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Low-temperature energy transfer in FMO trimers from the green photosynthetic bacterium Chlorobium tepidum

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Abstract

The pump-probe kinetics of the slowest spectral equilibrations between inequivalent BChl a Qy states in FMO trimers from Chlorobium tepidum are decelerated by nearly two orders of magnitude when the temperature is lowered from 300 K to 19 K. The pump-probe anisotropy decays are also markedly slower at 19 K than at 300 K. Singlet-singlet annihilation in FMO trimers is negligible at the laser powers used here. However, reduced temperatures greatly accentuate the probability of singlet-triplet annihilation, due to accumulation of metastable BChl a states under high laser repetition rates.

Abbreviations: BChl – bacteriochlorophyll; FMO – Fenna-Matthews-Olson; fwhm – full width at half maximum; PB – photobleaching; SE – stimulated emission

Introduction

The bacteriochlorophyll a-protein complex from the green sulfur bacterium Prosthecochloris aestuarii was the first photosynthetic pigment-protein complex successfully studied by X-ray crystallography (Matthews et al. 1979; Matthews and Fenna 1980; Tronrud et al. 1986). This trimeric complex (termed the FMO trimer after R. E. Fenna, B. W. Matthews, and J. M. Olson, who contributed extensively to its early characterization) subsequently attracted many spectroscopic, theoretical and kinetic studies (see van Grondelle et al. 1994; Blankenship et al. 1995 for recent reviews). The 21 BChl a pigments in this strongly coupled antenna complex give rise to Qy exciton components spanning from ~ 770 to 830 nm (Johnson and Small 1991; Lu and Pearlstein 1993; Pearlstein 1992). Since the room-temperature energy transfer kinetics of FMO trimers occur largely in the femtosecond regime, they were relatively little studied prior to the advent of mode-locked Ti:sapphire lasers. In a recent room-temperature pump-probe study of electronic energy transfers in FMO trimers from the green sulfur bacterium Chlorobium tepidum, we found that spectral equilibration between inequivalent states occurred with kinetics ranging from 100 to 900 fs (Savikhin and Struve 1994), depending on the pump and probe wavelengths used. (It is still unclear whether this process corresponds primarily to relaxation between Qy exciton components that are eigenstates of the supermolecule electronic Hamiltonian, incoherent Förster transfers between BChl a pigments with different Qy diagonal energies, or a combination of the two. Our use of the term 'states' will hereafter encompass all of these possibilities.) The one-color anisotropy decays exhibited major lifetime components of 75–135 fs and 1.4–2.0 ps. The latter anisotropy component (which resembles the 2.0 ps anisotropy decay observed at 814 nm by Lyle and Struve (1990) in FMO trimers from P. aestuarii) is considerably slower than the subpicosecond spectral equilibration time scales. It is thus likely dominated by energy transfers among equivalent, lowest-energy states belonging to different subunits within the trimer. In a 4.2 K hole-burning study of FMO trimers from P.
Figure 1. Steady-state Qy absorption spectrum of FMO trimers from *Cb. tepidum* at 50 K. Laser pump and probe pulse spectra for 812-829 nm two-color experiment (see text) are superimposed on the absorption spectrum.

*aestuarii*, Johnson and Small (1991) found spectrally broad zero-phonon holes in the higher-lying exciton components, signaling the presence of primary processes that occur with ~100 fs kinetics. The question thus arises whether these ~100 fs processes correspond to some of the 100–900 fs spectral equilibration steps that occur at room temperature (Savikhin and Struve 1994). Alternatively, they may stem from other processes (e.g. dephasing) that occur prior to spectral equilibration at low temperature. We have addressed this question by studying the pump-probe kinetics of FMO trimers from *Cb. tepidum* at three temperatures from 19 K to 130 K, in order to trace the temperature dependence of the major spectral equilibration and anisotropy lifetime components that appear at room temperature. Both the overall spectral equilibration and anisotropy decays prove to be decelerated into the picosecond regime at 19 K. However, we also find evidence for the occurrence of additional, subpicosecond processes at 19 K.

**Materials and methods**

FMO trimers from *Cb. tepidum* were isolated according to the procedure of Olson (1980), with the modifications described by Savikhin et al. (1994a). The samples ran as a single band on SDS-PAGE. The room-temperature absorption spectrum showed maxima at 809, 602, 371 and 267 nm, with absorbance ratios 5.4:1:2.6:1.6; the 50 K absorption spectrum is shown in Figure 1. The room-temperature spectral absorbance ratio $A_{267}/A_{371}$ was 0.6 for the samples used here. While the room-temperature Qy absorption spectrum of FMO trimers from *Cb. tepidum* is essentially featureless (Savikhin and Struve 1994), the 50 K spectrum exhibits clearly resolved bands arising from transitions to well-defined groups of exciton components.

The self-mode-locked Ti:sapphire laser and pump-probe optics have been extensively described elsewhere (Savikhin and Struve 1994; Savikhin et al. 1994c). The radio-frequency (RF) multiple modulation detector and electronics were replaced by a new design, in which the probe beam-detecting photodiode was incorporated into an RLC input loop tuned to the RF detection frequency (Savikhin 1995). Samples were housed between two optical flats spaced by 0.5 mm, in a window assembly in thermal contact with the end of a 2.25 cm diameter Cu cold finger in an Air Products (Allentown, PA) DE202 closed-cycle He expander module. The sample temperature was monitored directly using a calibrated Cu-constantan (type T) thermocouple placed at the center of the cell. Since quartz exhibits poor thermal conductivity, and since sapphire exhibits birefringence that can interfere with anisotropy studies, one window of each material was used. The laser beams traversed the quartz window prior to entering the sample, and then exited through the sapphire window. The latter contacted the Cu window mount through an In foil gasket. In this way, the temperature differential between the sample and the cold finger was only 6 K when the latter was 13 K; the temperature differential was reduced to 1 K at 100 K.

**Results and discussion**

Time-resolved absorption difference profiles are shown in Figure 2 for FMO trimers from *Cb. tepidum* at 19, 52 and 130 K. In these two-color experiments, the pump and probe pulse spectra (which were shaped using bandpass interference filters) were centered at 812 and 829 nm with 4.6 and 5.8 nm bandwidth, as shown in Figure 1. The cross-correlation was typically 200–250 fs fwhm. For these isotropic profiles, the pump and probe polarizations were separated by 54.7°. The probe spectrum overlapped the lowest-energy Qy exciton components in the steady-state absorption spectrum, while the pump spectrum excited components lying ~ 200–300 cm$^{-1}$ to the blue. For these pump and probe wavelengths, the negative-going absorption difference signals in Figure 2 are dominated at nearly all times by photobleaching (PB) and stimu-