Stark Hole-Burning Studies of Three Photosynthetic Complexes

M. Rätsep,[†] H.-M. Wu,[†] J. M. Hayes,[†] R. E. Blankenship,[‡] R. J. Cogdell,[§] and G. J. Small*,[†]

Ames Laboratory−USDOE and Department of Chemistry, Iowa State University, Ames, Iowa 50011, Department of Chemistry and Biochemistry, Center for the Study of Early Events in Photosynthesis, Arizona State University, Tempe, Arizona 85287-1604, and Division of Biochemistry and Molecular Biology, Institute of Biomedical and Life Sciences, University of Glasgow, G128 QQ, U.K.

Stark hole-burning spectroscopy at 1.8 K was used to determine the dipole moment changes $f\Delta\mu$ (f, the local field correction factor) for the B800 absorption band of the light harvesting 2 (LH2) complex of *Rhodobacter sphaeroides, Rhodopseudomonas acidophila* (strain 10050), and *Rhodospirillum molischianum.* Hole-burning values of $f\Delta\mu$ for the lowest energy exciton level (B870) associated with LH2's B850 band have recently been reported (Rätsep et al. *Spectrochim. Acta,* in press). Values for the lowest energy exciton level (B896) associated with the B875 band of the LH1 complex of *Rb. sphaeroides* (wild-type chromatophores and an LH1-only mutant) and the 825 nm band of the bacteriochlorophyll *a* (FMO) antenna complex of *Chlorobium tepidum* are also reported. For each band, $f\Delta\mu$ was determined for burn laser polarization parallel and perpendicular to the Stark field E_S and several burn frequencies. The dependencies on laser polarization and burn frequency are typically quite weak. Importantly, $f\Delta\mu$ values for the above bands are small, falling in the range ∼0.5−1.2 D, with the lowest and highest values associated with the 825 nm band of the FMO complex and B800 band of the LH2 complex, respectively. For the B896 band of the LH1 complex, $f\Delta\mu \approx$ 0.8 D. Such small values are consistent with the very weak linear electron−phonon coupling of antenna protein complexes as determined by hole-burning spectroscopy. Overall, the values for $f\Delta\mu$ from classical Stark modulation (CSM) studies (Gottfried et al. *Biochim. Biophys. Acta* **1991,** *1059,* 63; Beekman et al. *J. Phys. Chem. B* **1997,** *101,* 7293) are larger, in the cases of B850 and B875, by a factor of 3−4. (In CSM spectroscopy, one analyzes the response of the entire absorption band to the external field.) Discussion of the discrepancies between the two Stark techniques is given. It appears that difficulties inherent to the analysis procedure of CSM spectroscopy can lead to unreliable values for dipole moment and polarizability changes associated with absorption bands of photosynthetic complexes, especially when several excitonic levels contribute to the band, e.g., B850 and B875. An explanation for the small hole-burning values of $f\Delta\mu$ for the B870 and B896 levels associated with C_n cyclic arrays of strongly coupled BChl *a* dimers is given based on structural, symmetry, and energy disorder considerations. A key point is that, in the absence of energy disorder, the component of $\Delta\mu_j$ (j labeling the exciton level) perpendicular to the C_n axis is zero. Energy disorder, which destroys the C_n symmetry and leads to localization effects results in nonzero values which may depend on j when the protein-induced contribution to $\Delta\mu_j$ is taken into account.

1. Introduction

In the preceding paper[1] we presented absorption and hole-burning results on the effects of pressure and temperature on the B875 absorption band of the light harvesting complex 1 (LH1) of *Rhodobacter sphaeroides* (wild-type chromatophores and an LH1-only mutant). Of particular importance to this paper is that the linear pressure shifts of −0.60 and −0.67 cm⁻¹/MPa for the B875 band and the lowest exciton level (B896) of the B875 ring of BChl *a* molecules are very large, about 6−7 times larger than observed for weakly coupled BChl *a* molecules in protein complexes and $\pi\pi*$ states of isolated chromophores in polymers and proteins. (A relevant example of weakly coupled BChl *a* molecules is those responsible for the B800 absorption band of the LH2 complex of purple bacteria.) In that paper (referred to, hereafter, as I) it was argued that the above large pressure shifts cannot be explained in terms of electrostatic BChl *a*−BChl *a* interactions and BChl *a*−protein interactions. It was

estimated that, in combination, these interactions provide a shift of only about −0.2 cm⁻¹/MPa, a value that is supported by the pressure shift data for the B800 band of the LH2 complex and the Q_y bands of the BChl *a* antenna complex of *Chlorobium tepidum.* It was concluded that electron-exchange interactions between nearest neighbor BChl *a* molecules of the B875 ring are mainly responsible for the largeness of the pressure shifts and that such interactions are also important for the B850 ring of the LH2 complex (see I for descriptions of the structural arrangement of the BChl *a* molecules in the cyclic (C_n) B850 and B875 rings; the lowest energy exciton level of the B850 BChl *a* ring is often referred to as B870. Like B896, it carries A symmetry in the absence of disorder.) Electron-exchange leads to charge-transfer (CT) states which mix with the neutral $Q_y(\pi\pi*)$ states of the cyclic arrays as discussed by Alden et al.[2]

Accepting that CT states are important for understanding the exciton band structures of the B850 and B875 rings, a natural next step is to study the effect of external electric (Stark) fields on the B850 and B875 absorption bands and the exciton levels

---
[†] Iowa State University.
[‡] Arizona State University.
[§] University of Glasgow.

TABLE 1: Earlier Stark Results for the LH2 Complex

complex	band	$f\Delta\mu_{\|}$ (D)[a]	$f\Delta\mu_{\perp}$ (D)[a]	band	$f\Delta\mu_{CSM}$ (D)[b]	$f^2\text{Tr}(\Delta\alpha)$ (Å³)[b]
Rb. sph.	B870	1.10 ± 0.1	1.44 ± 0.1	B850	4.2 (3.3)[c]	619
Rps. acid.	B870	←1.0 ± 0.05→		B850	3.2	1250
Rs. moli.	B870	←1.2 ± 0.1→		B850	3.2	1420
Rb. sph.	B800	1.1 ± 0.2	0.8 ± 0.2	B800	1.1 (0.9)[c]	5

[a] Results from ref 29. [b] Results from ref 13 except for c. [c] Ref 30. CSM ≡ Stark modulation spectroscopy at 77 K. Typical stated uncertainties for $\Delta\mu_{CSM}$ are ±0.1 D.

that contribute to them. That is, a significant CT contribution to the exciton wave functions can lead to large dipole moment changes ($\Delta\mu$) for $S_1(Q_y) \leftarrow S_0$ transitions.[3] $\Delta\mu$ can be viewed as the sum of $\Delta\mu_0$ and $\Delta\mu_{ind}$, where $\Delta\mu_0$ is the molecular component and $\Delta\mu_{ind}$ the matrix-induced component. Classical Stark modulation (CSM) spectroscopy, in which the response of an absorption band to the applied field is determined, has been extensively applied to the special pair band of the bacterial reaction center (see, for example, refs 4−6). A value of $f\Delta\mu_{CSM}$ = 5.2 D at 77 K for the special pair (P) band of *Rb. sphaeroides*, P870, was determined[6] using the theory of Liptay[7] for analysis. f is the local field correction factor.[8] The analysis also led to a large polarizability change, $f^2 \text{Tr}(\Delta\alpha) \sim 1000$ Å³, which is over 1 order of magnitude larger than the value for monomeric BChl *a*.[9] The large polarizability change, together with the observation that $f\Delta\mu_{CSM}$ = 5.2 D is a factor of 2−3 times larger than the values for absorption bands of the reaction center associated with weakly coupled BChl *a* and BPheo *a* cofactors, led to the conclusion that the strong response of the special pair band is the result of P* (asterisk denoting excited state) carrying significant CT character. CSM studies of isolated BChl *a* molecules in polymer films[4] which yielded values for $f\Delta\mu_{CSM}$ in the range of ∼2.0−2.5 D also figured into this conclusion. That the CT character of P* leads to a large dipole moment change is consistent with the results of hole-burning experiments that showed that the P870 band is characterized by strong linear electron−phonon coupling with the Huang−Rhys factor S equal to 1.8 and 1.5 for modes centered near 30 and 120 cm⁻¹. These couplings lead to a large optical reorganization energy of 230 cm⁻¹.[10] The reader is referred to ref 11 for details. In that work it was emphasized that the electron−phonon coupling for the B875 and B850 bands (also the B800 band of the LH2 complex) is a factor of 20−30 times weaker, meaning that the BChl *a* molecules and protein are essentially blind to the optical excitations associated with these bands.

Results from CSM studies at 77 K of the B800, B850, and B875 bands of *Rb. sphaeroides*, *Rps. acidophila*, and *Rs. molischianum* have recently been reported.[12,13] Values of $f\Delta\mu_{CSM}$ and $f^2 \text{Tr}(\Delta\alpha)$ for B850 and B800 of the LH2 complexes are given on the right side of Table 1. Although the $f\Delta\mu_{CSM}$ values for B850 are about 30% smaller than that of P870, they are considerably larger than those for B800 of *Rps. acidophila* and *Rb. sphaeroides*. Furthermore, the B850 polarizability changes are large and comparable to the value for P870. It was concluded that, like P870, CT states make a significant contribution to the neutral $\pi\pi*$ exciton levels of the B850 ring.

The resolution of Stark spectroscopy is significantly improved by utilizing spectral hole burning. This combination has proven to be powerful for analysis of $\Delta\mu$ associated with the $S_1 \leftarrow S_0$ transitions of isolated molecular chromophores in amorphous polymer films at liquid helium temperatures.[14−27] Stark hole burning has also been reported for mesoporphyrin-IX in horseradish peroxidase.[28] Because the zero-phonon holes (ZPH) of the chromophores in these systems are very narrow (≲1 GHz) at liquid helium temperatures, modest field strengths (few tens

of kV/cm) could be used to determine the matrix-induced ($\Delta\mu_{ind}$) and molecular ($\Delta\mu_0$) contributions to the change in dipole moment between the ground and excited electronic states. In some of these studies the Stark cell configuration allowed for propagation of the laser burn and read beams perpendicular to the external field (E_S) so that the Stark effect on the ZPH could be determined with burn laser polarization parallel and perpendicular to E_S. Use of both polarizations is important for determination of $\Delta\mu_{ind}$ and $\Delta\mu_0$. We note that a dependence of $\Delta\mu_{ind}$ on the location of the burn wavelength within the inhomogeneously broadened absorption band yields additional information on the polarizability of the chromophore and the internal electric field (E_{int}) it experiences.[17]

Stark hole burning has recently been applied to the LH2 complex of purple bacteria.[29] The results for $f\Delta\mu_{\|}$ and $f\Delta\mu_{\perp}$ are given on the left side of Table 1. The subscripts on $\Delta\mu$ denote laser polarization parallel and perpendicular to the Stark field, E_S. The significance of a difference between the values of $f\Delta\mu_{\|}$ and $f\Delta\mu_{\perp}$ is discussed in section 4. Although the agreement between the Stark hole-burning and CSM values for $\Delta\mu$ of B800 (*Rb. sphaeroides*) is good, the values for B850 of all three species are very different. (We remind the reader that B870 is the lowest energy exciton level of the B850 BChl *a* ring.) Possible explanations for the large discrepancies are given in section 4. We note at this point, however, that the small value of $\Delta\mu \sim 1.1$ D for B870 determined by hole burning is consistent with weak electron−phonon coupling.

CSM spectroscopic values of $f\Delta\mu$ and $f^2 \text{Tr}(\Delta\alpha)$ for the B875 band of the LH1 complex have also been reported.[12] They are similar to the CSM values for the B850 band; see sections 3 and 4. In this paper we report $\Delta\mu$ values from hole burning for the B896 exciton level of wild-type and LH1-only mutant chromatophores of *Rb. sphaeroides*. In addition, values for the B800 band of *Rps. acidophila* and *Rs. molischianum* and the lowest energy Q_y band (825 nm) of the Fenna−Matthews−Olson (FMO) BChl *a* antenna complex of *Chlorobium tepidum* are reported for comparison against CSM values.[30]

2. Experimental Section

The reader is referred to I (section 2 and references therein) for the procedures used to obtain wild-type (WT) and LH1-only mutant chromatophores of *Rb. sphaeroides* and details concerning the glycerol/water glass-forming solvent and detergents used. Samples of the FMO complex from *Cb. tepidum* were prepared as described in Savikhin et al.[31] Complexes were dissolved in a glycerol/water mixture (70:30 by volume).

Samples were contained in gelatin capsules (0.13 mL/o.d. = 4.5 mm) purchased from Torpac Inc. Prior to insertion into the Stark cell, the gelatin capsule *filled* with sample was allowed to soften for about 5 min at room temperature so that it could be mechanically squeezed by the two copper electrodes of the Stark cell. This procedure yielded an optical path length perpendicular to the applied field of ∼6 mm with a distance of ∼2 mm between the electrodes. Teflon spacers were used to set the distance between the electrodes (±0.05 mm). The Stark

Stark Hole-Burning Studies

J. Phys. Chem. B, Vol. 102, No. 20, 1998 **4037**

field could be applied parallel or perpendicular to the burn laser polarization by positioning a polarizer placed in front of the Stark cell. The probing light (unpolarized) was collinear with the burning beam. A Trek Inc. model 610 C dc high-voltage power supply (0 to ± 10 kV) was used. By changing the polarity of the power supply, a maximum Stark field of ~ 100 kV/cm was achievable. Holes were initially burned at the highest field with a chosen polarity.

All measurements were made at 1.8 K in a Janis 10 DT liquid helium cryostat. With the sample immersed in pumped liquid helium, arcing never occurred at our maximum voltage. With an optical path length of ~ 6 mm, the concentration of the chromatophores and complexes was sufficiently low to ensure formation of high-quality glasses. In this regard, use of gelatin capsules was important. Optical densities of the samples were adjusted to ~ 0.5 in the spectral regions where hole burning was performed.

The burn laser was a Coherent CR899-21 Ti:sapphire laser (line width of 0.07 cm^{-1}) pumped by a 15 W Coherent Innova 200 Ar-ion laser. The preburn and postburn absorption spectra were obtained by using a Bruker IFS120 HR Fourier transform spectrometer, the difference of such spectra being the persistent nonphotochemical hole-burned spectrum. Spectral holes for B896 of the B850 ring were burned with linearly polarized light to fractional OD depths in the range 0.06−0.14. Typical burn intensities were ~ 200 mW/cm^2 with burn times of ~ 120 s. Holes in the lowest energy band of the FMO complex at ~ 825 nm were burned to fractional depths of 0.2−0.3. Typical burn intensities and burn times were ~ 50 mW/cm^2 and ~ 10 s.

3. Results and Analysis

We present first the Stark hole-burning results for the BChl *a* antenna complex of *Cb. tepidum* and then the results for the LH1 and LH2 complexes. Before doing so we present a brief review of some basic physics for an isolated chromophore in a matrix. The Stark shift of the optical transition frequency of the absorber is given by[15]

$$\Delta\omega = -\hbar^{-1}\left[(\Delta\mu_0 + \Delta\alpha E_{\text{int}})fE_{\text{S}} + \frac{1}{2}(fE_{\text{S}})\Delta\alpha(fE_{\text{S}})\right] \quad (1)$$

where, for simplicity, we take the local field correction factor f to be a scalar. $\Delta\mu_0$ is the molecular dipole moment change. E_{int} is the matrix field experienced by the absorber and $\Delta\mu_{\text{ind}} = \Delta\alpha E_{\text{int}}$, with $\Delta\mu_{\text{ind}}$ the dipole moment change induced by the matrix field. $\Delta\alpha$ is the molecular polarizability difference tensor. The first and second terms in square brackets depend linearly and quadratically on the Stark field. As discussed in ref 29, E_{int} in molecular systems is large, $\gtrsim 10^6$ V/cm (see also refs 6, 17, 18), 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 and all isolated chromophores in polymer, glass, and protein matrixes.[14−29,32,33] Thus, the second term in eq 1 is of no importance for Stark hole burning with the field strengths we employ.

Considering further the case of isolated molecules in glassy matrixes, let γ be the angle between the molecular dipole moment difference vector $\Delta\mu_0$ and the transition dipole vector $\square$. Linearly polarized light preferentially burns out those molecules with $\square$ parallel to the polarization vector e of the light. The experimentally observed dipole moment change can be written as $\Delta\mu = \Delta\mu_0 + \Delta\mu_{\text{ind}}$, where $\Delta\mu_{\text{ind}}$ is the matrix-induced contribution. When $\Delta\mu_0$ is dominant, the photoselection phenomenon enables one to probe molecules for which the angle

between $\Delta\mu_0$ and e is well-defined (shallow hole limit). As discussed in refs 15 and 28, Stark splitting of the hole can be observed for an angle between e and E_{S}, which depends on the value of γ. For example, for $\gamma = 0$ or π Stark splitting occurs for parallel polarization, while broadening occurs for perpendicular polarization. The situation is reversed for $\gamma = \pm\pi/2$. However, when $\Delta\mu_{\text{ind}}$ is dominant, only Stark broadening is expected for both polarizations. This is because the orientation of $\Delta\mu_{\text{ind}}$ relative to $\square$ or e is random for a glassy matrix; that is, the matrix field varies significantly from site to site.

The assumption of random orientations for $\Delta\mu_{\text{ind}}$ for Chl molecules in photosynthetic complexes is questionable since the structure of the protein around these chromophores is well-defined even though the Q_y absorption bands do suffer from significant inhomogeneous broadening. For example, Gafert et al.[28] 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. However, for the B800 and B870 bands of the LH2 complex, Stark broadening of the ZPH was observed for both parallel and perpendicular laser polarizations.[29] Further discussion of differences between isolated chromophores in glasses and Chl molecules in photosynthetic complexes is given later.

BChl *a* Antenna Complex of *Cb. tepidum*. The 4.2 K Q_y absorption spectrum of this BChl *a* (FMO) complex from *Prosthecochloris aestuarii* and *Cb. tepidum* exhibits prominent bands at 825, 814, and 805 nm.[34,35] Although the X-ray structure for the former species has been known for many years,[36] the structure for *Cb. tepidum* was only recently reported.[37] For both, the complex is a C_3 trimer of subunits which contain seven symmetry inequivalent BChl *a* molecules. Differences in the relative orientations of the BChl *a* molecules and distances between them for the two structures are minor.[37] Some potentially significant differences in H-bonding and BChl *a*−protein residue distances are observed. Spectral hole-burning results led to the conclusion that the 825 nm band is contributed to by two closely spaced, ~ 40 cm^{-1}, exciton levels of the trimer associated with the lowest energy level of the subunit with the lowest level at 827 nm.[34] This finding led to excitonic structure calculations that yielded results that provide some support for this conclusion[38,39] (there are, however, differences between the overall results of these two theoretical works; see section 4). It should be noted that it was assumed in refs 38 and 39 that the three subunits of the trimer are identical; that is, energy disorder due to structural heterogeneity was ignored. The implications of energy disorder for the above trimer interpretation for the 825 nm band are considered in section 4. In this subsection, Stark hole-burning data are presented for the 825 nm band of the FMO complex (*Cb. tepidum*). Stark hole burning is not possible for exciton levels higher in energy than the 825 nm band due to hole broadening from subpicosecond interexciton level relaxation processes.

Stark hole burning was performed at several burn frequencies (ω_{B}) between 12 161 and 12 076 cm^{-1}, from the high- to low-energy sides of the 825 nm band. No dependence of the Stark results on ω_{B} was observed. Some hole profiles obtained with $\omega_{\text{B}} \approx 12\ 115$ cm^{-1} are shown in Figure 1 for laser polarization perpendicular and parallel to the Stark field, E_{S}. These polarizations lead to hole broadening and splitting, respectively. To the best of our knowledge, this is the first time Stark hole splitting for a photosynthetic complex has been observed. The data are plotted in Figure 2, where it is seen that the Stark splitting is linear in E_{S}. For $E_{\text{S}} \gtrsim 40$ kV/cm, the broadening of
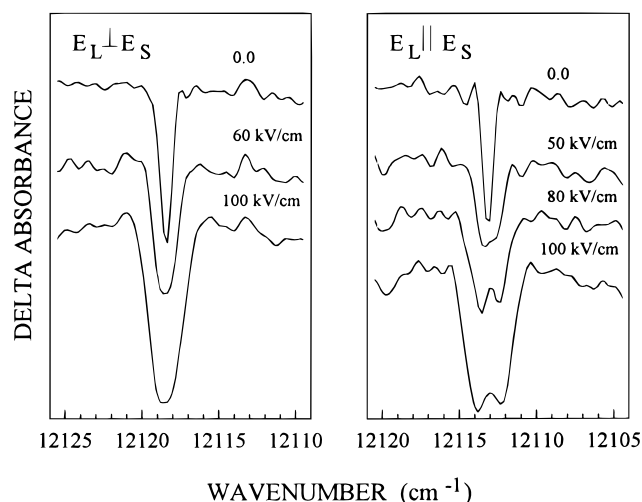
Figure 1. Stark effect on zero-phonon holes burned into B825 of the BChl *a* antenna complex from *Cb. tepidum*. Left and right frames are for burn laser polarization perpendicular and parallel to the Stark field, E_S, respectively.
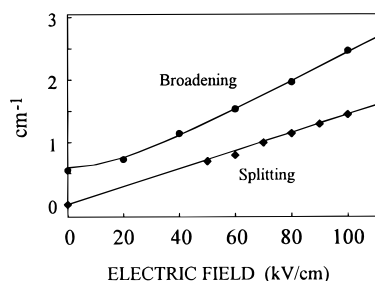

Figure 2. Dependence of the holewidth and Stark splitting on the zero-phonon holes of B825 of *Cb. tepidum* on electric field for perpendicular (circles) and parallel (diamonds) burn laser polarization. The burn frequencies were 12 119 cm^{-1} and 12 113 cm^{-1}, respectively. The solid curves are theoretical fits calculated using eq 4 with $f\Delta\mu_\perp = 0.72$ D (broadening) and eq 2 with $f\Delta\mu_{||} = 0.42$ D (splitting).
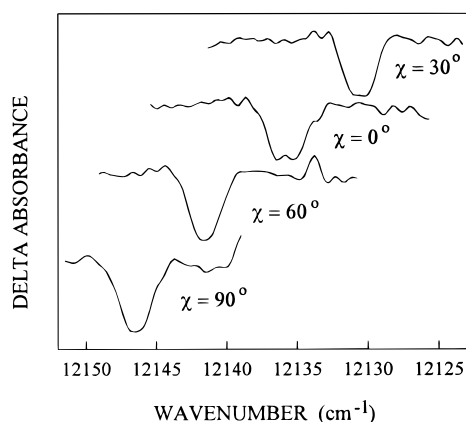

Figure 3. Stark effect on the hole profile of B825 of *Cb. tepidum* for angles (χ) of 0, 30, 60, and 90° between burn laser polarization and electric field of $E_S = 100$ kV/cm.

the hole profile varies linearly with E_S. The observation of Stark splitting for $e \parallel E_S$ but not for $e \perp E_S$ means that the angle γ between $\Delta\mu$ and the transition dipole $\varnothing$ of B827 is smaller than 45°.[28] To arrive at an upper limit for γ, the Stark effect on the hole profile for angles (χ) of 0°, 30°, 60°, and 90° between e and E_S was determined with $E_S = 100$ kV/cm, Figure 3. Stark splitting is not observed for $\chi = 60°$, and the splitting for $\chi = 30°$ is considerably smaller than the splitting for $\chi = 0°$ (1.1 and 1.4 cm^{-1}, respectively). Comparison of the hole profiles in Figure 3 with the results of theoretical simulations in ref 40

led to $\gamma \lesssim 15°$. The smallness of γ and the observation that the Stark splitting ($\Delta\omega_{SS}$) is maximum for $\chi \approx 0°$ means that in the well-known expression (see, for example, ref 41)

$$\Delta\omega_{SS} = (29.8)^{-1} f\Delta\mu E_S \cos\theta \qquad (2)$$

the angle, θ, between $\Delta\mu$ and E_S is $\lesssim 15°$ so that $\cos\theta \gtrsim 0.97$. In eq 2 the units of $\Delta\omega_{SS}$, $\Delta\mu$, and E_S are cm^{-1}, debye, and kV/cm. This equation with the data in Figure 2 yields $f\Delta\mu_{||} = 0.42 \pm 0.05$ D, where $\Delta\mu_{||}$ is the total dipole moment change. That the Stark effects do not depend on ω_B means that statistical fluctuations in structure from complex to complex are too small to be reflected in $\Delta\mu$. The Stark splittings plotted in Figure 2 were measured directly from the hole profiles. Since the Stark split components overlap and broaden with increasing field strength, it is necessary to simulate the hole profiles so as to arrive at a more accurate value for $f\Delta\mu_{||}$. The simulations with identical Gaussian line shapes for the split components led to $f\Delta\mu_{||} = 0.51 \pm 0.06$ D (the 70, 80, 90, and 100 kV/cm profiles were used), which is slightly larger than the 0.42 D value.

The solid curve through the Stark broadening data in Figure 2 is a theoretical fit obtained using the theory of Kador et al.,[16] which is valid when the variance of $\Delta\mu$ is small.[42] They define the parameter

$$F = \frac{2f\Delta\mu E_S}{\hbar(\gamma' + \gamma_d')} \qquad (3)$$

where γ' is the homogeneous width of the zero-phonon line and γ_d' the additional width associated with artifacts such as saturation broadening. The sum $(\gamma' + \gamma_d')$ is the width at zero field. When $F \lesssim 3.5$, their general expression for Stark broadening simplifies considerably to

$$\Gamma(F) = 2(\gamma' + \gamma_d')(1 + F^2)^{1/2} \qquad (4)$$

The above inequality is satisfied in our experiments. For sufficiently large F, $\Gamma(F) \propto F$, which depends linearly on E_S. It should be noted that the theory, as applied to isolated protein complexes in a glass, takes into account their random orientations. This randomness is responsible for the Stark broadening with $e \perp E_S$. The fit in Figure 2 yielded $f\Delta\mu_\perp = 0.72 \pm 0.06$ D, which is slightly larger than the value of 0.51 D for $f\Delta\mu_{||}$ (see section 4 for discussion of this difference).

LH1-Only Mutant Chromatophores. Stark hole burning was performed with ω_B values between 11 074 and 11 169 cm^{-1}, a range that spans the B896 absorption band due to the lowest energy exciton level of the B875 ring of BChl *a* molecules; see I for details. No dependence on ω_B was observed. Furthermore, symmetric Stark broadening of the holes with no shifting was observed for e parallel as well as perpendicular to E_S. No difference in the field dependence of the hole profile on laser polarization was observed. Hole profiles ($e \perp E_S$) obtained with $\omega_B = 11\,169$ cm^{-1} are shown in Figure 4. The broad holes displaced ~16 cm^{-1} to the right (red) of the ZPH are pseudophonon sideband holes. Holewidth as a function of field strength is given in Figure 5. The solid curve is the fit obtained using eq 4 with $f\Delta\mu = 0.78 \pm 0.06$ D. The data clearly point to a linear Stark effect. The absence of Stark splitting and identical Stark broadening for both laser polarizations indicate that the angle γ between $\Delta\mu$ and the transition dipole $\varnothing$ varies from complex to complex in the ensemble studied. From Table 1 one observes that $\Delta\mu$ for B896 of the LH1 mutant is slightly smaller than the values for the B870 exciton level of the B850 ring of the LH2 complexes.
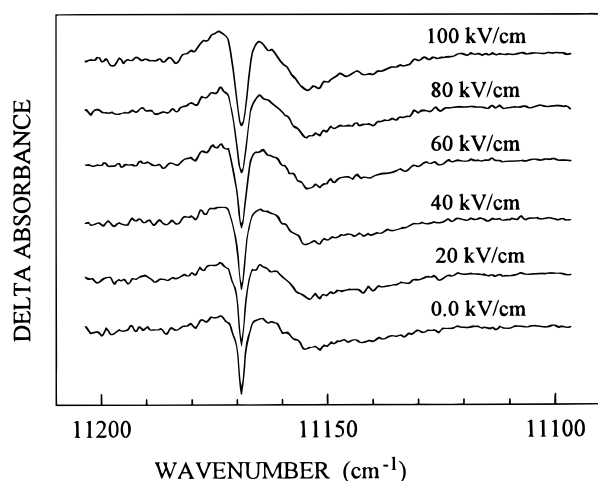
Stark Hole-Burning Studies

J. Phys. Chem. B, Vol. 102, No. 20, 1998 **4039**

Figure 4. Stark effect on zero-phonon holes burned into B896 of *Rb. sphaeroides* LH1-only mutant. Burn laser polarization was perpendicular to the Stark field. The spectra were obtained in order from top to bottom; the field strengths $E_S = 0-100$ kV/cm are indicated.
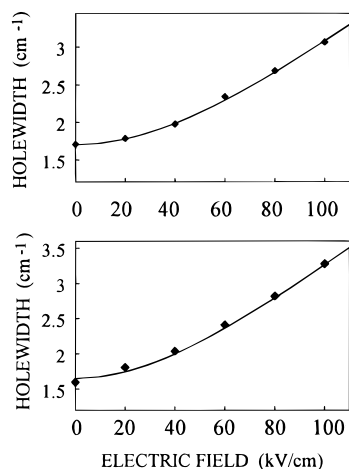

Figure 5. Dependence of the B896 holewidth of *Rb. sphaeroides* on electric field. Upper frame: LH1-only mutant, burn frequency 11 169 cm^{-1}, laser polarization perpendicular to electric field. Lower frame: wild-type chromatophore, burn frequency 11 124 cm^{-1}, laser polarization parallel to electric field. The solid curve is the theoretical fit calculated using eq 4 with $f\Delta\mu = 0.78$ D (upper frame) and $f\Delta\mu = 0.84$ D (lower frame).

LH1 of Wild-Type Chromatophores from *Rb. sphaeroides*. Stark hole burning was performed with ω_B values between 11 056 and 11 155 cm^{-1} located within the B896 band, which lies ~40 cm^{-1} to the red of B896 in the LH1 mutant. As in the case of the mutant, symmetric Stark broadening with no shifting was observed for both e parallel and perpendicular to E_S, with the broadening being independent of ω_B and laser polarization. Typical results are shown for $\omega_B = 11\ 124$ cm^{-1} and e parallel to E_S in the bottom frame of Figure 5. The solid curve through the data points is a theoretical fit, vide supra. Averaging over the fits to all data resulted in $f\Delta\mu = 0.84 \pm 0.06$ D, which, within experimental uncertainty, is the same as the value for the LH1 mutant.

B800 Band of *Rps. acidophila* and *Rs. molischianum*. Because of the large variation in the $\Delta\mu_{CSM}$ values for the B800 band of the LH2 complex of the above two species and *Rb. sphaeroides*, Table 1, we performed Stark hole-burning experiments on isolated complexes of *Rps. acidophila* (strain 10050) and *Rs. molischianum*. The 4.2 K absorption spectra were identical to those shown in refs 43−45. Hole burning was

performed at several burn frequencies ranging in value from close to the maximum of the B800 band to about 100 cm^{-1} lower in energy. The values for $f\Delta\mu_\perp$ and $f\Delta\mu_\parallel$ are given in Table 2. Values for B800 of chromatophores and the isolated LH2 complex of *Rb. sphaeroides* are also given. All values for $f\Delta\mu_\perp$ and $f\Delta\mu_\parallel$ in Table 2 were obtained by averaging the values obtained with different burn frequencies.

The main results from this section are summarized in Table 2. For comparison, results from CSM studies are also included.

4. Discussion

825 nm Band of the FMO Complex. The Stark splitting and broadening data of Figure 2 for laser polarization parallel and perpendicular to E_S led, respectively, to $f\Delta\mu_\parallel = 0.51$ D and $f\Delta\mu_\perp^{42} = 0.72$ D. (Both values are considerably smaller than the CSM value for the B825 band, Table 2.) Observation of Stark splitting means that the angle γ between $\Delta\mu$ and the transition dipole is quite well-defined. The results of Figure 3 led to an upper limit for γ of 15°. That $f\Delta\mu_\perp$ is somewhat larger than $f\Delta\mu_\parallel$ indicates that there may be a random contribution to the matrix field. Consistent with this is that the two components of the Stark split hole profile broaden with increasing field strength, vide infra.

Interpretation of the above results requires an understanding of the excitonic structure of the FMO trimer. The presence of seven symmetry inequivalent BChl a molecules in the subunit complicates the problem since they have different site (excitation) energies. Pearlstein arrived at his site energies by fitting calculated absorption, circular dichroism (CD), and hole-burned spectra to the experimental spectra.[38] (BChl a−BChl a electrostatic coupling energies were calculated using the point-monopole approximation with a static dielectric constant ϵ of 1.) BChl a 7 (numbering scheme of ref 36) was assigned the lowest site energy. The Q$_y$ transition dipole of BChl a 7 makes an angle of 11° with the C_3 axis. Calculations for the trimer led to the result that the 825 nm band is contributed to by two exciton levels at 826.4 and 824.1 nm. These levels are associated primarily with BChl a 7 (there is some contribution from BChl a 6) and the symmetry equivalent BChl a molecule in each of the other two subunits. The two levels carry E and A symmetry, respectively, with the latter (polarized parallel to the C_3 axis) carrying almost all of the absorption intensity of the 825 nm band. Subsequently, linear dichroism (LD) spectra for *P. aestuarii* were published[46] that indicate that the 825 nm band is polarized close to perpendicular to the C_3 axis.[47] Using Pearlstein's coupling energies, Gülen performed calculations in which the site energies were assigned by fitting to the absorption, LD, and triplet−singlet (T−S) spectra.[39] BChl a 6 was assigned the lowest site energy. Its Q$_y$ transition dipole makes an angle of 69° with the C_3 axis. Results for the trimer led to the 825 nm band being contributed to by E and A levels at 825.2 and 824.8 nm associated with BChl a 6 and to a lesser extent BChl a 5 (transition dipole angle with C_3 of 94.3°). The E level at 825.2 nm carries almost all of the absorption intensity. Based on the absorption, LD, and T−S spectra, the calculations of Gülen would appear to be the more reliable, although she made no attempt to fit the CD spectrum. Again, both works were influenced by the hole-burning results of ref 34, which led to the assignment of the holes at 827.1 and 824.4 nm to "partner" A and E trimer levels. (In ref 34 a symmetry assignment for these two levels was not possible.)

The calculations of Pearlstein and Gülen and the interpretation of the two-hole structure observed for the 825 nm band given in ref 34 assumed that the subunits of a given trimer are

Table 2:[a] New Stark Hole-Burning Results and Previously Published Classical Stark Modulation Results

complex	band	$f\Delta\mu_{\|}$ (D)	$f\Delta\mu_{\perp}$ (D)	band	$f\Delta\mu_{CSM}$ (D)	$f^2 \text{Tr}(\Delta\alpha)$ (Å³)
Rb. sph., LH1 (mutant)	B896	0.78 ± 0.06		B873	3.5[b]	1800[b]
Rb. sph., chromat.	B896	0.84 ± 0.08				
Rb. sph., chromat.	B800	0.8 ± 0.1	0.7 ± 0.1			
Rb. sph., LH2	B800	0.9 ± 0.1	0.7 ± 0.1	B800	1.1[c] (0.9[d])	5[c]
Rsp. acid., LH2	B800	0.62 ± 0.06	0.55 ± 0.06	B800	1.5[c]	
Rs. moli., LH2	B800	1.2 ± 0.2		B800	2.8[c]	290[c]
FMO, *Cb. tepidum*	B825	0.51 ± 0.06	0.72 ± 0.04	B825	1.4[d]	
				B813	1.9[d]	
				B805	1.9[d]	

[a] Chromat. ≡ chromophore; all other complexes in the table are isolated. [b] Ref 12. [c] Ref 13. [d] Ref 30.

energetically equivalent. Inhomogeneous broadening of the Q_y absorption bands was viewed as being due to glasslike fluctuations in structure from trimer to trimer in the ensemble being studied. As mentioned in the Introduction to I, considerable progress has recently been made in understanding how energy disorder leads to localization effects for the states of coupled BChl molecules in photosynthetic complexes. For example, in ref 48, which builds on refs 49 and 50, we studied localization-extendedness for the exciton levels of the B800 and B850 BChl *a* rings of the LH2 complex of purple bacteria (see also ref 2). Of relevance to the FMO complex is that the nearest neighbor coupling energy of the B800 ring is weak, -25 cm^{-1} ($\epsilon = 2.0$).[51] Inclusion of an experimentally acceptable level of energy disorder led to the conclusion that the levels of the B800 ring are essentially completely localized on single BChl *a* molecules. The calculations of Pearlstein on the FMO complex identified only two pairwise couplings between monomers belonging to different subunits of any significance. One involves BChl *a* 7 with its symmetry equivalent BChl *a* molecules in the other two subunits: coupling of 8 cm^{-1} for $\epsilon = 2$. The other involves BChl *a* 2 with BChl *a* 5 of another subunit: coupling of 10 cm^{-1} for $\epsilon = 2$. Given the smallness of these couplings and that the inhomogeneous broadenings of the 825 nm band of the FMO complex and the B800 band are comparable (~100 cm^{-1} in the low-temperature limit), it seems most likely that the states of the FMO trimer are localized on single subunits.[52] The recent calculations of Buck et al. are consistent with this.[53] It follows from the structure of the complex and the results of Gülen that our measured $f\Delta\mu_{\|}$ value for the 825 nm band can be viewed as being representative of a single BChl *a* molecule for either her model or that of Pearlstein. During this writing we became aware of very recent work by Louwe et al.[54,55] in which LD absorption detected magnetic resonance and LD T−S results are reported. Excitonic calculations[56] tailored to these new results and the absorption, LD, and CD spectra led to significantly improved overall fittings. It was found that the seven states of the subunit are highly localized on individual BChl *a* molecules, with the 825 nm band attributed to BChl *a* 3, whose Q_y dipole makes an angle of 109° with the C_3 axis of the trimer. The results of Louwe et al. provide additional support for our $f\Delta\mu_{\|}$ value of 0.51 D for the 825 nm band being mainly associated with a single BChl *a* molecule of the subunit.

In what follows we consider that $f\Delta\mu_{\|} = 0.5$ D is representative of the value for $f\Delta\mu$ of a BChl *a* molecule in the FMO subunit. One has that $f\Delta\mu = f(\Delta\mu_0 + \Delta\mu_{ind})$. The observation of Stark splitting proves that the angle γ between $\Delta\mu$ and the transition dipole is well-defined. The data indicate that $\gamma \lesssim 15°$. If $\Delta\mu_{ind}$ is random, as in glasses, this means that $\Delta\mu_0 \gg \Delta\mu_{ind}$ so that $\Delta\mu_0 \approx 0.5$ D. CSM data for monomer BChl *a* in a poly(methyl methacrylate) (PMMA) film led to $\gamma \approx 12°$.[4] For the monomer the optical transition is generally taken to be close to parallel with the in-plane *y*-axis, i.e., Q_y. INDO

TABLE 3: Summary of Previously Determined $f\Delta\mu_{Ind}$ Values for the $S_1(\pi\pi^*)$ States of Relevant Molecules Isolated in Amorphous Hosts

molecule	matrix[a]	ϵ	$f\Delta\mu_{ind}$ (D)	ref
Zn-tetrabenzoporphine	PVB	3.0	0.29	16
tetra-*tert*-butylphthalocyanine	PVB	2.55	0.27	62
tetraphenylporphine	PS	2.48	0.21	19
	PVB	2.55	0.10	19
	PMMA	2.71	0.15	19
tetrapropylporphycene	PS	2.48	0.18	19
	PVB	2.55	0.15	19
	PMMA	2.71	0.17	19
octaethylporphine	PS	2.5	0.11	17
	PS	2.56	0.10	18
	PVB	3.02	0.17	18
	PMMA	3.45	0.22	18
chlorophyll *a*	PVB	2.55	0.33	20
mesoporphyrin-IX	glass		0.06	28
	protein		0.05 $f\Delta\mu_0 = 0.15$	
chlorin	PVB	3.0	0.17 $f\Delta\mu_0 = 0.47$	15

[a] PVB, PS, and PMMA denote poly(vinyl butyral), poly(styrene), and poly(methyl methacrylate).

quantum chemical calculations of the type performed in ref 57, model C, predict that $\Delta\mu_0$ and the Q_y transition dipole for all seven BChl *a* molecules are parallel to within a couple of degrees.[58] However, a protein is not a glass and, thus, the assumption of randomness for $\Delta\mu_{ind}$ is questionable, vide supra. Of course, one would like to have a reliable value of $\Delta\mu_0$ for the BChl *a* monomer. A value for $f\Delta\mu$ of 2.4 ± 0.2 D at 77 K for BChl *a* monomer in a PMMA film has been determined by CSM spectroscopy.[4] (The value in PS films is the same within experimental uncertainty.[63]) This is a factor of 5 larger than our value for the FMO complex and over a factor of 2 larger than the value for B800 of the LH2 complex of *Rb. sphaeroides*, Table 1. For Chl *a* monomer in a poly(vinylbutyral) (PVB) film, however, a value for $f\Delta\mu_{ind}$ from Stark hole burning at 2 K has been reported. It is 0.33 ± 0.01 D.[20] It was also determined in the same work that $f\Delta\mu_0 \lesssim 0.15$ D, Table 3. CSM studies of monomer Chl *a* in PMMA at 77 K resulted in $f\Delta\mu = 0.7$ D[59] and in 2-propanol (118 K), 0.95 D.[60] Again, there is a discrepancy between the Stark hole-burning and CSM values. The discrepancy cannot be attributed to a significant difference between the static dielectric constants (ϵ) of PVB and PMMA. Very precise measurements of ϵ between 2.5 and 300 K for 23 polymers, including PVB and PMMA, have been reported.[19] For all polymers, the temperature dependence of ϵ below ~100 K is negligible. The ϵ values for PVB and PMMA in the low-temperature limit are 2.55 and 2.71, respectively. The local field corrections, as judged by $f = (\epsilon + 2)/3$, are too small to account for the above discrepancy. Temperature effects appear to be negligible. (As discussed in I, structural changes due to thermal expansivity are negligible below about 100 K.)

The value of 0.33 D for $f\Delta\mu_{ind}$ (Chl *a*) was determined from Stark hole-broadening data. It was shown that this value is consistent with

$$\Delta\mu_{ind}(D) = -0.04 + 8.8 \times 10^{-24}\Delta\alpha/MW; \quad r = 0.975 \quad (5)$$

which was arrived at on the basis of Stark hole-burning data for 11 isolated π-electron chromophores (see upper part of Table 3 for examples) in PVB and values of $\Delta\alpha$ from solvent shift measurements.[61] MW is the molecular weight of the chromophore (the phytyl tail of Chls is excluded). The unit of $\Delta\alpha$ is Å^3. $r = 0.975$ is the linear regression coefficient. For Chl *a*, $\Delta\alpha = 18 \pm 3$ Å^3. With MW = 598, eq 5 yields $f\Delta\mu_{ind} = 0.34$ D, which is quite close to the experimental value. We note that with $\Delta\alpha = 18$ Å^3, $\epsilon = 2.55$, and $f\Delta\mu_{ind} = 0.33$ D, the internal (matrix) field of PVB is 4×10^6 V/cm, which can be compared with the value of $(0.9-1.9) \times 10^6$ V/cm for polystyrene.[17]

Solvent shift data for BChl *a* led to $\Delta\alpha = 16 \pm 3$ Å,[39] which, within experimental uncertainty, is the same as the value for Chl *a*. Thus, in PVB, the value of $f\Delta\mu_{ind}$ for BChl *a* from eq 5 is also ~0.3 D. The upper part of Table 3 lists the value of the Stark hole-burning values for $f\Delta\mu_{ind}$ for centrosymmetric molecules in PVB and in some cases also PMMA and polystyrene (PS). Note that the values are in the range ~0.1–0.3 D. The value for chlorin in PVB is 0.17 D ($f\Delta\mu_0 = 0.47$ D). For mesoporphyrin in horseradish peroxidase, $f\Delta\mu_{ind} \sim 0.05$ D ($f\Delta\mu_0 = 0.15$ D). In view of these results and the applicability of eq 5 to the class of similar molecules considered, we consider a value of 0.3 D for $f\Delta\mu_{ind}$ of BChl *a* in PVB or PMMA to be a reasonable estimate.

What are the implications of this value for the CSM value of $f\Delta\mu = 2.4$ D for BChl *a* in PMMA and PS? With $f\Delta\mu_{ind} \approx 0.3$ D, $f\Delta\mu_0 \approx 2$ D, the intrinsic or molecular dipole moment change. The problem is that if $f\Delta\mu_0$ is that large (more than a factor of 10 larger than the value for Chl *a*, vide supra), one would expect to observe a Stark splitting of holes burned into the B800 band of the LH2 complex.[64] Stark splitting was not observed for either of the two laser polarizations, only broadening. The broadening data for the three species studied led to values of $f\Delta\mu$ in the range 0.6–1.2 D, Table 2. The absence of Stark splitting indicates that $\Delta\mu_{ind}$ is at least a factor of 2 larger than $\Delta\mu_0$,[28] which means that $f\Delta\mu_0 \lesssim 0.5$ D. Thus, it might appear that a value for $f\Delta\mu$ of 2.4 D for BChl *a* in PMMA and PS from CSM spectroscopy is unphysically large. However, such a conclusion would be incorrect were it to be the case that in polymer films the geometry of the BChl *a* molecule is distorted from that in protein complexes (rotation of the acetyl group of ring I and out-of-plane deformations are two possibilities). In a polymer there would presumably be a distribution of distortions, which further complicates the situation. If this possibility is correct, then it would be the case that $f\Delta\mu$ values for BChl *a* in polymer films are of little use in interpreting Stark data for photosynthetic complexes. It is important, therefore, to perform Stark hole-burning experiments on BChl *a* in polymer films Such experiments are planned.

To conclude this subsection, we consider further the values of 0.51 and 0.72 D for $f\Delta\mu_{||}$ and $f\Delta\mu_{\perp}$ of the 825 nm band of the FMO complex. It was noted that the two Stark split components for $e \parallel E_S$, which yielded a dipole moment change of 0.51 D, broadened with increasing field strength. The broadening suggests that there is a random contribution to $\Delta\mu_{ind}$ in addition to a contribution to the induced dipole moment change vector that is relatively well-defined with respect to the molecular frame. (We consider it unphysical to assume that

the 0.51 D dipole moment change is entirely molecular, i.e., equal to $f\Delta\mu_0$). The well-defined contribution might be associated with the "inner shell" of the protein matrix with the random contribution coming from the "outer shell" and, perhaps, even the glass-forming solvent. Fitting of the Stark broadening data with eq 4 led to $f\Delta\mu_{ind}$ (random) ≈ 0.5 D. Whether or not the model in which $\Delta\mu_{ind} = \Delta\mu_{ind}$(nonrandom) $+ \Delta\mu_{ind}$(random) can explain why $f\Delta\mu_{\perp}$ is slightly larger than $f\Delta\mu_{||}$ is unclear. We note that one is dealing with vectorial quantities, i.e., $f\Delta\mu_{||} = f\Delta\mu_0 + f\Delta\mu_{ind}$(nonrandom). Although it is reasonable to assume that $\Delta\mu_{ind}$(nonrandom) lies mainly in the plane of the BChl *a* molecule, it is not obvious that it is parallel to $f\Delta\mu_0$. Assuming that they are and that the prediction $f\Delta\mu_{ind} \approx 0.3$ D is correct, then $f\Delta\mu_0 \approx 0.2$ D, which is close to the upper limit for Chl *a* determined by Stark hole burning.

LH1 and LH2 Antenna Complexes. There are two findings from the Stark hole-burning experiments that were unanticipated. First, the values of $f\Delta\mu$ for the B800 band and the B875 and B896 bands of the B850 and B875 BChl *a* rings are not very different, Tables 1 and 2. The values fall in the range ~0.6–1.2 D. Such values are not much larger than those of the 825 nm band of the FMO complex. Second, the CSM values for $f\Delta\mu$ are significantly larger than the hole-burning values. This is also the case for the 825 nm band of the FMO complex, vide supra. Potential problems with the application of CMS spectroscopy to photosynthetic complexes are considered in the following subsection. In this subsection we consider only the results from Stark hole burning.

As discussed, the optical excitation of the B800 rings is most likely mainly localized on single BChl *a* molecules. That $f\Delta\mu$ for B800 is somewhat larger than that of the 825 nm band of the FMO complex, Table 2, might be due to the hydrophilic protein environment (charged residues) of the B800 molecules,[65,66] which results in a higher internal field ($f\Delta\mu_{ind}$) than exists in the FMO complex. The question arises as to why for both laser polarizations, $e \parallel E_S$ and $e \perp E_S$, only Stark broadening is observed when Stark splitting for the 825 nm band of the FMO complex is observed for $e \parallel E_S$. The answer might simply be the result of the intrinsic 4–5 cm^{-1} ZPH widths of the B800 band being larger than the ~0.6 cm^{-1} ZPH widths of the 825 nm band. With reference to Figure 1, one can see that if the Stark split components were broadened by a factor of about 3, the splitting would be unobservable. Thus, the model for the 825 nm band in which $\Delta\mu_{ind} = \Delta\mu_{ind}$(nonrandom) $+ \Delta\mu_{ind}$(random) may apply to B800. Of course, the possibility that the random component for B800 is relatively larger than for the 825 nm band cannot be excluded. In any event, we view the values of $f\Delta\mu$ obtained for both bands as representative for localized BChl *a* Q_y transitions in proteins.

The smallness of $f\Delta\mu$ for the B870 and B896 bands[67] was unanticipated because the BChl *a* molecules of their associated rings are strongly coupled and because of the large CSM values of $f\Delta\mu$ and f^2 Tr($\Delta\alpha$) for the B850 and B875 bands. Furthermore, the high-pressure results of I strongly indicate that electron exchange (CT states) is an important ingredient of the coupling. (We note that with f^2 Tr($\Delta\alpha$) ≈ 1000 Å^3, representative CSM value from Tables 1 and 2, it follows that $f\Delta\mu_{ind} \approx 3$ D when $\Delta\alpha$ is taken to be disklike, a value much larger than our values for B870 and B896.) A key question is why the $f\Delta\mu$ values for B870 and B896 are so small. In an attempt to answer this question we further[48] examine the implications of symmetry (C_n) and symmetry breaking on the Stark spectroscopy of the B850 and B875 rings (bands). Following the notation of refs 48–

50, let $|\alpha\rangle$ be the wave function of the BChl a dimer,[68] this wave function being associated with the absorption band of interest, B850 or B875. We may assume that $|\alpha\rangle$ is corrected for CT character from electron exchange between the two monomers of the dimer. In the absence of energy disorder the states associated with the absorption band are delocalized,[69]

$$|j\rangle = n^{-1/2} \sum_{\alpha=0}^{n-1} B^{j\alpha*}|\alpha\rangle, \quad j = 0, ..., n-1 \qquad (6)$$

where $B = \exp(i2\pi/n)$, with α labeling the dimer of the C_n ring. For the B850 and B875 bands, only $j = 0$ (A), $j = \{1,n-1\}$ (E_1), and $j = \{2,n-2\}$ (E_2) are relevant.[50] The above wave function is deficient in that it does not account for electron exchange between neighboring dimers. Let $|\alpha,\alpha+1\rangle_{CT}$ denote the charge-transfer wave function associated with electron exchange between dimers α and $\alpha+1$. The corresponding delocalized wave functions are given by

$$|j\rangle_{CT} = n^{-1/2} \sum_{\alpha} B^{j\alpha*}|\alpha,\alpha+1\rangle_{CT} \qquad (7)$$

Since the Hamiltonian is totally symmetric, mixing of $|j\rangle$ and $|j\rangle_{CT}$ is restricted to levels with the same j value. Thus, when mixing is taken into account, the delocalized wave functions are given by

$$|\Psi_j\rangle = n^{-1/2} \sum_{\alpha} B^{j\alpha*}[C_j|\alpha\rangle + D_j|\alpha,\alpha+1\rangle_{CT}] \qquad (8)$$

It follows easily that the dipole moment for state j is

$$\langle\Psi_j|d|\Psi_j\rangle \equiv \mu_j = n^{-1} \sum_{\alpha}[C_j^2\mu_\alpha + D_j^2\mu_{\alpha,\alpha+1}] \qquad (9)$$

when optical transitions between neutral Q_y states and CT states and between different CT states are neglected. (Given the structures of the B850 and B875 rings, this neglect is reasonable. For example, in the optical spectra of the LH1 and LH2 there is no evidence for CT optical transitions originating from the ground electronic state.) In eq 9, $C_j^2 + D_j^2 = 1$. The key point we wish to make follows when one recognizes that μ_α and $\mu_{\alpha+1}$ can be written as μ_α^{ip} and μ_α^{op}, where ip and op designate the components perpendicular and parallel to the C_n axis. For the cyclic C_n arrays it follows from eq 9 that $\mu_j^{ip} = 0$. This is also true for the ground electronic state. Actually, the vanishing of the ip component is dictated by symmetry. Based on Stark spectroscopic data, electronic structure calculations (vide supra), and the structures of the B850 and B875 rings (see Figure 1 of I), one expects that both μ_α and $\mu_{\alpha,\alpha+1}$ should be close to perpendicular to the C_n rotation axis; that is, the op contribution should be small. That μ_j or $\Delta\mu_j$ should be close to zero in the absence of energy disorder is consistent with the results of recent quantum chemical calculations that take into account the CT states.[70] It was shown by Wu and Small[48] how experimentally acceptable levels of energy disorder can lead to nonzero values for $\Delta\mu_j^{ip}$ due to localization effects. In that work only the first term in square brackets of eq 9 was considered, but inclusion of the second term does not affect their conclusions. It was found that the $j = 0$ (A or B870) level of the B850 ring undergoes single "clump" or arc-like localization, whereas the split components of the strongly allowed E_1 level undergo double-clump localization with their localization patterns complementing each other.[71] It was found that the values of $\Delta\mu^{ip}$ for the two components can be significantly different and, just as important, that the vectorial senses of their dipole moment

changes can differ by 180°. Thus, the assumptions made in interpretation of the CSM data for the B850 and B875 bands[12,13] deserve scrutiny. They are that the components that contribute to these bands carry the same value of $\Delta\mu$ and vectorial sense. As expected, the results of ref 48 show that the energy disorder-induced value for $\Delta\mu^{ip}$ of B870 is larger than those of the E_1 components, which, in turn, are larger than those of the adjacent and higher energy components of the E_2 level. The E_2 components may be responsible for the high-energy tailing of the B850 and B875 bands.[50] One possible reason for why Stark broadening is observed for both laser polarizations is that the extent of energy disorder varies from complex to complex in the sample, which lead to distributions of values for $\Delta\mu^{ip}$ and vectorial sense.

The above symmetry-based conclusions concerning μ_j^{ip} or $\Delta\mu_j^{ip}$ apply when the matrix (protein)-induced contribution to the dipole moments is taken into account. However, the treatment given above, which leads to eq 9, does not take this contribution into account. Determination of the matrix-induced contribution would involve calculation of the polarizability changes $\Delta\alpha_j$ of the exciton levels with respect to the ground electronic state. One could start with a delocalized excited-state basis set based on the Q_y, Q_x, and B $\pi\pi^*$ states of the BChl a molecule. (Lower energy CT states which couple most strongly with the Q_y $\pi\pi^*$ states would need to be included.) In calculating the polarizabilities α_j of the levels associated with the B850 and B875 bands it would be necessary to take into account the two-exciton transitions associated with the Q_y manifold as well as transitions between the one-exciton states of that manifold.[72] The important question that could be addressed by such calculations is whether the dependence of $\Delta\alpha_j$ on j is strong, with j labeling the levels that contribute to the B850 or B875 band, vide infra.

Stark Hole-Burning and Classical Modulation Spectroscopies. The results of Tables 1−3 show that the values of $f\Delta\mu$ determined by CSM are generally significantly larger than those determined by hole burning. In attempting to understand the apparent discrepancies it is important to know that with CSM spectroscopy the theory of Liptay[7] is used, in approximate form, to analyze the response of an absorption band to the external field. This involves fitting the Δ-absorbance (ΔA) spectrum to the sum of the first and second derivatives of the absorption spectrum and, when necessary, also the zeroth derivative. These derivatives relate, respectively, to changes in the polarizability, dipole moment, and oscillator strength. This requires an absorption spectrum with very high S/N ratio. In refs 12 and 13 the low S/N ratios for the B800, B850, and B875 bands necessitated fitting each band with a sum of skewed Gaussians. By necessity, the Gaussians of each band are assumed to have identical $\Delta\alpha$ and $\Delta\mu$.[73] We have argued that this assumption for the B850 and B875 bands is doubtful because of their underlying exciton level structures and energy disorder. With Stark hole burning, as reported on here and in the references cited, one is concerned only with the linear Stark effect, and the high resolution of the technique allows one to probe single excited states. Furthermore, the theory used to analyze the data was designed for the problem at hand, i.e., inhomogeneously broadened bands across which a variation in $\Delta\mu$ is taken into account. The ability to study this variation is an important attribute of Stark hole burning. A disadvantage of the technique is that, with typical attainable field strengths, it cannot be applied to states with ultrashort lifetimes (e.g., the E_1 and E_2 levels of the B850 and B875 bands) because of excessive hole broadening.

Stark Hole-Burning Studies

J. Phys. Chem. B, Vol. 102, No. 20, 1998 **4043**

If agreement between the Stark hole-burning and CSM values for $f\Delta\mu$ was to be reached, one might have expected it for the 825 nm band of the FMO complex and the B800 band of the LH2 complex. Table 2 reveals, however, that this is not the case. Given the disagreements between the B800 results for *Rps. acidophila* and *Rs. molischianum*, it may be that the quite close agreement for *Rb. sphaeroides* is fortuitous. Interestingly, the hole-burning value of 1.2 D for B800 of *Rs. molischianum* is larger than those for the other two species, as is the case for CSM. Beekman et al.[13] have suggested that the large CSM value of 2.8 D for *Rs. molischianum* relative to the 1.5 D value for *Rps. acidophila* might be due to the central Mg of their B800 BChl *a* molecules being ligated, respectively, by a formyl-methionine and aspartate (charged). The reader is referred to that reference for discussion of other structural differences which may lead to different matrix fields. It should be noted, however, that the hole burning value of $f\Delta\mu$ for B800 of *Rb. sphaeroides* is larger than that of *Rps. acidophila*, the opposite of what is observed by CSM. Furthermore, the hole-burning value of 1.2 D for *Rs. molischianum* cannot be viewed as large, especially if one accepts the CSM value of ~2.5 D for $f\Delta\mu$ of monomer BChl *a* in PMMA and PS films. For reasons given earlier, however, we think that the CSM values for monomer BChl *a* and Chl *a* are too high.

The discrepancies between the $f\Delta\mu$ values from the two techniques are even more striking for the B850 and B875 bands, Tables 1 and 2. The CSM values are 3−4 times higher. As mentioned, the assumptions made in the analysis of the CSM spectra of these complex bands are questionable. However, the hole-burning values are for the lowest energy ($j = 0$) exciton level, B870 and B896, respectively. With reference to the last sentence of the preceding section, it is possible that the polarizability of this level is smaller than those of the split (by energy disorder) components of the E_1 level which carry most of the transition dipole strength for absorption from the ground state. However, the square of the transition dipole from the ground state to an exciton level j is only one of many terms, vide supra, that enter into the expression for the polarizability tensor. Electronic structure calculations, with and without energy disorder, followed by detailed simulations of the CSM profiles of the B850 and B875 bands appear to be required for a firm understanding of the hole-burning and CSM results. The reader is referred to Somsen et al.[72] for theoretical discussion of the problems associated with applying the conventional CSM analysis procedure to bands contributed to by closely spaced exciton levels.

References and Notes

(1) Wu, H.-M.; Rätsep, M.; Jankowiak, R.; Gogdell, R. J.; Small, G. J. *J. Phys. Chem. B* **1988**, *102*, 4023.

(2) Alden, R. G.; Johnson, E.; Nagarajan, V.; Parson, W. W.; Law, C. J. Cogdell, R. G. *J. Phys. Chem. B* **1997**, *101*, 4667.

(3) We will refer to the S_1 states as Q_y even when they are an admixture of neutral $\pi\pi^*$ and CT states.

(4) Lockhart, D. J.; Boxer, S. G. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 107.

(5) DiMagno, T. J.; Bylina, E. J.; Angerhofer, A.; Youvan, C. D.; Norris, J. R. *Biochemistry* **1990**, *29*, 899.

(6) Middendorf, T. R.; Mazzola, L. T.; Lao, K.; Steffen, M. A.; Boxer, S. G. *Biochim. Biophys. Acta* **1993**, *1143*, 223.

(7) Liptay, W. In *Excited States*; Lim, E. C., Ed.; Academic Press: New York, 1974; pp 129−229.

(8) The Lorentz isotropic local field correction, $f = (\epsilon + 2)/3$, has been used for photosynthetic complexes with a static dielectric constant ϵ of about 2.

(9) Renge, I. *J. Phys. Chem.* **1993**, *97*, 6582.

(10) Lyle, P. A.; Kolaczkowski, S. V.; Small, G. J. *J. Phys. Chem.* **1993**, *97*, 6924.

(11) Small, G. J. *J. Phys. Chem.* **1995**, *197*, 239.

(12) Beekman, L. M. P.; Steffen, M.; van Stokkum, I. H. M.; Olsen, J. D.; Hunter, C. N.; Boxer, S. G.; van Grondelle, R. *J. Phys. Chem. B* **1997**, *101*, 7284.

(13) Beekman, L. M. P.; Frese, R. N.; Fowler, G. J. S.; Picorel, R.; Gogdell, R. J.; van Stokkum, I. H. M.; Hunter, C. N.; van Grondelle, R. *J. Phys. Chem. B* **1997**, *101*, 7293.

(14) Bogner, U.; Schätz, P.; Seel, R.; Maier, M. *Chem. Phys. Lett.* **1983**, *102*, 267.

(15) Meixner, A. J.; Renn, A.; Bucher, S. E.; Wild, U. P. *J. Chem. Phys.* **1986**, *90*, 6777.

(16) Kador, L.; Haarer, D.; Personov, R. *J. Chem. Phys.* **1987**, *86*, 5300.

(17) Kador, L.; Jahn, S.; Haarer, D.; Silbey, R. *Phys. Rev. B* **1990**, *41*, 12215.

(18) Meixner, A. J.; Renn, A.; Wild, U. P. *Chem. Phys. Lett.* **1992**, *190*, 75.

(19) Altmann, R. B.; Renge, I.; Kador, L.; Haarer, D. *J. Chem. Phys.* **1992**, *97*, 5316.

(20) Altmann, R. B.; Haarer, D.; Renge, I. *Chem. Phys. Lett.* **1993**, *216*, 281.

(21) Johnson, L. W.; Murphy, M. D.; Pope, C.; Foresti, M.; Lombardi, J. R. *J. Chem. Phys.* **1987**, *86*, 4335.

(22) Renn, A.; Bucher, S. E.; Meixner, A. J.; Meister, E. C.; Wild, U. P. *J. Lumin.* **1988**, *39*, 181.

(23) De Caro, C.; Renn, A.; Wild, U. P.; Johnson L. W. *J. Lumin.* **1991**, *50*, 309.

(24) Orrit, M.; Bernard, J.; Zumbusch, A.; Personov, R. I. *Chem. Phys. Lett.* **1992**, *196*, 595.

(25) Vauthey, E.; Holliday, K.; Wei, C.; Renn, A.; Wild, U. P. *Chem. Phys.* **1993**, *171*, 253.

(26) Vauthey, E.; Voss, J.; De Caro, C.; Renn, A.; Wild, U. P. *Chem. Phys.* **1994**, *184*, 347.

(27) Altmann, R. B.; Kador, L.; Haarer, D. *Chem. Phys.* **1996**, *202*, 167.

(28) Gafert, J.; Friedrich, J.; Vanderkooi, J. M.; Fidy, J. *J. Phys. Chem.* **1995**, *99*, 5223.

(29) Rätsep, M.; Wu, H.-M.; Hayes, J. M.; Small, G. J. *Spectrochim. Acta*, in press.

(30) Gottfried, D. S.; Stocker, J. W.; Boxer, S. G. *Biochim. Biophys. Acta* **1991**, *1059*, 63.

(31) Savikhin, S.; Zhou, W.; Blankenship, R. E.; Struve, W. S. *Biophys. J.* **1994**, *66*, 110.

(32) Köhler, M.; Gafert, J.; Friedrich, J.; Falk, H.; Meyer, J. *J. Chem. Phys.* **1996**, *100*, 8567.

(33) Jackson, J. B.; Goodwin, M. G. *Biochim. Biophys. Acta* **1993**, *1144*, 199.

(34) Johnson, S. G.; Small, G. J. *J. Phys. Chem.* **1991**, *95*, 471.

(35) Reddy, N. R. S.; Jankowiak, R.; Small, G. J. *J. Phys. Chem.* **1995**, *99*, 16168.

(36) Fenna, R. E.; Matthews, B. W. *Nature* **1975**, *258*, 573.

(37) Li, Y.-F.; Zhou, W.; Blankenship, R. E.; Allen, J. P. *J. Mol. Biol.* **1997**, *271*, 456.

(38) Pearlstein, R. M. *Photosyn. Res.* **1992**, *31*, 213.

(39) Gülen, D. *J. Phys. Chem.* **1996**, *100*, 17684.

(40) Schätz, P.; Maier, M. *J. Chem. Phys.* **1987**, *87*, 809.

(41) Hochstrasser, R. M. *Acc. Chem. Res.* **1973**, *6*, 263.

(42) In arriving at a value for $f\Delta\mu_\perp$ that part of the theory of ref 16 that assumes that the variance of $\Delta\mu$ is zero was used. However, in that reference it is shown that the introduction of a Gaussian distribution for $\Delta\mu$ makes only a small difference when F of eq 3 is $\lesssim 3.5$.

(43) Wu, H.-M.; Reddy, N. R. S.; Cogdell, R. J.; Muenke, C.; Michel, H.; Small, G. J. *Mol. Cryst. Liq. Cryst.* **1996**, *291*, 163.

(44) Wu, H.-M.; Reddy, N. R. S.; Small. G. J. *J. Phys. Chem. B* **1997**, *101*, 651.

(45) Wu, H.-M.; Rätsep, M.; Jankowiak, R.; Cogdell, R. J.; Small. G. J. *J. Phys. Chem. B* **1997**, *101*, 7641.

(46) van Mourik, F.; Verwijst, R. R.; Mulder, J. M.; van Grondelle, R. *J. Chem. Phys.* **1994**, *98*, 10307.

(47) The same polarization is observed for *Cb. tepidum*: Melkozernov, A. N.; Olson, J. M.; Blankenship, R. E. To be published.

(48) Wu, H.-M.; Small, G. J. *J. Phys. Chem. B* **1998**, *102*, 888.

(49) Wu, H.-M.; Small. G. J. *Chem. Phys.* **1997**, *218*, 225.

(50) Wu, H.-M.; Rätsep, M.; Lee, I.-J.; Cogdell, R. J.; Small, J. J. *J. Phys. Chem. B* **1997**, *101*, 7654.

(51) Sauer, K.; Cogdell, R. J.; Prince, S. M.; Freer, A.; Isaacs, N. W.; Scheer, H. *Photochem. Photobiol.* **1996**, *64*, 564.

(52) The double hole structure of the 825 nm band can be understood in terms of energy transfer from two higher energy subunits to the lowest energy subunit.

(53) Buck, D. E.; Savikhin, S.; Struve, W. S. *Biophys. J.* **1997**, *72*, 24.

(54) Louwe, R. J. W.; Vrieze, J.; Aartsma, T. J.; Hoff, A. J. *J. Phys. Chem. B* **1997**, *101*, 11273.

(55) Louwe, R. J. W.; Vrieze, J.; Hoff, A. J.; Aartsma T. J. *J. Phys. Chem. B* **1997**, *101*, 11280.

(56) Coupling energies of Pearlstein were uniformly reduced by about a factor of 2. It was argued that the energy disorder relative to the excitonic couplings between subunits of the trimer is sufficiently large to cause localization on a single subunit.

(57) Gudovska-Nowak, E.; Newton, M. D.; Fajer, J. *J. Phys. Chem.* **1990**, *94*, 5795.

(58) Fajer, J.; Newton, M. Private communication.

(59) Boxer, S. G. Private communication.

(60) Krawczyk, S. *Biochim. Biophys. Acta* **1991**, *1056*, 64.

(61) Renge, I.; Mölder, U.; Koppel, I. *Spectrochim. Acta A* **1985**, *41*, 967.

(62) Altmann, R. B Diploma Thesis, Bayreuth University, 1988.

(63) Lockhart, D. J.; Boxer, S. G. *Biochemistry* **1987**, *26*, 664.

(64) The energy disorder calculations of ref 48 indicate that the Q_y excitations of the B800 ring are essentially localized on single B800 molecules. The situation for the B850 and B875 rings is different, vide supra.

(65) Freer, A.; Prince, S.; Sauer, K.; Papiz, M.; Hawthornthwaite-Lawless, A.; McDermott, G.; Cogdell, R.; Isaacs, N. W. *Structure* **1996**, *4*, 449.

(66) Koepke, J.; Hu, X.; Muenke, C.; Schulten, K.; Michel, H. *Structure* **1996**, 4, 581.

(67) That the values of $f\Delta\mu$ for B870 of *Rps. acidophila* and *Rs. molischianum* are essentially identical establishes that ring size (C_9 vs C_8) is of little importance. That the values of $f\Delta\mu$ for B870 of the isolated LH2 complex and WT chromatophores of *Rb. sphaeroides* are so similar suggests that the isolation procedure has, at best, a weak effect on E_{int}.

(68) Here we are free to associate the dimer with the basic α,β polypeptide pair.

(69) Hochstrasser, R. M. *Molecular Aspects of Symmetry*; W. A. Benjamin: New York, 1966.

(70) Parson, W. W.; Johnson, E. Private communication.

(71) These findings for localization are valid for weak energy disorder, which means that the disorder-induced matrix elements between energetically different exciton levels are comparable to or smaller than the spacings between the levels. Low-temperatuare absorption and hole-burned spectra are consistent with weak energy disorder.[48]

(72) Somsen, O. J. G.; Chernyak, V.; van Grondelle, R.; Mukamel, S. *J. Phys. Chem. B*, submitted.

(73) This assumption is made even when the S/N ratio of the absorption band is high enough to yield acceptable first and second derivatives.