Comparison of the Structural Requirements for Bacteriochlorophyll Binding in the Core Light-Harvesting Complexes of *Rhodospirillum rubrum* and *Rhodobacter sphaeroides* Using Reconstitution Methodology with Bacteriochlorophyll Analogs[†]

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Received August 1, 1995; Revised Manuscript Received December 28, 1995[⊗]

ABSTRACT: Bacteriochlorophyll (BChl) structural requirements for formation of the core light-harvesting complex (LH1) and its structural subunit complex were examined by reconstitution with BChl analogs and the α- and *β*-polypeptides of *Rhodospirillum rubrum* and *Rhodobacter sphaeroides*. Comparable results were obtained with most of the BChl analogs and the polypeptides of each bacterium, indicating the conservation of BChl binding sites. These systems showed the following common requirements for formation of the subunit complex and LH1: (1) Mg or a metal of similar size and coordination chemistry (e.g., Zn, Cd, Ni), (2) a bacteriochlorin oxidation state of the macrocyclic ring, (3) a 13^2-carbomethoxy group, and (4) an intact ring V. Some structural features were not as critically important. For example, the subunit complex and LH1 could be formed with both sets of polypeptides and BChl *b*, as well as with analogs containing either short (ethanol) or long (phytol) esterifying alcohols. Two derivatives were identified that behave differently with the two sets of polypeptides. The 3-acetyl group is required to form LH1 in both bacteria, although a subunit-type complex was readily formed with [3-vinyl]BChl *a* and the polypeptides of *Rs. rubrum* but formed only slightly under special conditions with polypeptides of *Rb. sphaeroides*. [13^2-OH]BChl a_p formed both subunit- and LH1-type complexes with the α- and *β*-polypeptides of *Rb. sphaeroides* but not with those of *Rs. rubrum*. Thus, some subtle differences in the BChl binding sites exist in the LH1 complexes of these two bacteria.

The light-harvesting (LH)[1] complexes of photosynthetic bacteria, which consist of integral membrane protein, bacteriochlorophyll (BChl), and carotenoid, exhibit a highly associated structure which contains multiple copies of two small polypeptides. These polypeptides occur in a 1:1 stoichiometry and bind two or three BChl molecules per α*β* pair, depending on whether the light-harvesting complex is a core (LH1) or an accessory (LH2) complex (Cogdell, 1986; Drews, 1985; Thornber, 1986; Zuber & Brunisholz, 1991). All wild-type bacteria contain LH1, and many contain one or more types of accessory BChl-containing light-harvesting complexes. On the basis of analytical determination of BChl to reaction center (RC) ratios, native LH1 of *Rs. rubrum* was estimated to contain 12 copies of each of the α- and *β*-polypeptides, 24 BChl, and 12 carotenoid (Loach & Sekura, 1968; Loach, 1980; Miller et al., 1987). According to a recent study using cryoelectron microscopy, it has been proposed that there are 16 α- and *β*-polypeptides, 32 BChl, and 16 carotenoid per RC (Karrasch et al., 1995). Structurally, LH1 is thought to consist of a repeating subunit, $\alpha_1\beta_1$·2BChl, which has been isolated and characterized from seven different bacteria (Loach et al., 1985; Miller et al., 1987; Heller & Loach, 1990; Chang et al., 1990a; Meckenstock et al., 1992; Jirsakova & Reiss-Husson, 1994; Parkes-Loach et al., 1994; Kerfeld et al., 1994). This isolated subunit complex can be reassociated to form native LH1 as well as reversibly dissociated to its individual components (Miller et al., 1987; Parkes-Loach et al., 1988; Ghosh et al., 1988; Loach et al., 1994; Davis et al., 1995). Thus, reconstitution of the subunit complex and LH1 from BChl *a* and isolated α- and *β*-polypeptides provides an important tool for analysis of structure−function relationships.

Recently, a crystal structure of LH2 has been reported (McDermott et al., 1995). Many of the structural features of LH2 undoubtedly have relevance to the structure of LH1 and its subunit complex discussed in this paper. In the LH2 structure, there are two concentric cylinders of helical protein subunits which enclose the pigment molecules. At one "end" of the cylindrical structure, nine BChl molecules are positioned between the outer helices constituted by the *β*-polypep-

[†] This research was supported by a grant to P.A.L. from the U.S. Public Health Service (Grant GM11741).

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[⊗] Abstract published in *Advance ACS Abstracts,* February 15, 1996.

[1] Abbreviations: BChl, bacteriochlorophyll; BChl a_p, BChl a_{gg}, BChl a_m, BChl a_e, and BChl a_{eg}, bacteriochlorophyll *a* esterified with phytol, geranylgeraniol, methanol, ethanol, and ethylene glycol, respectively; BChl *b*, bacteriochlorophyll *b*, BPh, bacteriopheophytin; HmIX DME, hematophorphyrin IX dimethyl ester; LH1, the core light-harvesting complex (also called B873); subunit complex (also referred to as B820), the subunit form of LH1 consisting of native α- and *β*-polypeptides with the same stoichiometry of $\alpha_1\beta_1$·2BChl as LH1; subunit-type complex, a reconstituted complex exhibiting absorption and CD spectra highly similar to those of the native subunit complex but containing a BChl analog; subunit-type (*β*) complex, a reconstituted complex exhibiting absorption and CD spectra highly similar to those of the native subunit complex but containing only the *β*-polypeptide and BChl; LH1-type complex, a reconstituted complex containing a BChl analog and displaying absorption and CD spectra highly similar to those of native LH1; RC, reaction center; PRC, photoreceptor complex consisting of the RC and LH1; CD, circular dichroism; OG, *n*-octyl *β*-D-glucopyranoside; HFA, hexafluoroacetone trihydrate; near-IR, near infrared.

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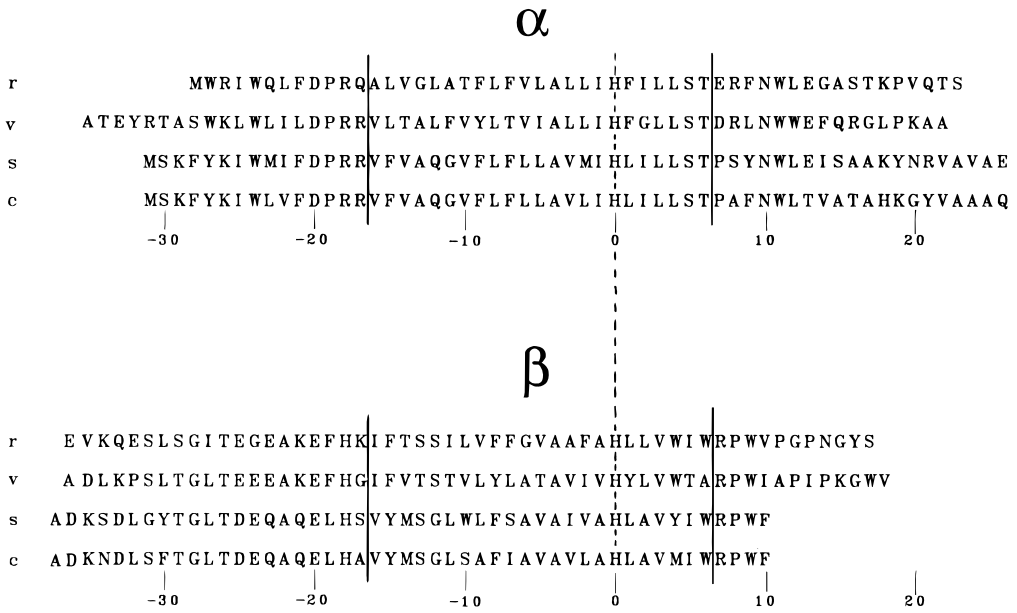
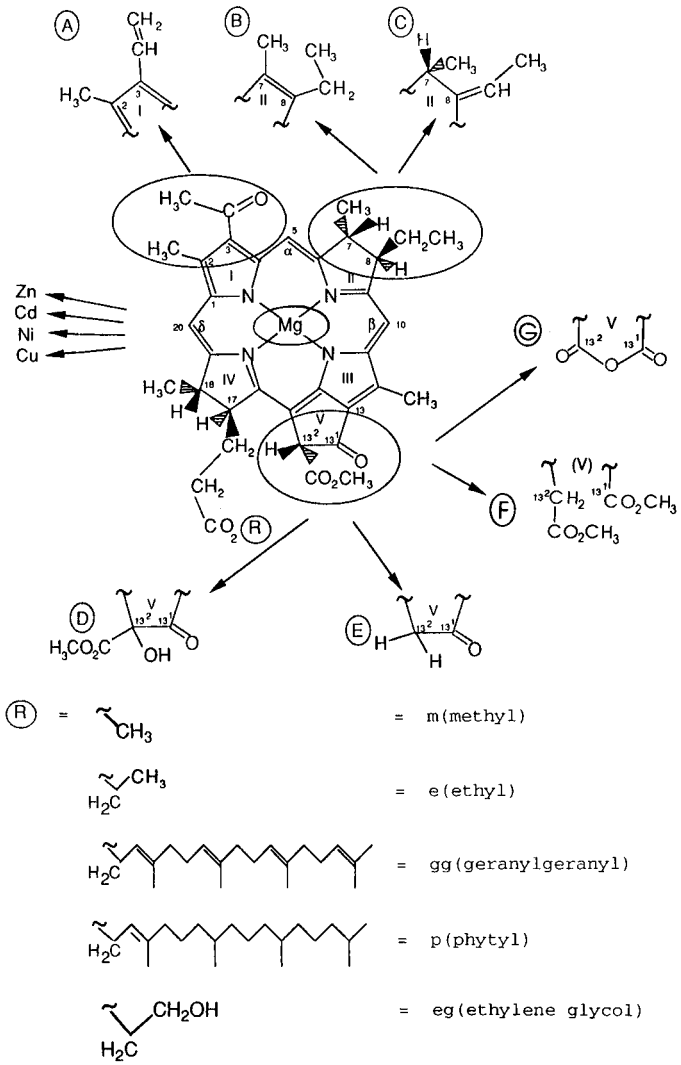


FIGURE 1: Amino acid sequences of the α- and *β*-polypeptides of four bacteria. r, *Rs. rubrum* (Brunisholz et al., 1981; Gogel et al., 1983; Brunisholz et al., 1984; Bérard et al., 1986); v, *Rps. viridis* (Brunisholz et al., 1985; Wiessner et al., 1990); s, *Rb. sphaeroides* (Theiler et al., 1985; Kiley et al., 1987); c, *Rb. capsulatus* (Tadros et al., 1984, 1985; Youvan et al., 1984). For ease of comparison, the common histidine residue nearest the C-terminus, which is the likely Mg-binding residue, was used for alignment and defined as the zero position.

tides, with the bacteriochlorin rings positioned perpendicular to the transmembrane helix axis. At the other "end" of the cylindrical structure, an additional 18 BChl *a* molecules are sandwiched between the two rings of helices and form a continuous overlapping ring. The BChl in this latter ring are thought to be responsible for the 850 nm absorption band of LH2 and are believed to exist in a structural environment with some similarity to those of the BChl in LH1. A similar but somewhat larger ring of $\alpha_1\beta_1$·2BChl units has also been proposed for LH1 structure based on electron cryomicroscopy measurements (Karrasch et al., 1995). The BChl thought to be responsible for the 850 nm absorbance of LH2 are coordinated to His 0 (see Figure 1 for the amino acid numbering scheme) of the α- and *β*-polypeptides of LH2 as predicted (Zuber & Brunisholz, 1991; Robert & Lutz, 1985). Two different repeating $\alpha_1\beta_1$·2BChl units can be considered in the LH2 structure, one of which is remarkably similar to a recent suggestion for the subunit structure of LH1 (see Figures 5A and 5C of Loach & Parkes-Loach, 1995). The only apparent hydrogen bonds to BChl observed in the 850 nm component of LH2 were between the 3-acetyl oxygens (see Figure 2 for the structure of BChl and the numbering system used for its atoms) and Tyr 13 and Trp 14 of the α-polypeptides (McDermott et al., 1995). A role for these amino acids in hydrogen bonding to BChl had previously been shown from site-directed mutagenesis and resonance Raman studies (Fowler et al., 1994). From resonance Raman spectroscopy (Sturgis et al., 1994) and reconstitution experiments with BChl analogs (Parkes-Loach et al., 1990) and selectively-modified polypeptides (Meadows et al., 1995; Loach & Parkes-Loach, 1995), additional hydrogen bonding is expected to exist in LH1 and its subunit complex. The fact that it has so far not been possible to isolate a stable subunit complex from LH2 also suggests that additional binding elements exist in LH1 to stabilize its subunit structure.

Using the reconstitution methodology, several analogs of BChl *a* were tested for their ability to bind to the α- and *β*-polypeptides of *Rhodospirillum rubrum* and form subunit-type and LH1-type complexes (Parkes-Loach et al., 1990). From these studies, several structural requirements were observed: the Mg atom is required, presumably for coordinating a ligand provided by the protein, the acetyl group at position C3 is important, presumably for participating in hydrogen bonds with the protein, the carbomethoxy group at position C13[2] is important for hydrogen bonding, either by influencing the binding of the 13[1]-carbonyl group or by influencing the overall geometry, and the bacteriochlorin oxidation state of the macrocycle was required, suggesting a highly restrictive overall geometry of the binding site. In a somewhat different approach, Berger et al. (1992) reconstituted LH1 with the polypeptides from *Rs. rubrum* and BChl *a* analogs using DMSO/HPLC methodology. Of the analogs they examined, only BChl a_p and BChl *b* formed LH1-type complexes. In competition studies, they found that analogs such as pyro-BChl a_{gg} and [13[2]-OH]BChl a_{gg} bound to the protein but did not form red-shifted species.

Some of the amino acid side chain groups responsible for binding BChl have been tentatively identified. On the basis of resonance Raman data, histidine was proposed to provide the coordinating ligand to each Mg (Robert & Lutz, 1985; Chang et al., 1990b). Consistent with this view, mutants in which His 0 of the α-polypeptide was changed to other amino acids failed to form LH1 complexes *in vivo* (Bylina et al., 1988). In addition, from the crystal structure of LH2, His 0 of the α- and *β*-polypeptides have been shown to provide the ligands to the BChl assigned to the 850-nm component (McDermott et al., 1995). From reconstitution studies with shortened LH1 *β*-polypeptides, His 0 (and not His −18) of the *β*-polypeptide has also been assigned this role for the LH1 complex as well (Meadows et al., 1995). The requirement for the 3-acetyl and the 13[2]-carbomethoxy groups implies that the protein supplies specific hydrogen bond donors. From site-directed mutagenesis experiments utilizing resonance Raman measurements, evidence was obtained that in LH1 Trp 11 is involved in hydrogen bonding to the C3[1] carbonyl group of one of the BChl molecules (Olsen et al., 1994). Finally, from comparative reconstitution studies, Tyr



Abbreviation	Macrocycle modification(s)	Central metal	Esterifying alcohol
BChl a_p	unmodified	Mg	p
BChl a_{gg}	unmodified	Mg	gg
BChl a_e	unmodified	Mg	e
BPh a_p	unmodified	none	p
Cd(II)BPh a_p	unmodified	Cd	p
Zn(II)BPh a_p	unmodified	Zn	p
Ni(II)BPh a_p	unmodified	Ni	p
Cu(II)BPh a_p	unmodified	Cu	p
BChl b_p	C	Mg	p
pyro-BChl a_p	E	Mg	p
pyro-BChl a_{eg}	E	Mg	eg
CHl a_p	A + B	Mg	p
Chl b_p	A + B[1] ([1]where 7-CH_3 is replaced by CHO)	Mg	p
BChl e_7	F	Mg	tme[2]
[3-vinyl]-BChl a_p	A	Mg	p
[13_2-OH]-BChl a_p	D	Mg	p
[3-acetyl]-Chl a_p	B	Mg	p
13^1a-Oxa-[13^2-Oxo]-BChl a_m	G	Mg	m

[2]tme = trimethyl ester

FIGURE 2: Structures of BChl *a* and its analogs used in these experiments.

4 of the β-polypeptide of *Rhodobacter sphaeroides* or Trp 4 of the β-polypeptide of *Rs. rubrum* has also been implicated as providing a hydrogen for hydrogen bonding to BChl (Loach et al., 1994).

In this paper, the examination of polypeptide interactions with BChl analogs has been extended to include the α- and β-polypeptides of *Rb. sphaeroides*. This bacterium was chosen because it has only about 30% amino acid sequence identity in its LH1 and reaction center (RC) polypeptides compared to those of *Rs. rubrum*, indicating substantial divergence on an evolutionary time scale. Also, it has a secondary LH complex, LH2, whereas *Rs. rubrum* does not, and the absorption spectra, circular dichroism (CD) spectra, and resonance Raman spectra (Robert & Lutz, 1985) of LH1 are somewhat different from those of *Rs. rubrum* (Kramer et al., 1984; Cogdell & Scheer, 1985; Chang et al., 1990b). New derivatives of BChl have also been examined in reconstitution with the α- and β-polypeptides of both *Rs. rubrum* and *Rb. sphaeroides*. The new BChl analogs include BChl with modifications of the side chain at C3, modifications of ring V, or bacteriopheophytin (BPh) containing metals other than Mg. The results illuminate common elements of binding in these two divergent photosynthetic bacteria as well as uncover some interesting features unique to each species. A preliminary report of some of these results has been given (Parkes-Loach et al., 1995).

MATERIALS AND METHODS

n-Octyl β-D-glucopyranoside (OG) was obtained from the Sigma Chemical Co. All solvents were obtained from Burdick and Jackson and were HPLC grade. Hexafluoroacetone trihydrate (HFA) was purchased from Aldrich Chemical Co. BChl *a* containing phytol as the esterifying alcohol (BChl a_p) was purchased from the Sigma Chemical Co. and also was isolated from the R26 mutant of *Rb. sphaeroides* following the procedures of Berger et al. (1987) and Michalski et al. (1988) as modified in Meadows et al. (1995). BChl *a* containing geranylgeraniol as the esterifying alcohol (BChl a_{gg}) was similarly isolated from the G-9 carotenoidless mutant of *Rs. rubrum*.

The preparations of the polypeptides used in these studies and all reconstitution assay procedures were as previously described (Parkes-Loach et al., 1990; Loach et al., 1994; Davis et al., 1995). All BChl analogs used were obtained as reported before (Parkes-Loach et al., 1990) except for the following: [3-vinyl]BChl a_p and [13^2-OH]BChl a_{gg} were prepared as described in Struck et al. (1990a) and Berger et al. (1992), respectively; BChl e_7 trimethyl ester and 13^1a-oxa-[13^2-oxo]BChl a_m were prepared as described in Struck et al. (1990b); and metal substituted BPh derivatives were prepared as described in Hartwich et al. (1995).

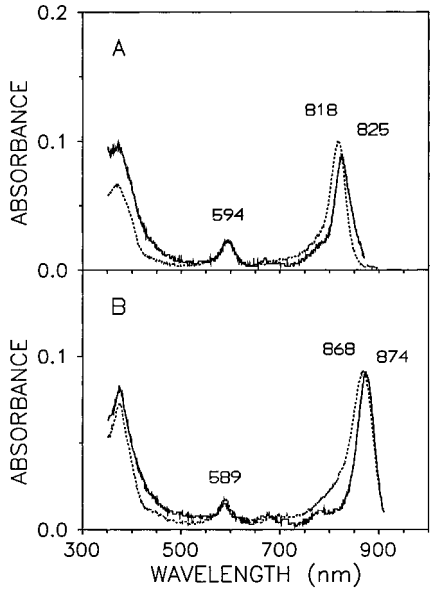


FIGURE 3: Absorption spectra of the reconstituted subunit-type (A) and LH1-type (B) complexes of *Rs. rubrum* (dashed line) and *Rb. sphaeroides* (solid line). Bchl a_{gg} dissolved in acetone was added to the polypeptides in 0.90% OG to give a final concentration of 1 μM under subunit-forming conditions. The λ_{max} of BChl a_{gg} in 0.90% OG without protein was at 777 nm (not shown). The concentration of each of the α- and β-polypeptides of *Rs. rubrum* was 0.009 mg/mL, while that of each of the α- and β-polypeptides of *Rb. sphaeroides* was 0.013 mg/mL. The concentration of OG was 0.66% for the *R. rubrum* samples and 0.60% for the *Rb. sphaeroides* samples. LH1 was formed by cooling the samples at 4 °C overnight. For further details, see Loach et al. (1994).

Absorption spectra were recorded with a Shimadzu UV-160 spectrophotometer interfaced to either an Apple IIe or Goldstar microcomputer. To reduce the effects of scattered light, opal glass was placed between the sample and the detector. CD spectra were recorded using a Jasco J500C spectropolarimeter interfaced to a Leading Edge microcomputer.

RESULTS

Reconstitution experiments were performed using low concentrations (1−10 μM) of both the BChl analogs and α- and β-polypeptides of *Rs. rubrum* or *Rb. sphaeroides*. Several criteria were used for evaluating the formation of native-like complexes. Successful reconstitution of the subunit-type complex was assumed (i) if the absorption spectrum in the near-IR exhibited a red shift of the Q_Y band comparable to BChl *a* (645 cm^{-1}) under subunit complex-forming conditions (compared to the pigment's Q_Y absorption maximum in the absence of protein) (Figure 3) and (ii) if it displayed a CD spectrum characteristic of that observed with the native subunit complex. Similar absorption (Figure 3) and CD criteria were applied to evaluating the formation of LH1-type complexes upon chilling or diluting the subunit-type complexes. Control experiments were conducted in all cases under identical conditions in the absence of protein. No evidence was found for formation of aggregated BChl species with red-shifted Q_Y bands under the conditions of the experiments except for the cases indicated below.

For most experiments, a standard set of concentration conditions were chosen to attempt reconstitution with BChl analogs: 3 μM each of the α- and β-polypeptides and a concentration of BChl or analog between 1 and 3 μM. These conditions result in nearly quantitative formation of the subunit complex with native BChl and the α- and β-polypeptides of either *Rs. rubrum* or *Rb. sphaeroides* (Miller et al., 1987; Chang et al., 1990a; Loach et al., 1994). For a quantitative comparison of the relative association constants of bound analogs, absorption spectra were always recorded at 0.90% and 0.75% OG. In cases involving analogs where the subunit-type complex was incompletely formed under these conditions, such formation could often be enhanced if desired, by using higher concentrations of protein and analog.

The term stable will be used to indicate when a complex is readily formed as indicated by a complete shift of the Q_Y band to the proper location of either a subunit-type or a LH1-type complex under the standard conditions used. A large K_{Assoc} of the order of magnitude of the native system (Loach & Parkes-Loach, 1995; Davis, 1995) is implied in this case. The term kinetically stable will be used to indicate that the absorption spectrum of the subunit-type complex does not change on standing for at least 24 h and a LH1-type complex does not change at 4 °C for several days. When a subunit-type complex is kinetically unstable, the Q_Y absorption band-shift to shorter wavelengths is typically irreversible. A LH1-type complex may be kinetically unstable upon warming to room temperature and show a blue shift of the Q_Y band to that indicative of subunit complex formation. These changes are usually completely reversible upon cooling the sample to 4 °C. On the other hand, LH1-type samples that are kinetically unstable and undergo a Q_Y band shift to that characteristic of free BChl (777 nm) are typically irreversible.

Analogs Forming Subunit-Type and LH1-Type Complexes. Of all the analogs tested for their ability to support reconstitution, only two types of changes could be made without decreasing the ability to form subunit-type and LH1-type complexes. Three BChl analogs with different esterifying alcohols at position C17[4] (phytyl, geranylgeranyl, ethyl; see Figure 2) formed native-like subunit and LH1 complexes with either the α- and β-polypeptides of *Rs. rubrum* or *Rb. sphaeroides* (Table 1). With both sets of polypeptides, the analog with the shortest esterifying alcohol (ethanol) formed a less stable subunit-type complex as evidenced by the significant amount of absorbance near 780 nm indicating free BChl (Figure 4). All three derivatives formed stable LH1-type complexes, although a small amount of absorbance at 780 nm persists for the system containing BChl a_e. This is likely due to the rapid degradation of BChl a_e (compared with BChl a_p or BChl a_{gg}) which occurred during formation of the subunit complex.

The other analog with which subunit-type and LH1-type complexes were formed with both sets of polypeptides was BChl *b*. As can be noted in Table 1, the red shifts observed upon forming subunit-type and LH1-type complexes with the α- and β-polypeptides of *Rb. sphaeroides* are the same as those with the α- and β-polypeptides of *Rs. rubrum* and represent the same magnitude of shifts in energy as observed with BChl *a* (Parkes-Loach et al., 1990, 1994). This points to a special interaction of the LH1 polypeptides from the BChl *b*-containing bacterium *Rhodopseudomonas viridis*, which displays a red-shifted Q_Y band to 1015 nm.

Analogs Unable To Form Subunit-Type and LH1-Type Complexes. Many analogs failed to show evidence of formation of subunit-type and LH1-type complexes (Table 1), even when the difference between the analog and BChl *a* was minimal. Among these were compounds such as

Table 1: Reconstitution Assay for Formation of Subunit- and LH1-Type Complexes with BChl *a* Analogs and the α- and *β*-Polypeptides of Various Photosynthetic Bacteria

		successful recon[a]		near-IR λ_{max}		
analog	polypep	subunit	LH1	0.90% OG (free pigment)	subunit conditions[b]	LH1 conditions[b]
BChl a_{gg}	*rub*	+	+	777	818	868
	sph	+	+	777	825	874
BChl a_p	*rub*	+	+	777	818	869
	sph	+	+	777	822	874
BChl a_e	*rub*	+	+	774	815	869
	sph	+	+	774	823	866
BChl b_p	*rub*	+	+	811	852	891
	sph	+	+	811	853	901
BPh a_p	*rub*	−	−	751	751, 851	752, 851
	sph	−	−	751	849	848
pyro-BChl a_{eg}	*rub*	−	−	778	777	779
	sph	−	−	777	777	779
13^1a-oxa-[13^2-oxo]BChl a_m	*rub*	−	−	840, 1007	839, 1010	839, 1010
	sph	−	−	834, 1009	835, 1009	849, 1009
BChl e_7 trimethyl ester	*rub*	−	−	771	772	770
	sph	−	−	771	771	771
[3-vinyl]BChl a_p	*rub*	+	−	738	762	762
	sph	−	−	738	744	742
	vir	−	−	738	742	743
[13^2-OH]BChl a_{gg}	*rub*	−	−	774	775	773
	sph	+	+	774	813	858
	cap	−	−	774	782	779
Zn(II)BPh a_p	*rub*	+	+	771	805	852
	sph[c]	+	+	771	813	831
Cd(II)BPh a_p	*rub*	+	+	772	804	855
	sph[c]	+	+	772	812	852
Ni(II)BPh a_p	*rub*	+	+	785	805	853
	sph[c]	+	+	785	812	865
Cu(II)BPh a_p	*rub*	−	−	776	779	781
	sph[c]	−	−	776	777	nd
Mn(II)HmIXDME	*rub*	−	−	nd[d]	nd	nd
	sph	−	−	427[e]	427[e]	nd
Chl *a*	*rub*	−	−	668	669	668
	sph	−	−	668	670	670
Chl *b*	*rub*	−	−	651	651	651
	sph	−	−	651	651	651
[3-acetyl]Chl *a*	*rub*	−	−	688	688	688
	sph	−	−	688	688	687

[a] A successful reconstitution is defined in the text (first paragraph under the Results) and indicated with a plus sign (+). A dash signifies that the requirements for reconstitution were not met. [b] See text for definition of conditions. [c] These experiments were reconstituted with the *β*-polypeptide of *Rb. sphaeroides* and the α-polypeptide of *Rs. rubrum*. [d] Not determined. [e] Soret band.

[3-acetyl]Chl a_p (indicating the importance of the bacteriochlorin oxidation state), pyro-BChl a_{eg} (hydrogen atom in place of the carbomethoxy group at position $C13^2$ and ethylene glycol as esterifying alcohol), and BChl e_7 trimethyl ester and 13^1a-oxa-[13^2-oxo]BChl a_m (both indicating the importance of ring V). Most of these analogs, while not showing spectral evidence for formation of a subunit-type complex, did competitively inhibit formation of the native complexes when BChl *a* was also present.

Except for BPh *a*, pyro-BChl a_p and 13^1a-oxa-[13^2-oxo]-BChl a_m, the control experiments (no protein) with BChl *a* analogs showed little spectral evidence of aggregate formation under subunit-forming conditions. On the other hand, these three exhibited extensive red shifts of their Q_Y bands under subunit- and LH1-complex-forming conditions without protein, essentially reflecting the same behavior as with protein (Table 1). Because aggregation of pyro-BChl a_p was severe and red-shifted species formed readily without protein, no data are reported on this derivative in Table 1. Instead, pyro-BChl a_{eg} (esterified with ethylene glycol) was used since this derivative did not exhibit self-association under the conditions employed. Also noteworthy are BPh whose facile oligomerization has been previously noted (Scherz & Parson, 1984; Scheer et al., 1985) and 13^1a-oxa-[13^2-oxo]BChl a_m. The latter compound is particularly interesting in that the aggregated species displays an extensively red-shifted Q_Y band (1010 nm) (Parkes-Loach et al., 1995), the furthest red band reported for protein-free, porphyrin-based aggregates, comparable to the red shift observed for LH1 in the BChl *b*-containing *Rps. viridis* (1015 nm). In the experiments with protein present, conditions were chosen to minimize aggregate formation (low concentrations of BChl *a* analog, room temperature, dilution to LH1-forming conditions), so that any red-shifted absorption bands could be unambiguously attributed to formation of subunit or LH1-type complexes. Because of the extensive self-aggregate formation in the cases of BPh, pyro-BChl a_p, and 13^1a-oxa-[13^2-oxo]BChl a_m, interaction with the protein may have been precluded.

Analogs Forming Subunit-Type Complexes but Not LH1-Type Complexes. The ability, under selected conditions, to prepare a stable subunit complex without forming LH1 indicates that interactions in addition to those that stabilize the subunit complex must occur to further stabilize LH1. Additional binding interactions to form LH1 from the subunit are also indicated from experiments where the *β*-polypeptides

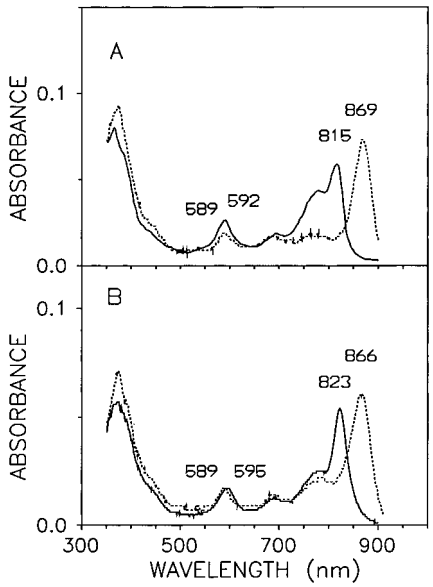


FIGURE 4: Absorption spectra of reconstituted subunit-type (solid line) and LH1-type (dashed line) complexes with BChl a_e and the α- and β-polypeptides of *Rs. rubrum* (A) and *Rb. sphaeroides* (B). The λ_{max} of BChl a_e in 0.90% OG without protein was at 777 nm. (A) The concentration of the α- and β-polypeptides of *Rs. rubrum* was 0.04 mg/mL each. The subunit-type complex was measured at 0.73% OG, and the LH1-type complex was formed upon cooling this sample at 4 °C overnight. (B) The concentration of α- and β-polypeptides of *Rb. sphaeroides* was 0.013 mg/mL each for formation of the subunit complex, which was measured at 0.60% OG. LH1 was formed by diluting the subunit complex to 0.40% OG. For comparative purposes, the spectral amplitudes were corrected for dilution.

of *Rs. rubrum*, *Rb. sphaeroides*, and *Rb. capsulatus* form a subunit-type complex without a corresponding α-polypeptide (see Table 2) but the α-polypeptides are also required for LH1 formation. If the additional interaction to form LH1 involves additional binding to BChl, then it seems reasonable that a BChl analog could be found that would form a stable subunit-type complex but would not form an LH1-type complex even with both the α- and β-polypeptides. While none of the BChl analogs originally tested seemed to fulfill this expectation (Parkes-Loach et al., 1990), the newly tested [3-vinyl]BChl *a* analog does show such behavior with the α- and β-polypeptides of *Rs. rubrum* (Figure 5). The Q_Y absorption band shifted from 739 to 762 nm and the CD spectrum was very similar, albeit blue-shifted, to those of the subunit complex with BChl *a*, but no further red shift was observed under LH1-forming conditions. When reconstitution with the [3-vinyl]BChl *a* analog was tested with only the β-polypeptide of *Rs. rubrum*, only a small red shift was observed (Table 2). Thus, the α-polypeptide is required to stabilize the subunit-type complex with this analog. Because the [3-vinyl]BChl *a* derivative is closely related to BChl *g*, we re-examined our earlier data obtained with this latter derivative and the α- and β-polypeptides of *Rs. rubrum* (Parkes-Loach et al., 1990); a small red shift (from 4 to 10 nm) was observed with BChl *g* and the α- and β-polypeptides of *Rs. rubrum*. However, because BChl *g* so readily rearranges to a structure with the chlorin oxidation state (which does not form a subunit-type complex), the red shift was only transiently observed. In the case of attempted complexation between the α- and β-polypeptides of *Rb. sphaeroides* and [3-vinyl]BChl *a*, no complex formation could be observed under our standard conditions, of either a subunit- or LH1-type. Some evidence for formation of a subunit complex was obtained (as indicated by a shoulder on the long-wavelength side of the band at 742 nm) with the α- and β-polypeptides of *Rb. sphaeroides* at higher pigment concentrations and using the detergent Mega 10 (data not shown). The reconstitution experiments with the [3-vinyl]BChl *a* analog (under our standard conditions) were extended to include the α- and β-polypeptides of *Rps. viridis*. The amino acid sequences of the α- and β-polypeptides of LH1 of this bacterium have substantially greater homology to those of *Rs. rubrum* than do those of *Rb. sphaeroides*. Even so, a subunit-type complex was not formed under our standard conditions, although a slight shoulder suggested that some subunit could perhaps be formed at higher protein and [3-vinyl]BChl *a* concentrations.

Analogs Forming Subunit-Type Complexes with the Polypeptides of Only One Bacterium. Because the amino acid sequences of the α- and β-polypeptides of *Rs. rubrum* and *Rb. sphaeroides* exhibit a low degree of amino acid identity, it might be expected that BChl analogs could be found that would bind to one set of polypeptides either not at all, or very weakly, compared to the other polypeptides. One example of this was given above in the case of the [3-vinyl]BChl *a* derivative. A second example where such differentiation was observed was with the derivative [13^2-OH]BChl *a*. The results of reconstitution with this derivative and the α- and β-polypeptides of *Rb. sphaeroides* are shown in Figure 6A. Although the red shifts are smaller than with native BChl *a*, both subunit-type and LH1-type complexes were formed. On the other hand, as reported earlier (Parkes-Loach et al., 1990), [13^2-OH]BChl *a* does not form stable subunit-type or LH1-type complexes with the α- and β-polypeptides of *Rs. rubrum*. Of interest is the formation of a subunit-type complex with only the β-polypeptide of *Rb. sphaeroides* (Figure 6B). Mimicking results observed with native BChl *a*, a more stable subunit-type complex (greater K_{Assoc}) is formed with only the β-polypeptide of *Rb. sphaeroides* and [13^2-OH]BChl *a* than when both α- and β-polypeptides are present.

Interestingly, although subunit-type and LH1-type complexes could be formed with the α- and β-polypeptides of *Rb. sphaeroides* and [13^2-OH]BChl *a*, no such complexes could be demonstrated with the α- and β-polypeptides of *Rb. capsulatus* (Table 1). Since these two bacteria are much closer on an evolutionary time scale and share a high percentage of amino acid sequence identity, especially in the core region of their sequence (87% and 82% for the α- and β-polypeptides, respectively), the difference in behavior is of considerable interest to understanding BChl binding.

Metal Analogs. Because a major factor in stabilizing the binding of BChl to protein in RC and LH complexes is the ligand coordination to Mg (Coleman & Youvan, 1990; Scheer & Struck, 1993; Loach & Parkes-Loach, 1995), incorporation of metals other than Mg into BPh can provide analogs with which to probe the ligand coordination site as well as to use in future structure−function studies. The Mg geometry in BChl *a* of LH1 and its subunit complex is square-pyramidal (5-coordinate) (Cotton, 1976; Cotton & van Duyne, 1981; Robert & Lutz, 1985; Chang et al., 1990b). Selection of other metals that display similar and different coordination behavior would be of interest. Four metal derivatives of BPh (Zn, Cu, Ni, and Cd) were prepared and tested in reconstitution with the α- and β-polypeptides of

Table 2: Reconstitution Assay for Formation of Subunit- and LH1-Type Complexes with BChl *a* Analogs and the β-Polypeptide Only of *Rs. rubrum* and *Rb. sphaeroides*

		successful recon[a]		near-IR λ_{max}		
analog	polypep	subunit	LH1	0.90% OG (free pigment)	subunit condition[b]	LH1 conditions[b]
BChl a_{gg}	*rub*	+	−	777	819	780, (819)
	sph	+	−	777	824	823
BChl a_p	*rub*	+	−	777	818	(818)
	sph	+	−	777	825	825
BChl b_p	*rub*	+	−	809, 841	809, 846	809, 846
	sph	+	−	nd[c]	nd	nd
[3-vinyl]BChl a_p	*rub*	(+)	−	738	747	741
	sph	−	−	738	740	742
[13^2-OH]BChl a_{gg}	*rub*	−	−	774	783	774
	sph	+	−	774	815	na[d]
	cap	−	−	774	782	774
Zn(II)BPh a_p	*rub*	+	−	771	807	na
	sph	+	−	771	812	na
Cd(II)BPh a_p	*rub*	+	−	772	799	na
	sph	+	−	772	813	na
Ni(II)BPh a_p	*rub*	+	−	785	793	na
	sph	+	−	785	812	na
Cu(II)BPh a_p	*rub*	−	−	776	779	na
	sph	−	−	776	779	na

[a] See Table 1. [b] See text for definition of conditions. [c] Not determined. [d] Not applicable.

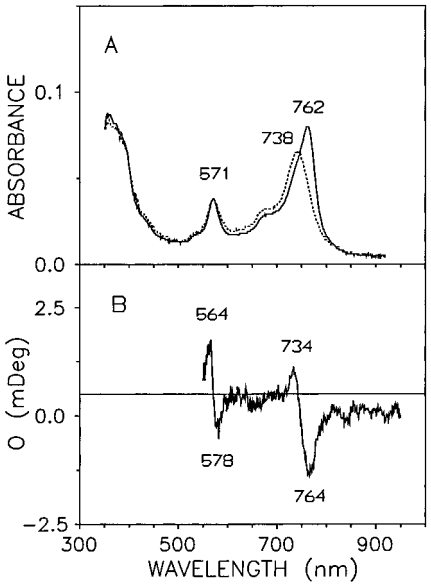


FIGURE 5: Absorption (A) and CD (B) spectra of the reconstituted subunit-type complex formed with [3-vinyl]BChl a_p and the α- and β-polypeptides of *Rs. rubrum*. (A) Dashed line, [3-vinyl]BChl a_p in buffer containing 0.64% OG in the absence of protein; solid line, [3-vinyl]BChl a_p reconstituted with 0.02 mg/mL each of the α- and β-polypeptides of *Rs. rubrum* in 0.64% OG. (B) [3-Vinyl]-BChl a_p reconstituted with 0.06 mg/mL each of the α- and β-polypeptides of *Rs. rubrum* in 0.70% OG. Average of four scans, 1 cm path length cuvette. Absorbance of the sample at 762 nm was 0.274.

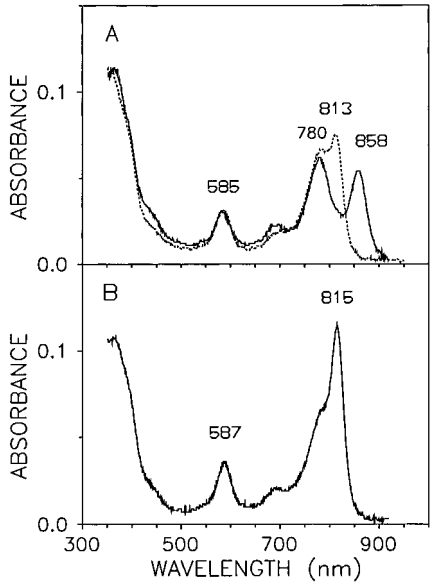


FIGURE 6: (A) Absorption spectra of the reconstituted subunit-type (dashed line) and LH1-type (solid line) complexes formed with [13^2-OH]BChl a_{gg} and the α- and β-polypeptides of *Rb. sphaeroides*. The concentration of each polypeptide was 0.013 mg/mL. The subunit-type complex absorption spectra were measured at 0.60% OG, and the LH1-type complex was formed upon cooling this sample at 4 °C overnight. (B) Absorption spectra of the reconstituted subunit-type complex formed with [13^2-OH]BChl a_{gg} and the β-polypeptide of *Rb. sphaeroides*. The concentration of the polypeptide was 0.026 mg/mL, and the spectrum was measured at 0.60% OG. The λ_{max} of [13^2-OH]BChl a_{gg} in 0.90% OG without protein was at 774 nm.

both *Rs. rubrum* and *Rb. sphaeroides*. According to extensive research, all but Cu(II) are reported to form a large variety of stable 5-coordinate complexes (Cotton & Wilkinson, 1980), and 5-fold coordination of Ni(II)BPh and Zn(II)BPh has recently been demonstrated for the $B_{A,B}$ binding sites of RC from *Rb. sphaeroides* (Chen et al., 1995). In keeping with expectations, Cd(II)BPh and Zn(II)BPh readily formed subunit-type and LH1-type complexes with the α- and β-polypeptides of either *Rs. rubrum* or *Rb. sphaeroides* (Figures 7 and 8). While formation of the subunit-type complex readily occurred with a K_{Assoc} as large as for the native system, LH1 was not as completely formed in either case and was kinetically unstable. It should be noted that the λ_{max} values of the Q_Y bands in both of these subunit-type and LH1-type complexes are less red-shifted than with BChl. The CD spectra of the subunit-type complexes formed are very similar to that of native subunit complexes (data not shown). In the results shown in Figures 7 and 8 for reconstitution with the β-polypeptide of *Rb. sphaeroides*, a heterologous combination of polypeptides was used in which the α-polypeptide was that of *Rs. rubrum*. We employed this heterologous combination because it has been demon-

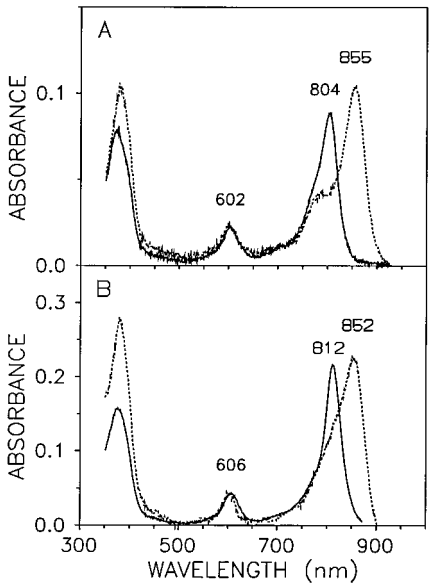


FIGURE 7: Absorption spectra of the reconstituted subunit- (solid line) and LH1-type (dashed line) complexes formed with Cd(II)-BPh a_p and the α- and β-polypeptides of *Rs. rubrum* (A) and the α-polypeptide of *Rs. rubrum* together with the β-polypeptide of *Rb. sphaeroides* (B). The λ_{max} of Cd(II)BPh a_p in 0.90% OG without protein was at 772 nm. (A) The concentration of the α- and β-polypeptides of *Rs. rubrum* was 0.09 mg/mL each. The subunit-type complex absorption spectra were measured at 0.75% OG and the LH1-type complex formed by cooling this sample at 4 °C overnight. (B) The concentration of the α-polypeptide of *Rs. rubrum* and the β-polypeptide of *Rb. sphaeroides* was 0.05 mg/mL each. The subunit-type complex was measured at 0.75% OG and the LH1-type complex formed by cooling this sample at 4 °C overnight and diluting while cold to 0.30% OG. For comparative purposes, the spectral amplitudes were corrected for dilution.

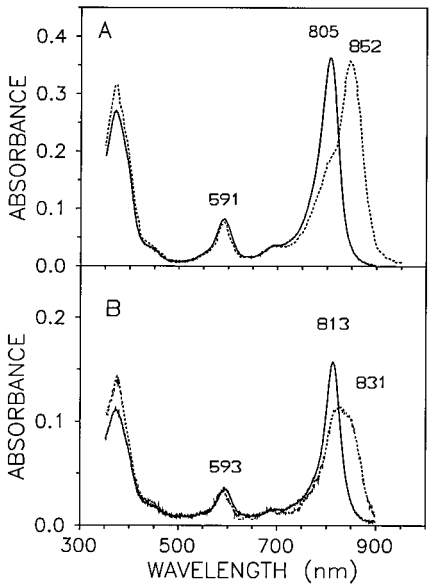


FIGURE 8: Absorption spectra of the reconstituted subunit- (solid line) and LH1-type (dashed line) complexes formed with Zn(II)-BPh a_p and the α- and β-polypeptides of *Rs. rubrum* (A) and the α-polypeptide of *Rs. rubrum* together with the β-polypeptide of *Rb. sphaeroides* (B). The λ_{max} of Zn(II)BPh a_p in 0.90% OG without protein was at 771 nm. (A) The concentration of the α- and β-polypeptides of *Rs. rubrum* was 0.02 mg/mL each. The subunit-type complex was measured at 0.90% OG and the LH1-type complex formed by cooling this sample at 4 °C overnight. (B) The concentration of the α-polypeptide of *Rs. rubrum* and the β-polypeptide of *Rb. sphaeroides* was 0.04 mg/mL each. The subunit-type complex was measured at 0.75% OG and the LH1-type complex formed by cooling this sample at 4 °C overnight and diluting while cold to 0.30% OG. For comparative purposes, the spectral amplitudes were corrected for dilution.

strated that heterologous combinations between the β-polypeptides of *Rb. sphaeroides*, *Rb. capsulatus*, or *Rps. viridis* and the α-polypeptide of *Rs. rubrum* form LH1-type complexes even more readily than those prepared with the homologous polypeptides (Loach et al., 1994). In addition, the α-polypeptide of *Rs. rubrum* is more stable and soluble than that of *Rb. sphaeroides*, it is easily available in pure form without subjecting it to HPLC isolation, and its use makes an interesting comparison to the homologous reconstitution with *Rs. rubrum* polypeptides. Reconstitution experiments with these metal analogs and the homologous α- and β-polypeptides of *Rb. sphaeroides* were also performed, with results similar to those shown in Figures 7B and 8B, but the LH1-type complex was not always as readily formed.

Ni(II)BPh also interacted with both sets of polypeptides to form subunit-type and LH1-type complexes (Tables 1 and 2), but these were not as stable as those formed with the Cd(II)BPh, Zn(II)BPh, or native BChl *a*. Their CD spectra were similar to the native subunit complex spectrum. The LH1-type complexes were less completely formed than in the case of the Cd(II) and Zn(II) complexes and were not kinetically stable.

In the absence of strong ligands to occupy both the 5th and 6th coordination positions, Cu(II) prefers a square-planar geometry and is rarely found to be in a square-pyramidal environment (Cotton & Wilkinson, 1980). In reconstitution experiments conducted with Cu(II)BPh and either the α- and β-polypeptides of *Rs. rubrum* or *Rb. sphaeroides*, no evidence was found for formation of subunit-type or LH1-type complexes. Although the dimeric structure of a hypothetical Cu(II)BPh subunit-type complex might not show significant changes in its absorption spectrum, one would expect to see a unique CD spectrum with bands of comparable intensity to BChl *a* dimers, as in other subunit complexes, but none was observed.

Complexes Formed with Only β-Polypeptides. Because a subunit-type complex is readily formed with only the β-polypeptide of *Rs. rubrum*, *Rb. sphaeroides*, or *Rb. capsulatus* (Loach et al., 1994), many of the BChl analogs were also tested with these β-polypeptides (Table 2). In all cases where a subunit-type complex was formed with the α- and β-polypeptides of *Rb. sphaeroides*, a subunit-type (β) complex was formed even more readily with only the β-polypeptide of *Rb. sphaeroides* (Table 2 and Figure 6), as indicated by the more complete subunit formation. Analogs forming subunit complexes with the α- and β-polypeptides of *Rs. rubrum* sometimes formed subunit-type complexes with only the β-polypeptide of *Rs. rubrum*, but in all cases the complexes were less stable (e.g., [3-vinyl]BChl *a*) than when both α- and β-polypeptides were present. Both of these above sets of results mirror those obtained with native BChl *a*. In no case was an LH1-type complex observed with the β-polypeptide-only systems of these three bacteria.

DISCUSSION

Common Binding Requirements for LH1 Formation in Rs. rubrum and Rb. sphaeroides. *Rs. rubrum* and *Rb. sphaeroides* are sufficiently evolutionarily divergent that their RC and LH1 proteins have only about 30% identity in their amino acid sequences (Brunisholz & Zuber, 1988, 1992). It is therefore significant that the structural requirements for

BChl binding to form the subunit complex and LH1 are highly specific and largely similar for both bacteria. These requirements include the following: (1) the Mg atom or a similarly coordinated central metal of appropriate size able to form stable 5-coordinate complexes with nitrogen-containing ligands, (2) bacteriochlorin oxidation state of the macrocyclic ring, and (3) an intact ring V. The two bacteria are also similar in requiring an esterifying alcohol longer than ethyl to form a stable subunit complex, but apparently any length suffices to form a stable, native-like LH1. Furthermore, the 3-acetyl group is required to form LH1 in both bacteria, although a subunit-type complex was readily formed with [3-vinyl]BChl *a* with polypeptides of *Rs. rubrum* but formed only slightly under special conditions with polypeptides of *Rb. sphaeroides*. Finally, the 13^2-carbomethoxy group is required to form LH1 as well as a stable subunit complex in both bacteria.

Except for one or two cases as noted in the next section, the specific structural requirements for BChl to form a subunit complex are the same as those required for forming LH1. This fact makes it likely that most of the interactions between BChl and the protein in the BChl binding sites are similar in the subunit complex and in LH1. A significant consequence of these observations is that LH1 must be formed by a simple association of the subunit complex without major changes in the BChl binding site. The spectral properties of LH1 are thus derived from some additional interactions of BChl with the protein and the interaction of excitonically-coupled BChl dimers of closely associated subunits. This conclusion is consistent with spectral characterizations of the subunit complex and LH1 (Chang et al., 1990b; Visschers et al., 1991; 1993a,b, 1994; van Mourik et al., 1991, 1992, 1993). On the basis of these results and a variety of experiments in which the role of amino acids in the α- and *β*-polypeptides has been evaluated, structural models of the subunit complex have been suggested (Loach & Parkes-Loach, 1995). The suggestions for the structure of the subunit complex of LH1 are strengthened by analogy to the recently published crystal structure of LH2 (McDermott et al., 1995). In this latter structure, hydrogen bonding to the 3-acetyl group of both BChl in the 850 nm protomer unit is important in the binding of this pigment. This result is consistent with the results of Olsen et al. (1994) and the present study, indicating the importance of the 3-acetyl group in LH1 formation. However, no other hydrogen bonding to the BChl of the 850-nm component was observed in the structure of LH2. Because the reconstitution with BChl analogs reported herein as well as previously (Parkes-Loach et al., 1990) indicates that the 13^2-carbomethoxy group is important for complex formation, and because resonance Raman data suggest that the 13^1-carbonyl group in ring V is involved in binding (Lutz & Robert, 1985; Sturgis & Robert, 1994), this aspect of the structure of LH1 would appear to be different from that of LH2 and predicts that additional hydrogen bonding is important in stabilizing the LH1 complex.

It may be useful to examine the known amino acid sequences of the α- and *β*-polypeptides of LH1 found in various photosynthetic bacteria and address the question as to which groups in these polypeptides might be involved in forming specific bonds to BChl (the amino acid sequences of polypeptides from four different bacteria are shown in Figure 1). This evaluation can be simplified by considering the results of recent experiments with truncated polypeptides in which it was shown that the N-terminus through amino acid residue −19 of the native *β*-polypeptides of *Rs. rubrum* and *Rb. sphaeroides* are not required for formation of subunit- and LH1-type complexes, although the LH1 complexes were not completely formed and were kinetically less stable than when formed with native polypeptides (Meadows et al., 1995). Furthermore, the even shorter *β*-polypeptide of *Rs. rubrum* beginning with residue −16 for its N-terminus and continuing to the normal C-terminus was shown to be sufficient for forming a native-like subunit complex without the α-polypeptide, although it was again less stable (Meadows et al., 1995). Likewise, amino acid residues of the α-polypeptide of *Rs. rubrum* from the normal N-terminus through residue −20 and from residue +13 to the end of the normal C-terminus were not required for subunit formation. As was observed in the N-terminal truncations of the *β*-polypeptide, the stability of the reconstituted LH1-type complex was decreased when the N-terminal truncated α-polypeptide was used. Experiments with the C-terminal-truncated α-polypeptide of *Rs. rubrum* and the native *β*-polypeptide formed LH1-type complexes as completely as those observed with the native α- and *β*-polypeptides.

If it is assumed that His 0 of each polypeptide provides its imidazole group for coordination to the central Mg of BChl, which is consistent with the crystal structure of LH2 (McDermott et al., 1995), then the possibilities can be further reduced in considering what conserved groups of the protein are in the correct proximity to provide specific interaction (presumably hydrogen bonding) with the 13^2-carbomethoxy group, 13^1-carbonyl group, and 3-acetyl groups of BChl. Finally, if it is assumed that the polypeptides remain α-helical throughout the BChl binding region (from about residue −19 to about residue +7), which would in analogy be consistent with the structure of LH2 (McDermott et al., 1995), then hydrogen bonding to BChl from the polypeptide backbone in this region is unlikely. This leaves in the *β*-polypeptide the conserved Trp(Tyr) +4, Trp +6, Arg +7, and Trp +9 and possibly Ser −13 whose side chains can provide hydrogen for hydrogen bonds to BChl, while in the α-polypeptide only the conserved Ser +5, Thr +6, Asn +10, and Trp +11 might participate. Trp +11 of the α-polypeptide of *Rb. sphaeroides* is clearly important in LH1 stabilization as indicated in recent experiments with site-directed mutant strain in which this residue was substituted by Phe and the Q_Y λ_{max} was blue-shifted to 853 nm (Olsen et al., 1994). Furthermore, the hydrogen bonding role of Tyr +13 and Trp +14 in the α-polypeptide of LH2 from *Rps. acidophila* strain 10050 (Fowler et al., 1994; McDermott et al., 1995) would suggest a possible parallel role for the highly conserved Asn +10 and Trp +11 of the α-polypeptides of LH1. Thus, the amino acids +4, +6, +7 and +9 in the *β*-polypeptides of LH1 and +5, +6, +10, and +11 in the α-polypeptides of LH1 will be of considerable interest to further evaluate in future studies.

Because BChl is very likely bound within the membrane but near the aqueous interface (Loach et al., 1985), hydrogen bonding between water molecules and the oxygen atoms of BChl is also important to consider. From this perspective, it should be noted that BChl is protected from deleterious interactions with oxygen, protons, and hydroxide ions in LH1, and to some extent in the subunit form, relative to BChl in OG (Davis, 1995). Such marked stability to extremes of

pH and oxygen presumably means that the C-terminal portion of the polypeptides covers the otherwise exposed edge of the BChl that would be at the aqueous interface and, as a consequence, protects it from interacting with water. Indeed, if the crystal structure of LH2 in the C-terminal region (McDermott et al., 1995) is indicative of the structure of LH1 in this region, then both the α- and β-polypeptides may bend near the C-terminal end at the presumed aqueous interface as suggested by Olsen and Hunter (1994) and fold over the edge of the BChl, providing the protection needed. Even so, a sequestered water molecule may be important in stabilizing the binding site.

Difference in Binding Requirements for the Subunit and/or LH1 in Rs. rubrum and Rb. sphaeroides. Two BChl analogs have been examined in which differences were observed in their interaction with the α- and β-polypeptides of *Rs. rubrum* compared to those of *Rb. sphaeroides*. One of these is a derivative that replaces the 3-acetyl group with a vinyl group, and in the other, the hydrogen atom at $C13^2$ is replaced with a hydroxyl group. With the [3-vinyl]BChl *a* derivative, a subunit-type complex readily formed with the α- and β-polypeptides of *Rs. rubrum* (Figure 5) but could not be observed with the α- and β-polypeptides of *Rb. sphaeroides* under the standard set of conditions selected. However, no LH1-type species could be observed with the *Rs. rubrum* system, nor could either subunit-type or LH1-type complexes be demonstrated with polypeptides from *Rb. sphaeroides* or *Rps. viridis*. These results imply that a hydrogen bond from the protein to the acetyl group at C3 is important for LH1 formation, in each of these bacteria. Resonance Raman data have suggested that such interaction occurs in LH1 and is altered in the subunit complex of *Rs. rubrum* (Chang et al., 1990b; Visschers et al., 1993b; Sturgis & Robert, 1994). The result that LH1 cannot be formed with only the native β-polypeptide of *Rs. rubrum*, *Rb. sphaeroides*, or *Rb. capsulatus* could well be explained by assuming that it is the α-polypeptides which contain an amino acid residue with a hydrogen bond-forming side chain that are important for interaction with the carbonyl function of the acetyl group at the C3 position for LH1 formation. Interestingly, hydrogen bonding between an amino acid side chain of the α-polypeptide and the 3-acetyl group of BChl has also been implicated by Olsen et al. (1994) for LH1 and by Fowler et al. (1994) and McDermott et al. (1995) for LH2.

The fact that a subunit-type complex forms with [3-vinyl]-BChl *a* and the *Rs. rubrum* α- and β-polypeptides, but was hardly observable with those of *Rb. sphaeroides*, may have an explanation other than hydrogen bonding. That is, when the 3-acetyl group is changed to the more hydrophobic vinyl group, the additional amino acid residues at the C-terminus of the β-polypeptide of *Rs. rubrum*, relative to the short C-terminal region of the β-polypeptide of *Rb. sphaeroides*, may better stabilize this side chain in the subunit form by sequestering it from water. According to resonance Raman measurements, the environment of at least part of the 3-acetyl group changes substantially in the *Rs. rubrum* subunit complex compared to that in LH1 (Chang et al., 1990b; Visschers et al., 1993b; Sturgis & Robert, 1994), but such information is not yet available for the subunit complex of *Rb. sphaeroides* in which the requirements for binding to the 3-acetyl group appear to be even more strict than is true for the *Rs. rubrum* system.

The results with the [13^2-OH]BChl a_p analog are especially interesting. This derivative readily forms a subunit-type complex with the α- and β-polypeptides of *Rb. sphaeroides*, or with only the β-polypeptide of *Rb. sphaeroides* (Figure 6), but does not interact significantly with the α- and β-polypeptides of *Rs. rubrum*. An LH1-type complex can also be formed with this derivative and the α- and β-polypeptides of *Rb. sphaeroides*, but no such complex can be observed with polypeptides of *Rs. rubrum*. For this particular analog, we have extended our reconstitution studies to include the α- and β-polypeptides of *Rb. capsulatus*. As with the polypeptides of *Rs. rubrum*, no subunit-type or LH1-type complexes could be formed. This result allows us to focus our attention on the more modest differences in the amino acid sequences of *Rb. sphaeroides* and *Rb. capsulatus*. Since there is a high percentage of amino acid sequence identity between the proteins of these two bacteria (Brunisholz & Zuber, 1988, 1992), there are only a few residues that are different in the region of interest (Figure 1). These are a Ser vs Ile at position −7, an Ile vs Val at position −3, a Val vs Leu at position −2, and a Tyr vs Met at position +4 in the β-polypeptides of *Rb. sphaeroides* vs *Rb. capsulatus*. For formation of the subunit-type complex, the hydroxyl group at the $C13^2$ position might (1) present a steric problem, more so with the polypeptides of *Rs. rubrum* and *Rb. capsulatus* than those of *Rb. sphaeroides*, or (2) interact by hydrogen bonding in a way that is more permissible with the polypeptides of *Rb. sphaeroides*. In the latter case, Ser −7 or Tyr +4 of *Rb. sphaeroides* would seem to be of special interest because, of the four differences, these are the only amino acids with side chain groups capable of hydrogen bonding. This additional hydrogen bonding of [13^2-OH]-BChl *a* (by the 13^1-carbonyl, 13^2-carbomethoxy, or 13^2-hydroxy) could stabilize the complex, whereas this does not occur with the α- and β-polypeptides of *Rs. rubrum*. From another perspective, the existence of a hydrogen atom at $C13^2$ enables a hypothetical enol structure in ring V to form whereas a hydroxyl group at $C13^2$ would not. If the enol form of ring V were important in binding BChl in *Rs. rubrum* and *Rb. capsulatus*, then the lack of binding of [13^2-OH]-BChl *a* would be predicted. However, there is presently no evidence for any significant amount of enol being formed in (B)Chl proteins.

Mg Binding Site Requirements. The geometry of the Mg site in all known BChl- and Chl-containing proteins is 5-coordinate, square-pyramidal (Cotton & van Duyne, 1981; Robert & Lutz, 1985; Tronrud et al., 1986; Deisenhofer & Michel, 1991; Kühlbrandt et al., 1994). In many cases, the 5th ligand is from a His residue in the protein. However, in the bacterial RC, a Gln residue, as well as other amino acids (Ser, Thr), has been substituted for His by site-directed mutagenesis experiments without affecting the function of the RC (Coleman & Youvan, 1990). Although the 5th ligand is not known with certainty in LH1 complexes, resonance Raman measurements (Robert & Lutz, 1985) as well as the crystal structure of LH2 (McDermott et al., 1995) suggest that His is an appropriate assignment of the amino acid donating the coordinating ligand. The binding energy due to ligand coordination can be estimated to be approximately 5 kcal mol^{-1} for each BChl (Cotton, 1976), or approximately 10 kcal mol^{-1} for a presumed subunit stoichiometry of $\alpha_1\beta_1$•-2BChl. This is approximately half of the total energy stabilizing the complex (Loach & Parkes-Loach, 1995). Thus,

the inability to form a complex with BPh is presumably due to the loss of coordinating ligands as stabilizing interactions which are not offset by the other interactions including possible hydrogen bonding involving the pyrrole secondary amine groups of BPh.

In the 5-coordinate BChl structure, the Mg atom is approximately 0.5 Å to one side, out of the plane of a domed bacteriochlorin macrocycle (Barkigia & Fajer, 1993). It would be expected that several other metal-containing analogs of BChl should form similar complexes. Among the metals we have studied that have coordination properties and ion size similar to Mg(II) are Zn(II), Ni(II), and Cd(II). Each of these BChl analogs have been successfully synthesized and form reasonably stable derivatives, and three of them can replace the native monomeric BChl at sites B_A and B_B in RC from *Rb. sphaeroides* (Hartwich et al., 1995; Chen et al., 1995). In reconstitution experiments with the α- and *β*-polypeptides of *Rs. rubrum* and *Rb. sphaeroides*, all three metal derivatives formed subunit-type and LH1-type complexes consistent with their ability to form 5-coordinate complexes with nitrogen-containing ligands. It is interesting that the subunit-type complexes of Cd(II)BPh and Zn(II)-BPh were as stable as, or more stable than, subunit complexes containing BChl *a*, but the Ni(II)BPh subunit-type complex was slightly less stable. It is likely the Ni causes some distortion in the macrocycle due to its somewhat less than optimal size, and Ni(II) also does not frequently form 5-coordinate complexes. LH1-type complexes were formed in each of the three analog cases, with Q_Y band λ_{max} values significantly less red-shifted than those for BChl *a*-containing complexes. In addition, the complexes were less well formed and less kinetically stable. These results suggest that subtle differences such as the extent to which the metal is out of the macrocycle plane or slight differences in bond strength or distances between the metal and imidazole groups are important in the associated and tightly-packed LH1 complex. This is consistent with the structural environment of the 850-nm component of LH2 which consists of a closely packed and apolar environment (McDermott et al., 1995).

The Cu(II)BPh analog provides an interesting test of the importance of the geometry around the metal. Although Cu(II) readily forms square-planar, octahedral, and distorted tetrahedral complexes, 5-coordinate complexes are rare (Cotton & Wilkinson, 1980). We were unsuccessful in our attempts to form subunit-type and LH1-type complexes with this derivative and the α- and *β*-polypeptides of either *Rs. rubrum* or *Rb. sphaeroides*. Apparently, the binding site for BChl cannot be sufficiently distorted to accommodate a stable bond from His to Cu(II), again reflecting the high specificity of the binding site.

Conclusions. All LH1 complexes appear to have a subunit structure, with several groups from the protein involved in binding BChl. The subunit binding site is highly specific, resulting in unique BChl exciton dimers. It is likely that, in LH1, the binding site for BChl is similar to that in the subunit but exhibits additional interactions with the protein and that the characteristic LH1 spectral properties result from some additional interactions between the multiple BChl exciton dimers with each other and the protein. The structure of the site has been largely conserved through evolution so that even divergent species appear to have highly homologous structures. Groups proposed to be directly interacting with BChl include His 0 of both polypeptides as well as two or more amino acids whose side chains are capable of providing hydrogen atoms for hydrogen bonding to BChl. The most likely candidates for such amino acids are Trp(Tyr) +4, Trp +6, Arg +7, and Trp +9 of the *β*-polypeptides and Ser +5, Thr +6, Asn +10, and Trp +11 of the α-polypeptides.

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BI951777L