The Role of βArg$_{-10}$ in the B800 Bacteriochlorophyll and Carotenoid Pigment Environment within the Light-Harvesting LH2 Complex of *Rhodobacter sphaeroides*[†]

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ABSTRACT: Previous work has suggested that the βArg$_{-10}$ residue forms part of the binding site for the B800 bacteriochlorophyll in the LH2 complex of *Rhodobacter sphaeroides* [Crielaard, W., Visschers, R. W., Fowler, G. J. S., van Grondelle, R., Hellingwerf, K. J., Hunter, C. N. (1994) *Biochim. Biophys. Acta 1183*, 473−482], and this is consistent with the X-ray crystallographic data that have been subsequently obtained for the related LH2 complex from *Rhodopseudomonas acidophila* [McDermott, G., Prince, S. M., Freer, A. A., Hawthornthwaite-Lawless, A. M., Papiz, M. Z., Cogdell, R. J., Isaacs, N. W. (1995) *Nature 374*, 517−521]. Therefore, in order obtain more information about the B800 binding site and its effect on the B800 absorption band, βArg$_{-10}$ was replaced by residues Met, His, Asn, Leu, and Lys (in addition to the Glu mutant described in our previous work); these residues were thought to represent a suitable range of amino acid shape, charge, and hydrogen-bonding ability. This new series of βArg$_{-10}$ mutants, in the form of LH2 complexes in the native membrane, has been characterized using a variety of biochemical and spectroscopic techniques in order to determine the ways in which the mutants differ from wild-type (WT) LH2. For example, most of the mutant LH2 complexes were found to have blue-shifted B800 absorption bands ranging from 794 to 783 nm at 77 K; the exception to this trend is the βArg$_{-10}$ to Met mutant, which absorbs maximally at 798 nm. These blue shifts decrease the spectral overlap between the "B800" and B850 pigments, which allowed us to examine the nature of the B800 to B850 transfer step for the βArg$_{-10}$ mutant LH2 complexes by carrying out a series of room temperature subpicosecond energy transfer measurements. The results of these measurements demonstrated that the reduced overlap leads to a slower B800 to B850 transfer, although the alterations at βArg$_{-10}$ were found to have little effect on the efficiency of internal energy transfer within LH2. Similarly, carotenoid to bacteriochlorophyll energy transfer was largely unaffected, although shifts in the excitation spectra in the carotenoid region were noted. These βArg$_{-10}$ mutant complexes provide an opportunity to investigate the structural requirements for the binding of monomeric bacteriochlorophyll and to examine the basis of the red shift seen for bacteriochlorophyll in photosynthetic complexes, in addition to providing new information about the environment of the carotenoid pigments in this complex.

The peripheral light-harvesting complex (LH2) of the photosynthetic bacterium *Rhodobacter (Rb.) sphaeroides* absorbs photons and transfers the energy to a core light-harvesting complex (LH1) which is connected in turn to the reaction center (RC),[1] where photochemistry occurs. LH2 is characterized by two absorbance bands in the near-infrared region that are located at approximately 800 and 850 nm. The positions of the near-infrared (NIR) bands of the bacteriochlorophyll (Bchl) pigments in LH2 are considerably shifted from their absorbance maxima in apolar solvents (∼770 nm), and it has been shown that both pigment−pigment and pigment−protein interactions account for this red shift [see, for example, Scherz and Parson (1984) and Fowler *et al.* (1992, 1994)].

The existence of these two absorbance bands within the same complex but with widely differing red shifts makes LH2 an interesting system for study, since the environments of B800 and B850 are expected to differ substantially. Early clues as to the environment around B800 were provided by a comparison of aligned sequences of LH2β polypeptides which showed that βArg$_{-10}$ is highly conserved, although not in LH1 complexes, which contain no B800 pigment (Brunisholz & Zuber, 1988). [Previously the LH2 residues have been numbered from the N-terminus, but for this work the system described by Loach *et al.* (1994) is used, in which the residues on the N-terminal side of the B850 His ligand are given a negative number (and those after His$_0$ a positive number); hence βArg$_{30}$ is now named βArg$_{-10}$.] This residue was therefore targetted for mutagenesis (Crielaard *et al.*, 1994; Visschers *et al.*, 1994), and one of the conclusions of this work, that the βArg$_{-10}$ residue forms part of the binding site for the B800 bacteriochlorophyll pigment in LH2, was consistent with the X-ray crystallographic data subsequently obtained on the related LH2 complex from *Rhodopseudomonas* (*Rps.*) *acidophila* (McDermott *et al.*, 1995). This showed that a strong hydrogen bond is formed between the 2-acetyl

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[1] Abbreviations: LH, light harvesting; RC, reaction center; Bchl, bacteriochlorophyll; WT, wild type.

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carbonyl group (ring B) of the B800 Bchl pigment and the βArg_{-10} residue, an observation that is strengthened by Raman spectroscopy studies on LH2 complexes (Sturgis *et al.,* 1995). However, it is interesting to note that the recently published structure for the LH2 complex from *Rhodopseudomonas molischianum* (Koepke *et al.,* 1996) indicates that such an Arg residue is not essential for B800 binding. It was therefore thought necessary to design experiments that further probed the structural requirements for the B800 binding site in the *Rb. sphaeroides* LH2 complex.

The energy transfer dynamics of the LH2 complex have been thoroughly investigated in recent years (Sundström & van Grondelle, 1994; van Grondelle *et al.,* 1994). Transfer among the spectrally inhomogeneous B800 molecules has been investigated by absorption anisotropy in the B800 absorption band at room temperature (Hess *et al.,* 1993) and at 77 K (Hess *et al.,* 1995a), wavelength-selective isotropic one-color absorption measurements of B800 (Hess *et al.,* 1995a; Monshouwer *et al.,* 1995), and hole burning (van der Laan *et al.,* 1993; de Caro *et al.,* 1994).

B800 to B850 transfer has been studied by monitoring either the decay of the B800 excited state (Hess *et al.,* 1995a; Monshouwer *et al.,*1995) or the formation of the B850 excited state using transient absorption measurements (Hess *et al.,* 1995b; Shreve *et al.,* 1991). The same process has also been monitored by time-resolving the rise of the B850 fluorescence following B800 excitation (Jimenez *et al.,* 1996) and by spectral hole burning of B800 (Reddy *et al.,* 1991; van der Laan *et al.,* 1993). It appears that the transfer of energy from the B800 to the B850 pigments takes ~0.7 ps at room temperature and 1.2−1.6 ps at 77 K and is somewhat slower (~2 ps) at 4 K. The dynamics within the B850 pigments have been examined by fluorescence (Jimenez *et al.,* 1996) and absorption (Pullerits *et al.,* 1996) anisotropy and are concluded to be very fast, on a 100 fs time scale. Transient absorption spectra of B850 were measured and interpreted to suggest that the elementary unit of B850 is a miniexciton extending over 4 ± 2 Bchl molecules (Pullerits *et al.,* 1996). Spectral hole burning of B850 at 4 K demonstrated slow transfer in the red wing of the B850 absorption band and fast relaxation (100 fs) in the blue and central parts of the spectrum which was interpreted as exciton relaxation (Reddy *et al.,* 1991).

In previous work we examined the mechanism of B800 to B850 energy transfer by investigating a series of LH2 mutants in which the B850 absorption band had been gradually blue shifted to 839 nm and then 826 nm by means of site-selective mutagenesis of residues αTyr_{+13}, Tyr_{+14} (Fowler *et al.,* 1992; Hess *et al.,*1994). In these mutants, the B800 pigment was largely unaffected by the changes at αTyr_{+13}, Tyr_{+14}. It was found that the variations produced in the transfer rate at 77 K could be explained by using the Förster energy transfer model, since the spectral overlap between B800 and the B850, B839, or B826 pigments steadily decreased, along with the transfer rate. A complementary approach would be to use mutagenesis to vary the absorption maximum of the B800 pigment, without changing the B850 absorption, which is one objective of the work reported below. In addition, mutagenesis of this residue provides important information on the binding site for a monomeric Bchl without any complications arising from the close excitonic coupling among the B850 ring of Bchls, which tend to "smear out" local spectral effects. Finally, there is the interaction of carotenoid and B800 molecules to consider, which is already known to be influenced by changing βArg_{-10} to Glu (Crielaard *et al.,* 1994; Visschers *et al.,* 1994). The structural data obtained for the *Rps. acidophila* LH2 complex were found to be consistent with this observation and showed that at least one of the carotenoid molecules makes close van der Waals contacts with the B800 bacteriochlorophyll pigment (Freer *et al.,* 1996).

Therefore, in order to obtain more information about the B800 binding site and its effect on the B800 absorption band, a range of mutations at the βArg_{-10} residue was engineered. The replacement residues Met, His, Asn, Leu, and Lys (in addition to the Glu mutant described in our previous work) were chosen, as they were thought to represent a suitable range of amino acid shape, charge, and hydrogen-bonding ability. In the present work the new series of βArg_{-10} LH2 mutants is characterized using a variety of biochemical and spectroscopic techniques in order to determine the ways in which they differ from wild-type (WT) LH2; for example, most of the mutant LH2 complexes were found to have blue-shifted B800 absorption bands. As a result it was decided to investigate further the nature of the B800 to B850 transfer step by carrying out a series of room temperature subpicosecond time-resolved energy transfer measurements for the βArg_{-10} mutant LH2 complexes; steady-state spectroscopic measurements at 77 K were also carried out in order to examine carotenoid to bacteriochlorophyll energy transfer.

EXPERIMENTAL PROCEDURES

Media, Antibiotics, and Growth Conditions. *Escherichia coli* strains were grown in Luria broth. *Rb. sphaeroides* strains were grown under semiaerobic/dark conditions at 34 °C on M22+ medium agar (Hunter & Turner, 1988); the M22+ medium was supplemented with 0.1% casamino acids for growth in liquid culture. For *E. coli*, tetracycline was used at a concentration of 10 μg/mL. For *Rb. sphaeroides*, antibiotic concentrations were as follows: tetracycline, 1 μg mL^{-1}; neomycin, 20 μg mL^{-1}; and streptomycin, 5 μg mL^{-1}.

Bacterial Strains and Plasmids. The *E. coli* strains used in this work include S17-1 [*thi pro hsdR*$^-$ *hsdM*$^+$ *recA RP4-2* (Tc::mu km::Tn7)] used for conjugative transfer of mobilizable plasmids into *Rb. sphaeroides* (Simon *et al.,* 1983). The *Rb. sphaeroides* strains used were DD13 and DD13/G1 (genomic deletion of both *pucBA* and *pufBALMX*; insertion of Sm^R and Km^R genes, respectively) and DBCΩ (genomic deletion of *pucBA* genes; insertion of Sm^R genes leaving only the native LH1-RC core, wild-type carotenoids) (Jones *et al.,* 1992). The two LH1-minus, RC-minus *Rb. sphaeroides* strains, DD13 and DD13/G1, differ in that while the DD13 mutant strain contains the normal complement of carotenoids, spheroidene and spheroidenone (red in appearance) found in WT *Rb. sphaeroides*, the DD13/G1 mutant strain carries a mutation in the *crt*D gene that alters the normal complement of carotenoids to neurosporene and derivatives (green in appearance). The mobilizable plasmids used were based on pRKCBC1 (Tc^R; derivative of pRK415; insertion of a 4.4 kb fragment encompassing *pucBAC*); briefly, this expression vector, described by Jones *et al.* (1992), contains the *pucBA* genes as a 420 bp *Kpn*I−*Bam*HI insert.

Conjugative Crosses. The constructs were introduced into *Rb. sphaeroides* by conjugative transfer. The mobilizable

plasmids to be introduced into *Rb. sphaeroides* were first transformed into *E. coli* strain S17-1 (Simon *et al.*, 1983). Matings were then performed as described in Hunter and Turner (1988). Transconjugants were grown aerobically in the dark on plates of M22+ medium supplemented with appropriate antibiotics. In the case of matings performed to complement deletion/insertion mutants *in trans* using plasmids based on pRKCBC1, tetracycline was included in the growth medium.

Site-Directed Mutagenesis. Construction of mutated *pucBA* genes in which specific codons at the βArg_{-10} site were altered was performed using a plasmid created using the pALTER-1 vector supplied by the Promega Corp. (Madison, WI). A *pucBA* fragment flanked by engineered *Kpn*I and *Bam*HI sites upstream and downstream of the *pucBA* genes, respectively, was cloned from pRKCBC1 (Jones *et al.*, 1992) into pALTER-1. Oligonucleotides corresponding to the noncoding strand were then used to alter the βArg_{-10} CGC codon according to the procedure supplied by Promega Corp. to the codons for Glu, His, Asn, Lys, or Leu. The mutagenized *Kpn*I−*Bam*HI fragment was then recloned into pRKCBC1 in place of the WT genes.

Preparation of Intracytoplasmic Membranes. Membranes were prepared from cells grown semiaerobically in the dark by disruption in a French pressure cell and were purified by harvesting from the interface of sucrose step gradients (15%/40% w/w) after centrifugation.

Steady-State Spectroscopy. Following conjugative transfer, antibiotic-resistant colonies were screened for the presence or absence of LH2 light-harvesting complexes using a Guided Wave Model 260 fiber optic spectrophotometer (Guided Wave Inc., 1590 Golden Foothill Parkway, El Dorado Hills, CA 95630). The fluorescence spectra were recorded on a Spex fluorolog spectrofluorometer (Spex Industries, Inc., 3880 Park Ave., Edison, NJ 08830). For recording the excitation spectra, an excitation slit width of 9 nm and an emission slit width of 18 nm were used. Low-temperature measurements were performed using a cryostat (Oxford Instruments Ltd.) cooled with liquid nitrogen. Samples were made up in 50% glycerol so that they formed a clear glass upon cooling to 77 K. Gaussian deconvolution analyses of absorption spectra were carried out using the Datamax software package of the Fluorolog spectrofluorometer.

Time-Resolved Spectroscopy. The direct transfer of energy from B800 to B850 at room temperature was investigated by measuring the transient absorption kinetics at selected wavelengths covering the B850 band. The system has been described elsewhere [see, for example, van der Laan *et al.* (1993), Hess *et al.* (1995a,b), and Pullerits *et al.* (1996)], but briefly all experiments were performed by using a femtosecond spectrometer with 100−140 fs pulses generated in an amplified Ti:Sa laser system. Amplified 795 nm pulses of 1.5×10^{14} photons cm^{-2} $pulse^{-1}$ energy at the sample and 5 kHz repetition rate were split into two parts, one for excitation of the B800 band and the other for generation of the white-light continuum for probing the absorbance changes induced by the excitation.

For room temperature measurements the samples were diluted with a Tris buffer solution (pH 8) to an optical density of approximately 0.4 and kept in a stationary cell of 1 mm path length, as has been described in detail previously (Sundström *et al.*, 1986; van Grondelle *et al.*, 1987). For 77 K spectra, membrane samples were made up in Tris buffer solution (pH 8) containing 65% glycerol which when frozen gave a clear glass. Due to the lability of some of the LH2 samples (see Figure 1), the absorption spectra of each sample were recorded before and after each experiment to ensure that no bleaching of the absorption bands had occurred. In case of the βArg_{-10}Leu LH2 mutant, for example, the very act of freezing and thawing the membrane samples in the course of transporting them led to some loss of the B800 band, but no extra pigment loss was observed during the kinetic studies. The transient absorption kinetics were analyzed as a sum of exponentials, in either single-trace or global analysis, yielding lifetimes and amplitudes. The finite width of excitation and probe pulses was accounted for by convoluting the fitted decay function with the apparatus response. All kinetic parameters were obtained using the Spectra Solve software package, either in single-trace and global analysis.

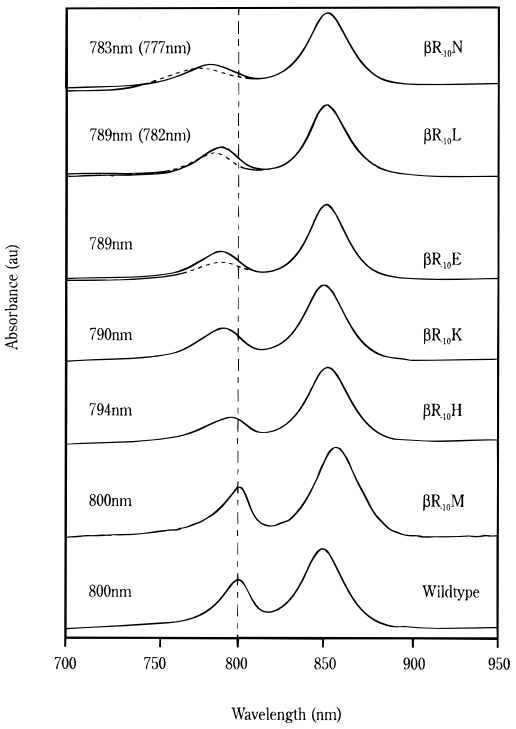

FIGURE 1: Near-infrared 77 K absorbance spectra of membranes of LH2 complexes containing site-directed mutations at the βArg_{-10} residue. The absorbance maximum for each B800 is given above each spectrum. For the mutant LH2 complexes in which βArg_{-10} is changed to an Asn, a Leu, or a Glu residue, the lability of the B800 band is illustrated by showing the band both in an unaltered state (solid line) and after exposure to light and/or freezing/thawing (dashed line, wavelength value in parentheses).

RESULTS

Absorbance Spectra of the βArg_{-10} LH2 Mutant Complexes. The mutagenesis and expression system used here produces strains in which LH2 is the major membrane protein; the LH1 and RC complexes have been removed by prior deletion of the appropriate genes. All of the work described below was carried out on membranes (rather than, for example, detergent-solubilized or purified complexes). The near-infrared absorbance spectra of membranes prepared from LH2-only transconjugant strains of *Rb. sphaeroides* recorded at 77 K are shown in Figure 1. The spectra clearly demonstrate that there are differences between each of the

mutant βArg$_{-10}$ LH2 complexes compared both to each other and to the wild-type (WT) LH2 complex. The most noticeable difference is seen for the B800 bands, which are attenuated and which show blue shifts ranging from 7 nm for the βArg$_{-10}$His LH2 complex to 17 nm for the βArg$_{-10}$Asn complex. The exception to this trend is the βArg$_{-10}$Met mutant which has a pseudo-WT B800 band. The Q_x absorbance band at ~590 nm was previously shown to be slightly red shifted for the βArg$_{-10}$Glu mutant (Crielaard *et al.*, 1994), and in the present work similar shifts (~1−3 nm) were observed for the other βArg$_{-10}$ mutants.

Some of the spectra in Figure 1, those for βArg$_{-10}$Leu, βArg$_{-10}$Asn, and βArg$_{-10}$Glu, are shown with two superimposed B800 peaks, one showing the peak in an unaltered state (plain line) and the other showing the same peak after exposure to light and/or freezing/thawing (dotted line); in most cases this loss of the B800 band was simultaneously associated with it being further broadened and blue shifted compared to WT. Although the other βArg$_{-10}$ LH2 mutant complexes also showed some lability in their B800 bands, βArg$_{-10}$Leu and βArg$_{-10}$Asn were the most affected.

A second difference between the spectra of the LH2 complexes shown in Figure 1 is seen in the shapes of the individual B800 bands. As has been noted elsewhere, the B800 band of the WT LH2 complex is nearly Gaussian on the red side of the band with a much shallower blue tail (Visschers *et al.*, 1994). The βArg$_{-10}$Met and βArg$_{-10}$His LH2 complexes show B800 bands shaped similarly to those of the WT LH2, if slightly more symmetrical; a Gaussian deconvolution analysis confirmed these observations (data not shown). In contrast, the βArg$_{-10}$Glu LH2 complex, as has been observed previously (Visschers *et al.*, 1994), has a much more symmetrical B800 band (with a less steep red side) than the WT; similarly symmetrical B800 bands are seen for the βArg$_{-10}$Lys, βArg$_{-10}$Asn, and βArg$_{-10}$Leu LH2 mutants.

Finally, the near-infrared spectra of a couple of the βArg$_{-10}$ mutants differed from WT LH2 in that their B850 bands were found to be red shifted compared to WT at 77 K, an effect which was most noticeable for the βArg$_{-10}$Met complex (Figure 1). No equivalent B850 red shift was observed for the βArg$_{-10}$Met complex at room temperature.

Functional Test for Energy Transfer within the βArg$_{-10}$ LH2 Mutant Complexes from B800 to B850. As has been previously observed (Crielaard *et al.*, 1994), the functionality of the βArg$_{-10}$Glu mutant LH2 with respect to "B800" to B850 energy transfer is unimpaired. Excitation of the complex into either the Bchl Q_x band or into the B800 Q_y band at 789 nm gives an emission at ~870 nm, the emission peak of LH2. Fluorescence emission spectra were recorded for all of the βArg$_{-10}$ mutant LH2 complexes and were all found to be similar to those for WT LH2 and the βArg$_{-10}$Glu mutant (data not shown). Likewise, fluorescence excitation spectra for the βArg$_{-10}$ mutant LH2 complexes measured at both 77 K and room temperature (monitoring the LH2 emission at ~870 nm; data not shown) showed profiles that matched both of the absorption spectra (see Figure 1), with equivalent B800 and B850 nm near-infrared peaks. Therefore, for all of the βArg$_{-10}$ mutant LH2 complexes, B800 to B850 energy transfer is fully functional as judged by steady-state fluorescence measurements, despite the presence of blue-shifted B800 bands.

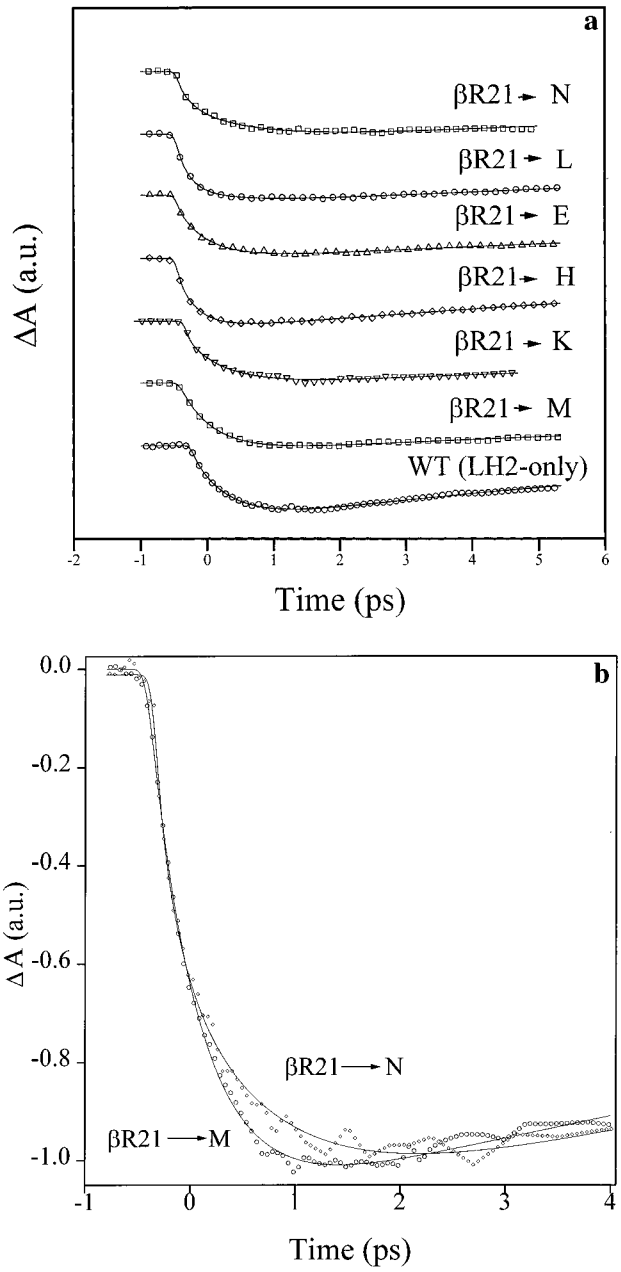

FIGURE 2: (a) Room temperature two-color pump−probe transient absorption measurements of membrane-bound LH2 complexes containing site-directed mutations at the βArg$_{-10}$ residue. In order to measure B800 to B850 energy transfer within LH2, the kinetic traces were obtained by probing at 860 nm after excitation at 795 nm. The absorption spectra of the samples were taken before and after each experiment to ensure that no bleaching of the absorption bands had occurred. (b) Room temperature two-color isotropic measurements of the βArg$_{-10}$ mutant LH2 with the least blue-shifted B800 band, βArg$_{-10}$Met, as compared with one of the βArg$_{-10}$ LH2 mutants having the most blue-shifted B800 band, βArg$_{-10}$Asn. The experiments were performed by excitation of the B800 bands at 795 nm and the arrival of energy to B850 monitored by probing at 860 nm by measuring the B850 bleaching/stimulated emission. The measured kinetics were normalized to $\Delta A = -1$ in order to emphasize the difference in the rise time between the two LH2 complexes (see text).

Measurement of Subpicosecond Time Scale B800 to B850 Energy Transfer within the βArg$_{-10}$ LH2 Mutant Complexes at Room Temperature. In order to probe the B800 to B850 energy transfer characteristics for these complexes further, it was decided to carry out subpicosecond measurements. Figure 2a shows the room temperature transient absorption

Table 1: Global Kinetic Analysis of Energy Transfer at Room Temperature from the B800 to the B850 Pigments in LH2 Complexes Containing Site-Directed Mutations at the βArg$_{-10}$ Residue

LH2 complex	λ_{max}^{B800}/nm	τ_1/ps	τ_2/ps	τ_3/ps	χ^2
wild-type *R. sphaeroides*	800	0.14	0.57	19	1.657
βArg$_{-10}$ (WT)	800	0.12	0.58	17.6	1.775
βArg$_{-10}$Met	798	0.11	0.6	23	1.720
βArg$_{-10}$His	793	0.20	0.72	21.5	1.83
βArg$_{-10}$Lys	790	0.10	0.65	22.7	1.350
βArg$_{-10}$Glu	789	0.17	0.83	16.6	1.195
βArg$_{-10}$Leu	782	0.12	0.95	20.5	1.749
βArg$_{-10}$Asn	782	0.11	0.98	19.8	1.019

kinetics for the energy transfer from B800 to B850 in the six βArg$_{-10}$ mutant LH2 complexes, while Table 1 summarizes the results of the global analyses performed on the kinetics at several wavelengths probed in the 830−860 nm range.

The kinetic traces shown in Figure 2a were measured at 860 nm after the B800 pigments were excited at 795 nm. The signal measured at 860 nm is known to be a combination of both ground-state bleaching and stimulated emission of B850 (Hess *et al.*, 1995a,b), and in all cases it was seen that the 795 nm excitation pulse induced a biphasic rise of the 860 nm signal followed by a slow decay. The kinetics for each mutant complex could be fitted using a sum of three exponentials, by either single-trace or global analysis, including the measurements obtained from probing at several wavelengths in the 830−860 nm range. These three components consisted of a fast rise time component ($\tau_1 \sim$ 0.2 ps) of small amplitude, a slower rise time of dominant amplitude ($\tau_2 \sim 0.5-1.0$ ps), and a very long decay time ($\tau_3 \sim 20$ ps) (Table 1). The fast component, τ_1, is almost invariable in the different mutants and has been previously assigned either to exciton relaxation within a strongly coupled B850 aggregate or to energy equilibration among the spectrally inhomogeneous B850 pigment molecules (Bradforth *et al.*, 1995; Hess *et al.*, 1995a; Pullerits *et al.*, 1996). Similarly, the slow component, τ_3, although not very well characterized on this short time scale, is attributable to the decay of the B850 excited state and shows a similar value ($\sim$20 ps) for all the samples. The $\sim$20 ps lifetime of τ_3 given here only represents the initial part of the slow (several hundred picoseconds; Bergström *et al.*, 1988) B850 excited-state lifetime and is most likely due to some excitation annihilation caused by the excitation pulse intensity used in the present experiments.

Of particular interest to the present work are the values of τ_2 for the βArg$_{-10}$ mutant LH2 complexes; for the WT LH2 complex these have been shown to be directly related to the energy transfer time from B800 to B850 (Hess *et al.*, 1995a,b). As can been seen from Table 1, there is a direct correlation between increasing B800 to B850 energy transfer times and the degree of blue shift of the individual B800 bands, increasing from 0.6 ps for a WT B800 to 0.98 ps for a B800 band blue shifted by 18 nm (βArg$_{-10}$Asn). This increase in B800−B850 transfer times for the blue-shifted B800 mutants is further emphasized in Figure 2b by comparing the kinetics of the least blue-shifted mutant, βArg$_{-10}$Met, with that of one of the most blue-shifted mutants, βArg$_{-10}$Asn. In order to emphasize the difference between the slower components of the biphasic rise time for the two LH2 complexes, the kinetic data have been normalized to $\Delta A = -1$ (Figure 2b); this slower component is thought to represent B800 to B850 energy transfer. In contrast, the very fast and slow decay components seen in the kinetic analysis remain almost invariable between the two complexes (Figure 2b, Table 1). The absorption of each sample at the excitation wavelength (795 nm) was approximately 0.5 while the signal intensity was of the order of 0.02−0.03. In order to ascertain that the measured kinetics were not affected by intensity-dependent nonlinear processes (for example, annihilation excitation), the measurements were repeated at 10-fold lower excitation energy; within experimental error the results were identical to those obtained at higher intensities (data not shown).

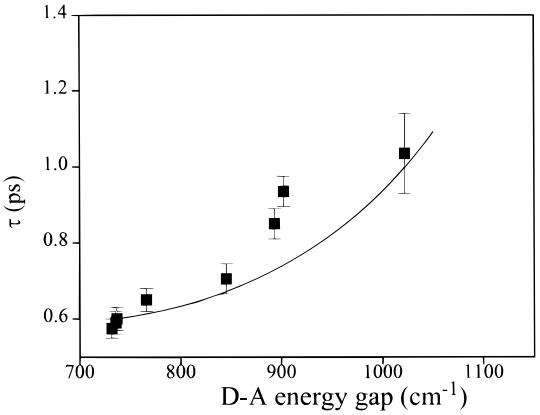

FIGURE 3: Room temperature B800 to B850 energy transfer times (τ_2) obtained from analyses of the kinetic traces similar to those shown in Figure 2, as a function of the donor−acceptor energy gap between the B850 and B800 bands for the βArg$_{-10}$ LH2 mutants. The samples were probed at several wavelengths within the B850 band after excitation at 795 nm; each point represents the τ_2 values obtained from a global analysis, while the bars correspond to the spread of τ_2 values obtained in the single trace analyses. The solid line displays the results of calculating the rate of B800−B850 energy transfer at room temperature (solid line) for this series of mutants, using the expressions derived by Kolaczkowski *et al.* (1994).

Thus it can be seen that the B850 signal for the βArg$_{-10}$Met mutant LH2 peaks at approximately 1.2 ps, while the maximum of the βArg$_{-10}$Asn mutant LH2 occurs considerably later at about 2.3 ps, directly illustrating the slower formation of the B850 excited state following B800 to B850 energy transfer in the βArg$_{-10}$Asn complex.

The variation of transfer time (as represented by τ_2) plotted against the increasing energy gap between the B800 and B850 absorption bands is shown in Figure 3. In order to examine the reproducibility and stability of the fitted lifetimes, both single-trace and global analyses were performed on the kinetics measured at several wavelengths over the B850 absorption band. The symbols in Figure 3 represent the results from the global analysis, while the bars for each point cover the spread of lifetimes obtained from the single-trace analysis at all measured wavelengths. Although the excited-state absorption from B850 was seen to make an additional contribution to the measured kinetics at detection wavelengths shorter than 860 nm, this did not alter the trends shown by the kinetic analysis (data not shown). The results show that the increase of the B800−B850 transfer time is directly correlated to the B800−B850 spectral separation and significantly larger than even the most conservative error limits provided by the spread of the single-trace analysis lifetimes.

Quantitative Analysis of the B800 → B850 Spectral Overlap. Figure 3 also displays the results of calculating the rate of B800−B850 energy transfer at room temperature (solid line) for this series of mutants, using the expressions derived by Kolaczkowski *et al.* (1994) for the calculation of energy transfer rates, using the microscopic homogeneous and inhomogeneous properties of the spectral bands.

The transfer rate (k_{DA}) averaged over the inhomogeneous distribution of donor and acceptor energies is given by

$$\langle k_{DA} \rangle = 2\pi\eta^{-4}V^2(1 - e^{-\tilde{S}})(2FC_{loc})[2\pi(\Gamma^2 + \Sigma^2)]^{-1/2} \times \exp\{-[(\Omega_o - \omega_{loc} - S\omega_m)^2/2(\Gamma^2 + \Sigma^2)]\} \quad (1)$$

where V is the electronic coupling, the term $(1 - e^{-\tilde{S}})$ is the Franck−Condon factor of the phonons, FC_{loc} and ω_{loc} are the Franck−Condon factor and frequency of the Bchl acceptor modes, Γ^2 is the variance of the donor−acceptor electronic energy gap, Σ^2 is the variance of the homogeneous broadening factor, and Ω_o is the donor−acceptor energy gap. $\tilde{S}$ and Σ are temperature dependent and given by

$$\tilde{S}(T) \sim S \coth(\hbar\omega_m/2kT) \quad (2)$$

$$\Sigma^2(T) \sim S(T)(\sigma^2 + \omega_m{}^2) + (\Gamma_h/2)^2 \quad (3)$$

where S is the Huang−Rhys factor for the phonons, ω_m is the mean frequency of the low-frequency phonons involved as acceptor modes, σ^2 is the variance of the frequency distribution of the phonons coupled to the energy transfer, and Γ_h is the homogeneous broadening of the B850 band (Reddy *et al.*, 1996).

In our calculations of the B800 to B850 transfer rate at room temperature, we have used the values of S, σ, ω_m, Γ_h, FC_{loc}, Γ, and Ω_o suggested by Reddy *et al.* (1991) and later used by Reddy *et al.* (1996) and Wu *et al.* (1996) to calculate the B800 to B850 transfer rate within the LH2 complexes of *Rb. sphaeroides* and *Rps. acidophila*. For Bchl acceptor modes ω_{loc}, we have used the B850 vibronic hole structures obtained from hole burning (280, 340, 560, 750, and 920 cm^{-1}). The Franck−Condon factors used were 0.05 for the mode absorbing at 750 cm^{-1} and 0.01 for all the other modes, as obtained in the analysis by Wu *et al.* (1996). The total contribution made by these five Bchl modes to the transfer rate for the mutant and WT complexes was calculated by using eq 1. It was assumed that, by shifting the B800 band, only the B800−B850 energy gap changes and that other possible variables, such as the electronic coupling and the inhomogeneous broadening of B800, remain constant. The ratio between the transfer time calculated for the WT (normalized to the experimental value) and each mutant is plotted as a function of the B800 → B850 energy gap of each mutant and compared to the experimental data in Figure 3. The points with error bars represent the measured energy transfer times from B800 to B850, as already mentioned in the previous section, while the line is the result of the calculations. It can be seen that these calculated energy transfer times are in reasonable agreement with the experimental data and reproduce the slowing down of the B800 → B850 transfer process with increasing energy gap.

Spectral Properties and Functionality of the Carotenoid Pigments in the βArg_{-10} LH2 Mutant Complexes. For this section of the experimental work, the βArg_{-10} LH2 mutations were introduced into two different null backgrounds (LH2-minus, LH1-minus, RC-minus), one of which, DD13, synthesizes WT carotenoids spheroidene and spheroidenone which are red in appearance and the other of which, DD13/G1, synthesizes neurosporene and derivatives and is green in appearance. In this way it was hoped to determine how the effects of the βArg_{-10} mutations on LH2 are modulated by these two types of carotenoid.

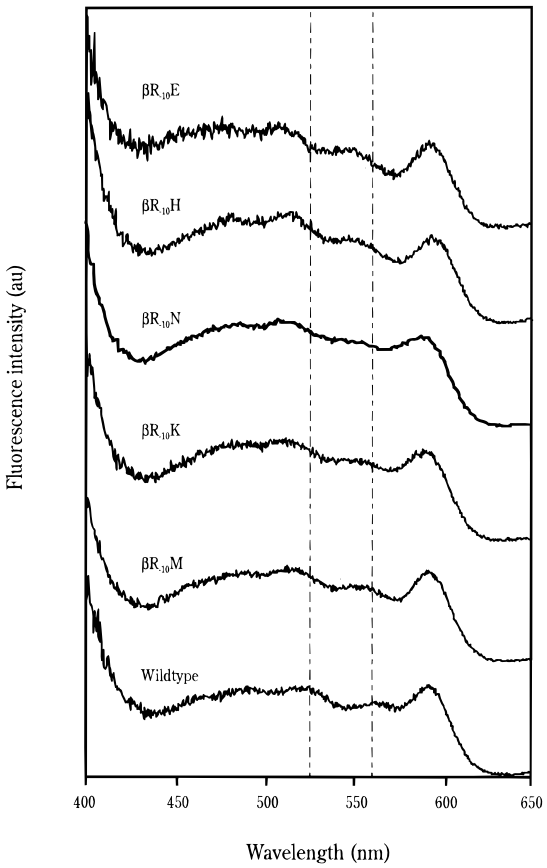

FIGURE 4: 77 K excitation spectra in the 350−650 nm region for the βArg_{-10} LH2-containing membranes expressed in the red DD13 *Rb. sphaeroides* strain. The spheroidene/spheroidenone excitation bands (for example, found at 526 and 561 nm for WT LH2) show the ability of these carotenoids present to elicit fluorescence from the Q_y bands of the bacteriochlorophylls, which emit at 860 nm. The spectra are normalized at the 590 nm Bchl Q_x band excitation peak, which can be taken as representing fully efficient transfer from the Bchl Q_x band at 590 nm to the Q_y band at 860 nm. Examination of the absorption spectra from these complexes at 77 K suggests that the carotenoid excitation peaks generally match those found in the underlying absorption peaks; since, however, the carotenoid absorption peaks for the red DD13 strains are rather ill-defined even at 77 K [see, for example, the spectrum for WT LH2/DD13 membranes given in Fowler *et al.* (1995)], the excitation spectra are presented instead.

Figure 4 compares the 77 K excitation spectra in the 350−650 nm region for the βArg_{-10} LH2-containing membranes expressed in the red DD13 *Rb. sphaeroides* strain and measures the ability of the spheroidene/spheroidenone carotenoids present to elicit fluorescence from the bacteriochlorophylls, detected at 860 nm. The spectra are normalized at the 590 nm excitation peak, which can be taken as representing fully efficient transfer within the Bchl molecule, from the Q_x band at 590 nm to the Q_y band, which emits at 860 nm. From the normalized carotenoid excitation peaks shown in Figure 4, it can be seen that the energy transfer process from the carotenoid pigments to the Bchl pigments

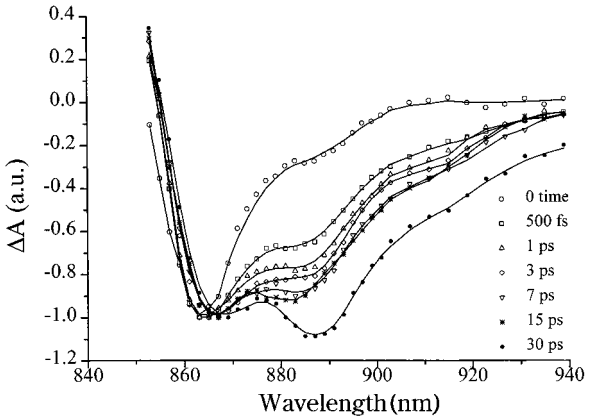

FIGURE 5: Transient absorption spectra resulting from experimental (symbols) and Gaussian fits (lines) for the βArg$_{-10}$Leu mutant, measured with 80 fs time resolution at 77 K. The spectra are normalized to the maximum of the negative signal corresponding to the bleaching of B850. For other parameters see the text. The B800 pigments were excited at 790 nm with a pulse of about 80 fs and probed with femtosecond continuum light in the 850−940 nm wavelength region at different delay times after excitation. The symbols represent the measured data at intervals of 2 nm, and the solids lines are the multi-Gaussian fits.

is efficient for both WT and βArg$_{-10}$ mutant LH2 complexes in DD13. Surprisingly, however, a closer examination of the carotenoid region (450−550 nm) of the spectra in Figure 4 shows that the carotenoid excitation peaks from the βArg$_{-10}$ mutant LH2 complexes (in the red DD13 *Rb. sphaeroides* strain) are blue shifted relative to the WT LH2. The shifts range from 4 to 5 nm for the βArg$_{-10}$Lys, βArg$_{-10}$Met, and βArg$_{-10}$Leu LH2 complexes, through 7 nm for the βArg$_{-10}$Asn LH2 complex, up to a remarkable 10−11 nm excitation blue shift for the βArg$_{-10}$His and βArg$_{-10}$Glu mutants.

This is different from the situation found for the same βArg$_{-10}$ mutants expressed in the green neurosporene-containing DD13/G1 *Rb. sphaeroides* strain, where there is little difference from the absorption and excitation spectra of WT LH2. It has already been noted in our previous work that, for the βArg$_{-10}$Glu mutant LH2 expressed in strain DD13/G1, there is a very slight (1 nm) blue shift of the carotenoid absorption peaks at ∼459.5 and ∼491.5 nm with no associated band broadening, relative to WT LH2 (Crielaard *et al.,* 1994); this was found generally to be the case for the other βArg$_{-10}$ mutants. Similarly, efficient carotenoid−Bchl energy transfer was found for the LH2 complexes in the green neurosporene-containing DD13/G1 *Rb. sphaeroides* strains, as indicated by matching fluorescence excitation and absorption spectra (data not shown).

Probing Energy Transfer from the βArg$_{-10}$ LH2 Mutants to WT LH1 Complexes. The role of the peripheral LH2 antenna is to transfer energy to the core LH1 complex, a process that can be monitored by recording time-resolved transient absorption difference spectra for each mutant. For this work we exploited the flexibility of the mutagenesis system, which can provide either a null background for examination of the LH2 complex alone or an LH1−RC background for examination of LH2−LH1 interactions. In Figure 5 we show the transient absorption spectra of one mutant, βArg$_{-10}$Leu, measured at 77 K; the other mutants were also measured (data not shown), and in each case the results were similar. These spectra illustrate the progressive evolution of bleaching and stimulated emission in the LH1 complex, which results from transfer of energy from the B850 pigments of LH2 to LH1.

DISCUSSION

Bacteriochlorophyll Absorption Bands. The results show that the βArg$_{-10}$ LH2 mutant complexes show a range of different spectroscopic properties. The near-infrared absorption bands for the complexes show blue shifts ranging from ∼7 to ∼17 nm, except for the βArg$_{-10}$Met LH2 complex which does not have an altered B800 band. It has already been suggested that a strong hydrogen bond is formed between the 2-acetyl carbonyl group of the B800 Bchl pigment and the βArg$_{-10}$ residue (McDermott *et al.,* 1995; Sturgis *et al.,* 1995), while elsewhere a correlation has been established between blue shifts in the B850 Bchl absorption maxima and hydrogen-bonding patterns in the LH2 complex from *Rb. sphaeroides* (Fowler *et al.,* 1994).

However, judging from the data in Figure 1, there is no straightforward correlation between the degree of B800 blue shift and the ability of the residue replacing βArg$_{-10}$ to form hydrogen bonds. His and Lys, for example, are capable of forming hydrogen bonds given the appropriate environment and structural geometry, and yet changing βArg$_{-10}$ to these amino acids leads to B800 blue shifts of 7 and 10 nm, respectively. If a strong hydrogen bond from βArg$_{-10}$ to the 2-acetyl carbonyl of Bchl is responsible for some of the ∼23 nm red shift of the B800 pigment from monomeric Bchl at ∼777 nm, then the indication is that His and Lys are incapable of replacing βArg$_{-10}$ in this role. This is not as unreasonable as it at first appears, given that a very precise geometry is required for successful formation of a strong hydrogen bond. For example, in the recently published structure for the LH2 from *Rps. molischianum* (Koepke *et al.,* 1996), an Asn residue in this complex is unable to form an H-bond to the 2-acetyl carbonyl group of the B800 pigment due to an unfavorable orientation between the two groups, even though they are within H-bonding distance of each other. Instead, this role is probably carried out by a β'Thr$_{23}$ (βThr$_{-12}$) residue.

Similarly intriguing is the LH2 complex in which βArg$_{-10}$ is changed to a Met (βArg$_{-10}$Met) which has a wild-type B800 band. Methionine is almost certainly incapable of forming a direct H-bond to the B800 2-acetyl carbonyl group, and yet the WT B800 band logically requires that such a bond exists, given the factors dictating the B800 absorbance properties mentoned above. One possibility is that introduction of the slightly smaller Met residue in place of the βArg$_{-10}$ residue creates a space within which a water molecule can sit, allowing it to restore the H-bond to the B800 2-acetyl carbonyl group (Figure 6). A similar role for water has been seen in the *Rps. acidophila* LH2 structure, where a water molecule is situated in the polar environment between the βHis$_{12}$, the 10-ester carbonyl (ring D), and the α-terminal formyl group in the B800 binding site (McDermott *et al.,* 1995). A second possibility is that the replacement of βArg$_{-10}$ with Met leads to some global rearrangement of the LH2 complex which brings an alternative H-bonding residue into close proximity to the B800 2-acetyl carbonyl group; however, it is not immediately obvious from either the *Rb. sphaeroides* amino acid sequences or the *Rps. acidophila* LH2 structure which residue this might be. None of the spectroscopic properties previously examined for the

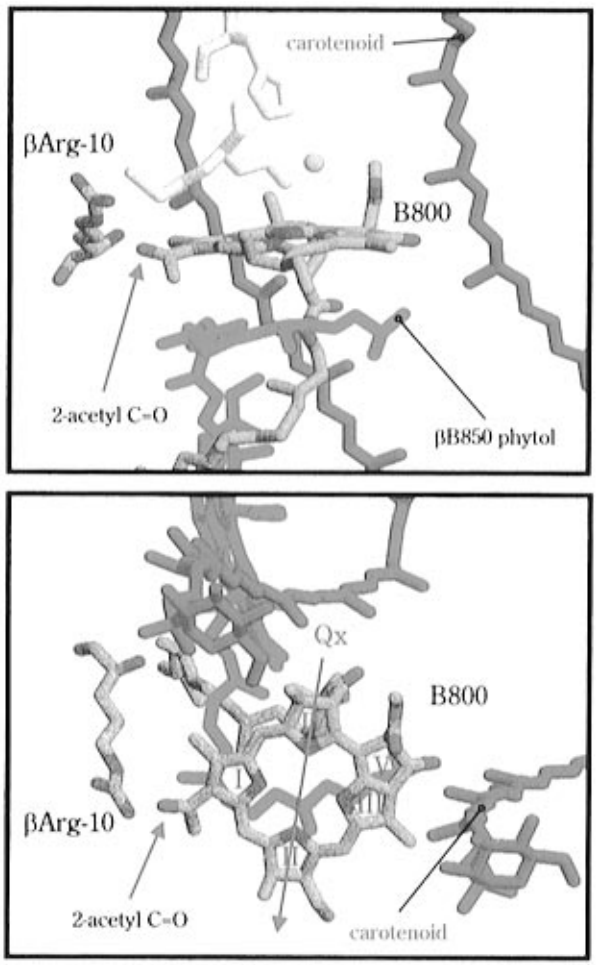

FIGURE 6: Schematic diagram of the LH2 B800 binding site showing the proximity of the βArg$_{-10}$ residue to the 2-acetyl carbonyl side group of the B800 bacteriochlorophyll pigment. Also shown are the phytol tail of the βB850 bacteriochlorophyll pigment (in green) and the carotenoid pigments (in red); two identical carotenoid pigments from neighboring $\alpha\beta$ dimers are shown. The yellow molecule represents a buried water molecule (McDermott *et al*., 1995). This figure was made using the 1995 crystal structure coordinates of the *Rps. acidophila* LH2 complex (N. W. Isaacs and R. J. Cogdell, personal communication) and a Rasmol Molecular Enhancer graphics package (UC Regents, 1995).

βArg$_{-10}$Glu LH2 mutant, including the near-infrared circular dichroism of the B850 Bchls, show any large-scale rearrangement to have taken place (Visschers *et al.,* 1994).

For at least one of the LH2 mutants, βArg$_{-10}$Asn, it seems that there are more effects than just the possible breakage of an H-bond to account for. The βArg$_{-10}$Asn mutant shows a minimum B800 blue shift of 18 nm, rising to 23 nm when the LH2 membranes are exposed to light or freeze/thaw cycles. Previous work on the shifts in the near-infrared peaks of the Bchl pigments in LH2 and LH1 suggests that the maximum shift that can be attributed to the breaking of a protein−carbonyl H-bond of this type is of the order of 10−12 nm (Fowler *et al.,* 1994; Olsen *et al.,* 1994). A possible contributor to this large blue shift in B800 could be rotation of the 2-acetyl carbonyl group; in the reaction center of *Rhodopseudomonas viridis*, for example, such an alteration in the orientation of a carbonyl group is thought to be able to modulate the Bchl absorption maximum over 50 nm (Parson & Warshel, 1987), an effect that has also been confirmed to some extent by theoretical calculations (Hanson *et al.,* 1987). Although this raises the possibility that replacing βArg$_{-10}$ with Asn leads to the formation of an H-bond that twists the 2-acetyl carbonyl group, and hence to the 18 nm B800 blue shift, the lability of the B800 in this complex makes this unlikely since the continued presence of an H-bond would presumably maintain B800 stability. None of these speculations rule out the possibility that replacing βArg$_{-10}$ with Asn leads to some global rearrangement of the LH2 complex. It is unlikely, however, that the βArg$_{-10}$Asn mutant and the βArg$_{-10}$Met mutant undergo similar rearrangements, for example, given the differences in their absorption spectra (Figure 1). In order to clarify this point, work has already been carried out on the Raman spectroscopy of these βArg$_{-10}$ complexes in order to ascertain the effect the various mutations have on H-bonding to the B800 Bchl pigment (A. Gall, G. J. S. Fowler, C. N. Hunter, and B. Robert, submitted for publication).

With respect to the shape of the B800 bands for the different LH2 complexes, it can be seen that at 77 K the wild-type (WT) LH2 B800 band has a steep red side with a shallower blue side. At 4 K the shape of the LH2 B800 band is partly dictated by the presence of phonon side bands and low-frequency vibrations which contribute to the blue wing of the band, as has been shown by hole burning experiments (van der Laan *et al.,* 1990). Computer simulations of the electron−vibronic spectra of photosynthetic systems indicate that as the temperature is raised from 0 K to room temperature the zero phonon line disappears rapidly so that by 77 K the band can be well approximated by a single Gaussian (Pullerits *et al.,* 1995), and so this is unlikely to be the explanation for the asymmetry of the WT B800 band at this temperature. However, another factor dictating the B800 band shape might be the upper exciton levels of the B850 band, which could result in an additional contribution which would lie to the blue of the B800 band maximum, but still within the B800 band (Reddy *et al.,* 1996). Such a contribution has recently been discussed for the LH2 antenna complex of *Rps. acidophila* 10050 (Wu *et al.,* 1996).

Examination of Figure 1 shows that the blue-shifted B800 band for the βArg$_{-10}$Lys LH2 complex suggests that this band is more symmetrical than that of WT LH2, an observation that is supported by Gaussian analysis (data not shown). If the B800 band is blue shifted so that it lies above the contribution from the upper exciton levels of the B850 band, then the shallow blue wing seen for WT LH2 would no longer be visible, and this appears to be a possible partial explanation for the symmetrical shape of the βArg$_{-10}$Lys B800 band. Similarly, the B800 band of the βArg$_{-10}$Met LH2 complex appears, on Gaussian analysis, to be slightly more symmetrical than that of WT LH2. The βArg$_{-10}$Met mutant appears to have a red-shifted B850 band at 77 K, and this is presumably matched by a red shift in the band due to the upper exciton levels of the B850. Such a red-shift would decrease the contribution to the shallow blue wing of the B800 band of the βArg$_{-10}$Met LH2 mutant, making it more symmetrical in shape.

In the future it is hoped to carry out more detailed spectroscopic measurements such as steady-state fluorescence line-narrowing or spectral hole-burning experiments in order to determine whether the different spectral components that make up the B800 bands of these βArg$_{-10}$ LH2 complexes can be more firmly identified.

B800 to B850 Energy Transfer. In general, excitation energy transfer within LH2 involves a number of steps which

are directly related to the properties of the Bchl absorption bands. These steps include (a) excitation transfer inside the spectrally inhomogeneous B800 pigments, (b) transfer from 800 to 850 absorbing pigments, and (c) exciton relaxation within B850. Our previous subpicosecond studies on the B800 to B850 energy transfer within αTyr_{+13},Tyr_{+14} LH2 mutants with blue-shifted B850 bands have shown that as the spectral overlap between the B800 and B850 bands increases, the rate of energy transfer also increases (Hess *et al.*, 1994). The results in the present work show that this is also true for βArg_{-10} LH2 mutants with blue-shifted B800 bands and that there is a clear correlation between energy transfer and the spectral separation of the B800 and B850 absorption bands. Remarkably even for βArg_{-10} LH2 mutant samples in which the absorption band is so blue shifted as to be only about 5 nm away from that of monomeric Bchl, namely, βArg_{-10}Asn and βArg_{-10}Leu, both steady-state and time-resolved fluorescence measurements indicate that the efficiency of B800 to B850 energy transfer is unimpaired. This observation is consistent with B800 to B850 energy transfer measurements for LH2 complexes that have been mistreated either with light or by freeze/thaw cycles and which have attenuated or broadened B800 bands (van der Laan *et al.*, 1993).

Assuming that mutation of the βArg_{-10} residue does not induce any major changes of orientation of the B800 bacteriochlorophyll molecules, the energy transfer within the Förster model is controlled by spectral overlap between the B800 fluorescence and the B850 absorption bands; the gradual blue shift of the B800 band through the series of mutants suggests that this overlap decreases. However the spectral overlap is determined not only by the peak positions of the bands involved but also by the B800 band shapes and their detailed spectral position. Although a more detailed analysis of the problem by low-temperature kinetic measurements and computer simulations is necessary, the results of an analysis of the room temperature data were presented in Figure 3, which show that the calculated energy transfer times are in reasonable agreeement with the experimental data and reproduce the trend of the measurements which show a decrease in the rate of the B800 $\rightarrow$ B850 transfer process with increasing energy gap. However, although these data indicate that the degree of spectral overlap is an influence on B800 $\rightarrow$ B850 transfer, the contribution of other factors such as an upper exciton state of B850 (Reddy *et al.*, 1996; Wu *et al.*, 1996) must also be taken into account.

Effect of Mutations at βArg_{-10} on LH2 Carotenoids. The results shown in Figure 4 suggest that different βArg_{-10} LH2 mutant complexes have different responses (as assessed by their spectroscopic properties) to different carotenoid backgrounds, something that was not apparent in our earlier studies on the βArg_{-10}Glu LH2 mutant (Crielaard *et al.*, 1994; Visschers *et al.*, 1994). The βArg_{-10}Glu LH2 mutant, for example, is very similar to WT LH2 in a green neurosporene background with respect to the absorption maxima of its carotenoid pigments. In a red spheroidene/spheroidenone background, however, these carotenoid absorption bands show a 11 nm blue shift.

Comparatively little is known about the binding environment of the carotenoid pigments in the LH2 complex of *Rb. sphaeroides*. A recent paper on the *Rps. acidophila* LH2 structure indicates that there are probably two different rhodopin glucoside carotenoid molecules that are closely associated with the B800 Bchl pigment in this complex (Freer *et al.*, 1996). The first carotenoid molecule makes close van der Waal contacts with the phytyl tails of the bacteriochlorophyll pigments, while carbon atoms C7 to C11 of the conjugated chain of the carotenoid make a number of contacts with rings III and V of the B800 Bchl macrocycle (Freer *et al.*, 1996). Two such carotenoid molecules are depicted in Figure 6, from neighboring units in the ring. However, this carotenoid does not pass very close to either the 2-acetyl carbonyl group on the other side of the Bchl macrocycle on ring I or to the βArg_{-10} residue. The second carotenoid molecule thought to be associated with B800 has not been fully defined and does not therefore appear in Figure 6; although the structure for the bottom third of the molecule near the B850 pigments has been resolved, the structure near the B800 Bchl is less well characterized (Freer *et al.*, 1996). Extrapolation of the available data suggests that the end of the conjugated system of this second carotenoid passes edge-on to the B800 Bchl, aligned with the Bchl Q_x transition. Therefore, it is logical to conclude, given the other structures that approach the B800 Bchl, that the second carotenoid could pass the Bchl ring quite close to either the βArg_{-10} residue or the 2-acetyl carbonyl group of the B800 Bchl. This, of course, assumes that the *Rb. sphaeroides* LH2 and the *Rps. acidophila* LH2 have similar structures in this region. There are at least two differences between these LH2 complexes; first, the *Rb. sphaeroides* LH2 does not contain rhodopin glucoside but instead has carotenoids from the spheroidenone series, and second, the βArg_{-10} residue in the *Rps. acidophila* LH2 is involved in an Asp−Arg salt bridge (McDermott *et al.*, 1995), a structural feature which is probably not possible in the *Rb. sphaeroides* LH2 due to the lack of an equivalent Asp residue.

Therefore, with respect to the present work, it can be seen from structural studies that it is possible to view the B800 Bchl pigment, the βArg_{-10} residue, and at least one of the carotenoids in the *Rps. acidophila* LH2 as a "unit", with all three molecules closely associated with each other. It is not unreasonable to suggest, therefore, that alteration of the B800 binding site by mutagenesis of the βArg_{-10} residue would also lead to alteration of the environment of one of the carotenoid molecules. However, the surprising thing about the results in the present paper is that the spheroidene/spheroidenone carotenoids seem to be more affected by changing the βArg_{-10} residue than is neurosporene. This suggests that the βArg_{-10} residue is more important for binding the red carotenoids than the green and provides information about the different binding requirements for the different carotenoids in LH2.

It is not easy to see a trend in the blue shifts seen in the carotenoid absorption/excitation bands (Figure 4). Previous studies on bacteriorhodopsin, for example, have suggested that at least part of the absorbance shift seen for the carotenoid-like chromophore retinaldehyde is due to nearby charged residues such as arginine (Spudich *et al.*, 1986). Although it is tempting to attribute the carotenoid band shifts described in the present work to the presence of charged residues such as Arg, His, Glu, and Lys, there does not appear to be any pattern. It is also hard to see why this effect is apparently more pronounced in the red spheroidene/spheroidenone carotenoid background than in the green neurosporene background. Therefore, in order to clarify the link between the type of residue at βArg_{-10} and the

spectroscopic behavior of the carotenoids in these mutant LH2 complexes, it is planned to measure the carotenoid to bacteriochlorophyll energy transfer rates at 77 K using time-resolved spectroscopic techniques.

LH2 to LH1 Energy Transfer. In each of the mutants studied, the LH2 complex is able to transfer energy to the native LH1−RC complex. The time scale and extent of this transfer are very similar to the corresponding processes in *Rb. sphaeroides* WT (M. Chachisvilis, T. Pullerits, W. H. J. Westerhuis, C. N. Hunter, and V. Sundström, submitted for publication), showing that the energy transfer and trapping functions of the mutants are intact. In some ways this is not surprising since for most of the βArg_{-10} complexes the B850 pigments (which are mainly those that transfer energy to LH1) are unaltered. This is complementary to previous results found for the αTyr_{+13}, Tyr_{+14} LH2 mutants with blue-shifted LH2 bands in which LH2 to LH1 transfer is equally unimpaired (Hess *et al.*, 1995). Given the location of βArg_{-10} on the "outside" of the nonamer ring of *Rps. acidophila* (McDermott *et al.*, 1995), it is possible that further rounds of mutagenesis coupled with subpicosecond energy transfer studies will reveal more about the factors that control this process.

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