$(T,Y) = (0,0)$, $(\frac{1}{2}, \pm 1)$, $(1,0)$, it is $(0,0)$ that contains no magnetically charged state and therefore is a natural candidate for the lowest-lying type of excitation. If both long-lived ψ particles are labeled $T(\text{mag.}) = Y(\text{mag.}) = 0$, they must be distinguished by quantum numbers associated with the group U_3(el.), in analogy with the well-known 1^- neutral particles ρ^0, ω, and ϕ. We indicate these options for the ψ particles as $\rho^{0\prime}$, ω', ϕ'. At the moment, I favor the view that $\psi(3.1)$ is a mass-degenerate superposition of $\rho^{0\prime}$ and ω' (ρ^0 and ω differ in mass by only 14 Mev), while ψ (3.7) is identified with ϕ'. The latter assignment is attractive in the following way. Although transitions of $\psi(3.1)$ and $\psi(3.7)$ to "normal" hadrons are largely forbidden by U_3(mag.) invariance, this would not inhibit the decay $\psi(3.7) \rightarrow \psi(3.1)$ + normal hadrons, except that the same mechanism which restrains the decay of ϕ into pions should also operate here. That mechanism was long ago (*8*) interpreted within the framework of U_3 invariance as signifying the unity of 9 rather than 8+1 unit spin mesons. The degeneracy of $\rho^{0\prime}$ and ω' is also quite important in attaining a thorough suppression of this coupling. Another point on behalf of the ϕ' status of $\psi(3.7)$ is the absence of appreciable production in proton-nucleon collisions (*9*), as compared with $\psi(3.1)$, which seems to be produced by hadronic rather than electromagnetic interactions (*10*).

There is a different argument pointing to the possibility that some suppression of quadratic ψ couplings with normal hadrons is a general feature. Through the direct coupling of the ψ particles to photons, such a quadratic ψ interaction implies the decay of a ψ particle to a photon and normal hadrons. This decay will occur too rapidly if the quadratic ψ interaction is of normal strength; the appropriate coupling constant must be roughly an order of magnitude smaller. Here is an indication that the internal rearrangements necessary to convert the magnetic states of two ψ particles to an invariant configuration occur with some difficulty. A suggestion of confirmatory evidence appears in photoproduction experiments, which can be interpreted to show that the ψ-nucleon scattering cross section is quite small on the normal hadronic scale (*11*). Finally, we remark that ψ particles more massive than $\psi(3.1)$ and $\psi(3.7)$ are unlikely to be as long-lived as the latter two. If they are members of a similar multiplet, the decay down to either $\psi(3.1)$ or $\psi(3.7)$ is impeded only by the magnetic rearrangement effect. Or, if they are magnetically neutral members of a multiplet other than (0,0), the large splittings anticipated within such a multiplet should lead to a considerable violation of U_3(mag.) symmetry, with a consequent loosening of the restraints against direct decay into normal hadrons.

A speculative model, such as the one we have outlined, can be useful if the impressionistic picture that it paints suggests more sharply focused phenomenological descriptions. Since we have already provided a phenomenology of the ψ particles in the areas of electromagnetic and weak interactions, the challenge is posed to establish contacts with the speculative model that could be broadened into specific hints concerning, for example, the forms of symmetry-breaking interactions, including charge and parity (CP) violation, and also guide the search for magnetically charged particles.

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Picosecond Kinetics of Events Leading to Reaction Center Bacteriochlorophyll Oxidation

Abstract. *A transient absorption spectrum has been measured in* Rhodopseudomonas spheroides *R26 reaction centers. Its salient features indicate that both the bacteriopheophytin and bacteriochlorophyll chromophores play a role in the excited state. Decay of this state yields a rise time for oxidation of the reaction center complex of about 150 picoseconds.*

Much of the work on our basic concepts of how photosynthetic systems may handle incident light energy and convert it into electrochemical potential energy within the photosynthetic membrane has come from studies on photosynthetic bacteria (*1–3*).

In the photosynthetic bacterium, *Rhodopseudomonas spheroides*, an array of light-harvesting bacteriochlorophyll (antennas) and carotenoid molecules function to capture photons. The energy contained in the excited "antenna" molecules is funneled into a special bacteriochlorophyll complex generally called the reaction center protein. This protein in photosynthetic bacteria is readily isolatable from the membrane and from the other pigments by use of detergents. Current investigations in several laboratories reveal that the reaction center protein is comprised of four magnesium porphyrins (bacteriochlorophyll), two hydrogen porphyrins (bacteriopheophytin), a ubiquinone, and an iron (nonheme) moiety (*4–6*). The principal absorption bands are found at 865, 800, 760, 600, and 530 nm; the bands at 865, 800, and 600 nm are generally considered to arise from bacteriochlorophyll absorption, while those at 760 and 530 nm seem to come from bacteriopheophytin absorptions. However, the possible existence of exiton interaction (*2*) makes a unique assignment of these bands difficult.

Excitation of the reaction center bacteriochlorophyll complex results in the transfer of an electron to the primary acceptor. Removal of the electron or the chemical oxidation of the reaction center bacteriochlorophyll complex results in major changes in the spectrum. These include bleaching of the bands at 865 and 600 nm and a small hypsochromic (blue) shift of the 800-nm band; there is also a small optical increase apparent at 1250 nm (*7*). Thus far no detectable optical changes have been identified with the primary electron acceptor in photosynthetic bacteria (*8*).

We have reported the bleaching of the 865-nm band after excitation into the 530-nm bacteriopheophytin band (*9*); this indicates rapid energy transfer between the two reaction center chromophores. We

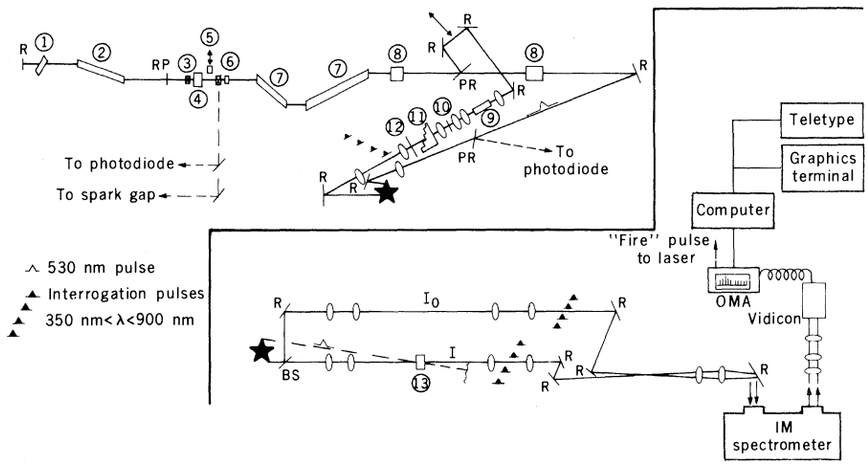


Fig. 1. Double-beam picosecond spectrometer utilizing a silicon vidicon detector. Components: 1, mode-locking dye cell; 2, laser oscillator rod; 3, calcite polarizer; 4, Pockels' cell; 5, translatable 90° polarization rotator for 1060-nm radiation; 6, fixed position 90° polarization rotator; 7, laser amplifier rod; 8, second harmonic (530 nm) generating crystal; 9, 20-cm octanol cell for generating the interrogation wavelengths; 10, ground-glass diffuser; 11, index matched glass echelon for producing picosecond optical delays between the stacked interrogation pulses; 12, vertical polarizer; 13, sample cell; R, reflector; PR, partial reflector; BS, beam splitter; OMA, optical multichannel analyzer.

now report on our efforts to resolve the light-induced processes in the reaction center which precede the bacteriochlorophyll oxidation. We have measured a transient absorption spectrum in reaction centers of *Rhodopseudomonas spheroides* R26, which indicates that both the bacteriopheophytin and bacteriochlorophyll chromophores play a role in the excited state. When photochemistry is blocked, the spectral changes persist rather than decay, leading us to believe that we have observed the state responsible for the initial light-induced charge separation. The decay rate of this spectrum when photochemistry can proceed yields a rise time for oxidation of the reaction center complex of ~ 150 psec.

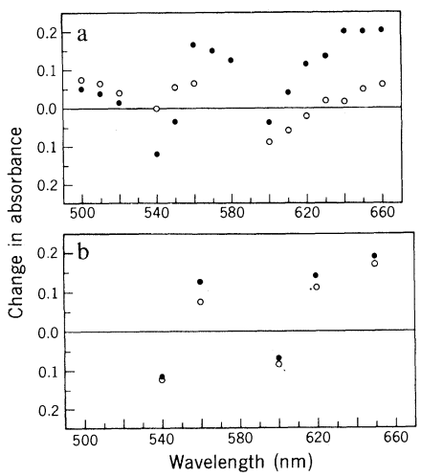
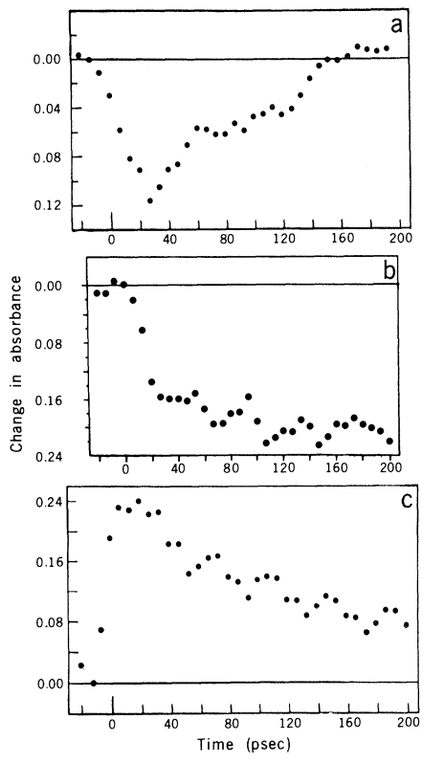


Fig. 2 (left). Laser-induced spectrum of *Rps. spheroides* R26 reaction centers. Absorbance change after 13 psec (●) and 250 psec (○). (a) Reaction centers at ~ +200 mv. (b) Reaction centers at < −200 mv. Fig. 3 (right). Kinetics of laser-induced absorbance changes in *Rps. spheroides* R26 reaction centers. (a) At 540 nm; E_h ~ +200 mv. (b) At 540 nm; E_h < −200 mv. (c) At 640 nm; E_h ~ +200 mv.



The picosecond laser apparatus used is quite similar to that described previously (*10, 11*) (Fig. 1), which utilized a double beam I and I_0. The output signal was detected with an optical multichannel analyzer (SSR Instruments) which was coupled with a minicomputer for data reduction and averaging. A photodiode (ITT F4000) monitored the 530-nm photolysis pulse. The double beam permitted accurate adjustment for shot-to-shot variations of the laser and the continuum probing pulse. The sensitivity and reliability of this apparatus allows the measurement of optical density changes as small as 0.03. To minimize possible effects arising from molecular reorientation, the sample was excited with circularly polarized light produced by inserting a quarter wave plate in the photolysis beam.

The *Rps. spheroides* R26 reaction centers were prepared in the usual manner (*4*), except for the use of column chromatography (Whatman DE52 cellulose) and that LDAO detergent (*12*) was replaced by 0.1 percent Triton during the column procedures. The electrochemical cell with a 2-mm optical path length has been described (*13*), and all experiments were accordingly performed under an atmosphere of argon.

Two redox potentials (E_h ~ +200 mv and < − 200 mv) were used. The more positive potential is essentially the condition of the reaction centers as they are prepared. In some experiments, 1 μM diaminodurol was added to verify that the ambient redox potential was in the +200-mv region; the addition had no effect on kinetics or spectra. For negative potential work microliter quantities of freshly prepared dithionite solution (made up in 1 M morpholinopropane sulfonate buffer, pH 7) were added, and stable redox potential readings in the range of − 300 to − 200 mv were established. All experiments were performed at pH 7.0 in 50 μM morpholinopropane sulfonate buffer and 0.1 percent Triton X-100. The optical density of the sample was adjusted to 3.2 at 860 nm for the 2-mm path length of the electrochemical cell for the experiments in the visible region of the spectrum, and to 0.8 to 1.2 for experiments in the near-infrared portion of the spectrum.

Kinetics in the 500- to 600-nm region. The spectral response of *Rps. spheroides* R26 reaction centers measured 13 and 250 psec after excitation is shown in Fig. 2a. At a potential of ~ +200 mv, the bacteriochlorophyll (E_m, +400 mv at pH 7.0) is essentially reduced, and the primary acceptor (E_m, −50 mv at pH 7.0) is essentially oxidized before activation. The system therefore is capable of the photochemical

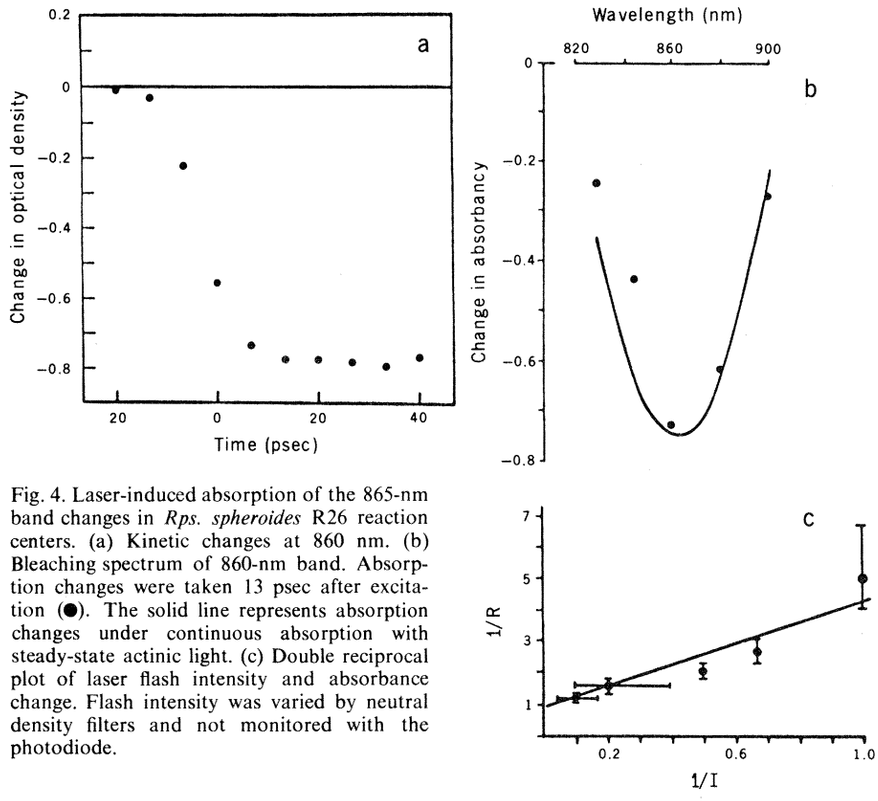


Fig. 4. Laser-induced absorption of the 865-nm band changes in *Rps. spheroides* R26 reaction centers. (a) Kinetic changes at 860 nm. (b) Bleaching spectrum of 860-nm band. Absorption changes were taken 13 psec after excitation (●). The solid line represents absorption changes under continuous absorption with steady-state actinic light. (c) Double reciprocal plot of laser flash intensity and absorbance change. Flash intensity was varied by neutral density filters and not monitored with the photodiode.

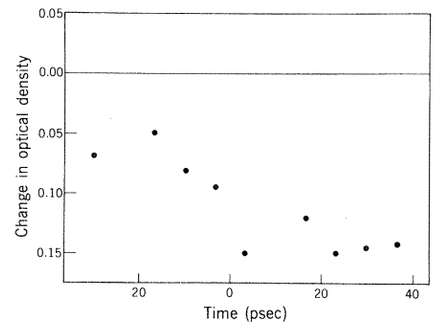


Fig. 5. Kinetics of laser-induced absorption changes of *Rps. spheroides* R26 reaction centers at 760 nm. Reaction centers at < −200 mv.

electron transfer from the reaction center bacteriochlorophyll to its acceptor. The features recorded 13 psec after excitation include a broad absorption throughout the region of observation between 500 and 660 nm with the exception of two regions of bleaching centered at 540 and 600 nm. The former (540 nm) we consider as originating from the bacteriopheophytin and the latter (600 nm) from the bacteriochlorophyll. The decay kinetics of the bleaching at 540 nm and the positive absorbance change at 640 nm are shown in Fig. 3, a and b.

Figure 2b also shows the spectral response of the reaction centers, but here the ambient redox potential was lowered to a value sufficient (E_h < − 200 mv) to chemically reduce the primary electron acceptor. Under these conditions, the electron transfer from the reaction center bacteriochlorophyll to its acceptor was prevented. Thus any optical transitions accompanying the oxidation of the bacteriochlorophyll were eliminated. Within experimental error, the initial 13-psec response (Fig. 2b) was the same as that encountered where the conditions were such that electron transfer could proceed (Fig. 2a). However, at 250 psec the relaxation of the broad band in the visible region did not occur. In contrast, we have data indicating that the 540-nm bleaching may in fact increase somewhat (Fig. 3b).

Kinetics in the near-infrared. Figure 4a shows a confirmation of the bleaching at 860 nm previously reported (*9*); Fig. 4b shows the spectrum for the bleaching of the 865-nm band measured 13 psec after activation. When dithionite was added in order to reduce the primary acceptor, no alteration in the bleaching kinetics at 860 nm could be resolved. The flash intensity profile of the bleaching (Fig. 4c) is presented as a double reciprocal plot. Experiments at the 760-nm (bacteriopheophytin) band indicate (Fig. 5) some bleaching. Details of the kinetics of these bands in the long time (250 psec) scale and data pertaining to the 800-nm band are not yet available.

Despite a great deal of experimental work, little progress has been made in elucidating the events that precede the transfer of an electron from the excited reaction center bacteriochlorophyll to its primary electron acceptor. Estimates from fluorescence quantum yield measurements (*14*) indicate that the lifetime of the excited singlet may only be ~ 10 psec. This value however can only be regarded as an order of magnitude estimate because of errors arising from the determination of the intrinsic radiative lifetime. Seibert *et al.* (*15*) have observed directly two separate fluorescence lifetimes—10 and 320 psec—in chloroplasts. Unfortunately, a direct correlation between lifetimes in plants and photosynthetic bacteria cannot be made.

Parson and co-workers have excited *Rps. spheroides* R26 reaction centers in which the primary acceptor has been reduced with 10- to 20-nsec laser pulses at various wavelengths (*3, 16*). They observed two distinct absorption spectra which differ significantly from each other, particularly at 540 and 420 nm. The first state, named P^f by Parson *et al.*, was reported to have its rise time and a decay time limited by the ~ 20-nsec lifetime of the exciting flash at both room and cryogenic temperatures. This state was shown to form with a quantum yield near unity. The second state, designated P^r, was found to have a low quantum yield at room temperature where it had a decay half-time of 6 μsec. The state P^f has a very broad absorption in the visible portion of the spectrum with the exception of bleaching at 380 and 540 nm. Little or no net absorption change at 600 nm was observed, and this was attributed by Parson *et al.* (*16*) to be due to a cancellation between the new broad band absorption and the bleaching of the bacteriochlorophyll.

The spectral changes shown in Fig. 2, a and b, agree within experimental error with those attributed to the P^f state. The rapid bleaching of the 865-nm band (Fig. 4a) coupled with the persistence of spectral changes observed 250 psec after excitation when primary acceptor is reduced (Fig. 2b) confirm that the bleaching at 540 and 760 nm shown in Figs. 2a and 5, respectively, are not merely due to excitation of the bacteriopheophytin but to an excited state of the reaction center which must involve both the bacteriopheophytin and bacteriochlorophyll chromophores.

The decay of the excited state when photochemistry can proceed (Fig. 2a) to the oxidation of the reaction center bacteriochlorophyll, and the persistence of the observed spectrum (Fig. 2b) when the primary acceptor is reduced, leads us to believe that this state could be the one re-

sponsible for transfer of the electron to the primary acceptor. If this is the case, from the kinetics derived from Fig. 3, a and c, we can deduce a rise time of approximately 150 psec for the oxidation of the reaction center bacteriochlorophyll. However, to confirm this, it will be necessary to measure the appearance of the 1250-nm band, since this is generally accepted as being associated with the oxidized reaction center bacteriochlorophyll.

If the proposed P^f state were a pure excited singlet, then the previous 10-psec singlet lifetime estimate made from fluorescent yield experiments (*14*) would have to be more than an order of magnitude in error. A lifetime of several hundred picoseconds would be necessary to explain the relatively small three- to fivefold increase in fluorescence yield on reduction of the primary acceptor. In contrast, Parson *et al.* (*16*) estimated the P^f lifetime to be a few nanoseconds when the primary acceptor was reduced. This estimate should be regarded as an upper limit because activation was performed with a 20-nsec flash. A pure singlet lifetime of several hundred picoseconds corresponds to a quantum yield for oxidation of reaction center bacteriochlorophyll in the 0.6 and 0.8 range. This is less than the value determined by Wraight and Clayton (*17*). At the present, there is no evidence to confirm or rule out with any certainty the various other possible assignments to this state, including the possibility of a triplet, as has been suggested by several workers, or perhaps a charge transfer dimer (*10, 18*) in which the bacteriopheophytin chromophore may actually play a role or undergo an electrochromic shift. We expect that fluorescence decay measurements on photosynthetic bacteria will promote spectroscopic identification of this state.

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A Tunneling Spectroscopy Study of Molecular Degradation due to Electron Irradiation

Abstract. *Electron tunneling spectroscopy has been used to characterize the degradation of β-D-fructose after electron bombardment in a scanning electron microscope. The decrease in intensity of various vibrational bands is correlated with structural changes in the molecule, thereby providing a detailed picture of the degradation process.*

In high-resolution electron microscopy the action of fast electrons on condensed molecular films has been of great interest, since it is now realized that it is specimen degradation and not the resolving power of the instrument that has limited the atomic imaging of biological molecules (*1*). Energy loss spectroscopy (*2*) and mass loss spectroscopy (*3*) have provided some information about changes in molecular specimens under the electron beam. However, changes in the physical structure of the molecules are difficult, if not impossible, to ascertain from such studies. The application of tunneling spectroscopy to molecular degradation induced by electron beams has been suggested as a possibly useful way of examining such changes (*4*); we present here preliminary results that not only identify the changes in the molecular structure but also indicate for a particular electron irradiation what fraction of the molecules has undergone a specific change.

Inelastic tunneling spectroscopy (*5*) reveals the vibrational modes of organic compounds included in the insulating layer of a metal insulator–metal tunneling junction. A vibrational mode of energy $\hbar\omega$ ($\hbar$ is Planck's constant, and ω is the frequency) is observed as a small change (<1 percent) in the electrical resistance of the junction at a voltage $V = \hbar\omega/e$ (e is the electronic charge). Both infrared and Raman active vibrational modes can be observed with a resolution of 16 cm^{-1} at a junction temperature of 4.2 K (4 cm^{-1} at 1 K) over a spectral range of 300 to 4000 cm^{-1}. The effect of sandwiching the molecules between the metal electrodes apparently does not deform the physical structure of the molecules, since the vibrational frequencies from tunneling spectroscopy correspond quite well to those from neat infrared and Raman spectra (*6*).

Crossed film tunnel junctions (Al-Al_2O_3-Pb) were fabricated in a clean, oil-free, high-vacuum evaporator and liquid-doped with β-D-fructose by general procedures described in detail elsewhere (*6*). In outline, a thermally oxidized Al strip (0.2 mm wide) on a glass slide was uniformly doped with a solution of β-D-fructose in water (0.5 mg/ml). After any excess solution had been spun off, the slide was returned to a high-vacuum evaporator where four Pb strips (0.2 mm wide, 2000 Å thick, and 1.5 mm apart) were evaporated across the doped, oxidized Al strip. Thus we obtained four closely spaced junctions, on the same Al strip, with nearly the same characteristics.

Electron irradiations of the junctions (and thus of the sandwiched molecule) were carried out in a scanning electron microscope (ETEC Autoscan). The exposed area was slightly larger than the junction area, thereby ensuring the even exposure of the entire junction. In most cases three of the four junctions on each substrate were given different electron fluences (*7*) and the fourth was left unexposed. Typical irradiation parameters were as follows: electron beam voltage, 30 kev; beam current, 0.1 to