

Red Shift of the Zero Crossing in the CD Spectra of the LH2 Antenna Complex of *Rhodospseudomonas acidophila*: A Structure-Based Study

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The published crystal structure of the LH2 complex of *Rhodospseudomonas acidophila* is used to calculate absorption and CD spectra of this system. An important parameter, the zero crossing of the CD spectrum with respect to the absorption maximum, is shown to be sensitive to structural changes. The changes in spectral properties as a function of varying excited state energy, variation in the dielectric properties of the surrounding medium, and changes in relative orientations of the chromophores are also investigated. It is shown that the experimentally observed red shift of the zero crossing can be explained by lifting the degeneracy of the excited state of the monomers and taking into account more than half of the LH2 ring.

1. Introduction

In photosynthesis, two fundamental and ultrafast processes play a key role in converting solar energy into free chemical energy: energy transfer in light-harvesting antennae and charge separation in the reaction center.^{1,2} A highly organized structure of membrane-bound pigment proteins, reaction centers, and antenna proteins is responsible for the observed high efficiency of photosynthesis.

Recently, the structure of the peripheral light-harvesting antenna of the photosynthetic bacterium *Rps acidophila* was resolved up to 2.5 Å resolution.³ One of the main challenges of our research is to establish the relation between the structure, its spectroscopic properties, and its function.

In a previous paper⁴ we have shown that the combination of absorption and circular dichroism (CD) spectroscopy can be helpful to elucidate the geometrical structure of a subunit of the core antenna of photosynthetic bacteria, the B820 bacteriochlorophyll (BChl) dimer. An extremely sensitive probe of structure in the asymmetry of the Q_y part of the CD spectrum. Because of the geometrical arrangement of the BChl molecules, the nearly resonant Q_y – Q_y coupling makes only a small contribution to the CD; the contributions from nonresonant coupling become equally important.

In this paper, we extend these calculations to the much larger ring system of BChls in the LH2 antenna complex of *Rps. acidophila*. The LH2 complex consists of two rings, one comprising 9 pairs of BChl molecules, sandwiched between an outer ring of β and an inner ring of α polypeptides, believed to absorb mainly in the 850 nm range, the other consisting of 9 BChl molecules with principal absorption in the 800 nm.^{5–7} The main absorption band in the 850 nm region is called the B850 band, although the actual absorption maximum in *Rps. acidophila* at 4 K is close to 870 nm.

Although the structure of this complex is known to within a resolution of 2.5 Å, which gives good insight in the position

and orientation of the chromophores,³ many questions remain regarding the electronic interactions, the spectroscopy, and energy transfer mechanisms within the system. Since the energy transfer, both within the complex and eventually to the special pair in the photosynthetic reaction center, is an important step in the photosynthetic process as a whole, it is of interest to investigate the nature of the electronic states of LH2. Knowledge of the structure enables us to calculate the coupling matrix elements between the BChl monomers to a reasonable approximation and hence to find the electronic states of the system by a simple diagonalization procedure. We will describe the model in detail in section 2.

One of the fundamental questions often raised is regarding the size of the spectroscopic unit of the system. On the basis of the structure of the B850 absorption band and depolarization experiments, it has been suggested that the spectroscopic unit is a dimer.^{8–10} In this model, excitation results in a localized dimer excited state that transfers its energy to neighboring dimers (presumably by a Förster-type transfer), and this random walk finally delivers the excitation via LH1 to the special pair. In the other extreme situation, the rings are coherently excited as a whole,^{11,12} and this stationary excitation “leaks” into the special pair of the reaction center where an electron transfer process eventually occurs. Intermediate models, with excitonic lengths ranging anywhere from 4 to 8 monomers have been proposed^{13–17} mainly based on pump–probe experiments.

As in the case of the B820 dimer, the combination of the absorption and CD spectrum provides a rather sensitive measure of structural and other changes in the system. The absorption spectrum itself can be fitted with almost any model; it is rather insensitive to specific values of the parameters. The CD spectrum by itself is already more sensitive to changes, but the combination of the two is crucial for our presentation. If we briefly consider a degenerate dimer (B820 provides a good example, but also a dimer taken from the ring structure will do), it is easy to see that the zero crossing of the CD spectrum is always to the blue of the absorption maximum. Most of the dipole strength of the Q_y moments is in the transition to the lower energy state, thus giving a red shift of the absorption maximum compared to the monomer position whereas the zero crossing of the CD spectrum is at the monomer transition energy. In fact we have found no way for a dimer to get a red shift of

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the zero crossing, unless the structure is changed drastically. If LH2 were a true collection of strongly coupled dimers, the CD spectrum would exhibit this blue shift property as was shown previously.^{4,18} In fact we will show that we need to take at least one-half of the ring system, *i.e.*, five dimers or more, into account before this property of the OD and CD combination can be explained.

Even though the geometric structure of LH2 is known, there are still a large number of parameters not available for the system. First of all, the structure does not need to be, and in fact is likely not, rigid. Second, the surroundings of the BChl can vary. This is certainly the case for the chromophores bound to the different α and β proteins. We have therefore investigated the spectroscopic properties of the system for a variety of situations, including variations in monomeric transition energies, local changes in the environment by variation of the dielectric properties of the system, small changes in the orientation of the chromophores, and the size of the spectroscopic unit.

In the next section we give a description of the model, and in section 3 we calculate the dipole and rotational strength as a function of the spectroscopic unit which we let range from a dimer to the complete LH2 ring. We break the symmetry of the ring to study the effect of the size of the spectroscopic unit on the red shift of the CD spectrum. In this process only the number of BChls in the spectroscopic unit are varied; their orientations and positions are as in the crystal structure. We are particularly interested in the minimal size of this unit to understand the localization effect of the exciton on a particular part of the ring for instance due to energy disorder.

The red shift of the zero crossing in the CD spectrum was noted previously by Sauer et al.,¹⁸ who pointed out that it could be explained by including the entire BChl ring in the model. However, the relative amplitudes of the positive and the negative CD bands were not calculated very successfully. Therefore, we are varying protein–pigment interactions as well as the dielectric properties of the medium to gain insight in the general behavior of the desired quantities. The key parameter in the calculations will turn out to be a static energy mismatch between the α -bound BChls and the β -bound ones.

We concentrate mainly on the B850 band, but some effects due to the interaction with the B800 ring are also discussed. The subsequent section is devoted to the complete LH2 system.

The experimentally observed red shift in the B800–850 complex, *i.e.*, LH2, is much larger than the shift seen in case of the B800–820 complex (*cf.* Table 1). Our calculations show that the size of the red shift can be understood in terms of the geometry of the BChls. This is the subject of section 5.

The problem of defining and using the concept of exciton length, and its relation to spectroscopy, is illustrated in section 6.¹⁹ In the last section we summarize the conclusions.

2. The Model

In the appendices of ref 4 we gave an extensive description of the calculation of absorption and CD spectra for coupled N -level systems. For the details and derivation of the expressions we refer to that paper. Here we can use a slightly simplified notation, since we only consider two-level systems and neglect the small (higher order) effects of the inclusion of doubly excited states. For a complex of n monomers we can denote the singly excited states as $|i\rangle$ where the number i denotes the excited chromophore.

The excitonic interaction energy between molecules i and j in the point dipole approximation is equal to

$$V_{ij} = \frac{1}{4\pi\epsilon R_{ij}^3} \vec{\mu}_i \cdot \left(1 - 3 \frac{\vec{R}_{ij} \vec{R}_{ij}}{R_{ij}^2} \right) \cdot \vec{\mu}_j \quad (1)$$

where $\vec{\mu}_i$ denotes the transition dipole moment of monomer i from the ground state to the excited Q_y state. The direction of this dipole moment depends of course on the orientation of the monomer. The vector \vec{R}_{ij} connects the centers of the monomers.

Knowledge of the coordinates and orientation of the BChls in the crystal structure of the LH2 antenna system *Rps. acidophila*,³ and assuming the structure to be rigid, allows us to calculate all the interaction terms. This presents us with an $n \times n$ matrix, the diagonal elements of which are the excitation energies. This $n \times n$ matrix can be considered the Hamiltonian of the system under consideration.

The use of the point dipole approximation for the interaction can be questioned. It implies that the distance between the charges in a monomer is much smaller than the distance between the monomers. This condition is likely to be violated for nearest-neighbor BChl molecules in the LH2 ring. The point dipole interaction may overestimate the interaction between neighboring molecules by about 25%, and the interaction between next nearest neighbors by about 8%.¹⁸

If such a detailed calculation is in fact warranted, it is questionable in view of the following. Charge distributions were calculated for isolated BChls molecules,²⁰ and these were used in the calculations of Sauer,¹⁸ and more recently in ref 21 in a more sophisticated calculation where charges are not only put on the nuclei but also on the bonds.

The results of Stark spectroscopy experiments on antenna systems suggest that the LH2 ring is highly polarizable.^{22,23} In addition, the surrounding amino acid groups have different polarizabilities. It is well established that molecular charge distributions in a polarizable surrounding can differ dramatically from the charge distributions in isolation.²⁴ Thus, without detailed knowledge of the charge densities of BChl within the protein structure, charge-distributed models are not necessarily more accurate than the point dipole approximation. On the other hand, it must be mentioned that certain results, *cf.* section 3, are rather sensitive to changes in the (next) nearest neighbor interaction.

The interaction energy also depends on the dielectric properties of the surroundings of the interacting molecules. In the proteins the high-frequency dielectric screening factor is thought to be ≈ 1.4 , but this must be considered an average. The dielectric permittivity is a macroscopic quantity, and its local value can be different; we could therefore replace the quantity ϵ in eq 1 by ϵ_{ij} . In this paper we present some results where we use a different ϵ within $\alpha\beta$ unit of the LH2 complex. It should be noted that variations in ϵ add to the uncertainty in the distributed charges mentioned above and that changes of 25% in ϵ , and therefore changes in the interaction energy by that amount, are not likely.²⁵

In this paper we vary the number n of monomers from 2 to the total ring (18) in the B850 band to investigate the minimum of monomers needed to explain some crucial features in the absorption and CD spectra. All interactions within the unit are considered, not just nearest-neighbor interaction. The interactions between the molecules in different units are neglected. We still have different choices, however. This can be illustrated using the dimer as a unit. The LH2 system can be thought of as constructed of $\alpha\beta$ units, which means that the interaction of BChls within such a unit is different from the interaction

between BChls belonging to different units. This is borne out by explicit calculation of the interaction V_{ij} for those cases: both the distance and the relative orientation are different. Another logical possibility, taking into account the interaction between the B800 and the B850 band is a trimer consisting of the $\alpha\beta$ unit and the nearby B800 BChl, in which case n of course can be as large as 27.

The eigenvalues and the eigenfunctions of the spectroscopic unit are calculated using numerical diagonalization routines.²⁶ For two-level monomers the number of one-exciton levels is equal to the number of molecules in the unit.

Denoting the new eigenstates as $|\psi_k\rangle$, with

$$|\psi_k\rangle = \sum_{i=1}^n C_i^k |i\rangle \quad (2)$$

and the (unmodified) ground state as $|0\rangle$, the absorption spectrum is proportional to

$$I(E_k) = \left| \sum_{i=1}^n \langle 0 | \hat{\mu}_i | \psi_k \rangle \right|^2 \quad (3)$$

$$I(E) = \sum_{k=1}^n I(E_k) P(E_k) \quad (4)$$

where in the last equation we broadened each stick at $E = E_k$ by a Gaussian $P(E_k)$, to make the spectrum resemble a true absorption spectrum.

The rotational strength is equal to

$$R(E_k) = \frac{\pi}{2\lambda_k} \sum_{i,j=1}^n \vec{R}_{ij} \cdot \langle 0 | \hat{\mu}_i \times \hat{\mu}_j | \psi_k \rangle, \quad \text{with } \sum_k R(E_k) = 0 \quad (5)$$

$$R(E) = \sum_{k=1}^n R(E_k) P(E_k) \quad (6)$$

where λ_k is the wavelength of the transition to level k .

As stated earlier, the absorption spectrum by itself is not a very discriminating experimental quantity, but it can be used, for a given set of parameters, to calibrate the width of the Gaussians used in the above expressions and peak positions. With the values obtained, we then simulate the CD spectrum. Using this procedure, it turns out that it is rather hard to obtain a red shift of the zero crossing of the CD spectrum with respect to the absorption maximum, and for that reason we investigated which parameter set and/or size of the above-defined spectroscopic unit is needed to actually generate that result.

A more sophisticated model to account for the line shape is the so-called diagonal or E -disorder model.^{17,27} Use of this model changes the values of the parameters, but it appears to leave the main conclusions with respect to the size of the spectroscopic unit in tact.

One might further consider variations in distance and orientation of the monomers as a source of nondiagonal disorder. Preliminary calculations on the B820 dimer show, however, that the line shapes resulting from this type of disorder do not even remotely resemble experimental line shapes (see also Fidler *et al.*²⁸).

The exciton length can be defined as

$$L(k) = \sum_{i=1}^n |C_i^k|^4 \quad (7)$$

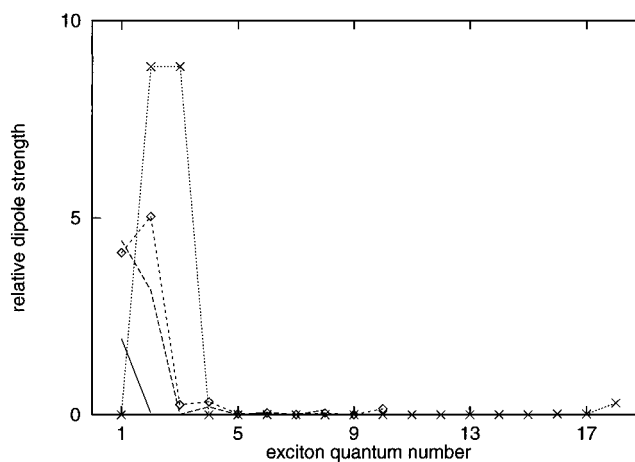


Figure 1. Dipole strength per excitonic state as a function of the spectroscopic unit size. The dipole strength per state is scaled relative to the dipole strength of the monomeric Q_y transition (40 D^2). The monomer excitation energy is here the same for the α - and the β -bound BChl and chosen to be 832 nm. The dielectric constant is $\epsilon = 1.2$. Shown are unit sizes $n = 1$ (dimer) (—), $n = 4$ (---), $n = 5$ (⋄), and $n = 9$ (×).

This makes the exciton length dependent on the exciton level of interest. The participation number $\% \mathcal{P}(k)$, *i.e.*, the number of molecules over which the excited state is delocalized, is then defined as the inverse of the exciton length $\% \mathcal{P}(k) = L^{-1}(k)$.

3. Dipole and Rotational Strength and the Nature of Spectra

Our first concern is to find the conditions for which the zero crossing of the CD spectrum is shifted to the red of the absorption maximum. To that end we calculate the dipole strength and the rotational strength of a collection of monomers for varying size of the spectroscopic unit, or the number n .

We will use the dimer as a building block. This seems natural in view of the fact that LH2 is built out of $\alpha\beta$ pairs. Initially, we only study the B850 band. The rationale for this is that the interaction between the B800 band and the B850 band mainly modifies the high-energy part of the B850 band. It is the low-energy part which shows the interesting red-shift behavior, and although the precise value of the parameters used may vary slightly upon turning on the interaction with the B800 band, the general behavior remains the same as was shown by Sauer *et al.*¹⁸

In this section we begin with showing the dipole and the rotational strength per state, because these distributions reveal the origin of the red shift. The actual size of the red shift depends on the site energies, the line width, and the interaction energies. These effects are discussed in the various subsections.

Dipole Strength per State in the B850 Band. We start our calculations with a degenerate system. That is, all monomers have the same excitation energy, of about 832 nm. With a dielectric constant $\epsilon \approx 1.2$ this yields a lowest excitonic state around 870 nm and the highest around 800 nm.

The magnitude of the dipole strength per state in the exciton band depends on the size of the spectroscopic unit. We denote spectroscopic units as $(\alpha\beta)_n$, where n now denotes the number of dimers taken into account. Thus $n = 1$ corresponds to the dimer itself, and $n = 9$ to the complete ring system.

The relationship between dipole strength and unit size is shown in Figure 1.

The dipole strength of the lowest excited state energy level initially grows when the size of the spectroscopic unit is increased. It reaches a maximum when $n = 4$, which is of

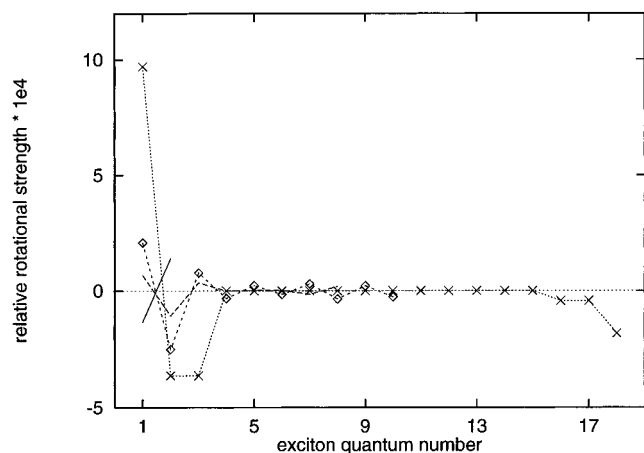


Figure 2. Rotational strength per excitonic state as function of spectroscopic unit size. Parameters and scaling are the same as in Figure 1. Shown are unit sizes $n = 1$ (dimer) (—), $n = 4$ (---), $n = 5$ (⋄), and $n = 9$ (×).

course just smaller than half the ring. When n is increased further, the dipole strength in the lowest state starts to decrease and shifts to the lowest exciton state but one. The reason is that the Q_y dipoles, which are almost in the plane of the ring, start to cancel each other. This drop in dipole strength continues with increasing n and reaches its minimum upon completion of the total ring.

For the complete ring the lowest exciton state has very little dipole strength left, less than 1% of the total, and the two next lowest levels have become degenerate, a reflection of the 9-fold symmetry.

Rotational Strength per State in the B850 Band. The precise distribution of the rotational strength over the excitonic states is very sensitive to the angles between the dipoles, the angles between the dipoles and the plane of the ring, the magnitude of the interaction energies and the size of the spectroscopic unit. There are a few general properties, however, which we comment upon here.

Figure 2 shows the distribution of the rotational strength over the exciton states for increasing spectroscopic unit size $(\alpha\beta)_n$.

In contrast to the dipole strength, which for this geometry is concentrated in the lower excitonic states, the rotational strength is distributed over both the highest and lowest excitonic states, with a significant contribution to the intermediate states. Upon closure of the ring only the lowest and the three highest exciton levels have appreciable rotational strength.

We draw attention to the fact that the rotational strength for all transitions is rather small, *i.e.*, does not increase very much compared to the dimer, even in the lowest exciton level. In addition, the behavior of the rotational strength appears to be rather erratic. For instance, the second lowest level first starts decreasing, then changes sign, subsequently increases in magnitude, starts decreasing again, and finally almost vanishes upon closure of the ring, where it becomes degenerate with the next level. It is easier to understand this behavior if the monomer degeneracy is lifted, as we will show in the next subsection.

3.1. Site Energy Dependence of the Rotational Strength.

The LH2 structure consists of 18 plus 9 chemically identical BChls in a protein environment. The BChl molecule can form hydrogen bonds with the protein²⁹ and the LH2 complex is polarizable.^{22,23} Obviously there is an excitation energy difference between BChls from the B800 and the B850 band,¹⁸ but also in the B850 band the monomers are not necessarily equivalent: the structure of LH2 and the protein sequence of strain 10500 suggest possible variations the hydrogen bonding

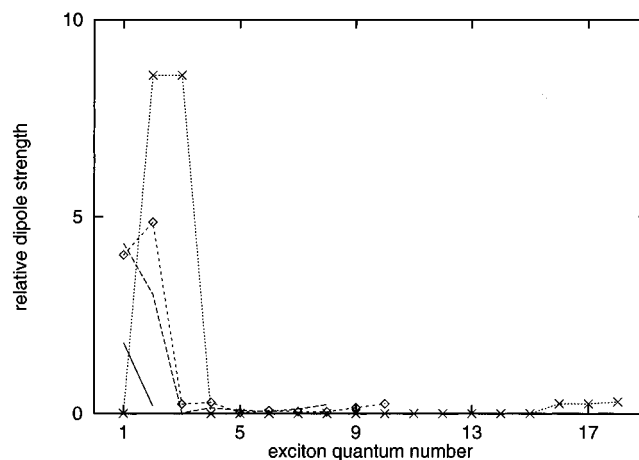


Figure 3. Dipole strength as function of unit size for a nondegenerate system. The dipole strength per state is scaled relative to the dipole strength of the monomeric Q_y transition (40 D^2). The α -bound BChl has an excitation energy of 822 nm, and β -bound BChl 843 nm. The dielectric constant is $\epsilon = 1.2$. Shown are unit sizes $n = 1$ (dimer) (—), $n = 4$ (---), $n = 5$ (⋄), and $n = 9$ (×).

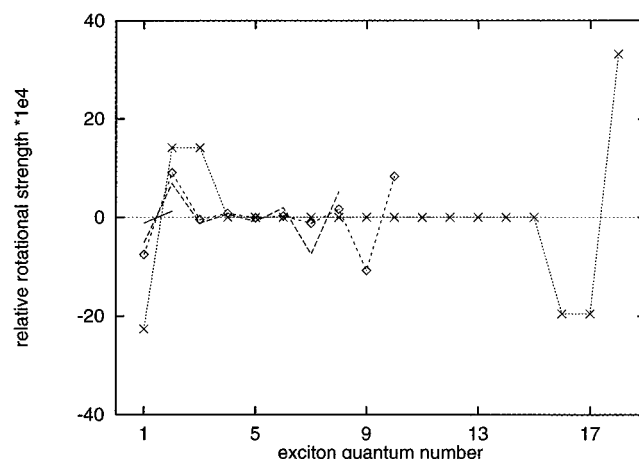


Figure 4. Rotational strength as function of unit size for a nondegenerate system. Parameters and scaling as in Figure 3. Shown are unit sizes $n = 1$ (dimer) (—), $n = 4$ (---), $n = 5$ (⋄), and $n = 9$ (×).

of the α - and β -bound BChl.³⁰ We therefore lifted the degeneracy of the BChl molecules by systematically varying the excitation energies of α - and β -bound BChls.

The energy difference was chosen to be about 20 nm; the β -bound molecules, which are slightly at the outer side of the ring, are assumed to have the smaller excitation energy; *i.e.*, the excited state energy level is more stabilized than that of the α bound BChls. We kept that mean energy at 832 nm, as in the previous subsection. The energy difference of 480 cm^{-1} corresponds with a spectral shift equal to 20 nm, similar to that between monomeric BChla, B777, and the dimer B820, and can thus easily be accounted for by a change in environment. Moreover, the experiments by Fowler et al.^{5,31} show a 15 nm shift of the absorption maximum due to the removal of a H bond in the LH2 complex of *Rb. sphaeroides*. The magnitude of the energy mismatch is somewhat arbitrarily chosen; its exact value should be revealed from mutant studies.

The dipole strength (Figure 3) is only marginally different from the previously discussed case. Its general behavior is the same, including the drop in dipole strength in the lowest level going from $n = 4$ to $n = 5$. The rotational strength per state, however, shows large differences (Figure 4). First of all, we note that there is a significant increase in rotational strength for larger unit size compared to the dimer. In addition, the

erratic behavior noted in the previous subsection has vanished: the lowest exciton level has a steadily larger negative rotational strength, and the next two levels show an increase. Apart from the magnitudes, for the closed ring the situation is approximately the same: only the lowest exciton levels (around 850 nm) and the highest (around 800 nm) show appreciable rotational strength. In addition, the rotational strength has become nearly conservative in both the 800 and the 850 nm bands: the ratio of the lowest but one levels with respect to the lowest level is 1.13:–1 for the B850 band. Note that the levels 2 and 3 have become degenerate, so their rotational strengths have to be added to get the peak ratio of the spectrum.

The reverse situation, giving the α -bound BChl a higher excitation energy, yields the same picture, except for the signs of the rotational strength, which are now the opposite. Comparison with the experimental spectrum shows that, within the context of this model, it must be the β -bound bacteriochlorophylls which are relatively red-shifted.

3.2. Permittivity Dependence of the Rotational Strength.

The dielectric constant modifies the exciton interaction between the monomers. It is, however, a macroscopic quantity, and due to different amino acid residues in the direct environment of a BChl the effective ϵ between BChl pairs can deviate from this average value.

If the dielectric constant within an $\alpha\beta$ unit is smaller, the interaction strength is larger, and consequently one would expect a more dimeric nature of the system. We used a value of $\epsilon = 1.0$ between the monomers of a unit, and 2.0 between all other monomers. This difference is rather extreme, but very illustrative.

The dipole strength per state is rather insensitive to the changes in the values of ϵ . The rotational strength per state, however, is more sensitive to a change in the electric permittivities. For the complete ring in the degenerate case, considerably more rotational strength is found in the combined exciton levels 2 and 3 for different values for ϵ in respect to the situation in which the inter- and intradimer ϵ have equal values. The ratio of the combined exciton levels 2 and 3 to the lowest level changes from about –1:25.0 to –2.9:1. In addition, the magnitudes increase slightly.

The changes in the nondegenerate case are minor: the ratio changes to about 0.9:–1, compared to 1.13:–1 in the previous case; the absolute magnitudes are still in the same range.

However, we noted in the Introduction that the point dipole approximation probably overestimates the interaction between the nearest neighbors by about 25%.¹⁸ To investigate the effect of a smaller interaction between nearest neighbors, we used a value of 3.0 for ϵ within an $\alpha\beta$ unit. Again the changes are most pronounced for the rotational strengths in the degenerate case; dipole strengths are almost unaffected by these changes. As in the previous case it is mainly the combined exciton levels 2 and 3 which are affected most and gain considerable rotational strength in the degenerate system.

For the nondegenerate system, the changes are minor; for instance, the ratio of the sum of the rotational strengths of the levels 2 and 3 to the strength of level 1 increases to about 1.3:–1, from 1.13:–1, which is unlikely to be observable in a measured spectrum.

The red shift of zero crossing also depends on the energy difference between the lowest exciton states. This energy difference depends on the magnitude of ϵ . The change of ϵ between the monomers of a unit from 1.0 to 3.0 leads to a reduction of this energy difference equal to 13 cm^{-1} , corresponding to 1 nm on the wavelength scale.

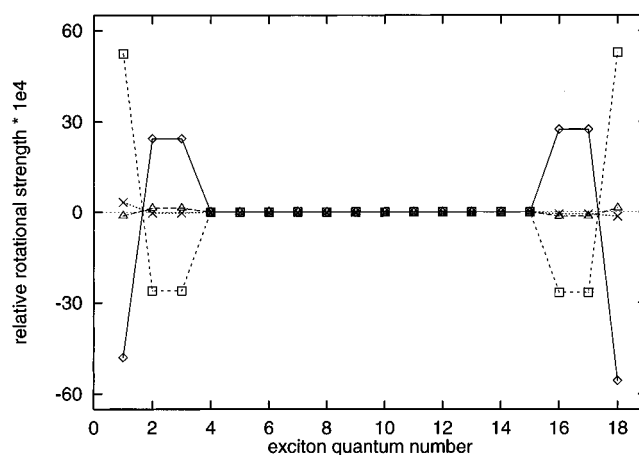


Figure 5. Contributions to the rotational strength from (\square) α -bound BChls, (\diamond) β -bound BChls, (\triangle) cross terms. The total rotational strength is also given (\times). Parameters as in Figure 1.

On the basis of these calculations and those of the previous subsection, we can draw a few conclusions. The sensitivity of the distributions of rotational strengths for the degenerate system to changes in either energy levels or interaction energies, suggests that the small rotational strengths are in fact the result of two larger contributions which almost cancel. This is indeed the case as Figure 5 shows. Here we calculated the rotational strengths for the complete ring.

We separated the contributions due to α -bound chromophores, β -bound chromophores, and the cross terms. It is clear from this figure that both the α -bound and β -bound BChls have large contributions of opposing sign, whereas the contribution from unlike pairs is generally small. If we now introduce a nondegeneracy, for instance make the excited state energy of the α BChls lower, then the lowest exciton state will have a relatively large contribution of these chromophores and hence a large positive rotational strength. The opposite is true if the β 's have a lower excited state energy.

The implication is that if all chromophores are degenerate, precise charge distributions and the local environment are needed to get a reasonable approximation to the CD spectrum: small changes have large effects. On the other hand, if the system is nondegenerate to the extent that we described above, these changes are of relatively little importance, errors due to the point dipole approximation have only minor effects on the results, and we do not have to worry too much about local values of the dielectric constant either.

3.3. Origin and Magnitude of the Red Shift. We now turn to the absorption and CD spectra resulting from the above calculations. The procedure is simple: sticks of magnitude dipole strength at the position of the corresponding eigenvalues multiplied by Gaussians give the absorption spectrum, and a similar procedure with the rotational strengths yields the CD spectrum.

To see when a red shift occurs, it is easiest to start with the nondegenerate system (Figures 3 and 4). For the dimer we note that the most of the dipole strength is in the lowest level, and the rotational strengths are the same for the two levels, so that the CD spectrum is symmetric, and the zero crossing is to the blue of the absorption maximum. For the complete ring the situation is different: the dipole strength has shifted to the next higher level,¹⁸ whereas the rotational strength is still evenly divided over the lowest two levels (at least if we look at the 850 nm region, note that there is also some strength in the 800 nm region). This means that the zero crossing is exactly in the middle of the two lowest eigenvalues as in the case of the dimer,

but the maximum of the absorption spectrum has shifted to the blue, resulting in a red shift of the zero crossing with respect to the absorption maximum. It is therefore clear that such a red shift can only be observed once the lowest exciton level starts losing dipole strength, which is the case for a unit size larger than four dimers.

From this we infer that a red shift can be observed in the following cases:

1. The lowest exciton level loses dipole strength to a higher level. This results in a blue shift of the absorption maximum, which is the effective cause of the relative red shift of the zero CD crossing. None of the calculations above shows such a shift unless more than half the complete ring is used as a spectroscopic unit. If we take a larger spectroscopic unit, the effect is larger and reaches its maximum for the complete ring.

2. The rotational strengths of the two lowest states have opposite signs. Obviously, if this is not the case, there is no zero crossing in the CD spectrum at all. There are few cases, and only if the interaction with the B800 BChls is turned on where this occurs, but it is related to the following.

3. The peak ratio of the rotational strength at the lowest states is not too large. A large difference between the two rotational strength also leads to a vanishing zero crossing, due to line-width effects: the large contribution on one side "eats" the much smaller contribution at the other side. This does occur for the B850 system, for instance in the degenerate model; cf. Figure 2. It implies that the magnitude of the red shift can be dependent on the line width, at least for a nonconservative CD spectrum and/or unequal line widths of the transitions. For a conservative CD and equal line widths the shift is independent of the line width: it is always exactly in the middle. We note that the CD spectrum becomes more conservative upon closing of the ring.

From this, and the other numerical results presented in this section, we can draw the following conclusions. It is relatively easy to explain a red shift of the zero crossing for the nondegenerate system. For the complete ring a rather strong conservative CD is found, and although the red shift can be observed if just more than half the ring is taken into account, the effect becomes large for a larger system. There is no *a priori* reason for not taking the complete ring, once it is noted that at least half is necessary to explain the spectroscopy. Changes in dielectric constant, and probably modifications of the dipole-dipole interaction, will not modify this conclusion.

The situation is more complicated for the degenerate system in that it is possible to generate a red-shifted CD zero crossing, but the CD signals are weaker, and much more sensitive to small changes. Fortunately, the magnitude of the experimental CD signal points to the former, nondegenerate, model.

4. Absorption and CD Spectra of LH2

In this section we calculate the absorption and CD spectrum of the complete LH2 complex. We only present calculations where the complete ring system is taken into account. In the previous section we commented on the B850 spectrum resulting from the ring of 18 BChls which comprise the B850 ring, and although we did not discuss it, it is clear from Figures 2 and 4 that this system also gives rotational strength (and a little dipole strength) in the 800 nm region. There is some experimental evidence for rotational strength in the higher excitonic states, around 800 nm, of the separated B850 ring, albeit in a different species (*Rhodobacter sphaeroides*).^{32,33}

The other nine BChls absorb mainly in the 800 nm region, and due to coupling can also exhibit CD at that position. The CD spectrum in the 800 nm range is thus a combination of the

TABLE 1: Experimental Values of Parameters Used in This Paper⁷

system	abs max (nm)	fwhm (cm ⁻¹)	CD peak ratio	CD zero cross (nm)
B800-850	868	243	1.3:-1	875
B800-820	822	302	0.85:-1	824

CD resulting from the B850 and the B800 rings. The interaction between the B800 BChls and the eigenstates in the B850 band is small, since it is nonresonant in addition to weakly coupled. The resonant interaction in the 800 nm region, between the B800 BChls and the upper exciton components of the B850 ring, does lead to changes in the combined system, which are minor, since the coupling is weak: small transition dipoles for the higher exciton states combined with a rather large distance between B800 and B850 chromophores. Therefore, the changes in the 800 nm CD spectrum are mainly due to the superimposing of the spectra of the B800 chromophores and the B850 BChls. Separate calculation of the CD spectrum of the B800 chromophores shows that indeed the line splitting is small, and of the same order of magnitude as the splitting of the two highest exciton levels of the B850 band, and that the CD spectrum has the opposite sign to the CD spectrum of the B850 chromophores in the 800 nm range. This may explain the rather weak CD signal observed in the LH2 complex in that area.

The experimental CD spectrum consists two pairs of lines, one pair centered around 802 nm, where the zero crossing is not shifted with respect to the absorption maximum, and one pair around 875 nm, where zero crossing is shifted 7 nm to the red side of the absorption maximum (Table 1). The signs of the CD lines are with increasing wavelength: - + + -. The line width of the "850 peak", at 868 nm, in the absorption spectrum is almost twice that of the "800 peak". The line intensities of the pair in the CD spectrum in the 800 nm area have a ratio equal to -0.7:1, *i.e.*, the ratio of the bluest line versus the bluest but one lines, and its line-width ratio is 0.75:1. The lines around 875 nm have a peak ratio of 1.3:-1, *i.e.*, the ratio of the red-most line but one in respect to the reddest line, and the ratio of the line widths is also 1.5:1. The lines in the CD spectrum are narrower than the lines in the absorption spectrum, indicating contributions with opposite sign.

In order to mimic the experimentally obtained spectra we convoluted the sticks with Gaussians. This is rather unsatisfactory; a better model is the diagonal disorder model which can account for differences in line widths but for the purpose of this paper we stick to Gaussians with equal width.

Although we performed calculations similar to the ones presented in the previous section for varying unit size, but now including B800 chromophores, so that the basic building block is a trimer, we only present the calculated spectrum for the complete system. The reason is that general behavior is exactly the same: more than half the complete system has to be taken into account for a red shift of the CD spectrum to occur. This is not surprising, since it is the 850 nm band, which is only weakly coupled to the other ring, which exhibits this feature.

The shift of the zero crossing in the experimental CD spectrum is enhanced by line-width effects. Since the CD spectrum is slightly nonconservative, the finite line width produces an extra shift. Precise values of parameters are therefore dependent on the model used to generate lines of finite width.

In the previous section we showed that the excitation energies of the α - and β -bound BChls are likely to be different; when they are degenerate the CD spectrum does not show a zero crossing at all. The values we used in this section are 822 and 843 nm, which produces lines at 868 and 800 nm. We used a

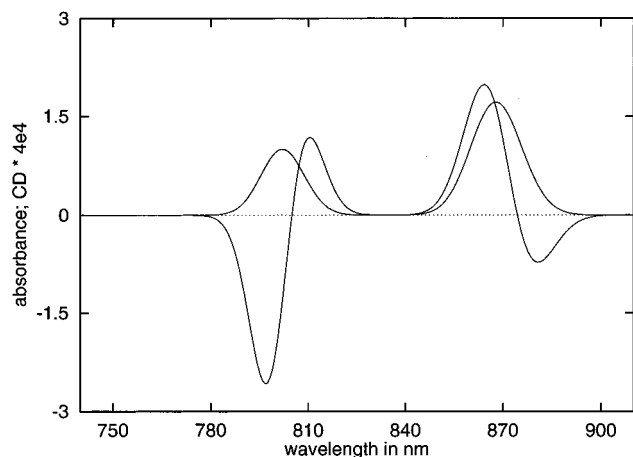


Figure 6. Calculated absorption and CD spectrum of the B850–800 complex. The CD spectrum is proportional to 4 times the rotational strength. Parameter values are as in Figures 3 and 4. The fwhm of the Gaussian peaks is equal to 243 cm^{-1} . The spectra are scaled relative to the OD of the B800-BChls equal to 1.0.

value of $\epsilon = 1.2$ and made no distinction between inter- and intra- ϵ mainly since for a nondegenerate B850 ring changes connected with different ϵ values are small. The value of ϵ and the degeneracy chosen are related: if ϵ becomes larger, the interaction becomes weaker, so a larger energy difference is needed to get the resulting lines at the right position. Since the B800 molecules are all in the same environment, we took them to be degenerate, with transition wavelengths of 800 nm.

The resulting spectra are presented in the next figure (Figure 6). The width is derived from the experimental absorption spectrum: a fwhm of 243 cm^{-1} for the 868 peak. The calculated red shift in this case is 7 nm, which reproduces the experimental value.

5. The B800–820 Complex

Depending on growth conditions, another LH2-like antenna complex can be produced in *Rps. acidophila*, which absorbs in the 800–820 nm area and is therefore called the B800–820 complex. The general features of the B800–820 antenna system are likely to be very similar to those of the B800–850 complex.³⁴ The absorption and CD spectra of both complexes show similarities, although the lines in the B800–820 are closer together, and the red shift of the CD zero crossing relative to the OD maximum around 822 nm is only 2 nm. There is experimental evidence that these differences can be attributed to a difference in hydrogen bonding of the chromophores to the α and β proteins.^{5,6,35} The breaking of hydrogen bonds can be the cause of a shift in excited state energies, and/or a change in orientation of the BChls. We show in this section that a change in excited state energies in addition to a rotation of the α -bound BChls can be sufficient to explain the spectral differences observed.

The rotational strength per state presented in section 3, using the crystal structure of the B850 ring, shows rotational strength at the high-energy part of the one-exciton manifold. This distribution is probably hidden under the lines originating from the B800 molecules in the LH2 spectra, and as a consequence their sum resulted in the B800–850 CD spectrum in the 800 nm range, as discussed in the previous section. If the B850 spectrum were just shifted to the 822 nm range, the CD lines resulting from this, and the CD lines from the B800 ring, would no longer coincide, and the shifted high-energy B850 lines should become visible, in contrast to observation.

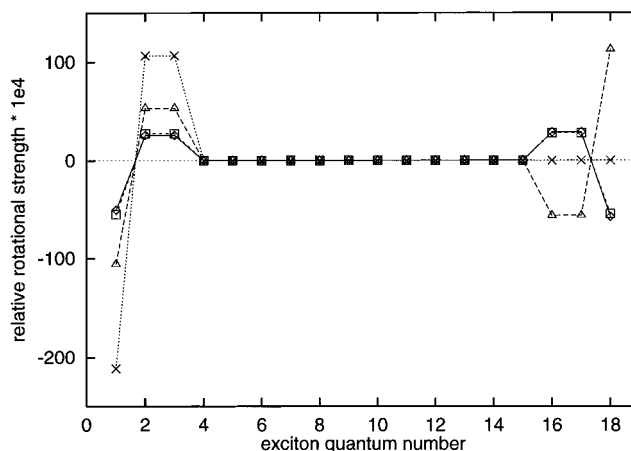


Figure 7. Contributions to the rotational strength from (\square) α -bound BChls, (\diamond) β -bound BChls, and (\triangle) cross terms for the B800–820 system. Parameter values used are described in the text. The total rotational strength is also given (\times). Scaling as in Figure 1.

We noted above that the rotational strength can be viewed as the result of different contributions and that the interplay is rather subtle in that large cancellation effects can occur. Although we did not perform an extensive search of the available phase space for the BChls, coupled to what is known of the protein structure, we did find an orientation for which the rotational strength disappears in the high-energy exciton levels. If the α -bound Q_y moment is rotated to point toward the other side of the ring, this is exactly what happens. The β -bound chromophores are unchanged. Admittedly, this is a rather large change; it means an overall rotation over about 15° for the α -bound chromophores, through the plane of the ring. We note that the interaction strength does not change very much in this new arrangement. Calculations indicate changes of about 10%.

In Figure 7 we show the rotational strength for this configuration, where we used degenerate BChls in the B820 ring. It is clear from this picture that in the lower exciton states contributions due to different pairs now have the same sign, so that the sensitivity to small changes in parameters in that area is now absent. Also the peak ratio is almost exactly 1:–1. On the other hand, the high-energy rotational strength is now the result of canceling contributions.

The spectra calculated with this orientation are reproduced in Figure 8. Another consequence of the change in angles is that there remains somewhat more dipole strength in the lowest exciton level, compared to the B850 ring. This of course contributes to the smaller red shift of the zero crossing of the B800–820 CD spectrum.

The main conclusion of this section is that the size of the red shift is correlated with the orientations of the BChls in the complex, as was illustrated using the modified crystal structure. It is not an attempt to propose a structure of the B800–820 complex.

6. Remarks about the Exciton Length

Antenna systems are used by bacteria to harvest sunlight and transport the excitation to the special pair in the reaction center. It is often assumed that the mechanism of this transport is such that an excitation is only localized on a small part of the antenna, for instance a dimer, and that subsequently a random walk takes place, with transition probabilities given for instance by the Förster formula, which eventually delivers the excitation at the special pair.^{8,36,37}

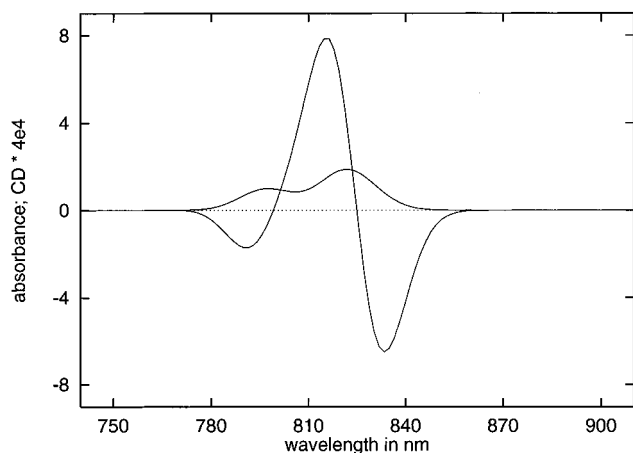


Figure 8. Calculated absorption and CD spectrum of the B820-800 complex. Parameter values are 795 nm for the monomer transition energies (degenerate); dielectric constant 1.4 and a fwhm of 302 cm^{-1} for the Gaussian peaks. Scaling as in Figure 6.

Although this is an attractive model to understand the energy transfer dynamics, we have presented evidence, based on the combination of absorption and CD spectra, that to understand the spectra of these complexes we must assume excitonic interactions involving more than half the ring of the antenna complex.

Another way of putting this could be the following: even though the average wave function could have an extension over only a few monomers, this does not mean that we could neglect the remainder of the system to calculate this wave function: calculating the wave function of these few monomers would certainly lead to a different result. Apparently, in the combination of absorption and circular dichroism the nonlocal properties of the system are probed, which makes it particularly sensitive to the global structure, and consequently average wave functions are not the correct objects to form the basis of calculations of spectra. All observables involve the square of the wave function, and it is well-known that the average of a square can deviate dramatically from the square of an average.

There appear to be two different definitions of exciton localization in the literature. The first is due to Anderson,^{38,39} who investigated under which circumstances an excitation remains localized, even if $t \rightarrow \infty$. In his model disorder plays a large role, in that for a system with interaction decreasing more rapidly than dipole-dipole coupling, and a certain amount of disorder, excitations can indeed be localized. Obviously, this is not what happens, and the excitation has to be transferred. Diagonal disorder is the key word here, and it could be argued that our method does not take this into account properly. However, it is hard to see how, if disorder would localize the excitation in the Anderson sense, the CD spectrum can still exhibit the crucial feature of a red-shifted zero crossing.

Another common definition of exciton length is given in eq 7 of ref 27. Taking a higher power of the coefficients of the wave function increases the differences in magnitude among sites, but it does not necessarily lead to a localized Anderson state. Consider a state with two nodes and two maxima: Taking the fourth power of the coefficients increases the maxima, and makes the excitation appear more local, thus for instance giving a participation number of 2, but the monomers (or dimers) participating in the "localized state" would be at opposite sides of the ring. A case in point is the participation number (inverse of the exciton length) for the lowest but one excitonic state of the complete ring. A simple calculation gives 12, but obviously we need the complete ring to find this state, and we could not

possibly say that 12 monomers are sufficient to reproduce the spectra that were calculated with the full number of monomers. In addition, this measure gives exciton lengths which depend on the total chain length, as Fidler^{27,28} shows. The exciton length becomes only independent of the chain length for very long chains (much longer than 18), and then only at the band edges. In addition, we would like to point out that the LH2 ring cannot be considered a linear chain by almost any standard.

7. Conclusions and Remarks

The main conclusion of this paper is that the key parameter in calculating the red shift of the zero crossing in the CD spectrum is the energy mismatch between α - and the β -bound BChls using the pigment arrangement as was revealed in the crystal structure. The transitions of the β -bound BChls are red-shifted in respect to transition in the α -bound BChls. At this point the shapes of the resulting spectra are rather arbitrary; to calculate these shapes, line-broadening mechanisms should be incorporated. The local dielectric properties affect the Coulombic interactions but have little effect on the size of the red shift in the nondegenerate case. The point dipole approximation introduces errors, but these do not affect the main results. Another conclusion of this paper is that, in order to explain the combination of absorption and CD spectra, it is imperative to take more than one-half of LH2 ring into account which means that the minimal size of the spectroscopic unit is five dimers. Smaller spectroscopic units, which may or may not be identifiable with localized excitons, are simply not sufficient to calculate the absorption and the CD spectra simultaneously. Furthermore, our calculations show a correlation between the size of the red shift of the zero crossing and the orientations of the BChls.

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