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THE STRUCTURE AND FUNCTION OF THE LH2 (B800-850) COMPLEX FROM THE PURPLE PHOTOSYNTHETIC BACTERIUM *RHODOPSEUDOMONAS ACIDOPHILA* STRAIN 10050

RICHARD J. COGDELL,* NEIL W. ISAACS,‡ ANDY A. FREER, ‡ JUAN B. ARELLANO,* TINA D. HOWARD,* MIROSLAV Z. PAPIZ, † ANNA M. HAWTHORNTHWAIT-LAWLESS† and STEPHEN PRINCE‡

*Departments of Biochemistry and Molecular Biology, University of Glasgow, Glasgow G12 8QQ, U.K.

†CCL Daresbury Laboratory, Daresbury, Warrington WA4 4AD, U.K.

‡Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, U.K.

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I. INTRODUCTION

The major light-absorbing pigments in purple photosynthetic bacteria are the bacteriochlorophylls (*a* and *b*) (BChl) and the carotenoids. These pigments are noncovalently attached to two types of integral membrane protein forming the reaction centers and the light-harvesting or antenna complexes (Hawthornthwaite and Cogdell, 1991; Hunter, 1995; Zuber and Cogdell, 1995). Photosynthesis in purple bacteria usually begins with the absorption of a photon in the light-harvesting system. The absorbed energy is then rapidly (in less than ~100 ps) and efficiently transferred to the reaction center (~95% quantum efficiency). In the reaction center this energy is used to drive the initial charge separation reaction and the energy is then "trapped" (Feher and Okamura, 1978; Feher *et al.*, 1989; Deisenhofer *et al.*, 1995). The combination of antenna complexes with a reaction center constitutes the photosynthetic unit (PSU). For most commonly studied purple bacteria the number of PSUs per cell and their size are variable. Depending on such factors as the light-intensity at which cells are grown, the size of the PSU can vary from about 30 BChls per reaction center up to 200-300 BChls per reaction center (Aagaard and Siström, 1972; Drews, 1985). This arrangement of reaction centers surrounded by an antenna system ensures that each reaction center is kept well supplied with incoming solar energy and effectively acts to increase their cross-sectional area for photon capture. It is interesting to note that in most species the same pigments are found in both reaction centers and antenna complexes, and it is the protein that determines which function a given pigment is destined to fulfil.

When BChl *a* is dissolved in an organic solvent such as 7:2 v/v acetone:methanol its NIR absorption band is located at 772 nm. This is the typical Q_y absorption band of monomeric BChl *a*. However, when the BChl *a* is non-covalently bound into an antenna complex, this NIR absorption band is red shifted between 800-940 nm, depending on the species (Fig. 1) (Thorner *et al.*, 1978; Hawthornthwaite and Cogdell, 1991). In most species this red shift is associated with an increase in spectral complexity, with several peaks/shoulders clearly visible in the *in vivo* absorption spectrum. This red shift arises from pigment-pigment and pigment-protein interactions within the antenna complexes and is regularly used to both identify them and judge their integrity.

Since they are integral membrane proteins, the isolation of a purple bacterial antenna complex begins with the solubilization of the photosynthetic membrane with a suitable detergent (Cogdell and Thorner, 1979;

Hawthornthwaite and Cogdell, 1991). Very often the solubilized complexes are then initially fractionated by sucrose gradient centrifugation (Fig. 2). In most species this fractionation reveals two types of antenna complex, called LH1 and LH2. LH1 forms the so called "core" complex. It is closely associated with the reaction center and forms a stoichiometric complex with it (usually ~32 BChls per reaction center (Gall, 1995; Karrasch *et al.*, 1995; Zuber and Cogdell, 1995). LH2, also sometimes called the "variable" or "peripheral" antenna complex, is the topic for the remainder of this review. Readers who want to obtain more information on the overall subject of the structure and function of the bacterial PSU should consult the following general reviews (Somsen *et al.*, 1993; Blankenship *et al.*, 1995; Loach and Parkes-Loach, 1995; Cogdell *et al.*, 1996; Papiz *et al.*, 1996).

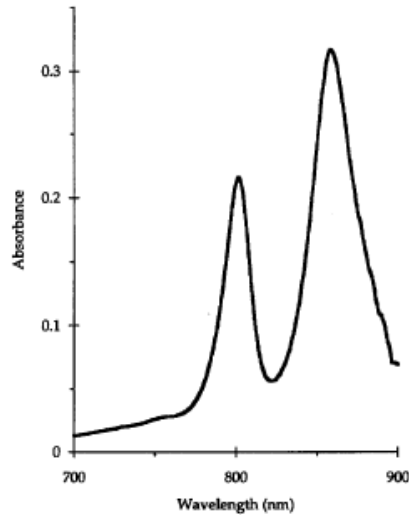


Fig. 1. The near infra-red absorption spectrum of chromatophores from *R. acidophila* 10050. Chromatophores are membrane vesicles of the photosynthetic membrane, formed when whole cells are mechanically disrupted.

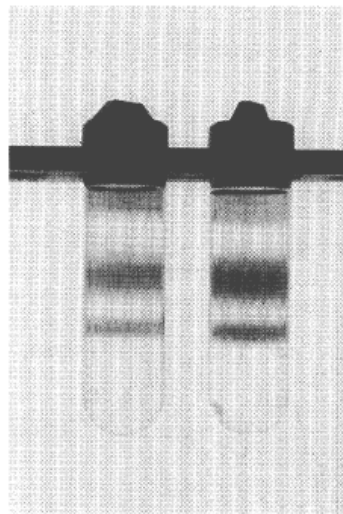


Fig. 2. Solubilized antenna complexes from *R. acidophila* strain 10050 separated on a sucrose gradient. The upper orange band is a little free pigment from denatured complexes. The major middle band, is LH2 and the bottom band is the LHI-RC "core" complex. For full details of this method see Hawthornthwaite and Cogdell (1991).

II. THE COMPOSITION OF THE LH2 COMPLEX FROM *RHODOPSEUDOMONAS ACIDOPHILA* STRAIN 10050

Figure 3a shows the absorption spectrum of the LH2 complex from *R. acidophila*. In the NIR, two strong absorption peaks due to the Q_y absorption of BChl *a* are seen, one at 801 nm and the other at 863 nm

(even though these peaks are not exactly at 800 and 850 nm, this complex is also given the generic name B800-850). The other absorption bands due to BChl *a* (Q_x at ~595 nm and the Soret peak at ~380 nm) are also indicated.

The three peaks between 450 and 550 nm are due to the carotenoid rhodopin-glucoside (Hawthornthwaite and Cogdell, 1991), which has 11 conjugated double bonds. These pigments are non-covalently attached to two low molecular weight apoproteins, called α and β (Bissig *et al.*, 1988). These apoproteins have been sequenced (Bissig *et al.*, 1988; Zuber and Cogdell, 1995) and contain 53 amino acids and 41 amino acids, respectively. In the intact complex these apoproteins are present in a 1:1 ratio and bind three BChl *a*s and two carotenoids per α/β pair. The native complex is an oligomer of this minimal unit (Hawthornthwaite and Cogdell, 1991).

Before going on to describe the detailed structure of this LH2 complex as revealed by X-ray crystallography it is useful to set the scene by summarizing what was known before this structural determination. This is important since many of these established facts were used to help interpret the electron density map obtained in the crystallographic studies.

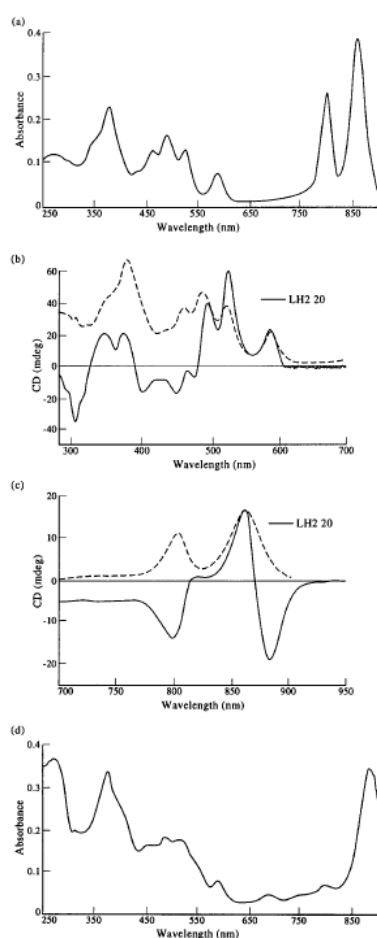


Fig. 3. Spectroscopic properties of LHI and LH2 complexes from *R. acidophila* strain 10050. (a) The room temperature absorption spectrum of a purified sample of LH2. The absorption bands at 863, 802, 595 and ~380 nm are due to BChl *a*, the three peaks between 450 and 550 nm are due to the carotenoid rhodopin glucoside and the broad peak at ~270 nm is due to aromatic residues in the apoproteins. (b)/(c) The room temperature CD spectrum of LH2. (b) 300-700 nm, (c) 700-950 nm. The CD spectrum is shown as a continuous line and the corresponding absorption spectrum is shown as a dashed line. These spectra were recorded at the BBSRC CD facility in Sterling University, UK. (d) The room temperature absorption spectrum of the LHI-RC "core" complex. The absorption bands at 875, 595 and ~380 nm are due to the antenna BChl *a*, while those at ~800 and 760 nm are due to the reaction centres. The complicated peaks between 450 and 570 nm are due to carotenoids.

As a result of many studies since the early 1980s, mainly due to a concerted effort from Zuber's laboratory in Zurich, a large database of primary structures of LH2 apoproteins, from a range of different purple bacteria, was accumulated (Hawthornthwaite and Cogdell, 1991; Zuber and Brunisholz, 1991). It was therefore possible to inspect this and to look for conserved features that might have functional significance. For the purposes of this review it is important to highlight three of these (Fig. 4a,b). All of the apoproteins sequenced show a "tripartite" structure. The N- and C-termini are polar (typically; α -apoprotein; N-, 12-14 residues; C-, 16-20 residues; β -apoprotein; N-, 13-23 residues; C-, usually about five residues but a few examples are larger) while the central region (21-23 residues) is hydrophobic. This immediately led to the suggestion that these apoproteins should lie across the membrane. Moreover, if this central hydrophobic region was a single transmembrane α -helix, then the apoproteins were so small that they could cross the membrane only once. This would put their N- and C-termini on opposite sides of the membrane. These predictions were tested and verified in several topological studies (reviewed in Hunter *et al.*, 1989; Zuber and Brunisholz, 1991; Zuber and Cogdell, 1995). IR and far-UV CD spectroscopy were also used to show that approximately the required α -helical content was indeed present (50-60%) (Cogdell and Scheer, 1985; Zuber and Brunisholz, 1991). As far as the detailed sequence was concerned, the major conserved feature was three rather invariant His residues, one in α - and two in β -. These were highlighted, of course, because they were good candidates for the fifth ligand to the central Mg^{2+} in the bacteriochlorin ring of BChl *a* (Olsen and Hunter, 1994). It was therefore proposed that the three BChl *as* bound per $\alpha\beta$ pair of apoproteins might be liganded to those His residues. Two of these His residues (one in α - and one in β -) are located two-thirds of the way across the central hydrophobic domains of the α - and β - apoproteins, such that if "helical rods" of the two apoproteins were modeled, these His residues would lie at the same level in this putative transmembrane domain. The CD spectrum of LH2 for *R. acidophila* is shown in Fig. 3b,c. BChl *a* itself as a monomer in organic solvent shows a weak negative CD band in the region of its Q_y absorption due to its inherent chirality. If, however, BChl *a* aggregates to form closely interacting oligomers (exciton-coupled) then the CD spectrum is enhanced and has both positive and negative peaks. On this basis, since the 800 nm BChl gives only a negative peak and the 850 nm absorbing ones give a strong, conservative positive and negative peak, it was proposed that the B850 absorption band represented a pair of exciton-coupled BChls while the B800 absorption band represented a monomeric BChl (Kramer *et al.*, 1984; Cogdell and Scheer, 1985). It was therefore further suggested that these two His residues bound the two B850 nm absorbing BChl *as* present per $\alpha\beta$ pair. The nature of the fifth ligand to the central Mg^{2+} of BChl *a* can be investigated with resonance Raman spectroscopy. In a series of papers Robert and Lutz (e.g., Robert and Lutz, 1985) showed clearly that the 850 nm absorbing BChl *as* had imidazole ligands (i.e., His ligands), but that this was not the case for the B800 absorbing BChl *as*. It was therefore suggested that the binding site for the B800 BChl *as* was close to the third conserved His residue but did not involve it as a direct ligand. Early, rather drastic site-directed mutagenesis studies on the LH2 complex from *R. capsulatus* (Bylina *et al.*, 1988) showed that deletion of the His residues postulated to be involved in binding the 850 nm absorbing BChl *as* resulted in a complex which failed to assemble; thereby showing again the essential nature of these two His residues. The third important conserved feature is rather more subtle, but also very important. Energy transfer within the PSU is not random. The absorption spectra of LH2 and LH1 are different (i.e., LH2 800 and 850 nm and LH1 875-890 nm) so that energy transfer proceeds in an "energy downhill" direction from LH2 to LH1 and then to the reaction center (Fig. 3d). Moreover, there are spectral variants of LH2 where the 850 nm absorption band is shifted to 820 nm. Careful inspection of LH2 sequences identified several key, C-terminally located, aromatic residues, the presence or absence of which strongly correlated with this shift in absorbance from 863 to 820 nm (Zuber and Brunisholz, 1991; Fowler *et al.*, 1992, 1994). When these residues were α Tyr44 and α Trp45 in *R. acidophila* the complex absorbed at 863 nm, and when they were \sim Phe44 and α Leu45 the complex absorbed at 820 nm. It is clearly one of the important issues to understand how the protein can modulate where a given BChl *a* molecule absorbs light.

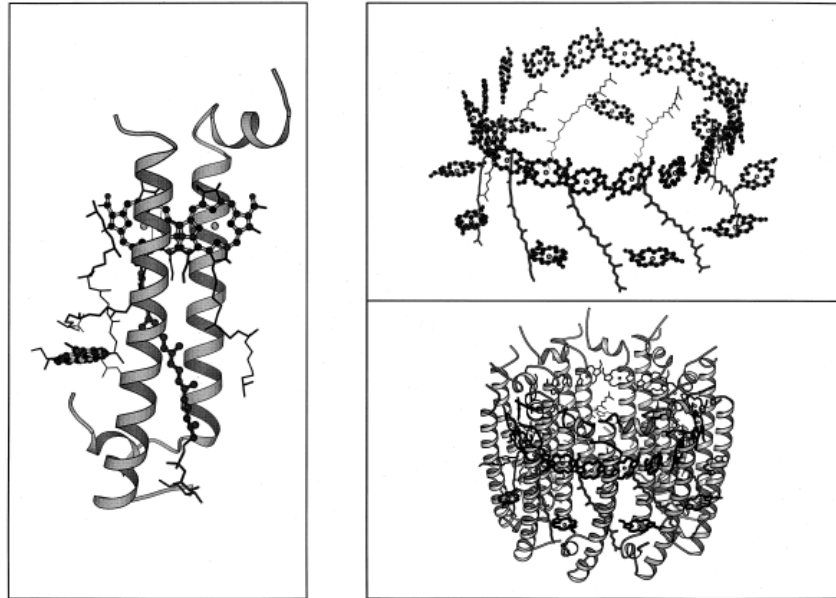


Fig. 5. The overall architecture of LH2 from *R. acidophila* strain 10050. Lower right--A schematic representation of the whole complex. The apoproteins are shown as ribbons. The BChl *as* are in bold. The well-ordered carotenoids are red. One $\alpha\beta$ apoprotein pair is shown colored and is picked out in the left-hand panel. Upper right--A schematic representation of the disposition of the chromophores. Left--A single $\alpha\beta$ protomer together with its associated pigments.

3.2. The Structure of the Apoproteins

As described above, topological experiments carried out on LH complexes from several species of purple bacteria have shown that the N-termini of both the α - and β -apoproteins are located on the cytoplasmic side of the photosynthetic membrane and both C-termini on the periplasmic side. The 3D structure confirms that both apoproteins are indeed oriented across the complex in the same direction.

In the α -apoprotein the N-terminal residue is buried about 9 Å, from the presumed cytoplasmic surface of the complex and coordinates the Mg^{2+} atom at the center of the BChl *a* macrocycle (for the nine BChl *a* molecules which lie between the β -apoprotein α -helices). This residue was identified by protein sequencing as a methionine (Bissig *et al.*, 1988; Zuber and Brunisholz, 1991). However, in the electron density map there is an extension which is best fitted by assuming that this residue is in fact a N-formyl methionine. The fifth ligand of the Mg^{2+} atom is then the "formyl" oxygen. The position of the sulphur atom in the Met is known accurately from a selenomethionine derivative (McDermott *et al.*, 1995). The presence of the formyl group is currently being looked for by carefully resequencing the α -apoprotein. Residues 2-9 form a short 3_{10} helix along the presumed membrane surface. There is then a turn at residues 11 and 12 (Asn and Pro, respectively) followed by the membrane spanning α -helix which runs from residues 11 to 36. At Thr 38 there is then another turn which then leads to a second amphipathic helix, this time lying along the presumed periplasmic surface of the complex. The general organization of the β -apoprotein is similar to that of the α -apoprotein. The first five residues at the N-terminus of the β -apoprotein lie on the presumed cytoplasmic surface of the complex in a rather extended conformation. Two turns of an irregular helix then lead to the transmembrane 0_t -helix which runs from residues 11 to 36. At residues 37 and 38 (Thr and Pro, respectively) there is a turn which leads to a short C-terminal tail at the presumed periplasmic side of the complex. Figure 5 shows the overall fold of the α - and β -apoproteins in one $\alpha\beta$ pair.

One clear feature of the structure is that the α - and β -apoproteins interact only at their N- and C-termini. There is no $\alpha\beta$ helix-helix contact within the transmembrane portion of the complex. This is the main reason why most of the detailed models of LH2 prior to the determination of the crystal structure failed (see below). However, it is interesting to note that the modeling of the conformation of the individual apoproteins,

see for example Olsen and Hunter (1994), was actually rather good with respect to the positioning of the α -helices and the location of the turns.

It is also important to point out some of the detailed contacts between large aromatic residues at the C-termini of both apoproteins. Residues α Trp40, Trp45, Tyr44 and β Trp39 are all involved in binding the apoproteins together through hydrogen bonds and hydrophobic interactions, which involve both radial and adjacent α - and β -apoproteins. In this way the structure is strongly "interlocking".

3.3. The Structure and Arrangement of the BChl *a*s

The BChl *a* molecules are organized into two clearly defined groups (Figs 5 and 6). Nine, well separated, more peripherally arranged ones lying between the β -apoprotein helices; and 18 tightly coupled ones, liganded to the conserved His residues, forming a closely interacting ring. Based upon the previous characterization of this LH2 complex by absorption and CD spectroscopy (described above) and on which ones are liganded to the His residues, these two groups could be assigned to the two spectroscopic species, i.e. those absorbing at \sim 801 (B800) and \sim 863 nm (B850). The nine, monomeric BChl *a*s have therefore been identified as the 801 nm absorbing ones and the group of 18 as the 863 nm absorbing ones.

The 18 B850 BChl *a* molecules form an overlapping ring (Fig. 6), but within this structure their individual environments and conformations are not all equivalent. Within an $\alpha\beta$ apoprotein pair the two B850 BChl *a*s have a Mg^{2+} - Mg^{2+} distance of 9.6 Å, whereas the Mg^{2+} - Mg^{2+} distance to the next B850 BChl *a* in the ring, outside this $\alpha\beta$ pair, is 8.9 Å. The face of the bacteriochlorin ring of the α -bound B850 BChl *a* which is presented to the inside of the complex is opposite to that of the β -bound B850 BChl *a*. This means that the BChl *a*s alternate in their orientation going around the ring. Within an $\alpha\beta$ apoprotein pair the two B850 BChl *a* macrocycles overlap at rings C and E, while between adjacent $\alpha\beta$ apoprotein pairs the overlap is at ring A.

The configuration of each type of BChl *a* macrocycle in LH2 is shown in Fig. 7. The α -bound B850 Bacteriochlorin is almost planar, while the β -bound B850 bacteriochlorin is significantly distorted. The β -bound B850 bacteriochlorin is in a "saddle" conformation with significant "bowing" along the direction of the Q_y transition. The B800 bacteriochlorin is slightly "domed". Possible spectroscopic consequences of these asymmetries are discussed below.

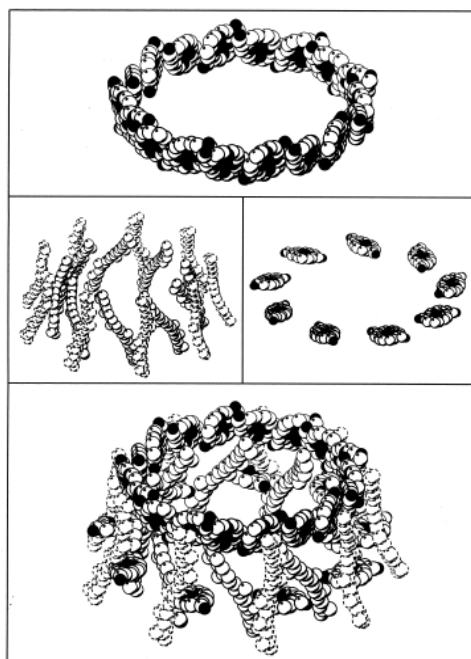


Fig. 6. A detailed Van der Waal's representation of the disposition of the chromophores in LH2. Top-The strongly coupled B850 array of BChl *a*s. Middle right-The monomeric B800 array of BChl *a*s. Middle left-The carotenoids (the putative second carotenoid chromophore extrapolated from the portion located in the crystallographic model is shown dotted). Bottom-The complete assembly.

To these asymmetries must be added those arising from the differential organization of the phytyl chains. The phytyl chain of the α -bound B850 BChl *a* is rather extended, while that of the β -bound B850 BChl *a* is bent allowing it to curve round and interact with the phytyl chain of the B800 BChl *a*, which is liganded to an adjacent α -apoprotein. This latter interaction is clearly important for binding the B800 BChl *a* into the complex.

Apart from the overall very hydrophobic environment of the B850-absorbing BChl *as*, it is worth pointing out some of the residues which are hydrogen bonded to the BChl *a* macrocycles. In the Introduction the importance of the exact wavelength at which the BChl *as* absorb for imparting directionality into the energy transfer process was pointed out. Comparison of the amino acid sequence of B800-850 and B800-820 forms of LH2 (Zuber and Brunisholz, 1991), together with the site-directed mutagenesis studies of Fowler *et al.* (1992, 1994), suggested a role of hydrogen bonds from α Tyr44 and α -Tryp45 to the BChl *a* macrocycle in modulating where those BChl *as* Q_y absorptions are located. The crystal structure clearly shows that α Tryp45 is hydrogen bonded to the acetyl carbonyl oxygen of ring 1 of the α -bound B850 BChl *a* and α Tyr44 is hydrogen bonded to the same carbonyl oxygen of ring 1 of the β bound B850 BChl *a* (Fig. 7). This nicely confirmed the previous studies.

The Mg^{2+} - Mg^{2+} distance from one B800 BChl *a* to the next is 21.23 Å. The binding pocket of these BChl *as* is very different from those of the B850 BChl *as*. It is rather hydrophilic. There is a water molecule that is located between β His 12 (the second conserved His residue found in LH2 β -apoproteins) and the ester carbonyl oxygen from ring C of the BChl *a* macrocycle. There is also a strong hydrogen bond between the acetyl carbonyl oxygen from ring A of the BChl *a* macrocycle to β Arg20.

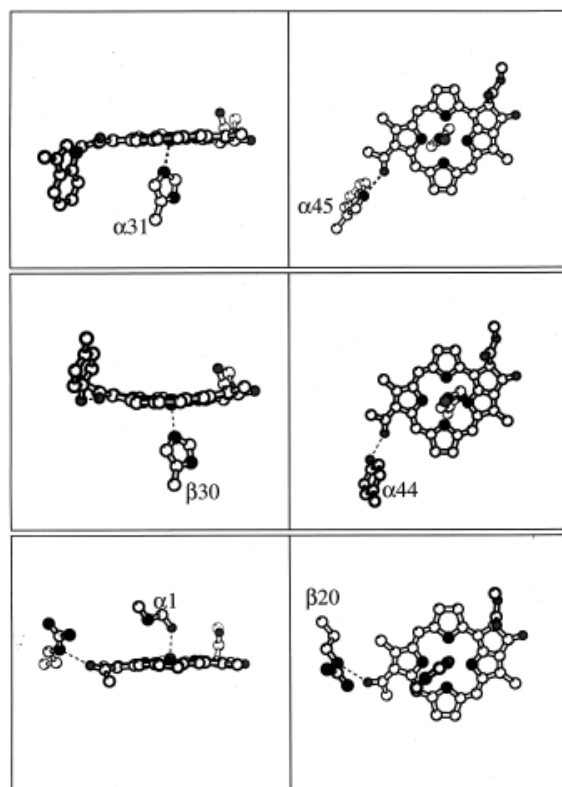


Fig. 7. A comparison of the detailed conformations and important ligands of the different BChl *as* in LH2 from *R. acidophila* 10050. The three independent BChl *a* molecules of the $\alpha\beta$ protomer are shown with their coordination contacts (labeled left) and acetyl hydrogen bonds (labeled right). Top; α -B850, middle; β -B850, and bottom; B800. The saturated ring substituents have been removed for clarity.

3.4. The Structure and Arrangement of the Carotenoids

In the initial description of the structure of LH2 (McDermott *et al.*, 1995) only one molecule of the carotenoid, rhodopin glucoside, per $\alpha\beta$ apoprotein pair was clearly identified in the electron density map. It has an extended, "S" shaped conformation and spans the whole depth of the complex (Fig. 5). This conformation is typical for all *trans*-carotenoids and confirms the previous assignment from resonance Raman spectroscopy (Robert and Lutz, 1985). The glucosyl ring interacts with polar residues on the N-terminal side (α Lys5 and α Thr9). The hydrocarbon chain then passes close to the edge of the B800 macrocycle, then passes on to run over the face of the α -bound B850 macrocycle in the adjacent $\alpha\beta$ pair of apoproteins. The closest approach of the carotenoid to the edge of the B800 macrocycle is 3.4 Å and to the B850 macrocycle is 3.68 Å. If this carotenoid is viewed down its long axis, from end to end, it can be seen to be twisted into about half a turn of a helix. This induced chirality may well be the origin of the strong CD seen in the carotenoid's absorption bands in the visible region of the spectrum (Fig. 3b). In this context it should be remembered that when this carotenoid is dissolved in organic solvent it shows no CD spectrum.

In the first description of the structure of the complex an additional portion of electron density was tentatively attributed to a bound molecule of the detergent β -n-octyl-glucoside. This; was incorrect (Freer *et al.*, 1996). The complex has subsequently been crystallized in LDAO. Under these conditions, when the complex has not been exposed to octyl-glucoside, the same extra electron density is present. The sugar head group here shows the same degree of "disorder" as that in the well-resolved carotenoid. It is probable therefore that this density represents another molecule of rhodopin glycoside per $\alpha\beta$ pair, but is only well-ordered for about 1/3 of its length. This second carotenoid has a rather similar conformation as the first one, but runs through the complex in the opposite direction (Fig. 5). In this case the sugar head group is located in the polar region on the C-terminal side of the complex. It then passes over the outer face of the α -bound B850 BChl *a* macrocycle. The closest approach to this ring is 4.7Å. This α -bound B850 BChl *a* macrocycle is therefore "sandwiched" between two carotenoid molecules. There is a groove on the outer surface of the LH2 complex into which the rest of the carotenoid could fit, running up to the B800 BChl *a* macrocycle. Interestingly, a 2D projection map of 2D crystals of LH2 from *R. sulfidophilus* shows extra density in this outer groove (Montoya *et al.*, 1995; Savage *et al.*, 1995). The authors of that study suggested that a second carotenoid could give rise to that density.

It is also important to point out here that the well-ordered carotenoid interlinks two $\alpha\beta$ apoprotein pairs, which again emphasizes the interlocking nature of the structure. It therefore appears to play a key role. This may explain why carotenoid deletion mutants of purple bacteria fail to assemble LH2 (Griffiths and Stanier, 1956; Zurdo *et al.*, 1993) and why in the absence of carotenoids, for example in *R. sphaeroides*, the LH2 apoproteins are synthesized but rapidly degraded (Lang and Hunter, 1994).

3.5. The Structure and Arrangement of the Phytyl Chains

The phytyl chains associated with BChl and chlorophyll molecules have largely been neglected when the function of these pigments have been considered. However, they are very important structural elements in the LH2 complex (Freer *et al.*, 1996) and play a key role in the alignment of the bacteriochlorin macrocycles. The phytyl chain of the B800 BChl passes up the inside of the complex, wraps around the phytyl chain of the β -bound B850 BChl and then goes to pass over the face of the β -bound BChl macrocycle. This phytyl-phytyl interaction is clearly important for holding the peripherally arranged B800 BChl firmly in the complex. The phytyl chains of the two B850 BChls pass down into the complex. The β -bound B850 phytyl chain, after winding around the B800 phytyl chain, passes over the periplasmic face of the B800 macrocycle. In contrast, the α -bound B850 phytyl chain is more or less fully extended and makes several close contacts with the well-ordered carotenoid molecule. The central region of the LH2 complex, between the two apoprotein rings, is entirely occupied by the pigments, forming a very hydrophobic core. However, there is one key place where β Phe22 protrudes into this. This Phe residue makes a series of strong interactions with a cluster of oxygen atoms from adjacent BChls and their phytyl ester oxygens. It appears that this Phe plays a key role in orienting the pigments in the complex (Freer *et al.*, 1996). In an antenna complex, the relative orientation of the BChl macrocycles is very important for energy transfer, where the efficiency is so dependent upon the relative direction of the Qy transitions. The phytyl chains provide protein with a convenient "handle" on the pigments with which to start this process. The pigments can then be finally "locked" into position by ligation of the Mg²⁺ and hydrogen bonding to the macrocycle.

3.6. Factors which Affect where the Qy Absorption of BChl *a* is Located--BChl *a* Site-energy

As pointed out above, one of the secrets that are locked up in the LH2 structure is what controls the site energy of the BChl *a* molecule. The term "site-energy" is used in this context to mean the energy of the Q_y transition of a given BChl *a* molecule if it was completely alone in its binding site, free of any interactions with other pigments, but with the protein structure otherwise unchanged. Some of the mechanisms by which proteins can modulate the site-energies of chlorophylls and BChls have recently been considered by Fajer and colleagues (Gudowska-Nowak *et al.*, 1990; Geutemann *et al.*, 1995). Broadly speaking, these can be put into two classes, "solvation effects", i.e. due to the local environment, and "chemical or structural changes" in the chromophore itself. Perhaps the best example of this type of analysis has been carried out on the BChl *a* antenna protein from *Prosthecochloris aestuarii* (Gudowska-Nowak *et al.*, 1990). This is a water soluble antenna complex whose structure has been determined by X-ray crystallography to a resolution of 1.9 Å (Tronrud *et al.*, 1986). It contains 7 BChl *a*s per protein monomer which have Q_y absorption maxima in the range of 793-825 nm at cryogenic temperatures. Using this structure Gudowska-Nowak *et al.* (1990) investigated the effects of axial ligands, orientation of the macrocycle ring substituent groups, metals, neighboring residues, macrocycle conformation, and tried to assess the relative importance of each of these factors in determining precisely where each BChl *a* molecule absorbs. They showed that the following were important. The torsion angle of the ring 1 acetyl group with respect to the plane of the macrocycle could produce shifts of up to ~300-400 cm⁻¹. Nearby charged amino acids, depending upon the sign of the charge could produce red or blue shifts of up to 2000 cm⁻¹. Further studies on model porphyrins have shown that distortions of the macrocycle such as "ruffling" will also cause significant shifts in the position of the Q_y absorption of up to ~1000 cm⁻¹ (Gudowska-Nowak *et al.*, 1990). Interestingly, in this latter case the excited state dynamics of the molecule are also dramatically altered.

At present, however, there are not enough examples of chlorophyll or BChl pigment-protein complexes whose structures are known to allow these effects to be fully quantified so that, given a detailed analysis of a pigment's structure and its local environment, its site-energy can be determined. It is most probable though that the protein will "tune" the position of the Q_y absorption by a subtle mixture of all these effects. Some strains of *R. acidophila* are able to alter the type of LH2 that is synthesized, depending upon the growth conditions (Cogdell *et al.*, 1983; Brunisholz and Zuber, 1988), in this case B800-850 or B800-820. This shift in the Q_y absorption of the 863 nm absorbing BChls to 820 nm has been correlated with the presence or absence of hydrogen bonds to the acetyl-carbonyl oxygen on ring I (Fig. 7) (Brunisholz and Zuber, 1988; Fowler *et al.*, 1994). It is not yet clear whether this is a direct effect of these hydrogen bonds or an indirect effect, where the hydrogen bonds are used to "fix" the BChl in a different structure where its "site-energy" is changed. B800-820 complexes have been crystallized (Guthrie *et al.*, 1992; Halloran *et al.*, 1995), but as yet these crystals do not diffract X-rays to high enough resolution to allow their structure to be determined and this idea tested.

The position of the Q_y absorption band of BChl is also sensitive to its aggregation state. In general, oligomerization produces red shifts, the amount of which depends upon the size of the aggregate and its detailed structure (see Katz *et al.*, 1991; Schertz *et al.*, 1991; for two reviews on this topic).

IV. COMPARISON WITH THE X-RAY STRUCTURE OF LH2 FROM *RHODOSPIRILLUM MOLISCHIANUM*

Recently, the X-ray structure of the LH2 complex from *Rhodospirillum molischanum* has been determined by a combination of molecular replacement using the coordinates from the *R. acidophila* structure and molecular modeling (Hu *et al.*, 1995; Koepke *et al.*, 1996). As with the LH2 complex from *R. acidophila*, the *R. molischanum* complex contains two apoproteins. The u-apoprotein has 56 amino acids and the α -apoprotein 45 amino acids (i.e. both longer than in *R. acidophila*). The current model of the *R. molischanum* LH2 structure includes all of the u-apoprotein residues and the first 43 of the α -apoprotein. Overall, the structure of the LH2 from *R. molischanum* is very similar to that of the *R. acidophila* complex but some interesting differences are revealed (Fig. 8).

The LH2 complex from *R. molischanum* is an $\alpha_8\beta_8$ octamer and not a nonamer as with the *R. acidophila* complex. As described above the α -apoprotein from the *R. molischanum* LH2 is rather longer than in *R. acidophila* and one of the consequences of this is that the Mg²⁺ atom at the center of the B800 BChl *a* macrocycle is coordinated to the γ_1 oxygen atom of α Asp6 (rather than a N-formyl methionine as in *R. acidophila*). The B800 macrocycle is also rotated compared with B800 in the *R. acidophila* complex.

In the *R. molischanum* structure the bacteriochlorin "ring" is no longer parallel to the presumed plane of the membrane but is "dipped" into it by about 20 ° and the macrocycle itself is rotated by 90 ° compared with the *R. acidophila* case. The C-terminal helix of the α -apoprotein is also longer than that seen in *R.*

acidophila. It is not yet clear from comparing the two structures why one is an octamer and the other a nonamer.

If the two structures are compared just at the level of their $\alpha\beta$ apoprotein pairs (Fig. 8), then they are really very similar. The same basic protein folds are seen even though the absolute sequence homology between *R. acidophila* and *R. molischianum* LH2 apoproteins is not very high (Fig. 4) (26% and 31% overall identity for the \sim - and fl-apoproteins, respectively).

Interestingly the CD spectrum of the LH2 complex from *R. molischianum* is strikingly different from that of *R. acidophila*. In this case the B800 band shows the strong double, conserved CD spectrum (B. Ücker and H. Scheer, unpublished observations). It remains to be seen how this can be explained.

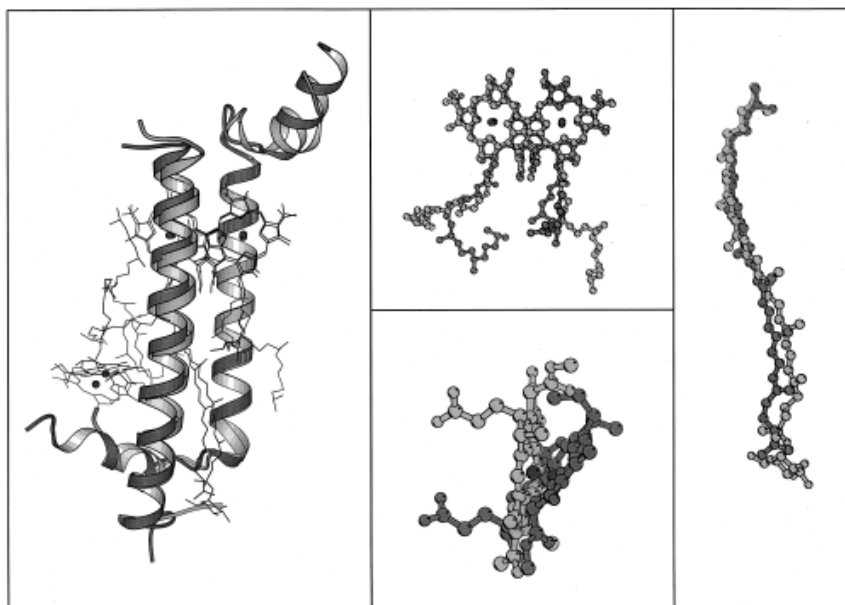


Fig. 8. A comparison of the LH2 protomers from *R. acidophila* strain 10050 and *Rhodospirillum molischianum*. The C_{α} atoms of residues α -22-29 from *R. molischianum* protomer have been optimized onto those of residues α -19-26 of the *R. acidophila* protomer (root-mean-square deviation 0.23 Å). Mg^{2+} ions are shown in magenta throughout. Elements of the protomer from the *R. molischianum* structure are highlighted in green, those from *R. acidophila* in yellow. Left- The complete optimized protomers. Upper middle-The B850 Bchl *as*. Lower middle-The B800 Bchl *as*. Right-The well-resolved carotenoids (lycopene in *R. molischianum*). We would like to thank Prof. H. Michel for access to the coordinates of the *R. molischianum* LH2 structure used in the preparation of this figure.

V. Bchl-Bchl ENERGY TRANSFER WITHIN LH2

There is extensive literature on energy transfer in photosynthetic systems. The following are a few recent reviews on this topic that interested readers may consult (van Grondelle *et al.*, 1994; Sundström and van Grondelle, 1995; Pearlstein, 1996; Pullerits and Sundström, 1996). We present here only a brief introduction to this topic in order to allow the non-specialist to cope with this section of our review.

Classically in photosynthesis (largely it has to be said in the absence of detailed structural information on the systems involved) singlet-singlet energy transfer, at least among the chlorophylls and BChls, has been discussed in terms of two limiting cases, Förster induced dipole-dipole resonance transfer (Förster, 1948) and Dexter electron exchange (Dexter, 1953). There are, of course, other cases, but for the purpose of this review we shall only consider further the Davydov (1962) treatment of exciton transfer.

The rate of energy transfer in the Förster weak interaction case is given by the following formula:

$$\text{rate} = K = \frac{9000(\ln 10) k^2 \Phi_d}{128 \pi^5 n^4 N \tau_d r^6} J$$

k = orientation factor
 Φ_d = donor fluorescence yield
 τ_d = donor fluorescence lifetime
 J = spectral overlap of the fluorescence emission of the donor with the absorption of the acceptor
 r = donor-acceptor distance
 n = refractive index
 N = Avogadro's number.

This type of energy transfer typically occurs between well separated pigments (20- 80 Å). It also formally requires that the excited state of the donor is thermally relaxed prior to the energy transfer step. In the Dexter electron exchange mechanism the rate of energy transfer is given by;

$$\text{rate} = K = \frac{4\pi}{n} Z^2 J$$

J = spectral overlap (as above)
 Z = is a distance parameter
 n = refractive index.

In this mechanism the pigments concerned must be close enough to each other so that there is electron exchange, i.e. within Van der Waal's contact. This is clearly a strong interaction case. Triplet-triplet energy transfer is believed to occur via this mechanism (Cogdell and Frank, 1987).

When there is strong Coulombic coupling of the transition dipoles of a group of pigments (such as may be the case for the B850 BChls) then energy transfer may occur via exciton transfer as described by Davydov (1962). In this case the energy transfer rate is proportional to the exciton energy of interaction, $E_{a,b}$.

$$E_{a,b} = \frac{C\mu_a\mu_b K}{R^3}$$

R = the intermolecular distance
 μ_a, μ_b are the transition dipoles of the two molecules a and b
 K = the orientation factor between the two transition dipoles and can vary between -2 and 2
 C = a constant which includes contributions from factors such as the dielectric constant.

Which of these, or indeed other, mechanisms operate in LH2 will depend upon which pigment groups are being considered. An initial estimate, based just on distance considerations, might lead to the conclusion that energy transfer from B800→B800 or from B800→B850 might go via the Förster dipole-dipole exchange mechanism, whereas B850→B850 (where the BChls are closely associated) may involve exciton exchange mechanisms. We shall now explore whether these first impressions are correct or not.

However, before this, it is important to consider how it is possible to distinguish between the various mechanisms. Clearly one way to test for the Förster mechanism is to vary the distance between the donor and acceptor and look for the $1/r^6$ dependence of the energy transfer rate. This is not possible in LH2 which has a fixed structure. Indeed, whereas it can be possible to show that a given mechanism is impossible from the structure; i.e. if the pigments concerned are too far apart, then electron exchange is impossible, it will be very difficult for practical reasons to prove that a given mechanism actually is taking place. It might be thought, given the structure, that the rate of energy transfer should be able to be calculated via the different mechanisms and then compared with the experimentally measured rates. Even this is not trivial. In many mechanisms, for example, the refractive index of the medium appears. This is a bulk phase, average parameter. What it means in terms of the specific, local environment between the donor and acceptor pigments in the protein is entirely unclear. These are therefore very real problems in critically distinguishing between possible mechanisms of energy transfer in a protein complex like LH2 compared with model systems in organic solvents.

Notwithstanding these inherent problems, we shall continue here to consider the BChl-BChl energy transfer processes going on within LH2 by starting with a simple model and then developing it in order to explain the actual experimental data. Since there is good spectral separation between the different pigment groups in LH2, it is possible to use timeresolved spectroscopic methods to investigate the energy transfer

processes. In this context though, not all the experiments we need to describe have yet been carried out in the LH2 from *R. acidophila* and so, where necessary, we shall also discuss data obtained with the LH2 complex from *R. sphaeroides*.

Initially we shall assume each BChl molecule can be considered as independent monomeric species. If LH2 is excited with a sub 100 fsec pulse at 800 nm, then at room temperature it takes ~0.7 psec for the energy to be transferred from B800 to B850 in *R. sphaeroides* (Hess *et al.*, 1995; Jimenez *et al.*, 1996; Soo *et al.*, 1996) and ~0.8 psec in *R. acidophila* (Ma *et al.*, 1996). Energy transfer among the nine B800 BChls during the lifetime of B800* can be explored by looking at the decay of the anisotropy of this excited state population, since each B800 BChl *a* molecule has a different orientation with respect to the direction of the exciting laser pulse. The time constant for the decay of the anisotropy of the B800* population was found to be ~0.3 psec (Hess *et al.*, 1996) which indicates a fast B800→B800 energy transfer is taking place. If it is assumed that the B800→B800 and B800→B850 energy transfers occur by the Förster mechanism and that only the distance is different in each case then, since the B800-B800 distance is 21.2 Å and the B800→B850 distance is ~18 Å, it might be expected that the B800→B850 transfer would be 2.7 times faster than the B800 to B800 transfer. However, the B800→B800 transfer is faster. From the structure it is possible to calculate the κ^2 orientation factors between these pigment groups (Table 1). The value of κ^2 for B800→B800 is 1.54, while it is only 0.61 for B800 to the α -bound B850 BChl. This then would reduce the expected difference in rates from a factor of 2.7 to 1.07. Furthermore, the spectral overlap term is also different, and so at present it is not possible to say with any real certainty whether or not the measured rates are consistent with the Förster mechanism.

Table 1. (κ^2)² values for the relative orientations of the BChl *a* chromophores in LH2

BChl <i>a</i> pairs	Mg ²⁺ -Mg ²⁺ distance (Å)	(κ^2) ² for the Q _y transition
α B850/ β B850 ^a	9.6	2.79
β B850/ α B850 ^b	8.9	1.42
B800/ α B850	17.4	0.01
B800/ β B850	18.2	0.01
B800/B800	21.2	1.54

^aWithin an $\alpha\beta$ protomer.

^bOutside an $\alpha\beta$ protomer.

Once the energy reaches the B850 ring of BChls in the absence of any other nearby antenna complexes the B850* excited state lasts for hundreds of psec (Sauer *et al.*, 1996). The anisotropy of B850* decays to half its initial value in about 300-500 fsec (Jimenez *et al.*, 1996). If this can be explained by the excited state "hopping" from BChl to BChl in the B850 manifold then the hopping time is < 50 fsec. However, it is very clear from the structure that B850 BChls cannot be considered as monomers. Given the structure of LH2 it should in principle be possible to then calculate the absorption and CD spectra of LH2. Attempts to do this are currently underway (Alden *et al.*, 1996; Koolhaas *et al.*, 1996; Sauer *et al.*, 1996) but these are now sufficiently advanced to allow some significant conclusions to be made. The B800 BChls do indeed behave as monomers. However, in order to get good agreement between theory and experiment the B850 BChls have to be considered as a single strongly coupled excitonic unit. In this regard it appears only possible to simulate the CD spectrum, where its zero crossing is to the red of the absorption maximum of B850, when strong, extensive exciton coupling among, if not all, then certainly most of the B850 chromophores is assumed. This then implies that the 18 molecules of BChl in the B850 ring must be viewed as a "supermolecule". This then calls into question the simple picture of energy transfer from B800 to B850 and the decay of the B850* anisotropy discussed above. This decay may therefore reflect relaxation among the various exciton states rather than BChl to BChl "hopping" of the energy. If the correct description of B850 is indeed a strongly exciton-coupled ensemble, then the details of the mechanism of energy transfer must include a description of the exciton states involved. Is there any other independent experimental evidence for or against this view?

If B850 BChls could be viewed as monomeric then, if a weak exciting pulse was delivered into B800 or the carotenoid absorption band (since the relative extinction coefficients of those bands are rather similar),

the extent of the bleaching seen at the excitation wavelength should be about the same as that seen at 850 nm. This, however, is not the case. The extent of the bleaching in the 850 nm band is always between three and five times as large as would be expected if the B850 absorption band was simply due to monomeric BChl (Abdourakhmanov *et al.*, 1989; Kennis *et al.*, 1994, 1996, Xiao *et al.*, 1994). This anomaly has been explained by assuming that the B580 absorption band is in fact due to a strongly exciton-coupled system. In this case then the larger bleaching seen at around 850 nm is exactly what would be predicted (Novoderezhkin and Razjivin, 1993, 1994, 1995). The Russian group in particular have provided a detailed explanation of this effect and show that even where more than 20 molecules of BChl contribute to the absorption band, the extra extent of bleaching will only be in the range of four to five times greater than that of a monomer. However, there has been some difference of opinion on this point (Monshouwer *et al.*, 1995; Jimenez *et al.*, 1996; Pullerits and Sundström, 1996) and several groups still favor the idea that the exciton is much more localized, maybe on one or two dimers of BChl only. If the correct description of the B850 "ring" is a fully delocalized one, then the κ^2 values calculated in Table 1 will not be the relevant ones. The direction of the transition dipoles of the exciton states involved in the energy transfer will be the important ones to know. As yet, however, there is no firm data on these.

There have also been some interesting studies aimed at investigating whether the rate of energy transfer from B800 to B850 depends upon the spectral overlap between these two groups of pigments in the way suggested by the Förster equation. In one recent report Hess *et al.* (1994) used a site-directed mutagenesis strategy to test this. LH2 from *R. sphaeroides* was engineered to produce complexes which had altered absorption maxima for the B850 band (i.e. 850, 839 and 826 nm) (Fowler *et al.*, 1994). The results of their pump-probe time-resolved study showed reasonable agreement with the behaviour expected from the Förster equation. On the other hand though, "hole-burning" studies (readers not familiar with "hole-burning" can consult Jankowiak and Small (1993) for a clear, concise account of what this technique is and what information can be extracted from it when using photosynthetic pigment-protein complexes) have suggested that the B800→B850 energy transfer cannot be simply explained by a Förster model which assumes that this energy transfer occurs from the Q_y 0-0 transition of B800 to the broad vibronic B850 absorption band (van der Laan *et al.*, 1993; De Caro *et al.*, 1994; Reddy *et al.*, 1996; Wu *et al.*, 1996). As the temperature is reduced, especially in the case of LH2 from *R. acidophila*, the B800 Q_y absorption band sharpens and the B850 Q_y absorption band shifts to the red. A similar, reversible red shift of the B850 Q_y absorption band can be achieved by the application of high pressure (up to 680 mPa). In both cases the "holeburning" experiments showed that the rate of energy transfer from B800 to B850 is largely independent of the gross spectral overlap. Two explanations for this have been suggested. The energy transfer could go either via higher energy vibronic bands of B850 or via higher energy exciton states. In both cases these are assumed to be rather coincident with the B800 Q_y absorption band itself. Further experimentation is required to sort out these two possibilities.

Very recently, Nagarajan *et al.* (1996) investigated the ultrafast relaxation of the B850 absorption band in LH2 complexes of *R. sphaeroides* when excited by a 35 fsec excitation pulse. Anisotropy measurements with this improved time resolution revealed an initial very high anisotropy of ~ 0.9 which decayed with a single time constant of ~ 20 fsec to an intermediate value of ~ 0.2 . These results have been interpreted to reflect dephasing of the exciton states. These authors conclude "we interpret the relaxation as internal exciton decay into a dark state. Given the circular arrangement of the BChl molecule, the combination of allowed higher-energy excited states and a dark lower state strongly supports the view that the exciton is delocalized over the entire ring."

Strong support for the extensive delocalization of the excitation energy in the B850 "ring" has also come from non-linear optical spectroscopy. These experiments with LH2 from both *R. sphaeroides* and *R. acidophila* have shown very large dipole moments for the transition between the ground state and the one-exciton band, as well as for the transition between the one- and two-exciton bands, corresponding to delocalization of the excitation energy over, if not all, then certainly most of the 18 BChl molecules of B850 (Leupold *et al.*, 1996; Stiel *et al.*, 1996).

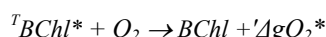
It is clear that there are some very subtle features of LH2 with regard to BChl-BChl energy transfer, which are only just beginning to be understood.

VI. ENERGY TRANSFER BETWEEN CAROTENOIDS AND BChL IN LH2

Energy transfer between carotenoids and BChl in LH2 takes place in both directions. There is triplet-triplet energy transfer from the BChl to the carotenoid and singlet-singlet energy transfer from the carotenoid to the BChl (Cogdell and Frank, 1987).

6.1. Triplet-Triplet Energy Transfer

If BChl *a* is excited by light then a proportion of the molecules will undergo intersystem crossing to produce BChl triplets ($^1\text{BChl}^*$). In the presence of molecular oxygen these triplets can sensitize the formation of singlet O_2 ($^1\Delta\text{g O}_2^*$), which is a very powerful and damaging oxidizing agent (Foote, 1968).



Singlet oxygen is potentially lethal and cells exposed to it are usually rapidly killed. Carotenoids prevent this harmful reaction in LH2 by quenching the $^1\text{BChl}^*$ to form $^1\text{Car}^*$, which is too low in energy to be able to sensitize the formation of singlet oxygen (Cogdell and Frank, 1987). This triplet-triplet exchange reaction has been studied with time-resolved pump probe optical spectroscopy, electron spin resonance and optically detected magnetic resonance (e.g., see Monger *et al.*, 1976; Frank *et al.*, 1987; Angerhöfer *et al.*, 1995). The results of these experiments can be summarized as follows. In the absence of carotenoids (e.g., LH2 from *R. sphaeroides* R26.1) $^1\text{BChl}^*$ produced in the antenna complex reacts with oxygen at essentially diffusion-limited rates (in practice, this means on the μsec time-scale). If, however, there are carotenoids present in LH2 then, following excitation of the BChl, $^1\text{Car}^*$ is formed in a few nsec. It then decays in a few μsec , releasing the excess energy harmlessly as heat. Angerhöfer *et al.* (1995) showed that in LH2 from *R. acidophila* strain 10050 the rate of car^1 formation was $\sim 6 \times 10^{-7}$ sec at room temperature and $\sim 3 \times 10^{-7}$ sec at 5 K (i.e. largely temperature independent). The rate of car^1 decay, $\sim 7 \times 10^{-4}$ sec, was also rather temperature independent. In these studies the LH2 samples were excited by light in the wavelength range 820-850 nm where only BChl absorbs. The protective effect is therefore due to the carotenoid quenching the $^1\text{BChl}^*$ three orders of magnitude faster than oxygen. As long as the number of conjugated double bonds in the carotenoid in LH2 is 10 or more the triplet-triplet exchange reaction is largely temperature independent down to 4 K (Angerhöfer *et al.*, 1995). It has always been assumed that this energy transfer process takes place via an electron exchange mechanism. It is comforting therefore that the carotenoids in LH2 are in Van der Waal's contact with the BChls.

6.2. Singlet-Singlet Energy Transfer

Understanding the carotenoid to BChl singlet-singlet energy transfer (the accessory light-harvesting role of carotenoids) in LH2 is by no means as straightforward as understanding the triplet-triplet exchange. The accessory light-harvesting role of carotenoids in LH2 is very easy to demonstrate by recording the fluorescence excitation spectrum of the B850 band (Cogdell *et al.*, 1981). Typical results are summarized in Table 2. Efficiencies for this process range from about 50 to 100%. Why is this difficult to understand? The problem arises because the quantum yield of fluorescence of the main types of carotenoid found in LH2 is very low. They are typically in the range 10^{-4} - 10^{-5} , which then translates into an excited state lifetime of 100-200 fsec (Frank and Cogdell, 1996; Koyama *et al.*, 1996). This is a very short time in which efficient singlet-singlet energy transfer can take place. Indeed, if you were designing a molecule to be an effective antenna pigment, you would definitely not select a pigment with such a short excited state lifetime! How then can carotenoids be such efficient accessory light-harvesters? Carotenoids have rather unusual photochemical properties (Fig. 9). The allowed singlet transition from the ground state which gives the carotenoids their strong absorption bands in the visible region of the spectrum is due to the second excited singlet state, S_2 , which is also called the $^1\text{Bu}^*$ state (Hudson *et al.*, 1982; Frank and Cogdell, 1996; Koyama *et al.*, 1996). The lowest excited singlet state, S_1 , is the $^2\text{Ag}^*$ state, which for symmetry reasons is essentially forbidden for a one-photon transition from the ground state (^1Ag). When carotenoids in organic solvents are excited by light the S_2 state is initially populated. Then in a few 100 fsec this relaxes internally to produce S_1^* . The S_1^* state then decays to the ground state in a few psec (Koyama *et al.*, 1996). The vibronic coupling between S_1 and the S_0 states is the most probable reason for the rapid relaxation of S_1 . This has been well characterized by psec Raman spectroscopy (Noguchi *et al.*, 1990; Kuki *et al.*, 1994), studying the C=C stretching Raman line of the S_1 state with a variety of bacterial carotenoids, both in organic solvents and in LH2 complexes.

Table 2. A representative compilation to illustrate the variation of the efficiency of carotenoid to BChl singlet-singlet energy transfer in some LH2 complexes

Species (strain)	Major carotenoid type	Energy transfer efficiency (%)	Refs.
<i>R. sphaeroides</i> strain 2.4.1	spheroidene	100	Cogdell <i>et al.</i> , 1981
strain GIC	neurosporene	95	
aerobic 2.4.1	spheroidenone	75	
<i>R. acidophila</i> strain 10050	rhodopin glucoside	~50	Angerhöfer <i>et al.</i> , 1986
strain 7050 (B800-820)	rhodopinal glucoside	~70-75	

The questions to be addressed, therefore, in LH2 are which of these two excited states donates its energy to the BChls, which BChls are involved and finally by which mechanism(s) does this energy transfer take place? This area has recently been reviewed (Frank and Cogdell, 1996; Koyama *et al.*, 1996) and is one of very active ongoing research. These questions have not yet been fully answered and so what will be presented here is only the current view. This view may well change substantially in the near future.

A detailed consideration of the singlet-singlet energy transfer from carotenoids to the BChl in LH2 must first consider the energy levels of the possible singlet states involved (Fig. 10). For the S_2 ($^1Bu^*$) state this is straightforward and can be determined from the absorption spectrum and the weak fluorescence emission spectrum. However, for the S_1 ($^2Ag^*$) this is not so easy. The symmetry forbiddance of the optical transition from S_0 to S_1 has hindered the direct determination of the S_1 energy level. This is a real problem since a major route of energy transfer is, in many systems, likely to involve the S_1 state of the carotenoid as an energy donor and it is therefore essential to know where this state is located on an energy scale relative to the BChl acceptor levels. A major obstacle has also been the lack of observable fluorescence from the S_1 state of carotenoids with longer than nine conjugated double bonds. This problem has recently been discussed in detail (Koyama *et al.*, 1996). The best estimate for the S_2 and S_1 energy levels of rhodopin glucoside (11 conjugated double bonds) are $\sim 19,500\text{ cm}^{-1}$ and $\sim 13,200\text{ cm}^{-1}$, respectively.

The energy levels of the possible BChl acceptors in LH2 are $\sim 17,000\text{ cm}^{-1}$ (Q_x), $\sim 12,700\text{ cm}^{-1}$ (B800 Q_y) and $\sim 11,800\text{ cm}^{-1}$ (B850 Q_y). Immediately then, on energy grounds (i.e. the spectral overlap term-see section 5), one might expect energy transfer from S_2 to go via the BChl Q_x band and from S_1 to go via the Q_y band.

If the carotenoid rhodopin glucoside (which is present in LH2 from *R. acidophila*) is dissolved in an organic solvent in which it absorbs at the same wavelength as in LH2 and is excited with a ~ 100 fsec pulsed light, its S_2 lifetime can be determined using the technique of fluorescence up-conversion (McPherson, A., Arellano, J., Gillbro, T. and Cogdell, R. J., 1996; unpublished observations). Under these conditions its decay time has been measured to be 135 fsec. In the LH2 complex this S_2 lifetime is reduced to 68 fsec, indicating that energy transfer (and therefore a shortening of the S_2 lifetime) has occurred to the BChls. The arrival of this energy at the BChl Q_y bands can also be looked for by monitoring the rise of the sensitized BChl fluorescence at 875 nm (B850) and 795 nm (B800) (again using the fluorescence up-conversion technique). The results are somewhat complicated. At 875 nm the fluorescence rise time is dominated by a fast phase, ~ 129 fsec ($\sim 70\%$), the rate of which is wavelength dependent. It is ~ 60 fsec at 850 nm and ~ 200 fsec at 900 nm. About 30% of the fluorescence at 875 nm had a slower rise time of ~ 875 fsec. The wavelength-dependent rise times may represent decay of the exciton states in the 850 nm absorption band that were described in the previous section. When the rise of the fluorescence at 795 nm was recorded, it too was found to be fast, with essentially a single rise component of ~ 156 fsec. The decay of the fluorescence at 795 nm (B800) reflected B800 \rightarrow B850, BChl \rightarrow BChl energy transfer. This corresponded to about 30% of the total carotenoid to BChl energy transfer, and may be the cause of most of the slow rise in fluorescence seen at 875 nm. It is clear, therefore, in this case that most of the energy transfer occurs from S_2 to B850 directly. For energetic reasons this probably goes via the Q_x band of the B850 BChls. Only a rather small fraction of the energy may therefore come via S_1 and this may explain why the efficiency of energy transfer from rhodopin glucoside to the BChl in LH2 is rather low.

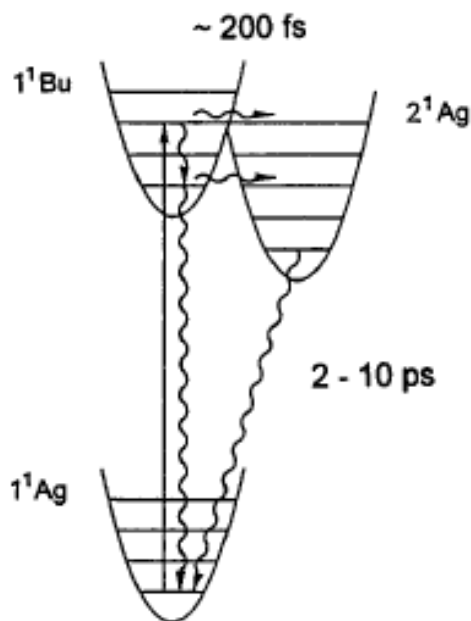


Fig. 9. A schematic representation of the S_2 and S_1 excited singlet states of carotenoids. The approximate times for the transitions $S_2 \rightarrow S_1$ and $S_1 \rightarrow S_0$ are also shown. The $S_0 \rightarrow S_2$ represents the allowed optical (one photon) transition that gives rise to the carotenoid's well known absorption spectrum in the visible region of the spectrum.

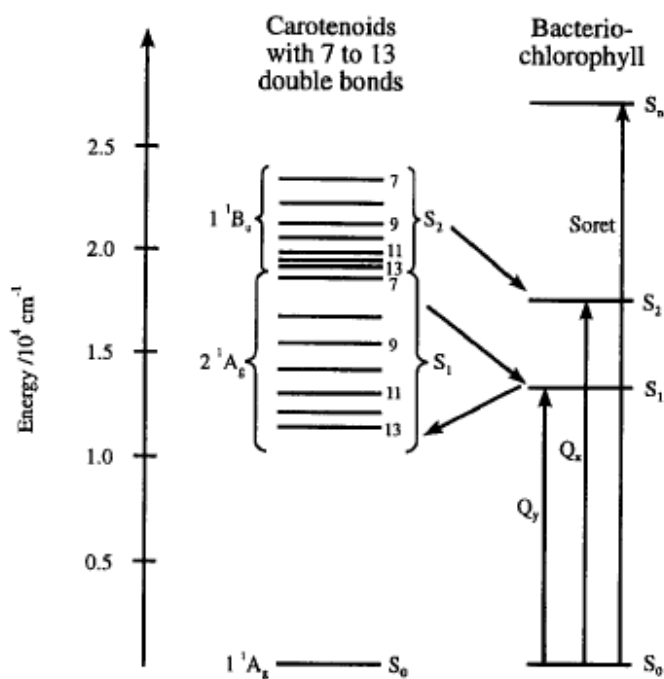


Fig. 10. A tentative energy level scheme for the important singlet states involved in carotenoid to BChl a singlet-singlet energy transfer. The data for this figure were taken from Frank and Cogdell (1993).

Figure 11 shows the relative orientation of the transition dipoles of the BChls in LH2 and the carotenoids. If it can be assumed that the transition dipoles of the carotenoid are essentially parallel to its long axis, then it can be seen that the Q_x transition of the B850 BChls is favorably oriented to accept energy from the carotenoid, while all the other BChl transition dipole moments are unfavorably oriented. This implies then that only energy transfer from S_2 to the Q_x of B850 could possibly go by the Förster mechanism. Energy transfer from S_1 (due to its lower energy) must go into the Q_y transition of the acceptor BChls, implying that mechanisms such as electron exchange or Coulomb coupling (Nagae *et al.*, 1993) must be involved for this part of the energy transfer process, since due to its forbidden nature energy transfer from S_1 by the Förster mechanism is not allowed. In other words, the exact mechanism of energy transfer will vary depending on which of the carotenoid donor's excited singlet states are involved, and this in turn can change with time.

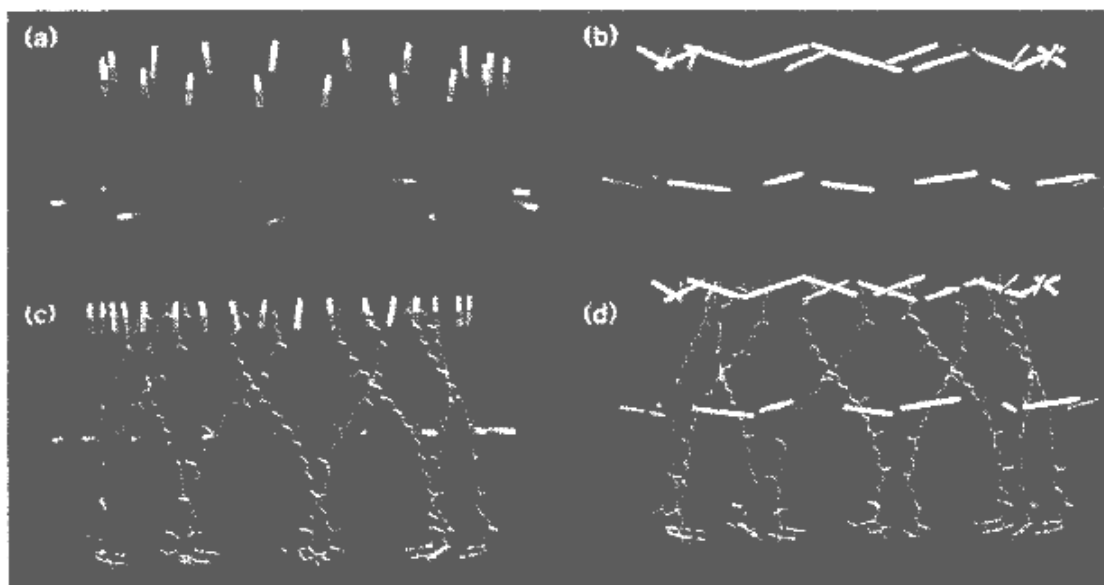


Fig. 11. The relative orientation of the transition dipoles of the pigments in LH2 from *R. acidophila* strain 10050. (a) the alignment of the Q_x dipoles (green/yellow). (b) The alignment of the Q_y dipoles (red/white). (c) The alignment of the well resolved carotenoid to the Q_x dipoles. (d) The alignment of the well resolved carotenoid to the Q_y dipoles. This figure was produced using O (Jones *et al.*, 1991).

In other antenna systems the balance between how much energy comes from S_2 and how much from S_1 will vary depending on which light-harvesting complex is being studied. Akimoto *et al.* (1996) monitored the rate of energy transfer from the carotenoid (peridinin) to chlorophyll *a* in the water soluble peridinin-Chl *a* antenna complex from a dinoflagellate, *Alexandrium cohorticula*. This energy transfer process is ~100% efficient. In this case the energy transfer only occurred from S_1 in ≤ 3 psec.

It is clear therefore that although several mechanisms have been discussed to explain carotenoid to BChl energy transfer (e.g., Shreve *et al.*, 1991; Frank and Cogdell, 1993) there are as yet no really definitive answers. Indeed, the exact mechanism may well depend precisely on which antenna complex is being considered and, moreover, as mentioned above, many actually change during the time-course in which it is occurring as the carotenoid S_2 state evolves into the S_1 state.

VII. FINAL REMARKS

For people interested in the process of light-harvesting in photosynthesis, now is a particularly exciting time. High-resolution structural information is available for a wide range of different types of antenna complexes, e.g., three types of water soluble antenna complexes, the BChl *a* antenna complex from *Prosthecochloris aestuarii* (the FMO-protein) (Tronrud *et al.*, 1986), a range of phycobiliproteins (Schirmer *et al.*, 1985, 1986; Fiener and Huber, 1993), and the peridinin-chl *a* antenna complex from the dinoflagellate *Amphidinium carterae* (Hofmann *et al.*, 1996), as well as for two types of integral membrane antenna complexes, the higher plant LHC II complex (Kühlbrandt *et al.*, 1994) and the purple bacterial LH2 complex (McDermott *et al.*, 1995; Koepeke *et al.*, 1996).

At first glance, however, it appears that, unlike the photosynthetic reaction centers, where there is a strong conservation of the basic structure across all types of reaction centers (Witt, 1996), the light-harvesting complexes all have very different structures. It is hard therefore to identify any general structural features which may have fundamental significance for the basic process of light-harvesting.

Why should this be? Are there any ways to understand the reasons for this diversity? In order to do this it must first be appreciated what an antenna complex is designed to achieve. A light-harvesting complex must be optimized for the following:

- (a) to absorb maximally in that region of the spectrum of solar radiation that is available to the organism concerned in its particular ecological niche;
- (b) to transfer that absorbed solar radiation rapidly and efficiently to either the reaction centers directly or the next antenna complex in the energy transfer pathway towards the reaction centers.

Different antenna complexes are clearly optimized for absorbing solar radiation in different spectral regions. In many cases this involves the use of different pigment molecules and this might be expected to impose different structural requirements on the pigment-binding apoproteins. Dinoflagellates, for example, in their marine environment "see" mainly blue light. In this case their major antenna complex, of which the peridinin-chl *a* complex is a typical example (Hofmann *et al.*, 1996), mainly contains carotenoids (in the peridinin-chl *a* complex there is a ratio of car:chl *a* of 4:1). These carotenoids absorb the blue light and with an efficiency of nearly 100% transfer it to the chlorophyll molecules, thereby making it available for photosynthesis (Song *et al.*, 1996). Purple bacteria, on the other hand, with their anaerobic pattern of photosynthesis are found in the lower levels of ponds and streams. They, then, only receive solar radiation after it has been filtered by passing through a layer of oxygenic photosynthetic organisms. They therefore are adapted to use either green light or light further to the red beyond where chlorophyll *a* absorbs. Hence, their antenna complexes use both carotenoids (absorbing in the green) and BChl *a* (absorbing in the NIR).

The water-soluble antenna complexes are in general extrinsic membrane proteins. They are located on the membrane surface and pass their excitation energy down into the membrane where the reaction centers and integral-membrane antenna complexes are located. It is not surprising, then, that they are quite different from LHCII and LH2. One might, however, expect that the integral membrane light-harvesting complexes should show some similarity of structure. In the case of LHCII and LH2 this is not the case. Here are two clear examples of antenna complexes which have evolved to optimize the flow of energy to their respective reaction centers. Though the core of the reaction centers appear to be structurally rather similar, comparing PS II with purple bacterial reaction centers (Michel and Deisenhofer, 1988), the higher plant reaction centers are essentially embedded in "core" antenna systems, which are densely packed with chlorophylls. LHCII has therefore to be able to pass its absorbed light energy to those complex "core" structures in which the chlorophylls are expected to be located at all levels across the membrane (Thornber *et al.*, 1994). This then is probably why in the structure of the LHCII the chlorophylls appear to be more randomly distributed throughout the protein, compared with the more regular arrangement seen in LH2. On the other hand, the purple bacterial reaction center is much less complex. The energy must reach the "special pair" of BChl *a* molecules in the reaction center and these are located on the periplasmic side of the photosynthetic membrane. LH2, and indeed LH1 (to which it transfers its energy on the way to the reaction center), has its donor-BChl *as* (the B850 "ring" in LH2 and the B875 "ring" in LH1) at the same level in the membrane as the "special pair" in the reaction center (Karrasch *et al.*, 1995; Papiz *et al.*, 1996). Therefore, the donor and acceptor BChl *as* can get as close as possible to each other to maximize the rate of energy transfer. The final point to appreciate in these systems is that excitation energy transfer is essentially temperature independent. In most cases the efficiency of energy transfer is hardly changed on going from room temperature to 4 K. This implies that the process is essentially equivalent to a "hard-wired solid state system". In order for this to occur, the antenna complexes must provide a rather rigid structural framework within which the light-absorbing pigments are precisely organized to promote rapid and efficient transfer of the excitation energy. It is clearly therefore crucial for function that the antenna complexes are "tailored" to match the structure of the pigment-protein complex to which the energy is to be transferred, since there is no time for gross motion during the process of energy transfer.

Probing the detailed mechanisms of energy transfer within the antenna complex for which there are structures is a very active ongoing area of research. We expect significant progress to be made in this area in the next few years, and if our experience with LH2 from *R. acidophila* is mirrored in the other antenna systems, then it is clear that we are only just beginning to understand the very beautiful and subtle ways in

which the various antenna complexes have been engineered by evolution to be such efficient harvesters of solar radiation.

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