Redox Effects on the Excited-State Lifetime in Chlorosomes and Bacteriochlorophyll *c* Oligomers

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ABSTRACT Oligomers of [E,E] BChl c_F (8,12-diethyl bacteriochlorophyll *c* esterified with farnesol (F)) and [Pr,E] BChl c_F (analogously, M methyl, Pr propyl) in hexane and aqueous detergent or lipid micelles were studied by means of steady-state absorption, time-resolved fluorescence, and electron spin resonance spectroscopy. The maximum absorption wavelength, excited-state dynamics, and electron spin resonance (EPR) linewidths are similar to those of native and reconstituted chlorosomes of *Chlorobium tepidum*. The maximum absorption wavelength of oligomers of [E,E] BChl c_F was consistently blue-shifted as compared to that of [Pr,E] BChl c_F oligomers, which is ascribed to the formation of smaller oligomers with [E,E] BChl c_F than [Pr, E] BChl c_F. Time-resolved fluorescence measurements show an excited-state lifetime of 10 ps or less in nonreduced samples of native and reconstituted chlorosomes of *Chlorobium tepidum*. Under reduced conditions the excited-state lifetime increased to tens of picoseconds, and energy transfer to BChl *a* or long-wavelength absorbing BChl *c* was observed. Oligomers of [E,E] BChl c_F and [Pr,E] BChl c_F in aqueous detergent or lipid micelles show a similar short excited-state lifetime under nonreduced conditions and an increase up to several tens of picoseconds upon reduction. These results indicate rapid quenching of excitation energy in nonreduced samples of chlorosomes and aqueous BChl *c* oligomers. EPR spectroscopy shows that traces of oxidized BChl *c* radicals are present in nonreduced and absent in reduced samples of chlorosomes and BChl *c* oligomers. This suggests that the observed short excited-state lifetimes in nonreduced samples of chlorosomes and BChl *c* oligomers may be ascribed to excited-state quenching by BChl *c* radicals. The narrow EPR linewidth suggests that the BChl *c* are arranged in clusters of 16 and 6 molecules in chlorosomes of *Chlorobium tepidum* and *Chloroflexus aurantiacus*, respectively.

INTRODUCTION

The majority of light-harvesting pigments in green photosynthetic bacteria are located in chlorosomes, which are large membrane-attached ellipsoid bodies with an average size of 100 nm × 30 nm × 10 nm (Olson, 1980; Amesz, 1991; Blankenship et al., 1995). Besides several types of carotenoids and small amounts of BChl *a*, these chlorosomes contain BChl *c*, *d*, or *e*, depending on the specific species (Amesz, 1991; Blankenship et al., 1995). Each chlorosome contains approximately 10,000 BChl *c*, *d*, or *e*, surrounded by a lipid monolayer (Olson, 1980). The pigment-protein ratio in chlorosomes is nearly two- to threefold higher than in light-harvesting antenna of other photosynthetic species (Schmidt, 1980; Amesz, 1991; Blankenship et al., 1995), suggesting that pigment-pigment interactions may prevail above pigment-protein interactions. Furthermore, the Q_y absorption maximum of BChl *c*, *d*, or *e* in vivo is shifted to longer wavelengths as compared to that in polar organic solvents, indicating that pigment-pigment excitonic interactions play a major role in these light-harvesting systems (Scherz et al., 1991; Katz et al., 1991). However, the role of protein in chlorosomes is still a subject of debate (Holzwarth et al., 1992; Niedermeier et al., 1992).

The molecular structures of BChl *c*, *d*, and *e* are particularly well suited to pigment-pigment aggregation. It was first shown by Bystrova et al. (1979) that BChl *c* spontaneously associates in oligomers absorbing at 750 nm in nonpolar solvents. This result was later confirmed by many other groups (Smith et al., 1983; Olson and Cox, 1991; Olson and Pedersen, 1990; Brune et al., 1987). The involvement of the central Mg atom in the self-assembly of 740 nm absorbing oligomers was shown by the lack of such self-assembly of Mg-free BPheo *c* (Bystrova et al., 1979; Smith et al., 1983). Self-assembly was also absent when pyrochlorophyll *a*, which lacks the $C3^2$ hydroxyl group of BChl *c*, was used, indicating that the $C3^2$ hydroxyl group is involved in self-oligomerization of BChl *c* as well (Brune et al., 1988). Several infrared and resonance Raman techniques showed that the $C13^1$ keto group is strongly bound in the BChl *c* oligomers (Bystrova et al., 1979; Lutz and van Brakel, 1988). These vibrational spectroscopic techniques also showed that the central Mg atom is likely 5-coordinated (Bystrova et al., 1979; Brune et al., 1988; Hildebrandt et al., 1991, 1994). Additional evidence for BChl *c* oligomers in chlorosomes has recently been provided by ^{13}C NMR studies on chlorosomes of *Chlorobium tepidum* and BChl *c* aggregates (Nozawa et al., 1994; Balaban et al., 1995).

Early work on long-wavelength-absorbing oligomers of BChl *c*, *d*, or *e* used only highly nonpolar solvents (Smith et al., 1983; Bystrova et al., 1979; Olson and Pedersen, 1990;

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Brune et al., 1987). Recently it was found that dispersion in water of a pigment-lipid extract from chlorosomes resulted in similar long-wavelength-absorbing oligomers, with an absorption spectrum strongly resembling that of isolated chlorosomes (Hirota et al., 1992; Miller et al., 1993). It was suggested that the lipids create a hydrophobic environment in which BChls accumulate and self-assemble into long-wavelength-absorbing oligomers. Recently, similar long-wavelength-absorbing oligomers were prepared with different types of purified BChl *c* in the presence of the major natural abundant lipid in chlorosomes, monogalactosyl diglyceride (MGDG) (Uehara et al., 1994). These types of aqueous oligomers create the possibility of studying BChl oligomers in a more natural environment.

Many studies have been performed on the molecular interactions in BChl *c* oligomers, but not much is known about their actual excited-state dynamics. This is especially interesting because of the major light-harvesting function of BChls in chlorosomes, which is absorption of light and transfer of the excitation energy to the BChl *a* baseplate (Olson, 1980; Amesz, 1991; Blankenship et al., 1995). Time-resolved fluorescence measurements on oligomers in hexane showed a decrease in the excited-state lifetime by at least a factor of 100 as compared to that of the monomer (Brune et al., 1987). Similar studies on different dimers and oligomers in CCl_4 hexane mixtures showed lifetimes of 1030 ps for large oligomers and hundreds of picoseconds for dimers and trimers (Causgrove et al., 1990, 1993).

It has been shown that the excited-state lifetime of BChl *c* in chlorosomes of anoxygenic green sulfur bacteria increases at low redox potential (van Dorssen et al., 1986; Wang et al., 1990; Blankenship et al., 1993). A potential explanation the prevention of photochemistry in the reaction center, which, under oxidized conditions, may already be oxidized and can therefore be damaged by an excess of excitation energy (Blankenship et al., 1993, 1995). It is not yet clear what the mediator of this redox-regulated quenching effect might be.

In this paper we report the steady-state spectra of oligomers of [E,E] BChl c_F (8,12-diethyl bacteriochlorophyll *c* esterified with farnesol (F)) and [Pr,E] BChl c_F (analogously, M methyl, Pr propyl; see Smith, 1994) in hexane and detergent/lipid micelles. The largest red shift upon oligomerization is observed in hexane, the smallest one in detergent micelles. Oligomers of [E,E] BChl c_F show consistently less red shift of their absorption maximum than those of [Pr,E] BChl c_F. The excited-state dynamics in these oligomers were studied by means of time-resolved fluorescence as a function of the redox potential, and the fluorescence lifetimes will be compared to those observed in native and reconstituted chlorosomes of *Chlorobium tepidum.* The fluorescence lifetimes in aqueous oligomers were strongly redox sensitive, comparable to the redox-controlled lifetimes observed in native chlorosomes. Electron spin resonance (EPR) spectroscopy shows that the redox-controlled excited-state dynamics can be well explained by the presence of BChl *c* radicals. The EPR linewidth is related to the number of molecules involved in oligomerization and shows that the differences in absorption maxima can be explained by the tendency to form smaller or larger oligomers.

MATERIALS AND METHODS

Cb. tepidum cells were grown phototrophically in a 90 liter container, using the medium described by Wahlund et al. (1991). After precipitation of the cells by the addition of 1 liter of saturated alum ($KAl(SO_4)_2 \cdot H_20$), the cells were harvested by centrifugation. Chlorosomes of *Cb. tepidum* and *Chloroflexus aurantiacus* were isolated according to the method described by Gerola and Olson (1986) and stored at −20°C until further use.

Pigment extraction

Pigments were extracted by sonication of whole cells in excess of methanol. Cell fragments were pelleted by centrifugation at 26,000 × *g* for 20 min. The supernatant, containing BChl *c*, BChl *a*, carotenoids, and presumably polar lipids, was dried under vacuum on a rotary evaporator. The dried film was dissolved in hexane, and BChl *c* aggregates were pelleted by centrifugation at 30,000 × *g* for 20 min. The supernatant, which contained mainly carotenoids, was discarded, and the pellet was dissolved in a minimal volume of methanol. Pigments were purified on reversed-phase high-performance liquid chromatography, as described by Cheng et al. (1993). An eluent mixture of methanol and water (96:4, v/v), a flow rate of 10 ml/min, and approximately 2000 psi gave a satisfactory separation of pigments. The chromatogram was in agreement with that published by Nozawa et al. (1991), who identified the two major pigment fractions as [E,E] BChl c_F and [Pr,E] BChl c_F. These two fractions were collected, dried under vacuum on a rotary evaporator, and stored under nitrogen at −20°C. If necessary, rechromatography was performed for additional purification.

Lipid extraction

Lipids were isolated by extraction of pigments and polar lipids from chlorosomes in an excess of methanol. The extract was centrifuged for 10 min at 3000 × *g*, resulting in a white pellet and green supernatant. The pellet, containing protein and nonpolar lipids, was dissolved in chloroform and centrifuged for 10 min at 12,000 × *g*. The supernatant, containing nonpolar lipids, was dried under vacuum and stored at −20°C. Thin-layer chromatography (TLC) showed no presence of MGDG lipids, but only one other type, probably a mixture of phospholipids (Schmidt, 1980), was present.

Oligomer preparation

Oligomers of [E,E] BChl c_F and [Pr,E] BChl c_F in hexane were prepared according to the method described by Brune et al. (1987). Protein-free reconstituted chlorosomes of *Cb. tepidum* were obtained according to the method described by Hirota et al. (1992). A pigment-lipid mixture of chlorosomes of *Cb. tepidum* was extracted in chloroform and dried under vacuum. The dried extract was redissolved in a small volume of methanol, and aliquots (less than 1% v/v) were injected into a vigorously stirred 10 mM potassium phosphate buffer (pH 7.4). In most cases, the initial absorption maximum was at 720 nm. Additional sonication for 10–20 min in a bath sonicator resulted in reproduction of the 740-nm absorption, as was reported previously for extracts of *Chlorobium limicola* (Hirota et al., 1992). Oligomers of [E,E] BChl *cF and* [Pr,E] BChl c_F in detergent micelles were prepared by dispersion of aliquots of purified pigments in methanol (less than 1% v/v) into a 0.15 mM β-*N*-dodecylmaltoside, 10 mM potassium phosphate buffer (pH 7.4) and 20 min of sonication in a bath sonicator. Oligomers in MGDG lipid were prepared according to the method described by Uehara et al. (1994); concentrated purified [E,E]

BChl c_F or [Pr,E] BChl c_F in methanol were mixed with MGDG in methanol, before injection into a vigorously stirred 10 mM potassium phosphate buffer. The total methanol concentration was kept at less than 1% (v/v).

Time-resolved fluorescence

Picosecond time-resolved fluorescence experiments were performed by means of single-photon counting with a synchronously pumped dye laser operating at 7.6 MHz, as described in detail by Causgrove et al. (1990). Samples were contained in a stationary cuvette with an optical path length of 1.5 mm, and the optical density was typically 0.5/cm or less. The excitation beam was focused into the cuvette by a 1-m focal length lens, and fluorescence was collected in 90° configuration. The excitation wavelength was at 590 nm. The instrumental time response function was about 50 ps at FWHM. Data were analyzed by means of global analysis, and the accuracy of the fit was determined by the χ^2 value.

EPR spectroscopy

EPR spectra were measured on a Bruker 300 E spectrometer at 125 K using a standard liquid nitrogen cryostat. Microwave power of 2.0 mW and 100 kHz modulation amplitude of either 0.9 or 1.9 Gauss were used, as indicated in the text. Each spectrum is an average of 32 scans.

Redox control

Samples referred to as "neutral" contained no added redox mediators. The average measured redox potential of these samples was 200 ± 50 meV, depending on the amount of oxygen present. Oxidized aqueous samples were prepared by the addition of 20 mM potassium ferricyanide ($K_3Fe(CN)_6$), resulting in a typical redox potential of 600 ± 50 meV. Aqueous samples were reduced by the addition of 10–30 mM sodium dithionite ($Na_2S_2O_4$) to a typical redox potential of −400 ± 50 mV.

RESULTS

Steady-state absorption spectra

Steady-state absorption spectra of chlorosomes of *Cb. tepidum* (*solid line*) and its pigment-lipid reconstitute (*dotted line*) are shown in Fig. 1 *A*. The final Q_y absorption of the reconstituted chlorosomes of *Cb. tepidum* tends to be shifted 10 nm to the blue, as compared to that of the original chlorosomes, as was also observed by Hirota et al. (1992) for chlorosomes of *C. Limicola*. The resemblance of the absorption spectrum of chlorosomes and its protein-free reconstitute is striking. Besides the blue-shifted Q_y maximum in reconstituted chlorosomes, the BChl *a* absorption appears to be broadened as compared to that in original chlorosomes, indicating disruption of the BChl *a* baseplate structure.

Fig. 1 *B* shows the absorption spectrum of [E,E] BChl c_F and [Pr,E] BChl c_F in an aqueous solution of MGDG lipids. The absorption maximum of [E,E] BChl c_F is at 729 nm, and that of [Pr,E] BChl c_F is at 740 nm. Both absorption maxima are in good agreement with previous data (Uehara et al., 1994). When the MGDG lipid and [E,E] BChl c_F or [Pr,E] BChl c_F were dissolved in dichloromethane instead of methanol, oligomers were still formed, but the inhomogeneous linewidth of the spectra was nearly two times larger. Because BChl *c* is probably dimeric in dichloromethane (Olson and Pedersen, 1990), this indicates that the final absorption spectra depend on the type of building block the oligomerization starts with.

MGDG is the major lipid in chlorosomes of green sulfur bacteria. To determine whether this lipid is unique in the formation of the oligomers we isolated nonpolar lipids from chlorosomes. Thin-layer chromatography showed that these lipids were not MGDG lipids, but probably a mixture of phospholipids. The spectra of [E,E] BChl c_F and [Pr,E] BChl c_F, mixed with this lipid mixture in methanol and subsequently dispersed in water, are shown in Fig. 1 *C*. Oligomers were formed with absorption maxima at 722 nm for [E,E] BChl c_F and 746 nm for [Pr,E] BChl c_F. This suggests that any amphiphilic molecule in aqueous solution that creates a hydrophobic environment for BChl *c* will stimulate the formation of oligomers. Oligomers of [E,E] BChl c_F and [Pr,E] BChl c_F in micelles of dodecylmaltoside are shown in Fig. 1 *D*. The absorption maxima are at 726 nm and 738 nm, respectively. Oligomers in detergent micelles have also been observed while using dodecyl sulfate as detergent (Niedermeier et al., 1992).

The absorption spectra of [E,E] BChl c_F (*dotted line*) and [Pr,E] BChl c_F (*solid line*) in the apolar organic solvent hexane are shown in Fig. 1 *E*. The Q_y absorption of [E,E] BChl c_F and [Pr,E] BChl c_F are 741 and 752 nm, respectively. The spectra show a shoulder on the blue side of the Q_y absorption band. This can be ascribed to small amounts of dimers or tetramers, which are present because of the addition of dichloromethane (Olson and Cox, 1991). Dichloromethane (3–5%) was added to prevent precipitation of the oligomers as reported by Brune et al. (1987).

Time-resolved fluorescence

The time-resolved decay-associated fluorescence spectra of chlorosomes, reconstituted chlorosomes, and aqueous BChl *c* oligomers were studied in the absence ("neutral" condition) or presence (reduced condition) of sodium dithionite. Fig. 2 *A* shows the excited-state lifetimes of chlorosomes of *Cb. tepidum*. In the absence of dithionite (Fig. 2 *A, upper panel*), we observed a major decay component of 9 ps and a minor one of 45 ps. None of these components showed a rise at 800 nm, indicating the absence of significant energy transfer from the bulk BChls *c* to the baseplate BChl *a*. Upon the addition of dithionite (Fig. 2 *A, lower panel*), the major fluorescence decay component increased to about 40 ps and showed a rise component around 800 nm, indicating that under reduced conditions excitation energy is transferred from BChl *c* to the BChl *a* baseplate with an overall time constant of about 40 ps. An additional main decay component of 540 ps is clearly red shifted compared to the 40-ps component. The apparent dual maxima in fluorescence at 790 and 810 nm suggest that this decay component may be ascribed to decay of excitation energy equilibrated

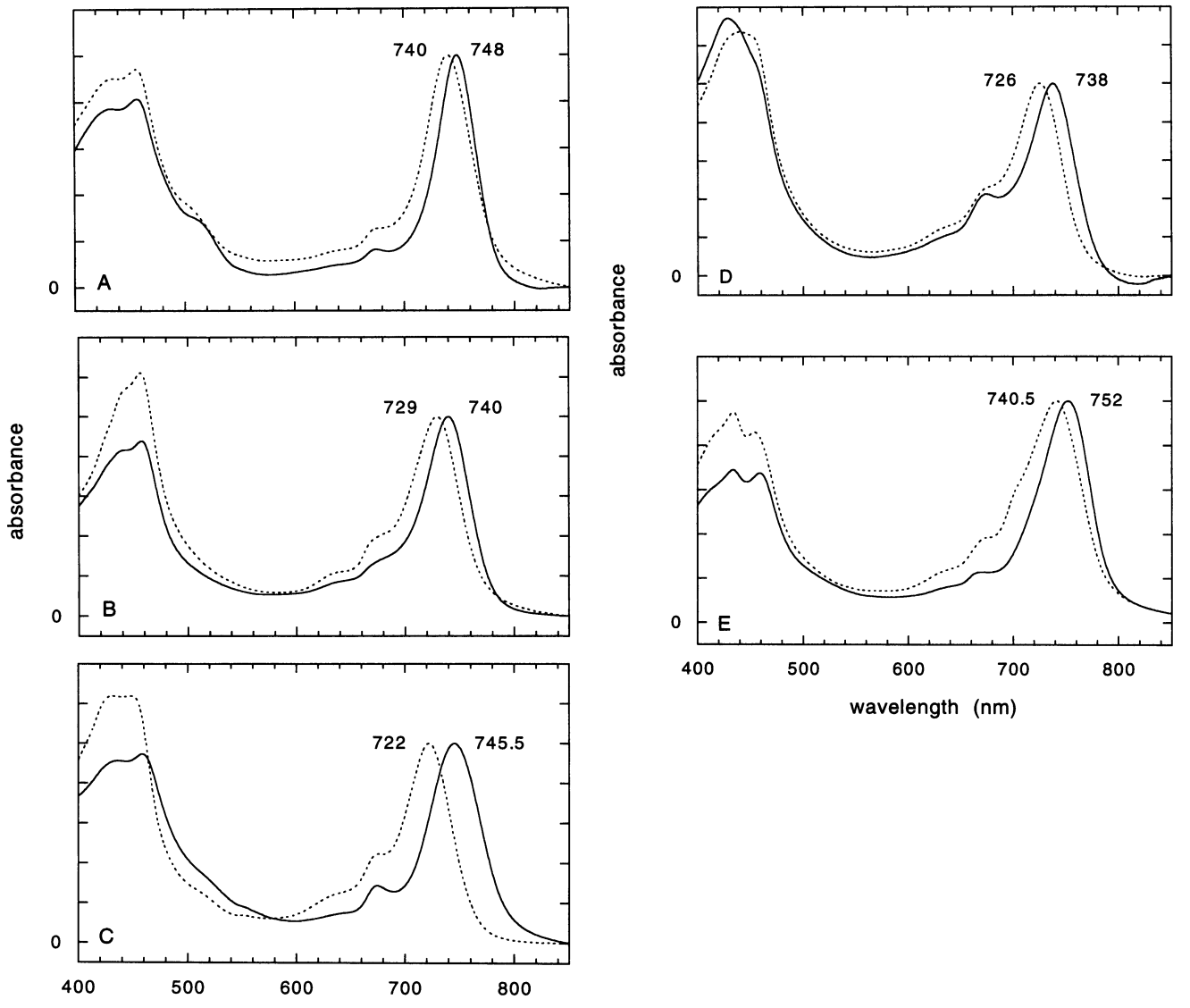

FIGURE 1 (*A*) Steady-state absorption spectra of native (——) and reconstituted protein free chlorosomes (– – –) of *Cb. tepidum*. Steady-state absorption spectra of purified [Pr,E] BChl c_F (——) and [E,E] BChl c_F (– – –) in (*B*) MGDG (*C*) nonpolar lipid extract of chlorosomes (*D*) dodecylmaltoside, and (*E*) hexane. The spectra were normalized to 1.0 in the Q_y absorption maximum.

between long-wavelength-absorbing BChl *c* and BChl *a* baseplate. The existence of such a long-wavelength-absorbing BChl *c* species in chlorosomes has also been suggested by other studies (Savikhin et al., 1994, 1995b; Mimuro et al., 1994; van Noort et al., 1994). Other longer-lived decay components with a maximum at 750 nm and 810 nm were ascribed to slow relaxation of BChl *c* and *a*, respectively.

The increase in fluorescence lifetime upon the addition of dithionite is in good agreement with steady-state fluorescence measurements (van Dorssen et al., 1986; Wang et al., 1990), in which 800-nm fluorescence is only observed at low redox potential. The fluorescence lifetimes in the presence and absence of dithionite in chlorosomes of *Cb. tepidum* are in good agreement with those observed by Causgrove et al. (1992) in chlorosomes of the related species *Cb. limicola*. The fluorescence excited-state dynamics of reconstituted chlorosomes are shown in Fig. 2 *B*. In the absence of dithionite (Fig. 2 *B, upper panel*) we observed a major decay component of 4 ps and a minor one of 45 ps, indicating that excitation energy in these reconstituted chlorosomes is highly quenched. Although the 4-ps component is at or beyond the time resolution of our apparatus, this short excitation lifetime is comparable to that of native chlorosomes under oxidized conditions. A similarity in excited-state dynamics of BChl *c* in native and reconstituted chlorosomes has also been reported by Savikhin et al. (1995a). Upon the addition of dithionite (Fig. 2 *B, lower panel*), the fluorescence lifetime of BChl *c* increased to about 22 ps and showed a rise at 800 nm, indicative of excitation energy transfer from BChl *c* to a longer-wavelength-absorbing species. Either longer-wavelength-absorbing BChl *c* oligomers or BChl *a* may act as acceptors of excitation energy. Similar

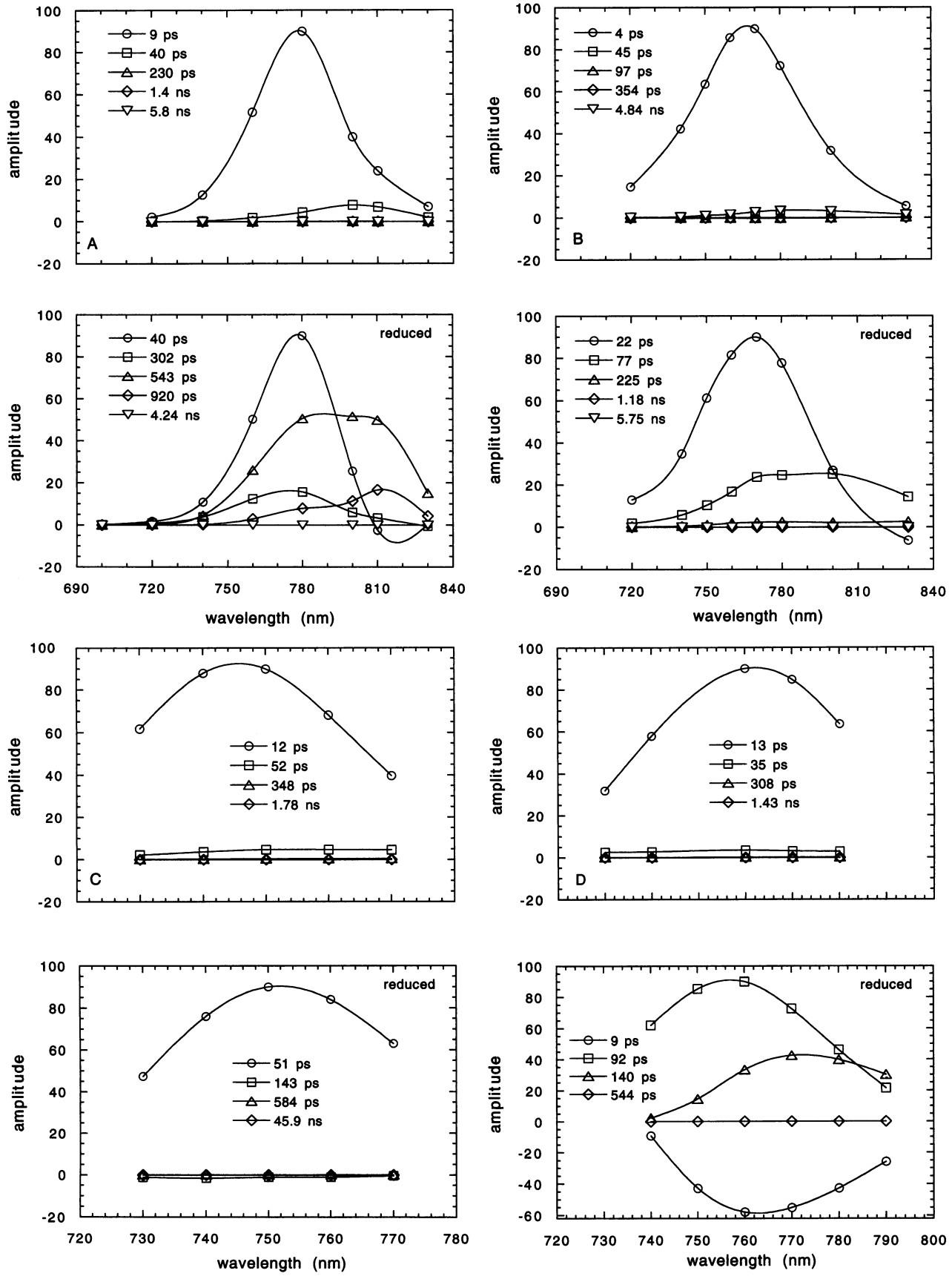

FIGURE 2 Decay-associated fluorescence decay spectra of (*A*) native chlorosomes (*B*) reconstituted chlorosomes (*C*) [E,E] BChl c_F in MGDG, and (*D*) [Pr,E] BChl c_F in MGDG in neutral (*upper panels*) and reduced (*lower panels*) condition. The excitation wavelength was at 590 nm.

energy transfer processes have been suggested by Miller et al. (1993) in reconstituted chlorosomes of *Cf. aurantiacus.*

Uehara et al. (1994) showed that long-wavelength-absorbing oligomers of BChl *c* can be prepared in the presence of MGDG. Although the structure of this lipid oligomer is not known, these preparations might reflect the in vivo structure of oligomers better than the oligomers in organic solvents. The decay-associated fluorescence spectra of [E,E] BChl c_F and [Pr,E] BChl c_F in MGDG are shown in Fig. 2, *C* and *D*, respectively. In the absence of dithionite we observed a major decay component of about 10 ps for oligomers of [E,E] BChl c_F (Fig. 2 *C, upper panel*) and [Pr,E] BChl c_F (Fig. 2 *D, upper panel*), indicating rapid quenching of excitation energy within these oligomers, similar to that observed in native and reconstituted chlorosomes. Upon the addition of dithionite the lifetimes of [E,E] BChl c_F and [Pr,E] BChl c_F MGDG oligomers increased significantly. In the case of [E,E] BChl c_F MGDG oligomers, the main fluorescence lifetime component increased from about 10 to 50 ps upon the addition of dithionite. The addition of dithionite to MGDG oligomers of [Pr,E] BChl c_F resulted in multiexponential relaxation kinetics. A rise component of about 10 ps with a maximum at 760 nm, a multiexponential decay with a major component of 90 ps showing a maximum at 760 nm, and a minor redshifted decay component of 140 ps with a maximum at 770 nm were observed. The accuracy of the 10-ps rise time is uncertain and depends on the selected time window for global analysis. However, a rise component was definitely needed to obtain a satisfactory fit of the data. The fluorescence decay at 670 nm showed a major decay component of 13 ps, suggesting that the observed rise in MGDG oligomers of [Pr,E] BChl c_F arises from energy transfer from a 670-nm-absorbing species to the main [Pr,E] BChl c_F MGDG oligomers. The absence of such a rise in [Pr,E] BChl c_F MGDG oligomers without dithionite can be explained by rapid quenching of excitation energy, preventing visualization of an apparent rise component. The multiexponential fluorescence decay from 740 to 790 nm indicates strong inhomogeneity in the MGDG [Pr,E] BChl c_F oligomers, which can mainly be ascribed to two types of [Pr,E] BChl c_F oligomers, fluorescent at 760 and 770 nm. The increase in fluorescence lifetime in MGDG oligomers of [E,E] BChl c_F and [Pr,E] BChl c_F upon the addition of dithionite are in good agreement with a two- to fivefold increase in the steady-state fluorescence (data not shown).

The redox effect on the excited-state lifetimes in BChl *c* oligomers is comparable to that observed in isolated chlorosomes, but cannot be explained by chlorosome-bound redox mediators. Traces of impurities during the isolation and purification of BChl *c* may account for the additional quenching of excitation energy in BChl *c* oligomers. We compared the increase in steady-state fluorescence of oligomers composed of freshly prepared BChl *c* with those of BChl *c* that had been stored. HPLC analysis showed that purified BChl *c* degrades during storage at −20°C into several degradation compounds. However, we observed no significant difference in the effect of dithionite on the steady-state fluorescence of oligomers composed of fresh or degraded BChl *c*, suggesting that the fluorescence quenching under oxidized conditions is not caused by the presence of trace impurities due to a small amount of degradation during isolation. Furthermore, neither the steady-state absorption nor the FT-Raman spectra of isolated chlorosomes (G. S. Jas, personal communication) show any significant differences upon the addition of dithionite, suggesting the absence of major structural differences upon the addition of dithionite.

Electron spin resonance

To study the potential formation of radicals, which may act to quench excitation energy in pigment oligomers (de Boer et al., 1987), we performed EPR experiments. EPR spectra of isolated chlorosomes of *Cf. aurantiacus* and *Cb. tepidum* under neutral, oxidized, and reduced conditions are shown in Fig. 3. Oxidized chlorosomes of *Cb. tepidum* (Fig. 3 *A*) show a clear EPR signal at a *g* value of 2.0025 and a linewidth of 5 Gauss. The *g* value at 2.0025 is characteristic for a free radical and is ascribed to oxidized BChl *c*. A

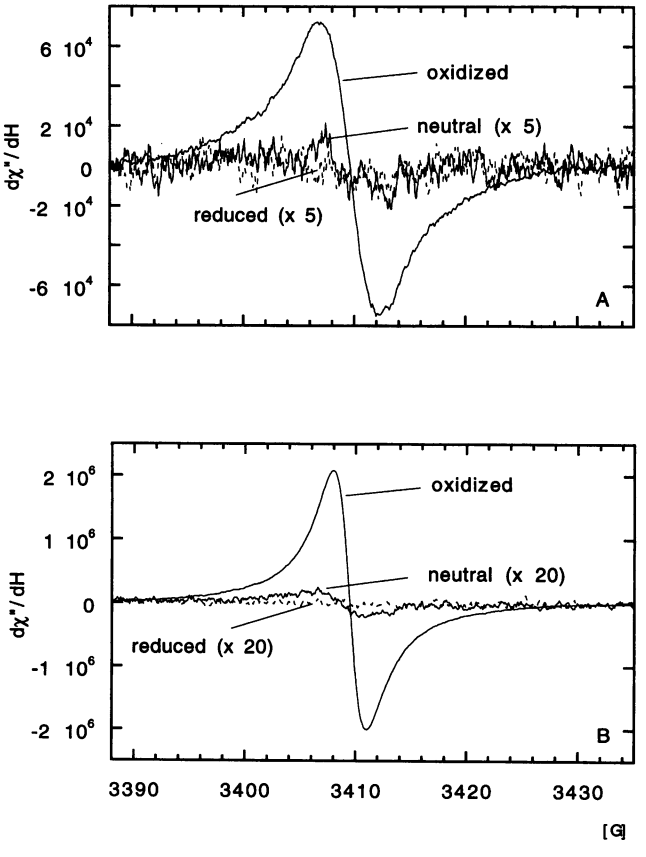

FIGURE 3 EPR spectra of chlorosomes of *Cb. tepidum* (*A*) and *Cf. aurantiacus* (*B*) in neutral, reduced, and oxidized conditions. The neutral and reduced spectra were enlarged for better comparison. The microwave power and modulation frequency were at 2.0 mW and 0.9 G, respectively. The temperature was 125 K.

similar small EPR signal is observed in neutral chlorosomes of *Cb. tepidum,* suggesting that a small fraction of BChls *c* is oxidized in this "neutral" state. When chlorosomes of *Cb. tepidum* were reduced by sodium dithionite, no significant EPR signal was observed, indicating that the traces of oxidized BChl *c* have been reduced by dithionite.

Oxidized chlorosomes of *Cf. aurantiacus* (Fig. 3 *B*) show a large EPR signal at the same *g* value as that observed for oxidized chlorosomes of *Cb. tepidum.* However, the linewidth of the EPR signal in chlorosomes of *Cf. aurantiacus* is about 3 Gauss. This linewidth is in good agreement with that observed by Betti et al. (1982) and somewhat smaller than that of chlorosomes of *Cb. tepidum.* Neutral chlorosomes of *Cf. aurantiacus* show a faint EPR signal. This indicates that neutral chlorosomes of *Cf. aurantiacus* contain minor traces of oxidized BChl *c*, as was also observed for neutral chlorosomes of *Cb. tepidum.* Reduced chlorosomes of *Cf. aurantiacus* show no trace of any EPR signal, indicating the complete absence of oxidized BChl *c*. After correction for differences in optical density, the EPR signal in neutral chlorosomes of *Cf. aurantiacus* is about 10 times larger than that in chlorosomes of *Cb. tepidum,* suggesting that radical formation may be more efficient in chlorosomes of *Cf. aurantiacus.*

Fig. 4 shows the results of EPR spectroscopy on oxidized [E,E] BChl c_F monomer and oxidized MGDG oligomers of [E,E] BChl c_F and [Pr,E] BChl c_F. Oxidized BChl *c* monomer (*upper signal*) shows an EPR signal at 2.0025 *g* with a linewidth of about 12 Gauss. Similar signals have been observed for radicals of monomeric BChl *a* and Chl *a* (Norris et al., 1971). Oxidized MGDG oligomers of [E,E] BChl c_F and [Pr,E] BChl c_F show EPR signals at the same *g* value, but with a linewidth of 8 Gauss and 5 Gauss, respectively. Because the optical densities of monomer and MGDG oligomers were equal in this experiment, the amplitudes of the EPR signals can be compared directly. This comparison suggests that radical formation by oxidation with $K_3Fe(CN)_6$ is least efficient in oligomers of [Pr,E] BChl c_F and somewhat more efficient in oligomers of [E,E] BChl c_F. In neutral oligomers of [E,E] BChl c_F and [Pr,E] BChl c_F we observed a similar EPR signal, indicating that traces of oxidized BChl *c* are present in these oligomers under so-called neutral conditions. The amplitude of the EPR signals in neutral oligomers was nearly 10 times smaller than those in oxidized conditions.

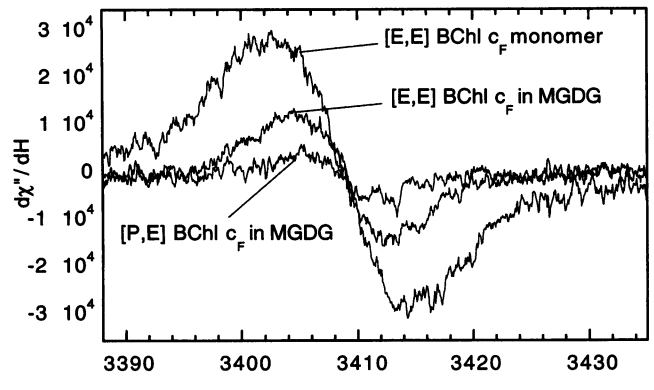

FIGURE 4 EPR spectra of [E,E] BChl c_F monomer in methanol/water (3:1 v/v), [E,E] BChl c_F, and [Pr,E] BChl c_F in MGDG. All samples were oxidized. The microwave power and modulation frequency were at 2.0 mW and 0.9 G, respectively. The temperature was 125 K.

DISCUSSION

The steady-state absorption maxima of [E,E] BChl c_F and [Pr,E] BChl c_F in hexane and aqueous lipid or detergent micelles are red shifted as compared to the 667 nm absorption of monomeric [E,E] BChl c_F and [Pr,E] BChl c_F in methanol. This indicates that excitonic interactions between molecules occur by the formation of oligomers. The final red shift of the absorption maximum of the excitonically coupled pigments depends on the intermolecular orientations and the size of the aggregate (Kasha, 1976; Alden et al., 1992). The larger the number of molecules involved in excitonic interaction, the larger the red shift of the final maximum absorption wavelength. Infrared spectroscopic studies on BChl *c* oligomers in organic solvents as well as in aqueous solution show similar vibrational bands, indicating analogous intermolecular interaction between molecules in these oligomers. This suggests that the observed increase in absorption maximum of [E,E] BChl c_F and [Pr,E] BChl c_F in dodecylmaltoside, MGDG, phospholipid, and hexane may be attributed to an increase in the size of the oligomers. This is corroborated by the observed precipitation of oligomers in hexane.

The steady-state absorption spectra (Fig. 1) also show that the absorption maximum of [E,E] BChl c_F oligomers is blue shifted compared to that of [Pr,E] BChl c_F in all solvents studied. A similar trend has been observed by Bobe et al. (1990) and may be ascribed to a slight variation in molecular excitonic interaction and/or oligomer size. At present there is no clear indication of variation in infrared vibrational bands of [E,E] BChl c_F and [Pr,E] BChl c_F oligomers (Uehara et al., 1994), suggesting that [E,E] BChl c_F oligomers are smaller than [Pr,E] BChl c_F oligomers. It is possible that an increase in lipophilicity of the alkyl groups at the 8 and 12 positions increases the hydrophobic interaction between porphyrin backbones, resulting in stronger interaction and more stable oligomers.

The fluorescence lifetime in chlorosomes of green sulfur bacteria strongly depends on the redox potential of the environment (van Dorssen et al., 1986; Wang et al., 1990; Blankenship et al., 1993). A similar redox effect is shown in chlorosomes of *Cb. tepidum* (Fig. 2 *A*). In the absence of sodium dithionite (so-called neutral condition), the major decay component in chlorosomes of *Cb. tepidum* has a time constant of 9 ps, and no energy transfer to BChl *a* can be observed. Upon the addition of sodium dithionite (reduced condition), the lifetime of the major decay component increases by up to 40 ps and can be ascribed to energy transfer to BChl *a*.

It has been suggested that the redox-dependent excited-state lifetime in chlorosomes is caused by redox-regulated quenchers that control the flux of excitation energy into the reaction center under oxidized or reduced conditions (Blankenship et al., 1993). A similar control mechanism has been observed in FMO proteins (Zhou et al., 1994), which has also been suggested to be a potential redox-controlled quencher of excitation energy. The excited-state dynamics of reconstituted chlorosomes (Fig. 2 *B*) also show a decrease in the excited-state lifetime, suggesting that a redox-controlled quencher in chlorosomes is also incorporated during the preparation procedure of reconstituted chlorosomes. Because these reconstituted chlorosomes are free of protein (Miller et al., 1993), the redox-controlled quencher is not a protein-bound species. This is corroborated by our observation that the excited-state lifetime of [E,E] BChl c_F and [Pr,E] BChl c_F in MGDG (Fig. 2, *C* and *D*) and dodecylmaltoside (data not shown) micelles is also redox dependent, indicating that the observed redox-dependent quenching of excitation energy in isolated chlorosomes is characteristic for BChl *c* oligomers.

The redox-dependent excited-state lifetime of the BChl *c* oligomers provides a different view of the mechanism of excitation quenching in chlorosomes. The absence of protein- or lipid-bound quenchers in BChl *c* oligomers suggests that the quenching is inherent to aggregated BChl *c* molecules. Our EPR data on chlorosomes of *Cb. tepidum* and *Cf. aurantiacus* show the presence of traces of free radicals under so-called neutral conditions. The *g* value of 2.0025 is very similar to that of oxidized BChl *c*, suggesting that some oxidized BChl *c* is indeed present. Under reduced conditions these radicals are absent in chlorosomes as well as in oligomers, indicating that the traces of oxidized BChl *c* may act as a potential quencher of excitation energy. Similarly, it is known from several antenna reaction center complexes that the oxidized primary donor, which is a BChl dimer, acts as an efficient quencher of excitation energy from the antenna.

The presence of oxidized BChl *c* radicals in chlorosomes of the green filamentous species *Cf. aurantiacus* suggests that the excited-state lifetime in these species may also be redox sensitive. However, previous steady-state fluorescence spectra did not show this effect (Wang et al., 1990), but more recent studies in isolated chlorosomes also show an increase in excited-state lifetime in this species upon reduction with sodium dithionite (Y. Zhu, personal communication). The cause of this difference in behavior between cells and isolated chlorosomes is still unclear.

The oxidation of chlorosomes of *Cb. tepidum* and *Cf. aurantiacus* results in an increase in the radical signal. The linewidth of the radical signal is narrow compared to monomeric oxidized BChl *c* (Fig. 4), indicating delocalization of the free electron among different BChl *c* molecules. Norris et al. (1971) showed in aggregates of Chl *a* that the linewidth of oxidized oligomers is proportional to the reciprocal of the square root of the number of pigments involved in delocalization of the free radical. Analogous to this relation, the number of pigments involved in delocalization of the radical was calculated to be about 16 and 6 in chlorosomes of *Cf. aurantiacus* and *Cb. tepidum*, respectively. This may suggest that oligomers in chlorosomes of the green filamentous species *Cf. aurantiacus* are larger than those in the green sulfur species *Cb. tepidum*. The EPR linewidth in oxidized chlorosomes of *Cf. aurantiacus* is in good agreement with previous studies of Betti et al. (1982). A similar number of closely connected molecules was reported by Smith et al. (1983) on oligomers of BChl *c* in hexane.

The EPR spectra of oxidized [E,E] BChl c_F and [Pr,E] BChl c_F oligomers also provide a good indication of the number of molecules involved in delocalization of the free electron and are therefore one measure of the size of the oligomers. The linewidths of 5 and 8 Gauss for oligomers of [E,E] BChl c_F and [Pr,E] BChl c_F indicate that approximately six and two BChl *c* molecules, respectively, are involved in delocalization of the free radicals in these oligomers. This number of closely connected molecules in oligomers is smaller than those in isolated chlorosomes of *Cb. tepidum* and *Cf. aurantiacus* and suggests that the blue shift of the steady-state absorption maximum of oligomers as compared to that of chlorosomes is an indication of a smaller number of molecules involved in oligomerization. Similarly, one may conclude that the consistently blue-shifted absorption maximum of [E,E] BChl c_F oligomers as compared to [P,E] BChl c_F oligomers is due to formation of smaller molecular clusters.

CONCLUSIONS

Oligomers of different types of BChl *c* spontaneously assemble in hydrophobic cavities created by amphiphilic molecules in aqueous solution. The red shift in absorption maximum due to aggregation of molecules is larger for [Pr,E] BChl c_F oligomers than for [E,E] BChl c_F oligomers, which can be ascribed to the formation of larger oligomers for [Pr,E] BChl c_F than for [E,E] BChl c_F, suggesting that lipophilic interactions between the alkyl groups at the 8 and 12 positions play a considerable role in the stability of oligomers. The time-resolved fluorescence study shows that the fluorescence lifetimes in native and reconstituted chlorosomes of *Cb. tepidum* and aqueous oligomers of [E,E] BChl c_F and [Pr,E] BChl c_F are redox controlled. This can be ascribed to the presence of BChl *c* radicals, which may act as a quencher for excitation energy and account for the short excited-state lifetimes in these systems.

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