Volume 239, number 2, 211–218 FEB 06447 November 1988

Subpicosecond characterization of the optical properties of the primary electron donor and the mechanism of the initial electron transfer in *Rhodobacter capsulatus* reaction centers

Christine Kirmaier and Dewey Holten

Department of Chemistry, Washington University, St. Louis, MO 63130, USA

Received 1 September 1988

The optical difference spectrum of the excited primary electron donor, P*, in *Rb. capsulatus* reaction centers has been measured 600 fs after a 350-fs flash at 870 nm. The spectrum is characterized by bleaching in the ground state absorption bands at 855 and 600 nm, and a weak featureless transient absorption in between. The lack of significant (if any) bleaching at 800 nm indicates that P does not contribute appreciable oscillator strength to the 800-nm ground state absorption band. The conversion of P* to $P^+BPh_L^-$ is accompanied by isosbestic points in the transient difference spectra at 765 and 798 nm. The existence of the longer-wavelength isosbestic point, occurring essentially at the near-infrared absorbance maximum of the accessory BChls, provides compelling evidence that if state $P^*BChl_L^-$ forms, its transient concentration is exceedingly small. At certain wavelengths in the near-infrared the absorption changes develop somewhat more slowly than the rate at which P* decays, a finding that may reflect a contribution from readjustments in the pigment-protein complex in response to electron transfer.

Reaction center; Electron transfer; Photosynthesis; Femtosecond spectroscopy

1. INTRODUCTION

The primary electron donor in bacterial photosynthetic reaction centers (RCs) is a dimer of bacteriochlorophyll (BChl) molecules, usually referred to as P. The photoexcited dimer (P*) disappears with a time constant of 3–4 ps following excitation, with the concomitant reduction of BPh_L, the bacteriopheophytin molecule associated with the L polypeptide. In a slower step requiring about 200 ps an electron from BPh_L^- is transferred to the primary quinone (Q_A), achieving charge separation in the form of state $P^+Q_A^-$ with a unity quantum yield (see [1] for a recent review).

Although the overall scheme of charge separation is fairly clear, there are many unresolved issues concerning this process and the fundamental electronic and optical properties of the RC. A key issue is whether electronic coupling among the six chromophores is sufficiently strong so as to be a principal determinant of the ground state and transient state spectra. The role of the accessory pigment $BChl_L$ in the initial photochemistry also is somewhat controversial; arguments both for [2,3] and against [4–8] $P^+BChl_L^-$ being a resolved intermediate state between P* and $P^+BPh_L^-$ have been presented from recent subpicosecond measurements. In order to address these issues, we have examined the initial electron transfer reaction in *Rb. capsulatus* RCs, using 350-fs 870-nm excitation flashes. The results yield important new information concerning the optical properties of P and the mechanism of charge separation.

Correspondence address: D. Holten, Department of Chemistry, Washington University, St. Louis, MO 63130, USA

2. EXPERIMENTAL

Rb. capsulatus wild-type strain U43 (pU2922) was provided by Dr D. Youvan. Cells were grown non-photosynthetically and RCs isolated as described elsewhere [9]. RCs in 10 mM

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

potassium phosphate buffer (pH 7.4)/0.05% LDAO were maintained at 10°C and flowed through a 2 or 3 mm pathlength optical cell during experiments. Transient absorption spectroscopy using 350-fs 870-nm excitation flashes, broad-band probe flashes, and dual-beam detection was performed as described elsewhere [10]. The 870-nm excitation flashes were polarized at 45° with respect to the probe flashes to avoid dichroism of the absorption changes, and were attenuated to produce ~30% bleaching in the 855-nm band of P.

3. RESULTS

Fig.1 shows absorption difference spectra measured 600 fs (solid) and 16.8 ps (dashed) after excitation of *Rb. capsulatus* RCs with a 350-fs flash at 870 nm. The 600-fs spectrum, which we assign to P*, contains bleaching of the 855-nm band of P, a weak featureless transient absorption between 810 and 610 nm, and bleaching of the 600-nm Q_X band of the dimer. A flat absorption across the visible and near-infrared, broken only by bleaching of the ground state absorption bands, is characteristic of the difference spectra of the $^1(\pi,\pi^*)$ and $^3(\pi,\pi^*)$ excited states of BChl and BPh in vitro [11,12], and of the excited triplet state of P [13]. Although the 600-fs spectrum certainly is consistent with it being due to a $^1(\pi,\pi^*)$ excited state of P, it cannot be deduced from this spectrum alone whether P* is a pure neutral exciton state or if in addition it contains some intra-dimer charge transfer character.

P* decays with a time constant of 3.4 ± 0.5 ps, as monitored by disappearance of the stimulated emission to the red of the 855-nm P-band bleaching [10,14]. Concomitant with this decay is the development of bleaching in the Q_X and Q_Y bands of BPh_L near 542 and 755 nm, respectively, resulting from $P^+BPh_L^-$ formation. The 16.8-ps $P^+BPh_L^-$ spectrum is further characterized by the broad BPh_L anion band centered near 665 nm, whose peak intensity is about 35% greater than the amplitude of the flat absorption due to P* present at earlier times (fig.1). The formation of $P^+BPh_L^-$ also is accompanied by an absorption decrease near 815 nm and an increase near 785 nm that together have the appearance of a band shift towards the blue. However, as discussed further below, the absorption changes in the 775- to 825-nm region are complex. As an example of this, we have found in this region that the absorption changes do not develop uniformly at all

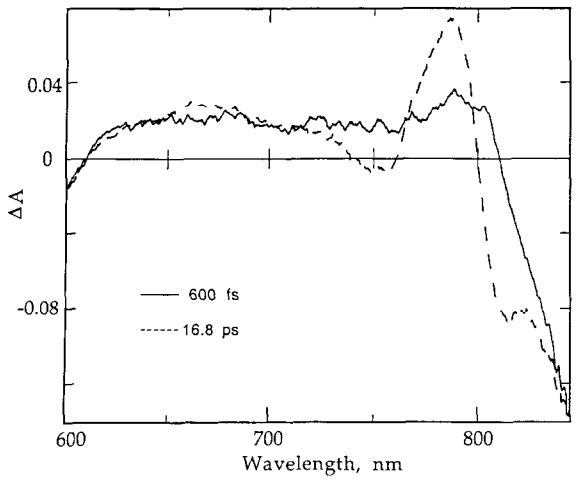

Fig.1. Absorption difference spectra measured 600 fs (solid) and 16.8 ps (dashed) after excitation of *Rb. capsulatus* RCs with a 350-fs 870-nm flash. Each spectrum represents the average of data acquired using ~1500 excitation flashes, and the absorption changes typically have a standard deviation in ΔA of ± 0.005.

wavelengths with the same rate at which P* decays. A time constant of 3.6 ± 0.5 ps is measured from 803 to 823 nm (in the absorption decrease), while a somewhat longer time constant of 7.6 ± 1.3 ps is

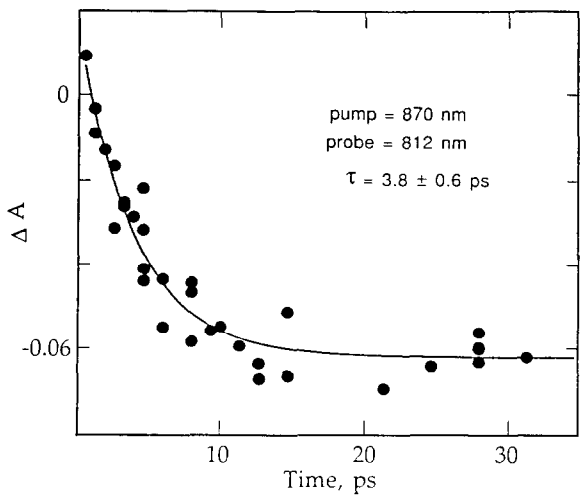

Fig.2. Kinetics for development of the absorption decrease centered near 810 nm, produced by excitation with an 870-nm flash. The solid curve is a fit of the kinetics of the absorption changes averaged between 808 and 816 nm to a constant plus an exponential, giving a 3.8 ± 0.6 ps time constant. Several measurements throughout the 803–823 nm region yield an average time constant of 3.6 ± 0.5 ps.

found from 782 to 790 nm, the peak of the absorption increase. Typical results are shown in figs 2 and 3. These same results are obtained when weak 582-nm excitation flashes are employed (not shown).

Fig.4 shows a closer examination of how the near-infrared absorption changes evolve as P* gives way to $P^+BPh_L^-$. The series of spectra spanning 600 fs to 16.8 ps (including numerous additional spectra taken during this interval) have the

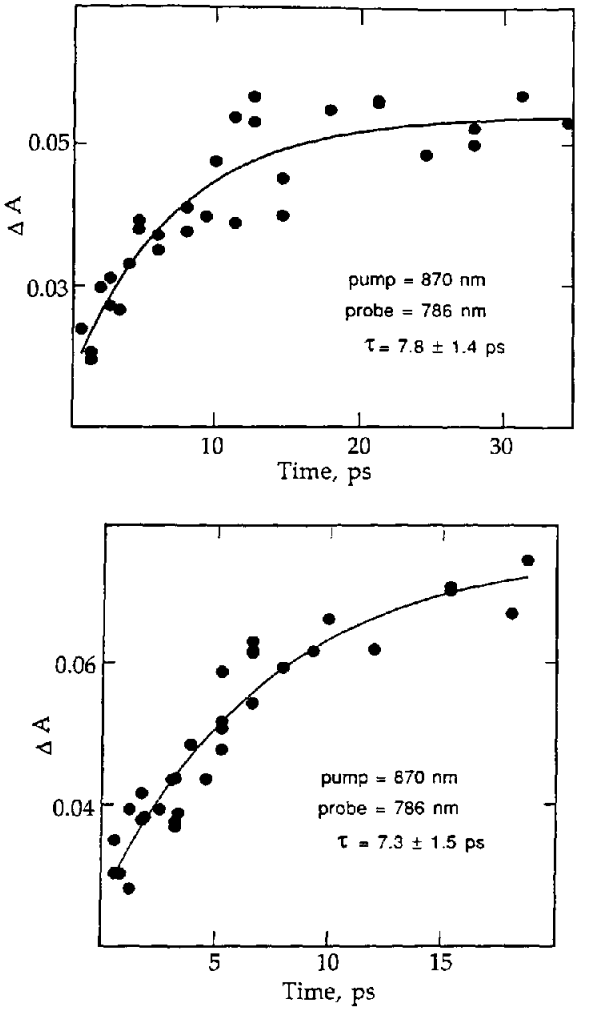

Fig.3. Kinetics of the absorption changes averaged between 782 and 790 nm produced by excitation with an 870-nm flash. The two panels show results from experiments on two different RC preparations. Data in each case are fitted to a constant plus an exponential (solid curves), giving the time constants shown. Several measurements in this region (the peak of the absorption increase) give an average time constant of 7.6 ± 1.3 ps.

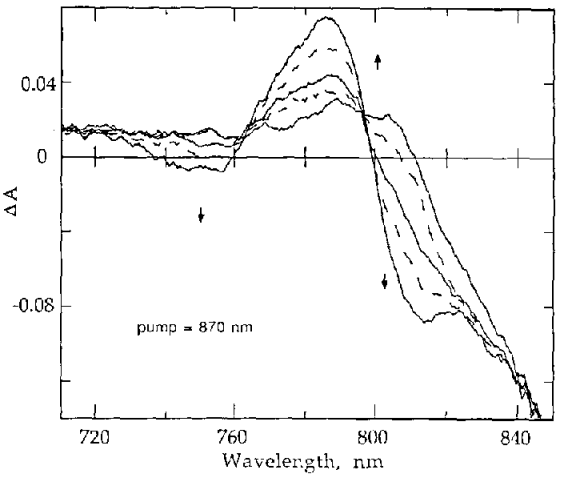

Fig.4. Time evolution of the near-infrared absorption changes accompanying conversion of P* to $P^+BPh_L^-$ produced by a 350-fs 870-nm excitation flash. The spectra were measured after 600 fs (solid), 1.6 ps (dashed), 3.2 ps (solid), 6.0 ps (dashed), and 16.8 ps (solid). The arrows mark how the absorption changes vary with increasing time. Other conditions as in fig.1.

same ΔA values at both 765 and 798 nm. These isosbestic points would be consistent with the buildup of $P^+BChl_L^-$ only if this state has the same differential extinction coefficients as P* and $P^+BPh_L^-$ at 765 and 798 nm. In view of the fact that 798 nm is nearly the peak of the strong Q_Y absorption of the accessory BChls, and this ground state band should bleach if $BChl_L$ is reduced, the concentration of $P^+BChl_L^-$ present throughout the time P* decays and $P^+BPh_L^-$ forms must be exceedingly small.

4. DISCUSSION

4.1. *Absorption spectrum of P*

The P* (600-fs) absorption difference spectrum of fig.1 provides insights into the contribution of P to the ground state absorption spectrum of the RC. This is so because P*, like the (π,π^*) excited states of BChl and BPh in vitro, provides an essentially flat transient absorption that serves as a baseline (albeit non-zero) for bleachings of the ground state absorption bands. Clearly seen in the 600 fs spectrum in fig.1 is bleaching of the 855-nm band of P, and of its weaker Q_X band near 600 nm. Obviously absent is any significant loss of

absorbance near 800 nm. If P were to contribute to the strong 800-nm ground state absorption band because of electronic coupling with the two accessory BChls, as suggested from several of the recent simulations of the absorption spectrum of *Rb. sphaeroides* RCs [15,16] (and of the related *Rps. viridis* RCs [17,18]), then one would expect that removal of P from the ground state would cause a bleaching near 800 nm in proportion to the amount of oscillator strength derived from the dimer. In the 600-fs spectrum there is only a hint of a dip at 800 nm in the otherwise featureless absorption increase. If one takes the entire dip to represent a true bleaching of P, then the loss of absorbance at 800 nm is at most 1/25 the amplitude of the bleaching at 855 nm. This indicates that P does not contribute significantly to the ground state absorption at 800 nm via electronic coupling between the dimer and the monomeric BChls. This point of view is supported by work on *Rb. sphaeroides* and *Rps. viridis*, including other recent spectral simulations [19,20], linear dichroism measurements [21] and the effects of temperature [22] and $NaBH_4$ treatment [23] on the ground state absorption spectrum.

Other interpretations for the small dip at 800 nm (if not simply noise) are possible. It could be due to the higher-energy exciton component of P, which is not expected to carry much oscillator strength because of the geometry of the dimer, assuming P in *Rb. capsulatus* is arranged as is P in *Rps. viridis* and *Rb. sphaeroides* [24–26]. (Alternatively, bleaching of this component could be buried under the blue edge of the 855-nm band bleaching.) Another possibility for the feature is the presence of some reduced $BChl_L$, either in the form of state $P^+BChl_L^-$ or a $P^+BChl_L^-$ charge transfer contribution to P*. Or, the small dip could simply represent the beginning of the absorption changes due to the formation of $P^+BPh_L^-$. A contribution from one or more of these other sources further limits the extent to which P, via electronic coupling with the BChls, contributes to the small dip at 800 nm in the 600-fs spectrum, and thus to the 800-nm ground state absorption band.

4.2. *Mechanism of the initial electron transfer reaction*

Our observation that conversion of P* to $P^+BPh_L^-$ is accompanied by isosbestic points at 765 and 798 nm severely constrains the amount of P^+BChl^- that forms, if this state forms at all. Previous arguments against the transient reduction of BChl in *Rb. sphaeroides* and *Rps. viridis* RCs have been based largely on the finding of identical kinetics for the decay of P* and the formation of $P^+BPh_L^-$ [4–8]. Our spectral data provide a new measure of the extent to which the conversion of P* to $P^+BPh_L^-$ may involve an intermediate state. Our results would allow a significant population of $P^+BChl_L^-$ during the lifetime of P* only if $P^+BChl_L^-$ fortuitously has exactly the same $\Delta\epsilon$ as P* and $P^+BPh_L^-$ at 765 and 798 nm. That this should be so is particularly unlikely at 798 nm, since $BChl_L$ has its main absorption band near 800 nm, and this band necessarily would bleach if $BChl_L$ is reduced. (It is important to note that we observe isosbestic behavior, and the same kinetics of the electron transfer reactions, when weak 582-nm excitation is employed [10].)

Our combined spectral and kinetic data restrict the participation of $BChl_L$ in the initial photoact to a role that requires no more than an extremely low concentration of the reduced pigment to be present at any time. A number of mechanisms have been proposed for the initial stage of charge separation [27–34]. Perhaps the simplest mechanism is a two-step process involving electron transfer from P* to $BChl_L$ followed by electron transfer from $BChl_L^-$ to BPh_L, with the second step much faster than the first (i.e. $k_2 \gg k_1$ in eqn 1).

$$P^* \xrightarrow{k_1} P^+BChl_L^- \xrightarrow{k_2} P^+BPh_L^- \qquad (1)$$

It has been estimated recently from analysis of the absorption changes and kinetics in the vicinity of 833 nm in *Rps. viridis* at low temperature that, with regard to the two-step model, $k_2 \sim 70\ k_1$ [35]. To calculate this ratio, values for a number of spectral parameters of the three states in eqn 1 had to be assumed. Our observation of isosbestic points in the transient spectra as P* converts to $P^+BPh_L^-$ provides for a more straightforward quantitative evaluation of the relative magnitudes of the two rates in the two-step model. This derives, again, from the fact that one of the isosbestic points is at 798 nm (fig.4), essentially at the peak of the $BChl_L$ ground state absorption. Hence, the maximum concentration of $P^+BChl_L^-$ that our data will tolerate during the entire time for

conversion of P* to $P^+BPh_L^-$ is related simply to our ability to determine the 798-nm isosbestic point. Thirty-two spectra measured between 600 fs and 16.8 ps give an average ΔA at 798 nm of 0.025 $\pm$ 0.005. The error in this measurement, i.e. ΔA = 0.005, reflects the limitations of detectability of $BChl_L^-$ in this experiment. Another measurement of the maximum extent of $BChl_L$ reduction is provided by the possible bleaching at 800 nm. The small dip at 800 nm in the 600-fs spectrum (fig.1) has $\Delta A = -0.006$ (referenced to the positive background absorption). One can construct an argument giving the maximum concentration of $P^+BChl_L^-$ on the basis of either the error in the isosbestic point, or the magnitude of the potential bleaching near 800 nm. Since essentially the same value is obtained from either assay, we take -0.006 with confidence as the maximum potential bleaching of $BChl_L$ contributing to the transient spectra at 798 nm.

The ratio of the maximum concentration of $P^+BChl_L^-$ to the initial concentration of P* is given by

$$[P^+BChl_L^-]_{max}/[P^*]_0 = X^{1/(1-X)} \quad (2)$$

where X is the rate ratio k_1/k_2 [29]. The initial concentration of P* is proportional to the magnitude of the bleaching in the 855-nm band in the 600-fs spectrum, which is $\Delta A = -0.15$ (figs 1 and 4). To use eqn 2 we must additionally convert the $\Delta A = -0.006$ maximum $BChl_L$ bleaching to a concentration of $P^+BChl_L^-$ relative to $[P^*]_0$. In view of our discussion of the P* spectrum given above, we assume that about 50% of the 800-nm ground state absorption band is derived from $BChl_L$. Since the ratio of the 800- and 855-nm ground state bands is approximately 2/1, this means that $BChl_L$ and P have roughly the same near-infrared peak extinction coefficient. Thus, an upper limit to the left side of eqn 2 is given simply by the ratio of the maximum bleaching at 800 nm to the bleaching at 855 nm, or $(-0.006)/(-0.15) = 0.040$. This means that $X < 0.047$, or that $k_2 > 21\ k_1$. Based on this result, it is easy to show that $P^+BChl_L^-$ should reach its maximum concentration (<4% that of $[P^*]_0$) prior to 520 fs and should decay essentially with the 3.4-ps lifetime of P*.

The finding of at least a twenty-fold difference between the two rates provides essential information needed for the further evaluation of the two-step model. In particular, the results imply a very large difference in the electronic and/or Franck-Condon factors for the two steps in eqn 1. A number of calculations [28,29,32] have indicated that the second step in eqn 1 would have an electronic factor about 3 times that of the first. Assuming that the Franck-Condon factors for the two steps are roughly the same (and recalling that the rate of electron transfer is proportional to the square of the electronic factor), one estimates that $k_2 \sim 9\ k_1$, and that P^+BChl^- should reach a concentration of about 0.1 $[P^*]_0$. This would translate into a ΔA of about -0.015 under the experimental conditions of figs 1 and 4. A bleaching of this size would have been observable in our data. Another calculation gives an electronic factor ratio of about 6 [33], implying that k_2 could be 36 times greater than k_1. In this case $P^+BChl_L^-$ would remain at a low enough concentration that we would not have detected its presence. Thus, this analysis of our results implies either that the two-step model is not a viable mechanism, or that the larger ratio of electronic factors is more correct. (Similarly, although we have not considered it explicitly, the model involving the transient formation of $BChl_L^+BPh_L^-$ [28,31] is subject to restrictions at least as severe as those our experiments have placed on the formation of $P^+BChl_L^-$.)

Additional considerations also imply that the two-step model may not be wholly appropriate. If $k_2 > 21\ k_1$ then the rate of the second step must be exceedingly fast: $k_2 > (160\ fs)^{-1}$, using $k_1 = (3.4\ ps)^{-1}$. Therefore, our results, and those obtained recently for *Rps. viridis* at low temperature [35], indicate that electron transfer from $BChl_L^-$ to BPh_L probably would have to compete with vibrational equilibration of $P^+BChl_L^-$. If so, then $P^+BChl_L^-$ probably should not be treated as a true chemical intermediate. Perhaps more appropriate in this regard, then, is the model in which electron transfer from P* to $BChl_L$ is nonadiabatic, while electron transfer from $BChl_L^-$ to BPh_L is adiabatic [30]. $P^+BChl_L^-$ would not be considered a thermally-equilibrated intermediate, and the $BChl_L$ ground-state depletion would not be sufficient for its observation. Like the two-step model, this mechanism requires that the electronic coupling between $BChl_L$ and BPh_L be much larger than that between $BChl_L$ and P. Whether the actual difference in the couplings is large enough for

adiabatic/nonadiabatic mechanism to be viable is not yet clear.

In the superexchange model, $P^+BChl_L^-$ lies above P* in energy, but couples P* and $P^+BPh_L^-$ [27,33,34]. There is some debate as to whether the superexchange electronic couplings are large enough to account for the rapid (~3 ps) time constant for electron transfer [27–34]. The superexchange model is certainly compatible with our results, since it does not require the formation of $P^+BChl_L^-$ as an intermediate state. This model has received support from recent measurements of the Stark effect on the fluorescence from *Rb. sphaeroides* RCs [36]. Superexchange is clearly consistent with the now seemingly overwhelming experimental evidence that if $BChl_L$ participates in the initial stage of charge separation, it must do so via a mechanism that tolerates the absence of a significant transient concentration of the reduced chromophore.

An additional finding that may have important mechanistic implications is that the kinetics observed in conjunction with the initial photochemistry in *Rb. capsulatus* do not appear to be uniformly the same at all wavelengths. We have measured a time constant of about 3.5 ps for decay of P* stimulated emission (860–920 nm) [10,14], for development of bleaching of the 755-nm BPh_L Q_Y band [10], and for appearance of the 810-nm absorption decrease (fig.2). However, we measure a somewhat longer time constant of 7–8 ps at the peak of the 785-nm absorption increase (fig.3). This result was reproducibly obtained in six different measurements on *Rb. capsulatus* RCs. We also have found the same phenomena manifest in *Rb. sphaeroides* RCs (a time constant of 3 ps for both decay of stimulated emission and for development of the 810-nm bleaching, but a value of 5–6 ps at the peak of the 785-nm absorption increase: Kirmaier, C. and Holten, D., unpublished).

The absorption changes between 775 and 825 nm are quite complex and resistant to simple interpretations. It is well known that this region of the transient state spectra in *Rb. sphaeroides*, for example, cannot be ascribed solely to a simple blue shift of a single pigment's absorption band [37–41]. Our data are consistent with this view, because we measure different kinetics at the extremes (i.e. the top and the bottom) of the apparent blue shift. There are certainly a number of ways in which our data on *Rb. capsulatus* can be visualized. Perhaps the simplest is the development of a blue shift with a 3.5 ps time constant, together with a slower component whose predominant contribution is near the peak of the 785 nm absorption increase. If the lifetime of the second component is not substantially longer than 3.5 ps (e.g. 10–15 ps), then the two components would not be easily separable and single exponential kinetics with an intermediate lifetime would be observed at wavelengths where they both contribute. Since the isosbestic behavior is maintained during the 3.5-ps lifetime of P*, the slow component either does not contribute to the absorption changes at 765 and 798 nm, or its contribution in the vicinity of these isosbestic points (particularly at early times) is too small to be manifest in the spectra. In this regard, it is important to note that the slower (~7.5 ps) time constant is measured near 786 nm, which is more than 10 nm removed from the 798 nm isosbestic point, and some 20 nm from the one at 765 nm.

A blue shift of the 800-nm BChl absorption with a 3.5-ps time constant undoubtedly reflects in part the changing charge distribution that $BChl_L$ experiences in its surroundings as an electron moves from P* to BPh_L. The slower phase contributing predominantly only to the absorption increase might involve, for example, a further alteration in the shifted absorption due to subsequent readjustment of the protein (e.g. breaking/forming hydrogen bonds), or readjustments of the pigments themselves (e.g. ring puckering or flattening). The absolute rate of this additional contribution may not be limited solely by the rate of charge separation, but by slow, low-frequency motions of the protein. One must also consider that the Q_Y absorption band of $BChl_M$ probably also blue shifts in response to the oxidation of P, and that P^+ itself may contribute to the absorption increase near 785 nm [37–41]. The situation becomes even more complex if there is a distribution of pigment-protein conformations prior to excitation which differ in their spectra and kinetics.

The finding that the kinetics which otherwise would be associated with a simple electron transfer process in the RC are not uniformly the same at all wavelengths is not without precedent. In reaction centers from several bacterial species we have

previously observed a detection-wavelength dependence of the ~200 ps kinetics associated with the $P^+BPh_L^- \longrightarrow P^+Q_A^-$ reaction [1,39,42,43]. The two possible contributions that we have considered to explain these earlier observations, and those of the present study, are supported by the results of other measurements. Namely, there may be structural changes in response to the new charge distribution resulting from electron transfer [44–46], and/or that there may be a distribution of protein conformations [47,48]. Both concepts are physically reasonable, and both may be manifest in various ways in the observed photochemistry. Which, if either, of these possibilities is responsible for our observations is as yet not clear. Low temperature measurements on RCs from several organisms are underway to help determine whether the time-evolution of the absorption changes at some wavelengths may be associated in part with dynamic processes in the pigment-protein complex that are triggered by electron transfer.

Acknowledgements: We thank Drs D. Youvan and E. Bylina for advice on the preparation of *Rb. capsulatus* RCs, L. McDowell for assistance with the RC preparation, and Drs R.M. Marcus and J. Rodriguez for helpful discussions. This work was supported by grant DMB 86-04693 from the National Science Foundation.

REFERENCES

[1] Kirmaier, C. and Holten, D. (1987) Photosynthesis Res. 13, 225–260.
[2] Chekalin, S.V., Matveets, Ya.A., Shuropatov, A.Ya., Shuvalov, V.A. and Yartzev, A.P. (1987) FEBS Lett. 216, 245–248.
[3] Chekalin, S.V., Matveets, Yu.A. and Yartsev, A.P. (1987) Rev. Phys. Appl. 22, 1761–1771.
[4] Woodbury, N.W., Becker, M., Middendorf, D. and Parson, W.W. (1985) Biochemistry 24, 7516–7521.
[5] Martin, J.-L., Breton, J., Hoff, A.J., Migus, A. and Antonetti, A. (1986) Proc. Natl. Acad. Sci. USA 83, 957–961.
[6] Breton, J., Martin, J.-L., Migus, A., Antonetti, A. and Orszag, A. (1986) Proc. Natl. Acad. Sci. USA 83, 5121–5125.
[7] Wasielewski, M.R. and Tiede, D.M. (1986) FEBS Lett. 204, 368–372.
[8] Breton, J., Martin, J.-L., Petrich, J., Migus, A. and Antonetti, A. (1986) FEBS Lett. 209, 37–43.
[9] Prince, R.C. and Youvan, D.G. (1987) Biochim. Biophys. Acta 890, 286–291.
[10] Kirmaier, C. and Holten, D. (1988) Israel J. Chem., in press.
[11] Holten, D., Gouterman, M., Parson, W.W., Windsor, M.W. and Rockley, M.G. (1976) Photochem. Photobiol. 23, 415–423.
[12] Holten, D., Hoganson, C., Windsor, M.W., Schenck, C.G., Parson, W.W., Migus, A., Fork, R.L. and Shank, C.V. (1980) Biochim. Biophys. Acta 592, 461–477.
[13] Parson, W.W., Clayton, R.K. and Cogdell, R.J. (1975) Biochim. Biophys. Acta 387, 265–278.
[14] Kirmaier, C., Holten, D., Bylina, E. and Youvan, D.G. (1988) Proc. Natl. Acad. Sci. USA, in press.
[15] Vasmel, H., Amesz, J. and Hoff, A.J. (1986) Biochim. Biophys. Acta 852, 159–168.
[16] Scherer, P.O.J. and Fischer, S.F. (1987) Biochim. Biophys. Acta 891, 157–164.
[17] Knapp, E.W., Scherer, P.O.J. and Fischer, S.F. (1986) Biochim. Biophys. Acta 852, 295–305.
[18] Parson, W.W. and Warshel, A. (1987) J. Am. Chem. Soc. 109, 6152–6163.
[19] Won, Y. and Friesner, R.A. (1988) J. Phys. Chem. 92, 2208–2214.
[20] Eccles, J., Honig, B. and Schulten, K. (1900) Biophys. J. 53, 137–144.
[21] Breton, J. (1985) Biochim. Biophys. Acta 810, 235–245.
[22] Kirmaier, C. and Holten, D. (1988) in: The Photosynthetic Reaction Center – Structure and Dynamics (Breton, J. and Vermeglio, A. eds) pp.219–228, Plenum, New York.
[23] Maroti, P., Kirmaier, C., Wraight, C., Holten, D. and Pearlstein, R.M. (1985) Biochim. Biophys. Acta 810, 132–139.
[24] Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1984) J. Mol. Biol. 180, 385–398.
[25] Chang, C.-H., Tiede, D., Tang, J., Smith, U., Norris, J. and Schiffer, M. (1986) FEBS Lett. 205, 82–86.
[26] Allen, J.P., Feher, G., Yeates, T.O., Komiya, H. and Rees, D.C. (1987) Proc. Natl. Acad. Sci. USA 84, 5730–5734.
[27] Bixon, M., Jortner, J., Michel-Beyerle, M., Ogrodnik, A. and Lersch, W. (1987) Chem. Phys. Lett. 140, 626–630.
[28] Fischer, S.F. and Scherer, P.O.J. (1987) Chem. Phys. 115, 151–158.
[29] Marcus, R.A. (1987) Chem. Phys. Lett. 133, 471–477.
[30] Marcus, R.A. (1988) Chem. Phys. Lett. 146, 13–22.
[31] Warshel, A., Creighton, S. and Parson, W.W. (1988) J. Phys. Chem. 92, 2696–2701.
[32] Creighton, S., Hwang, J.-K., Warshel, A., Parson, W.W. and Norris, J.R. (1988) Biochemistry 27, 774–781.
[33] Bixon, M., Jortner, J., Plato, M. and Michel-Beyerle, M.E. (1988) in: The Photosynthetic Reaction Center – Structure and Dynamics (Breton, J. and Vermeglio, A. eds) pp.399–419, Plenum, New York.
[34] Won, Y. and Friesner, R.A. (1988) Biochim. Biophys. Acta, submitted.
[35] Fleming, G.R., Martin, J.L. and Breton, J. (1988) Nature 33, 190–192.
[36] Lockhart, D.J., Goldstein, R.A. and Boxer, S.G. (1988) J. Chem. Phys., submitted.
[37] Vermeglio, A., Breton, J., Paillotin, G. and Cogdell, R. (1978) Biochim. Biophys. Acta 501, 514–530.
[38] Kirmaier, C., Holten, D. and Parson, W.W. (1983) Biochim. Biophys. Acta 725, 190–202.

[39] Kirmaier, C., Holten, D. and Parson, W.W. (1985) Biochim. Biophys. Acta 810, 33–48.
[40] Kirmaier, C., Holten, D. and Parson, W.W. (1985) Biochim. Biophys. Acta 810, 49–61.
[41] Breton, J. (1988) in: The Photosynthetic Bacterial Reaction Center – Structure and Dynamics (Breton, J. and Vermeglio, A. eds) pp.59–69, Plenum, New York.
[42] Kirmaier, C., Holten, D., Mancino, J.L. and Blankenship, R.E. (1984) Biochim. Biophys. Acta 765, 138–146.
[43] Kirmaier, C., Holten, D. and Parson, W.W. (1986) Biophys. J. 49, 586a.
[44] Kleinfeld, D., Okamura, M.Y. and Feher, G. (1984) Biochemistry 23, 5780–5786.
[45] Woodbury, N.T. and Parson, W.W. (1984) Biochim. Biophys. Acta 767, 345–361.
[46] Tiede, D.M., Kellogg, E. and Breton, J. (1987) Biochim. Biophys. Acta 892, 294–302.
[47] Parot, P., Thiery, J. and Vermeglio, A. (1987) Biochim. Biophys. Acta 893, 534–543.
[48] Hoff, A.J. (1988) in: The Photosynthetic Bacterial Reaction Center – Structure and Dynamics (Breton, J. and Vermeglio, A. eds) pp.89–99, Plenum, New York.