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Reversible photochemical holeburning in *Rhodopseudomonas viridis* reaction centers

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The change in absorption of the band assigned to the primary electron donor in *R. viridis* reaction centers (P960) has been measured at 1.4–2.1 K following narrow bandwidth excitation at many wavelengths within the P960 absorption band. In all cases nearly the entire band was photobleached (full width at half-maximum about 400 cm^{-1}). This result can be explained by either an ultra-fast decay process from the initially excited state or a substantial difference in the equilibrium nuclear configuration between the ground and excited state of the donor. The latter may arise if the excited state of the donor has substantial charge transfer character. The results suggest a charge separation step which precedes formation of a bacteriopheophytin *b* anion.

Photosynthesis *Reaction center* *Holeburning* (Rhodopseudomonas viridis)

1. INTRODUCTION

The rate of the initial charge separation step in photosynthesis has been the subject of intense investigation by transient spectroscopy during the past 10 years [1–5]. The recent structural determination of bacterial reaction centers (RCs) from *Rhodopseudomonas viridis* [6,7] has focused special attention on this species. The reaction scheme which describes the initial photochemistry in *R. viridis* RCs is shown in fig.1, along with the three-dimensional arrangement of the reactive components adapted from the crystal structure obtained by Deisenhofer and co-workers [6]. Photoexcitation of P960 at cryogenic temperatures leads to the formation of an intermediate characterized by transient absorption spectroscopy within about 5 ps [8]. Subsequent electron transfer to reduce the menaquinone occurs within about 170 ps at 5 K (C. Kirmaier and D. Holten, personal communication). The large absorption decrease in the band centered at 960 nm due to the initial excitation event is preserved until the charge recombination reaction, $\mathrm{P960^+\ MQ^-} \longrightarrow \mathrm{P960\ MQ}$, regenerates the ground state (1/*e* time 7 ± 1 ms at 1.5 K).

This long-lived change in absorption at cryogenic temperatures makes it possible to study the initial charge separation step in the frequency domain using the technique of photochemical holeburning as an alternative to conventional kinetic spectroscopy. The homogeneous linewidth (full width at half-maximum, $\Delta\nu_h$) is given in general by:

$$\Delta\nu_h = \frac{1}{\pi T_2} = \frac{1}{\pi T_2^*} + \frac{1}{2\pi T_1}$$

where T_1 characterizes the lifetime of the excited state, and $1/T_2^*$ is the time constant for pure dephasing. In the absence of pure dephasing (see below) the homogeneous linewidth is simply related to the excited state lifetime and can be measured directly by measuring the width of a hole burnt in the absorption band. Thus, the homogeneous linewidth for the P960 absorption band would be 1 cm^{-1} (holewidth 2 cm^{-1}) if the excited state lifetime were 5 ps. Our strategy,

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therefore, is to burn a hole in the P960 absorption band with a narrow bandwidth laser at various wavelengths within the P960 absorption band and probe the change in absorption (hole spectrum). This strategy was successfully applied to the primary electron donor in RCs from *R. sphaeroides*, denoted P870, and the results from these studies [9–11] are compared with those from *R. viridis*.

2. MATERIALS AND METHODS

R. viridis RCs embedded in approx. 0.1 mm thick polyvinylalcohol films were a generous gift from Professor W.W. Parson of University of Washington, Seattle, WA. The RCs were isolated using the method described in [8] with some improvements (D. Middendorf, personal communication). The absorbance at 960 nm was typically 0.2. The film was held between plexiglass plates and was immersed in a liquid He cryostat. The He was pumped below the lambda point and the temperature was determined from the vapor pressure of the He. At this temperature reduction of P960$^+$ by the high potential cytochrome that is associated with the RC is inhibited (there is also a low potential cytochrome associated with the RC that can reduce P960$^+$ at helium temperatures; this however is already oxidized in the samples used in our measurements). The sample was excited with moderately narrow bandwidth laser light obtained from a frequency doubled Nd:YAG pumped dye laser whose output was Raman shifted with high pressure H_2 gas to 1023–971 nm (linewidth 2 cm^{-1}, maximum energy density 25 mJ/cm^2, pulse width 5 ns). The change in absorption was probed with very weak light passed through a monochrometer (ultimate resolution 0.3 cm^{-1}).

3. RESULTS AND DISCUSSION

Light-minus-dark difference spectra (hole spectra) for excitation at wavelengths within the P960 absorption band are shown in fig.2A. Data obtained in a similar experiment on *R. sphaeroides* RCs [10] are shown for comparison in fig.2B. In both species very broad holes are observed (see figure legend). No variation in the hole spectrum with burn wavelength is observed for *R. viridis*, whereas a small variation is observed for *R. sphaeroides*. No variation with temperature was observed in the hole spectra for either species between 1.4 and 2.1 K. No narrow features were observed in the hole spectra; since the laser linewidth is comparable to the minimum possible holewidth of 2 cm^{-1}, the observed holes are not limited by instrumental resolution. At temperatures below 2 K the possibility of spectral diffusion during the 7 ms lifetime of the intermediate is very unlikely.

The magnitude and temperature dependence of the pure dephasing contribution to the homogeneous linewidth of organic molecules in glasses has been the subject of great interest during the past several years [12–17]. In glasses, the pure dephasing contribution depends strongly on temperature, being proportional to T^{α}, with α between 1 and 2 at liquid helium temperatures. We have observed no dependence of the holewidth in RCs on temperature over a wide range making it very unlikely that pure dephasing contributes significantly to the observed linewidth.

Inhomogeneous broadening of the absorption of organic molecules in glasses generally prevents the observation of resolved vibronic structure. For chlorophylls and porphyrins, absorption spectra in Shpolskii matrices and fluorescence line narrowing studies in glasses demonstrate that several low frequency vibronic bands are present within 400 cm^{-1} of the 0-0 transition [18–20]. Because the lowest energy P960 absorption band is due to a bacteriochlorophyll *b* dimer (fig.1), similar vibronic structure is also expected for this band. For this reason, it is not necessarily correct to simply convert the observed holewidth into a homogeneous linewidth for P960 because each vibronic band will have an associated homogeneous width (possibly different for different vibronic transitions). Nonetheless, because the vibronic line spacing for chlorophylls and other aromatic molecules at low frequency is as large as 50 cm^{-1}, the homogeneous linewidths for the underlying transitions would have to be broader than about 20–30 cm^{-1} in order to account for the complete absence of structure in the hole spectra (fig.2).

Taking the simplest view that the observed hole is uncertainty broadened by a very rapid decay process from the excited state, one obtains a lifetime shorter than 250 fs. We speculated several

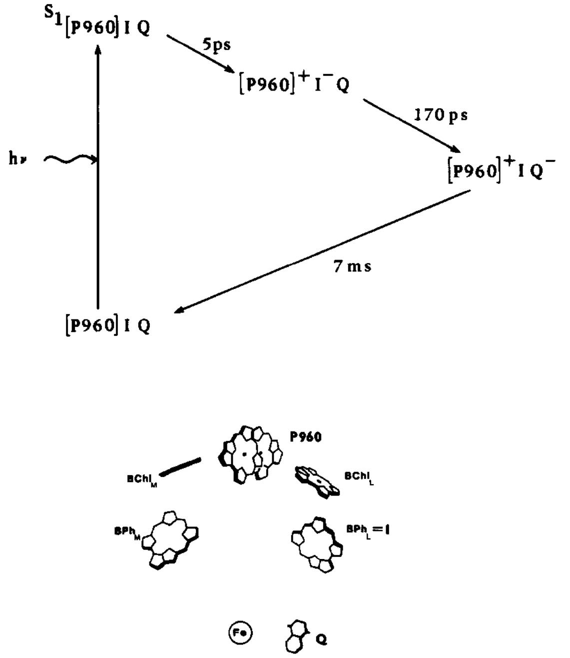

Fig.1. Kinetic scheme of the initial charge separation and recombination steps in RCs from *R. viridis* and the physical relationship of the reactive components adapted from the structure of Deisenhofer and co-workers [6]. P960 is the primary electron donor, I is an intermediate redox carrier and involves both the bacteriochlorophyll and bacteriopheophytin on the L side of the structure, and Q is menaquinone. The lifetimes of states are at cryogenic temperature.

years ago that such a short lifetime might explain the fact that the lowest energy P960 and P870 absorption band linewidths are considerably broader than those for the other chromophores in the RC [21]. Very recently, Wiersma and co-workers [11] have independently attempted to measure the homogeneous linewidth for P870 in *R. sphaeroides* using the accumulated photon echo technique. The echo decay was observed to be faster than the time resolution of their instrument (200 fs); this was interpreted as evidence for an excited state lifetime shorter than 200 fs.

We wish to propose an alternative explanation for the broad holes [10]. We postulate that excitation of P960 involves excitation to a state with considerable charge transfer character, either localized on the special pair dimer or involving all four bacteriochlorophylls. We do not consider reactions

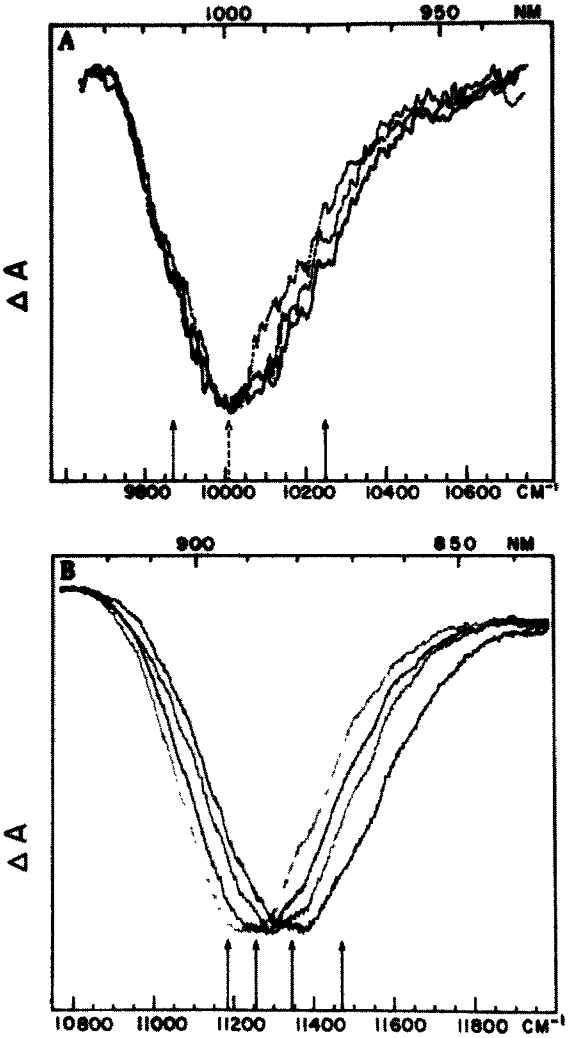

Fig.2. (A) Photochemical holes observed in *R. viridis* RCs at 1.4 K. The burn wavelengths (indicated with vertical arrows) are 10250, 10009 and 9870 cm^{-1}. The full width at half-maximum for the hole in each case is about 400 cm^{-1}. The maximum change in absorbance was 7%. (B) Photochemical holes observed in *R. sphaeroides* RCs at 1.5 K for comparison with (A). The burn wavelengths (indicated with vertical arrows) and associated holewidths are (all in cm^{-1}): 11470 (440); 11345 (420); 11253 (410); and 11180 (390). For both species the RCs were embedded in polyvinylalcohol films. The amplitudes of the hole spectra were scaled to the same magnitude to facilitate comparison of the lineshapes. Note that the wavelength scales in the two plots are offset, but the widths (in cm^{-1}) are identical.

involving photoreduction of the bacteriopheophytin, since recent transient absorption spectroscopic studies of both *R. viridis* [8] and *R. sphaeroides* [4,5] RCs demonstrate that this photoreduction does not occur earlier than a few picoseconds.

There have been several recent attempts to calculate and explain the absorption and circular dichroism properties of *R. viridis* RCs based on the crystallographic coordinates [22–24]. To date these calculations do not provide definitive information on the energies and oscillator strengths for charge transfer states or precise estimates for the amount of charge transfer character in the lowest excited state, although the work of Parson and coworkers [23] suggest that the excited state of P960 may have substantial charge transfer character. Preliminary Stark effect measurements on P870 suggest a substantial change in its electric dipole moment upon photoexcitation [25] which is consistent with our proposal. Considering the isolated chromophores in fig.1, there is no obvous distinction between the two bacteriochlorophylls comprising P960 or between the two monomer bacteriochlorophylls, the molecules within each pair being related by a C_2 axis [6]. Charge transfer states and the admixture of charge transfer character for a C_2 symmetric set of chromophores cannot distinguish one side of the structure from the other. On the other hand, the symmetry can be broken by an asymmetry in the environment around the chromophores due to local amino acid differences and differences in hydrogen bonds to the chromophores (the latter could lead to selective stabilization of a polar state by proton transfer). Both of these have been observed in the *R. viridis* X-ray structure (H. Michel and J. Deisenhofer, personal communication). Such interactions can favor the nascent charge separation along the L pathway (fig.1) which is required for efficient unidirectional electron transfer.

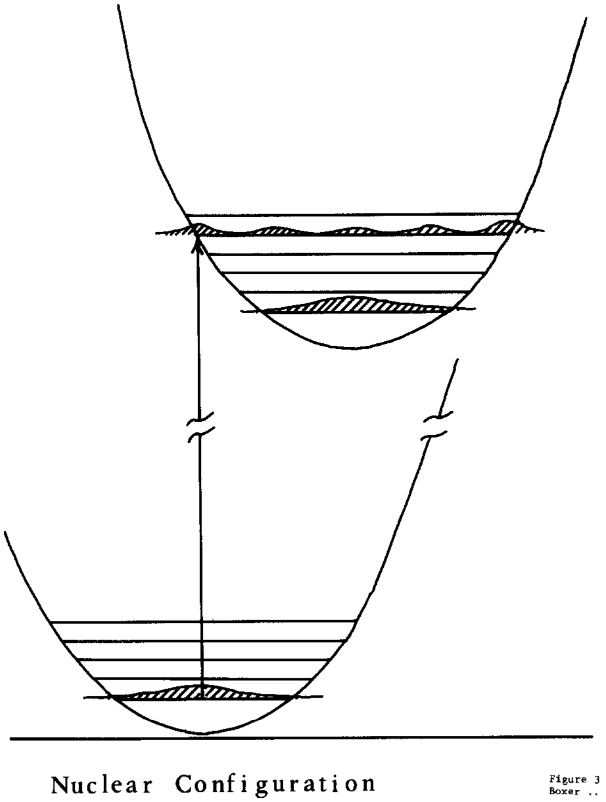

Fig.3. Schematic representation of an excitation process in which the excited state potential surface is displaced relative to the ground state surface. The level to which the transition occurs is determined by the overlap of the equilibrium ground and excited state wave functions. The level spacing in the potential wells is determined by the frequencies of coupled lattice motions, torsional oscillations of the molecules, internal vibrations, etc. The width of each level is determined by the rate of decay from that state. The level spacings, density of states and the widths are not necessarily uniform throughout the potential wells.

Direct excitation of a state with substantial charge transfer character offers an attractive alternative to an ultra-fast decay process as an explanation for the broad holes. The formation of such a state from the neutral ground state would involve a large difference in the equilibrium nuclear configuration between the ground and excited state. This is illustrated schematically in fig.3. Such a difference could lead to the suppression of the zerophonon line and result in broad phonon bands associated with each vibronic transition. Holeburning studies of molecular systems which undergo electron transfer or form charge transfer states at cryogenic temperatures are obviously required in order to test this mechanism.

In summary, photochemical holeburning provides a new approach to probing the initial step in photosynthesis. Very broad holes have been observed for two different species of bacteria, suggesting that this phenomenon is a general characteristic of the initial charge separation step.

Two explanations have been presented to account for the broad holes, both involving the formation of a charge transfer state. The first postulates that an initial pure singlet state decays within less than 200 fs to another state, possibly involving charge transfer. The alternative postulates direct excitation of a state with a very different equilibrium nuclear configuration than the ground state, such as a state with substantial charge transfer character, and that the holewidth is due to direct excitation into congested phonon and/or vibronic levels. In the limit of strong coupling between an initial excited state and a charge transfer state, these descriptions are very closely related. In either case, the holeburning experiments provide evidence for a charge separated state which precedes the reduction of bacteriopheophytin in both *R. viridis* and *R. sphaeroides.*

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