

PUMP PROBE ANISOTROPY STUDIES OF THE PHOTOSYNTHETIC REACTION CENTER

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(Received 8 April 1997)

The photosynthetic reaction center of *Rb. sphaeroides* is investigated by unique pump-probe anisotropy methods using 13 fs optical pulses tunable throughout the near IR absorption band. The time and wavelength resolved pump probe anisotropy transients yield detailed experimental descriptions of the energy levels, P_{y^+} and P_{y^-} of the excitonically coupled special pair (P), and dynamics prior to charge separation, particularly energy transfer from the accessory bacteriochlorophylla's (B) to the special pair (P).

Keywords: Photosynthesis; energy transfer; anisotropy

Although much work has focused on the dynamics prior to and during charge separation in the photosynthetic reaction center [1], many issues remain unresolved, including the nature of the upper excitonic state of the electronically excited special pair (P_{y^+} , where P is the special pair ground state) and its influence on energy and electron transfer [2, 3]. In this report, we present “two color” wavelength resolved pump-probe anisotropy measurements [4] of the photosyn-

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thetic reaction center (RC) of Rhodobacter Sphaeroides at room temperature. The major Q_y absorption bands are collectively excited using short (13–18 fs) pulses with a broad spectral range, or individually excited with longer duration, spectrally narrowed pulses that overlap with the transition(s) of interest. A broad-band probe pulse is used to record the response of the system with respect to wavelength, time, and polarization.

Four distinct contributions to the wavelength resolved anisotropy measurements are observed: (i) ground state bleach and (ii) stimulated emission components from B and P, (iii) excited state absorption from P, and (iv) a newly identified narrow bleach/stimulated emission band

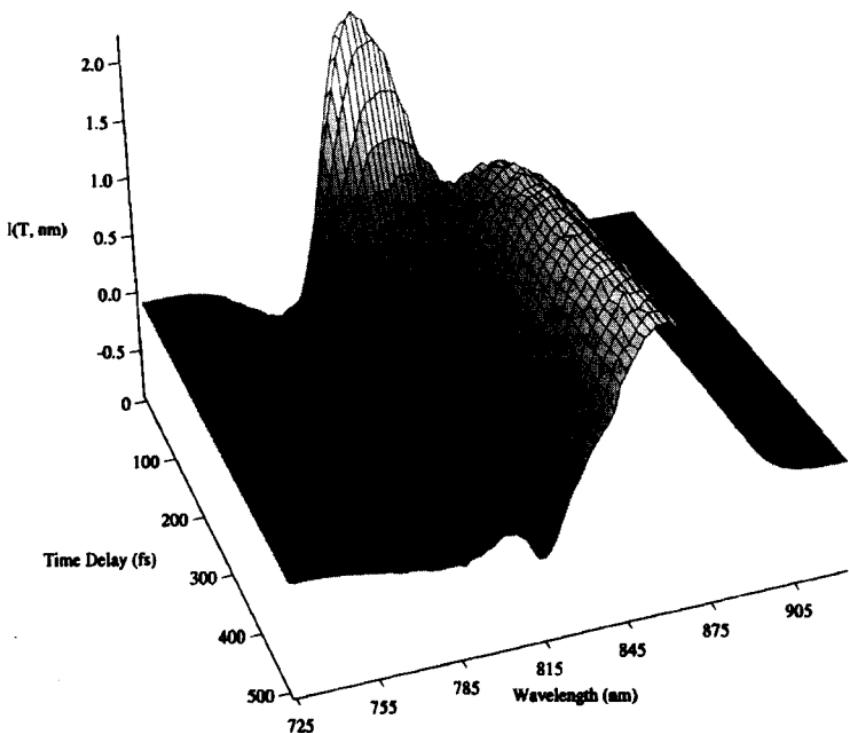


FIGURE 1 The entire temporal and spectral response of the photosynthetic reaction center of Rb. Sphaeroides following excitation of the accessory bacteriochlorophyll pigments (B). Here, a positive signal indicates a transient bleach, while a negative signal indicates a transient absorption. The surface plot shows the rapid decay of a bleach at 800 nm due to B, and a corresponding increasing bleach at 860 nm from P_y . A smaller component at about 825 nm, between these two dominant features, can also be observed and is associated with P_{y+} . The pump-probe relative polarization is at the magic angle.

at 825 nm, identified as P_{y^+} . This latter spectroscopic feature appears immediately upon exclusive (*i.e.*, spectrally narrowed) excitation of P_{y^-} and yields anisotropy values consistent with a transition dipole moment rotated 70° from the $P - P_{y^-}$ transition moment, in agreement with low temperature results for P_{y^+} [5, 6]. The absorption maximum of this band, however, occurs at longer wavelengths (825 nm *vs.* 810 nm at low temperature).

The wavelength resolved magic angle transient observed after excitation of B is shown in Figure 1. This three-dimensional plot clearly shows the rapid decay of the optically excited state of B (B^*) and the subsequent rise of P_{y^-} following an energy transfer event. The three-dimensional data are analyzed using *experimentally determined*

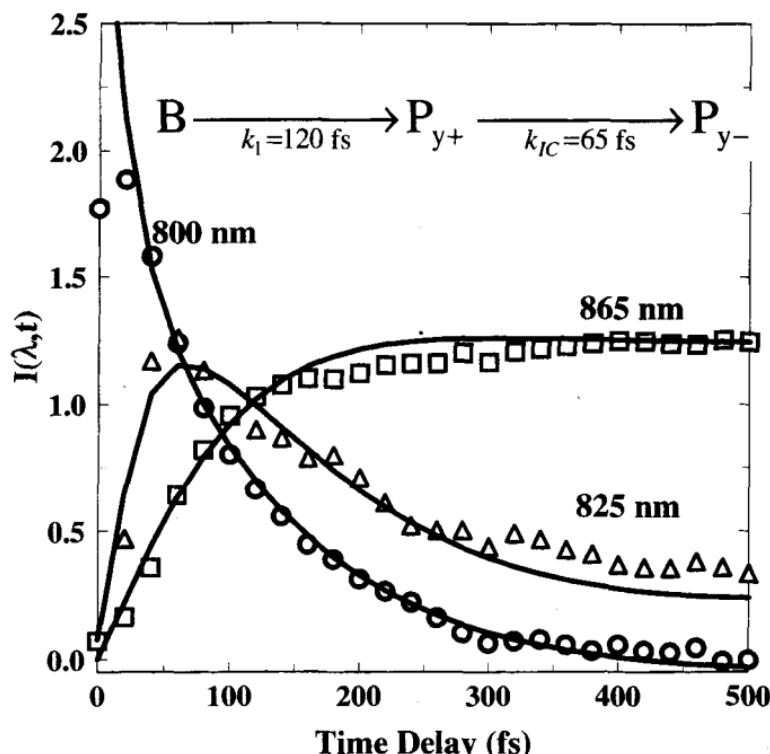


FIGURE 2 Kinetic analysis of the wavelength resolved magic angle pump probe spectra obtained after excitation at 800 nm. The model consists of a two-step energy transfer mechanism, $B \rightarrow P_{y^+} \rightarrow P_{y^-}$, where the two steps occur with $k_1 = 120$ fs and $k_{IC} = 65$ fs time constants, respectively, (where IC refers to internal conversion of P) and the spectra for each species are experimentally determined. Experimental data (symbols) and the calculated fits (solid lines) are shown for 800, 825 and 865 nm.

spectra and anisotropy values for B, P_{y^+} , and P_{y^-} . The detailed structure of the data is satisfactorily fit with a model wherein a very rapid red shift of the initially prepared excited state is followed by energy transfer from B to P_{y^-} with P_{y^+} serving as the intermediate. The results of this kinetic analysis are shown in Figure 2. The resulting time constants for the two steps of energy transfer are 120 fs for $B^* \rightarrow P_{y^+}$ and 65 fs for internal conversion of $P(P_{y^+} \rightarrow P_{y^-})$, the latter of which is independently measured by excitation of P_{y^+} . These time constants are substantially different than those reported by Jonas *et al.* [2]. The fast time constants for energy transfer imply substantial electronic overlap between B and P_{y^+} . Therefore, a supermolecular picture of the reaction center chromophores, *i.e.*, one in which the initial excitation may be delocalized over more than one cofactor, cannot be ignored [6, 7].

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