Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 957–961, February 1986
Biophysics

Femtosecond spectroscopy of electron transfer in the reaction center of the photosynthetic bacterium *Rhodopseudomonas sphaeroides* R-26: Direct electron transfer from the dimeric bacteriochlorophyll primary donor to the bacteriopheophytin acceptor with a time constant of 2.8 ± 0.2 psec

(photooxidation/charge separation/stimulated emission)

J.-L. MARTIN†, J. BRETON‡, A. J. HOFF‡§, A. MIGUS†, AND A. ANTONETTI†

†Laboratoire d'Optique Appliquée, Institut National de la Santé et de la Recherche Médicale U275-Ecole Polytechnique-Ecole Nationale Supérieure de Techniques Avancées, 91128 Palaiseau Cedex, France; ‡Service de Biophysique, Département de Biologie, Centre d'Etudes Nucleaires de Saclay, 91191 Gif-sur-Yvette Cedex, France; and §Department of Biophysics, Huygens Laboratory of the State University, P.O. Box 9504, 2300 RA Leiden, The Netherlands

Communicated by C. V. Shank, September 20, 1985

ABSTRACT **The primary light-induced charge separation in reaction centers from *Rhodopseudomonas sphaeroides* R-26 has been investigated after excitation with laser pulses of 150 fsec duration within the longwave absorption band of the primary donor at 850 nm. An excited state of the primary donor, characterized by a broad absorption spectrum extending over the whole spectral range investigated (545–1240 nm), appeared within 100 fsec and gave rise to stimulated emission in the 870- to 1000-nm region with a 2.8-psec lifetime. The photooxidation of the primary donor, as measured at 1240 nm, and the photoreduction of the bacteriopheophytin acceptor, monitored at 545 nm and 675 nm, have been found to proceed simultaneously with a time constant of 2.8 ± 0.2 psec. Kinetics of absorbance changes at other probe wavelengths gave no indication that an accessory bacteriochlorophyll is involved as a transient electron acceptor.**

The mechanism by which light is converted into chemical free energy involves a sequence of very fast events between the absorption of a photon by the antenna pigments and the completion of the primary charge separation in the chlorophyll–protein complex, called the reaction center. Picosecond spectroscopy on reaction centers isolated from photosynthetic bacteria has revealed the presence of short-lived intermediates in this electron transfer process (1–11). However, the first steps take place in a time shorter than the duration of the pulses generally used and therefore have not been kinetically resolved. A previous study using subpicosecond pulses suggests that an electron has been transferred in ≈4 psec (5). However, the use of an excitation at 610 nm, which could directly excite one of the possible initial acceptors (bacteriochlorophyll), made it difficult to reach definite conclusions. In the present work, the earliest events in the charge separation process occurring in the reaction center of *Rhodopseudomonas sphaeroides* R-26 have been investigated by using a femtosecond spectroscopic technique, which associates a 100-fsec time resolution with tunability of the excitation in the near-infrared region (12, 13).

Reaction centers from photosynthetic bacteria can be isolated in a functionally intact state and contain three polypeptides, four bacteriochlorophylls, two bacteriopheophytins, two quinones, and one non-heme iron atom (14). In the case of the reaction center from *R. sphaeroides* R-26, the main absorption bands of the pigments are located at 865, 800, 760, 590, and 540 nm. The 865-nm band, which bleaches upon (photo)oxidation of the reaction center, is ascribed to the primary donor (P). ENDOR data (15, 16) indicate that in oxidized P (P^+) the hole left by the removal of the electron is delocalized over a dimer of bacteriochlorophylls. Concomitant with the (photo)oxidation of P, the 800-nm band undergoes a change, which appears to be largely a blue shift. This shift is currently assigned to an electrochromic effect of P^+ on the Q_Y transition of the two other bacteriochlorophyll molecules (called B) whose absorptions overlap at 800 nm. The 760- and 540-nm bands are attributed to the Q_Y and Q_X transitions of the two bacteriopheophytins (called H), respectively, while the 590-nm band is assigned to the Q_X transitions of all the four bacteriochlorophyll molecules. Photodichroism experiments on the absorption changes linked to the photooxidation of P have demonstrated the existence of fixed geometries among the transition moments of the six pigment molecules (17). X-ray diffraction studies on crystals of the reaction center of the bacterium *Rhodopseudomonas viridis* have recently confirmed that P is a dimer and have shown details of the spatial organization of P, B, H, and the primary quinone acceptor Q_A in the polypeptide scaffold (18). In particular, the two bacteriochlorophylls constituting P, the two B and the two H molecules, appear organized with C_2 symmetry relative to an axis running from the center of P to the iron atom. This symmetry defines two "branches" of pigments extending from P, with only one of them directed toward Q_A. We will denote B_A and H_A the B and H molecules associated with the latter branch.

Previous studies (3, 4) have shown that excitation of the bacteriopheophytins with an actinic pulse at 530 nm leads to the appearance of the characteristic absorption band of P^+ at 1240 nm within 5–10 psec after the excitation pulse. This initial transfer induces the reduction of at least one intermediary electron acceptor that is identified as one of the bacteriopheophytins (H_A). The primary charge separation is completed by the transfer of the electron from H_A^- to Q_A in about 200 psec (1–5, 19).

While the involvement of one of the two H molecules as an intermediary acceptor has been clearly indicated, the occurrence of earlier electron transfer step(s) and the involvement of B_A in these processes is uncertain (6–11). Holten *et al.* (5) observed a very fast absorbance increase (instrument-limited rise time) prior to the 4-psec phase, which apparently corresponds to a precursor of the $P^+H_A^-$ species. The identity of this initial transient could not be determined and was assigned to either an excited state of P or the state $P^+B_A^-$. Shuvalov *et al.* (6, 9) and Kryukov *et al.* (20) reported

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

evidence for $P^+B_A^-$ as an intermediate step before electron transfer to H_A. However, Borisov *et al.* (10) were unable to detect in the 800-nm region a change associated with B_A reduction. In a recent review of these results, Kirmaier *et al.* (11) argue that there is no convincing evidence that $P^+B_A^-$ is a kinetically or spectrally resolved intermediary state in the charge separation process. Thus, while most of the above studies agree with a scheme in which $P^+H_A^-$ is formed in a few picoseconds, data on the possible involvement of B_A^- as an earlier intermediate are still strongly debated. The main reason for this lack of agreement appears to be due to a combination of unsatisfactory experimental conditions, such as ill-suited excitation wavelengths in which the excitation is not directly created on P but rather on H or B, excessive excitation energies that can lead to nonlinear processes (7), and pulses of a duration much longer than the phenomena under investigation. This has led to a tangle of ambiguous results and conflicting interpretations. Only with the advent of techniques to generate sufficiently intense pump pulses in the near-infrared with a duration of 100–200 fsec has it become possible to avoid the pitfalls described above.

MATERIALS AND METHODS

Generation and amplification of laser pulses of 150 fsec have been described (12, 13, 21, 22). Briefly, 80-fsec pulses at 620 nm were generated by a passively modelocked CW dye ring laser. Amplification of these pulses to powers in the gigawatt regime was achieved by using a four-stage dye amplifier pumped by a Q-switched frequency doubled Nd-Yag laser. This set up produced 1-mJ pulses at 10 Hz in the 100- to 200-fsec time range. The output pulses were split in two parts, both used to generate a broadband stable continuum. The pulse duration in the near-infrared region has been measured in a separate experiment by using a second harmonic frequency mixing technique and it was found to be close to 150 fsec. From one continuum, a selected part of 7 nm width centered at 850 or 870 nm was further amplified to a few μJ in a Styryl 9 dye flowing solution and used as the pump beam. In most experiments, parallel polarization of pump and probe beams was used. Handling of the data as well as analysis of the measured kinetics and fitting of the curves were carried out essentially as described (22).

The reaction centers, prepared according to ref. 14, were resuspended in 10 mM Tris·HCl buffer (pH 8.0) containing 0.1% lauryl-dimethylamine oxide to an absorbance of 0.7–0.8 *A* at 865 nm in a cuvette of a 0.1-cm light path. Under these conditions ≈20% of the reaction centers in the probed volume were excited on each laser pulse. During the experiment, the cuvette was moved in a plane perpendicular to the propagation of the light beams so that each pulse at 10 Hz excited a new region of the sample. Equilibrium spectra recorded before and after each run were identical.

RESULTS

Fig. 1*a* shows a measurement of the induced transmission at 870 nm after excitation at 850 nm. The bleaching at 870 nm corresponds to the disappearance of the ground state of P. This kinetics was found to have an instrument limited rise time and is well-fitted with a 150-fsec pulse duration. Fig. 1*b* represents the same kinetics on a larger time scale. This figure emphasizes a partial relaxation phase that has a time constant of 2.8 psec. The origin of this recovery process is discussed later.

Fig. 2 corresponds to the induced absorption at 1240 nm after photoexcitation at 850 nm. The 1240-nm absorption band has been assigned at equilibrium to P^+ (3). The kinetics is biphasic with an instantaneous contribution—i.e., <100 fsec time constant—and a 2.8-psec component. More precisely, the best fit corresponds to the sum of two species with different absorption coefficients; the first species appears instantaneously and populates in 2.8 psec the second one, whose lifetime is much larger than the time domain investigated.

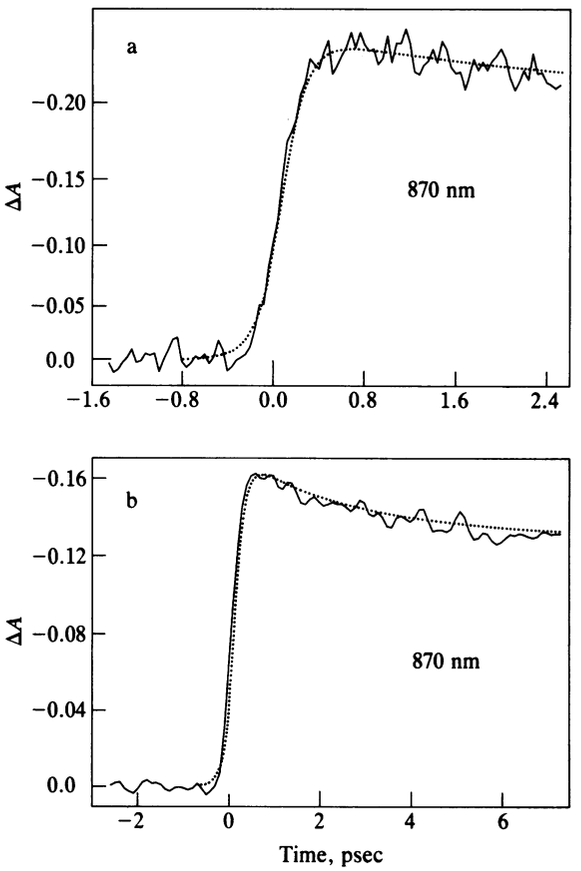

FIG. 1. (*a*) Kinetics of induced transmission at 870 nm after excitation of reaction centers of *R. sphaeroides* R-26 with a 150-fsec laser pulse at 850 nm. (*b*) A separate experiment, emphasizing the partial recovery due to the stimulated emission. The best fit (dotted line) corresponds to the sum of an instantaneous bleaching, which is stable on these time scales, and the stimulated emission, which relaxes in 2.8 psec. The ratio of the amplitudes of the former over the latter component is 4.5.

Fig. 3 gives the corresponding induced absorption at 675 nm, which has been attributed to the formation of H_A^- (5,

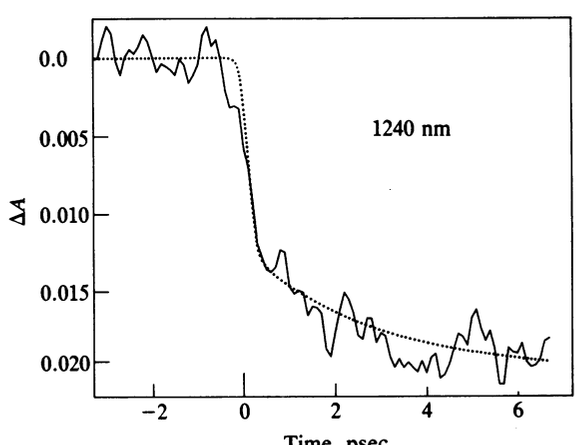

FIG. 2. Kinetics of induced absorption at 1240 nm. Conditions are the same as in Fig. 1*b*. The best fit (dotted line) corresponds to an absorption rising instantaneously and relaxing in 2.8 psec and an absorption increasing with a 2.8-psec time constant. The ratio of the amplitudes of the former over the latter component is 1.8.

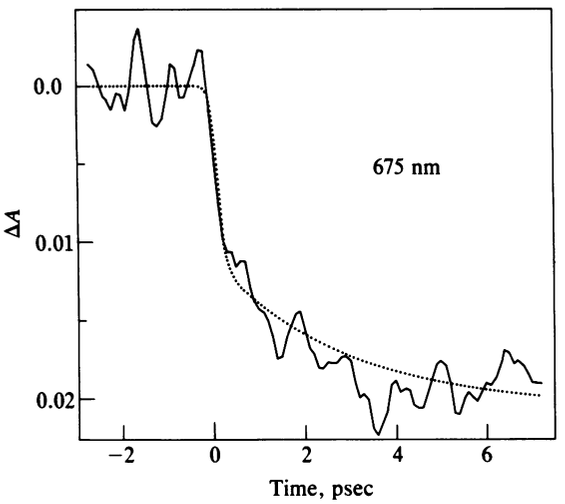

FIG. 3. Kinetics of induced absorption at 675 nm. Experimental conditions are the same as in Fig. 1*b*. The best fit (dotted line) corresponds to the one in Fig. 2 with a ratio of 2 for the amplitudes.

23, 24). Within our experimental uncertainty, this kinetics is identical to the one measured at 1240 nm. It reveals the presence of the same two components, an instantaneous one and a 2.8-psec one. The kinetics and amplitudes of the two components are identical within our experimental accuracy for parallel or perpendicular configuration of pump and probe beams.

The formation of H_A^- in the states $P^+H_A^-$ and PH_A^- is accompanied by a bleaching of the Q_Y and the Q_X bands of H_A at 760 and 545 nm, respectively. Fig. 4 shows the measurement at 545 nm after excitation at 850 nm. It reveals a biphasic response with an initial instantaneous absorption followed by a recovery phase that leads to an induced bleaching with a formation time of 2.8 psec. We found a similar result at 760 nm (not shown).

The measurement of the induced absorbance changes at 650 nm (Fig. 5) reveals a kinetics very close to the one observed at 675 nm (Fig. 3). The instantaneous initial increase in absorption is followed by a slower phase well-fitted by the 2.8-psec time constant for the rate of formation of H_A^-.

Fig. 6 shows that after an instantaneous absorbance increase, the blue shift of the 800-nm band occurring upon $P^+H_A^-$ formation (measured between 785 and 805 nm) appears with a 2.8-psec time constant. As is especially clear from the

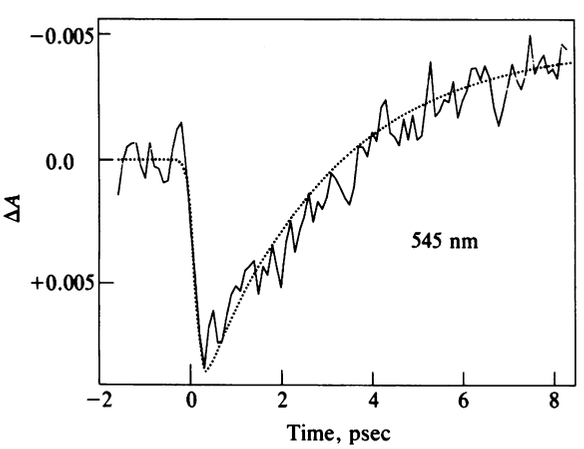

FIG. 4. Kinetics of absorption changes at 545 nm. Conditions are the same as in Fig. 1*b*. The best fit (dotted line) corresponds to an instantaneous absorption relaxing in 2.8 psec and a bleaching appearing in 2.8 psec. The ratio of the amplitudes of the two processes is 0.5.

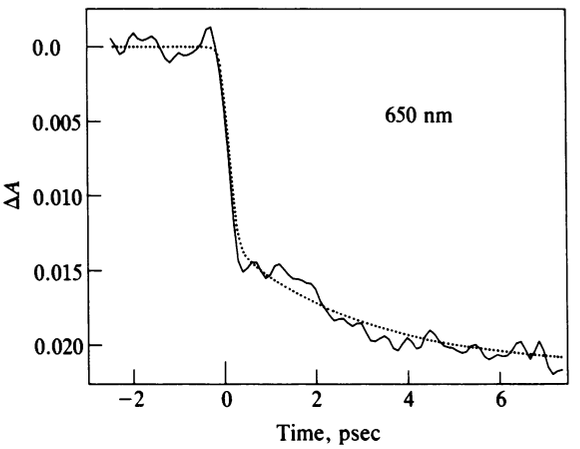

FIG. 5. Kinetics of induced absorption at 650 nm. Conditions are the same as in Fig. 1*b*. The best fit (dotted line) corresponds to the one in Fig. 2.

kinetics near the isosbestic point of the shift (Fig. 6 *b* and *c*), there is no evidence for the presence of a fast transient bleaching in the 100-fsec to 2-psec time domain.

The kinetics in the 920- to 950-nm region have negative signs—i.e., they appear to represent a bleaching (Fig. 7). However, no significant absorption is present at these wavelengths, especially above 930 nm. Consequently, the measured kinetics must be attributed to the presence of stimulated emission. The trace of the kinetics at 920 nm (Fig. 7*a*) has a negative asymptote at long times, which is due to the bleaching of the edge of the absorption band of P. At 930 nm (Fig. 7*e*) the asymptotic value is zero, whereas at 950 nm (Fig. 7*c*) the asymptote is positive, representing a long-lived induced absorption, which is attributed to P^+ (3). Apparently, 930 nm is the isosbestic point of the $P–P^+$ transition, in agreement with ref. 3. Taking into account the respective asymptotic values, the decay kinetics in Fig. 7 as well as in Fig. 1*b* are well fitted with a 2.8-psec time constant. The increase in the stimulated emission at 940 nm is displayed in Fig. 7*d*. The fits correspond to an instantaneous and to a 100-fsec rise time. The latter value, which is our instrumental time resolution, appears to represent an upper limit.

DISCUSSION

The $P^+H_A^-$ Species. The similarity of the kinetics of the absorbance changes at 1240 nm (Fig. 2) and 675 nm (Fig. 3), after exciting directly in the absorption band of the primary donor P, is strong evidence that P is oxidized simultaneously with the reduction of H_A with a time constant of 2.8 ± 0.2 psec. (This time constant of 2.8 psec corresponds to the value that gives the best fits for the kinetics observed at all the investigated wavelengths. The error bar of ±0.2 psec is estimated from the maximum and minimum values beyond which the fit is no longer satisfactory, at least at one wavelength.) This is confirmed by the 2.8-psec phase of the kinetics of the absorbance decrease at 545 and 760 nm after the instantaneous increase, and the same kinetics for the blue shift of the band as measured around 800 nm.

No Evidence for a $P^+H_A^-$ Transient. An important point is whether one of the accessory bacteriochlorophylls (B_A) is directly involved in electron transfer from P to H_A. Conflicting evidence has been presented in the literature (9–11). In the 650-nm region where the bacteriochlorophyll anion *in vitro* shows an absorption band (23), while the difference spectrum of P^+ minus P is practically zero, we found kinetics of induced absorption very close to the one observed at 675 nm, with no indication of the existence of an intermediate transient state in the time range larger than 100 fsec.

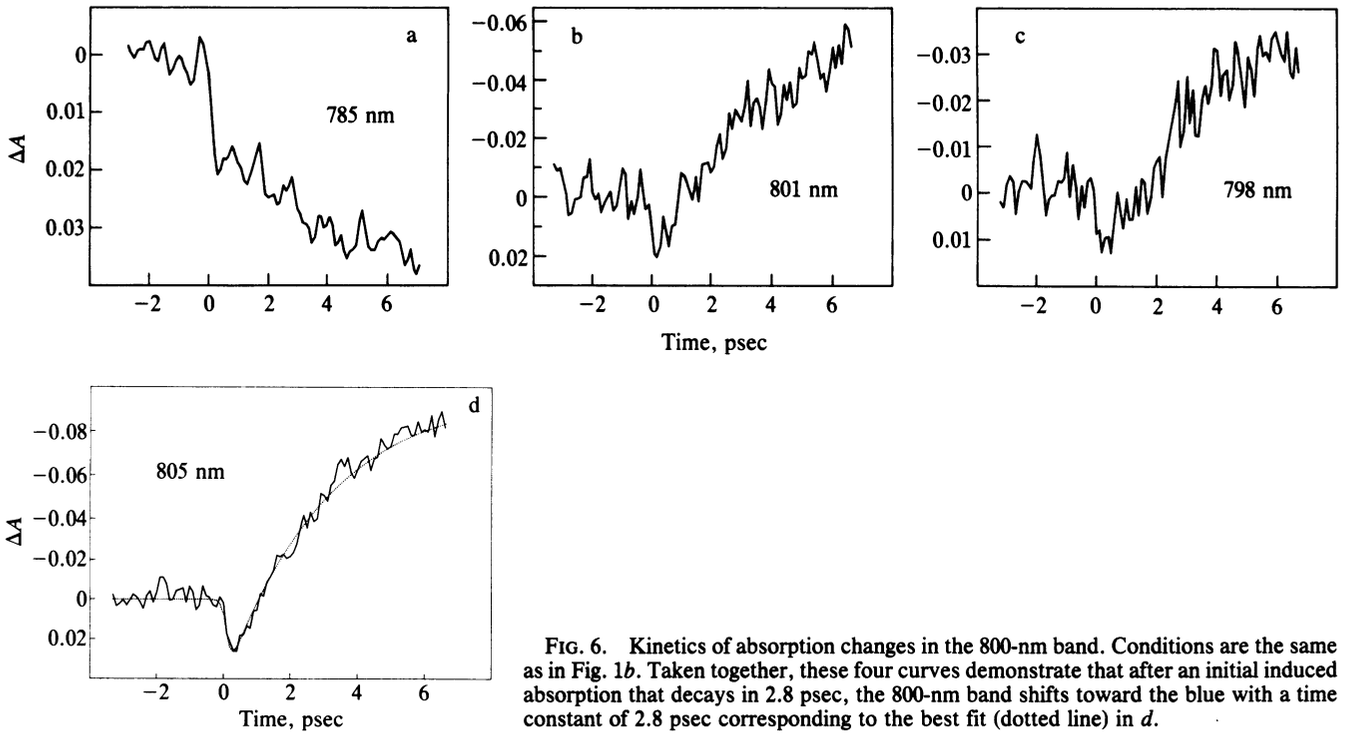

FIG. 6. Kinetics of absorption changes in the 800-nm band. Conditions are the same as in Fig. 1*b*. Taken together, these four curves demonstrate that after an initial induced absorption that decays in 2.8 psec, the 800-nm band shifts toward the blue with a time constant of 2.8 psec corresponding to the best fit (dotted line) in *d*.

However, the strongest evidence against the involvement of a transient $P^+B_A^-$ state comes from the measurements in the 785- to 805-nm spectral range. More precisely, the absence of significant bleaching in the 800-nm band, and especially near the isosbestic point observed for the blue shift around 798 nm, excludes the participation of B_A as an electron acceptor operating in the 100-fsec to 2.8-psec time range—i.e., between the appearance of the excited state of P and the reduction of H_A.

The above result contrasts the assertion of Shuvalov and Klevanik (9), based on a transient absorbance change at 798 nm measured with 30-psec pulses, that B_A is reduced in <1 psec and reoxidized in 7 psec. A recent reexamination by Kirmaier *et al.* (11) of the results of ref. 9 suggests that this discrepancy is caused by the data analysis procedure used by Shuvalov and Klevanik (9). Our work confirms the result of Borisov *et al.* (10) who, using a 4-psec pump and probe pulses, were unable to observe bleaching at 801 nm.

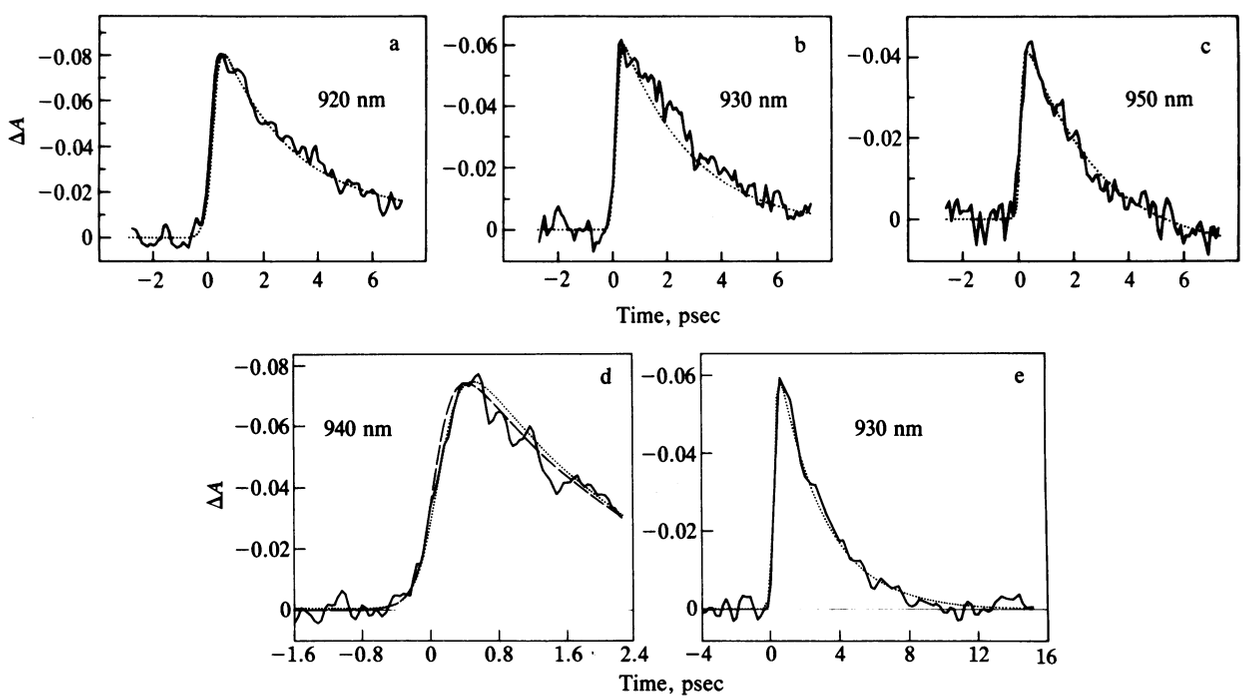

FIG. 7. Kinetics of stimulated emission from 920 to 950 nm. These curves taken in a region of initial low optical density demonstrate a net optical gain. All relaxations are best-fitted (dotted line) with a time constant of 2.8 psec, assuming some residual bleaching (*a*) or induced absorption (*c* and *d*) contribution. (*a*, *b*, *c*, and *e*) Curves were obtained under the same experimental conditions as in Fig. 1*b*. On curve *e*, taken on a 20-psec full scale, the relaxation phase is well-fitted with a single exponential decay of 2.8-psec time constant. Curve *d* was obtained under the experimental conditions used for the measurement in Fig. 1*a*: the fits correspond to an instantaneous (---) and a 100-fsec (·····) rise time.

The Precursor of $P^+H_A^-$ and the Stimulated Emission. At practically all wavelengths investigated, an instantaneous increase in absorbance was observed. An early increase was also seen by Holten *et al.* (5) in the wavelength region 480–570 and 650–680 nm, using 0.7-psec pulses at 610 nm. The observed increase is likely due to absorbance of an excited state, which is either the excited singlet state of P, denoted $^1P^*$, or an early charge transfer state $P^\pm$ within the dimer making up P, or a mixture of the two. It is this excited state (called P* for simplification) that apparently gives rise to the stimulated emission observed at 870 nm and beyond, a phenomenon that was also observed by Parson *et al.* (25). The peak of this emission is around 910 nm with a cross-section value of 2.8×10^{-16} cm^2. This relatively high emission cross-section does not mean that the radiative transition is a major pathway of relaxation of P*. In fact, the picture that emerges from these results is that the 2.8-psec lifetime of the stimulated emission corresponds to the rate of disappearance of the P* state into $P^+H_A^-$ as revealed by the similar time constant of 2.8 psec found at 1240 nm and 675 nm as well as for the blue shift of the band at 800 nm. As the "natural" radiative transition to the ground state is in the nanosecond time region, radiative decay accounts for $\approx 10^{-3}$ of the energy stored in P*. This is consistent with the known high quantum yield of energy conversion in reaction centers.

From the rise time of the stimulated emission at 940 nm (Fig. 7*d*), which is at the limit of our time resolution (the best fit gives 100 fsec), we conclude that if there is a precursor to the state responsible for both the stimulated emission and $P^+H_A^-$ formation, its lifetime should be <100 fsec. Such a short lifetime may correspond to vibrational relaxation within the $^1P^*$ singlet state.

The spectrum of the resolved excited state (Fig. 8), which in principle might include charge transfer states such as $P^\pm$ or $P^+B_A^-$, has been computed following a procedure to be described elsewhere (unpublished results). This broad-band spectrum, extending up to 1240 nm, is consistent with an assignment to either $^1P^*$ or to a mixture of $^1P^*$ and $P^\pm$ states. The rather flat character of the spectrum of Fig. 8 in the 800-nm region argues against a significant contribution of a $P^+B_A^-$ state composed of weakly interacting P^+ and B_A^- species. Note that for a strongly interacting species $P^+B_A^-$ or for a large contribution of $^1B_A^*$ to P*, one would expect a strong bleaching at 800 nm, contrary to observation. Hence, although we cannot exclude that B_A participates to a small extent in P*, we can rule out a role as transient electron acceptor. The presence of B_A might serve to facilitate electron tunneling from P* to H_A by lowering the energy barrier, as suggested by Parson *et al.* (25).

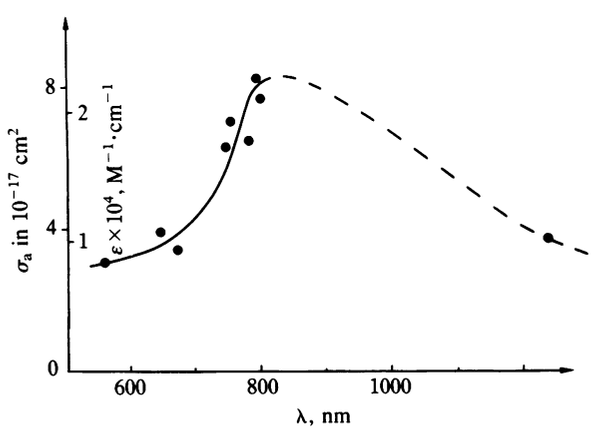

FIG. 8. Molecular extinction coefficient and absorption cross-section of the excited species P*, which decays with a 2.8-psec time constant. The dots are the experimental values (see text).

In conclusion, by using femtosecond laser spectroscopy with a time resolution of 100 fsec and exciting directly in the main absorption band of P, we have determined that the charge separated state $P^+H_A^-$ is formed in 2.8 ± 0.2 psec. This state results either directly from the excited singlet state of P, or from an initial excited charge transfer state $P^\pm$ within the primary donor that presumably is strongly mixed with the excited singlet state of P. The initial excited state gives rise to a stimulated emission, whose lifetime is identical to the characteristic time of electron transfer to H_A. We have found no direct evidence that an accessory bacteriochlorophyll is involved as a transient electron acceptor, as suggested by Shuvalov and Klevanik (9). Our data are essentially consistent with the results of Holten *et al.* (5), of Parson *et al.* (25), and the interpretation of Kirmaier *et al.* (11). We believe that from the spectral and kinetic properties of the initial excited state we can exclude a contribution of B_A^- to this state.

This paper is dedicated to the memory of Dr. E. Roux. We acknowledge financial support from Institut National de la Santé et de la Recherche Medicale and Ecole Nationale Supérieure de Techniques Avancées.

1. Kaufmann, K. J., Dutton, P. L., Netzel, T. L., Leigh, J. S. & Rentzepis, P. M. (1975) *Science* **188**, 1301–1304.
2. Rockley, M. G., Windsor, M. W., Cogdell, R. J. & Parson, W. W. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 2251–2255.
3. Dutton, P. L., Kaufmann, K. J., Chance, B. & Rentzepis, P. M. (1975) *FEBS Lett.* **60**, 275–280.
4. Moskowitz, E. & Malley, M. M. (1978) *Photochem. Photobiol.* **27**, 55–59.
5. Holten, D., Hoganson, C., Windsor, M. W., Schenck, C. C., Parson, W. W., Migus, A., Fork, R. L. & Shank, C. V. (1980) *Biochim. Biophys. Acta* **592**, 461–477.
6. Shuvalov, V. A., Klevanik, A. V., Sharkov, A. V., Matveetz, Y. A. & Kryukov, P. G. (1978) *FEBS Lett.* **91**, 135–139.
7. Akhmanov, S. A., Borisov, A. Y., Danielius, R. V., Kozlovskij, V. S., Piskarskas, A. S. & Razjivin, A. P. (1978) in *Picosecond Phenomena*, eds. Shank, C. V., Ippen, E. P. & Shapiro, S. L. (Springer, Berlin), pp. 134–139.
8. Klevanik, A. V., Kryukov, P. G., Semchishen, V. A. & Shuvalov, V. A. (1981) *Dokl. Akad. Nauk SSSR* **256**, 483–486.
9. Shuvalov, V. A. & Klevanik, V. A. (1983) *FEBS Lett.* **160**, 51–55.
10. Borisov, A. Y., Danielius, R. V., Kudzmauskas, S. P., Piskarskas, A. S., Razjivin, A. P., Sirutkaitis, V. A. & Valkunas, L. L. (1983) *Photobiochem. Photobiophys.* **6**, 33–38.
11. Kirmaier, C., Holten, D. & Parson, W. W. (1985) *FEBS Lett.* **185**, 76–82.
12. Migus, A., Martin, J.-L., Astier, R., Antonetti, A. & Orszag, A. (1982) in *Picosecond Phenomena*, eds. Eisenthal, K. B., Hochstrasser, R. M., Kaiser, W. & Laubereau, A. (Springer, Berlin), pp. 6–9.
13. Martin, J.-L., Migus, A., Poyart, C., Lecarpentier, Y., Astier, R. & Antonetti, A. (1983) *EMBO J.* **2**, 1815–1819.
14. Feher, G. & Okamura, M. Y. (1978) in *The Photosynthetic Bacteria*, eds. Clayton, R. K. & Sistrom, W. R. (Plenum, New York), pp. 349–386.
15. Norris, J. R., Druyan, M. E. & Katz, J. J. (1973) *J. Am. Chem. Soc.* **95**, 1680–1682.
16. Feher, G., Hoff, A. J., McElroy, J. D. & Isaacson, R. A. (1973) *Biophys. J.* **13**, 61 (abstr.).
17. Breton, J. & Vermeglio, A. (1982) in *Photosynthesis: Energy Conversion by Plants and Bacteria*, ed. Govindjee (Academic, New York), Vol. 1, pp. 153–194.
18. Deisenhofer, J., Epp, O., Miki, K., Huber, R. & Michel, H. (1984) *J. Mol. Biol.* **180**, 385–398.
19. Parson, W. W. (1982) *Annu. Rev. Biophys. Bioeng.* **11**, 57–80.
20. Kryukov, P. G., Letokhov, V. S., Matveetz, Y. A., Nikogossian, D. N. & Sharkov, A. V. (1978) in *Picosecond Phenomena*, eds. Shank, C. V., Ippen, E. P. & Shapiro, S. L. (Springer, Berlin), pp. 158–166.
21. Fork, R. L., Shank, C. V. & Yen, R. T. (1982) *Appl. Phys. Lett.* **41**, 223–225.
22. Martin, J.-L., Migus, A., Poyart, C., Lecarpentier, Y., Astier, R. & Antonetti, A. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 173–177.
23. Fajer, J., Brune, D. C., Davis, M. S., Forman, A. & Spaulding, L. D. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4956–4960.
24. Shuvalov, V. A. & Klimov, V. V. (1976) *Biochim. Biophys. Acta* **440**, 587–599.
25. Parson, W. W., Woodbury, N. W. T., Becker, M., Kirmaier, C. & Holten, D. (1985) in *Antennas and Reaction Centers of Photosynthetic Bacteria: Structures, Interactions and Dynamics*, ed. Michel-Beyerle, M. E. (Springer, Berlin), in press.