minus the ground state B_2IQ^- spectrum, lower spectrum Fig. 1. The top spectrum is the difference associated with the relaxed form of the $B_2I^-Q^-$ state. It is the difference of the top spectrum of Fig. 1 minus the lower spectrum of Fig. 1.

accessory BChl. The appearance of the band at 690 nm is consistent with the formation of a BPheo anion [5,24]. These spectral changes closely match those reported previously for the trapped B_2I^- state generated at temperatures below 100 K [14,34,35].

Warming the sample to near to 200 K was found to cause prominent changes in the absorbance spectrum for reaction centers in the B_2I^- state. These changes were not reversed by re-cooling to 110 K. The resulting spectrum at 110 K is shown in the top trace of Fig. 1. The warming treatment causes a decrease in the amplitude of the BChl absorption at 827 nm and the appearance of a new component at approximately the same position as the BPheo 810 nm absorption in the dark spectrum. The I^-I difference is now given by the top spectrum in Fig. 2. The Q_y region does not show a conservative shift in the BChl bands, but is characterized by net absorption losses in the BChl 835 nm and 850 nm bands. Although a prominent absorption decrease is no longer evident in the BPheo Q_y region, the 545 nm absorbance loss remains. A new band appears at 643 nm, and small changes near 550 nm are due to re-reduction of the cytochrome (e.g., see Ref. 25).

With the present cryostat we could not maintain constant temperatures between 100 K and 200 K, and could not investigate the kinetics for conversion of B_2I^- from low temperature to 'relaxed' forms at different temperatures. However, by boiling off liquid nitrogen in the reservoir (see Materials and Methods), the sample cuvette temperature could be changed from 110 K to 250 K in 5–10 min. No appreciable change was seen for the absorbance spectrum of reaction centers in the B_2I^- state until the sample reached 160 K–200 K. Then the spectrum relaxed quickly (less than 30 s) to its final form. The conversion seemed to be complete, since once the transition occurred allowing the sample to remain in this temperature range for an additional 1–2 min did not produce further noticeable changes. At temperatures above 200 K the B_2I^- state itself begins to decay to the starting state B_2I [7]. As described above, the spectrum of the relaxed state did not alter significantly upon recooling to 110 K. The initial, low-temperature B_2I^- state was stable for at least 1–2 h at 110 K. With a modified cryostat, current work is investigating the kinetics of the B_2I^- conversion in more detail.

Reaction centers in the B_2I^- state can be returned to the B_2I starting state by warming the sample to room temperature. Following this, a spectrum recorded at 110 K gave a spectrum indistinguishable from that shown in the lower trace in Fig. 1, and the entire sequence of absorbance changes described above could be regenerated. This rules out the possibility that the temperature-dependent changes in the B_2I^- spectra resulted from some sort of denaturation or irreversible change in the reaction center itself.

Comparison of the areas under the absorption spectra in the 760 nm to 900 nm region from preparations obtained both before and after crystallization showed that the absorption integral decreased by 20–25% upon going from the B_2I ground to B_2I^- trapped states. Only small differences (less than 10%) were sometimes seen between the integrals from the low temperature and relaxed forms of the B_2I^- state. For example this

was seen with the integrals for the spectra in Fig. 1, which gave values of 1.55, 1.30, 1.18 for the B_2I, low temperature and relaxed states of B_2I^- respectively, normalized to a value of 1.0 for the B_2 Q_y (1000 nm) band. These integrals indicate that the generation of the B_2I^- state causes the optical absorption in the 750–900 nm region to decrease by approx. one quarter. This is consistent with the interpretation that the spectrum in this region is principly due to the absorption of the four accessory pigments, and reduction of the acceptor I removes nearly a quarter of the total oscillator strength.

These experiments demonstrate that the B_2I^- state is first formed in one molecular configuration, which is frozen in below 150 K, but irreversibly converts to a 'relaxed' form upon warming above this temperature. The reappearance of an 810 nm absorption component in the relaxed B_2I^- without producing changes in the BPheo Q_x region prevents an easy interpretation of the spectral changes which accompany this conversion. The question to be determined is whether the new 810 nm component is equivalent to the BPheo Q_y absorption in the dark spectrum, or whether this component is a product of altered accessory pigment absorptions in the new reaction-center configuration.

Optical dichroism spectra

Optical linear dichroism studies have shown that the Q_y transitions of the accessory BChl and BPheo are oriented in different directions [5,14,25–27]. The accessory BChl (833 nm) and BPheo (810 nm) absorptions are found to lie approximately parallel ($\theta \approx 30°$) and perpendicular ($\theta = 90°$) to the B_2Q_y direction, respectively [5,14,25,26]. Comparison with the *Rps. viridis* crystal structure shows that these near infrared absorptions are approximately correlated with the I–III pyrrole ring directions for these pigments [27].

The dichroism of the absorption changes accompanying B_2I^- formation can be measured by photoselection. In this experiment, a randomly ordered, but immobilized (frozen) reaction center sample is excited with non-saturating, linearly polarized light within the B_2Q_y transition ($\lambda > 900$ nm) [25]. Photochemistry occurs selectively for those reaction centers having the B_2Q_y transition aligned along the direction of polarization. Absorption spectra are measured with light polarized parallel ($A_{\parallel}$) or perpendicular ($A_{\perp}$) to this direction. Initially $A_{\parallel}$ and $A_{\perp}$ are equivalent, but following photoselection dichroism is seen in the difference spectrum, $A_{\parallel} - A_{\perp}$.

Generation of the B_2I^- state by photoselection at 110 K produces the linear dichroism spectra, $A_{\parallel} - A_{\perp}$, shown in Fig. 3. The lower spectrum was obtained for reaction centers in the low temperature form of the B_2I^- state. The BChl band shift (lower trace, Fig. 2), which is seen as an absorbance loss at 837 nm and reappearance at 824 nm, yields a negative and positive dichroism, respectively. This shows that BChl Q_y transition moment is oriented along the $A_{\parallel}$ direction and that the band shift is not accompanied by a change in polarization. Conversely, the loss of the BPheo

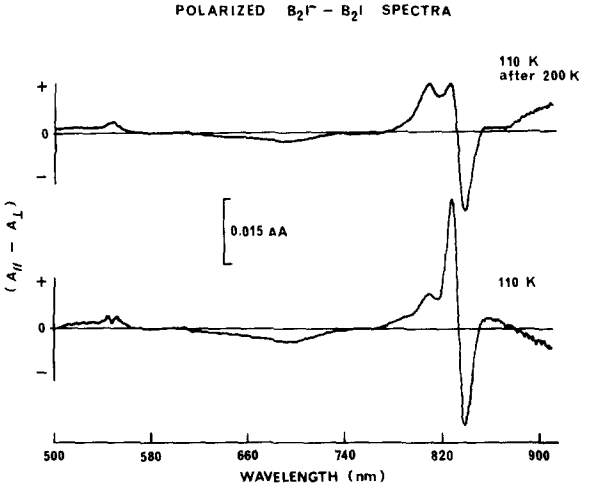

Fig. 3. Linear dichroism ($A_{\parallel} - A_{\perp}$) spectra of reaction centers in the B_2I^- state at 110 K. The lower spectrum is the dichroism for the low temperature form of the B_2I^- state, and the top spectrum is the dichroism spectrum for the relaxed B_2I^- state. The spectra were recorded sequentially on the same sample. The lower spectrum was recorded following illumination at 110 K with linearly polarized light ($\lambda > 900$ nm). The spectrum is the difference between absorbance measured with light linearly polarized parallel to the excitation polarization, $A_{\parallel}$, minus the absorbance recorded with light polarized perpendicular to the excitation polarization, $A_{\perp}$. The top spectrum was recorded in the same way, following a warming of the sample to 200 K and recooling to 110 K. The spectrophotometer used a photomultiplier with a S-20 response, so the spectra above 840 nm are not accurate. Other conditions were as described in Fig. 1.

absorption at 808 nm is associated with a positive dichroism, indicating a preferential $A_\perp$ absorption. A more quantitative analysis of this dichroism spectrum is given in Ref. 25.

The dichroism of the relaxed formed of the B_2I^- state, measured at 110 K, is shown in the top trace, Fig. 3. The most significant feature of this spectrum is that the amplitude of the positive dichroism at 808 nm has increased by about 35%. In contrast, the $I^- - I$ difference spectrum (top trace, Fig. 2) now shows only a small net absorption change in this region. This difference spectrum shows that the shoulder near 810 nm which appears in the absorption spectrum of the relaxed B_2I^- state, is nearly equivalent to the BPheo absorption before reduction of I. However, the fact that the appearance of this band does not reverse the BPheo dichroism generated by photoselection, demonstrates that the 810 nm absorption component in the relaxed B_2I^- state is not the BPheo Q_y transition seen in the ground B_2I state. In fact, the increase in the dichroism at 808 nm suggests that the new component has a preferential $A_\parallel$ absorption, while as described above, the BPheo Q_y band is polarized in the $A_\perp$ direction.

One possibility is that the 810 nm shoulder in the relaxed B_2I^- spectrum is the accessory BChl absorption which has been further blue-shifted in the relaxed B_2I^- state. This is consistent with the decrease in the 827 nm BChl absorption which accompanies the formation of the relaxed B_2I^- state, and with the $A_\parallel$ polarization of this band. If this interpretation were true, and the orientation of the BChl transition moment were unchanged by the electrochromic shift, then the dichroic ratio for this transition $(A_\parallel - A_\perp)/A$, calculated from the dichroism and absorption spectra, would remain constant upon converting the B_2I^- state from the low temperature to relaxed forms. In the experimental spectra, optical transitions from each of the pigments are strongly overlapping. However, net changes in pigment orientation can be detected by comparing the integrals of the dichroism spectrum (yielding a net dichroism for all the pigments) to the absorption integrals (yielding the net absorption for all of the pigments) for both B_2I^- states.

As described in the previous section, the integrals in the 750 nm–900 nm region showed that there was essentially no loss (less than 10%) in absorption intensity upon converting the B_2I^- state from the low temperature to relaxed forms. The absorption loss near 827 nm in the relaxed B_2I^- state was compensated by absorption increases in the 810 nm region. In contrast, integrations of the spectra in Fig. 3 show that the dichroism is not conserved. The integral of the positive portion of the dichroism spectrum decreases by about 30% upon converting the B_2I^- state from the low temperature to relaxed forms. Integrals of the negative components remain essentially unchanged. Inspection of the spectra in Fig. 3 show that the net loss of dichroism is due to a loss of the BChl dichroism at 827 nm in the relaxed B_2I^- state, which is not completely compensated by the increase in dichroism near 810 nm.

These data suggest that the new 810 nm com-

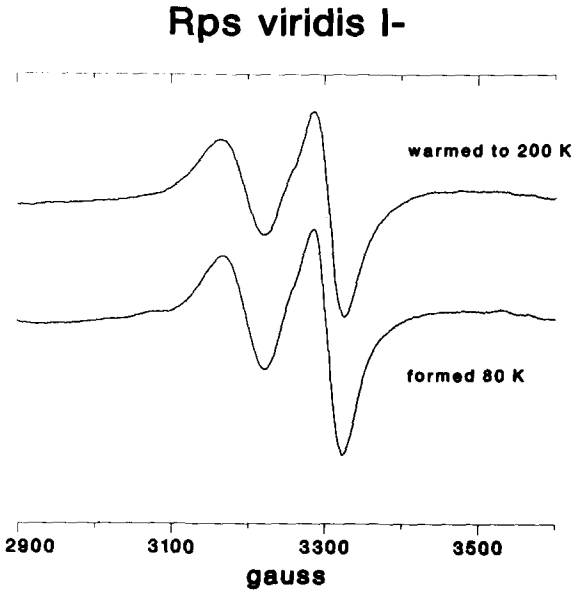

Fig. 4. Electron paramagnetic resonance spectra of reaction centers in the trapped B_2I^- states. The lower and top spectra were recorded for reaction centers in the low temperature and relaxed forms, respectively, of the trapped B_2I^- state. Spectra were recorded sequentially at 6 K, on the same sample. The lower spectrum was recorded following illumination at 77 K. The top spectrum was recorded after the sample was placed in a dry ice/acetone bath (200 K) for 10 min while in the dark, and recooled to 6 K. Other conditions were as describe in Fig. 1. The spectra were recorded with a modulation amplitude of 10 G and modulation frequency of 100 kHz, with a microwave power of 5 mW and microwave frequency of 9.1 GHz.

ponent in the relaxed state is more weakly $A_{\parallel}$ polarized than previous 827 nm BChl transition in the low-temperature form of the B_2I^- state. We conclude that the conversion of the B_2I^- state from the low temperature to relaxed forms causes a loss of the 827 nm BChl absorption and appearance of a new component near 810 nm, which must be a product of new accessory pigment absorption, since it cannot be attributed to either the original BPheo or BChl Q_y transition.

Electron paramagnetic resonance spectra

Fig. 4 shows EPR spectra of the B_2I^- states. The EPR absorption of I^- is split due to a weak magnetic exchange interaction with the anion semiquinone [7,15,28], whose spin states are mixed with those of the nearby high spin Fe^{2+} [29,30]. The magnetic exchange interaction operates through an overlap of the electron orbitals, and the strength of this interaction should be sensitive to changes in electron delocalization on I^-, or to changes in its molecular conformation. The spectra in Fig. 4 show that conversion of I^- to the relaxed form does not cause an appreciable alteration in the lineshape or splitting of the EPR absorption. This suggests that the unpaired spin has not moved a site more distant to the Q^- (Fe^{2+}), nor have conformational changes altered the extent of orbital overlap.

Discussion

The optical absorption spectra of reaction centers show that B_2I^- state exists in at least two forms. The low-temperature I^- state is characterized by a prominent bleaching of the BPheo Q_y band and nearly conservative blue shift of the BChl Q_y band. In the high temperature, relaxed form, the BPheo Q_y absorbance loss is not as evident (although the 545 nm bleaching is unchanged), and the BChl does not show a symmetrical band shift.

Similar temperature-dependent features have been reported in the transient $B_2^+I^-$ (P^F) spectra of *Rb. sphaeroides* [18]. In addition, the $I^-Q \rightarrow IQ^-$ electron-transfer rate in *Rb. sphaeroides* becomes temperature dependent above 100 K [19]. These analogies suggest that the nuclear relaxations which convert the trapped I^- state from low temperature to relaxed forms in *Rps. viridis* may also be functioning in the picosecond transient states in *Rb. sphaeroides*. In *Rps. viridis* the ($I^-Q - IQ^-$) difference spectra measured by subtraction of the transient $B_2^+I^-Q$ and $B_2^+IQ^-$ spectra exhibit only a poorly resolved BPheo Q_y bleaching at room temperature [31] compared to that seen at low temperature (Wasielewski, M. and Tiede, D., unpublished results; Kirmaier, C., Holten, D. and Parson, W.W., personal communication). This temperature dependence of the transient ($B_2^+I^-Q - B_2^+IQ^-$) difference spectra is analogous to that seen in the trapped ($B_2I^-Q^- - B_2IQ^-$) difference spectra, although the spectra differ in several respects, particularly in the BChl band shift. The discrepancies are likely to arise from the presence of the oxidized B_2^+ in the transient states which modifies the absorption of the accessory pigments in both the I^- and I states. The transient spectra may be modified to a lesser extent by the change in the redox state of Q which is not compensated in the transient different spectra. The analogous temperature dependencies of the transient ($B_2^+I^-Q - B_2^+IQ^-$) and the trapped ($B_2I^-Q^- - B_2IQ^-$) difference spectra suggest that nuclear relaxation may also be occurring to some extent in the ps transient states in *Rps. viridis* at room temperature. However, the $I^-Q \rightarrow IQ^-$ electron-transfer rate is found not to be temperature dependent in *Rps. viridis* [32]. This would suggest that if the nuclear relaxations are occurring during the picosecond electron transfer in *Rps. viridis*, they are not rate determining.

Significantly, the measurements on the trapped B_2I^- states show that the temperature-dependent optical absorption changes result from a conformational relaxation which is not reversible. This rules out the possibility [18] that I^- consists of a temperature-dependent equilibrium mixture of $BPheo^-$ and $BChl^-$. Shuvalov et al. have also reported a similar temperature-dependent conversion in the optical spectrum associated with the trapped B_2I^- states in reaction centers of *Rps. viridis*, and have proposed that the irreversible conformation change causes the electron distribution in I^- to shift from $BPheo^-BChl$ to $BPheoBChl^-$ [23]. This interpretation is based on the assignment of the 810 nm absorption band in the relaxed B_2I^- state to the ground state (B_2I)

BPheo absorption. However, companion absorption changes are not seen in the BPheo Q_x region (545 nm), and this interpretation requires that BChl and BPheo have identical absorption in this region [23].

The stability of the low temperature B_2I^- state (for at least 1 h at 110 K) suggests that an activation barrier separates the two forms of B_2I^-. At 160 K–200 K there is sufficient thermal energy (110–140 cm^{-1}) to cross the barrier within seconds. The irreversibility of the conformational relaxation indicates that the relaxed B_2I^- is lower in energy than than the initial, low temperature B_2I^- state. This prevents a repopulation of the low-temperature conformation upon recooling. A study of the kinetics of conversion at different temperatures can provide information on the activation energy separating these two states. These studies are currently being undertaken.

In this paper we have shown that the 810 nm component which appears in the relaxed B_2I^- state is not equivalent to the BPheo Q_y absorption in the ground state B_2I. Further, the conversion of B_2I^- to the relaxed form is found not alter the magnetic coupling between I^- and Q^- (Fe^{2+}). We conclude that the temperature-dependent changes in I^- do not change the redox state of the BPheo. Instead, we propose that the conversion of the B_2I^- from the low temperature to relaxed form alters the optical absorption of the accessory BChl. In the ground state B_2I, BChl Q_{xy} peaks are seen at 833 nm and 610 nm. Reduction of BPheo at 110 K causes these peaks to shift to 827 nm and 615 nm, respectively. The reduction apparently occurs without a complete solvent reorganization, and this conformation is 'frozen in' at temperatures below 150 K. A configurational change occurs upon warming the sample above this temperature. The BChl 827 nm and 615 nm peaks are lost, and a new component appears with absorption maxima at 810 nm and 643 nm (see also Figs. 2 and 3, Ref. 25). The polarization of the 810 nm component shows that it is not equivalent to the previous BPheo or BChl Q_y absorptions, and must be a product of accessory pigment absorption in the new reaction center configuration.

Recognition that I^- exists in at least two conformations will be important for calculating the electronic coupling between pigments in the reaction center. We are currently using curve fitting techniques to more quantitatively characterize the optical properties of reaction center in the I^- redox states.

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