Steady-state spectroscopy of zinc-bacteriopheophytin containing LH1—an in vitro and in silico study

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Abstract

By reversible dissociation of the light-harvesting complex 1 (LH1) of Rhodospirillum rubrum it is possible to (partly) exchange the bacteriochlorophyll (BChl) a by zinc-bacteriopheophytin (Zn-BPheo). After reassociation of the protein a complex is formed which can have different percentages of Zn-BPheo bound to the polypeptides [Biochemistry 39 (2000) 1091].

Low-temperature absorption spectra show a shift of the absorption maximum from 886 to 863 nm, when the native LH1 complex is compared to a modified complex containing 90% Zn-BPheo, whereas the positions of the absorption maxima of BChl a and Zn-BPheo differ by only 6 nm, when the pigments are bound to the isolated polypeptides. Using an exciton model with static disorder of site energies based on the ring-like structure of LH1 we can describe this shift by assuming a difference in the excitonic coupling originating solely from the different dipole strength of the exchanged Zn-BPheo compared with the original BChl a. We estimate that in LH1 the nearest-neighbour interaction energy of two BChl a molecules is around 400 cm−1 and the diagonal disorder is around 600 cm−1.

Furthermore, we determined if the energy transfer in pigment-modified complexes is similar to native LH1. This can be observed by selectively exciting electronic states depending on their energy and measuring the polarized emission spectra at low temperature. The native complex was compared to a complex containing 70% Zn-BPheo. The fluorescence anisotropy and the shift of the emission maximum in native LH1 and 70% Zn-BPheo-LH1 depending on the excitation wavelength can generally be described within the same disordered exciton model, extended in a simple way

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with line shapes. The model proved to be simple and robust when applied to these engineered light-harvesting complexes. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Photosynthesis; Pigment–protein complex; Light harvesting; Excitons; Modelling

Abbreviations: BChl, bacteriochlorophyll; Zn-BPheo, zinc-bacteriopheophytin; fwhm, full width at half-maximum; IDF, inhomogeneous distribution function; LH, light harvesting; PW, phonon wing; RC, reaction centre; Rps., Rhodopseudomonas; Rs., Rhodospirillum; ZPL, zero-phonon line

1. Introduction

In photosynthesis the primary steps are “light harvesting” and charge separation. For light harvesting all photosynthetic organisms have so-called antenna complexes. The function of these pigment–protein complexes is to absorb light and to transport the excited state energy finally to a special pigment–protein complex—the photosynthetic reaction centre (RC)—where charge separation occurs [1,2].

In photosynthetic purple bacteria the core light-harvesting complex (LH1) directly surrounds the RC. In most species also a peripheral light-harvesting complex (LH2) occurs. The basic building block of these light-harvesting complexes is a dimeric subunit of two small transmembrane polypeptides α and β, which have mainly α-helical structure. To these polypeptides the pigments—bacteriochlorophyll (BChl) and/or carotenoid—are non-covalently bound.

High-resolution structures from X-ray diffraction are available for the LH2 from the purple bacteria Rhodopseudomonas (Rps.) acidiphila [3,4] and Rhodospirillum (Rs.) molischianum [5]. The LH2 complex of Rps. acidiphila consists of 9 α and 9 β transmembrane helices and 27 BChl a molecules arranged in a ring-like structure with C₃ symmetry. The α and β polypeptides form an inner and outer cylinder of 36 and 68 Å diameter, respectively. Each of these polypeptides possesses a histidine residue in its transmembrane α-helical part, which ligates a BChl a molecule [6]. These 18 pigments form a ring structure that is responsible for the strong Qₐ absorption band around 850 nm (B850). The remaining 9 BChl a molecules are bound to the α polypeptides near the cytoplasmic surface, and are responsible for a strong Qₐ absorption band around 800 nm (B800); hence the name B800–850 complex for the LH2 complex. The LH2 of Rs. molischianum [5] is very similar to that of Rps. acidiphila but has C₈ symmetry; the main difference being that the B800 chromophores are rotated by 90° and tilted, causing a reversal of the 800 nm circular dichroism spectrum [7].

For the LH1 complex of Rs. rubrum a projection map of the structure has been determined by electron microscopy to 8.5 Å resolution [8]. The α and β polypeptides of this complex are arranged in a ring-like structure similar to LH2. However, the LH1 ring has C₁₆ symmetry, the 16 α-polypeptides forming an inner and the 16 β-polypeptides an outer cylinder of 68 and 116 Å diameter, respectively—large enough to contain the bacterial RC. In between these cylinders 32 BChl a molecules are located. In LH1 one BChl a molecule is bound to every polypeptide via ligation to a histidine residue, situated in the transmembrane α-helical part of the polypeptide [6]. The Qₐ absorption band of BChl a in LH1 of Rs. rubrum is usually located at 885 nm at room temperature. However, for most of the works performed on these complexes a strain unable to synthesize carotenoid was used. The position of the Qₐ transition of BChl a in carotenoid-less LH1 from Rs. rubrum is 873 nm.

The spectroscopic properties of BChl in light-harvesting complexes are influenced by the protein environment the pigments are embedded in. A protein is a very complex medium/solvent, because it shows dynamics on time scales ranging from femtoseconds to teroseconds (see Ref. [9] and references therein). The interactions of the pigments with the protein can be time dependent. Fluctuations which occur on a time scale longer than the chromophore’s excited state lifetime are not seen in the optical experiment, i.e. if we consider an en-
Table 1
Comparison of values found in the literature for disorder and excitonic coupling in light-harvesting complexes of purple bacteria

<table>
<thead>
<tr>
<th>Reference</th>
<th>Technique</th>
<th>Species</th>
<th>Complex</th>
<th>Temperature</th>
<th>Disorder cm⁻¹</th>
<th>Excitonic coupling cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>[18]</td>
<td>Pump-probe</td>
<td><em>Rhodobacter</em></td>
<td>LH1</td>
<td>RT³</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>[19]</td>
<td>Pump-probe</td>
<td><em>Rhodobacter</em></td>
<td>LH2⁶</td>
<td>4 K</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>[20]</td>
<td>Pump-probe</td>
<td><em>Rhodobacter</em></td>
<td>LH1</td>
<td>RT</td>
<td>430</td>
<td></td>
</tr>
<tr>
<td>[21]</td>
<td>Pump-probe</td>
<td><em>Rhodobacter</em></td>
<td>LH1 core⁶</td>
<td>77 K</td>
<td>440</td>
<td></td>
</tr>
<tr>
<td>[22]</td>
<td>Three-pulse photon echo</td>
<td><em>Rhodobacter</em></td>
<td>LH1</td>
<td>RT</td>
<td>530</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LH2</td>
<td></td>
<td>390</td>
<td></td>
</tr>
<tr>
<td>[23]</td>
<td>Superradiance</td>
<td><em>Rhodobacter</em></td>
<td>LH1</td>
<td>Independent</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LH2</td>
<td></td>
<td>400–600</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>[24,25]</td>
<td>Single-molecule spectra</td>
<td><em>Rhodobacter</em></td>
<td>LH2</td>
<td>1.5 K</td>
<td>125, 250⁶</td>
<td>300, 254</td>
</tr>
<tr>
<td>[26]</td>
<td>Absorption</td>
<td><em>Rhodobacter</em></td>
<td>LH2</td>
<td>RT</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>[13]</td>
<td>Circular dichroism</td>
<td><em>Rhodobacter</em></td>
<td>LH2</td>
<td>77 K</td>
<td>500</td>
<td>300</td>
</tr>
<tr>
<td>[27]</td>
<td>Absorption, fluorescence</td>
<td><em>Rhodobacter</em></td>
<td>LH1 fragments</td>
<td>77 K</td>
<td>300–400</td>
<td>200–260</td>
</tr>
<tr>
<td>[28]</td>
<td>Triplet-minus-singlet</td>
<td><em>Rhodobacter</em></td>
<td>B820⁶</td>
<td>77 K</td>
<td>240</td>
<td></td>
</tr>
</tbody>
</table>

* Given as fwhm of a Gaussian IDF.
* Given as nearest-neighbour interaction energy.
3 *Rhodobacter*.
4 Room temperature.
5 In this table LH2 refers to the B850 band.
6 Consisting of LH1 and RC.
7 Additionally a intercomplex heterogeneity of 120 cm⁻¹ was assumed.
8 B820 is a small subunit form of LH1 with an absorption maximum around 820 nm exhibiting the spectroscopic properties of a dimer [28,29], although it frequently appears to behave as a tetramer from a biochemical point of view [30,31].

A ensemble of a large number of molecules, the electronic transition frequencies of the individual pigments will be randomly distributed around a mean value. This broadening is called inhomogeneous broadening and the distribution is commonly referred to as inhomogeneous distribution function (IDF). Because the IDF is related to very slow dynamics in the surrounding protein, also the term static disorder is used. Rapid fluctuations, however, are related to vibrations of the pigments and/or the surrounding protein. These are spectroscopically reflected in the homogeneous line shape.

Furthermore, due to the relatively small distances between the pigments in such rings the interaction between the pigments will play an important role in their spectroscopy. The availability of crystallographic information for LH2 has triggered a lot of effort to calculate the spectroscopy of these systems, mainly on the basis of exciton models [10–17].

Several experimental techniques have been applied to determine the exciton coupling between the pigments and the amount of disorder in light-harvesting antenna complexes of purple bacteria. Depending on the technique, the species and the complex different values have been found. This is demonstrated by examples in Table 1. Trying to summarize, the disorder (given as full width at half-maximum (fwhm) of a Gaussian IDF) in LH1 and LH2 is between 125 and 600 cm⁻¹ and the nearest-neighbour coupling between 200 and 400 cm⁻¹, respectively. Clearly, additional information is required from independent experiments.

Recently, it has been possible to (partly) exchange the BChl a by zinc-bacteriopheophytin (Zn-BPheo) in the LH1 complex of *Rhodobacter* by the use of the well-characterized LH1/B820²/B777³ reversible dissociation process [34]. It was

² B820 is a small subunit form of LH1 with an absorption maximum around 820 nm exhibiting the spectroscopic properties of a dimer [28,29], although it frequently appears to behave as a tetramer from a biochemical point of view [30,31].
³ B777 consists of isolated z-helical z and β polypeptides, still retaining their bound BChl a molecule [32,33] and it has an absorption maximum at 777 nm.
shown that a complex is formed which can have different percentages of Zn-BPheo bound to the polypeptides, while the structure of the complex is not changed [34]. The modified complexes have a binding site structure identical to that of native LH1. Experimentally, a blue shift of the room temperature absorption spectrum with increasing amount of Zn-BPheo has been observed.

This pigment exchange opens up the unique possibility to test the robustness of the exciton model for light-harvesting complexes: for the exciton calculations the exchange of a pigment ideally corresponds with a change in the magnitude of the transition dipole moment (see below). When this change is known, it should be possible to extend the model from native to engineered light-harvesting complexes. In this study we try to answer the question if the spectral properties of LH1 complexes with different percentages of BChl \( \alpha \) and Zn-BPheo can be quantitatively described in one model. From this modelling we attempt to obtain an independent estimate of the coupling versus the disorder.

In this respect our problem is related to the study by Westerhuis et al. [27]. There, the spectral properties of a series of detergent isolated fragments of the LH1 complex from \textit{Rhodobacter sphaeroides} were investigated. Using an exciton model including static disorder the oligomeric-state dependent experimental features of these complexes could be described.

2. Materials and methods

2.1. Biochemistry

The preparation of native LH1 of \textit{Rs. rubrum} and of derived LH1 complexes containing different amounts of Zn-BPheo is described in Ref. [34]. Briefly, dissociation of the LH1 protein into B820 subunits is achieved by treatment with the detergent \( \beta \)-octylglucopyranosid. Then BChl \( \alpha \), Zn-BPheo or a mixture of both is added to B820. This solution is heated to allow the exchange of pigments. Afterwards, free pigments are removed on an anion exchange column. Finally, the protein is reassociated in its native form by dissolution of the detergent and overnight cooling at 4 °C.

For the spectroscopic measurements the samples were diluted in a buffer containing 50 mM ammonium bicarbonate (pH = 8.0) and about 60% (v/v) glycerol to form transparent glasses at low temperatures.

2.2. Absorption measurements at 30 K

Absorption spectra were recorded on a Cary 5E double-beam spectrophotometer (Varian plc, Sydney). The measurements have been performed in 2 mm optical length plastic cuvettes placed in a cryostat (SMC-TBT, France) cooled by a stream of He gas. The optical density in the \( Q_t \) transition was about 0.3.

2.3. Absorption and fluorescence at 6 K

Absorption and fluorescence spectra were measured at 6 K in a liquid helium cryostat (Utreks) in 1 cm optical length plastic cuvettes. The absorption was measured on a home-built spectrophotometer. The fluorescence spectra were measured using a cooled CCD camera (Chromex ChromCam 1) via a 1/2-m spectrograph (Chromex 5001S). As excitation source a cw titanium–sapphire laser (Coherent 890) pumped by an argon ion laser (Coherent Innova 310) was used. The excitation power was kept below 400 \( \mu \)W/cm².

2.4. Simulations

The absorption spectra were calculated on the basis of an exciton model using a Monte Carlo simulation [10,23]. In each Monte Carlo iteration a Hamiltonian was generated with the diagonal elements representing the site energies of the pigments and the other matrix elements the interactions between the pigments. This Hamiltonian was numerically diagonalized [35] and the eigenvalues and eigenvectors were used to calculate the energies and dipole strengths of the exciton states. Typically 50 000 iterations were done.

The site energies are distributed (static disorder) according to the IDF, which is generally taken to be a Gaussian, because of its statistical origin. The
site energies were randomly taken from this Gaussian distribution. The interaction $V_{nm}$ between the pigments $n$ and $m$ was presumed to scale as given by the formulas for dipole–dipole coupling in the point-dipole approximation:

$$V_{nm} \propto \frac{\mu_n \mu_m \kappa_{nm}}{r_{nm}^3}. \quad (1)$$

$\mu_n$, $\mu_m$ are the sizes of the transition dipole moments of the pigments $n$ and $m$ and $r_{nm}$ is the distance between them. The orientation factor $\kappa_{nm}$ is:

$$\kappa_{nm} = \hat{\mu}_n \cdot \hat{\mu}_m - 3(\hat{\mu}_n \cdot \hat{r}_{nm})(\hat{\mu}_m \cdot \hat{r}_{nm}) \quad (2)$$

with $\hat{\mu}_n$, $\hat{\mu}_m$ and $\hat{r}_{nm}$ being the unit vectors in the direction of the transition dipoles of the pigments $n$ and $m$ and the line joining the centres of the pigments, respectively.

The calculations presented below do not depend on the absolute values of the transition dipole moments and dielectric screening factors. The only scaling factor we use is implicitly contained in the value we choose for the nearest-neighbour interaction between two BChl $a$ molecules (see below). We do presume, though, that the dipole–dipole approximation gives a valid estimate for the ratio between the nearest neighbour and non-nearest-neighbour interactions. The largest non-nearest-neighbour interaction is about one order of magnitude smaller than the nearest-neighbour interaction. Therefore most of the calculations presented here are not very sensitive to non-nearest-neighbour interactions.

In our simulation we take the interactions between the $Q_0$ transitions of all the pigments into account, neglecting their mixing with $Q_1$, $B_2$, $B_3$ transitions and charge transfer states. For the calculation of the excitonic coupling positions and directions of the transition dipoles of the pigments are needed. However, there is no crystal structure available for the LH1 antenna of $Rs.\ rubrum$. From the electron micrographs of Karrasch et al. [8] it is known, that this LH1 is a ring of dimers with 16-fold rotational symmetry. Furthermore, there are strong indications that the dimer in LH1 is very similar to the dimer in the B850 complex of LH2. Therefore, using the crystal structure of the B850 dimer of the 9-fold symmetric ring of $Rps.\ acidiphila$, we have constructed a ring with 16-fold symmetry as model for LH1 of $Rs.\ rubrum$. This procedure results in a minor difference between intra- and interdimer nearest-neighbour interactions (intra refers to pigments on the same dimeric building block and inter to pigments on different dimers).

When BChl $a$ is changed into Zn-BPheo this will result in a different site energy and in a different coupling. When BChl $a$ and Zn-BPheo are bound to the monomeric polypeptides $\alpha$ and/or $\beta$ they absorb at 777 and 771 nm, respectively [34]. Therefore, a difference in site energy of 100 cm$^{-1}$ is assumed and the Gaussian distributions for both pigments are separated by that value. We use the same width for both distributions.

For the transition dipole moments it is supposed that the orientations for BChl $a$ and Zn-BPheo are equal at the same binding site. This is reasonable, because of the identical binding site structure for both pigments [34]; in other words, $\kappa_{nm}$ (see Eq. (2)) does not depend on the kind of pigment. What does depend is the dipole strength. We make use of the experimentally determined ratio of the extinction coefficients ($\varepsilon_{BChl\ a}$ and $\varepsilon_{Zn-BPheo}$) in diethylether [36]:

$$\mu_{Zn-BPheo}/\mu_{BChl\ a} = \sqrt{\varepsilon_{Zn-BPheo}/\varepsilon_{BChl\ a}} = 0.863. \quad (3)$$

When the interaction between two BChl $a$ molecules is denoted as $V$, Eq. (3) implies that if one of these BChl $a$ molecules is replaced by a Zn-BPheo the interaction will be 0.863$V$, and in case both BChl $a$ molecules are replaced by Zn-BPheo it will be 0.863$^2V$.

The relative amount of Zn-BPheo in the LH1 complex is taken into account in the simulations as follows. In every iteration it is determined for each pigment by a chance of $x\%$, if the respective pigment is Zn-BPheo, thus giving an average relative amount of $x\%$ Zn-BPheo in the complex. There-

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4 The positions of the nitrogen atoms of the BChl $a$ molecules were taken from the published structure of $Rps.\ acidiphila$ ([4] RCSB Protein Data Bank, PDB identifier 1KZU). For the position of each pigment the positions of its four nitrogen atoms were averaged. The direction of the $Q_0$ transition dipole moment was taken from N$_{N_0}$ to N$_{N_9}$ according to the nomenclature of the Protein Data Bank.
fore, in our model each site has equal preference for BChl \(a\) versus Zn-BPheo.

For the calculation of emission spectra some additional assumptions have to be made. The used model is in fact a combination of the described exciton model with the model of energy transfer in a cluster of pigments [37]. The relaxation in the exciton manifold is modelled as downhill exciton hopping from the excited exciton state(s) to the lowest exciton state.

First of all, line shapes are needed for all the exciton states. As line shape we take a zero-phonon line (ZPL) with a phonon wing (PW). As ZPL we take a sharp Gaussian with a fwhm of \(4\ \text{cm}^{-1}\), corresponding to \(kT\), as the measurements and simulations are done at/or very low temperature.

The PW is constructed from the 6 K emission spectrum, which is measured when the excitation is performed into the red edge of the absorption band. We assume that the mirror-image of the PW of this emission spectrum gives the PW of the homogeneous absorption spectrum. This experimental PW (relative to the excitation wavenumber) can be fitted to a simple analytic shape [38]:

\[
\text{PW}(v) = \begin{cases} 
  v \exp(-v/v_0), & v \geq 0 \\
  0, & v < 0
\end{cases}
\]

which is determined by a single parameter \(v_0\), characterizing the (relative) position of the maximum of the PW. From the experimental emission spectrum at red-wing excitation \(v_0\) is determined to be \(80\ \text{cm}^{-1}\).

The Huang–Rhys factor \(S\) reflects the relative areas of the ZPL \(\text{area}_{ZPL}\) and the PW \(\text{area}_{PW}\) according to \(\text{area}_{ZPL}/(\text{area}_{ZPL} + \text{area}_{PW}) = e^{-S}\) and characterizes the strength of the electron–phonon coupling. We will assume a relatively strong electron–phonon coupling \((S = 1)\), although hole-burning experiments have suggested a smaller value \((\sim 0.3)\) [39,40].

With the outlined procedure a simple line shape can be constructed. However, lifetime broadening leads to an additional broadening of this line. The lifetime of the lowest exciton state is in the order of 1 ns, whereas the lifetimes of the other states are \(\sim 100\ \text{fs}\) [18,22,40–42]. To take lifetime broadening into account, the line shapes of all but the lowest exciton states are derived by convoluting the simple line shape described above with a Gaussian with a fwhm of 50 cm\(^{-1}\) (a lifetime of 100 fs corresponds to a spectral width of about 50 cm\(^{-1}\) fwhm). Actually, a Lorentzian should be taken for the ZPL and therefore also for the line shape used for the “lifetime-broadening convolution”. However, it is known that a Lorentzian line shape will influence the anisotropy calculations in an unreasonable way: due to the long wings of a Lorentzian relatively strong excitation of a state can happen even if the centre of its ZPL is spectrally far away from the excitation wavelength. Because of the extreme sensitivity of the anisotropy to this “line shape artefact”, we have chosen a Gaussian for the ZPL.

With these assumptions the polarized emission spectrum can be calculated as a function of the excitation wavelength. The excitation on the \(k\)th exciton state after excitation at wavelength \(\lambda\), is weighted by the dipole strengths of this state and of the lowest exciton state (the emitting state). The relative intensity of the emitting lowest state as a function of excitation wavelength \(\lambda\) and polarization is calculated as:

\[
\begin{bmatrix}
I \\
I_{vv} \\
I_{vh}
\end{bmatrix}
= \sum_{k=1}^{N} \left( \int_{v=0}^{\infty} \text{laser}_\lambda(v) \varphi_{k}^{\text{exc}}(v)dv \right) \frac{m_k^{\text{exc}}}{\mu_k^{\text{exc}}} \left[ \begin{bmatrix} 1 \\ 1+2r_k \\ 1-r_k \end{bmatrix} \right] \times |m_k^{\text{exc}}|^{-2}.
\]

\(I\), \(I_{vv}\) and \(I_{vh}\) are the relative intensities of the emission for magic angle geometry, vertical excitation/vertical detection and vertical excitation/horizontal detection, respectively. \(\text{laser}_\lambda(v)\) is the spectrum of the excitation light at wavelength \(\lambda\); as a narrow banded laser is used as excitation source, we take a sharp peak (“delta function”). \(\varphi_{k}^{\text{exc}}(v)\) is the (normalized) line shape and \(|m_k^{\text{exc}}|^{-2}\) the dipole strength of exciton state \(k\). \(|m_k^{\text{lowest}}|^{-2}\) is the dipole strength of the lowest exciton state.

The anisotropy term \(r_k\) for the \(k\)th exciton state (averaged over all possible orientations in an isotropic sample) is:
\[ r_k = \frac{(3 \cos^2 \theta_k - 1)}{5}, \]

where \( \theta_k \) is the angle between the transition dipole moments of exciton state \( k \) and the lowest exciton state.

For every realization of disorder (and pigment composition), the outlined procedure results in stick spectra for the (polarized) emission. The stick spectra are convoluted with the single-site emission spectrum, where simply the mirror image of the line shape used for the lowest exciton state is taken. Repeating the procedure several times in a Monte Carlo simulation gives inhomogeneously broadened (polarized) emission spectra.

The “measurable” anisotropy spectrum \( r \) is finally calculated from the averaged convoluted spectra for vertical excitation/vertical detection and vertical excitation/horizontal detection as:

\[ r = \frac{\langle I_{vv, \text{convoluted}} \rangle - \langle I_{vh, \text{convoluted}} \rangle}{\langle I_{vv, \text{convoluted}} \rangle + 2\langle I_{vh, \text{convoluted}} \rangle}, \]

where the brackets indicate the Monte Carlo averaging.

### 3. Results and discussion

#### 3.1. Low-temperature absorption

Fig. 1 shows the experimental absorption spectra of pigment-exchanged LH1’s containing different percentages of Zn-BPheo measured at 30 K. Increasing the amount of Zn-BPheo results in a gradual blue shift and a small increase in the width of the spectrum. The absorption maximum of native LH1 is located at 886 nm and that of LH1 containing 90% Zn-BPheo at 863 nm. This shift is comparable to the shift measured at room temperature where native LH1 maximally absorbs at 873 nm and LH1 containing 90% Zn-BPheo at 854 nm [34].

The observed shift (~300 cm\(^{-1}\)) is clearly larger than what would be expected, if only the difference in site energy between BChl \( a \) and Zn-BPheo, bound to the monomeric polypeptides, would play a role (100 cm\(^{-1}\) [34]). Moreover, in that case, the spectrum of each modified complex would just be a superposition of a spectrum of LH1 with 100% BChl \( a \) and that of LH1 with 100% Zn-BPheo in the appropriate ratio. This would give considerably broader spectra for the partly pigment-exchanged complexes than is experimentally observed. Clearly, the absorption spectra themselves and their shift depending on the percentage of Zn-BPheo are indications for exciton interaction in the modified complexes. The size of the interaction will be quantified in a model later, but we can already make a crude estimate.

Based on the shown absorption spectra, we assume a linear shift of the absorption maximum depending on the relative amount of Zn-BPheo in the complex (see also Fig. 4). We extrapolate, that from 100% BChl \( a \) to 100% Zn-BPheo, the shift will be ~330 cm\(^{-1}\). The coupling in a Zn-BPheo–LH1 will be 0.86\(^2\) of that in a BChl \( a \)–LH1 (see Eqs. (1) and (3)).
The excitonic levels $\varepsilon_k$ of a circular aggregate of $N$ pigments are given by:

$$\varepsilon_k = E + 2V \cos \frac{2\pi k}{N}$$  \hspace{1cm} (8)

in the case that all site energies are equal ($E$), i.e. there is no disorder, and if there is only nearest-neighbour interaction of size $V$. Then all exciton levels are degenerate except two (for even $N$): $k = 0$ and $k = N/2$. For a circular aggregate only the exciton states $k = 0$ and $k = \pm 1$ have a non-zero transition dipole moment. Taking into account that the transition dipoles of the pigments in these bacterial light-harvesting complexes are nearly lying in the plane of the ring, the exciton states $k = \pm 1$ will have by far the largest transition dipole moment.

From this and from the difference in site energy of $\sim 100$ cm$^{-1}$ we can estimate the nearest-neighbour coupling in a 100% BCHl $\alpha$-LH1 of 32 pigments to be $\sim 450$ cm$^{-1}$.

3.2. Emission at 6 K

The results of the polarized fluorescence measurements on native LH1 and LH1 with 70% Zn-BPheo are shown in Figs. 2 and 3, respectively. For both kinds of complexes a similar behaviour can be observed. When the excitation is performed into the blue wing and the middle of the absorption band the position of the emission maximum stays constant and a low anisotropy is measured. When the excitation is tuned further to the red, a red shift of the emission maximum and an increase of the anisotropy with the excitation wavelength are observed.

These results are similar to those obtained on LH1 and LH2 of *Rhodobacter sphaeroides* [37,43] and *Rb. molischianum* [44]. A quantitative explanation of this effect has been given by van Mourik et al. [37] in terms of energy transfer in a cluster of weakly coupled pigments, which have inhomogeneously distributed site energies. Upon blue and main band excitation the excitation energy is transferred to pigments absorbing more to the red and emission will occur from the lowest energy pigment in each cluster. This leads to a low anisotropy and a constant position of the emission

maximum, reflecting the position of the distribution of lowest pigments per cluster. However, when the excitation is tuned to the red, an increasing fraction of lowest energy pigments in a cluster is excited. Thus, the anisotropy increases and the emission maximum shifts to the red with the excitation wavelength.
We will show that this model can still be used when the pigments’ pure electronic states are replaced by exciton states (see also e.g. Ref. [27]).

3.3. Simulation of absorption spectra

In our exciton model there are two parameters that determine the result of the simulation: (a) the intradimer coupling \( V_{\text{BChl}_a,\text{BChl}_a} \) (immediately determining all other interactions in the LH complex between two BChl \( a \) molecules and—by using Eq. (3)—also between BChl \( a \) and Zn-BPheo and between two Zn-BPheo molecules), and (b) the width of the IDF, \( \text{fwhm}_{\text{IDF}} \). When these values are chosen, the spectra can be calculated.

In Fig. 4 we show the calculated relative shift of the absorption maximum versus the relative amount of Zn-BPheo in the LH1 for different intradimer couplings \( V_{\text{BChl}_a,\text{BChl}_a} \) and \( \text{fwhm}_{\text{IDF}} \) and compare this to the experimental data. It can be seen that the coupling determines the slope of the curve. We conclude that the intradimer coupling \( V_{\text{BChl}_a,\text{BChl}_a} \) which fits the measured relative shift of the absorption maximum is \( \sim 400 \text{ cm}^{-1} \). However, from this figure we cannot conclude anything about the width of the IDF.

To estimate the width of the IDF, the simulated spectra (for an intradimer coupling \( V_{\text{BChl}_a,\text{BChl}_a} = 400 \text{ cm}^{-1} \), which fits the measured shift) are directly compared to the experimental spectra. In Fig. 5 this is shown for the spectra of native LH1 and of LH1 with 90% Zn-BPheo. As we have calculated (accumulated) stick spectra and did not take phonons into account, the simulated spectra have a smaller width than the experimental spectra. But the PW has only an effect on the shape of the blue wing of the spectrum and the ZPLs of the lowest exciton states are narrow. Therefore, the red flank of the spectra does not depend on this broadening effect and can be used for comparison. Although the differences are not large, it can be seen that the spectra calculated with \( \text{fwhm}_{\text{IDF}} = 600 \text{ cm}^{-1} \) fit the red flank of the measured spectra better than the spectra for the other widths.

Summarizing it can be concluded, that in the described exciton model the best agreement between experimental data and simulation is achieved by using \( 400 \text{ cm}^{-1} \) as intradimer coupling between two BChl \( a \) molecules in LH1 and a \( \text{fwhm}_{\text{IDF}} \) of \( 600 \text{ cm}^{-1} \). In Fig. 6 the comparison between a simulation using these values and the experiment is shown for LH1 complexes with different percentages of Zn-BPheo. We stress that all simulated spectra were shifted by the same value, which was overall determined by a linear fit of the position of the absorption maximum versus the

![Fig. 4. Relative shift of the absorption maximum versus the relative amount of Zn-BPheo incorporated into LH1. The experiment (solid line with circles) is compared with the simulations. The different panels show calculations for different values of \( \text{fwhm}_{\text{IDF}} \), which are indicated in the panels. The used intradimer couplings \( V_{\text{BChl}_a,\text{BChl}_a} \) were 300 cm\(^{-1}\) (dashed, thin line), 400 cm\(^{-1}\) (dashed, thick line), 500 cm\(^{-1}\) (dotted, thick line), 600 cm\(^{-1}\) (dotted, thin line). The vertical shift of each curve was determined by a linear fit.](image-url)
percentage of Zn-BPheo in the complex (see Fig. 4) and additionally by $-50$ cm$^{-1}$ to allow easy comparison of the red flank of the spectra (see Fig. 5). The shift of the absorption maximum and the shape (red flank) of the spectra are well described. The overall resemblance is reasonable, although for individual spectra the agreement between simulation and experiment can be better or worse. We note that the coupling estimated in the Monte Carlo simulation is quite close to the estimated value based on a circular aggregate without disorder (see Eq. (8)). However, the coupling is smaller than the fwhm of the IDF, underlining the importance of disorder in these light-harvesting complexes and the fact that disorder has to be included in the simulation.

3.4. Simulation of emission data

Using the parameters found from simulating the absorption spectra for LH1 complexes containing different percentages of Zn-BPheo, the emission can be calculated within the same exciton model as described in Section 2. This is done for native LH1 (see Fig. 7) and LH1 containing 70% Zn-BPheo (see Fig. 8).

In both cases, the general trend is simulated quite well. A low anisotropy is found when the excitation is performed in the blue wing and in the middle of the absorption band and the anisotropy increases when the excitation wavelength is tuned to the red. Especially the wavelength at which the increase actually starts is nicely reflected in the simulations.
Fig. 6. Comparison of experimental absorption spectra (solid lines) of LH1 containing different percentages of Zn-BPheo at 30 K and simulated spectra (dotted lines). The relative amounts of Zn-BPheo are indicated in the figure. 0% Zn-BPheo corresponds to native LH1. The simulations were done with an intradimer coupling $V_{BC_{34},BC_{34}} = 400 \text{ cm}^{-1}$ and a fwhm$_{DF} = 600 \text{ cm}^{-1}$. The spectra were normalized to the maximum absorption and for clarity of representation were given equidistant offsets. The simulated spectra were equally shifted on the wavelength scale corresponding to the shift (relative to the experimental spectra) in Fig. 5.

For a symmetric ring of pigments with no disorder and no interaction an anisotropy of 0.1 is expected for aselective excitation and excitation on the blue side of the absorption maximum, corresponding to a fully depolarized excitation. Our measured and calculated values are slightly lower. On one hand the exciton interaction causes delocalization of the excitation energy but on the other hand the large disorder has an opposite effect. In the result the anisotropy value for the disordered ring with exciton interaction is just slightly smaller than 0.1. In particular, this is surprisingly well reproduced by the model for native LH1.

The shift of the emission maximum is described in a less satisfying way. Although the constant position of the emission maximum for excitation in the blue and middle part of the absorption band and also the shift while exciting in the red wing can clearly be seen, the plateau in the simulation is clearly at much shorter wavelength than in the
experiment. One possible explanation for this effect might be ring-to-ring energy transfer. This energy transfer will give a further shift of the emission maximum to the red [18,45]. However, it will not lower the anisotropy, as an anisotropy value of ~0.1 already corresponds to a fully depolarized excitation.

3.5. Elliptical deformation?

Recently, there has been a lively discussion if the LH2 ring of *Rps. acidophila* is elliptically deformed [24,25,46]. This was concluded from single-molecule experiments, where the complexes were embedded in a polyvinyl-alcohol matrix. The recent model by Matsushita et al. [46] relates this C3 deformation to a special geometric arrangement of the pigments: upon going from the circular structure to the ellipse the geometrical angle of each pigment measured from the centre is kept constant, resulting in a larger distance of the pigments at the long axis of the ellipse (model C in Ref. [46]). The orientations of the transition dipole moments are adjusted to keep the same local tangent with the ellipse as in the undeformed case.

It is not the purpose of our study to analyse the single-molecule spectra. We only want to investigate, how an elliptical deformation effects the calculated spectral properties in our model. The elliptical deformation was done as described in model C of Ref. [46] (see above). Upon deformation the relative orientations of the transition dipole moments and their distances change. To calculate the interactions for the deformed ring the dipole–dipole approximation was used (see Eqs. (1) and (2)). The same scaling factor for the coupling as in the unperturbed case was taken. Using a fwhm_{IDF} of 600 cm\(^{-1}\) and an elliptical deformation of 8.5% (value taken from Ketelaars et al. [25]) yields a spectrum (not shown), which is too broad and has too much structure (due to the large splitting between the three lowest exciton states in the deformed case). Therefore, the fwhm_{IDF} and the deformation were adjusted to the experimental spectrum. A fwhm_{IDF} of 400 cm\(^{-1}\) and a deformation of 4% give a reasonable spectrum, which is shown in Fig. 9. We also compare the measured and calculated anisotropy. It can be seen that the calculated anisotropy on the blue side of the absorption maximum goes nearly to zero and is clearly lower than measured. On the other hand, tuning the excitation more and more to the red, the calculated anisotropy increases much faster than measured. However, as already mentioned, the measured anisotropy is well reproduced by the model for native, undeformed LH1 (compare Fig. 9 to Fig. 7).

These effects can be understood quite easily. In the elliptical case the second lowest exciton state is well split from the third lowest state. In the nomenclature of Matsushita et al. [46] these states are called \(k = k_{\text{low}}\) and \(k = k_{\text{high}}\), respectively. The state \(k = k_{\text{low}}\) is in the centre of the band. The state \(k = k_{\text{low}}\) is on the red side of the absorption band and is little split from the lowest exciton state (see Fig. 9). The contribution a laser-excited exciton state makes to the anisotropy is determined by the angle of its transition dipole moment with the transition dipole moment of the lowest exciton

![Figure 9](image_url)

Fig. 9. Comparison of experimental and simulated emission data for native LH1 with an elliptical deformation (4% deformation, fwhm_{IDF} = 400 cm\(^{-1}\), see text for further details). Shown are the fluorescence anisotropy (experiment: filled squares, simulation: thick solid line) versus excitation wavelength. The experimental absorption spectrum (thin solid line), the calculated spectrum (dotted line) and the absorption bands of the three lowest exciton states (dashed lines) are also shown in the figure.
state, from which the fluorescence occurs (see Eq. (6)). The distribution of these angles is shown for the exciton states \( k = 1_{\text{low}} \) and \( k = 1_{\text{high}} \), for both the undeformed and the elliptical case in Fig. 10.

The distribution of angles for state \( k = 1_{\text{high}} \) (dashed line) has a relatively sharp peak around 90° in the elliptical case, which results in a negative contribution to the anisotropy. Therefore the overall anisotropy becomes nearly 0, when this state is excited (blue side of the maximum of the absorption band). In the undeformed case, however, the angle distribution is much broader and washes out the effect, resulting in an anisotropy between 0.05 and 0.1.

On the other hand, the distribution of angles for the state \( k = 1_{\text{low}} \) (solid line) is sharply peaked around 0° in the elliptical case. This is the reason, why for the ellipse the anisotropy is already high before the red wing of the band is directly excited: the angle of 0° gives a maximal 0.4 contribution to the anisotropy. In the undeformed case, however, again this angle distribution is broader. Moreover it has its maximum around 90°. Therefore, the anisotropy increases slower while tuning the excitation wavelength to the red.

We note that these calculations were done for half of the deformation suggested in Refs. [25,46]. Using our model it is possible to exploit the full implications of a structural deformation on the fluorescence anisotropy. It is known from literature that the anisotropy in LH2 is very similar to LH1 [43,44]. As the measured data can fully be described by a circular aggregate, this certainly puts some constraints on elliptical deformations.

4. Conclusions

Spectroscopic properties of native bacterial light-harvesting complexes can reasonably be described in exciton models [10–17]. We have here presented experimental data on LH1 complexes, where the BCHl \( \alpha \) molecules are exchanged for Zn-BPheo in different percentages. Using the same exciton model as for the native complex we are able to describe the experimental data of these engineered light-harvesting complexes. The shift of the absorption maximum could be modelled depending on the relative amount of Zn-BPheo in the complex. Furthermore, we have extended the model to describe fluorescence data. Although this was done in a rather simplistic (and naive) way, we were able to describe the general behaviour of the fluorescence anisotropy and the shift of the emission maximum depending on the excitation wavelength for native LH1 and 70%-Zn-BPheo–LH1. The model has proved to be simple and robust to be applied to these complexes with modified pigments.

Moreover in Ref. [34] it was shown that the light-harvesting complexes with exchanged pigments have a very similar structure as the native

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Fig. 10. Distribution of the angle of the transition dipole moment of the lowest exciton state with the transition dipole moments of the second (solid line) and third (dashed line) lowest exciton states \( k = 1_{\text{low}}, k = 1_{\text{high}}, \) respectively. (A) Native LH1 and (B) elliptically deformed LH1 of Fig. 9.
complex. Our simulations are based on the same structure. The fact that the model gives a good description for both the native light-harvesting complex and the modified complexes with different percentages of Zn-BPheo is another argument for a similar structure.

For the coupling of two neighbouring BCHl a molecules within LH1 a value of 400 cm\(^{-1}\) was found. A fwhm of the IDF of 600 cm\(^{-1}\) gave the best description of the experimental data. Compared to values found in the literature, our values are on the high end of the scale. We want to mention, however, that the parameters estimated from extensive simulations of pump-probe data on the LH1 core complex of *Rps. viridis* are in very good agreement with our values, yielding 400 cm\(^{-1}\) for the coupling and 575 cm\(^{-1}\) for the disorder [47].

Our used coupling strength is certainly an “overall” value. It is known that in bacterial light-harvesting complexes besides coulombic, e.g. dipole–dipole, interactions also short-range interactions are involved, which depend on interchromophore orbital overlap [48]. An example of the latter are charge-transfer interactions. It was e.g. estimated, that their contribution to the total coupling in B850 of LH2 of *Rps. acidophila* is about 20% [48]. In the special pair of the RC of *Rps. viridis* their contribution to the coupling is probably even larger [49,50]. The contribution of charge-transfer states in LH1 may also be relatively large in view of the strong Stark effect of the near infrared transition [51,52].

Comparing the ab initio molecular orbital calculations [48] with a model using the dipole–dipole approximation [13] it can be stated that the differences in the finally estimated values are small, indicating that the simple model still can be used as approximation. In our approach for the circular LH1 ring the dipole–dipole approximation was used for relating the nearest-neighbour to the non-nearest-neighbour interactions. If we assume that our estimated nearest-neighbour coupling consists of coulombic and short-range interactions, then the non-nearest-neighbour interactions are slightly overestimated. But as these are small compared to the nearest-neighbour interactions this will not have much effect on the calculated properties.

But more than the actual values, it is noteworthy to realize the importance of disorder in these protein complexes. This disorder has to be included in exciton calculations.

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