# PRIMARY EVENTS IN PHOTOSYNTHESIS: PICOSECOND KINETICS OF CAROTENOID BANDSHIFTS IN *RHODOPSEUDOMONAS SPHEROIDES* CHROMATOPHORES

J. S. LEIGH, Jr.*, T. L. NETZEL, P. L. DUTTON* and P. M. RENTZEPIS
*Bell Laboratories, Murray Hill, New Jersey 07974 USA*

Received 16 September 1974

## 1. Introduction

In photosynthetic systems, band shifts of the endogenous bulk carotenoids [see refs. 1–10] have been considered by several authors to be a response to delocalized electric field changes [3–7]. Evidence for this interpretation has come from the induction of the shifts by light [3–5] and diffusion potentials from ion gradients [6,7]. Recently, however it has been shown [8], that carotenoid band shifts could also occur at the more localized *intermolecular* level within the membrane. This finding has recently been extended [see refs. 9,10] by detailed kinetic studies in the microsecond time range.

The rates of the early phases of the carotenoid bandshift in chloroplast membranes have been measured to be faster than 25 nsec [7,11]. In this letter, we report kinetic data of carotenoid bandshifts in *Rhodopseudomonas spheroides* mutant Ga chromatophores in the picosecond ($10^{-12}$ sec) range.

## 2. Materials and methods

*Rhodopseudomonas spheroides* Ga mutant was grown anaerobically in the light on succinate as a carbon source. Chromatophores were prepared by sonication [6] from two-day cultures. Bacteriochlorophyll concentration was estimated from absorbance at 590 nm, assuming $\epsilon = 20\ mM^{-1}\ cm^{-1}$. The dynamics of the carotenoid bandshifts were measured by the methods of Picosecond Spectroscopy [10–12]. The sample was excited by a single 530 nm; 10 psec pulse generated in a phased matched KDP crystal by a 1060 nm pulse from a $Nd^{+3}$ glass, mode-locked laser. It was interrogated by a Stimulated Stokes Raman light generated in a 20 cm cell containing n-octanol. The interrogation pulse train formed by an echelon consists of 9 pulses with an inter-pulse separation of 6.7 psec. It was split by an 8$\mu$ thick pellicle beam splitter into a reference beam, $I_0$, and a measuring beam, I. The intensity of I was approximately twice that of $I_0$.

The physical position of the I and $I_0$ beams is represented in fig. 1. A preselected pulse of the I echelon train was coincident with the photolysis single pulse; thus, the I pulses monitored the changes in optical density that the photolysis pulse generated in the sample cell. $I_0$ passed around the sample and was used to correct for shot to shot laser power variations. This was achieved by combining the two beams $I_0$ and I beyond the sample cell and with common optics imaging one above the other on the entrance slit of a monochromator. After exiting the monochromator, common optics imaged both beams, I and $I_0$, onto a silicon vidicon surface. An SSRI optical multichannel analyzer (OMA) [13] was used to store the resulting signals from the vidicon and display the intensity pattern generated by the two echelon beams on an oscilloscope. Figure 2a is a photograph of this oscilloscope display with the I and $I_0$ echelon patterns shown. Clearly distinguishable are two sets of nine peaks produced by the echelon segments. The left hand side I, passed through the sample solution and the set on the right, $I_0$, transversed the air above the cell. No

* Permanent address: Johnson Research Foundation, University of Pennsylvania, Philadelphia, Pennsylvania 19174 USA.

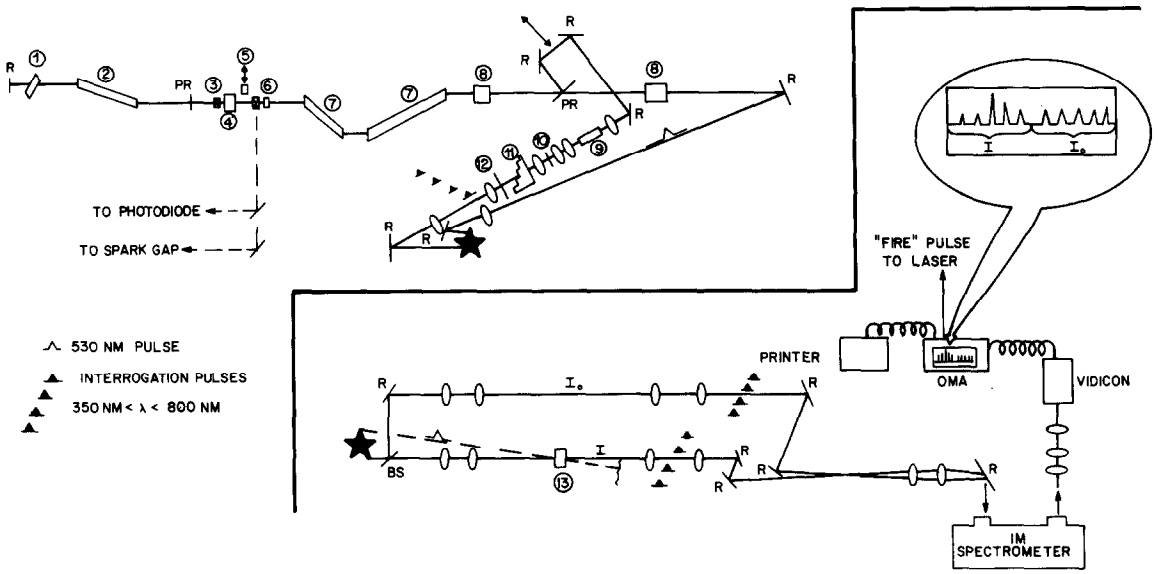


Fig. 1. Double-beam picosecond spectrometer utilizing a silicon vidicon detector. Components: 1). mode-locking dye cell, 2). laser oscillator rod, 3). calcite polarizer, 4). pockels cell, 5). translatable 90° polarization rotator for 1060 nm radiation, 6). fixed position 90° polarization rotator, 7). laser amplifier rod, 8). second harmonic (530 nm) generating crystal (KDP), 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.

530 nm excitation pulse was used for this run. Fig. 2b shows the analogous trace from a run with the 530 nm excitation pulse present. The excitation pulse is coincident with the fourth echelon segment peak of the I set. Changes in absorbance caused by the application of a 530 nm excitation pulse were calculated for each time segment as $\Delta A = -\log \frac{I^e I_0^u}{I_0 e I^u}$. The superscripts, u and e, denote unexcited and excited experimental runs, respectively.

## 3. Results

The optical density changes calculated from data such as that shown in fig. 2 are plotted as a function of time in picoseconds and presented in fig. 3. In this figure, the absorption intensity at 515 nm is seen to reach a maximum within the 10 psec width of the excitation pulse and remain essentially constant for at least 60 psec. This double beam method has made possible a quantitative comparison of the picosecond response with the longer time results in the millisecond and microsecond ranges. The O.D. changes, as a function of wavelength, 20 psec after excitation are plotted in fig. 4. One may note that: 1) the spectrum is similar to that observed in the microsecond and millisecond time ranges except for the increased absorbancy change at longer wavelengths. 2) The absorbancy changes of the picosecond experiments are ~ 20 times greater than that previously reported in longer time experiments. Additions of sodium dithionite (1–50 mM) did not alter the observed changes. On the other hand, preliminary measurements indicated that oxidation of P870 with 5 mM $K_3Fe(CN)_6$ abolished the observed absorbance changes except for a small transient effect. Picosecond studies with chromatophores prepared from the carotenoid-free mutant *Rps. spheroides* R26 did not show any spectral changes, although the concentration of the bacteriochlorophyll was equal to that in the Ga mutant experiments.

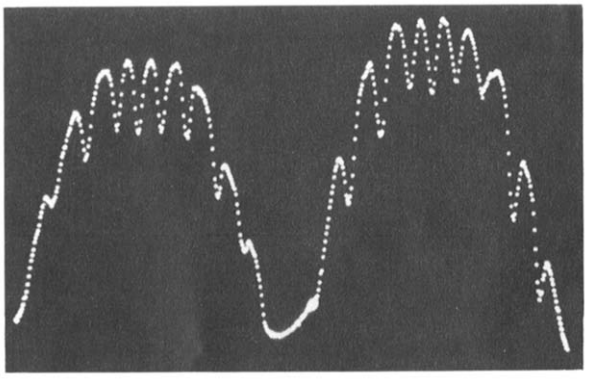

Fig. 2. Typical data from the double beam picosecond spectrometer as displayed on an oscilloscope. The upper photo is from an unexcited sample measurement and consists of two sets of nine peaks. The set on the left, $I_0$, monitors sample absorbance changes while the set on the right, $I_0$, provides a reference beam. The inter-peak time separation is 6.7 psec and the height is proportional to the light transmitted by each echelon segment. The lower photo displays the data for a sample that had the fourth segment of the I beam coincident with a 530 nm excitation pulse.

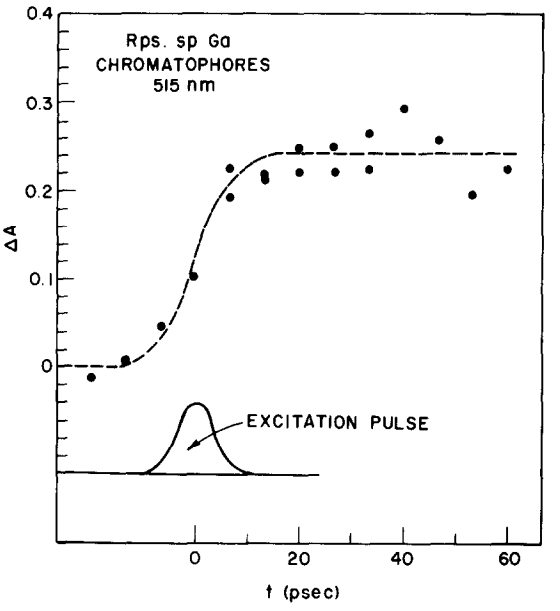


Fig. 3. Typical picosecond kinetic response for *Rps. sp.* Ga chromatophores at 515 nm. The sample was suspended in 10 mm Tris–HCl buffer, pH 8.0; BChl concentration, 150 μm; pathlength, 5 mm; spectral resolution, 2.4 nm; temperature, 22°C.

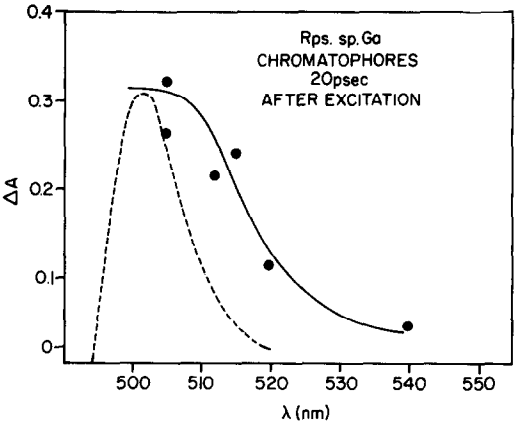


Fig. 4. Partial spectrum of absorbance changes in *Rps. sp.* Ga chromatophores measured 20 psec after excitation. Sample conditions as in fig. 3. Dashed line shows spectrum of carotenoid response measured 4 msec after excitation plotted at 20–fold increased amplitude.

## 4. Discussion

Two previously unknown experimental facts have emerged from this study: the ultrafast (< 10 psec) risetime of the 505 nm to 540 nm absorption changes attributed to carotenoid response, and the extraordinarily large magnitude of the absorbance change of this spectrum. These observations can be viewed as a response of the carotenoids to an induced charge separation generated in the reaction center immediately after picosecond excitation. Similar effects have been previously observed [14] in dimethylaminobenzonitrile whose large dipole ~ 22 Debye is found to induce spectral shifts of several hundred wavenumbers within the 7 psec width of the excitation pulse.

That the observed responses are due to carotenoids is demonstrated by the absence of any spectral changes in the carotenoid-free mutant *Rps. spheroides* R26. Appreciable direct excitation of carotenoids by the 530 nm excitation pulse is not likely because of the very low absorption by the carotenoids of the *Rps. spheroides* Ga mutant at 530 nm. The possibility that the picosecond carotenoid response is caused by interaction of carotenoids with the excited state of antenna bacteriochlorophyll is improbable due to the 'extended' lifetime (> 60 psec) of observed changes.* This interpretation is supported by the fact that oxidation of the reaction center with ferricyanide eliminated the carotenoid response. Thus, the carotenoids seem to be responding to the excitation of reaction center bacteriochlorophyll.

Similarity of the spectral characteristics of the picosecond response and the carotenoid band shifts induced by membrane electric field alterations [1–6] suggests that the picosecond response is similarly caused by electrochromic effects. The extremely rapid (< 10 psec) response may be caused by the generation of electric fields in the initial charge separation act of the excited reaction center bacteriochlorophyll complex (P–P870). Inhibition of electron transfer from P–P870 to the primary electron acceptor by the addition of dithionite does not alter the picosecond response. Thus it appears likely that the initial charge separation act takes place within the P–P870 complex itself, leading to the formation of an oriented $P^+$–$P^-$ state [15].

It is reasonable to identify the $P^+$–$P^-$ state with the reaction center 'triplet state' ($^3$P–P870) previously observed in low temperature ESR studies [16–18]. That is, a $P^+$–$P^-$ 'biradical' state would be expected to have the EPR spectral characteristics of a triplet state. The EPR lineshape may be analysed to obtain an average distance between the two spins. This leads to an estimation of ~ 4 Å for the 'average distance'* in the initial charge separation act.

Electric fields generated by the formation of the $P^+$–$P^-$ state should correspond to that of a unit dipole with a separation of ~ 4 Å in a medium of dielectric constant, $\epsilon \sim 2$, corresponding to the approximate electronic polarizability of the reaction center–membrane complex. The magnitude of these fields would be quite large, for example, fields of $\sim 3 \times 10^6$ V/cm would be expected at a distance of 20 Å from such a dipole. The instantaneous electronic polarization of the dielectric medium would be followed by molecular reorientations within the reaction center–protein complex and other nearby membrane components occurring in the nanosecond time range. During this period the effective field magnitude would decrease appreciably as the membrane dielectric became fully polarized, leading to the smaller carotenoid shifts which are found in the microsecond time range. It is possible that the electron transfer to the primary acceptor accompanies this form of dielectric relaxation. A large portion of the photon energy is 'stored' as dielectric polarization energy. A possible direct coupling of this dielectric energy to the free energy conservation (ATP synthesis) is an intriging possibility.

The magnitude of the carotenoid shifts ($\Delta A/A > 0.2$) with picosecond excitation indicates that a rather large portion, more than 20%, of the carotenoids are affected. This new data implies that at least that percentage of the carotenoids are 'bound' close to if

* The possibility that the carotenoids are responding to a higher level metastable state of the antenna bacteriochlorophyll complex has not been completely ruled out. Such a state might be induced by multi-photon processes caused by the brief but very intense laser excitation.

* The estimate of average distance between the $P^+$–$P^-$ charges is based upon observed zero-field splittings. Zero-field splitting caused by electron dipole–dipole interaction varies with the mean inverse cube of the distance between the two spins. Since these calculations tend to overemphasize close approaches of the two spins, it is likely to be a slight underestimate of the 'average distance'.

not directly to the reaction center protein in *Rps. spheroides* Ga. The actual magnitude of the bandshift seems to be about 10–20 nm. If the same number of carotenoids are involved in both microsecond and picosecond studies then the shift in the microsecond time range should be less than ~ 1 nm. These estimates agree quite well with the measurements of Schmidt et al. [21,22] on electrochromic effects of carotenoids in lipid multilayers. These model carotenoid shifts were quadratic in electric field strength. In order to explain the apparent linearity of the carotenoid bandshifts induced by imposed diffusion potentials [6,7], Schmidt et al. proposed the existence of a permanent 'bias' electric field in the chromatophore membrane. Using estimates of a 'bias' electric field of $\sim 2 \times 10^6$ V/cm in the unexcited ('resting') chromatophore membrane [21,22], one may estimate that the picosecond carotenoid response described here corresponds to an increase in the electric field by $2.6 \times 10^6$ V/cm as compared with $0.2 \times 10^6$ V/cm observed in the microsecond time range.

## References


[1] Smith, L., Baltscheffsky, M. and Olsen, J. M. (1960) J. Biol. Chem. 235, 213–218.
[2] Baltscheffsky, M. (1967) Biochem. Biophys. Res. Commun. 28, 270–276.
[3] Fleischman, D. E. and Clayton, R. K. (1968) Photochem. Photobiol. 8, 287–298.
[4] Junge, W. and Witt, H. T. (1968) Z. Naturforsch 23b, 244–254.
[5] Jackson, J. B. and Crofts, A. R. (1971) Eur. J. Biochem. 18, 120–130.
[6] Strichartz, G. and Chance, B. (1972) Biochim. Biophys. Acta 256, 71–84.
[7] Jackson, J. B. and Crofts, A. R. (1969) FEBS Lett. 4, 185–189.
[8] Dutton, P. L. (1971) Biochim. Biophys. Acta 226, 63–80.
[9] Jackson, J. B. and Dutton, P. L. (1973) Biochim. Biophys. Acta 325, 102.
[10] Case, G. D. and Parson, W. W. (1973) Biochim. Biophys. Acta 325, 441.
[11] Wolff, C., Buchwald, H. E., Ruppel, H., Witt, K. and Witt, H. T. (1969) Z. Naturf. 24b, 1038.
[12] Rentzepis, P. M. (1973) Adv. in Chem. Phys. 23, 189.
[13] Netzel, T. L., Struve, W. S. and Rentzepis, P. M. (1973) Ann. Rev. of Phys. Chem. 24, 473.
[14] Busch, G. E., Jones, R. P. and Rentzepis, P. M. (1973) Chem. Phys. Lett. 18, 178.
[15] Netzel, T. L., Rentzepis, P. M. and Leigh, J. S. Jr. (1973) Science 182, 238.
[16] Struve, W. S., Rentzepis, P. M. and Jortner, J. (1973) J. Chem. Phys. 59, 5014.
[17] Katz, J. J. (1971) Conf. on Primary Photochem. of Photosyn., Argonne.
[18] Dutton, P. L., Leigh, J. S. Jr. and Seibert, M. (1972) Biochem. Biophys. Res. Commun. 46, 406–413.
[19] Wraight, C. A., Leigh, J. S. Jr., Dutton, P. L. and Clayton, R. K. (1974) Biochim. Biophys. Acta 333, 401–408.
[20] Leigh, J. S. Jr. and Dutton, P. L., Biochim. Biophys. Acta (in press).
[21] Schmidt, S., Reich, R. and Witt, H. T. (1971) Proc. IInd Intern. Congr. on Photosyn., Stresa.
[22] Schmidt, S. and Reich, R. (1972) Ber. Bunsenges. Physik. Chem. 76, 1202–1208.