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The organization of bacteriochlorophyll *c* in chlorosomes from *Chloroflexus aurantiacus* and the structural role of carotenoids and protein

An absorption, linear dichroism, circular dichroism and Stark spectroscopy study

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Abstract

The organization of bacteriochlorophyll *c* (BChl *c*) molecules was studied in normal and carotenoid-deficient chlorosomes isolated from the green phototrophic bacterium *Chloroflexus aurantiacus*. Carotenoid-deficient chlorosomes were obtained from cells grown in the presence of 60 μg of 2-hydroxybiphenyl per ml. At this concentration, BChl *c* synthesis was not affected while the formation of the 5.7 kDa chlorosome polypeptide was inhibited by about 50% (M. Foidl et al., submitted). Absorption, linear dichroism and circular dichroism spectroscopy showed that the organization of BChl *c* molecules with respect to each other as well as to the long axis of the chlorosomes was similar for both types of chlorosomes. Therefore, it is concluded that the organization of BChl *c* molecules is largely independent on the presence of the bulk of carotenoids as well as of at least half of the normal amount of the 5.7 kDa polypeptide. The Stark spectra of the chlorosomes, as characterized by a large difference polarizability for the ground- and excited states of the interacting BChl *c* molecules, were much more intense than those of individual pigments. It is proposed that this is caused by the strong overlap of BChl *c* molecules in the chlorosomes. In contrast to individual chlorophylls, BChl *c* in chlorosomes did not give rise to a significant difference permanent dipole moment for the ground- and excited states. This observation favors models for the BChl *c* organization which invoke the anti-parallel stacking of linear BChl *c* aggregates above those models in which linear BChl *c* aggregates are stacked in a parallel fashion. The difference between the Stark spectrum of carotenoid-deficient and WT chlorosomes indicates that the carotenoids are in the vicinity of the BChls.

Abbreviations: OD (A) – absorption; LD – linear dichroism; CD – circular dichroism; BChl *c* – bacteriochlorophyll *c*; BChl a – bacteriochlorophyll *a*; HBP – 2-hydroxybiphenyl

Introduction

Chlorosomes are specialized photosynthetic antenna complexes formed exclusively by the green phototrophic bacteria. They differ considerably from all other photosynthetic light-harvesting complexes known (van Grondelle et al. 1994). They contain thousands of BChl *c*, *d* or *e* pigments which possess a high degree of order, and relatively little protein which is thought to be mainly located in the lipid monolayer around the chlorosome (Blankenship et al. 1995; Oelze and Golecki 1995). The chlorosomes from the

green filamentous bacterium *Chloroflexus aurantiacus* are rather uniform in size ($106 \pm 23 \times 32 \pm 10 \times 15 \pm 5$ nm^3). Besides BChl *c*, they also contain some BChl *a* in a molar ratio of about 20:1, depending on the growth conditions. Whereas BChl *c* molecules are spanning the chlorosomes, BChl *a* is thought to be located in the base plate which connects the chlorosome to the cytoplasmic membrane in which the reaction center is located. The envelope of the chlorosomes contains glycolipids and, according to more recently proposed models, three different polypeptides of 15.5, 11 and 5.7 kDa (Mr = 18 000, 10 800 and 3 700). Under physiological conditions the three polypeptides are formed in constant proportion to each other (Foidl et al. 1994). The role of the 5.7 kDa polypeptide has been subject to considerable controversy. Originally, it was proposed that this polypeptide was located within the chlorosome as a BChl *c* binding polypeptide (Wechsler et al. 1985) After localization of the 5.7 kDa polypeptide in the chlorosome envelope (Wullink et al. 1991), the above hypothesis was modified by assuming an influence of this peripheral polypeptide on the aggregation of BChl *c* molecules within the chlorosomes (Lehman et al. 1994; Blankenship et al. 1995). There is now wide agreement on the fact that BChl *c* molecules are self organized into rod-like structures which fill the chlorosomes and the diameter of these rods is close to 5 nm. Several models have been proposed in the literature for the BChl *c* organization. Whereas many previous models concentrated on the organization in linear aggregates, the latest models also take into account the organization of the linear aggregates into rod-like structures. Three recent models have in common that the linear aggregates are stacked upon each other in an antiparallel fashion (Nozawa et al. 1993; Matsuura et al. 1993; Mimuro et al. 1995) whereas in a model by Holzwarth and Schaffner the aggregates are organized in a parallel fashion (Holzwarth et al. 1994). The chlorosomes also contain carotenoids, mainly β- and γ-carotene and the BChl *c* to carotenoid molar ratio is close to 10 (Blankenship et al. 1995). Whereas, the properties of BChl *c* in chlorosomes have been extensively studied, relatively little is known about the properties of the carotenoids. It was reported by van Dorsen et al. (1986) that at 4 K excitation of the carotenoids led to fluorescence of BChl *c* and the transfer efficiency was calculated to be 55%, indicating close contacts between the carotenoids and the BChl *c* molecules. From a comparison of the absorption and the excitation spectra it was concluded that the carotenoid pool is spectroscopically slightly heterogeneous and the red-most absorbing carotenoids turned out to be more effective in transferring their excitations to BChl *c*.

It was recently demonstrated that chlorosomes can be obtained with almost no carotenoids as by growing *Chloroflexus aurantiacus* in a medium containing 2-hydroxybiphenyl (HBP) (M. Foidl et al., submitted). Nevertheless, these chlorosomes have a similar absorption spectrum and similar dimensions as 'normal' chlorosomes. In this study we compare in detail the absorption, linear dichroism (LD), circular dichroism (CD) and Stark spectra of both types of chlorosomes since these techniques provide a basis for analyzing the local and overall organization of the BChl *c* molecules. Many studies have appeared in which absorption, LD and CD spectra were reported. An overview of the results was for instance given by Blankenship et al. (1995). Some striking properties are the large red shift of the Q_y absorption band for chlorosomes as compared to that of individual pigments ($\sim$670 to $\sim$740 nm), the strong LD of the Q_y band, indicating a small angle (15–20°) between the corresponding transition dipole moments and the long axis of the chlorosomes. The CD spectra show a large variability and it was recently demonstrated that these large variations can be explained within the context of the above-mentioned rod-like models by small variations in the BChl c orientations within these rods (Somsen et al. 1996).

Materials and methods

Preparation of chlorosomes

Chlorosomes were isolated from *Chloroflexus aurantiacus* grown phototrophically in the absence and presence of 2-hydroxybiphenyl, respectively, as described (M. Foidl et al., submitted). Chlorosomes were suspended in 10 mM Tris·HCl, pH = 8.0 and for low-temperature measurements glycerol concentrations were used of 70% (v/v).

Spectroscopy

Absorption spectra were recorded on a Cary 219 spectrophotometer. CD- and LD-spectra were recorded on a home-built spectrapolarimeter. For recording LD spectra chlorosomes were oriented in polyacrylamide (PAA) gels containing 14.5% (w/v) acrylamide and 0.5% N,N′-methylbisacrylamide. After polymeriza-

tion of the gels with 0.05% (w/v) ammonium persulfate and 0.03% TEMED (Sigma) they were compressed in two perpendicular directions (*x*- and *y*-axis) with a factor of *n* = 1.19 and the gels expanded along the z-axis with factor of $(1.19)^2$. An equation was derived by Ganago et al. (1980) to correlate the amount of LD to the orientations of the transition dipole moments with respect to the long axis in the case of rod-like molecules. This equation was shown to be applicable for chlorosomes (Van Amerongen et al. 1991) and it is presented here in a slightly different form:

$$\frac{\Delta A}{3A} = \Phi(n)\frac{1}{2}\langle 3\cos^2\theta - 1\rangle \qquad (1)$$

The LD signal ΔA denotes the difference in absorption for light polarized along the *z*- and *y*-axis and A is the isotropic absorption. The orientation factor Φ is 0.22 for the experiments described below. θ denotes the angle between the transition dipole moments and the long axis of the chlorosomes. The brackets <.....> denote averaging over all orientations being present. When angles are calculated below it is for simplicity assumed that this angle is identical for all dipole moments that contribute to the absorption at a particular wavelength.

Stark spectroscopy

The experimental setup is discussed in detail by Boxer (1996). Details on our home-built setup are described by Beekman (1997a). Stark and absorbance spectra are recorded simultaneously. For randomly oriented and fixed molecules the Stark lineshape is described by a sum of zeroth, first and second derivative of the ground-state absorption spectrum (Liptay 1974; Mathies et al. 1976; Lockhart et al. 1987) and is given by Equation (2):

$$\begin{aligned}\Delta A(\nu) \;=\; & (fF_{ext})^2\left\{A_\chi A(\nu) + \frac{B_\chi}{15hc}\nu\frac{d}{d\nu}\left[\frac{A(\nu)}{\nu}\right]\right. \\ & \left. + \frac{C_\chi}{30h^2c^2}\nu\frac{d^2}{d\nu^2}\left[\frac{A(\nu)}{\nu}\right]\right\} \qquad (2)\end{aligned}$$

In Equation (2) ν is the energy in wavenumbers, *h* is Planck's constant and *c* is the speed of light. F_{ext} is the externally applied field and *f* is the local field correction factor which relates the applied electric field to the electric field at the site of the molecule. A_χ, B_χ and C_χ are weight factors depending on χ: the experimental angle between the electric vector of the linearly polarized probe light and the direction of the applied field. If we neglect the electric field effect on the optical transition moment (Middendorf et al. 1993) A_χ becomes zero and B_χ and C_χ are described by Equations (3) and (4), respectively:

$$\begin{aligned}B_\chi \;=\; & \frac{1}{2}Tr(\Delta\alpha)[5 + (3\cos^2\chi - 1)\cdot 3((p\cdot\Delta\alpha\cdot p)/ \\ & (Tr(\Delta\alpha))) - 1] \qquad (3)\end{aligned}$$

$$C_\chi = |\Delta\mu|^2[5 + (3\cos^2\chi - 1)\cdot(3\cos^2\zeta - 1)] \quad (4)$$

When we set χ at magic angle all angle dependencies vanish. The second derivative contribution scales with the size of the difference in permanent dipole moment, $|\Delta\mu|$, between the excited- and ground state of the molecule. The first derivative yields Tr($\Delta\alpha$) which is a measure for the difference polarizability between the excited- and ground state, $\Delta\alpha$. The zeroth derivative is a measure for the field dependent changes of the oscillator strength of the optical transition. Furthermore, the dependence of the signal on the angle, χ, between the applied electric field and the polarization direction of the light gives information on the orientation of $\Delta\mu$ and $\Delta\alpha$ with respect to the transition dipole, *p*. More details can be found in (Liptay 1974; Middendorf et al. 1993). In a combined analysis the Stark and absorbance spectrum are fitted simultaneously using a non-linear least squares fitting program. If the absorption spectrum is due to a homogeneously broadened single transition it can be fitted with a polynomial function and the Stark spectrum with the zeroth, first and second derivative of this function (Equation (2)). Thus we obtain estimates for $|\Delta\mu|$ and Tr($\Delta\alpha$). For the polynomial we chose Bsplines of order 6 (International Mathematical and Statistical Libraries, Houston, TX, routines DBSVAL, DBSDER, DBSLSQ) which guarantees that the second derivative is smooth. A more complex absorption spectrum can be fitted with a number of (skewed) Gaussian functions and the Stark spectrum with the derivatives of these functions. The molecules are embedded in a protein/lipid environment which may influence the value of the internal electric field, $F_{\text{int}} = f^* F_{\text{ext}}$. Since *f* is a parameter for which it is hard to give a proper estimate all values for $|\Delta\mu|$ and Tr($\Delta\alpha$) will be represented in terms of D/f and $\text{Å}^3/f^2$, respectively (1 $D = 3.34\times10^{-30}$ cm, 1 $\text{Å}^3 = 1.113\times10^{-40}$ cm^2/V). In this respect chlorosomes could represent a unique photosynthetic complex if there is no protein surrounding the BChls which would else cause some unknown variation in *f* for different pigments. Absorption, LD and CD measurements were performed at room tempera-

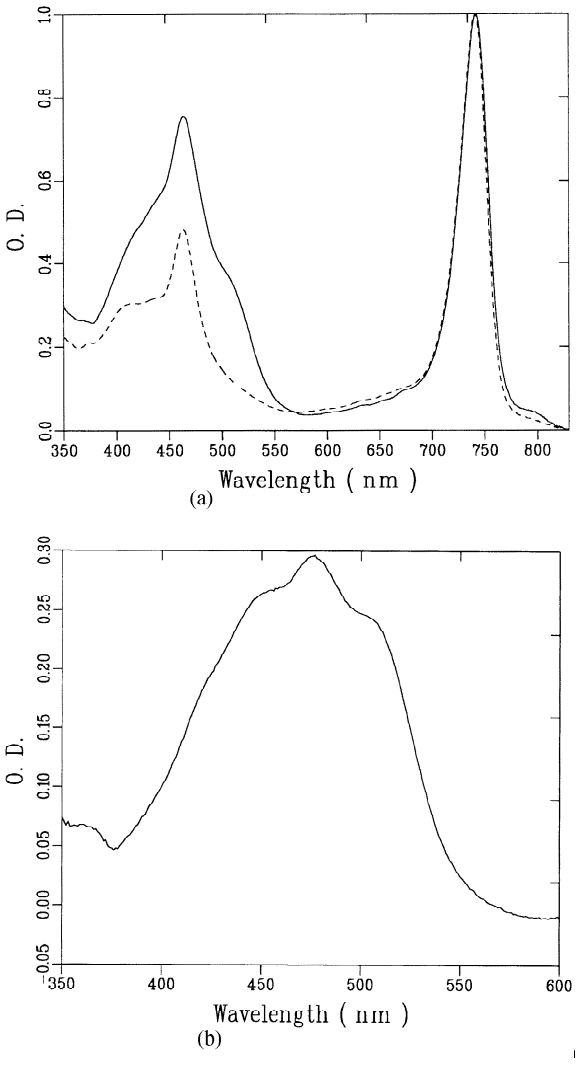

Figure 1. Absorption spectra of normal chlorosomes (solid) and carotenoid deficient chlorosomes (dash) recorded at room temperature with an optical bandwidth of 1.0 nm. The spectra have been normalized to the BChl *c* Q_y absorption maximum. (b) Difference between the two spectra given in Figure 1a.

ture. Stark and the accompanying absorbance spectra were measured at 77 K in liquid nitrogen.

Results and discussion

Absorption

The absorption spectra of normal and carotenoid-deficient chlorosomes at room temperature are given in Figure 1a. The spectra have been normalized in the peak of the Q_y band at 744 nm. No significant difference can be observed between the two spectra in the Q_y region and both the position of the maximum and the width of the band are very similar. A significant difference is observed between 400 and 550 nm where the carotenoids absorb. Such a difference is expected since the carotenoid-deficient chlorosomes contain (almost) no carotenoids. The difference spectrum of the two spectra is given in Figure 1b and it is a typical carotenoid absorption spectrum. In principle such a difference spectrum might contain a contribution from a change in the absorption of the BChl *c* absorption peak at 463 nm but there is no indication for such a change. Therefore, we conclude that the absorption properties of BChl *c* in chlorosomes are independent of the presence of the majority of the carotenoids. In good approximation the BChl *c* contribution to the chlorosome spectrum is equal to the spectrum of carotenoid-deficient chlorosomes whereas the shape of the carotenoid contribution is given by the difference spectrum.

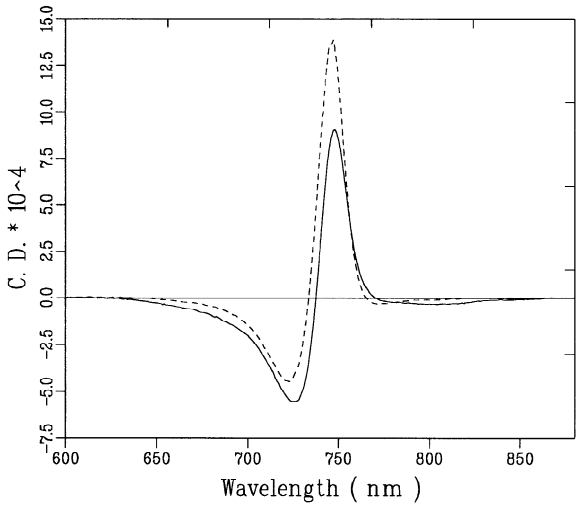

Figure 2. Circular dichroism spectra of normal chlorosomes (solid) and carotenoid deficient chlorosomes (dash) recorded at room temperature with an optical bandwidth of 3.0 nm. The spectra have been normalized to an OD of 1.0 for the BChl *c* Q_y transition.

Circular dichroism

Typical circular dichroism spectra of normal and carotenoid-deficient chlorosomes are given in Figure 2. The spectra have very similar features although they are not identical. However, the variation in the spectra of different batches of supposedly identical prepara-

tions is larger than the variations between the normal and carotenoid-deficient chlorosomes. The values of the maxima are $(1.2 \pm 0.3) \times 10^{-3}$ and $(1.5 \pm 0.3) \times 10^{-3}$ for normal and carotenoid-deficient chlorosomes, respectively, and the values of the minima are $(-0.6 \pm 0.2) \times 10^{-3}$ and $(-0.7 \pm 0.2) \times 10^{-3}$, respectively (after scaling to an OD of 1 in the BChl *c* Q_y peak). Many different CD spectra for chlorosomes have appeared in the literature. An extensive study by Griebenow et al. (1991) showed that for measurements on chlorosomes from one batch sometimes completely different CD spectra could be observed in some cases, even including a sign reversal. Almost all spectra could be described as a linear combination of two seemingly extreme kinds of spectra (type I and type II). The spectra that are given in Figure 2 are of the type II kind. Also their intensities fall within the ranges that have been reported in the aforementioned study. Given the rather large variability of previously published spectra, the spectra of normal and carotenoid-deficient chlorosomes are remarkably similar. The CD spectrum is very sensitive to the organization of the BChl *c* molecules with respect to each other. Model calculations demonstrated that even a small structural reorganization could lead to a transition from a predominantly type I to a type II kind of spectrum (Somsen et al. 1996). Therefore, the presented CD spectra do not indicate a significant difference in the organization of the BChl *c* molecules with respect to each other in the presence or absence of carotenoids.

Linear dichroism

The LD spectra at room temperature for both types of chlorosomes are given in Figure 3. Again the spectra have been normalized to a value for the OD = 1 in the peak at 744 nm. The shape in the Q_y region is very similar as expected since also the absorption spectra are similar in this region. Differences are observed in the Soret region. Around 510 nm the LD of the carotenoid-deficient chlorosomes becomes less positive which is due to the disappearance of some positive LD of the carotenoids in this region. The remaining LD is probably due to BChl *c*. The LD around the BChl *c* peak at 463 nm slightly increases. The reason for this may be twofold. (1) Some negative LD contribution of the carotenoids near this wavelength is absent for the carotenoid-deficient chlorosomes. (2) On the other hand it might be possible that the average orientation of the BChl *c* molecules with respect to the long axis of the chlorosomes is different for both chlorosome types due to which the relative height of the 744 and 463 nm peaks could differ. The intensities of the spectra of normal and carotenoid-deficient chlorosomes in the Q_y region are within the error identical. Besides, the organization of the BChl *c* molecules with respect to the long axis of the chlorosomes the height is expected to depend on the shape of the chlorosomes. For instance, going from a rod-like structure to a more globular structure is expected to lead to a decrease of the signal (Van Amerongen et al. 1988). Therefore, the LD spectra show that besides a similar internal organization of the different chlorosomes also their overall shape must be similar. From the height of the spectrum the average angle between the transition dipole moments that are responsible for the 744 nm absorption and linear dichroism bands and the long axis of the chlorosomes can be calculated according to Equation (1) ('Materials and methods'). This angle is $26 \pm 3°$ for both preparations. An angle of $15 \pm 10°$ was obtained with the same method (Griebenow et al. 1991) which is not in disagreement, given the presented error intervals. A smaller value of $17 \pm 4°$ was obtained with a slightly different method (Van Amerongen et al. 1988) which might point to a somewhat different organization of the chlorosomes. Also the relative height of the 463 nm band in the LD spectrum was higher in that study pointing to the same conclusion but it cannot be

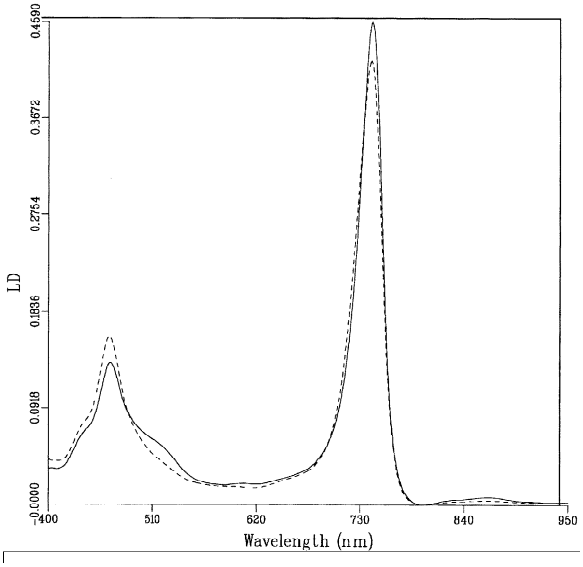

Figure 3. Linear dichroism spectra of normal chlorosomes (solid) and carotenoid deficient chlorosomes (dash) recorded at room temperature with an optical bandwidth of 1.0 nm. The spectra have been scaled to an OD of 1.0 for the BChl *c* Q_y transition.

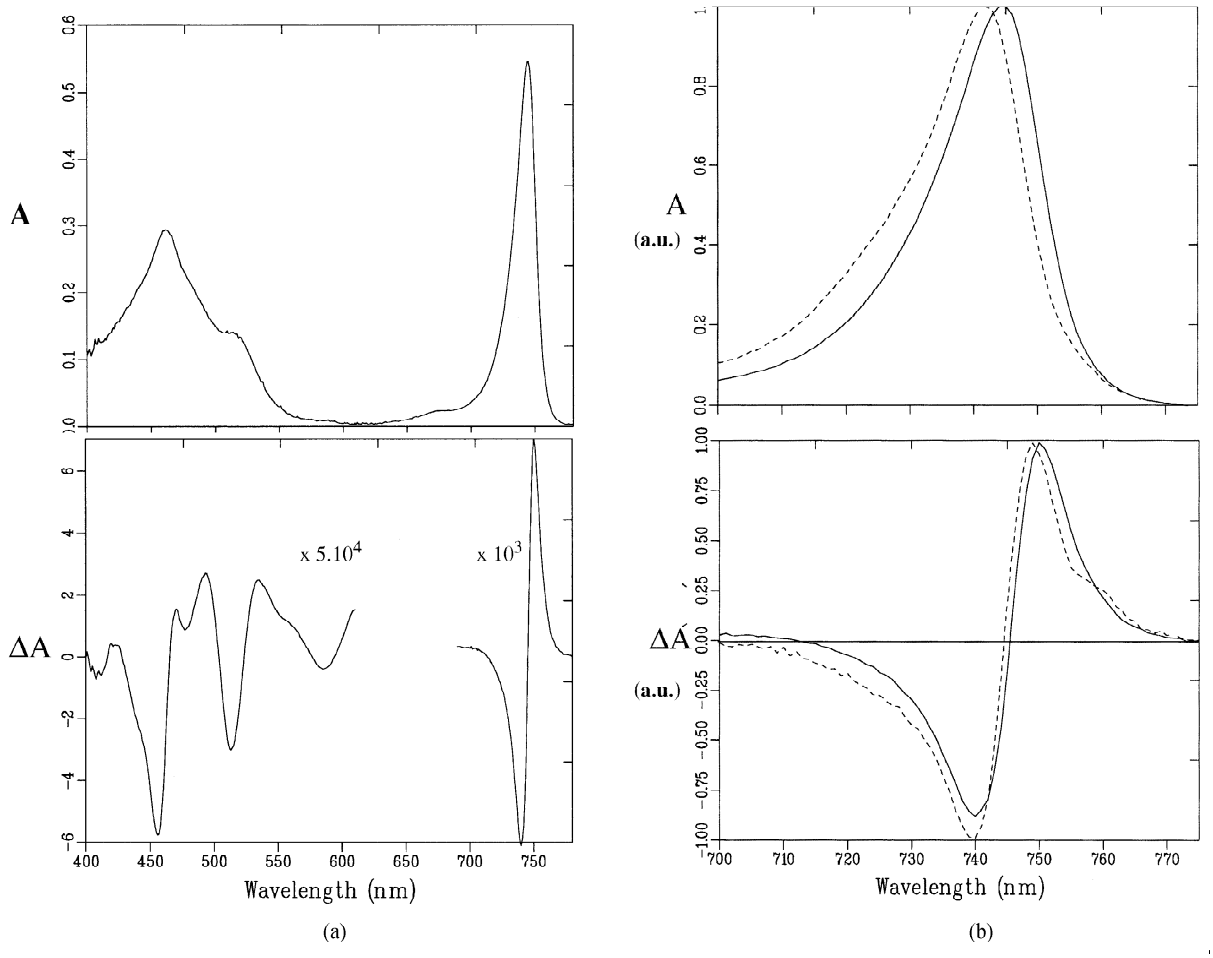

Figure 4. 77 K Absorption (top) and Stark spectra (bottom) of normal chlorosomes. The carotenoid-Q_x Stark spectrum is multiplied 5 times more for comparison reasons. The field strength is 10^5 V_{rms}/cm, thickness of the cell is 100 μm. χ is set at magic angle (54.7°). 77 K Q_y Absorption (top) and Stark spectra (bottom) of normal (solid line) and carotenoid deficient (dash) chlorosomes. The spectra are all normalized for comparison reasons.

completely ruled out that the different way of compressing the gels may have caused part of the difference. Nevertheless, we conclude that there is a general agreement between the results from different studies. It was argued above that the BChl *c* organization in normal and carotenoid-deficient chlorosomes is virtually identical. Therefore, the angle between the transition dipole moments responsible for the BChl *c* band at 463 nm can be best calculated for the carotenoid-deficient chlorosomes since there is hardly any contribution from the carotenoids. The obtained angle is 35 $\pm$ 3°. The carotenoids only give rise to a small LD contribution therefore their orientation with respect to the long axes of the chlorosome could be close to magic angle (54.7°). However, it is more likely that the carotenoids are not so well organized in the chlorosomes as the BChl c molecules and this would also lead to a small LD.

Stark spectroscopy

Stark spectra of chlorosomes have not been measured before. In Figure 4a the 77 K absorbance and Stark spectrum of normal chlorosomes is shown. The Q_y and Soret-carotenoid region are scaled differently for comparison reasons. In the Q_y region a very strong Stark signal is found with a first-derivative lineshape. The Soret-carotenoid Stark signal has a very complicated lineshape not resembling that of β-carotene in solution (Krawczyk et al. 1995) and most likely reflects

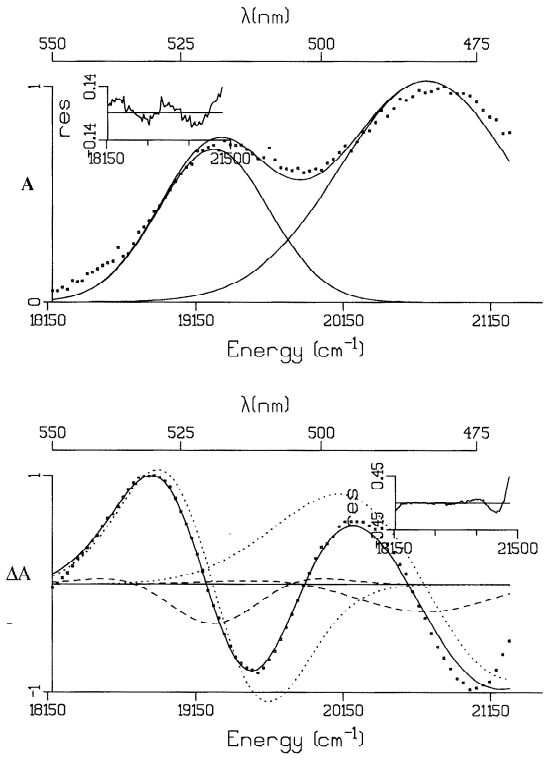

Figure 5. Simultaneous fit of 77 K carotenoid-Q_x region difference absorption (top) and difference Stark spectra (bottom) between normal and carotenoid deficient chlorosomes with two independent Gaussian functions for the absorption (top) and the zeroth, first and second derivative of these functions for the Stark spectrum (bottom). The squares are data points and the solid line represents the fit. The first and second derivative contribution to the fit of the Stark spectrum are dotted and dashed respectively. The insets show the residuals from the fits of the Absorption and Stark spectra.

a combination of Stark signals from different origin. The absorbance and the Stark spectrum of normal and carotenoid-deficient chlorosomes in the Q_y region at 77 K is shown in Figure 4b. The overall lineshape and peak-to-peak magnitude of the spectra are remarkably similar. The magnitude of the Stark signal can be determined within ∼10%. The main reason for this uncertainty is the variation in the distance between the electrodes. There are differences between the two preparations though: the carotenoid-deficient chlorosomes show a shoulder on the red wing of the Stark spectrum and the Q_y absorbance band is shifted 2–3 nm to the blue while the zero crossing of the Stark spectrum shifts less than 1 nm. This demonstrates that the carotenoids do influence the spectroscopic properties, and thus the organization of the BChl *c* molecules in a subtle way. Not every Stark measurement on the same

Table 1. Stark parameters for different preparations and fits

Species	Figure	$\Delta\alpha$(Å^3/f^2)	$\Delta\mu$ (D/f)
WT	6a	1606 (160)	2.1 (0.2)
	6b	1750 (170)	0
	6c	1606 (160)	fixed to 0
Carotenoid-deficient	7	1505 (150)	3.4 (0.3)
Carotenoid	5	1600-1900	4-7

Results from the simultaneous fitting routine of the Stark and absorption spectra for the different species and different ranges of fit types as shown in the corresponding figures. The estimated errors for the parameters are indicated in parentheses. No such errors are given for the carotenoid spectrum as this is a constructed difference spectrum.

batch of the carotenoid-deficient chlorosomes showed such a pronounced red shoulder but the shift of the zero crossing seems to be a general feature. A similar red shoulder is sometimes found for the LH2 complex from purple bacteria (Gottfried et al. 1991a; Beekman et al. 1997b). The origin is not clear though it has been speculated that it might arise from aggregation between several complexes. This is not unlikely as one uses very high OD samples in a Stark measurement. Another explanation might be that it reveals a lower energy exciton component separated from the main transition (Wu et al. 1997).

In the Soret region the spectra are very different. The difference between the two absorbance and Stark spectra after normalization at 744 nm together with the result of a simultaneous fitting procedure (see below) is given in Figure 5. Because the uncertainty for the magnitude of the difference spectra becomes higher approaching the UV region where our setup is less sensitive, we only used the two red-most bands for the analysis. Since the difference spectrum shows no clear contribution from BChl *c* it can be concluded that also in the Soret region the presence or absence of carotenoids does not influence the spectroscopic properties of the BChl *c* aggregates in a very profound manner.

By simultaneous fitting of the difference absorption spectrum and the difference Stark spectrum, applying Equation (2) ('Materials and methods'), with two independent Gaussian functions it is found that the $\Delta\mu$ values for the different carotenoid bands are between 4 and 7 *D*/*f* and Tr($\Delta\alpha$) values are found of 1750±150 Å^3/f^2. Due to a different slope in the baseline for the two different measurements it is not possible to give more exact numbers. These values are lower than those

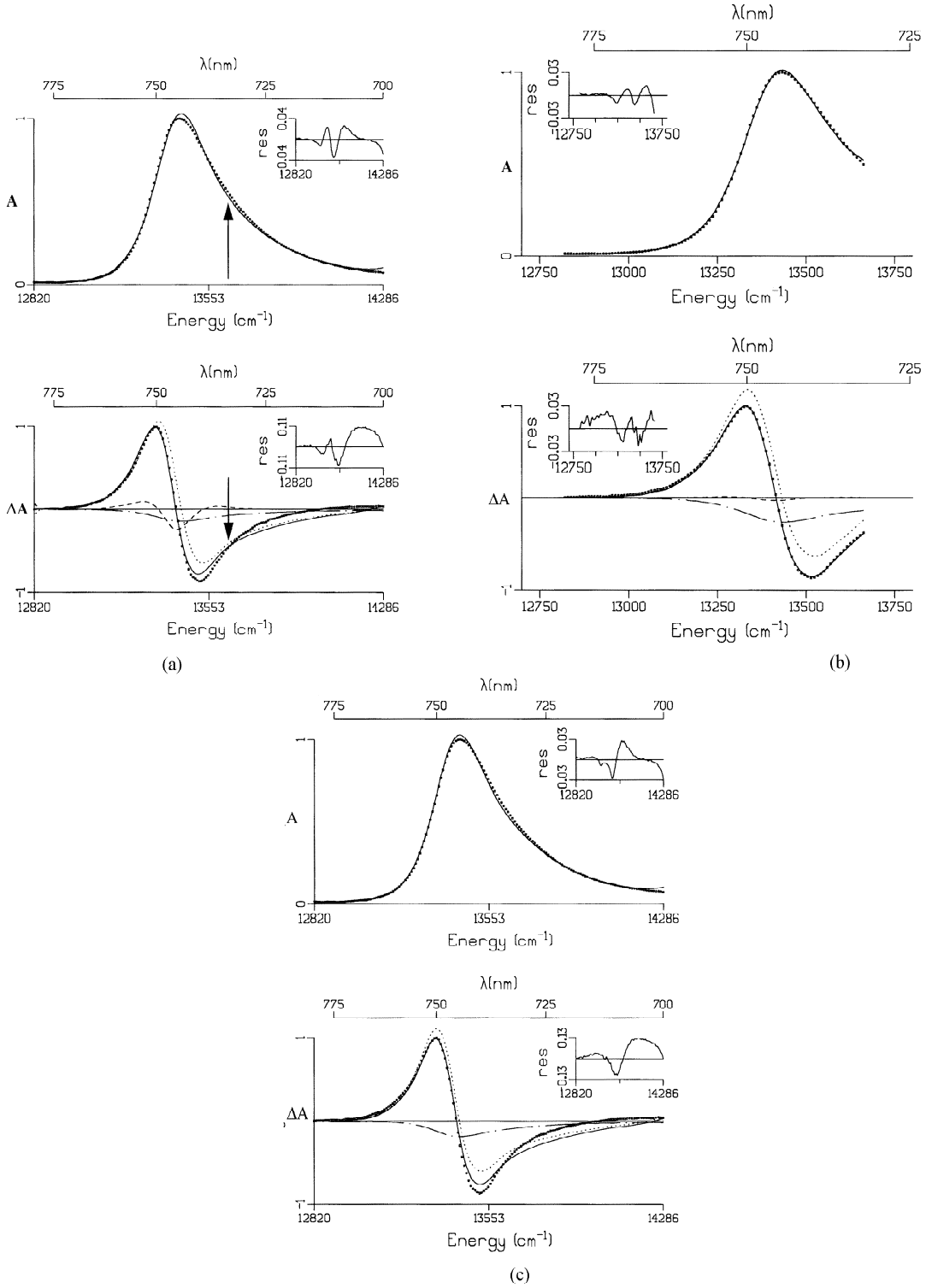

Figure 6. Simultaneous spline fit of the electronic and vibronic part of the BChl Q_y region of the absorption and Stark spectrum of WT chlorosomes using a spline curve for the absorption (top) and the zeroth, first and second derivative of this spline curve for the Stark spectrum (bottom). The squares are data points and the solid line represents the fit. The first, second and zeroth derivative contribution to the fit of the Stark spectrum are dotted, dashed and chain-dotted, respectively. The inset shows the residual from the fit of the absorption and Stark spectra. The arrows show cut-off points for Figure 6b. (b) Simultaneous spline fit of only the electronic part of the BChl Q_y region of the Absorption and stark spectrum of WT chlorosomes using one single band (spline) for the absorption and the zeroth, first and second derivative of this band for the Stark spectrum. The spectral range is 730–780 nm. Cut-off points are indicated by arrows in (a). (c) As Figure (a) but now only first- and zeroth derivative components are used.

obtained for carotenoids in other photosynthetic complexes (∼20 *D*/*f*) (Gottfried et al. 1991b) but of the order of β-carotene in solution ($\Delta\mu \sim 5$ *D*/*f*, Tr($\Delta\alpha$) $\sim$ 1200 Å^3/f^2) (Krawczyk et al. 1995). Carotenoids in photosynthetic complexes are thought to be very sensitive to the static electric field of the surrounding protein. Although the long polyenic chain should give rise to a high polarizability in the excited state the Stark spectrum of carotenoids in for instance the LH2 complex of purple bacteria is totally dominated by a second derivative contribution (Gottfried et al. 1991b). It is proposed that the non-uniform charge distribution in the protein produces a strong internal electric field which is converting the polarizability into an induced dipole-moment which then appears as a permanent dipole-moment with respect to the externally applied field. In contrast to this, β-carotene in solutions of different polarity did not show such a significant, induced dipole-moment, effect. Probably the polar solution molecules do not produce a strong non-uniform electric field as the fixed amino-acid residues in protein do. If this is correct then it explains why we find the same values for β-carotene in chlorosomes as in solutions: it is highly likely that the carotenoids in chlorosomes are in the vicinity of the BChls and (like the BChls) not near to protein. So the internal electric field inside the chlorosomal envelope is then generated by the protein in the lipid mono-layer which is distributed uniformly and thus giving rise to a small internal electric field.

An interesting feature of the Stark spectrum in the Q_y region shown in Figure 4b is that there is no indication of a contribution from a second derivative contribution of the absorption spectrum. Also for this region we used a simultaneous fitting program for the absorbance and Stark spectrum but now we fitted them with a polynomial function and the derivatives of this function, respectively. We thus regarded both spectra as arising from one single, homogeneously broadened band. The result is shown in Figure 6a and the Stark parameters for this fit are listed in Table 1. It is clear that for the electronic part of the spectra the fit is satisfactory whereas the vibronic tail (below 730 nm) is fitted poorly. Vibronic overlap can burden the analyses of Stark spectra. In this respect, we note that Equation (2), ('Materials and methods'), is essentially derived for single electronic transitions. The intensity of vibronic bands may depend on coupling to other states with possible other Stark parameters than the 0–0 transition so the vibronic tail can hinder the analysis of the 0–0 transition. Stark spectra of single vibronic

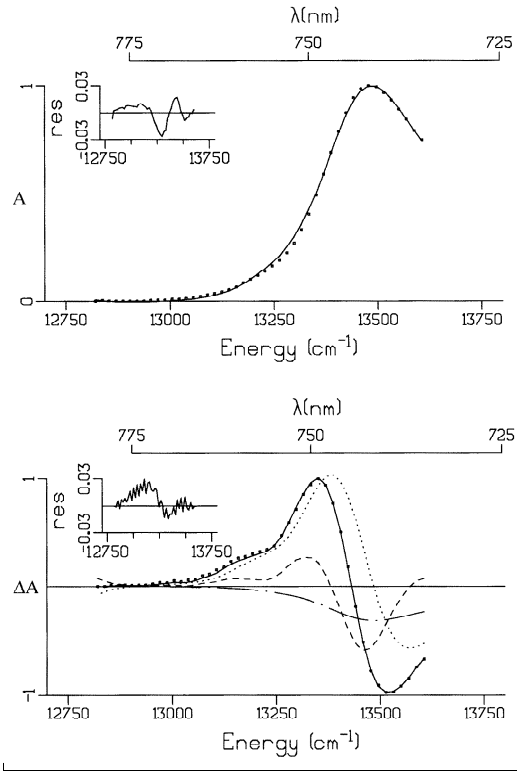

Figure 7. Simultaneous spline fit of the electronic and vibronic part of the BChl Q_y region of the absorption and stark spectrum of carotenoid depleted chlorosomes using a spline curve for the absorption and the zeroth- first- and second derivative of this spline curve for the Stark spectrum. Data-points: closed circles, fitting results: solid line. First, second and zeroth derivative contribution to the fit are dotted, dashed and chain-dotted respectively. The insets show the residual from the fit of the Absorption and Stark spectra

transitions are reported to be dominated by a second derivative contribution (Hush et al. 1995). When we cut off the vibronic part of the Stark and absorption spectrum (at 730 nm, cut-off points indicated by arrows in Figure 6a) an almost perfect fit is obtained, see Figure 6b. Remarkably, the second derivative contribution to this fit is negligible, although we did not restrain the fit in any way. Again, if the spectra with the vibronic part included are fitted but without a second derivative contribution restraint the fit was nearly as good as the one obtained with the second derivative contribution allowed, see Figure 6c. Note that the residues for both fits (Figure 6a and 6c) are similar thus the second derivative contribution in Figure 6c is only contributed to fit the vibronic part slightly better. Some negative zeroth contribution is used in all fits. This indicates some loss of oscillator strength due to the electric field and it is generally found for various photosynthetic

complexes. It also indicates a possible effect on the transition polarizability which is a component of the Tr($\Delta\alpha$) value (Middendorf et al. 1993). This component is neglected here in the calculation of Tr($\Delta\alpha$) and is an essential uncertainty in the calculation. The experimental and fitted spectra for the carotenoid-deficient chlorosomes are shown in Figure 7. In this case a second derivative contribution is needed to obtain a good fit even if we cut off the vibronic tail. Also, for the Stark spectra where the red-lying shoulder was not so pronounced, similar fits were obtained. The main reason for the $\Delta\mu$ to arise is the shift between the absorbance maximum and the zero-crossing of the Stark signal. It shows that in the absence of carotenoids the nature of the BChl *c* Q_y exited state is different and that the carotenoids are in the vicinity of the BChls.

The Stark parameters for both preparations resulting from the fits are listed in Table 1. All different analysis methods lead to a difference in polarizability $\Delta\alpha$ for ground- and excited state of 1650 ± 100 Å^3/f^2. This value is extremely large when compared to that of monomeric chlorophylls. For instance, for the Q_y band of monomeric Chl *a*, values between 1.5 and 4 Å^3/f^2 were obtained depending on the buffer used. This value increases significantly to approximately 90 Å^3/f^2 after dimerization but is still more than one order of magnitude lower than the value obtained here (Krawczyk 1991). For LH1 and LH2 complexes from various purple photosynthetic bacteria values ranging from approximately 500–1800 Å^3/f^2 were obtained (Beekman et al. 1997a, b) for the bands around 875 and 850 nm. The crystal structure of LH2 from *Rps. acidophila* was obtained at 2.5 Å resolution and it shows strong overlap of BChl *a* pigments which are organized in a ring-like structure. Electron micrograph pictures of LH1 indicate a very similar overlap. A rather analogous overlap between the BChls is thought to exist for chlorosomes as is illustrated in the different tubular models that have been proposed for the BChl *c* organization (Blankenship et al. 1995). In line with the Stark results on LH1 and LH2 our Stark experiments confirms such overlap models for chlorosomes. A large value for $\Delta\alpha$ is expected to cause a large solvent shift of the corresponding absorption maximum and this is a common feature of the 740 nm band of the chlorosomes (monomeric BChl *c* absorbs around 670 nm) and the 850 and 875 nm bands of LH2 and LH1, respectively. The BChl *a* pigments from LH2 complexes from different purple bacteria show a significant variation in their $\Delta\alpha$ values, despite large similarities in the LD and CD spectra, indicating that small structural differences may lead to relatively large changes in the Stark spectra.

The value for Tr($\Delta\alpha$) is among the highest found so far for photosynthetic complexes. The special pair from reaction centers from *Rb. sphaeroides* has a Tr($\Delta\alpha$) of 500 Å^3/f^2 and a $\Delta\mu$ of 5 *D*/*f*. These already high numbers are explained in the context of charge transfer states mixed in the excited (excitonic) state by the external electric field (Middendorf et al. 1993). Also part of the difference dipole-moment contribution to the Stark spectrum could be in fact a polarizability term converted to a dipole moment by the protein electric field. Then, the 500 Å^3/f^2 value is an average of 'surviving' components of the real Tr($\Delta\alpha$). The BChls in chlorosomes have no protein in their vicinity and most likely the internal electric field *in vitro* is small. Therefore, the $\Delta\alpha$-values reported here might reflect the intrinsic value for strongly excitonically coupled BChls.

In principle, the value of $\Delta\mu$ for excitonically coupled pigments can be much different from that of isolated pigments. In the case of LH2 or the special pair from bacterial reaction centers an increase of $\Delta\mu$ compared to monomeric BChls is found. This likely arises from the internal electric field generated by the protein which transforms the difference polarizability in a difference dipole-moment when a Stark experiment is performed. On the other hand, much smaller $\Delta\mu$ values are expected provided that the pigments have strongly contrasting orientations. Recently this is found to be the case for the LH1 antenna complex where all $\Delta\mu$s of the constituting BChls can be pictured in the plane of a ring thus cancelling the overall difference dipole-moment contribution (Frese et al., unpublished). The similarity between chlorosomes and LH1 complexes regarding the Stark lineshape and parameters is striking. When both complexes contain carotenoids the $\Delta\mu$ contribution vanishes likely because of an anti-parallel stacking motif. Also, for both complexes the Tr($\Delta\alpha$) values are very similar. When both complexes lack carotenoids, either by chemical inhibition (chlorosomes, this work) or in case of LH1 by reconstitution from dimeric units (Beekman et al. 1997a) a $\Delta\mu$-component is observed.

In the literature, several models have been proposed for the BChl *c* organization (Blankenship et al. 1995). In the model of Holzwarth and Schaffner the BChl *c* pigments are organized as rods consisting of parallel stacks of partially overlapping pigments. The transition dipole moments are close to parallel to the symmetry axis of the rod. In that case one does not expect important differences in the size of $\Delta\mu$ as com-

pared to individual pigments. Therefore, this model does not easily explain the apparent absence of $\Delta\mu$. On the other hand, the proposed models of Nozawa et al (1993) and Matsuura (1993) contain antiparallel stacks of partially overlapping pigments and exciton delocalization over different stacks would lead to a strong reduction or even disappearance of the $\Delta\mu$ contribution (second derivative) in the Stark spectra. Our Stark measurements appear to favor an antiparallel model.

Conclusions

With absorbance, LD, CD and Stark spectroscopy we have probed the structural arrangement of the BChls and carotenoids on different structural levels. All results show that the absence of carotenoids does not perturb the structural arrangement of the BChls strongly: to a large extent the disappearance of characteristic carotenoid absorption bands does not change the angles of the transition dipole moment relative to the long axis of the chlorosomes neither does it change the lineshape and type of the CD spectrum of which also the magnitude remains within a range as reported before. Also, the Stark spectra of the two preparations resemble each other in lineshape and magnitude apart from a shoulder and a shift in zero-crossing compared to the absorbance maximum in the HBP treated sample. The small difference between the Stark signals from the two different preparations might be caused by the disappearance of the contacts between the carotenoids and the BChls. The overall resemblance between the spectral properties of normal and HBP-treated preparations shows that the BChl *c* organization hardly depends on the presence of the 5.7 kDa protein. All together it seems that the BChls in the chlorosomes are selforganizing structures with no need for any other stabilizing force. We found from the Stark spectra that the difference dipole moments of the constituting BChls cancel, thus indicating an anti-parallel stacking motive.

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