

BATH FLUCTUATIONS FROM OPTICAL COHERENCE: LIQUIDS AND PROTEINS

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ABSTRACT

The vibrational and optical coherence responses of bacteriochlorophylla (BChla) in solution and within the bacterial reaction center protein of *Rh. Sphaeroides* are reported. Fourier transform analysis of the optically impulsively prepared vibrational coherences of BChla in solution show features in the 350-1300cm⁻¹ spectral range while the analogous response of the accessory bacteriochlorophyll's (B) in the protein exhibit the same features as well as low frequency modes in the 50-350cm⁻¹ region. A kinetic analysis of the response in the spectral region associated with B exhibits both prompt decay features and a 2.8ps rise. A dominant vibrational mode is observed in the pump-probe response of the special pair (P) that is presumably associated with vibrational motion of the dimer. 2-pulse echo signals from BChla in solution and from P in the protein both decay exponentially. The associated homogeneous linewidth obtained for P is in good agreement with low temperature hole burning results.

1. Introduction

Insight into the role of the bath on the function of biological molecules in their native (i.e. protein) environment can be obtained through comparative studies of the chromophore(s) in another environment. The (bacterio-)chlorophyll chromophores carry out their function of energy or charge transport in rather heterogeneous and structured protein environments.^{1,2} Specific polar and nonpolar bath interactions may be very important for proper function³⁻⁵ but can also complicate interpretation of the system-bath interaction. The molecule bacteriochlorophylla (BChla) functions in the light harvesting and energy transport processes of photosynthesis and also in a highly concerted way in bacterial reaction centers, where energy is converted into a trans-membrane potential.⁵ The initial charge separation and electron transfer process(es) occur on ultrashort timescales. The most selective part of this process, which is complete in about 3ps, involves charge separation and electron transfer down the L-side reaction center pigments [special pair dimer of BChla molecules (P), accessory BChla (B) and pheophytin (H)].⁶ The high quantum yield (almost 100%) and the large distance between P and H_L suggests the involvement of B_L in the charge transfer process. Elucidation of the spectral density of system-bath interactions of BChla's in both liquid and protein environments would be useful for better establishing the role of the protein in the reaction center's function.

Photon echo and pump-probe measurements reflect the evolution of optical and vibrational coherence of the chromophore along time axes τ and T, respectively.⁷ The variable τ refers to the interval between the first two matter-radiation field interactions and T the interval between the second and third. Recent 2-pulse,^{8,9} stimulated photon echo¹⁰⁻¹² and time-gated echo studies¹³⁻¹⁴ have shown the utility of these techniques to extract the spectral density^{9,10,12} and/or the correlation function^{11,13} describing system-bath interactions for various chromophores in liquids. In this paper we present third-order polarization responses of BChla monomers in pyridine and THF solutions, and from B and P in the photosynthetic reaction center of *Rh. Sphaeroides*. The echo decays are compared with the results of hole burning studies⁴ while the vibrational coherence responses are compared with resonance Raman results¹⁵ and other temporal studies.^{16,17}

2. Experimental

The short pulse source is a home-built cavity-dumped Kerr lens modelocked Ti:Sapphire laser¹⁸ of a unique design¹⁹ producing ≤ 13 fs duration gaussian pulses with 90–100 nm spectral bandwidth and 40 nJ energies. The light is split into three beams and focused onto the sample with all reflective optics. The sample is contained in a 0.5 mm path length spinning cell and the echo signal is detected in the $k_s = k_3 + k_2 - k_1$ direction. The pump-probe signal is in the forward direction of the k_2 beam with the third beam blocked. The system temporal response and the experimental zero-of-time are determined from the nonresonant scattering response of the respective pure solvents.

3. Results and Discussion

Figure 1 shows the $|P^{(3)}(t_1=0, t_2=0, t_3)|^2$ response of BChla in THF. (Each time variable refers to the delay of the respective optical field or pulse with respect to the zero of time.) Vibrational coherences are impulsively driven and detected in the nonlinear optical response; the Fourier absolute magnitude spectrum is shown in the inset. The vibrational frequencies observed in the figure (340, 460, 560, 740, 786, 900 and 1170 cm^{-1}) are in good agreement with line positions obtained from cw-Raman measurements.²⁰ The time constants for vibrational dephasing, determined by singular value decomposition analysis, range from 0.5 to 1.5 ps increasing with vibrational frequency. The vibrational dephasing reflects the force autocorrelation function of the bath projected onto the vibrational modes. Clearly, the timescale for vibrational dephasing is much shorter than the relaxation of the photo-bleach/stimulated emission²¹ contribution to the signal (i.e. dc-offset). The notable feature is the absence of low frequency vibrational modes, an effect observed by others using longer duration pulses (30–50 fs).^{21,22} Similar results are obtained in pyridine solution.¹² It is known that both THF and pyridine produce monomeric BChla solutions because of solvent ligation of the central Mg atom.

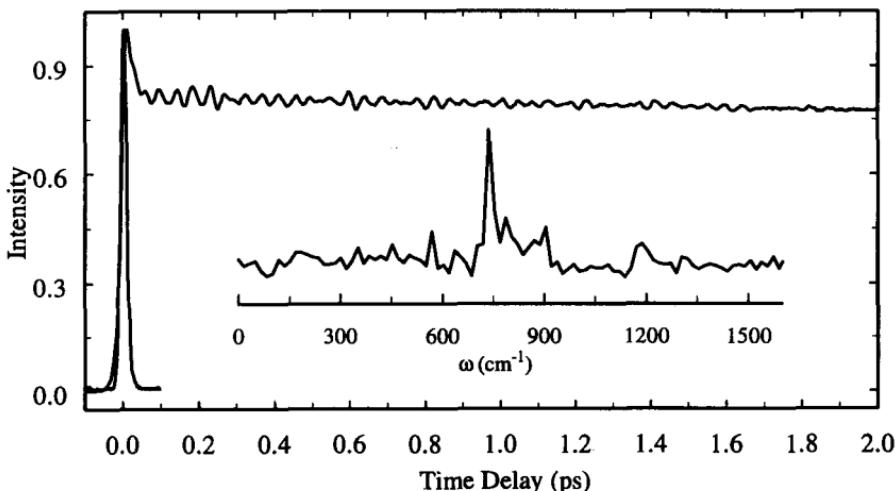


Fig. 1. Pump-probe response of BChla in THF solution and the autocorrelation response. Inset: Fourier spectrum. Fourier transformation is performed following the removal of a biexponential response to account for the prompt decay and the slow decay components observed in temporal waveform.

The $|P^{(3)}(t_1=0, t_2=t_3)|^2$ optical coherence response of BChla in THF is shown in semilog format in figure 2. The 2-pulse photon echo shape is very asymmetric with a significant time-shift (>10fs) from the zero-of-time point and broadened compared with the nonresonant solvent scattering response. The echo shift along with the spectral diffusion decay (not shown) indicates that the system-bath interaction is not in the homogeneous limit but has an inhomogeneous contribution.¹² The rapid decay of the 2-pulse echo signal indicates that optical homogeneous relaxation is, however, significant, with a "T₂" dephasing time of 64fs. This Bloch-type analysis implies a Markovian bath correlation function, which is known to be too simplistic for chromophore dephasing in solution.^{7-14,23} The dephasing time that is extracted, however, does give a basis for comparison with other experiments analyzed with a Bloch formalism.⁴ The 2-pulse photon echo response is well simulated using the solvent polarizability spectral density as a model for the bath fluctuations; these results are reported elsewhere.¹²

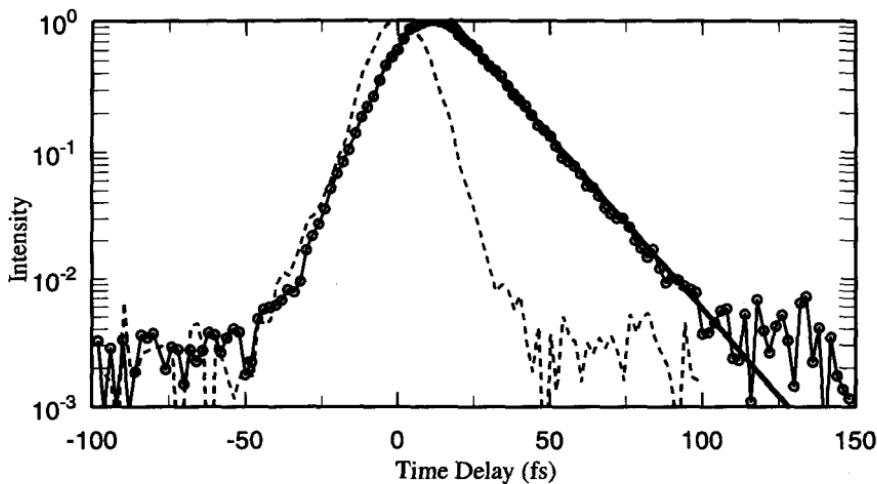


Fig. 2. 2-pulse photon echo signal of BChla in pyridine solution (connected open circles). Exponential decay of echo signal (straight line through points). Pyridine solvent scattering response (dashed).

The pump-probe response, $P^{(3)}(0,0,t_3)$, of the reaction center protein detected with post-sample spectral filtering (10nm FWHM bandpass @ 800nm) is shown in Figure 3. A complicated waveform is obtained that exhibits oscillations and positive and negative going features. The inset is a fit to the data using the kinetic (i.e. time constant) parameters reported by Fleming and co-workers.¹⁶ The fitted curve has significantly different amplitudes for each of the three components than those reported in ref. 16. This simply reflects the different excitation conditions between the two measurements. The change in the waveform due to the broadband excitation supports the idea that the long time (i.e. $\tau_3=2.8$ ps rise) component results from direct excitation of P* and subsequent charge transfer to form the charge-separated product. The prompt decay features ($\tau_1=100$ fs, $\tau_2=360$ fs) reflect the decay of B* by way of energy transfer to P* (τ_1) and other nonradiative processes. Oscillatory contributions to the waveform are also observed and are Fourier analyzed in the same manner as discussed above.

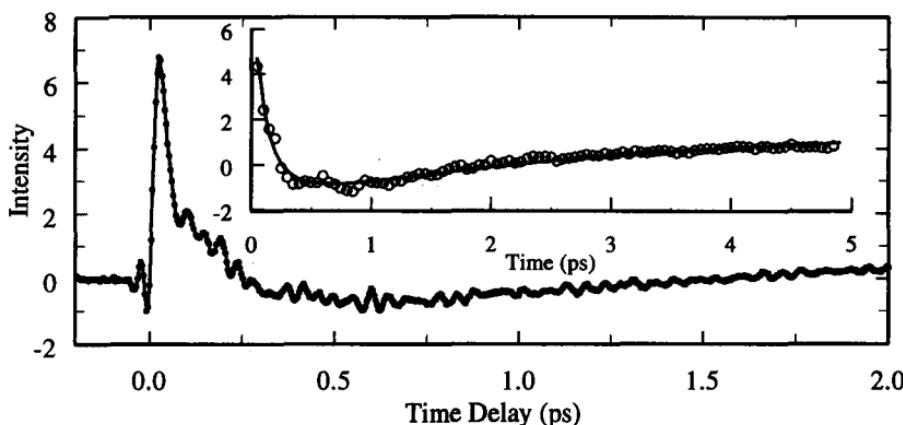


Fig. 3 Pump-probe data for the reaction center with spectral selection at 800nm. Inset: kinetic analysis with tri-exponential fit (solid) through the points.

The Fourier power spectra for three wavelength-resolved pump-probe data sets (10nm FWHM each @ 770, 800, 870) are shown in figure 4. The comparison of the spectra show the increasing coupling of the low-frequency modes to the electronic excitation coordinate. In particular, the response at 770, which is at the maximum of the pheophytin H \rightarrow H* absorption, shows no low frequency oscillations. This spectrum is very similar to BChla in solution. The signal at 800nm shows considerable amplitude in the low frequency modes ($50\text{--}350\text{cm}^{-1}$), which is in contrast with the solution results shown above. This spectrum is, however, in qualitative agreement with the resonance Raman spectrum of B reported by Mathies and co-workers.¹⁵ Finally, the spectrum of the pump-probe signal that is spectrally selected at 870 nm is dominated by a low frequency mode at 125cm^{-1} . This is the same frequency as the "marker mode" observed by Small and co-workers⁴ in low temperature hole burning studies of this reaction center.

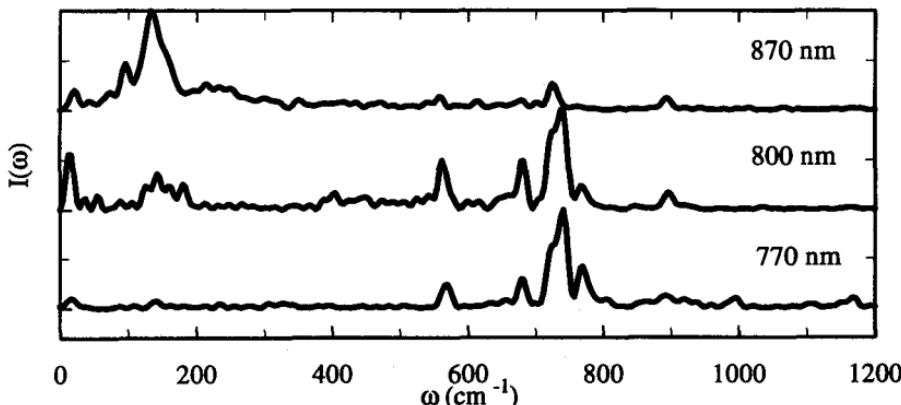


Fig. 4 Power spectra of pump-probe data obtained by spectral selection at 770, 800 and 870nm.

The absence of low frequency modes in the pheophytin spectrum (pheophytin lacks Mg) and with the lack of activity at low frequency modes of BChla in solution supports the conclusion that the protein environment causes a significant perturbation on the displacement of low frequency chromophore modes. The two solvents, pyridine and THF, coordinate to the Mg in BChla in both axial positions creating a fairly symmetric environment (and molecule-solvent potential of mean force) such that the low frequency motions that involve out of plane motions of Mg and other groups²⁴ are not displaced for Q₁ band excitation. Finally, contrasting the impulsive vibrational dynamics observed in BChla and B indicates that the protein environment above and below the plane of the porphyrin ring is not symmetric leading to different interactions between the chromophore and protein in the ground and excited electronic states and associated displacements of out-of-plane modes on optical excitation.

The peaks in the Fourier spectrum associated with vibrational coherences prepared in the P→P* electronic excitation are similar to those reported by Martin and co-workers¹⁷ and resonance Raman results.¹⁵ The Fourier spectrum shown in fig. 4 for P (i.e. at 870 nm) shows the most intense features at 125cm⁻¹, which does, however, differ from the Raman results. By contrast, the same prominent vibrational feature is the same frequency as the marker mode observed in hole-burning studies.⁴ They conclude that the dominant 125cm⁻¹ mode is associated with an intermolecular displacement of the monomers (i.e. Mg-Mg separation) in the special pair and that the Huang-Rhys factor is large (i.e. S=1.5).^{4,24} The vibrational dephasing time we obtain (300fs) for the same vibrational feature is quite close to the value (i.e. 200fs) estimated from simulations of the hole-burning spectra. This close agreement of dephasing times at low and high temperatures has interesting implications for the cause of vibrational dephasing.

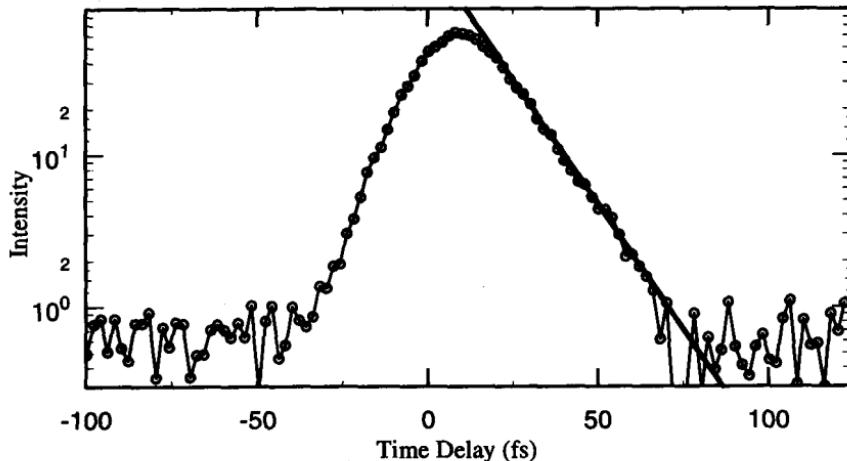


Fig. 5 2-pulse photon echo signal of the reaction center protein, spectrally gated at 870nm. Straight line is an exponential fit through the data points.

The primary goal of the present experiments, however, is to extract the optical dephasing spectral density, which will give the P→P* electronic transition energy-gap correlation function. Figure 5 shows the 2-pulse photon echo response of the special pair detected by wavelength selection at 870nm. The semilog plot indicates a significant shift of the echo maximum (8fs) from the zero-of-time and a large range of linearity in the decay of

the echo signal. The exponential decay of the echo, giving a T_2 time of 52fs, yields a homogeneous linewidth of 380cm^{-1} ; in good agreement with hole burning results.⁴ The dephasing is not purely homogeneous, however, since stimulated photon echo results show that spectral diffusion (i.e. slow bath fluctuations) occurs over several picoseconds.²⁵ Therefore, the $P \rightarrow P^*$ absorption is also "inhomogeneously" broadened. Several groups have calculated spectral densities for electronic energy fluctuations of various chromophores, but the results from Chandler's simulation²⁶ show both fast and slow modulations that are reflected in our experimental results; further analysis and experiments are required for a detailed comparison. Photon echo simulations that include intra-chromophore vibrational modes are currently being performed to establish a more general bath correlation function. These results will be reported elsewhere.

4. Conclusions

Numerical simulations incorporating the solvent polarizability spectral density and the multi-mode aspect of the chromophore response, i.e. intra-chromophore vibrational modes,⁹ are required for a more complete understanding of the various dephasing phenomena reported here. In the case of Bchl_a the experimental/simulation comparison will test both the utility of the pure solvent polarizability response for analysis of short time dynamics as well as the long-time spectral diffusion response.¹² In the case of the reaction center, the simulations will incorporate information from the pump-probe data of fig. 4 as well as displacements from hole burning studies, and resonance Raman results.

The comparison of stimulated photon echo results for Bchl_a in solution with numerical simulations employing the measured OKE spectrum of the solvent show that the amplitude of the low frequency portion of the solvent spectral density must be increased, indicating a stronger coupling of low frequency bath motions. The implication is that the optical coherence studies directly capture important contributions to the solute-bath interaction potential. The coherence studies in the reaction center also show evidence for significant low-frequency (i.e. glass-like) interactions²⁵ which may reflect the coupling to the phonon bath reported by Small.⁴ Time-gated stimulated photon echo studies will be performed on the biological systems discussed above. More complete interpretation of the optical dephasing dynamics and extraction of the optical dephasing spectral density can be better established by time-resolving the actual third-order polarization response, rather than its time integral. It will be of interest to see how far time domain techniques will help to elucidate the magnitude of electronic energy fluctuations and help to understand the mechanism of charge transfer.²⁶⁻²⁹

5. Acknowledgements

We thank Prof. Leslie Dutton for the reaction center samples and helpful conversations. This research was supported by grants from the National Science Foundation (CHE-9357424) and National Institutes of Health. DCA is a recipient of a Department of Energy GAANN fellowship. We acknowledge the support of the NIH sponsored Regional Laser and Biotechnology Laboratory at the University of Pennsylvania. NSF is the recipient of David and Lucile Packard and Arnold and Mabel Beckman foundation fellowships.

6. References

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