

PHOTOCHEMICAL HOLE-BURNING IN PHOTOSYNTHETIC REACTION CENTERS

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The change in absorbance (hole spectrum) of the primary electron donor (P870) in bacterial photosynthetic reaction centers has been studied at 1.5–2.1 K following narrow-band excitation at several wavelengths within the P870 absorption band. The hole width is very large, suggesting a homogeneous linewidth on the order of 200–300 cm^{-1} . Possible interpretations of this highly unusual result, including ultra-fast excited-state decay, are discussed.

In this communication we describe experiments designed to obtain information on the initial processes which follow photoexcitation of the primary electron donor in reaction centers of photosynthetic bacteria. Given the extraordinary rate and high efficiency of the initial charge separation steps in photosynthesis, we have chosen to study this process in the frequency domain using reversible photochemical hole-burning, rather than in the time domain using sub-picosecond kinetic methods. Related methods have been used to study photochemical and photophysical processes in simpler molecular systems; however, this approach has not previously been used to study electron transfer reactions.

The initial photochemistry in bacterial reaction centers (RCs) from *R. spheroides*, R-26.1 mutant, is shown in fig. 1. An essential feature for the experiments reported below is that this cycle functions at 1.5 K, as well as at room temperature. The primary electron donor, P870, a strongly interacting pair of bacteriochlorophylls, is photoexcited to its lowest singlet state 1S_1 [P870], followed by a very rapid electron

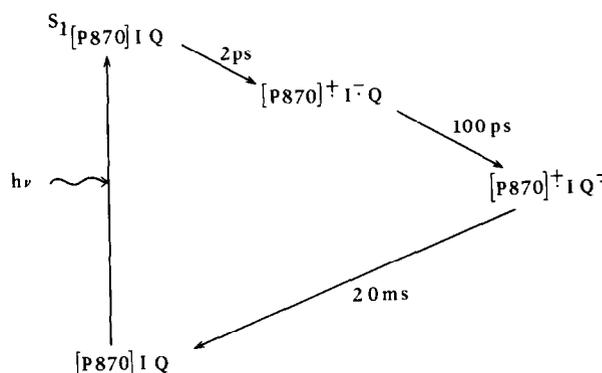


Fig. 1. Simplified kinetic scheme for initial charge separation steps and recombination in bacterial photosynthetic reaction centers at cryogenic temperatures. P870 is the primary electron donor, I is an intermediate electron acceptor, and Q is ubiquinone.

transfer reaction to form a charge-separated intermediate [1–5]. The electron moves on to a ubiquinone molecule within about 100 ps at cryogenic temperature [6], producing a moderately stable and well characterized ion pair, $\text{P870}^+ \text{Q}^-$ [7]. Subsequent movement of the electron to a secondary quinone can be blocked, causing $\text{P870}^+ \text{Q}^-$ to decay by charge recombination to the neutral ground state with a lifetime of about 20 ms ‡ . Following a saturating flash, the P870 absorption band is nearly completely photobleached, and this bleach decays with the $\text{P870}^+ \text{Q}^-$ decay rate.

* A preliminary account of this work was presented in the Proceedings of the Fifth International Seminar on Energy Transfer, Prague, July 1985.

‡ The notation 1S_1 [870] is used to denote the initial singlet state reached by excitation at wavelengths greater than 850 nm. The notation *P commonly used in the literature is avoided. The name P870 is retained by convention, although the peak wavelength is dependent on temperature and solvent [7].

Attempts to characterize the initial intermediate and time-resolve the rate of its formation have proven to be difficult. Very recently, two groups have provided convincing evidence that a species with absorbance features consistent with the formation of P870⁺ and the anion of a monomer bacteriochlorophyll or bacteriopheophytin (both present in the RC) is formed in about 5 ps at room temperature [4] and about 2 ps at cryogenic temperature [5], confirming earlier studies with poorer time resolution [1–3]. Since the RC contains six intensely absorbing chromophores, all of which may participate in the initial photochemistry, and since the absorption spectra of the singlet and triplet excited states and radical ions of all chromophores are not expected to be very different, assignments of transient absorption changes to specific chemical intermediates is not straightforward. This problem is compounded by the fact that the chromophores are in close physical proximity in the RC complex [9], so their absorption properties may depend on intermolecular contributions which are expected to change upon formation of excited states or charged intermediates [10]. A further complication is that most picosecond kinetic experiments employ excitation pulses at wavelengths shorter than the lowest singlet state of P870 and use high peak power pulses. The former leads to ambiguity due to competition between vibrational and/or electronic relaxation and electron transfer (this was not a problem in one of the recent experiments [4]), while the latter may produce artifacts when more than one chromophore per RC is excited.

Since the excited state of P870 lives for less than 5 ps due to rapid electron transfer, the homogeneous linewidth for excitation of P870 must be greater than about 1 cm⁻¹. The homogeneous linewidth (fwhm = $\Delta\nu_h$) is comprised of two terms, that due to pure dephasing (T_2^*) and that due to the lifetime of the excited state (T_1):

$$1/T_2 = 1/T_2^* + 1/2T_1, \quad \Delta\nu_h = 1/\pi T_2.$$

* The decay rate of P870⁺Q⁻ in the R-26 mutant at low temperature was reported as 33–38 s⁻¹ in the early literature [7]. More recent measurements on R-26. 1, including our result, show that the decay rate is somewhat faster [8]. The optically measured P870⁺Q⁻ decay rate exactly corresponds to the decay rate measured by EPR [7].

The pure dephasing contribution decreases with temperature. For typical aromatic guest molecules in a glassy host at 1.5 K, the homogeneous linewidth is primarily determined by the excited state lifetime, which is on the order of nanoseconds, giving linewidths on the order of 10⁻³ cm⁻¹ [11]. Extremely low temperatures (<1 K) are required to reach this situation for some molecules in some glasses [12,13]. In most cases studied to date, including photochemical hole-burning in chromophore–protein complexes [14,15], the measured linewidth is about 1 cm⁻¹ or less at temperatures below 2 K. This is in sharp contrast with the results we obtain in RCs.

The homogeneous linewidth can be measured either by photon echo methods or hole-burning [16,17]. Each method has advantages for a given set of experimental circumstances. In the case of RCs, photochemical hole-burning is a reasonable choice because the homogeneous linewidth is so large (wide infra). The principle of our experiment is the following: P870 is excited at temperatures below 2.1 K at various wavelengths within its absorption band with a laser whose bandwidth is comparable to the narrowest possible homogeneous linewidth given what is known about the initial charge-separation kinetics [4,5]. The resulting bleach is probed with a very weak, narrow bandwidth beam to determine whether the entire band is bleached or whether a narrower region (the hole) is bleached. Given the reaction scheme in fig. 1, the hole burned in the P870 absorption band is completely reversible within 0.1 s. We find that the entire band is not bleached, but that the hole is very broad. This can be interpreted as a very rapid (tens of femtoseconds) decay process from S¹ [P870], although other interpretations are possible. We note that studies of irreversible photodestruction in whole photosynthetic cells and photosystem I and II preparations have been reported [18,19]; although the apparent intent of these studies is similar to ours, the reaction mechanism and systems under investigation make any comparisons difficult.

2. Experimental

RCs containing one quinone were isolated by methods described in ref. [20], and were embedded in polyvinyl alcohol films with a typical optical

density at cryogenic temperatures of 0.3 at 882 nm (RC concentration 2×10^{-4} M, film thickness ≈ 0.1 mm). The films were mounted between quartz plates in a sample holder. Samples were cooled to 77 K in the dark over 10–15 h, followed by immersion in liquid He. The He was pumped below the lambda point and the temperature was monitored with a calibrated manometer (Validyne Engineering Corp.) with an accuracy of ± 0.05 K. The absorption spectrum was measured in situ before and after laser excitation; no degradation was observed following many hours of experiments at 1.5 K.

The hole-burning apparatus consists of a frequency-doubled Nd:YAG-laser-pumped dye laser whose output is converted into near infrared light by stimulated Raman scattering using H_2 gas (80–90 psi). This arrangement produces stable, tunable, moderate bandwidth ($3\text{--}4\text{ cm}^{-1}$) pulses throughout the near infrared region. Pulse power was measured with a Scientech power meter. Great care was taken to ensure that the beam was spatially homogeneous over the probed area (≈ 3 mm diameter). The absorption spectrum was probed with very weak ($\approx 30\text{ nW/cm}^2$) light passed through a $3/4$ m monochromator ($\approx 0.3\text{ cm}^{-1}$ ultimate resolution, calibrated with a standard neon lamp) and detected with a cooled S-1 photomultiplier tube. The output of the photomultiplier was measured with a lock-in amplifier at the 10 Hz repetition rate of the burn laser. The 10 Hz repetition rate and low burn power ensure that any changes in absorption due to the burn laser have fully recovered between excitation pulses. In some cases the burn laser was a cw multimode laser diode (peak wavelength 867 nm, power at sample 3 mW, 20 ms burn time).

Absorption spectra were obtained by separately measuring the intensity of the probe beam passing through the sample and through a film containing no RCs under otherwise identical conditions. Spectra were signal averaged for the weakest powers (five scans). Mechanical shutters were used to exclude the burn beam from the detection system (shutter opening and closing times typically about 2 ms). Hole spectra are defined as the light-minus-dark difference absorption spectra.

3. Results

The absorption spectra of RCs in the near infrared region in the absence and presence of photobleaching at 1.8 K are compared in fig. 2. The red side of the absorption band of P870 ($\lambda_{\text{max}} = 11335\text{ cm}^{-1}$ (882 nm) in PVA films at 1.8 K) was fit with a Gaussian lineshape function with fwhm 510 cm^{-1} . Overlap on the blue side with the 800 nm band (partially resolved at low temperature) complicates fits of the entire lineshape. Note also the change in the shape of the 800 nm band upon photobleaching which changes the degree of overlap (this has been discussed elsewhere [6,7]). The decay kinetics of absorbance changes within the P870 band were found to be wavelength independent and exponential with decay constant $60 \pm 3\text{ s}^{-1}$ at 1.8 K (see footnote ‡).

Hole spectra were obtained for scans between 10750 and 12000 cm^{-1} (930 and 833 nm). Initially great care was taken to scan very slowly at high resolution in the vicinity of the burn wavelength in order not to miss sharp features, but narrow holes were never observed.

The dependence of the hole spectrum on burn wavelength, power, and temperature were studied in detail. The burn wavelength dependence is shown for selected wavelengths in fig. 3 (in all cases the band was less than 15% bleached). It is observed that

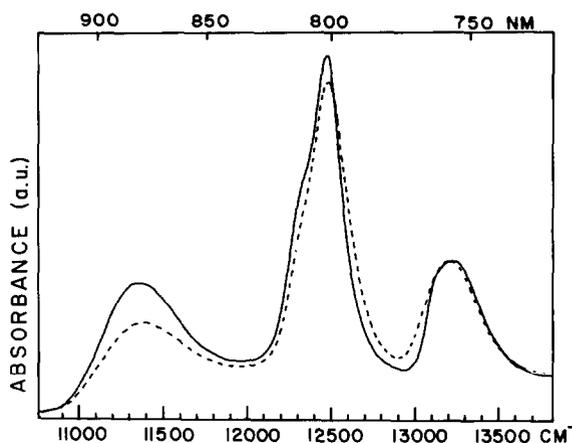


Fig. 2. Absorption spectrum of RCs in a PVA film at 1.8 K in the absence (—) and presence (---) of laser excitation at 11350 cm^{-1} (881 nm). In this example the P870 absorption band was 30% bleached for clarity of presentation.

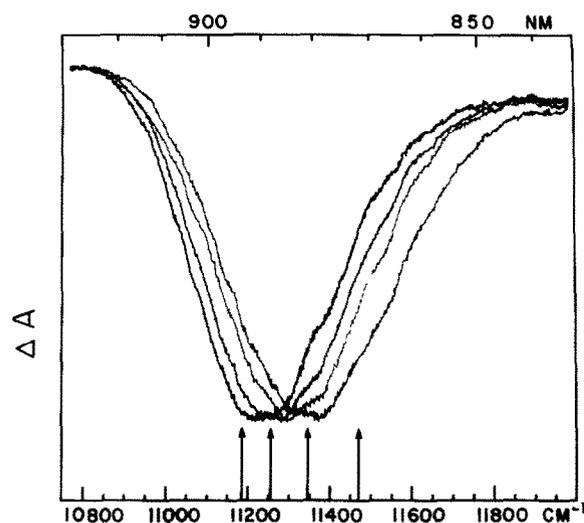


Fig. 3. Burn wavelength dependence of the hole spectrum at 1.5 K. The burn wavelengths are indicated with vertical arrows: 11470, 11345, 11253 and 11180 cm^{-1} , with the corresponding holes shifting from blue to red. The amplitudes of the hole spectra were scaled to the same magnitude to facilitate comparison.

the holes shift from red to blue as the burn wavelength is tuned from red to blue, but that the deepest point in the hole spectrum is not at the burn wavelength. The magnitude of this displacement increased as the burn beam was moved further from the center towards the red or blue edge of the absorption band. In all cases, the hole is narrower than the absorption line and is somewhat asymmetric. The origin of the asymmetry is, in part, the change in overlap on the blue side with the 800 nm absorption band (fig. 2). The red side of the holes fit well to Gaussian lineshape functions; the holewidths for several burn wavelengths are presented in table 1. They are meant only to provide a useful numerical value for comparison of the hole spectra and are discussed further below. It is seen that the holes broaden considerably as the excitation wavelength is shifted from red to blue.

The holewidth was studied as a function of excitation power by reducing the intensity of the burn beam with calibrated neutral density filters (fig. 4A). The degree of saturation was obtained by comparing the integrated absorption before and after burning. For all experiments reported here, the power of the burn beam was below the point at which the absor-

Table 1

The effect of burn wavelength on approximate holewidth ^{a)} and hole center

Burn wavelength (cm^{-1})	Holewidth ^{a)} (cm^{-1})	Hole center (cm^{-1})
11180	390	11235
11253	410	11270
11345	420	11310
11470	440	11350
11593	500	11410

^{a)} Holewidth is estimated by fitting the red side of the hole spectrum to a Gaussian lineshape. The hole spectrum is not symmetrical, so this is only meant for rough comparison. The uncertainty in the hole fits is approximately 10 cm^{-1} .

bance decrease no longer scales linearly with power. No change in holewidth was observed within the experimental error (the energy density at the lowest burn intensity was approximately 40 $\mu\text{J}/\text{cm}^2$). Possible effects of the high peak power of the pulsed laser were checked by comparison of the holewidth for excitation with the cw laser diode. The measured holewidths were identical to within the experimental error, despite a decrease of more than five orders of magnitude in peak power. The independence of the holewidth on temperature between 1.5 and 2.1 K is shown in fig. 4B. The independence of the holewidth on temperature and power was demonstrated for each burn wavelength shown in fig. 3.

4. Discussion

The simplest interpretation of the results above is that the excited-state lifetime of P870 is very short, leading to a very broad hole. The widths reported in table 1 translate into decay rates on the order of $5 \times 10^{13} \text{ s}^{-1}$ (lifetime on the order of 15–25 fs). This interpretation provides no information on the possible state(s) into which ^{S1} [P870] decays.

In the following we consider experimental and conceptual issues which may lead to other interpretations of these results. One potential experimental problem is power broadening. This concern was tested by studying the holewidth at a series of low powers

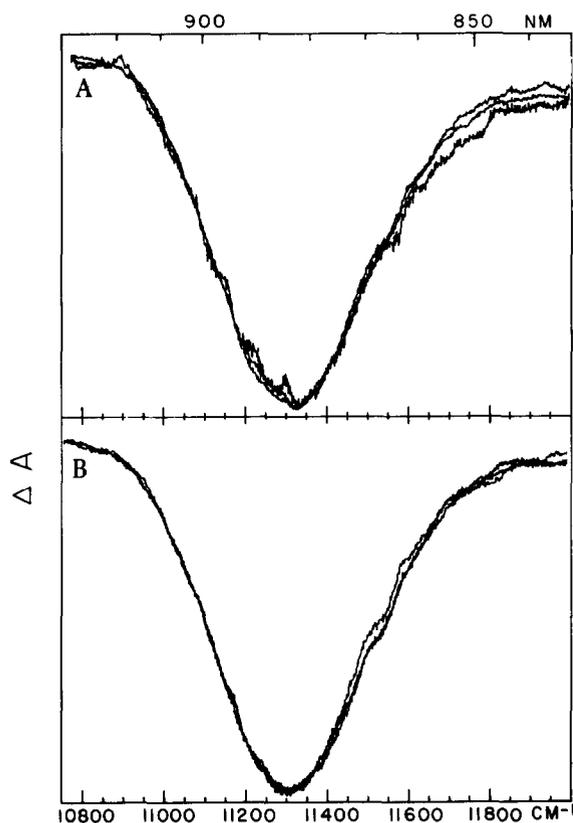


Fig. 4. (A) Energy density dependence of the hole spectrum at 1.5 K. The curves correspond to 1.5, 0.9 and 0.3 mJ/cm^2 , with the highest energy density giving the curve with the best signal to noise. Otherwise the hole spectra are indistinguishable within the experimental error as was the hole spectrum for an energy density one order of magnitude smaller. The amplitudes of the hole spectra were scaled to the same magnitude to facilitate comparison. (B) Temperature dependence of the hole spectrum. Data are presented at 1.5, 1.8 and 2.1 K. The curve which appears slightly narrower on the blue side is at 2.1 K; however, the holewidths and areas are indistinguishable to within the experimental error.

and by using the cw laser diode as the excitation source. No variations in the holewidth were observed within the experimental error. Another possibility is that the holewidth changes with time, either following the initial step or during charge recombination. The latter would manifest itself as a variation in the $\text{P870}^+ \text{Q}^-$ recombination decay kinetics (bottleneck decay) at different wavelengths within the band which was not observed within the experimental error. The

former is more difficult to rule out as we do not observe the change in absorbance immediately after the burn. The following argument can be used to suggest that the holewidth could not be much narrower: making the reasonable assumption that the hole area is conserved, a postulated initial narrow hole will be much deeper than the wider hole we observe. If this were the case, the hole amplitude would begin to saturate and give a non-linear response with increasing burn intensity while the observed hole is still shallow. Since linear behaviour is observed up to at least 15% observed bleach, this indicates that this is not the case.

In order to deal qualitatively with our data we have adopted the grossly oversimplified picture that the P870 absorption band is comprised of a Gaussian distribution of Lorentzian components each with a 200–250 cm^{-1} linewidth (this linewidth was varied). This ignores vibronic structure, phonon sidebands, and variations in the homogeneous linewidth across the band and assumes a stochastic decay process. Calculations for this model system show that the holes should not be symmetrical except for burns in the center of the band (the calculated hole shape is neither Lorentzian nor Gaussian) and that the deepest point in the hole is expected to be displaced from the burn wavelength towards the center of the band for burns on the red or blue side. The trend of a smaller holewidth as the burns are shifted from the blue towards the red side of the band obviously cannot be duplicated in a symmetrical model. Although crude, a comparison of this simple model with our data demonstrates that the homogeneous linewidth cannot be smaller than about 200 cm^{-1} . A detailed analysis of the observed hole shape as a function of excitation wavelength is in progress.

In the following, we consider other mechanisms which may lead to broad holes. It is possible that the holewidth is a measure of vibrational relaxation within P870; however, vibrational relaxation in aromatic molecules under comparable conditions is generally slower than 10^{13} s^{-1} [21]. We are presently limited by our apparatus to burns to the blue of 11180 cm^{-1} , and it is conceivable that very weak absorption further on the extreme red side of the P870 band represents excitation of the 0–0 band or that there are new absorption features further to the red (e.g. unusually weak charge transfer bands). A second possible source of broadening is residual pure

dephasing. The magnitude and origin of the temperature dependence of the pure dephasing contribution to the homogeneous linewidth in glasses is a very active area of current experimental [11,13] and theoretical work [22–24]. In general, the pure dephasing contribution for aromatic molecules (including free-base porphyrin dimers [11]) in soft glasses has a T^α dependence, where α is between 1 and 2. Such a dependence would be easily observed in our experiment, but the holewidth and area showed no temperature dependence between 1.5 and 2.1 K (fig. 4B). One cannot rule out novel dephasing mechanisms for multichromophoric or protein systems such as the RC complex, however, there is no precedent for such mechanisms.

A third possible explanation is that the zero-phonon line is entirely suppressed and the absorption band and hole are made up of an inhomogeneous distribution of phonon bands, possibly convolved with low-frequency vibronic structure. In such a situation the holewidth and shape are due to the distribution of phonon states, the probability distribution for transitions from the ground electronic state to each phonon state, and the rate of decay from each state. The zero-phonon line dominates hole and fluorescence line-narrowing spectra of porphyrins and chlorins in glasses [11], but at the present time we have very little information on the nature of phonon sidebands for the chromophores in the RC. We are not aware of previous examples of complete suppression of the zero-phonon line in the hole-burning literature, but this situation could be produced by a very large displacement of the potential energy surface of S^1 [P870] relative to ground state P870 making the Franck–Condon factor for the 0–0 transition very small relative to its value for higher phonon levels. A possible mechanism would be direct excitation to a charge-transfer state. This interpretation is appealing because it would not invoke new ultra-fast processes which go undetected by sub-picosecond kinetic spectroscopy, and there have been experimental [25] and theoretical [5] suggestions that the P870 absorption has charge-transfer character.

Finally, we return to the simplest interpretation, that S^1 [P870] decays by some very rapid process such that S^1 [P870] is not present to any appreciable extent after about 100 fs. Such a rapid change may indicate that the process should not be viewed in

the conventional sense of a stochastic relaxation characterized by a rate constant. This problem has been considered in some detail for very different molecular systems [26,27], and such an analysis may also be appropriate for describing ultrafast reaction dynamics in RCs. Irrespective of the underlying conceptual framework, we can speculate on the nature of the dynamics as this suggest possible experimental tests. An obvious possibility is charge separation between the bacteriochlorophylls comprising P870 [4,28,29], or charge separation involving other chromophores (this is not ruled out by the sub-picosecond absorption data which show many unexplained absorption changes). A second possibility is a structural change involving P870, for example, a change in the intermolecular separation or orientation, changes in the interaction between the central Mg atoms and the ligands, or changes in hydrogen bonds.

In summary, we have observed very broad holes in the absorption band of the primary electron donor of bacterial RCs. This result can be explained either by a large and unusual difference between the potential energy surfaces of the ground and excited states of P870 or an ultra-fast dynamic process following excitation. At the present time there are only limited hole-burning precedents for three key features of the RC system: the primary photochemistry is reversible electron transfer, the molecular species is a strongly coupled pair of chromophores, and the host medium is a protein. We have developed model systems for each feature [30–32], and further hole-burning experiments are in progress.

Note added

Following submission of this communication we received a preprint from Professor Wiersma in Groningen describing related experiments. Meech, Hoff and Wiersma [33] attempted to measure T_2 for P870 directly using the accumulated phonon echo technique. The echo decay rate was instrument limited (about 200 fs).

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