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Identification of the Upper Exciton Component of the B850 Bacteriochlorophylls of the LH2 Antenna Complex, Using a B800-Free Mutant of *Rhodobacter sphaeroides*[†]

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ABSTRACT: In this paper, we report the circular dichroism (CD) spectra of two types of LH2 -only mutants of *Rhodobacter sphaeroides*. In the first, only the wild type LH2 is present, while in the second, the B800 binding site of LH2 has been either destabilized or removed. For the first time, we have identified a band in the CD spectrum of LH2, located at ~780 nm, that can be ascribed to the high exciton component of the B850 band. The experimental spectra have been modeled by theoretical calculations. On this basis, the average interaction strength between monomers in the B850 ring can be estimated to be approximately 300 cm^{-1}. In addition, we suggest that in LH2 of *Rb. sphaeroides* the angles made by the Q_y transitions of the B850 BChls with respect to the plane of the ring are slightly different from those calculated from the crystal structure of the *Rhodopseudomonas acidophila* LH2 complex.

In photosynthesis, (solar) photons are absorbed by a light-harvesting antenna and are efficiently transported to a reaction center where they are used to drive a charge separation (*1, 2*). Following the elucidation of the structure of the peripheral light-harvesting complex (LH2 or B800−850) of *Rhodopseudomonas acidophila* (*3*), defining the relationships between the structure, the spectroscopic properties, and the energy transfer dynamics of this complex presents a major challenge.

The most prominent absorption band of LH2, around 850 nm, arises from the B850 ring of 18 BChla molecules, and since the publication of the structure, an important issue has been how to describe the absorption and CD features of this ring. Several attempts have since been made to calculate the CD and OD spectra of the B850 ring from the structure (*4−6*). However, in such calculations, there is one major problem, which originates from the estimate of the amount of dipolar coupling between neighboring pigments which so far has varied between 100 and 700 cm^{-1} (*7−9*). Therefore, it is essential that the position of the high-energy exciton band is known for such calculations. However, due to the

symmetric ring structure and the almost in-plane orientation of the transition dipoles, this band is almost forbidden, and is therefore difficult to observe. On the basis of a variety of indirect experiments, it has been suggested that it is in the 800 nm region (*10, 11*), but so far, there have been no direct measurements of this band.

In a previous paper (*6*), we presented calculations of the absorption and CD spectra of the LH2 antenna system of *Rps. acidophila*, based on the known geometrical structure of that system. A rather prominent feature of the experimental spectra is the zero crossing of the CD spectrum, which is up to 7 nm to the red of the absorption maximum. The main conclusion of ref 6, in agreement with earlier suggestions by Sauer et al. (*4*), was that it is necessary to take into account all the interactions in the full ring in order to explain this red shift. Furthermore, we concluded that the mean excitation energies of the chromophores connected to the α and β proteins had to be different.

Variations of other parameters, such as the dielectric constant, within reasonable limits, were shown to have only minor effects on the spectra.

One result of these calculations is that the CD spectrum in the 800 nm region is the combination of two contributions. The first contribution comes from the ring comprising the 800 nm BChls. Since the distance between the chromophores in this ring is rather large, the interaction is weak, and the resulting CD spectrum is small. The second contribution is from the chromophores in the B850 ring, which interact much more strongly. For an ideal ring system, i.e. no disorder, it can be shown that only three exciton levels can have oscillator strength and rotation strength, but that these can contribute in both the 850 and 800 nm region if the BChls attached to the α and β peptides are nondegenerate (*6, 12*). It is likely a coincidence that the additional levels have energies precisely in the 800 nm region. The observation of a CD signal from the B850 BChls in the 800 nm region using a mutant LH2 lacking B800 BChls would provide a crucial test of these model calculations.

In this paper, we present the experimental evidence for the existence of an upper exciton band of B850 which is located at approximately 780 nm.

MATERIALS AND METHODS

The spectroscopic measurements were performed on membranes isolated from a strain of *Rhodobacter sphaeroides* which synthesizes only the LH2 complex (DD13/G1) (*13*). This was used as a basis for the construction and characterization of an LH2 mutant in which the α polypeptide was shortened at the N terminus by two amino acids (referred to as the α Thr$_{29}$-Asn$_{28}$ truncation mutant). A second mutant, in which the N-terminal region of the LH2 α polypeptide was exchanged for the corresponding region of the LH1 α polypeptide, was also used. Thus, the sequence αMTNGKIW was exchanged for αMSKYKIW. In the first mutant, the B800 binding site has been destabilized; in the second, it has been removed.

The two LH2 *puc*A mutants were constructed using polymerase chain reaction (PCR). For both of the mutagenic changes, one of the pair of PCR oligonucleotides was designed to be homologous to the 3′ end of the *puc*A gene and included the *Bam*HI site found beyond the end of the
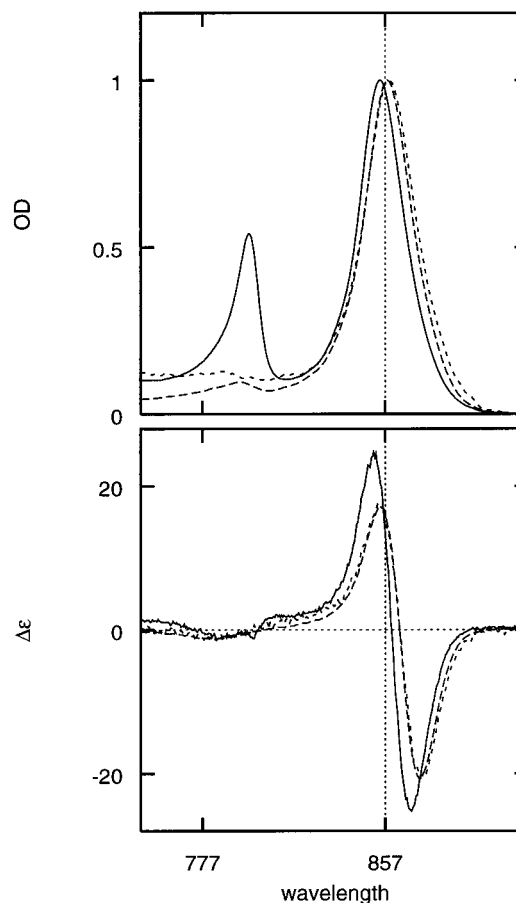

FIGURE 1: Experimental absorption and CD spectra ($\times 10^4$) of α Thr$_{29}$-Asn$_{28}$ ($- - -$), α N$_{LH1}$ (- - -), and the wild type LH2 of *Rb. sphaeroides* ($-$). All spectra were recorded at 77 K and are scaled to the absorption maximum equal to 1 of the B850 absorption maximum. Values related to these spectra are collected in Table 1.

LH2 genes. The second oligonucleotide for each PCR was designed to include the necessary changes to the 5′ end of the *puc*A gene and included a *Hin*dIII site upstream of the gene. The mutant *Hin*dIII−*Bam*HI-ended mutant *puc*A gene fragments were cloned alongside *Kpn*I−*Hin*dIII-ended wild type *puc*B fragments into the pUC19 cloning vector and sequenced. In order to verify that the new LH2 genes were expressed in the *Rb. sphaeroides* strains, the plasmids containing these genes were recovered from *Rb. sphaeroides* and retransformed into *Escherichia coli*, and then the plasmid DNA was prepared and sequenced for the second time. This verified that no alteration of the *puc* genes had occurred and that the LH2 complexes contained the desired mutations. These and other mutants will be described in detail elsewhere (*14*).

Membranes containing LH2 complexes were suspended in 10 mM Tris-HCl (pH 8.0), and for the low-temperature measurements, glycerol concentrations of 70% were used (v/v). CD spectra were recorded on a homemade spectrapolarimeter, at a temperature of 77 K (*15*).

RESULTS

The absorbance (OD) and CD spectra of the two different membrane samples containing B800-free LH2 complexes are shown in Figure 1. The CD/OD spectra are all scaled to an OD of 1.0 of the B850 Q_y transition. The B850 bands of the two B800-less mutants are both red-shifted to about 858

Table 1: Numerical Values Derived from the Spectra of Figure 1[a]

	absorbance		circular dichroism ($\Delta\epsilon$)			
LH2 mutant	B850	B800	zero	+max	−min	800
WT	855	797	860	852	868.5	784
		0.54		24.7	−25.2	−1.1
α Thr$_{29}$-Asn$_{28}$	858	782	863	856	872	782
				17.1	−20.7	−1.1
α N$_{LH1}$	859		863.5	854.0	873.5	783
				20.3	−17.7	−1.3

[a] The +max and −min columns for CD give both the positions and relative magnitudes of the signals at their peak positions ($\times 10^4$). The 800 columns in absorbance and circular dichroism give the values of positions and intensities in the 800 nm region. For the wild type (WT), there is a contribution from the B800 ring. For the mutants, these are contributed to Davidov splitting. The WT CD in the 800 nm region is very noisy; hence, the error in position is rather large (5 nm).
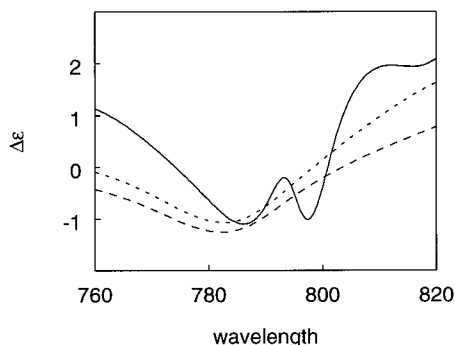

FIGURE 2: Enlargement and fit of the 760−820 nm CD spectra of α Thr$_{29}$-Asn$_{28}$ (− − −), α N$_{LH1}$ (- - -), and the wild type LH2 of *Rb. sphaeroides* (—). The WT spectrum is likely a superposition of the B850 upper exciton component and the CD of the B800 ring.

nm at 77 K. The small absorption features around 780 nm are most likely the result of contamination with a small amount of free BChla, in the case of the α Thr$_{29}$-Asn$_{28}$ truncation mutant, although this is not the case for the α N$_{LH1}$ mutant. The CD spectra of the two mutants are essentially similar, with the two B800-less mutants slightly red-shifted. Detailed comparison of the CD spectra of the two mutants shows that the overall CD line shape and the zero crossing of the CD signal relative to the absorbance maximum are almost identical.

Compared to the CD spectra of *Rps. acidophila* (*5, 6*), the intensity in the 800 nm region of the WT *Rb. sphaeroides* is weaker, which indicates some interspecies variability of the LH2 antenna, possibly reflecting small changes in structure. In addition, the spectra depend on temperature and on the amount of detergent used. For one particular sample, however, the spectra are consistently the same.

In all spectra, a broad negative feature in the CD spectrum, centered around 780 nm, can be observed; for the WT LH2 spectrum, an additional signal around 800 nm is superimposed on the broad negative band. For the two B800-less complexes, the position and intensity of the small negative CD signal in the 780 nm region of each spectrum are similar (Table 1). In Figure 2, we show a cubic spline fit of the CD spectra in the 800 nm region, which shows these features more clearly.

The small negative CD signal around 780 nm can have different origins.

(1) It could be attributed to some remaining B800 pigments. This is unlikely since the signal is much different from the signal that originates from the B800 of the WT LH2 complex of *Rb. sphaeroides*.

(2) There could be free or monomeric protein-bound BChla present; the truncation mutant contains some free BChla as shown in the absorption spectrum of Figure 1. However, the CD of BChla is a small positive band, rather than the negative one observed in Figure 1, and in any case, there is no indication of free BChla in the absorbance spectrum of the α N$_{LH1}$ mutant (*16*).

(3) It could be the high exciton component of the B850 ring, which arises from the splitting, due to the dimeric character of the unit cells in the ring of B850 BChla's, of the B850 band. In the next section, we give the theoretical background and show some results which support this possibility.

ANALYSIS

The B850 part of the LH2 antenna complex can be considered a ring of coupled dimers (*17*), or alternatively as two interacting rings of monomers: one ring of chromophores bound to α and the second ring bound to β proteins. This dimer model does not mean that the spectroscopic unit can be a dimer, merely that the unit cell in the ring is not a monomer. Excitonic states should be used as a basis for description of both of these rings.

The starting point for our calculations is the Hamiltonian

$$H = \sum_{n=1}^{9} \sum_{\mu=\alpha,\beta} \epsilon_\mu |n\mu\rangle\langle n\mu| + \sum_{n,m=1}^{9} \sum_{\mu,\nu=\alpha,\beta} V_{n\mu,m\nu} |n\mu\rangle\langle m\nu| \tag{1}$$

In this equation, $|n\mu\rangle$ denotes the state where monomer μ in dimer n is excited, and $V_{n\mu,m\nu}$ is the (dipolar) interaction energy between the monomers. All interactions are taken into account, not just nearest neighbors. The site energies ϵ_μ can be different for the monomers within a dimer (*5*).

The conversion to excitonic states $|\psi_k,\mu\rangle$ is now effected by

$$|\psi_k,\mu\rangle = \frac{1}{3}\sum_{n=1}^{9} e^{2\pi i n k/9} |n\mu\rangle, \quad \mu=\alpha,\beta \tag{2}$$

which transforms the above Hamiltonian into

$$H = \sum_{k=1}^{9} H_k \equiv \sum_{k=1}^{9} \sum_{\mu=\alpha,\beta} [[\epsilon_\mu + \tilde{V}_{\mu\mu}(k)]|\psi_k,\mu\rangle\langle\psi_k,\mu| + \tilde{V}_{\mu\bar\mu}(k)|\psi_k,\mu\rangle\langle\psi_k,\bar\mu|] \tag{3}$$

with

$$\tilde{V}_{\mu\nu}(k) = \sum_{n=1}^{9} V_{1\mu,n\nu} e^{2\pi i(n-1)k/N}, \quad \mu,\nu=\alpha,\beta \tag{4}$$

In eq 3, $\bar\mu$ has the value α (β) when μ has the value β (α). This equation also shows that for every value of k we have to diagonalize the Hamiltonian H_k, which is a 2 × 2 problem for two-level systems. If we want to take more transitions into account, for instance to Q_x, the method remains the same, only the resulting H_k is then also a three-level Hamiltonian.
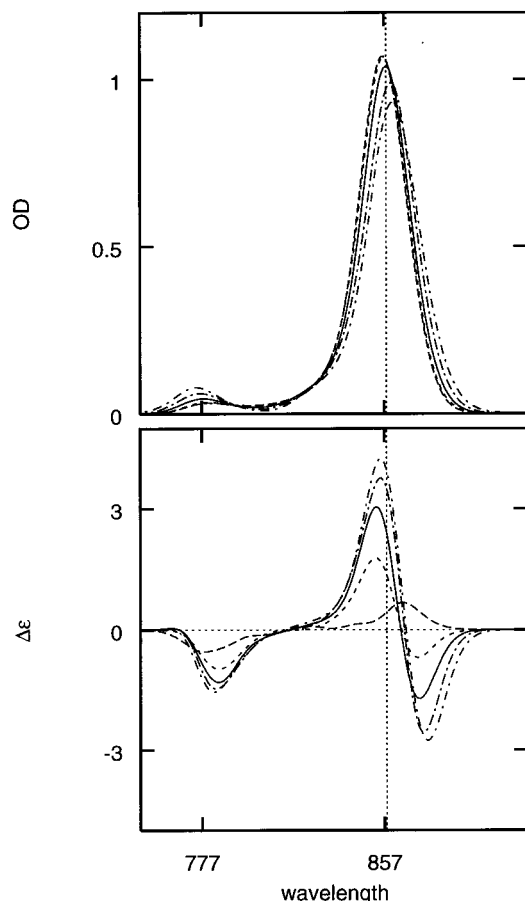
FIGURE 3: Dependence of the absorption (OD) and CD spectra on the site energy difference ΔE ($= E_\alpha - E_\beta$) between α and β chromophores. The mean site energy is kept constant. The theoretical fit to the *Rps. acidophila* spectra, for which we used $\Delta E = 300$ cm^{-1}, is used as the reference (—). The other curves are for energy differences of 0 cm^{-1} (— — —), 150 cm^{-1} (- - -), 450 cm^{-1} (—•—), and 600 cm^{-1} (-•-). We have not scaled the OD spectra to 1 at the maxima to show their dependence on the parameter ΔE.

For LH2, the effects of coupling to higher electronic states turned out to be small.

We note that the interactions between the α (or β)-bound chromophores make the levels nondegenerate even if $\epsilon_\alpha = \epsilon_\beta$. The magnitude of the energy separation due to this effect, the $\tilde{V}_{\mu\mu}$ terms in eq 3 which are approximately 80 cm^{-1}, may account for part of the red shift of the LH2 ring to 850 nm.

The eigenvalues and eigenvectors of the LH2 system can easily be calculated. Every k level is split into two levels (Davidov splitting), which gives rise to the OD and CD features from the B850 ring in the 800 nm region.

Since in the case of negligible disorder only the $k = 9$ and the degenerate $k = 1$ and 8 excitonic states have nonzero transition dipole moments, the first one small and perpendicular to the plane of the ring and the second one much larger and in the plane, these are the only states of the rings that interact with an external field. Introduction of disorder complicates the calculation of the eigenstates but has only minor effects on the predicted CD spectrum (*18*).

For the calculations presented in this paper, we used values of 6.3 D for the magnitude of the transition dipole moment of a monomer and a value of 1.2 for the dielectric constant. The directions of the transition moments were taken to be along the N1–N3 axes of the BChla molecules. This gives

a value of about 280 cm^{-1} for the mean magnitude of the nearest neighbor interaction, based on the angles and distances for the BChls in LH2 of *Rps. acidophila* (*6*).

The states resulting after diagonalization of each H_k turn out to be almost equal mixtures of the excitonic $|\psi_k,\alpha\rangle$ and $|\psi_k,\beta\rangle$ states. The interaction energy is dominated by the positive nearest neighbor $\beta-\alpha$ interaction. This means that the transition moments connected with these states are added in the low-energy state and subtracted in the high-energy state. Since the transition dipole moments connected with the above excitonic states are also almost equal, this explains the virtual absence of oscillator strength in the 800 nm region.

We have shown in ref 6 the extreme sensitivity of the CD signal to small changes in the angles the chromophores make with the plane of the ring and to the energy difference between the α and β chromophores. The energy difference was needed to obtain a proper red-shifted CD crossing with respect to the absorption maximum, when homogeneous and inhomogeneous broadening are also present. Small changes in the angles do not greatly change the CD spectrum in the 800 nm region but have a rather large effect on the part of the spectrum around 850 nm.

On this basis, we have attempted to obtain a "best fit" of the experimental spectrum. An important feature of the experimental CD spectrum is the ratio of the peak heights at 780 and 870 nm, which is 1:10. Starting from the geometrical structure of *Rps. acidophila*, we found that we can change this ratio from 3:1 (assuming an energy mismatch of 150 cm^{-1}) to 1:2 at a mismatch of 600 cm^{-1} (Figure 3). This is clearly not sufficient to explain the observed ratio. In addition, the position of the zero crossing of the CD spectrum with respect to the absorption maximum limits the value of this energy mismatch to about 300 cm^{-1}.

To fit the spectrum, we used values for the homogeneous line width and inhomogeneous line width of 200 and 500 cm^{-1}, respectively (*19–21*). Changes in these values do not change the peak ratio appreciably.

This leaves the possibility of a change in the direction of the transition dipole moments with respect to the plane of the ring. The ratio of peak heights is extremely sensitive to such changes. In Figure 4, we show how the spectrum varies as a function of the angle that the α and β transition dipoles have with respect to the ring plane. Rotating either the α chromophore toward the plane of the ring or the β chromophore away from the plane can lead to the desired peak ratio. The mutant spectra can be reproduced by changing both angles by approximately 5°, leading to angles of 2 and 13° for the angles of the transition moments of the α and β chromophores with the ring plane, respectively. Since there are two angles that can be altered, these are not the only possibilities. It is the direction of the transition dipole moments that is relevant here, so this does not necessarily imply a change in geometry, although the protein structure appears to allow for geometric changes in the indicated direction.

Once the orientation of the transition dipole moments has been modified, the spectrum becomes less sensitive to changes in the site energies, a consequence of the absence of canceling effects (*6*). Studies on LH2 mutants, involving the breakage of H bonds to the C2 acetyl carbonyls of the α and β B850 BChls, show no great variations in the overall
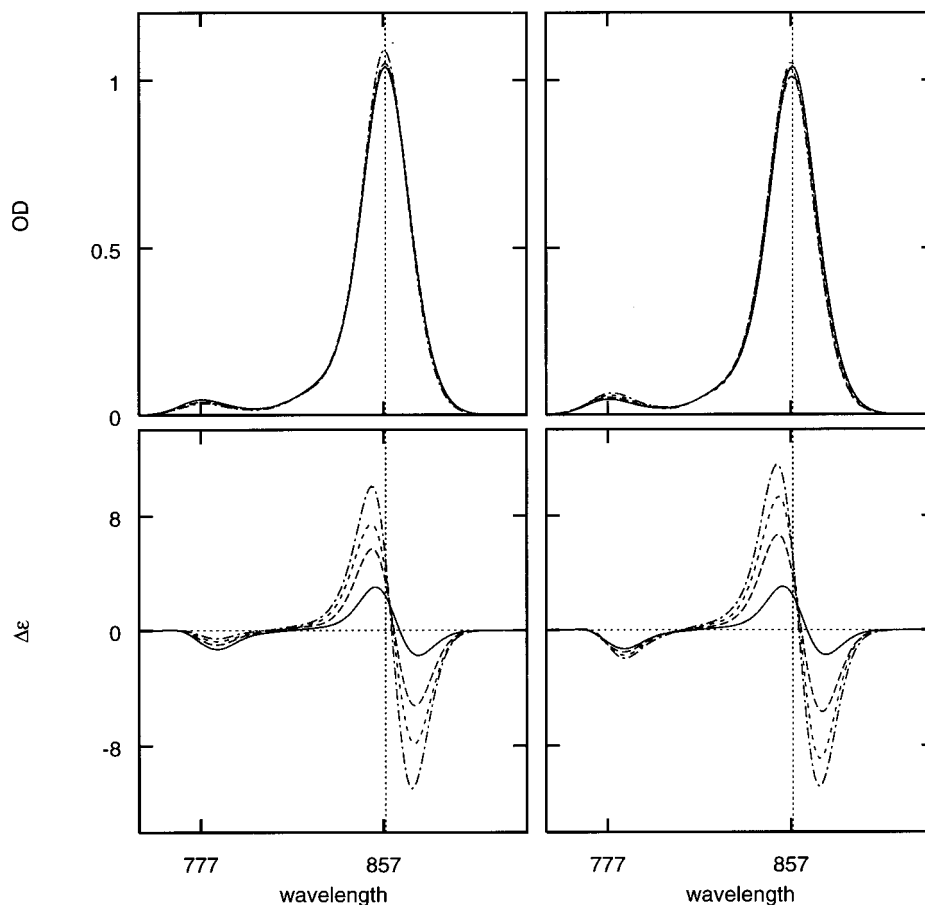
FIGURE 4: Dependence of the OD and CD spectra on the angles of transition moments with the plane of the ring. The theoretical spectrum of *Rps. acidophila* is again used as the reference (—). On the left, we show the change when the direction of the α BChla is tilted toward the ring by 3° (— — —), 5° (- - -), or 7° (—·—), while the β chromophore angle is kept at its original value of 7°. On the right side, the direction of the β BChla is tilted away from the plane of the ring by 3° (— — —), 5° (- - -), or 7° (—·—), while the direction of the α BChla is kept at its original value of 7°. In these figures, the energy mismatch ΔE is kept fixed at 300 cm^{-1}.

features of the CD spectrum, in agreement with these observations (*22, 23*).

For the parameters used, the CD intensities and the position of the CD zero crossing are also in good agreement with the experimental values. The oscillator strength of the upper exciton (blue) component is approximately 6% of the lower (red) component. This component is shifted to the blue compared to the CD at 780 nm, due to line width effects which cancel the blue part of the CD spectrum.

To summarize, we used values of 803 and 823 nm as the transition wavelengths for the α- and β-bound chromophores, respectively, a dielectric constant of 1.2, and a FWHM of 500 cm^{-1} for the inhomogeneous and 200 cm^{-1} for the homogeneous line width. In addition, the values for transition dipole moments lead to nearest neighbor interactions of 300 and 233 cm^{-1} between α and β and β and α chromophores. This leads to a disorder−interaction ratio of about 1.8, in agreement with super-radiance studies (*24*).

CONCLUSIONS

The starting point of our calculations is the known geometrical structure of the LH2 system of *Rps. acidophila* (*3*). The structure of the B800-less LH2 of *Rb. sphaeroides* has not been determined by crystallography but is assumed to resemble the structure of the B850 part of the *Rps.*

acidophila LH2 closely. Calculations of the CD, however, indicate that a much better fit, especially of the magnitude in the 850 nm region, can be obtained by slightly changing the angles of the transition dipole moments with respect to the ring. There appears to be enough leeway in the protein structure to accommodate the chromophores with the angles given in the previous section.

From the observed CD spectrum, we obtain a direct estimate of the excitonic interaction in the B850 ring. In the Davidov splitting model, the energy difference between the levels is approximately $2V \cos(\pi/9)$ cm^{-1}, where V is the average nearest neighbor interaction. Using values obtained from the experimental spectrum, this leads to a V of ≈300 cm^{-1}, which agrees well with the value used in section 3, estimated from the structure, assuming dipole− dipole coupling, a transition moment of 6.3 D, and a dielectric constant of 1.2.

In conclusion, we can state that we have shown that we can quantitatively account for the total observed CD spectrum of LH2. We have located the high exciton component of the B850 ring system around 780 nm, which corresponds to a value of approximately 300 cm^{-1} for the nearest neighbor interaction in the excitonic interaction matrix.

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