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Picosecond Kinetics of the Initial Photochemical Electron-Transfer Reaction in Bacterial Photosynthetic Reaction Centers†

N. W. Woodbury, M. Becker, D. Middendorf, and W. W. Parson*
Department of Biochemistry, University of Washington, Seattle, Washington 98195
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ABSTRACT: The absorption changes that occur in reaction centers of the photosynthetic bacterium *Rhodopseudomonas sphaeroides* during the initial photochemical electron-transfer reaction have been examined. Measurements were made between 740 and 1300 nm at 295 and 80 K by using a pulse–probe technique with 610-nm, 0.8-ps flashes. An excited singlet state of the bacteriochlorophyll dimer P* was found to give rise to stimulated emission with a spectrum similar to that determined previously for fluorescence from reaction centers. The stimulated emission was used to follow the decay of P*; its lifetime was 4.1 ± 0.2 ps at 295 K and 2.2 ± 0.1 ps at 80 K. Within the experimental uncertainty, the absorption changes associated with the formation of a bacteriopheophytin anion, Bph^-, develop in concert with the decay of P* at both temperatures, as does the absorption increase near 1250 nm due to the formation of the cation of P, P^+. No evidence was found for the formation of a bacteriochlorophyll anion, $Bchl^-$, prior to the formation of Bph^-. This is surprising, because in the crystal structure of the *Rhodopseudomonas viridis* reaction center [Deisenhofer, J., Epp, O., Miki, K., Huber, R., & Michel, H. (1984) *J. Mol. Biol. 180*, 385–398] a Bchl is located approximately in between P and the Bph. It is possible that $Bchl^-$ (or $Bchl^+$) is formed but, due to kinetic or thermodynamic constraints, is never present at a sufficient concentration for us to observe. Alternatively, a virtual charge-transfer state, such as P^+Bchl^-Bph or $PBchl^+Bph^-$, could serve to lower the energy barrier for direct electron transfer between P* and the Bph.

Reaction centers from *Rhodopseudomonas sphaeroides* contain three polypeptide chains with a total M_r of about 80000, four bacteriochlorophylls (Bchl), two bacteriopheophytins (Bph), one or two ubiquinone molecules (Q_A and Q_B) depending on the preparation, and a non-heme iron atom. Recently, the three-dimensional structure of the reaction center from a related bacterium, *Rhodopseudomonas viridis*, has been solved to 3-Å resolution (Figure 1) (Deisenhofer et al., 1984). Two of the Bchls are associated to form a special pair (P). The other two Bchls ($Bchl_L$ and $Bchl_M$) are placed symmetrically on either side of P, followed by the two Bph molecules (Bph_L and Bph_M) and the quinones. When the reaction center is excited with light, an electron is transferred from P to Bph_L, forming the radical-pair state P^+Bph^- in about 5 ps (Zinth et al., 1985; Shuvalov et al., 1978; Holten et al., 1980; Dutton et al., 1975; Shuvalov & Klevanik, 1983; Parson & Ke, 1982). An electron then moves from Bph^- to Q_A in about 200 ps (Parson & Ke, 1982).

The crystal structure (Figure 1) suggests that the initial charge separation proceeds by transfer of an electron first from P to $Bchl_L$ and then from there to Bph_L. In agreement with this hypothesis, pulse–probe measurements using 30-ps pulses have suggested that the transient state P^+Bchl^- may be created prior to P^+Bph^- (Shuvalov et al., 1978; Shuvalov & Klevanik, 1983). However, the evidence on this point has been ambiguous (Kirmaier et al., 1985a; Borisov et al., 1983), and the role of $Bchl_L$ in the electron-transfer sequence is not yet clear.

In this work we use picosecond pulse–probe techniques to investigate the kinetics and spectra of absorbance changes between 740 and 1300 nm at 295 and 80 K. We follow the decay of an excited singlet state of P (P*) by monitoring stimulated emission and find that this decay occurs with the same time constant as the formation of the state P^+Bph^-. We find no evidence for the formation of the state P^+Bchl^- between P* and P^+Bph^-. Preliminary data have been presented previously (Parson et al., 1985a).

Materials and Methods

Reaction centers were prepared from *Rps. sphaeroides* as described previously (Schenck et al., 1982) and were suspended in 0.1% lauryldimethylamine oxide (LDAO) and 0.01 M tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 8.0, for measurements at 295 K. For some low-temperature measurements, 56% (v/v) glycerol was added. For most low-temperature measurements, reaction centers were placed in polyvinyl alcohol (PVA) films essentially as described (Schenck et al., 1981a). For measurements involving reduction

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*Author to whom correspondence should be addressed.

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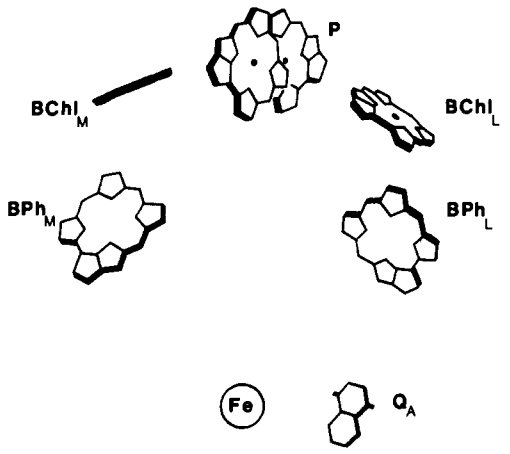

FIGURE 1: Approximate arrangement of the chromophores in *Rps. viridis* reaction centers [redrawn from Deisenhofer et al. (1984)]. Abbreviations as in text. Q_A is a menaquinone in *Rps. viridis* and a ubiquinone in *Rps. sphaeroides*. In the reaction center preparation used for the X-ray studies, Q_B was absent.

of Q_A, reaction centers in 0.1% LDAO were brought to 0.05% Triton X-100 and dialyzed against 0.05% Triton X-100 and 50 mM Tris-HCl, pH 8.0, for several days.

The 610-nm light pulses of 0.8-ps (full width at half-maximum) duration were produced by a synchronously pumped dye laser (Spectra Physics Model 375 with rhodamine 590 dye). These pulses were amplified by a four-stage optical amplifier (Ippen & Shank, 1979) with one stage pumped transversely and three stages pumped longitudinally by a neodymium YAG laser (Quantel Model 481) operating at 10 Hz. Approximately 5% of the light output from the amplifier was amplified further by reflection back through the last stage to obtain an excitation pulse on the order of 10 μJ. The remaining light was focused into a 10-cm cell of either water or methanol for continuum generation. Methanol, which produces a broad, relatively intense band in the 1260-nm region, was used only for the kinetic measurements shown in parts B and C of Figure 4. The continuum was filtered to remove residual 600-nm light and was split into two roughly equal beams, which were focused to small (about 300-μm) spots on the sample. The two transmitted beams passed through a monochromator and were monitored by separate photodiodes. For the measurements shown in Figure 4, the monochromator bandwidth was 20 nm, and germanium photodiodes (Hamamatsu, B 1918-01) were used for detection. In all other measurements, the bandwidth was 5 nm, and silicon photodiodes (EG & G, UV-444) were used.

The 610-nm excitation light passed down a variable delay line and was focused to a 1-mm spot aligned with one of the two probe beams on the sample. The excitation caused approximately a 20% bleaching of the 860-nm absorption band of P. (Decreasing the excitation intensity by a factor of 2 caused a similar decrease in the 860-nm bleaching.) At 295 K, a 2-mL sample was flowed through a 1 mm thick cell rapidly enough so that the excitation region was cleared during the 100-ms interval between flashes. At 80 K a 1-mm cell filled with reaction centers in glycerol/water or a PVA film of reaction centers was clamped to a metal holder in a quartz dewar.

To analyze the kinetics of the absorbance changes, an excitation function, $E(t)$, was obtained by inverting and differentiating the signal measured at 860 nm. (Bleaching of the ground-state absorption band at 860 nm presumably reflects the time course of P excitation.) Signals measured at other wavelengths were fit, with a nonlinear least-squares algorithm,

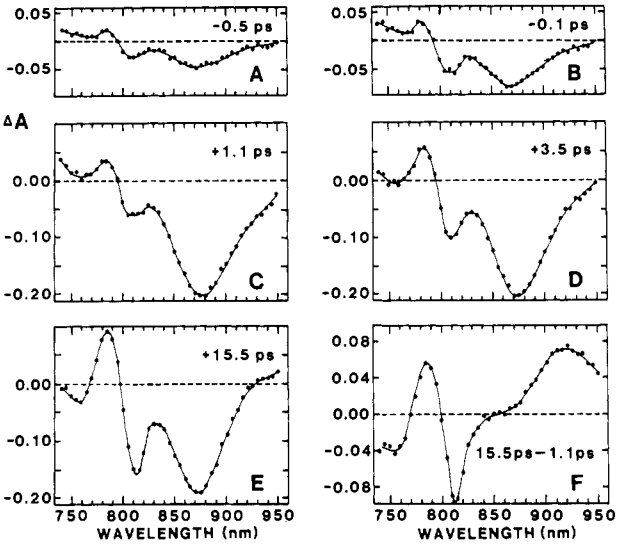

FIGURE 2: Absorbance changes measured at 295 K between 740 and 950 nm. Each spectrum is an average of five independent measurements (1500 flashes/point total). Zero picoseconds is the time when the absorbance decrease at 860 nm reaches half its final value. The reaction centers had an optical density of 1.4 at 802 nm. (A–E) Absorbance changes at the indicated times with respect to the absorbance at −10 ps. (F) The difference between the spectra in panels E and C.

to the theoretical function obtained by convoluting $E(t)$ with the expression $A \exp[-(t - \Phi)/\tau] + B$, where A, B, τ, and Φ are free parameters (Woodbury & Parson, 1984). The uncertainties stated for the time constant τ were calculated as described (Bevington, 1969). The phase shift Φ was included to allow for small temporal shifts between probe pulses of different wavelengths. Such shifts result from the wavelength dependence of the refractive index of the continuum-generating medium. The phase shifts required to fit the data between 755 and 922 nm were less than 0.5 ps and were in the expected directions. The kinetic traces in Figures 3 and 6 and the spectra in Figures 2 and 5 show the raw data, without corrections for the phase shift. As expected, probe light in the 1260-nm region was shifted more strongly. Measurements of the initial absorbance changes with an infrared dye (Kodak dye 26, exciton) showed phase shifts of 1.4 ± 0.1 ps at 1140 and 1260 nm relative to 860 nm. The kinetic trace at 1260 nm shown in Figure 4C has been shifted by this amount.

Results and Discussion

Transient Absorption Spectra at 295 K. Spectra of the absorbance changes caused by the 610-nm excitation flash were measured between 740 and 950 nm at five times during and after the excitation (Figure 2). The 15.5-ps spectrum (Figure 2E) is essentially identical with spectra published previously (Kirmaier et al., 1985b) for the absorbance changes associated with the formation of P^+Bph^-. The major absorption band of P at 870 nm is bleached due to the conversion of P to P^+. The 760-nm band of the Bph also shows a bleaching, which probably is due primarily to formation of the Bph anion radical (Kirmaier et al., 1985b). In addition, there is an absorbance increase in the 785-nm region and a decrease in the 810-nm region. The latter changes probably reflect the effects of P^+ and Bph^- on the other two Bchl molecules, which absorb near 800 nm (Kirmaier et al., 1985b).

The 1.1-ps spectrum (Figure 2C) shows the absorbance changes immediately after the end of the excitation pulse. The 860-nm bleaching is approximately the same size as it is at 15.5 ps, indicating that P has been removed from its ground state. However, the 755-nm bleaching has not yet developed,

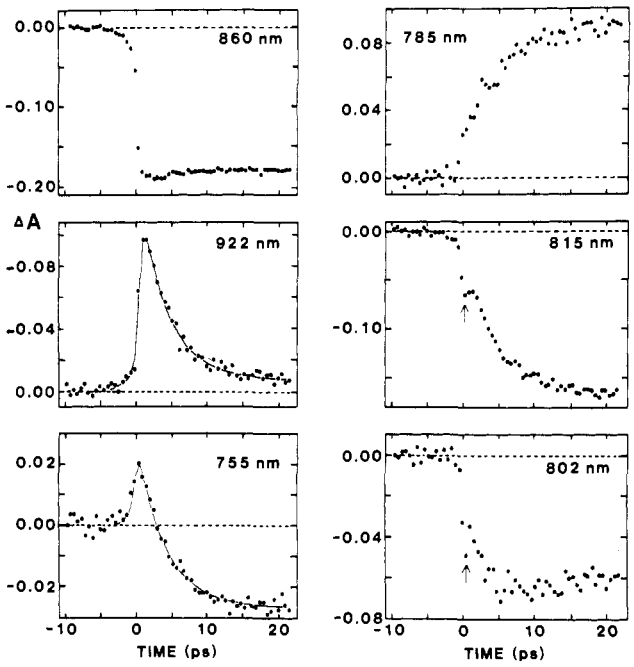

FIGURE 3: Kinetics of absorbance changes at 295 K. Conditions as in Figure 2. Measurements were made every 0.53 ps at the indicated wavelengths. Typically, 25 time scans were made per trace (500 flashes/point total). The signal at 922 nm is expressed as an absorbance change, but is inverted. The solid curves shown for the 922- and 755-nm traces are single-exponential fits of the data. The time constants are 4.1 ± 0.2 and 3.8 ± 0.2 ps, respectively.

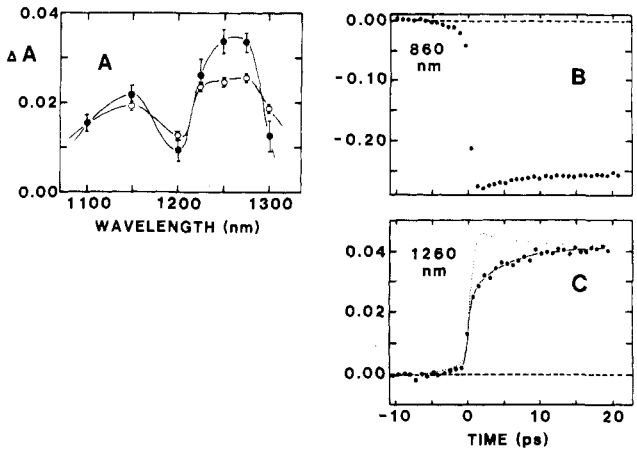

FIGURE 4: (A) Absorbance changes at 295 K between 1100 and 1300 nm. Conditions as in Figure 2 except that the sample optical density at 802 nm was increased to 1.9. Open circles, measurements made at 1 ps; closed circles, measurements made at 16 ps. The error bars indicate the standard errors of the mean calculated from 12 independent measurements at each point (3600 flashes/point total). (B and C) Kinetics of absorbance changes at 860 (B) and 1260 nm (C). Conditions as in (A). Time points every 0.80 ps. Each trace is an average of 150 time scans (4500 flashes/point total). The dotted line in the lower panel is an inverted and scaled smooth curve drawn through the points in the upper panel. The overshoot in the initial signal at 860 nm reflects the inclusion of some stimulated emission in the signal, due to the use of a larger spectral band-pass here than in Figure 3 (see Materials and Methods). The 1260-nm data have been shifted in time by 1.4 ps (see Materials and Methods). The solid line in (C) is a fit with the trace in (B) used to generate the excitation profile. The time constant for the slow portion of the absorbance increase is 4.9 ± 0.6 ps.

and the absorbance changes around 785 and 810 nm are much smaller than they are at 15.5 ps. Between 740 and 795 nm there are absorbance increases similar to the absorbance changes associated with the formation of an excited triplet state of P (Shuvalov & Parson, 1981) or the transient state seen in reaction centers with the Bph reduced (Schenck et al., 1981b). Thus at 1.1 ps, the reaction center appears to be in a transient state that precedes the formation of P^+Bph^-.

*Stimulated Emission from an Excited State, P**. A major new feature of the 1.1-ps spectrum that is not seen in the 15.5-ps spectrum is an apparent absorbance decrease centered around 920 nm. This can be seen most clearly by plotting the difference between the spectra measured at the two times (Figure 2F). The spectrum of the 920-nm component is similar to the fluorescence emission spectrum of reaction centers (Woodbury & Parson, 1984), suggesting that this feature may be due not to an absorbance decrease but to stimulated emission from an excited singlet state, P*. This interpretation is supported by the fact that the apparent absorbance decrease of about 0.08 at 920 nm is greater than the sample's total initial absorbance here (0.05). Stimulated emission is expected to depend on the concentration of P* in just the same way as absorbance changes associated with the formation of this species. Signals due to emission should predominate over those due to absorbance changes at wavelengths near 920 nm, where the optical cross section for emission is greater than that for absorption. The 920-nm signal thus appears to provide a direct means to measure the lifetime of P*.

Kinetics of P Decay and Bph^- Development at 295 K.* Figure 3 shows the absorption changes as a function of time at 755, 785, 802, 815, 860, and 922 nm. The stimulated emission at 922 nm decays with a time constant of 4.1 ± 0.2 ps. The bleaching at 755 nm develops with a similar time constant (3.8 ± 0.2 ps), suggesting that P^+Bph^- is formed as P* decays. The absorbance changes at 785 and 815 nm also show similar kinetics, although there are indications of a faster component at 815 nm (see below).

Spectra and Kinetics of Absorbance Changes near 1250 nm. Spectra of the absorbance changes between 1100 and 1300 nm were measured at 1 and 16 ps after excitation (Figure 4A). Between 1100 and 1200 nm the absorbance changes are similar at the two times, indicating that the state present at 1 ps has approximately the same extinction coefficient as the state present at 16 ps. Between 1200 and 1275 nm, where P^+ has a distinct absorption band (Dutton et al., 1975; Clayton, 1973), there is a significant increase in absorbance after 1 ps. At 1300 nm there is a small decrease in absorbance with time.

The time course of the absorbance increase at 1260 nm is given in Figure 4C. Kinetic analysis (see Materials and Methods) indicates that about 70% of the absorbance increase at this wavelength develops with the same time course as the bleaching at 860 nm. The remaining 30% develops with a time constant of 4.9 ± 0.6 ps, which is, considering the experimental uncertainty, similar to the time constant for the decay of the stimulated emission at 922 nm. This is strong evidence that at least a portion of P^+ is created with kinetics that match the decay of the stimulated emission from P* and the formation of Bph^-.

Effect of Reducing Q_A on the Lifetime of the Stimulated Emission. The lifetime of the stimulated emission at 922 nm increased by 32% from 4.1 ± 0.2 ps in Triton X-100 dialyzed reaction centers (see Materials and Methods) to 5.4 ± 0.1 ps after reduction of Q_A by addition of approximately 0.5 mg/mL sodium dithionite (data not shown). This is in agreement with the 25% increase in the yield of "prompt" spontaneous fluorescence observed upon reduction of *Rps. sphaeroides* reaction centers (Woodbury & Parson, 1984). Further addition of dithionite had little or no effect.

Transient Absorption Spectra and Kinetics at 80 K. Figure 5 shows spectra measured at 5 times during and after exci-

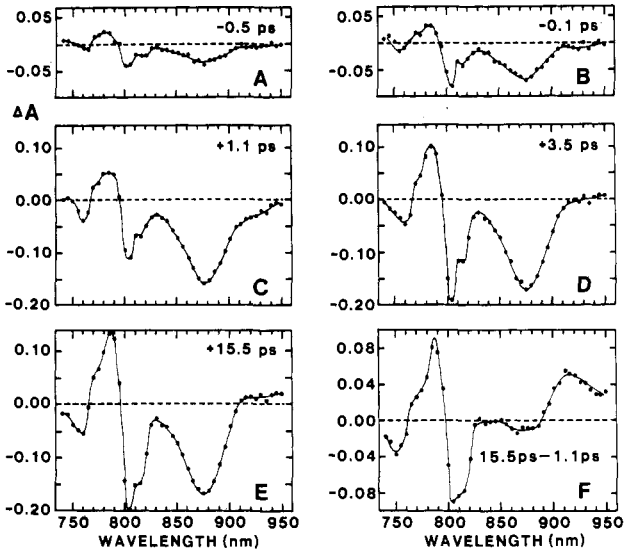

FIGURE 5: Absorption changes measured at 80 K between 740 and 950 nm. Each spectrum is the average of four independent measurements (1200 flashes/point total). Reaction centers were in polyvinyl alcohol films at an optical density of 1.3 at 802 nm (measured at 295 K). (A–E) Absorbance changes at the indicated times with respect to the absorbance at −10 ps. (F) The difference between the spectra in panels E and C.

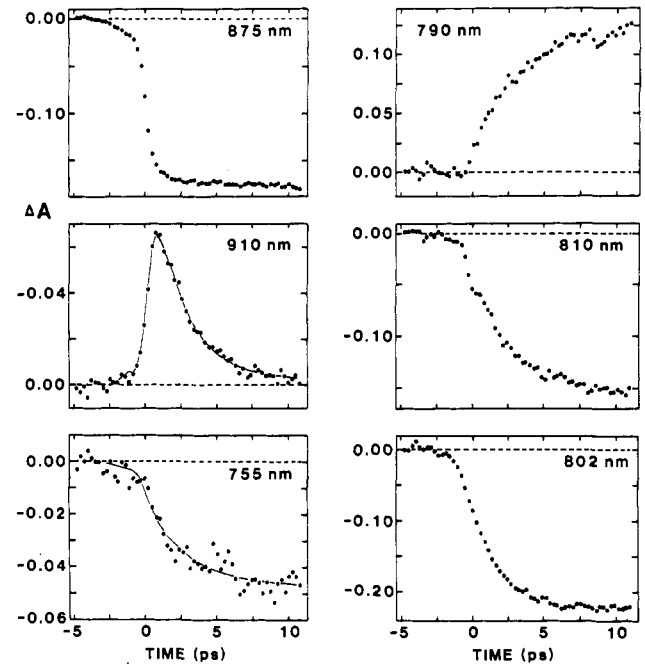

FIGURE 6: Kinetics of absorbance changes at 80 K. Conditions as in Figure 5. Measurements were made every 0.27 ps at the indicated wavelengths. Typically, 25 time scans were made per trace (500 flashes/point total). Fits to the data at 910 and 755 nm are shown. The time constants are 2.2 ± 0.1 and 3.1 ± 0.8 ps, respectively. The 875-nm time course was used to generate the excitation profile.

tation of reaction centers in PVA films at 80 K. The basic features are similar to those observed at room temperature. One notable feature of the low-temperature spectra is a splitting of the absorbance decrease between 800 and 825 nm into two bands. This effect has been observed previously in the P^+Bph^- spectrum (Kirmaier et al., 1985b). In addition, the bleaching around 755 nm is better resolved from the complicated absorbance changes between 770 and 825 nm, and the 910-nm stimulated emission is better separated from the bleaching of the ground state band at 875 nm.

Figure 6 shows the time courses of the absorbance changes measured at 80 K at six wavelengths. The decay of the stimulated emission at 910 nm has a time constant of 2.2 ± 0.1 ps, nearly a factor of 2 shorter than that measured at 295 K. The bleaching at 755 nm develops with a time constant of 3.1 ± 0.8 ps, which is, within experimental error, indistinguishable from the decay time of the stimulated emission. The absorbance changes at all other wavelengths have qualitatively similar kinetics or consist of a very fast component in addition to an absorbance change with a 2–3-ps time constant.

Similar kinetic measurements at 80 and 295 K were made with reaction centers in 56% (v/v) glycerol. At 295 K the time constant for the decay of the stimulated emission was 4.0 ± 0.1 ps, the same as that obtained when reaction centers without glycerol were used. At 80 K the stimulated emission decayed with a lifetime of 1.2 ± 0.2 ps, significantly faster than the decay at the same temperature in PVA films. Thus, the decrease in the lifetime of the stimulated emission between 295 and 80 K (Figures 3 and 6) is not due simply to a difference in the solvent systems used at the two temperatures; the transfer of an electron from P* to Bph evidently increases in speed by a factor of 2 to a factor of 4 (depending on the solvent system) with decreasing temperature. Electron transfer from Bph^- to Q_A also speeds up by a similar factor (Kirmaier et al., 1985b; Schenck et al., 1981a).

Relationship of the Lifetime of P to the Quantum Yield of Fluorescence.* The quantum yield of fluorescence from P* should be given by the ratio of the lifetime of P* to the natural radiative lifetime, provided that the vibrational substates of P* rapidly achieve thermal equilibrium. The radiative lifetime is about 13 ns at both 295 and 80 K (Woodbury & Parson, 1984; N. W. Woodbury and W. W. Parson, unpublished results). With the 4.1-ps lifetime of P* at 295 K, the calculated fluorescence yield is 3.2×10^{-4}, in good agreement with the yield $4.0 \pm 1.5 \times 10^{-4}$ measured previously (Zankel et al., 1968).

Decreasing the temperature to 80 K reduces the lifetime of P* by nearly a factor of 4 in 56% (v/v) glycerol, whereas the quantum yield of fluorescence does not change appreciably (Schenck et al., 1982; Woodbury & Parson, 1984). However, at the lower temperature the fluorescence spectrum is somewhat broader than would be expected from the absorption spectrum (N. W. Woodbury and W. W. Parson, unpublished results). Heterogeneous broadening of the fluorescence spectrum could result if a small fraction of the reaction centers froze in a conformation in which P* decayed with a long lifetime. This could increase the fluorescence yield from P* without greatly affecting the measurements reported here.

Formation and Decay of Excited States of $Bchl_L$ and $Bchl_M$. Indications of the transient excitation of the two Bchls that are not part of P can be seen in Figures 2 and 5. In the 295 K spectra the ratio of the 805-nm peak to the 860-nm peak is greater at −0.1 ps than at 1.1 ps (Figure 2). Similar results are evident in the 80 K spectra, though the effect is less obvious because the overall absorbance changes are faster. The room temperature kinetic traces in the 800-nm wavelength region show the same effect. At 815 and 802 nm there is a significant deviation from an exponential time course at about 0 ps (arrows, Figure 3), though this is obscured by the low signal to noise ratio at 802 nm. At 80 K, the kinetic trace at 802 nm shows a substantial fast absorbance decrease followed by a slower decrease with a time constant similar to those found at 810, 755, 790, and 910 nm.

A quantitative kinetic analysis at wavelengths between 785 and 815 nm was not attempted. Qualitatively, however, the kinetics and spectra in this region indicate that a component that absorbs here is probably bleached as rapidly as P is (as

measured at 860 nm) but recovers with a time constant of less than about 1.5 ps. These measurements are not consistent with a large, rapid bleaching that recovers with a time constant equal to that found for the formation of Bph^-, as might be expected for the formation and decay of the state P^+Bchl^-. We cannot, however, exclude a small contribution from such a component.

The simplest interpretation is that the fast component in the 800-nm region reflects the transient presence of excited singlet states of $Bchl_L$ and $Bchl_M$ in some of the reaction centers and that those Bchls transfer the excitation to P within about 1.5 ps. Although the 610-nm excitation light probably is absorbed preferentially by P, it is likely to excite $Bchl_L$ and $Bchl_M$ to some extent.

Direct excitation of P near 860 nm is required for an unambiguous interpretation of the absorbance changes between 785 and 815 nm. After the measurements described here were finished, we learned that Martin et al. (1986) had made similar measurements using 850-nm excitation. They observed an initial absorbance *increase* at 800 nm, which they attributed to P*. This is consistent with the view that the initial absorbance decrease we observed near 800 nm was due to excited states of $Bchl_L$ and $Bchl_M$. At other wavelengths, Martin and co-workers obtained results similar to ours, except that their time constant for the decay of the stimulated emission at 295 K (2.8 ps) was shorter. This could be due to differences in sample preparation or bacterial strains.

Possible Electron-Transfer Mechanisms. The results presented above indicate that the decay of P* and the formation of P^+ and Bph^- occur with essentially identical kinetics. These results were reproducible from measurement to measurement with several different reaction center preparations. The simplest conclusion is that an electron moves from P* directly to Bph_L. However, the three-dimensional structure of the *Rps. viridis* reaction center shows $Bchl_L$ located approximately between P and Bph_L (Figure 1) (Deisenhofer et al., 1984). This Bchl would seem likely to play some role in electron transfer between P and Bph_L. There are several possible ways to reconcile these observations. First, the arrangements of pigments in the reaction centers of *Rps. sphaeroides* and *Rps. viridis* could be different. This seems unlikely, in view of the functional and spectroscopic similarities between the two types of reaction centers. Second, it is possible that $Bchl^-$ is an intermediate in the electron-transfer reaction but is not kinetically resolvable, perhaps because electron transfer from $Bchl^-$ to Bph is faster than that between P* and Bchl. Alternatively, suppose that the state P^+Bchl^-Bph lies above P* in energy, as suggested by recent molecular orbital calculations (Parson et al., 1985b), and that equilibrium between these states is achieved on a subpicosecond time scale. The Bchl anion could then transfer an electron more slowly to the Bph. This would explain some of the room temperature observations: P* would persist in equilibrium with P^+Bchl^--Bph, and additional P^+ would form with the same time constant as the electron transfer from $Bchl^-$ to Bph. In this model, however, the overall rate of electron transfer between P* and Bph should decrease as the temperature is lowered. Instead, an increase in the rate constant is observed.

Another consideration is that the excited state that we have symbolically called P* probably includes excitonic contributions from the excited $\pi\pi^*$ states of the neighboring Bchls, in addition to $\pi\pi^*$ and charge-transfer states of the two Bchls that make up P (Parson et al., 1985b). Spreading of the excitation onto $Bchl_L$ could allow direct electron transfer from this Bchl to the Bph. This would create the state $PBchl^+Bph^-$, which might not be kinetically resolved if it decayed quickly to $P^+BchlBph^-$.

Finally, it is possible that P^+Bchl^-Bph (or $PBchl^+Bph^-$) participates only as a virtual state with no finite lifetime. Even if P^+Bchl^-Bph is above P* in energy, it still could serve to lower the potential energy barrier between P and Bph. Such a decrease in the barrier height would facilitate direct electron tunneling from P* to Bph (Parson et al., 1985a). We presently favor this mechanism because it is consistent with all of the observations and does not invoke the transient population of a state for which we find no direct evidence.

Acknowledgments

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Islet Activating Protein Inhibits Physiological Responses Evoked by Cardiac Muscarinic Acetylcholine Receptors. Role of Guanosine Triphosphate Binding Proteins in Regulation of Potassium Permeability†

Jennifer M. Martin, Dale D. Hunter, and Neil M. Nathanson*
Department of Pharmacology, University of Washington, Seattle, Washington 98195
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ABSTRACT: The involvement of GTP binding proteins in muscarinic acetylcholine receptor (mAChR) mediated responses of cultured chick embryonic cardiac muscle cells was studied by using islet activating protein (IAP) from *Bordetella pertussis*. Incubation of cells for 24 h with IAP resulted in inhibition of subsequent IAP-catalyzed incorporation of [α-^{32}P]ADP-ribose into membrane proteins of M_r 39 000 ($N_{o\alpha}$) and 41 000 ($N_{i\alpha}$); treatment of cultures with 5 ng/mL IAP was sufficient to ADP-ribosylate all available $N_{o\alpha}$ and $N_{i\alpha}$. Inhibition of forskolin-stimulated cAMP accumulation by the muscarinic agonist carbachol was abolished in cultures pretreated with IAP. The affinity of carbachol for the mAChR in membranes from IAP-treated cells was considerably decreased compared to control membranes and was not further decreased by addition of guanyl-5′-yl imidodiphosphate. In contrast, the affinity of carbachol for the mAChR on intact cells was not affected by pretreatment with IAP. To investigate the involvement of N_o and/or N_i in mAChR-mediated increases in K^+ permeability, the effect of IAP treatment on mAChR stimulation of $^{86}Rb^+$ efflux was determined. Treatment of cultures with 5 ng/mL IAP for 24 h completely blocked the stimulation of $^{86}Rb^+$ efflux evoked by carbachol. Because previous work has shown that mAChR regulation of K^+ permeability is independent of changes in cAMP levels, these results suggest a role for N_o and/or N_i in coupling the mAChR directly to K^+ channels in the heart.

Activation of muscarinic acetylcholine receptors (mAChR)[1] in the cardiac muscle membrane causes a decrease in beating rate due to an increase in K^+ permeability (Hutter & Trautwein, 1955). Although muscarinic agonists inhibit the synthesis of cAMP by adenylate cyclase in the heart (Watanabe et al., 1978; Jakobs et al., 1979; Hazeki & Ui, 1981), electrophysiological studies have shown that mAChR-mediated increases in K^+ conductance are independent of changes not only in cyclic nucleotide levels (Trautwein et al., 1982; Nargeot et al., 1983) but also of any cytosolic diffusable second messengers (Sakmann et al., 1983). At present, the molecular mechanism of mAChR coupling to K^+ channels is not known.

Agonist binding to mAChR in the heart results in a guanine nucleotide dependent inhibition of adenylate cyclase activity (Watanabe et al., 1978). Guanine nucleotides also regulate the affinity of the mAChR for agonists in the heart (Berrie et al., 1979). These guanine nucleotide effects can be blocked by treatment with islet activating protein (IAP) from *Bordetella pertussis* (Hazeki & Ui, 1981; McMahon et al., 1985). IAP catalyzes the ADP-ribosylation of 39- and 42-kDa polypeptides in chick heart (Halvorsen & Nathanson, 1984), which correspond in molecular weight to the α subunits of the GTP binding proteins N_o and N_i in bovine brain.[2] Both of these proteins are capable of regulating agonist binding to the mAChR (Florio & Sternweis, 1985). Halvorsen & Nathanson (1984) have demonstrated that the appearance of the mAChR-mediated negative chronotropic response in developing embryonic chick heart correlates well with the physical and functional maturation of the GTP binding proteins N_o and N_i. This suggests a role for guanine nucleotide regulatory proteins in coupling the mAChR to physiological responses in chick heart. In the present study we use IAP to investigate the involvement of N_o and N_i in physiologic responses mediated by the mAChR in cultured chick cardiac cells. In contrast to the recent report of McMahon et al. (1985), we show here that IAP treatment of cells abolishes both guanine nucleotide regulation of agonist binding to the mAChR and mAChR-

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[1] Abbreviations: mAChR, muscarinic acetylcholine receptor(s); IAP, islet activating protein; $N_{o\alpha}$, 39-kDa α subunit of the GTP binding protein ADP-ribosylated by IAP; $N_{i\alpha}$, 41-kDa α subunit of the inhibitory GTP regulatory protein of adenylate cyclase ADP-ribosylated by IAP; N proteins, N_o and N_i; Gpp(NH)p, slowly hydrolyzable GTP analogue guanyl-5′-yl imidophosphate; IC_{50}, concentration that inhibits 50%; EC_{50}, concentration producing 50% effect; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; QNB, quinuclidinyl benzilate; NMS, *N*-methylscopolamine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NAD, nicotinamide adenine dinucleotide; kDa, kilodalton(s); Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

[2] Also referred to as G_o and G_i by Florio & Sternweis (1985).

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