High-Pressure and Stark Hole-Burning Studies of Chlorosome Antennas from *Chlorobium tepidum*

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ABSTRACT Results from high-pressure and Stark hole-burning experiments on isolated chlorosomes from the green sulfur bacterium *Chlorobium tepidum* are presented, as well as Stark hole-burning data for bacteriochlorophyll *c* (BChl *c*) monomers in a poly(vinyl butyral) copolymer film. Large linear pressure shift rates of -0.44 and -0.54 cm^{-1}/MPa were observed for the chlorosome BChl *c* Q_y-band at 100 K and the lowest Q_y-exciton level at 12 K, respectively. It is argued that approximately half of the latter shift rate is due to electron exchange coupling between BChl *c* molecules. The similarity between the above shift rates and those observed for the B875 and B850 BChl *a* rings of the light-harvesting complexes of purple bacteria is emphasized. For BChl *c* monomer, $f\Delta\mu = 0.35$ D, where $\Delta\mu$ is the dipole moment change for the Q_y transition and f is the local field correction factor. The data establish that $\Delta\mu$ is dominated by the matrix-induced contribution. The change in polarizability ($\Delta\alpha$) for the Q_y transition of the BChl *c* monomer is estimated at 19 Å^3, which is essentially identical to that of the Chl *a* monomer. Interestingly, no Stark effects were observed for the lowest exciton level of the chlorosomes (maximum Stark field of 10^5 V/cm). Possible explanations for this are given, and these include consideration of structural models for the chlorosome BChl *c* aggregates.

INTRODUCTION

Green sulfur bacteria, such as *Chlorobium* (*Cb.*) *tepidum* and *Cb. limicola*, and green nonsulfur bacteria, such as *Chloroflexus* (*Cf.*) *aurantiacus*, are separated by a large evolutionary distance according to 16S rRNA analysis (Woese, 1987; Olsen et al., 1994). Their distinctiveness is reflected in the different types of reaction centers and membrane-bound antenna complexes found in the two families, as well as their entirely distinct metabolic and ecological characteristics (see Blankenship et al., 1995; Olson, 1998, and references therein). Interestingly, they both contain chlorosomes; an antenna complex of flattened ellipsoidal shapes of $\sim 100 \times 30 \times 10$ nm^3 for the green nonsulfur bacteria and $\sim 200 \times 70 \times 12$ nm^3 for the green sulfur bacteria. Chlorosomes consist of up to ~10,000 aggregated bacteriochlorophyll (BChl) *c*, *d,* or *e* molecules surrounded by a monolayer lipid envelope and a relatively small amount of protein. BChl *a* molecules are associated with the baseplate of the chlorosomes (Blankenship et al., 1995; Olson, 1998), which serves as an intermediate in the excitation energy transfer from the chlorosomes to BChl *a*-containing antenna complexes in both types of microorganisms: the B808–866 complex for the green nonsulfur bacteria and the Fenna-Matthews-Olson (FMO) complex for the green sulfur bacteria. Fig. 1 is a schematic of the arrangement of photosynthetic complexes in green sulfur bacteria. The molar ratio of BChl *c* (*d* or *e*) to BChl *a* in the chlorosomes varies with species and growth conditions. Molar ratios of 20:1 for chlorosomes from *Cf. aurantiacus* (Schmidt, 1980) and 90:1 for those from *Cb. limicola* (Gerola and Olson, 1986) have been reported.

Compared with other antenna complexes, chlorosomes have relatively low protein to BChl *c*, *d,* or *e* ratios, ranging from ~0.5 to ~2, depending on the species and growth conditions (Cruden and Stanier, 1970; Schmidt, 1980; Feick and Fuller, 1984; Chung et al., 1994; Blankenship et al., 1995). It is generally accepted that pigment-pigment interactions within chlorosomes are mainly responsible for the pigment organization, rather than pigment-protein interactions, as is commonly found in other antenna complexes. BChl *c*, *d,* or *e* can readily form aggregates in nonpolar organic solvents (Bystrova et al., 1979; Smith et al., 1983; Brune et al., 1987; Olson and Pedersen, 1990) or aqueous lipid/detergent micelles (Hirota et al., 1992; Miller et al., 1993; van Noort et al., 1997) in vitro with spectroscopic properties similar to those observed in chlorosomes. That is, the Q_y band of the BChl *c* monomers at ~670 nm is red-shifted to the ~720–760-nm region because of the formation of aggregates. Based on resonance Raman, Fourier transform infrared spectroscopy, and absorption studies, the BChl *c* molecules are essentially pentacoordinated, and the 3^1 hydroxyl and 13^1 keto groups are involved in the aggregation (Brune et al., 1988; Lutz and van Brakel, 1988; Nozawa et al., 1990; Hildebrandt et al., 1994). It is believed that the aggregated BChl *c*, *d,* or *e* molecules form rods 5–10 nm in diameter within chlorosomes, as was seen in freeze-fracture electron micrographs of *Cb. limicola* and *Cf. aurantiacus* cells (Staehelin et al., 1978, 1980; Golecki and Oelze, 1987). Various pigment aggregation models have been proposed for the chlorosome aggregates that are based on antiparallel chains (Brune et al., 1988; Nozawa et al., 1994; Minuro et al., 1995), parallel stepped chains (Brune et

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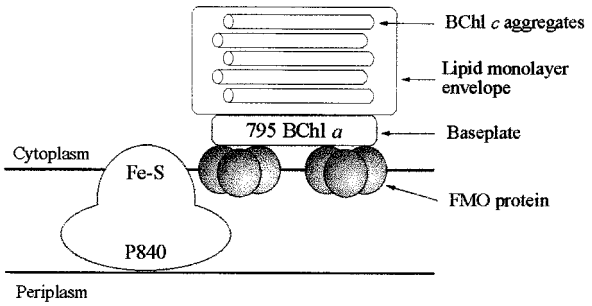

FIGURE 1 Schematic model for the arrangement of the chlorosome, the FMO complex and the reaction center in green sulfur bacteria. Chlorosomes consist of BChl *c* aggregates in the form of rods surrounded by a lipid monolayer with the BChl *a*-containing baseplate attached. The FMO complex and reaction center (P840) bind BChl *a* molecules, and the reaction center contains an iron-sulfur cluster.

al., 1988; Mizoguchi et al., 1998), and cylindrical arrangements of BChl molecules (Somsen et al., 1996). Larger scale structural models for the rod elements, aided by quantum chemical calculations or molecular modeling, have also been proposed (Holzwarth and Schaffner, 1994; Buck and Struve, 1996; Fetisova et al., 1996).

Over the past 20 years nonphotochemical hole-burning (NPHB) spectroscopy has been applied to chromophores embedded in disordered host media whose optical spectra suffer from significant inhomogeneous broadening. Photosynthetic protein complexes with an inhomogeneous broadening contribution of $\sim$100 cm^{-1} to the absorption bandwidths are good candidates for NPHB spectroscopy, which can improve spectral resolution by two to three orders of magnitude. Information that is obtainable includes the inhomogeneity of the protein complex, excited-state lifetimes, exciton-level structures, the electron-phonon coupling strength of optical transitions and frequencies, and Franck-Condon factors of chlorophyll (Chl) modes. For general reviews covering NPHB and its applications, see Moerner (1988) and Jankowiak et al. (1993). For reviews pertaining to NPHB studies of photosynthetic complexes, see Johnson et al. (1991), Reddy et al. (1992a), and Jankowiak and Small (1993).

Recently, NPHB, in combination with high pressure and electric (Stark) fields, has been applied to several photosynthetic protein complexes. The results obtained led to new insights into excitation energy transfer dynamics and the Q_y electronic structures of Chl molecules. Of particular relevance to this study are the high-pressure- and Stark-NPHB experiments of Reddy et al. (1996), Wu et al. (1996, 1997a, 1998), and Rätsep et al. (1998a,b) on light-harvesting (LH) complexes from purple bacteria. Not only are the results of those studies consistent with the structure of LH2 as determined by x-ray crystallography; they also provide benchmarks for electronic structure calculations by establishing that electron exchange coupling between nearest-neighbor pigments and energy disorder associated with the cyclic LH2 and LH1 rings cannot be ignored. This is important because a firm understanding of the Q_y states of Chl molecules bound to the proteins of LH complexes is essential for understanding their excitation energy transfer/relaxation dynamics. The usefulness of the external field/NPHB combination (or external field/optical spectroscopy combination in a broader sense) in the study of dynamics, structures, interactions, and functions is not limited to photosynthetic complexes. In principle, any system with a probe is an ideal candidate for such a study. An example of a probe intrinsic to the system is the heme group in hemoglobin, but artificial labeling is also possible, such as the labeling of human serum albumin with hypericinate ions (Falk and Meyer, 1994). An example of an external field/optical spectroscopy combination study that is nonbiologically oriented is the photon echo investigation of optical dephasing in pentacene-doped polymethyl methacrylate under high pressure (Berg and Chronister, 1997). The reader interested in the effects of high pressure and Stark fields on the spectroscopic properties of pigments bound to proteins is referred to the review articles of Kohler et al. (1998) and Fidy et al. (1998).

The chlorosome Q_y bands of *Cf. aurantiacus* and *Cb. limicola*, which have absorption maxima at 742 and 751 nm, respectively, have been investigated by NPHB in the fluorescence excitation mode at low temperature (Fetisova and Mauring, 1992, 1993). The results were consistent with the chlorosome pigments being excitonically coupled. The lowest exciton level of the Q_y band was characterized by zero-phonon hole (ZPH) action spectroscopy and found to carry an inhomogeneous full width at half-maximum (FWHM) of $\sim$100 cm^{-1} with a band center at $\sim$752 nm for *Cf. aurantiacus* and $\sim$774 nm for *Cb. limicola*. The ZPH action spectrum is generated by burning a series of ZPHs across the absorption band with constant laser fluence and is a powerful method for obtaining the width, center, and intensity of an inhomogeneously broadened distribution mixed with other absorption contributions. An example of such a study on photosynthetic antenna complexes is the identification of the lowest exciton level (B870) of LH2 complex from *Rhodopseudomonas acidophila* (Wu et al., 1997c). Broad nonresonant holes resulting from fast inter-exciton-level relaxation from the higher exciton components, which carry most of the oscillator strength, were also observed. Recently, ZPH action spectroscopy was applied in a study of the lowest exciton level of chlorosomes of *Cb. tepidum* (Psencik et al., 1998). The lowest exciton level was found to lie in the range of 760–800 nm and to be centered at $\sim$780 nm. The fractional hole depth of the most intense hole was 0.04 in the action spectrum. Different redox conditions were also employed to examine their effects on hole widths, as the excited-state lifetime of BChl *c* aggregates and energy transfer to the baseplate were known to be regulated by the redox potential for green sulfur bacteria.

The ZPH width was 1.8 cm^{-1} under anaerobic conditions and 4.0 cm^{-1} under aerobic conditions and was independent of wavelength and temperature up to 25 K. Psencik et al. (1998) proposed that the ZPH width is due to the BChl *c*-to-BChl *c* energy transfer. A width of 1.8 cm^{-1} corresponds to a BChl *c*-to-BChl *c* energy transfer time (T_1) of 5.8 ps, whereas a width of 4.0 cm^{-1} corresponds to a transfer time of 2.7 ps. The shortening to 2.7 ps may be due to an additional quenching mechanism, involving one or both of the quinone molecules chlorobiumquinone and menaquinone-7 under oxidizing conditions (Frigaard et al., 1997).

Currently the exact structural arrangement of the pigments within the chlorosomes is unknown, but models generally include J-aggregate-like long chains arranged circularly to form the rod elements observed by electron microscopy. The homogeneous line shift and broadening of pseudoisocyanine (PIC) J-aggregates under pressure (<6.5 MPa) have been investigated by hole-burning spectroscopy at 4.2 K (Hirschmann and Friedrich, 1992). The holes within the J-band were found to shift with a linear rate of about -0.3 cm^{-1}/MPa, which depends on the burn frequency within the band. The hole broadening induced by pressure was found to be surprisingly small. The largest linear broadening rate observed was ~ 0.025 cm^{-1}/MPa; a dependence on the burn frequency (color effect) was observed. Hirschmann and Friedrich argued that the low hole-broadening rate is the result of motional narrowing of structural heterogeneity due to large exciton coherence lengths.

Before this study, Stark hole burning was applied to the J-band of aggregated pseudoisocyanine iodide (PIC-I) at 2 K (Wendt and Friedrich, 1996). The aggregation of PIC-I reduced the linear Stark effect by more than two orders of magnitude compared to the monomer. At a field strength of 300 kV/cm, the quadratic Stark effect was significant for the J-aggregate (comparable to the linear contribution). The large difference in the polarizability between the ground and excited states, which is responsible for the quadratic Stark effect, was argued to be due to the aggregation of PIC-I molecules on the basis of exciton theory. Wendt and Friedrich argued that certain structural constraints such as a helical pigment organization could explain the large reduction in the linear Stark effect of the aggregates.

Recently, conventional Stark modulation spectroscopy at 77 K was used in a study of chlorosomes from *Cf. aurantiacus* (in both wild-type and carotenoid-deficient cells) (Frese et al., 1997). The Stark spectrum of the Q_y band resembles a first-derivative-type lineshape, consistent with there being a large difference in polarizability between the ground and excited states ($\mathrm{Tr}(\Delta\alpha) = 1650 \pm 100$ $Å^3/f^2$, where $\mathrm{Tr}(\Delta\alpha)$ denotes the trace of the polarizability difference tensor). The second-derivative contribution to the Stark spectrum was found to be negligible, which indicates that the dipole moment change between the ground and excited states is very small. Frese et al. suggested that the aggregated BChl *c* in chlorosomes adopt an antiparallel structure, as opposed to various parallel models proposed by others (Brune et al., 1988; Alden et al., 1992; Mizoguchi et al., 1998).

We present here the results of high-pressure and Stark hole-burning experiments on isolated chlorosomes from BChl *c* containing *Cb. tepidum,* as well as Stark hole-burning results for BChl *c* monomers in a polymer film. The motivations for the experiments were to gain additional insights into the arrangement of BChl *c* molecules in chlorosomes and the exciton level structure of the aggregates (with emphasis on the lowest Q_y exciton level) and to provide benchmarks for electronic structure calculations based on structural models for the BChl *c* aggregates.

EXPERIMENTAL

Cb. tepidum cells were grown at ~40°C in a 90-liter clear plastic vessel in a modified version of the CH1 medium reported by Olson et al. (1973) and illuminated with four banks of fluorescent tube lights ($\approx$ 60 μEinsteins m^{-2} s^{-1}) over 3–4 days with gentle stirring. Cells were harvested using a continuous flow centrifuge and used in the preparation of chlorosomes.

Chlorosomes were isolated following the method of Gerola and Olson (1986) with minor revisions. Four grams of cell paste was mixed with 16 ml of chlorosome buffer (10 mM sodium phosphate (pH 7.4), 10 mM sodium ascorbate, and 2 M sodium thiocyanate) until an even suspension was achieved. Cells were broken by sonication in the presence of DNase I, and the sonicate was centrifuged to remove cell debris. The dark green supernatant was layered on top of a 20–50% continuous sucrose density gradient. The sucrose gradient was spun at 45,000 rpm for 18 h at 4°C, and the chlorosome fraction was removed. Chlorosomes (FMO depleted) equilibrate between 25% and 30% sucrose.

BChl *c* pigments used in the Stark hole-burning experiment were extracted from whole cells of *Cb. tepidum* according to the procedure described by van Noort et al. (1997). BChl *c* was dissolved in methanol, which prevents the formation of aggregates, and was added to poly(vinyl butyral-co-vinyl alcohol-co-vinyl acetate) (Aldrich, Milwaukee, WI)/dichloromethane solution (0.5 g of the polymer with an average molecular weight of 90,000–120,000 in 15 ml dichloromethane). The solution was dried overnight in a flat Petri dish covered by a glass plate (to reduce the drying rate). The dried film was kept in an oven under vacuum at 40°C for ~2 h to remove the residual solvent. Typically the film separated from the Petri dish after it was placed in a darkened closed box with a wet towel for ~2 days. The resulting OD and FWHM of the BChl *c* absorption band at 667 nm at room temperature were 0.07 and $\approx$ 400 cm^{-1}, respectively.

Hole-burned spectra of chlorosomes were detected in the absorption mode (Lyle et al., 1993). A Ti:sapphire ring laser (model 899-21, linewidth ~0.07 cm^{-1}; Coherent, Santa Clara, CA) pumped by a Coherent 15-W argon ion laser was used to burn holes in the Q_y absorption band. The spectra before and after burning were recorded with a Bruker IFS 120 HR Fourier transform spectrometer (Bruker, Billerica, MA). Burn fluence and spectral reading resolutions are given in the figure captions.

Because of the smaller ZPH width, spectral hole burning of BChl *c* monomers was detected in the fluorescence excitation mode with an apparatus described by Reinot et al. (1996) and Kim et al. (1995). The burn and read laser was a Coherent 699-21 ring dye laser (linewidth <30 MHz) pumped by a 6-W Coherent Innova 90 Ar ion laser stabilized by a LS 100 power stabilizer (Cambridge Research and Instrumentation, Cambridge, MA). Fluorescence was detected by a GaAs photomultiplier tube (RCA C31034) and a photon counter (SR-400; Stanford Research Systems, Sunnyvale, CA). Scattered laser light was eliminated with cutoff filters at 750 nm. Laser intensities used for hole burning were in the range of ~10

μW/cm^2, and hole depths were typically ~25%. For hole reading, the duration of which was ~300 s, the laser was attenuated by a factor of ~60.

High pressures of up to 1.5 GPa were generated by compressing helium gas with a Unipress U11 three-stage compressor (Warsaw, Poland). The sample was contained in a gelatin capsule and placed in a high-pressure cell (rated for pressures less than 800 MPa) with two sapphire windows installed. A custom-made liquid helium cryostat (Janis, Wilmington, MA) was used to achieve low temperatures for the high-pressure hole-burning experiments. For further details, see Reddy et al. (1995).

A Trek model 610 C dc high-voltage power supply (0 to ± 10 kV; Trek, Medina, NY) was used to generate the Stark field. By changing the polarity of the power supply, we were able to achieve a maximum Stark field difference of 100 kV/cm for two copper electrodes separated by 2 mm. For the quadratic Stark effect, however, the highest field achievable was 50 kV/cm, because the positive and negative polarities of the power supply would result in the same effect. The gelatin capsule containing the sample was allowed to soften at room temperature for 5 min before being squeezed into the space between the electrodes (optical path length ~6 mm). To avoid dielectric breakdown, all measurements were performed at 1.8 K in a Janis 10 DT liquid helium cryostat. This setup allowed for study of the Stark effect with varying laser polarization relative to the external field. The interested reader is referred to Rätsep et al. (1998a) for further details.

The Stark cell used in the fluorescence excitation mode for the BChl *c* monomer study consisted of two vertical Teflon walls and two copper electrodes perpendicular to the walls (Milanovich et al., 1998). A separation distance of 5 mm between electrodes was maintained by placing them in the grooves on the inside of the Teflon walls. A slit was made in one of the Teflon walls to allow the laser access to the sample. Fluorescence was collected at a 90° angle relative to the incident laser light through an opening on the other Teflon wall in the Stark cell. A polarizer placed in front of the Stark cell allowed for control of the laser polarization relative to the applied field direction.

RESULTS

Pressure-dependent studies of the Q_y band and its lowest exciton level

Fig. 2 shows the near-IR absorption spectrum of chlorosomes at 15 and 752 MPa (1 MPa ≈ 10 atm) at 100 K. A red shift of 320 cm^{-1} (from 13,250 ± 5 to 12,930 ± 5 cm^{-1}) and a band broadening of 95 cm^{-1} (from 550 ± 5 to 645 ± 5 cm^{-1}) with increasing pressure are observed. Fig. 3 plots the absorption band positions and widths versus pressure. Within the pressure range employed, the red shift and broadening are reversible and linear with pressure (see the regression lines in Fig. 3). The linear pressure shift and broadening rates were −0.44 and 0.12 cm^{-1}/MPa, respectively.

As in the studies of LH2 and LH1 antenna complexes of purple bacteria (Reddy et al., 1992b, 1993; Wu et al., 1997c, 1998), the lowest exciton level of the chlorosome Q_y band was studied by means of ZPHs burned at the red edge of the band. This level was determined by ZPH action spectroscopy at ambient pressure to have an inhomogeneous distribution width (FWHM) of ~100 ± 10 cm^{-1} and was centered at ~12,900 ± 10 cm^{-1} (775 nm), consistent with the findings of Psencik et al. (1998) for *Cb. tepidum*. The pressure shifting of the lowest exciton level of the Q_y band at 12 K is shown in Fig. 4. Five ZPHs were burned at 15

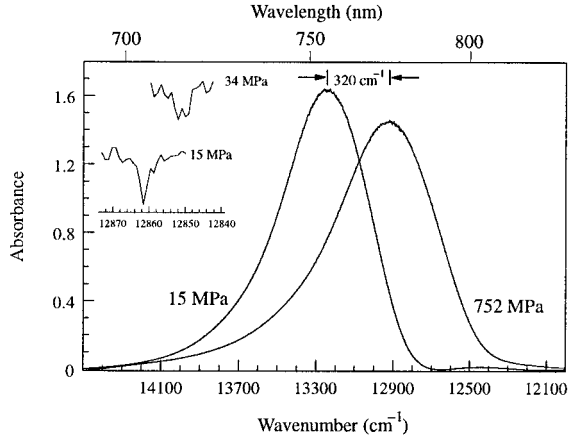

FIGURE 2 Absorption spectra (100 K) of the Q_y band of isolated chlorosomes from *Cb. tepidum* at 15 and 752 MPa. As pressure increases the Q_y band shifts from 13,250 cm^{-1} (755 nm) to 12,930 cm^{-1} (773 nm), and the bandwidth increases from 550 to 645 cm^{-1}. See Fig. 3 for the pressure shift and broadening rates. The inset shows the zero-phonon hole burned at 12,861.8 cm^{-1} at 15 MPa and 12 K and its shift and broadening when the pressure was increased to 34 MPa. The hole detection was made with a Fourier transform infrared spectrometer in the absorption mode. The burn fluence and read resolution are 100 J/cm^2 and 2 cm^{-1}, respectively.

MPa in this spectral region, which shift to the red with increasing pressure. The inset of Fig. 2 shows the ZPHs burned at 12,862 cm^{-1} at 12 K and 15 MPa. The shifting and broadening of that hole resulting from an increase in pressure to 34 MPa are also shown. Because of the solidification of the pressure-transmitting medium, helium gas, at ~75 MPa at 12 K, as well as rapid hole filling and broadening, no data were obtained beyond 57 MPa. As with the absorption band, the shift of ZPHs is linearly dependent on the pressure (see the regression lines and their slopes in Fig. 4) with an averaged linear pressure shift rate of −0.54 cm^{-1}/MPa. Unlike the lowest exciton level (B870) of B850

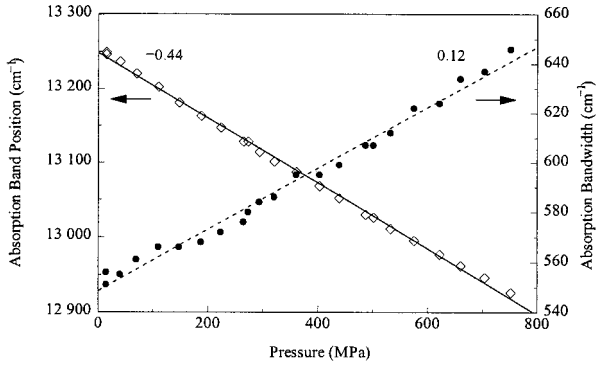

FIGURE 3 Pressure shift (◇) and broadening (●) of the Q_y absorption band of isolated chlorosomes from *Cb. tepidum* at 100 K. As indicated by the two regression lines, the pressure dependence of the band shifts and widths is linear within the pressure range employed. The linear shift and broadening rates are −0.44 ± 0.01 cm^{-1}/MPa and 0.12 ± 0.01 cm^{-1}/MPa, respectively. No irreversible pressure-induced effects were observed.

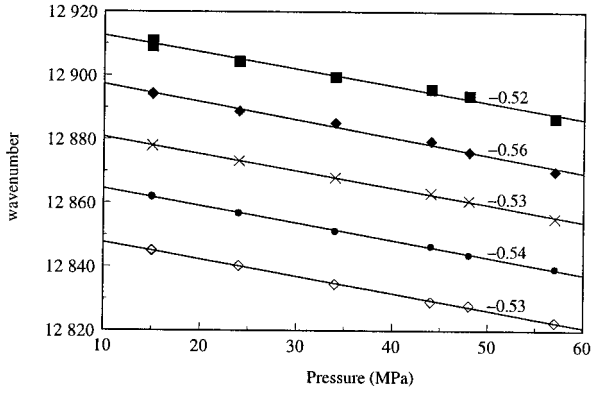

FIGURE 4 Linear pressure shifting of zero-phonon holes (holes were read in the absorption mode with a read resolution = 2 cm^{-1}) burned on the low-energy side of the Q_y absorption band of chlorosomes from *Cb. tepidum*. Initially, five holes were burned at 12,910.9, 12,894.3, 12,877.9, 12,861.8, and 12,844.9 cm^{-1}, at 15 MPa and 12 K. One of the ZPH profiles is shown in the inset of Fig. 2. A burn fluence of 100 J/cm^2 was used. All holes shifted linearly (see the *solid lines*) to the red with increasing pressure with rates (in cm^{-1}/MPa) indicated in the figure. The uncertainties of the shift rates are ±0.03, ±0.02, ±0.01, ±0.01, and ±0.01 cm^{-1}/MPa from top to bottom. No irreversible pressure-induced effects were observed.

molecules of LH2 from *Rps. acidophila* and *Rb. sphaeroides* (Wu et al., 1997a), which exhibits an increase in shift rate in going from the high-energy to low-energy sides of the band, the shift rate of the lowest exciton level of the chlorosome Q_y band is essentially constant.

Stark-hole burning studies

The Stark shift of the optical transition frequency of the absorber is given by

$$\Delta\omega = -\hbar^{-1}[(\Delta\underline{\mu}_0 + \Delta\underline{\underline{\alpha}}\underline{E}_{int})f\underline{E}_s + \tfrac{1}{2}(f\underline{E}_s)\Delta\underline{\underline{\alpha}}(f\underline{E}_s)]. \quad (1)$$

f is the local field correction factor and is taken to be a scalar. $\Delta\underline{\mu}_0$ is the molecular dipole moment change, and $\Delta\underline{\underline{\alpha}}_0$ is the molecular polarizability difference tensor. $\underline{E}_{int}$ is the matrix field experienced by the absorber and the induced dipole moment $\Delta\underline{\mu}_{ind} = \Delta\underline{\underline{\alpha}}\underline{E}_{int}$. The first and second terms in the square brackets depend linearly and quadratically on the Stark field E_s, respectively. As reviewed in Rätsep et al. (1998b), E_{int} in molecular systems is large, $\gtrsim 10^6$ V/cm (see also Kador et al., 1990; Meixner et al., 1992; Middendorf et al., 1993), which is an order of magnitude larger than the maximum Stark fields used in Stark hole-burning experiments. As a result, only the linear Stark effect was observed for the B800 and B870 bands of the LH2 complex, the B896 band of the LH1 complex, the 825-nm band of the FMO complex, and all isolated chromophores in polymer, glass, and protein matrices (see Rätsep et al., 1998a, and references therein). (The B870 and B896 bands correspond to the lowest exciton level of the LH2 and LH1 BChl *a* rings.) However, a large quadratic Stark effect was observed in the J-aggregate, in which $\Delta\alpha$ was enhanced because of aggregation (Wendt and Friedrich, 1996).

Considering further the case of isolated molecules in glassy matrices with the domination of the linear Stark effect, let γ be the angle between the molecular dipole moment difference vector $\Delta\underline{\mu}_o$ and the transition dipole vector $\underline{d}$. Linearly polarized light preferentially burns out those molecules with $\underline{d}$ parallel to the polarization vector $\underline{e}$ of the light. The experimentally observed dipole moment change can be written as $\Delta\underline{\mu} = \Delta\underline{\mu}_o + \Delta\underline{\mu}_{ind}$, where $\Delta\underline{\mu}_{ind}$ is the matrix-induced dipole moment change equal to $\Delta\underline{\underline{\alpha}}\underline{E}_{int}$. When $\Delta\underline{\mu}_o$ is dominant, photoselection enables one to probe molecules for which the angle between $\Delta\underline{\mu}_o$ and $\underline{e}$ is well defined (shallow hole limit). As discussed by Meixner et al. (1986) and Gafert et al. (1995), Stark splitting of the hole can be observed for an angle between $\underline{e}$ and $\underline{E}_s$, which depends on the value of γ. For example, for $\gamma = 0$ or π, Stark splitting occurs for parallel polarization, while symmetrical broadening occurs for perpendicular polarization. The situation is reversed for $\gamma = \pm\pi/2$. However, when $\Delta\underline{\mu}_{ind}$ is dominant, only Stark broadening is expected for both polarizations. This is because the orientation of $\Delta\underline{\mu}_{ind}$ relative to $\underline{d}$ or $\underline{e}$ is random for a glassy matrix, i.e., the matrix field varies significantly from site to site.

The assumption of random orientations for $\Delta\underline{\mu}_{ind}$ for Chl molecules in photosynthetic complexes is questionable because the structure of the protein around these chromophores is well defined, although the Q_y absorption bands do suffer from significant inhomogeneous broadening. For example, Gafert et al. (1995) observed Stark hole splitting for two of the three sites of mesoporphyrin substituted in horseradish peroxidase. For the same molecule in a glass, only Stark broadening was observed for both laser polarizations. Gafert et al. introduced the notion of random and nonrandom protein contributions to $\Delta\mu_{ind}$, i.e., $\Delta\mu_{ind}$(random) and $\Delta\mu_{ind}$(nonrandom). Recently, Rätsep et al. observed Stark splitting and broadening with parallel and perpendicular laser polarizations, respectively, for the lowest exciton level of the FMO complex (Rätsep et al., 1998a). However, for the B800 and B870 bands of the LH2 complex and B896 of the LH1 complex, Stark broadening of the ZPH was observed for both parallel and perpendicular laser polarizations (Rätsep et al., 1998a,b).

Stark hole-burning studies of the Q_y band of BChl c monomers

Fig. 5 shows the Stark broadening of a ZPH burned at 670.2 nm (14921 cm^{-1}) into the Q_y absorption band of BChl *c* monomers in a poly(vinyl butyral) copolymer film at 1.8 K for laser polarization perpendicular to the Stark field. (Hole broadening was also observed for laser polarization parallel to the Stark field.) The FWHM of the hole at zero field is 1.1 GHz (0.036 cm^{-1}) and increases symmetrically to 1.7

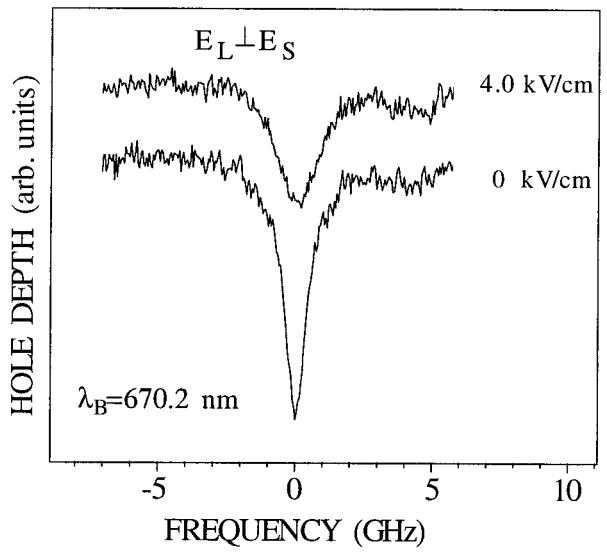

FIGURE 5 Stark effect for a zero-phonon hole burned at 670.2 nm in the Q_y band of BChl *c* monomer in a poly(vinyl butyral) copolymer film at 1.8 K. The burn fluence used was 1.3 mJ/cm^2. Holes were read in the fluorescence excitation mode. The laser polarization is perpendicular to the Stark field. The horizontal axis is offset to center the hole at zero. The dependence of the hole width on the electric field is shown in Fig. 6.

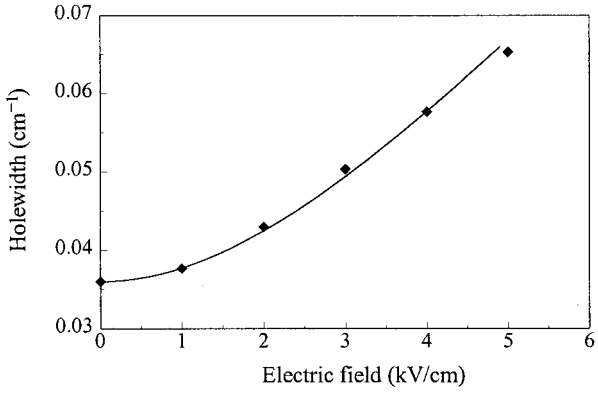

FIGURE 6 Stark broadening of a zero-phonon hole burned at 670.2 nm in the Q_y band of the BChl *c* monomer in a poly(vinylbutyral) copolymer film, $T = 1.8$ K. Holes were read in the fluorescence excitation mode. The solid curve is the theoretical fit obtained using the theory of Kador et al. (1987), with $f\Delta\mu = 0.35$ D.

GHz (0.058 cm^{-1}) at 4 kV/cm. Fig. 6 shows the dependence of the FWHM of the hole burned at 670.2 nm on electric field (*diamonds*) and the theoretical fit (*solid curve*) obtained using the theory of Kador et al. (1987). The calculated dipole moment change $f\Delta\mu$ for the Q_y transition based on the symmetrical hole-broadening fit is 0.35 ± 0.03 D near the absorption band maximum (670.2 nm). It increases to 0.48 ± 0.05 D at the red side of the band (677 nm) (results not shown). Changing the laser polarization did not affect the broadening rate and hole shape.

Stark hole-burning studies of chlorosomes

Stark hole-burning experiments on the lowest exciton level of the chlorosome Q_y band were conducted at 1.8 K. The widths of the ZPH at zero field were ~2 cm^{-1}, as detected in the absorption mode (results not shown). This width, if interpreted in terms of energy transfer from the lowest exciton level to the baseplate, corresponds to a transfer time (T_1) of 5 ps. However, the possibility that the width is due to pure dephasing from exciton-defect scattering cannot be excluded. However, no Stark effect (i.e., broadening, splitting, or shift) was observed. Possible reasons for the absence of Stark effects will be discussed in the following section.

DISCUSSION

Absorption spectra

Compared with the 4.2 K absorption bands of other photosynthetic antenna complexes such as B800 (FWHM = 125 cm^{-1}) and B850 of LH2 (FWHM = 200 cm^{-1}) of purple bacteria and the three partially resolved Q_y bands (FWHM ≈ 100 cm^{-1}) of the FMO complex, the chlorosome Q_y band is considerably broader (FWHM ≈ 550 cm^{-1}) and clearly asymmetrical (Fig. 2). Given that the inhomogeneous broadening of the lowest exciton level of the chlorosome is only ~100 cm^{-1}, a significant fraction of the 550 cm^{-1} width is most likely due to higher energy exciton levels. These levels are inhomogeneously and homogeneously broadened because of structural heterogeneity and downward interexciton level relaxation, as proposed in the earlier hole-burning papers discussed in the Introduction.

Before discussing the spectral heterogeneity of chlorosomes, it is useful to discuss the exciton level structure of a J-aggregate. For a linear chain of N identical molecules, the Hamiltonian in the absence of energy disorder is (Fidder et al., 1991; Alden et al., 1992)

$$H_0 = e\sum_{\alpha}|\alpha\rangle\langle\alpha| + \sum_{\alpha,\beta(\alpha\neq\beta)} V_{\alpha\beta}|\alpha\rangle\langle\beta|, \qquad (2)$$

where $|\alpha\rangle$ label the states in which molecule α ($\alpha = 1, 2, \ldots N$) is excited and all others are in the ground state. e is the excitation energy of the chromophore, and $V_{\alpha\beta}$ is the coupling energy between molecules α and β. Under the nearest-neighbor coupling approximation $V_{\alpha,\alpha+1} \pm V_{\alpha,\alpha-1} = -V$, the Hamiltonian can be diagonalized exactly to give the eigenfunctions and eigenenergies as

$$|j\rangle = \left(\frac{2}{N+1}\right)^{1/2}\sum_{\alpha}^{N}\sin\left(\frac{\pi j\alpha}{N+1}\right)|\alpha\rangle \qquad (3)$$

and

$$E_j = -2V\cos\left(\frac{\pi j}{N+1}\right), \qquad (4)$$

where $j = 1, 2, \ldots, N$. For simplicity, e of Eq. 2 has been set equal to zero. From Eq. 3 the transition dipole strength of $|j\rangle$ is found to be (Fidder et al., 1991)

$$f_j = \left(\frac{2\mu_{mon}^2}{N+1}\right)\cot^2\left(\frac{\pi j}{2(N+1)}\right), \quad j = \text{odd} \tag{5}$$

$$f_j = 0, \quad j = \text{even}. \tag{6}$$

μ_{mon} denotes the transition dipole moment of the chromophore. It follows from Eq. 5 that the $j = 1$ state carries the largest transition dipole strength. It also follows that f_j decreases with increasing j and that the larger the value of N, the smaller is the percentage of the total transition dipole strength carried by the $j = 1$ state. In the limit of large N, 81% of the total transition dipole strength is carried by the $j = 1$ state.

For J-aggregates, the coupling energy $-V$ between the nearest neighbors must be negative. As a result, the lowest exciton level ($j = 1$) carries most of the transition dipole strength in the absence of energy disorder. However, the J-aggregate picture is inconsistent with NPHB studies of chlorosomes which establish that the lowest exciton level is only weakly allowed (see Results and Fetisova and Maurring, 1992, 1993, and Psencik et al., 1998). Buck and Struve (1996) proposed that this issue can be resolved by introducing a tubular arrangement of J-aggregates and diagonal energy disorder to explain the exciton level structure of chlorosomes. Their tubular arrangement leads to the lowest exciton level being forbidden in the absence of energy disorder. Energy disorder endows this level with some intensity stolen from the allowed levels. This situation is similar to those of B870 of LH2 and B896 of LH1 of purple bacteria (Reddy et al., 1992b, 1993; Wu et al., 1997b,c; Wu and Small, 1997, 1998). The picture that emerges is that the broad and asymmetrical Q_y band of chlorosomes is a superposition of exciton levels carrying different absorption intensities and, possibly, bandwidths. However, the tailing on the blue side of the Q_y absorption band seen in Fig. 2 may be contributed to by weak intramolecular vibronic transitions of the BChl *c* molecules (Cherepy et al., 1996).

Spectral equilibration among different components of chlorosome BChl *c* molecules of *Cb. tepidum* was observed in the femtosecond pump-probe experiments of Savikhin et al. (1995). A red shift in the absorption difference spectrum was observed upon 720-nm excitation. The one-color and two-color isotropic data also confirmed the existence of spectral heterogeneity within the chlorosome absorption band. Similar results have been obtained for chlorosomes from BChl *e*-containing *Cb. phaeovibrioides* and BChl *c*- and *d*-containing *Cb. vibrioforme* by picosecond pump-probe laser spectroscopy (van Noort et al., 1994). Interestingly, chlorosomes from *Cf. aurantiacus*, which contain BChl *c* and trace quantities of BChl *d* molecules, do not show such downhill energy transfer within BChl aggregates. At room temperature, the FWHM of the chlorosome Q_y band of *Cb. tepidum* and *Cf. aurantiacus* is 800 and 530 cm^{-1}, respectively, suggesting greater heterogeneity for *Cb. tepidum*. As mentioned in the Introduction, the chlorosomes from green sulfur bacteria such as *Cb. tepidum* are larger in size and have higher BChl *c*/BChl *a* ratios than those of green nonsulfur bacteria such as *Cf. aurantiacus*. This, together with the spectral equilibration data obtained by Savikhin et al. (1995), suggests that more than one pool of BChl aggregates exists in green sulfur bacteria such as *Cb. tepidum* to compensate for the lower energy transfer efficiency to the baseplate due to larger chlorosome sizes and lower amounts of BChl *a* molecules. The different pools of BChl aggregates may be due to variations in the size of the rod elements and/or the relative numbers of BChl *c*, *d*, and *e* molecules and their homologs.

High-pressure studies

Pressure shifting of the Q_y band

According to earlier high-pressure studies on isolated chromophore systems and protein complexes, it was found that a pressure shift rate larger than $\sim$0.2 cm^{-1}/MPa is an indication of strong interactions between pigments (Reddy et al., 1996; Wu et al., 1997a, 1998). The shift and broadening rates of the chlorosome Q_y band are comparable to those of the strongly exciton-coupled B850 and B875 rings of LH2 and LH1 from purple bacteria (Freiberg et al., 1993; Tars et al., 1994; Reddy et al., 1996; Wu et al., 1997a, 1998) and are much larger than those of the monomer-like B800 molecules of LH2 (Tars et al., 1994; Reddy et al., 1996; Wu et al., 1997a, 1998).

Laird and Skinner (1989) developed a theory to account for the pressure broadening and shift of hole-burned spectra of isolated chromophores in isotropic, homogeneous amorphous solids. High-pressure hole-burning studies showed that the Laird-Skinner theory also works well for the monomer-like B800 molecules in LH2 (Reddy et al., 1996; Wu et al., 1997a) and the FMO complex from green bacteria (Reddy et al., 1995). Although in these systems the protein matrix is not isotropic, homogeneous, and structurally random, the weak BChl-BChl electrostatic coupling (defined as the dipole-dipole interaction) of a few tens of cm^{-1} and negligible electron exchange coupling permit use of the Laird-Skinner theory. That is, the pressure shifting appears to be dominated by attractive pigment-protein interactions, as required by the theory.

Despite the success of the Laird-Skinner theory in accounting for the pressure shift rates of the above systems, it is inapplicable to strongly coupled BChl in chlorosomes that exhibit a pressure shift rate of ~ -0.5 cm^{-1}/MPa. Currently there is no microscopic theory for pressure shifting of strongly exciton-coupled systems. Thus, in what follows, we use the simple analysis procedure developed for the

B850 band of LH2 and the B875 band of LH1 (Wu et al., 1998). In the point dipole-point dipole approximation, the contribution of electrostatic coupling (EC) between pigments to the pressure shift rate $\partial\nu/\partial p$ is

$$\left(\frac{\partial \nu}{\partial p}\right)_{EC} \approx -2V\kappa, \tag{7}$$

where the nearest-neighbor coupling (V) approximation with only two nearest neighbors is assumed. κ is the compressibility. Based on the red shift of $\sim$1400 cm^{-1} for the aggregated BChl *c* in chlorosomes relative to monomeric BChl *c*, the value of V is $\sim$700 cm^{-1}. This value has been used in theoretical calculations and modeling of chlorosomes (Buck and Struve, 1996; Fetisova et al., 1996). It is obtained under the assumption that the BChl *c*-protein interactions are negligible and do not contribute to the red shift of the chlorosome absorption band. Such an assumption is reasonable on the basis of the low protein/pigment ratio in chlorosomes and BChl *c in vitro* aggregation behavior. For J-aggregates, the reported V values range from 500 to 1200 cm^{-1} (Kopainsky et al., 1981; van Burgel et al., 1995; Lindrum and Chan, 1996). For B850 molecules of LH2 antenna from purple bacteria, the value of V has been argued to be $\sim$500 cm^{-1} (Wu et al., 1998). Using $V = 700$ cm^{-1} and $\kappa = 0.1$ GPa^{-1} (Perepechko, 1980), Eq. 7 yields a pressure shift rate due to electrostatic couplings of -0.14 cm^{-1}/MPa. For the pressure shift rate of chlorosomes, the total contribution of -0.14 cm^{-1}/MPa from electrostatic couplings and -0.1 cm^{-1}/MPa from dispersion interactions (Wu et al., 1998) falls short of the measured value of ~ -0.50 cm^{-1}/MPa by -0.26 cm^{-1}/MPa. Thus, as in the case of the strongly coupled B850 and B875 molecules of LH2 and LH1, it appears that electron exchange interactions between the aggregated BChl *c* molecules in chlorosomes should not be neglected in the analysis. We note that electron exchange coupling leads to charge transfer states that interact with the $\pi\pi^*$ states.

The contribution of electron exchange (EE) interactions to the pressure shift rate is approximately given by

$$\left(\frac{\partial \nu}{\partial p}\right)_{EE} \approx -(2/3)A\kappa R_{EE}, \tag{8}$$

where R_{EE} is the effective BChl-BChl distance controlling EE coupling and A is defined as $\Delta V_{EE}/\Delta R_{EE} = -$A for $\Delta R_{EE} < \pm$ 0.1 Å. ΔV_{EE} is the change in coupling due to a change in ΔR_{EE}. Utilization of Eq. 8 requires a value for R_{EE} that, in the absence of a x-ray structure of chlorosomes, cannot be estimated, although it may correspond to the interplanar separation between neighboring BChl *c* molecules. For the B850 molecules of LH2, R_{EE} was taken to be 3.5 Å, based on the x-ray structure (Freer et al., 1996; Koepke et al., 1996). For what follows, we use a R_{EE} value of 3.5 Å. With $\kappa = 0.1$ GPa^{-1}, an A value of 110 cm^{-1}/0.1 Å is obtained from Eq. 8 to account for the discrepancy of -0.26 cm^{-1}/MPa in the pressure shift rate mentioned above. Using the same method and $R_{EE} = 3.5$ Å, an A value of 85 cm^{-1}/0.1 Å for the B850 molecules of the LH2 complex and 126 cm^{-1}/0.1 Å for the B875 molecules of the LH1 complex of purple bacteria were obtained (Wu et al., 1998).

It is possible, however, that R_{EE} in chlorosomes is shorter than that of the B850 molecules interacting with histidine ligands and that it approaches the van der Waals contact limit of $\sim$3 Å. That is, in the absence of interactions with histidine residues, the BChl *c* molecules in chlorosomes may adopt a more compact arrangement.

Pressure broadening of the Q_y band

As summarized in table 1 of Wu et al. (1997a), the pressure broadening of the B800 band of LH2 is negligible, while for the B850 band the broadening rate is 0.13–0.23 cm^{-1}/MPa, depending on the species. For the Q_y band of isolated chlorosomes, the broadening rate is 0.12 cm^{-1}/MPa (Fig. 3). Pressure broadening can be qualitatively understood in terms of the underlying exciton level structure and the pressure shifting rates of the levels. Different exciton levels may shift at different rates, and the rates can have different signs, leading, therefore, to pressure broadening. This reasoning is supported by the observation that the shift rate of -0.54 cm^{-1}/MPa for the lowest exciton level is larger than the shift rate of -0.44 cm^{-1}/MPa for the origin absorption band of the chlorosome Q_y transition, as determined from the band maximum. One can view the shift rate of the entire absorption band to the red as the average of the larger rate of the lowest exciton level and smaller rates of other levels that contribute to the Q_y band. That the exciton level lying lowest in energy tends to shift faster can be qualitatively understood in terms of perturbative arguments involving dispersion interactions, electrostatic and electron-exchange interactions, and energy disorder. Support for this line of argument is also provided by the observation that the broadening rate for the P960 special pair band of *Rps. viridis*, to which only one exciton level contributes, is small, 0.02 cm^{-1}/MPa (Reddy et al., 1996).

Stark hole-burning studies

Stark hole burning of BChl c monomers in polymer films

That the hole broadening for BChl *c* monomers is independent of laser polarization suggests that the random contribution from the amorphous polymer matrix is the dominating factor in determining the BChl *c* Stark effect. The absence of a hole shift or asymmetrical hole broadening suggests that the quadratic Stark effect is negligible for the BChl *c* monomers. Using the theory of Kador et al. (1987), $f\Delta\mu$ at the center of the absorption band is 0.35 D, which,

within experimental uncertainty, is equal to the value of 0.33 D for Chl *a* monomers obtained by Stark hole-burning (Altmann et al., 1993). A somewhat larger $f\Delta\mu$ value of 0.6 D for Chl *a* monomers was obtained by Small and co-workers (Rätsep et al., 2000). Spectral properties of BChl *c* monomers are generally believed to be similar to those of Chl *a* because ring B is unsaturated in both BChl *c* and Chl *a*. That the Q_y absorption bands for BChl *c* and Chl *a* monomers are both located at ~670 nm is consistent with this. Stark hole burning has also been performed on the Chl *a*-containing photosystem II and Chl *a*/*b*-containing light-harvesting complex II from higher plants (Rätsep et al., 2000). Those studies indicate that $f\Delta\mu$ values for weakly coupled Chl *a* molecules in photosynthetic complexes are ~0.6 D.

The polarizability change of BChl *c* can be estimated using the following empirical formula, which was arrived at on the basis of Stark hole-burning and solvent shift data for 11 π-electron chromophores isolated in poly(vinyl butyral) (Altmann et al., 1993):

$$\Delta\mu_{ind}(D) = -0.04 + 8.8\Delta\alpha/MW. \quad (9)$$

MW is the molecular weight of the chromophore (the phytyl tail of the BChl molecule is excluded). The unit of $\Delta\alpha$ is Å^3. For *Cb. tepidum*, the two major BChl *c* homologs found in the chlorosomes both have an ethyl group at the 12 position, and each has a *n*-propyl and ethyl group at the 18 position. The average molecular weight for these two homologs is 608. We assume that the measured $f\Delta\mu$ of 0.35 D contains only the matrix-induced component, because the laser polarization-independent and symmetrical hole broadening suggests little contribution from $\Delta\mu_0$. With $f = 1.5$ and MW = 608, Eq. 9 yields $\Delta\alpha = 19 \pm 2$ Å^3 for monomeric BChl *c* molecules, which essentially is the same as the Chl *a* value of 18 Å^3 obtained by Altmann et al. (1993).

Stark hole burning of isolated chlorosomes from Cb. tepidum

In contrast to BChl *c* monomers, no Stark effect was detected for the lowest exciton level of the chlorosome Q_y band at the maximum field strength of 100 kV/cm. It should be emphasized that the relatively shallow saturated hole depths (usually less than ~0.05 fractional depth) and broad holewidths observed in chlorosomes (~2 cm^{-1}, compared with ~0.04 cm^{-1} for the BChl *c* monomer) would hinder the observation of small Stark effects. Previous studies by Blankenship and co-workers (Causgrove et al., 1990; Wang et al., 1990; Blankenship et al., 1993) indicated the existence of redox-activated regulation of energy transfer efficiency and excited-state lifetimes of chlorosomes from green sulfur bacteria. Such a mechanism serves to protect the low-potential iron sulfur center from oxidative damage. The addition of dithionite to keep the chlorosomes in a reducing environment increased the hole depth only slightly, and the holes were slightly narrower (1.4 cm^{-1} at 775 nm; results not shown). Experiments were performed on whole cells of *Cb. tepidum* in the presence of dithionite, but the results (holewidth = 1.6 cm^{-1} and fractional hole depth ≈ 0.04 at 777 nm) are similar to those of isolated chlorosome complexes. As for the difficulties in obtaining deeper ZPHs in chlorosomes, there was evidence (results not shown) suggesting that this may be due to partial hole filling induced by the white light of the Fourier transform spectrometer employed in the experiments. Holes with a fractional hole depth of 0.1 have been reported for the lowest exciton level of *Cb. limicola* detected in the fluorescence excitation mode (Fetisova and Mauring, 1993).

The absence of observable Stark effects in this study restricts us to an estimate of the upper limit for the total dipole moment change $f\Delta\mu$. Under the reasonable assumption that a minimum symmetrical hole broadening of 0.3 cm^{-1} is necessary for the observation of the linear Stark effect at 100 kV/cm on a hole with an initial holewidth of 2 cm^{-1}, $f\Delta\mu$ must be less than 0.3 D (based on equation 15 of Kador et al., 1987). The value of 0.3 D is essentially equal to the value of 0.35 D for BChl *c* monomer in a polymer, *vide supra*, a value that was concluded to be due mainly to $f\Delta\mu_{ind}$, because Stark splitting was not observed for laser polarization parallel or perpendicular to the electric field $\underline{E}_s$; i.e., the molecular contribution $f\Delta\mu_0$ to 0.35 D is small. It follows from exciton theory that $f\Delta\mu_0$ for the BChl *c* aggregate cannot be larger than that of the monomer (Wu and Small, 1998). Furthermore, the structure of the aggregated BChl *c* molecules could lead to a further reduction in the magnitude of $f\Delta\mu_0$. This possibility is suggested by the paper of Wendt and Friedrich (1996), in which a helical structure was invoked to explain the marked reduction in $f\Delta\mu_0$ of the J-aggregate relative to that of the monomer. It follows that a helical type structure could also lead to a reduction in the value of $f\Delta\mu_{ind}$(nonrandom).

This brings us to the question of why $f\Delta\mu_{ind}$ is no greater than ~0.3 D, given that Frese et al. (1997) concluded that $f^2\mathrm{Tr}(\Delta\alpha)$ for chlorosomes is 1600 Å^3 on the basis of classical Stark modulation experiments at 77 K. With $E_{int} = 10^6$ V/cm, which is close to the values measured for polystyrene and *n*-hexane crystals (Kador et al., 1990; Gradl et al., 1992), $f\Delta\mu_{ind} \approx 5$ D, which is a factor of ~10 greater than the upper limit determined above. An $f\Delta\mu_{ind}$ of 5 D would have been easily detected in our experiments. One explanation for this discrepancy is that $\Delta\mu_{ind}$ is dominated by the nonrandom contribution and that structural constraints, such as a helical structure or a tubular arrangement (Buck and Struve, 1996), leads to a cancellation effect. Frese et al. (1997) suggested an antiparallel arrangement of BChl *c* molecules to explain why a $f\Delta\mu$ signal was not observed in their Stark spectra. A second explanation suggested in that work is that E_{int} is small because of the low amount of protein residues near the BChl *c* aggregate. A value of

$E_{int} \approx 10^5$ V/cm would explain the absence of a linear Stark effect in our experiments. Such a small value, however, seems difficult to reconcile, given that it is an order of magnitude smaller than those of polystyrene and *n*-hexane. A third possibility is that $\Delta\alpha$ of the lowest and weakly allowed exciton level probed by Stark hole-burning is much smaller than those of the strongly allowed levels, which dominate the optical responses in classical Stark modulation spectroscopy. This possibility can be investigated using the theory of Somsen et al. (1998) once the chlorosome structure has been determined.

Finally, we address the question of why a quadratic Stark effect was not observed in our experiments. The condition for its observation is (Rätsep et al., 1998a)

$$E_{int} \leq fE_s/2 \tag{10}$$

when $\Delta\mu_0$ is small relative to $\Delta\mu_{ind}$, which, given our results for BChl *c* monomer, is a reasonable assumption. The above condition is not satisfied with $E_{int} = 10^6$ V/cm, because our maximum E_s value was 50 kV/cm (a value close to 1.5 is often used for f). Furthermore, it may not be satisfied even if E_{int} is as small as 10^5 V/cm.

SUMMARY

The strongly coupled nature of BChl *c* molecules in chlorosomes was confirmed by the large linear pressure shift rate of the Q_y band (-0.44 cm^{-1}/MPa) and the lowest exciton level (-0.54 cm^{-1}/MPa) at low temperature. According to the analysis presented, about half of the contribution to the shift rate is from electron exchange interactions, which require a rather compact arrangement of pigments. This important finding serves as a guideline for electronic structure calculations of the chlorosome in the absence of knowing its exact structural arrangement of pigments.

The dipole moment change ($f\Delta\mu$) and polarizability change ($\Delta\alpha$) for the Q_y transition of BChl *c* monomers in a polymer film were determined to be 0.35 D and 19 Å^3, respectively. $\Delta\mu$ is dominated by the matrix-induced contribution. The absence of any Stark effect in the lowest exciton level of chlorosomes led us to conclude that pigments in chlorosomes possibly adopt certain structural constraints to attain small dipole moments and polarizability changes. The basic tubular models put forth by Buck and Struve (1996) and Somsen et al. (1996), as well as the helical structure proposed by Wendt and Friedrich (1996), are all in agreement with the Stark hole-burning results, as long as the orientations of the pigments result in the reduction of $\Delta\mu_0$ and $\Delta\alpha_{ind}$.

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