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Picosecond Kinetics of Reaction Centers Containing Bacteriochlorophyll

T. L. Netzel, P. M. Rentzepis, J. Leigh

After photoexcitation of bovine rhodopsin, several intermediates have been identified. The first spectral change, considered to indicate the primary photochemical event leading to geometrical isomerization of the polyene chromophore, has been interpreted as evidence of the formation of an intermediate, prelumirhodopsin. In that study rhodopsin was excited with the second harmonic (530 nanometers) picosecond pulse of a mode-locked neodynium (Nd^{3+}) glass laser. The rate of formation of the metastable isomer, prelumirhodopsin, was detected through the use of the stimulated Stokes Raman emission from benzene at 561 nm. The time-dependent absorption of the 561-nm emission was monitored in the picosecond range by the use of an echelon which consisted of a stack of glass slides arranged in a

The technique of picosecond (*1*) spectroscopy was used to study the ultrafast kinetics of the reaction center protein of *Rhodopseudomonas spheroides* strain R-26 (*2*). Picosecond spectroscopy has provided the means for the direct measurement of kinetics in the picosecond range in several kinds of molecules (*3*). It has been used to measure vibrational relaxation in liquids (*4*) and intersystem crossing in molecules such as benzophenone (*5*). Also, for very fast reactions the technique has yielded simultaneous time and frequency resolved spectra (*6*). To our knowledge the first application of picosecond spectroscopy to a biological system was the direct measurement of the rate of formation and decay of prelumirhodopsin at room temperature (*7*).

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staircase manner. This echelon divided the initial 6-picosecond 561-nm pulse into many incrementally delayed pulses. This made it possible to observe absorbance changes at many time positions, with a single pulse from the Nd^{3+} laser. It was found that the formation of the prelumirhodopsin took place within 6 picoseconds and exponentially decayed with a lifetime of ~ 30 nanoseconds. The limiting factor in these experiments was that the prelumirhodopsin's absorption could not be monitored throughout its frequency width.

In the experiments reported here we have utilized the picosecond continuum (*8, 9*) generated by the intense laser pulse in water to measure the ultrafast kinetics of reaction centers containing bacteriochlorophyll. In this article we present the experimental method and results concerning the picosecond kinetics of the bleaching of the 865-nm absorption band interrogated by the 865-nm spectral portion of the picosecond continuum. A pheophytin band with a maximum at 535 nm was excited with the second harmonic (530 nm) of the mode-locked Nd^{3+} glass laser, and the kinetics of the bleaching of the bacteriochlorophyll band at 865 nm were measured directly in the picosecond range.

The laser oscillator consisted of a Nd^{3+} glass rod (½ × 7 inches), whose ends were cut at Brewster's angle and placed between two reflectors. The oscillator's output was mode locked by bleachable dye (Kodak 9860) in a cell placed at Brewster's angle between the glass rod and the cavity's 100 percent reflector. A train of approximately 120 pulses, separated by nearly 6 nanoseconds, was coupled out through the ~50 percent transmitting front mirror of the oscillator cavity. This horizontally polarized pulse train then passed through a Pockels cell and into a quartz cylinder that was cut so that it would produce a 90° rotation in the polarization of any 1060-nm light passing through it. A prism polarizer placed between the quartz rotator and the amplifier Nd^{3+} (glass rod, ¾ × 12 inches) was oriented to reject vertically polarized light into a pressurized spark gap (nitrogen at ~26 pounds per square inch) which was charged to 16 kilovolts. The "rejected" light pulses caused the spark gap to break down and a high voltage pulse of less than 6 nanoseconds in duration was applied to the Pockels cell, thereby inducing a birefringence. The laser pulse passing through the Pockels cell at this time was rotated by 90° and emerged from the quartz rotator with a horizontal polarization so that it alone was transmitted through the polarizer and into the amplifier rod.

The single extracted pulse was then amplified by a factor of ~15 and sent into an angle-phase-matched KDP (potassium dihydrogen phosphate) crystal generating the second harmonic (530 nm). A dielectric reflector, which reflected light in the 820- to 900-nm region but at the same time allowed 530- and 1060-nm light to be transmitted, was placed after the KDP crystal to eliminate flash lamp or other extraneous light in the 820- to 900-nm region. This provided sufficient 530- and 1060-nm light, but prevented the scattered 864-nm light from interfering with subsequent measurements at these wavelengths. The laser output was now focused into a 20-centimeter-long cell containing water. The interaction of this very intense laser pulse with the medium resulted both in stimulated Stokes and anti-Stokes Raman scattering and in the generation of a broadband continuum, which has been shown (*8*) to have the same time duration as the exciting laser pulse. We have used the 864-nm spectral region of this picosecond continuum as the absorption source to measure the picosecond kinetics of the reaction centers. A beam splitter placed (as shown in Fig. 1) after the H_2O cell reflected the remaining 530-nm light while transmitting the 864-nm interrogating light. The interrogating light monitors the absorbance changes in the sample. The 530-

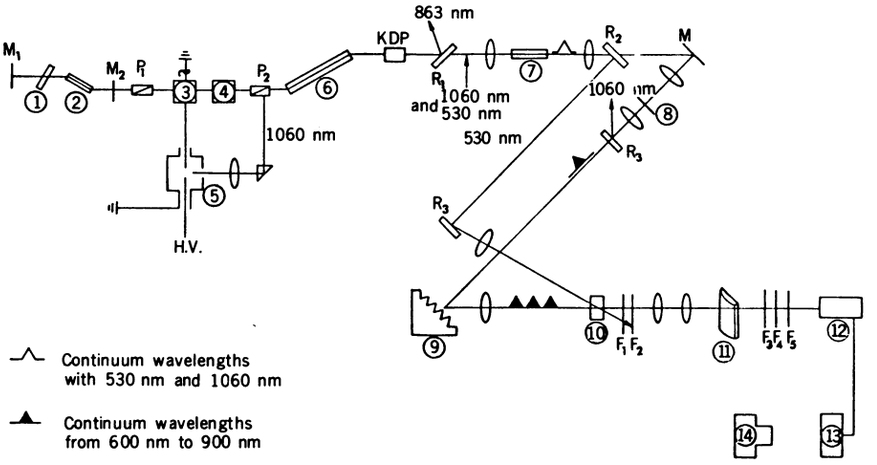
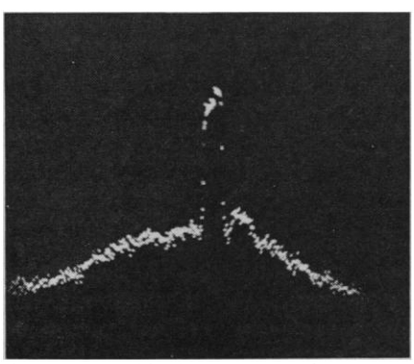


Fig. 1. Experimental apparatus to measure the picosecond kinetics of bacteriochlorophyll-containing reaction centers from *Rhodopseudomonas spheroides* strain R-26 chromatophores. The components are: (1) bleachable dye solution, (2) Nd^{3+} glass rod, (3) Pockels cell, (4) quartz 90° polarization rotator, (5) laser triggered spark gap, (6) Nd^{3+} glass amplifier rod, (7) 20-cm cell containing H_2O, (8) ground-glass "diffuser," (9) reflecting echelon, (10) 1-cm sample cell, (11) cylindrical lens, (12) silicon-vidicon, (13) cathode-ray tube, (14) camera, (M) mirror, (P) polarizer, (R) dielectric reflector, (F) filter, and (HV) high voltage. The angle formed by the exciting and interrogating optical light paths in the sample cell is very small.



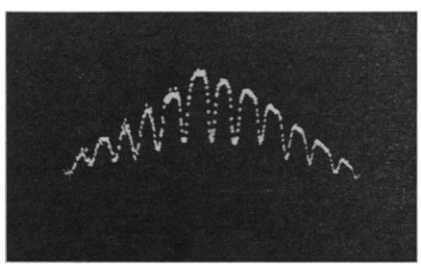


Fig. 2 (left). Photograph showing that only the seventh echelon segment is transmitted (Fig. 3) when the sample is replaced by the carbon sulfide "picosecond light gate." This demonstrates the temporal width as well as spatial coincidence of the exciting and interrogating pulses (~ 6 picoseconds per segment). Fig. 3 (right). Echelon image as detected by the vidicon when no reaction center particles are placed in the sample cell (time proceeds from left to right).

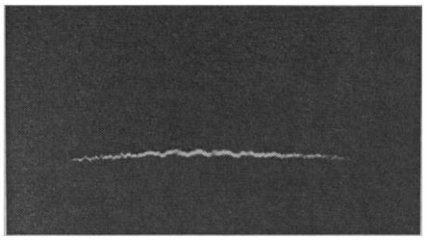


Fig. 4 (left). Echelon image when the reaction centers are present but the 530-nm exciting pulse is blocked from entering the sample cell.

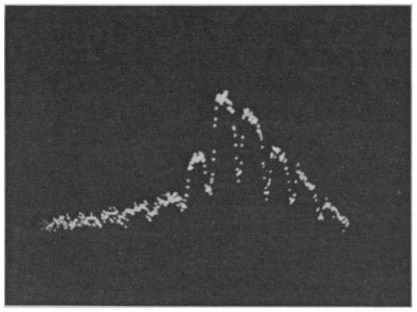


Fig. 5 (right). Echelon image when both the reaction centers and the 530-nm exciting pulse are present. The exciting pulse is coincident with the seventh echelon segment (see Fig. 3). The reaction centers at a concentration of 15 μM were suspended at room temperature in a solution consisting of 0.1 percent LDAO (lauryldimethylamine-*N*-oxide), 10 m*M* tris-Cl at *p*H 8.0. They were isolated from *Rhodopseudomonas spheroides* R-26 with the use of 1 percent LDAO and purified by DEAE cellulose (Whatman DE52) column chromatography.

nm light traversed the optical path designated in Fig. 1 and proceeded into the 1-cm cell containing the reaction center suspension where it initiated the photochemical event. Its arrival in the sample cell determined the zero time ($t = 0$) for the kinetic measurement.

The 864-nm light, meanwhile, followed its own optical path and, by means of a reflecting echelon, was transformed into a set of pulses which could interrogate the reaction centers immediately before and after the excitation by the 530-nm pulse. This transformation was effected by first focusing the 864-nm light onto a ground-glass diffuser to ensure uniform illumination of the echelon (*10*). Then the diffused light pulse was collimated onto a stack of glass slides aluminized on the front surface. The distance of the reflected light is thus increased in constant steps with the net result that the single pulse of the 864-nm light that impinged upon the echelon was transformed into a set of pulses separated from each other by a distance determined by the thickness of the slides. In this experiment, the slides were 1 millimeter thick corresponding to a separation of 6 picoseconds between the interrogation pulses. The 864-nm light from the echelon was then focused into a 1-cm cell containing the suspended reaction centers. The synchronization of the excitation (530 nm) and interrogation pulses was achieved through the use of carbon disulfide in a 1-cm cell, placed between crossed polarizers that were oriented at 45° with respect to the polarization vector of the laser pulse. The shutter checked the temporal coincidence as well as spatial coincidence of the interrogating (echelon pulses) and exciting beams within the sample cell.

The 530-nm exciting pulse induced a transient birefringence in the liquid carbon disulfide (*11*), which has a reorientation time of 1.8 picoseconds. This allows the light from the echelon segment, which is coincident with the exciting pulse, to be transmitted through the crossed polarizers (Fig. 2). The optical paths of the exciting and interrogating portions of the experiment were adjusted so that the seventh segment (slide), as shown from the left in Fig. 3, was coincident with the 530-nm pulse. The zero time of the experiment is therefore at this segment. The detector consisted of a silicon-vidicon photodiode surface, with a quantum efficiency of ~ 60 percent at 800 nm, coupled to an optical multichannel analyzer. A triple lens combination, including a cylindrical lens to collapse the echelon image into a segmented line, transferred the interrogating light that had traversed the sample, onto the vidicon's photodiode surface. The output of the vidicon was displayed on a cathode-ray tube and showed intensity as a function of segment position (6 picoseconds per segment). A set of red transmitting filters was used in conjunction with a narrow-band (12.0 nm) interference filter with peak transmittance at 864 nm to ensure that only the 864-nm light was detected by the optical multichannel analyzer. To prevent 1060-nm light from entering the sample, we inserted a dielectric reflector (> 99.9 percent reflectance from 900 to 1100 nm) between the diffuser and the reflecting echelon.

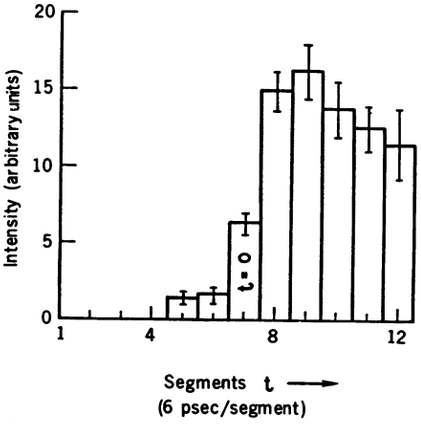


Fig. 6. A histogram of normalized intensities as a function of segment position for the data shown in Fig. 5.

In the case where the experiment was performed without reaction center particles in the cell, 12 echelon segments were transmitted over a period of ~ 70 picoseconds in time (see Fig. 3). When the reaction centers were placed in the sample cell (absorbancy, 1.9 at 865 nm) and the 530-nm exciting pulse was blocked from entering the cell, the 864-nm echelon pulses were absorbed (Fig. 4). The reaction centers of *Rhodopseudomonas spheroides* strain R-26 are known to have an absorption band at 864 nm (*12*). This band bleaches when the reaction center is photooxidized. This experiment measures the ultrafast kinetics of this bleaching. We adjusted the 530-nm pulse to be coincident with the seventh echelon segment from the left and thereby established $t = 0$ (as shown in Fig. 3). The first six segments that traversed the cell before the excitation pulse are absorbed by the reaction centers as expected. This is illustrated by the absence of the first six segments in Fig. 5. The seventh segment, which is coincident with the excitation pulse, is partially absorbed, an indication that the bleaching at 864 nm is not complete within the first 6 picoseconds. After 12 picoseconds maximum bleaching was observed as shown (Fig. 5) by the complete transmission of the eighth echelon segment. We conclude therefore that the 530-nm light absorbed by a bacteriopheophytin chromophore bleached a bacteriochlorophyll band whose maximum is at 865 nm within 7 ± 2 picoseconds.

Discussion

To account for the fact that all of the interrogating echelon segments are not of the same intensity a normalization of the data was performed. Analysis of the deviations in the correction factor values obtained from different echelon images and different baselines

(straight line and peak-to-valley) showed that these errors only slightly affected the final determination of the normalized peak height, as shown by the error bars in Fig. 6. Therefore, we can be confident that the intensity profile of the interrogating pulse train is quite reproducible. Experimental studies have shown that this reproducibility was absent if a "diffuser" was not used. A histogram of normalized intensity as a function of segment position under conditions that resulted in a favorable signal-to-noise ratio is shown in Fig. 6. A slight decrease in intensity of transmitted segments at later times may be indicated.

It is important to realize that the measurements described in this article were made with only a single picosecond pulse. Because the bleaching of the bacteriochlorophyll band at 865 nm (P 865) has a millisecond recovery time, the use of the whole laser pulse train would have shown only that all of the echelon segments could be transmitted when the sample was excited. Therefore no kinetic information could have been obtained. In studies in which an entire laser train is used to excite a sample and subsequent fluorescence in the picosecond range is monitored, great care must be taken to ensure that long-lived intermediates do not contribute artificial results.

This work has enabled us to observe the very first steps in photosynthesis. What was found was that photooxidation of the reaction center is not an instantaneous process when it is excited with green light. Rather an incubation period of a few picoseconds is needed to transfer the energy to P 865. If the bleaching of P 865 is concomitant with the electron ejection, then this work is a direct measure of the rate of photooxidation. However, the strong interactions between the bacteriopheophytins and the bacteriochlorophyll (P 865) are clearly demonstrated. This confirms a general belief that could not be directly substantiated with previous experimental techniques.

Current experiments are oriented toward directly measuring the rate of photooxidation itself by studying the kinetics of the 1250-nm band of these reaction centers. These results in conjunction with those in this article and previously reported quantum yield measurements (*13*) are expected to definitely answer the important question as to whether or not the triplet state of bacteriochlorophyll (P 865) plays an active role in photosynthesis.

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Millikelvin Temperatures Measured with a Noise Thermometer

Brownian motion in electrical circuits has been used to measure temperatures as low as 2 millikelvins.

John C. Wheatley and R. A. Webb

The temperature region below a few thousandths of a degree absolute is distinguished as much by its inaccessibility as by the abundance of important scientific problems present there. Here in particular we refer to the recent observations (*1*) that liquid ^{3}He transforms into new and remarkably different phases over a broad range of pressures at temperatures below 0.0026 K (2.6 mK). These discoveries have emphasized the need for a thermometer capable of accurate measurement of absolute or thermodynamic temperature in this temperature regime. We describe here one approach to the thermometry problem.

There are few thermometers for which the absolute temperature may be obtained from the empirical temperature with confidence and simplicity in the millidegree range. Neither "ideal gas" nor vapor-pressure thermometry is possible since all gases have condensed with negligible vapor pressure at much higher temperatures. Analogs of these methods are possible in principle either by measurement of the osmotic pressure of solutions of ^{3}He in superfluid ^{4}He or by measurement of the melting pressure of ^{3}He, but discussion of these techniques is beyond the scope of this article. Magnetic thermometers, for which the thermometric parameter is the magnetic susceptibility, χ, are used quite commonly. If χ obeys Curie's law, $\chi = C/T$, where C is the Curie constant and T is the absolute temperature, then not only is T determined directly by C/χ but also the sensitivity increases as T decreases. Nuclear magnetism in some metals ought to obey Curie's law in the millidegree range, but nuclear susceptibilities are not as easily measured as electronic susceptibilities, for which the strength of the magnetism is much greater. Measurement of nuclear magnetism usually requires a substantial externally applied magnetic field

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