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Published in:
Journal of the American Chemical Society

DOI:
10.1021/ja3065478

2012

Document Version:
Peer reviewed version (aka post-print)

Coherent picosecond exciton dynamics in a photosynthetic reaction center

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KEYWORDS: quantum coherence, bacterial reaction center, photosynthesis, multidimensional spectroscopy.

ABSTRACT: Photosynthetic reaction centers convert sun light into a transmembrane electrochemical potential difference, providing chemical energy to almost all life on earth. Light energy is efficiently transferred through chromophore co-factors to the sites with the lowest transition energy, where charge separation occurs. We apply two-dimensional electronic spectroscopy to assess the role of coherences in the photoresponse in the bacterial reaction center of *Rhodobacter sphaeroides.* By controlling the polarization of the laser beams we are able to unambiguously assign oscillatory dynamics to electronic (intermolecular) coherences. The data shows that these coherences are sustained for more than one picosecond, indicating that the protein coherently retains some excitation energy on this timescale. Our finding provides a mechanism for effective delocalization of the excitations on the picoseconds timescale by electronic coherence, setting the stage for efficient charge separation.

Photosynthesis provides energy to most life on earth by conversion of sun light into chemical energy. The light is absorbed by pigment-rich antenna proteins and transferred to reaction center proteins, where charge separation occurs. All photosynthetic reaction centers contain a conserved functional core, which in this study is represented by the reaction center of the purple bacteria *Rhodobacter sphaeroides* (RC_{sph}). The RC_{sph} comprises, among other cofactors, four bacteriochlorophylls and two bacteriopheophytins. These chromophores form an assembly (see Figure 1a), with three distinctive absorption bands peaking at 760 nm, 805 nm and 860 nm (Figure 1b, red line), respectively. We use H, B, and P to denote the excitonic states that give rise to these bands and whose major contributions are from the bacteriopheophytins, the accessory bacteriochlorophyll, and the dimeric bacteriochlorophyll (spatial pair), respectively. It is generally accepted that the photoexcitations are transferred from H over B to P within 200 fs.[1] Subsequently, charge separation occurs within a few picoseconds.[2]

In order to further elucidate the photoresponse of photosynthetic reaction centers, it is important to assess the role of quantum coherences (superpositions) between the excited states. This has been made possible by the advent of two-dimensional (2D) optical spectroscopy in the visible spectral range.[3,4] Excited state coherences were first studied in photosynthetic antenna proteins and have been shown to live for several hundreds of femtoseconds in complexes from bacteria,[5,6] higher plants,[7] and marine algae.[8] The implications of these findings for the photophysical function of the proteins are today vividly debated. Some theoretical studies suggest that coherent, wavelike motion of the excitations may be responsible for the high quantum yield of excitation energy transfer among the antenna pigments,[9,10] but the mechanism causing the long-lived quantum coherences is today not well understood.[11-16] For reaction center proteins, information on quantum coherence dynamics is sparse. In the reaction center of photosystem II, electronic coherences could not be assigned unambiguously by the 2D electronic spectroscopy.[17] For RC_{sph}, a two-color photon echo experiment has indicated a decay time of 440 fs for coherent interaction between the B and the H exciton at 77 K,[18] but a direct observation of the electronic coherence by oscillatory dynamics has so far remained elusive. It is therefore highly desirable to directly assess the electronic coherences in reaction center proteins.

In this work we use 2D electronic spectroscopy to simultaneously probe the population and coherence dynamics of excitations in detergent solubilized RC_{sph} with a chemically oxidized P at 80 K. The absorption spectrum of this sample is shown in Figure 1b, blue line. The chemical modification blocks charge transfer and strongly reduces the absorption strength of P,[19] but leaves B, H, and the energy transfer to P unaffected.[20] A typical 2D spectroscopy map at a waiting time t_2 = 40 fs is shown in Figure 2a. The B and H bands are clearly observed on the diagonal, as is the cross-peak (marked 'HB') below the diagonal. The corresponding upper cross-peak is masked by the negative excited state absorption signal from B.

The 2D spectroscopy experiment can probe the evolution of populations, vibrational (intramolecular) coherences, or electronic (intermolecular) coherences as a function of t_2. Population dynamics give rise to smoothly evolving signals, whereas coherences are observed as oscillatory signals. Typically, this leads to convoluted traces, where vibrational and electronic coherences as well as population dynamics contribute and which are difficult to assign. An example is seen in Figure 2b, where the t_2-dependencies of the H- and B-diagonal peaks and the HB lower cross-peak (all-parallel polarization conditions) reveal a decay of populations overlaid with small oscillatory signals.

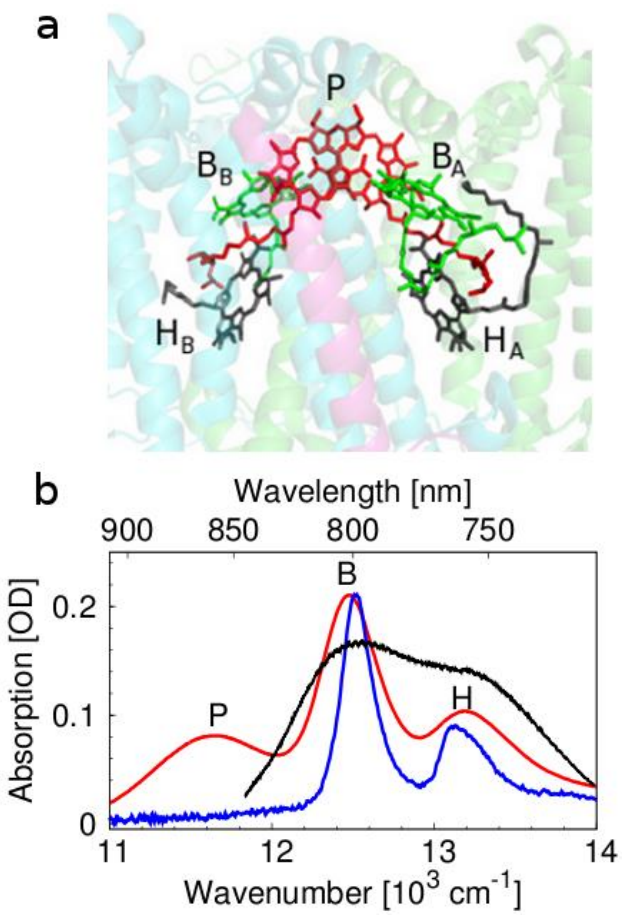

Figure 1. Structure and absorption spectra of RC_{sph}. (a) The molecular arrangement of H, B and P (see text for abbreviations) in the RC_{sph} binding pocket. (b) The linear absorption spectra of RC_{sph} at 294 K (red) and RC_{sph} with oxidized P at 80 K (blue) are shown together with the laser spectrum (black).

In order to experimentally identify coherences with electronic character, we use a special combination of linearly polarized pulses that dramatically suppresses all other but oscillating intermolecular signals.[7] This strategy is borrowed from 2D infrared spectroscopy, where a number of polarization schemes are used to suppress or enhance certain pathways.[21,22] In our configuration, the polarization orientation of the pulses 1 to 4 was set to $\pi/4$, $-\pi/4$, $\pi/2$, and 0, respectively. This selects, after orientational averaging, only those interaction pathways which evolve during t_2 as a superposition of two excited states with different transition dipole moment orientations, which we term 'intermolecular' or 'electronic' coherences in the remainder of the manuscript. The configuration strongly suppresses population dynamics and coherences between vibrational states, termed 'intramolecular' or 'vibrational' coherences, because these evolve as a superposition of excited states with parallel transition dipole moments during t_2. Taking into account all experimental considerations in the measurements discussed here, we estimate the suppression ratio of the population dynamics and vibrational coherences to be ~85 in the ($\pi/4$, $-\pi/4$, $\pi/2$, 0) polarization configuration compared to the all-parallel configuration.

The effectiveness of this acquisition strategy is documented in Figure 2b, where the t_2-dependence of the HB cross-peak is shown for the ($\pi/4$, $-\pi/4$, $\pi/2$, 0) and the all-parallel polarization configurations as magenta and green traces, respectively. Clearly, populations dynamics are suppressed in case of the ($\pi/4$, $-\pi/4$, $\pi/2$, 0) configuration, where an offset-free oscillating signal is observed. This signal must be from a coherence that involves two excited states with different transition dipole orientations and we therefore assign it to coherences with electronic character. Furthermore, the Fourier transforms of these kinetics, shown in Figure 2c, reveal 645 cm^{-1} as the major frequency component for both the ($\pi/4$, $-\pi/4$, $\pi/2$, 0) and the all-parallel polarization conditions. This frequency corresponds exactly to the difference between the transition energies of B and H, further strengthening our assignment.

Figure 2d shows the Fourier transform amplitude of the oscillatory signals in the decay of the B-diagonal peak, measured with all-parallel pulse polarizations. Oscillations are observed at 90 cm^{-1}, 190 cm^{-1}, 220 cm^{-1}, 310 cm^{-1}, 390 cm^{-1}, and 710 cm^{-1}. All these modes have vibrational origin, as they match quite well published resonance Raman frequencies of B.[23] The peak at 575 cm^{-1} is observed in all measurements presented in Figure 2c and Figure 2d and is thus likely of mixed vibrational and electronic origin.[12] A more detailed analysis of these vibrational coherences will be presented in a forthcoming publication.

In summary this analysis shows that the t_2-dependence of the lower cross peak HB, measured with ($\pi/4$, $-\pi/4$, $\pi/2$, 0) polarization conditions, is a direct and clean signature of coherence beatings between H and B with electronic character. Remarkably, this coherence lives significantly longer than the 1 ps time window probed here (Figure 2b, magenta trace) and its lifetime exceeds the 440 fs reported earlier based on the two-color photon echo experiment.[18,24] In contrast to the long lived coherence between H and B, Figure 2b also illustrates that most of the population on H and on B decays with an effective time constant of ~94 fs and ~151 fs, respectively, in agreement with previous reports.[20] Clearly, the observed coherence between H and B has a much longer decay time than the population dynamics of H and B.

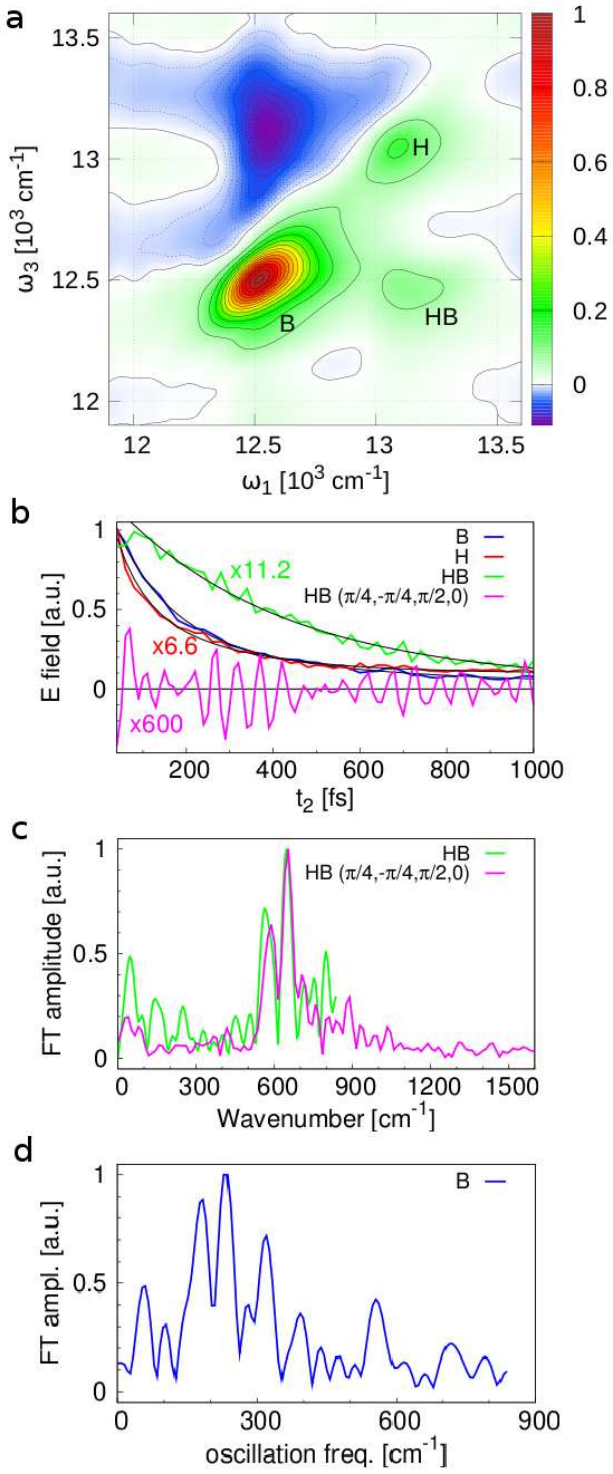

Figure 2: Two-dimensional absorption spectroscopy of oxidized RC_{sph} at 80 K. (a) A representative 2D spectrum at waiting time t_2 = 40 fs is shown. ω_1 and ω_3 are the Fourier transform frequencies of the coherence and detection time (t_1 and t_3), respectively. (b) The integrated intensity of the diagonal peaks H and B and the cross peak HB are shown as a function of t_2. The grey lines are fits of multiexponential decay functions to the data. The effective decay times calculated from the two major decay rates are 94 fs and 151 fs for H and B, respectively. (c) The Fourier spectrum of the t_2-dependence of the integrated lower cross-peak HB is shown for all-parallel and ($\pi/4$, $-\pi/4$, $\pi/2$, 0) polarization conditions. (d) The Fourier spectrum of the t_2-dependence of the integrated diagonal peak B is show. All panels: if not indicated otherwise in the figure legends, all beams were polarized in parallel.

This finding is unexpected. Considering the total molecular system dynamics in terms of population relaxation rates (γ_H and γ_B) and coherence dephasing rates, the total HB coherence dephasing rate is given by

$$\Gamma_{BH} = \frac{1}{2}(\gamma_H + \gamma_B) + \Gamma_{BH}^{pure}, \qquad \text{(eq 1)}$$

where Γ_{BH}^{pure} is the pure electronic dephasing rate of the electronic coherence between H and B. From this argument it follows that the observed long-lived coherence between H and B cannot survive without corresponding excitation populations on H and B. We note that this statement reflects a very general property of the reduced density matrix of molecular aggregates, σ_{ij}, where off-diagonal and diagonal elements represent coherences and populations on sites *i,j*. By definition,

$$\sqrt{\sigma_{ii}\sigma_{jj}} \geq \left|\sigma_{ij}\right|, \qquad \text{(eq 2)}$$

further demonstrating that coherences cannot exist without corresponding populations.[25]

Indeed, close inspection of the population decays (Figure 2b, see also Figure 2 in Ref. 20) reveals small, but long-lived (more than a picosecond) population components on both H and B. This behavior is surprising because the driving force for excitation energy transfer (EET) from H to B and P vastly exceeds the available thermal energy. Within the framework of incoherent EET, Boltzmann statistics would therefore predict negligible back transfer from P to H and B, and the populations of H and B should decay to 0 within the time window probed. This, together with our finding of the long-lived coherences between H and B, shows that EET to P is incomplete. The protein coherently retains some excitation energy on higher energy chromophores on a picosecond timescale.

For molecular aggregates in the weak or intermediate coupling regime, electronic coherence effectively delocalizes excitations over one or several chromophores, whereas dephasing leads to localization of the energy. This is exemplified in multichromophoric light-harvesting proteins, where long-lived electronic coherences have previously been observed.[5-8] In contrast to the findings for antenna proteins, we find here that electronic coherences apparently outlive the majority of excitation populations and that this leads to incomplete energy transfer. We therefore suggest that the protein uses long-lived electronic coherences to delocalize excitation energy over H, B, and likely P on picosecond timescales. This mechanism is in agreement with efficient charge generation in the wild type RC_{sph}, because intermolecular excited state delocalization benefits efficient charge transfer.

ASSOCIATED CONTENT

Supporting Information

Supplementary information on experimental details and the protocol for the overproduction and purification of RC_{sph} is supplied in a separate file. This material is available free of charge via the Internet at http://pubs.acs.org.

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ACKNOWLEDGMENT

SW and DZ acknowledge financial support from the Swedish Research Council. The authors thank Prof. R. Neutze for access to the SweGen protein production facilities and the protocols for the overproduction procedure.

ABBREVIATIONS

RC_{sph}: Reaction center protein from *Rhodobacter sphaeroides.*

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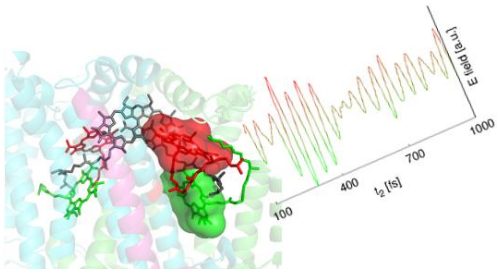

Figure TOC

Supplementary Material for

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by

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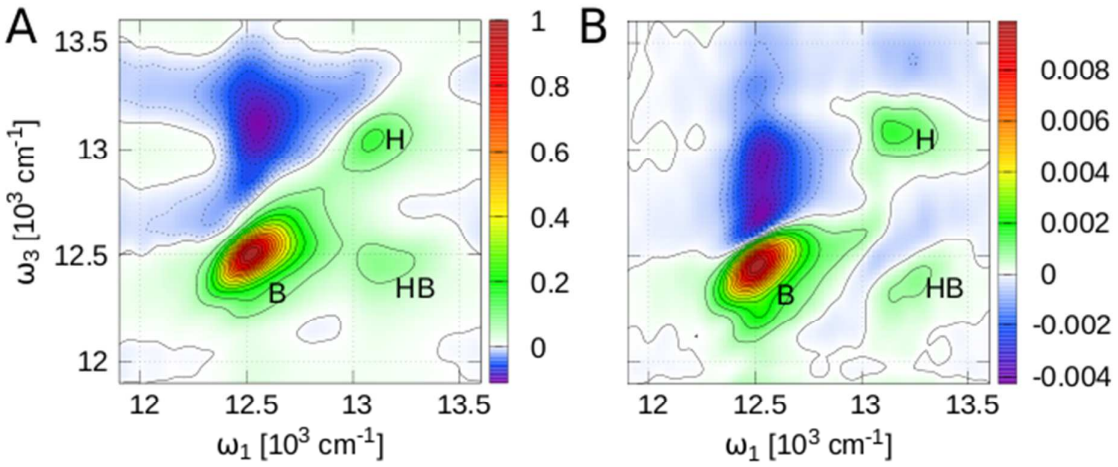

Fig. S1: Two-dimensional absorption spectroscopy of oxidized RC_{sph} at 80 K – comparison of polarization conditions. **A** is the same as Fig 2a of the main paper and **B** is a two-dimensional absorption spectrum recorded with the (*π/4, -π /4, π/2, 0*) polarization arrangement at t_2 = 40 fs (**B**). Note that the signal strength for data recorded with the (*π/4, -π /4, π/2, 0*) polarization arrangement is much weaker than signal recorded with the pulses polarized all parallel.

2D electronic spectroscopy

The double frequency modulation electronic 2D spectrometer used in the experiments is described in Ref. *2*. Shortly, it is based on the KGW amplified laser system ”Pharos” (Light Conversion Ltd) operating at 20 - 200 kHz repetition rate. The amplifier pumps a home-built non-collinear optical parametric amplifier (NOPA) producing pulses centered at 770 nm with a pulse length of 17 fs FWHM. The NOPA output is split into four beams, which are arranged in a boxcar geometry. The population delay t_2 is controlled by a mechanical delay line and the coherence time delay t_1 is controlled by inserting fused silica wedges into the first and second beams. These beams are chopped separately by two optical choppers at different frequencies, while the signal is detected at the difference and sum frequencies of the choppers. In order to set the beam polarizations independently, we use an achromatic quarter-wave plate together with four wire grid polarizers (typical contrast ratios >800) – one in each of the beams. We estimate the accuracy of the polarization to be $\pm 1°$.

All four beams were focused and overlapped in the sample that was contained in a 0.5 mm optical path demountable cell and cooled to 80 K in a continuous-flow cryostat (Janis). The third order polarization signal emitted from sample in the photon-echo phase matching direction is heterodyned with the fourth beam and spectrally resolved in a spectrograph. The resulting interferograms are detected by a CCD camera (PIXIS, Princeton Instruments). The 2D spectra are extracted from the raw data by procedures outlined in (3). Linear absorption spectra were recorded before and after the measurements to monitor the photo-stability of the samples.

Preparation of the reaction centers (RC)

The RC from *Rb. sphaeroides* R-26 were prepared as described previously (4) with the following modifications. The membrane solubilisation was performed for 10 min at room temperature, in darkness and a N,N-dimethyldodecylamine N-Oxide (LDAO) concentration of 0,8% was used. Furthermore, the RC solution was desalted prior to loading it onto the DEAE column, which was eluted with a continuous NaCl gradient. For the spectroscopy measurement the RC concentration was approximately 2 mg/ml in 10 mM Tris-HCl, 1 mM EDTA, 0.1% LDAO, pH 8 buffer. The oxidizing agent, $K_3Fe(CN)_6$ and glycerol concentrations were 150 mM and 85% (v/v), respectively.

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