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Biochimica et Biophysica Acta 1185 (1994) 318–326

BBA Biochimica et Biophysica Acta

Spectral alterations in *Rhodobacter capsulatus* mutants with site-directed changes in the bacteriochlorophyll-binding site of the B880 light-harvesting complex

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(Received 9 December 1993)

Abstract

Site-directed mutagenesis has suggested that conserved histidine and alanine residues in the α-subunit of the B880 (LHI) antenna complex of *Rhodobacter capsulatus* (αHis32 and αAla28) form part of the bacteriochlorophyll binding site (Bylina, E.J., Robles, S.J. and Youvan, D.C. (1988) Isr. J. Chem. 28, 73–78). Spectroscopic characterization of chromatophores from αAla28 mutants at 77 K revealed: (i) red shifts in B880 absorption and emission maxima of ~6 and 10 nm, respectively, with a serine exchange; (ii) red shifts of 3 nm with a glycine exchange; (iii) and no significant shifts with a cysteine exchange, despite a reduction of ~50% in B880 level. The strains with the serine and glycine exchanges showed characteristic fluorescence polarization increases over the red-edge of the B880 band, suggesting that the absorption red shifts arose from altered pigment-protein interactions rather than from increased oligomerization states that would be expected to show markedly diminished and red shifted rises in polarization (Westerhuis, W.H.J., Farchaus, J.W. and Niederman, R.A. (1993) Photochem. Photobiol. 58, 460–463). Excitation spectra of strains with αHis32 to glutamine and αAla28 to histidine exchanges, thought to be depleted in B880, revealed low levels of a 'pseudo-B880' complex with blue-shifted maxima and fluorescence polarization rises; when excited directly into this component, the former strain showed an emission spectrum similar to that of B880. An essentially wild-type electrochromic carotenoid response was observed only in the B880-containing mutants, since membranes isolated from the B880-depleted strains exhibited an increased permeability to ions.

Key words: Antenna; Bacteriochlorophyll-protein complex; Photosynthesis; Energy transfer; Fluorescence polarization; Site-directed mutagenesis (*R. capsulatus*)

1. Introduction

The ICM of *Rhodobacter capsulatus* contains two integral light-harvesting pigment-protein complexes, designated as B880 and B800-850 on the basis of their respective near-IR absorption maxima. B880 together with the photochemical reaction center, in a constant proportion of ~25 mol B880 BChl/mol reaction center, forms the cores of the photosynthetic units [1]. These core structures are surrounded and interconnected by the peripheral B800-850 antenna which is synthesized in amounts that vary inversely with light intensity. Radiant energy harvested by B800-850 is transferred to B880 which directs these excitations to the reaction center BChl special pair where they are rapidly transduced into a transmembrane charge separation. This initiates a cycle of electron transfer reactions involving primary and secondary UQ acceptors, the cytochrome bc_1 complex and cytochrome c_2, that results in re-reduction of the photooxidized special pair and conservation of the light energy as an electrochemical proton gradient coupled to the synthesis of ATP [2].

The B880 complex of *R. capsulatus* is composed of an $\alpha\beta$-heterodimer of 6.6 and 5.3 kDa subunits, respectively [3–5]. Their amino acid sequences, as well as

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Abbreviations: BChl, bacteriochlorophyll *a*; B880, B800-850, light harvesting complexes identified by near-IR absorption maxima designated also as LHI and LHII, respectively; αHis32 → Gln, etc.: His, wild-type residue; α, subunit designation; 32, position of residue in respective subunit; Gln, newly incorporated residue; ICM, intracytoplasmic membrane.

SSDI 0005-2728(94)00009-T

those of the core and peripheral antenna complexes from a number photosynthetic bacteria [6], conform to a common tripartite structure, with predominantly polar N- and C-terminal regions separated by a hydrophobic domain of ~ 22 amino acid residues thought to form a transmembrane α-helix. Proteolytic digestion studies have suggested that the N-terminal domains of the B880 polypeptides are exposed at the cytoplasmic surfaces of the ICM, with the C-termini directed toward the periplasm [7].

Although structures at the level of atomic resolution are not yet available for these integral antenna proteins, sequence alignments of core and peripheral antenna polypeptides from several species have facilitated the assignment of a putative site at which their non-covalently associated BChls are believed to be liganded [6,8]. For the core antenna heterodimers, the consensus sequences at this site are:

	(G)
α-polypeptides	-A-X-X-I-H-
	(V) (V)
β-polypeptides	-A-X-X-A-H-

which are derived from the primary structures of α- and β-polypeptides from eight different species, representing the purple sulfur bacteria (*Rhodobacter*, *Rhodopseudomonas* and *Rhodospirillum* spp.) and the families Chlorobiaceae, Chromatiaceae and Ectothiorhodospiraceae. The conserved histidine residues (αHis32 and βHis38 for the *R. capsulatus* B880 protein) are thought to be located at the same relative position within the periplasmic leaflet of the membrane bilayer, providing axial ligands for the central magnesium atoms of each of the two BChl chromophores. Moreover, the conserved alanine at the histidine N-4 position was proposed to be within van der Waals distance of the tetrapyrrole ring [9]. Nearly identical consensus sequences have been identified for the peripheral antenna polypeptides, in which the histidine and N-4 alanine residues are invariant, with the exceptions of the B800-850-α polypeptide of *R. sphaeroides* which has a serine at the N-4 position [10] and the *Rp. palustris* B800-850-α_2 isoform where a valine was found [6].

A critical test of this model has been provided by site-directed mutagenesis which produced changes in the αAla28 and αHis32 residues of the *R. capsulatus* B880 complex [11]. The B880 complex appeared to be lost from the membrane when αAla28 was replaced with aspartate or side chains larger than valine (i.e., glutamate, histidine, or phenylalanine residues), or with all αHis32 substitutions (i.e., arginine, asparagine, aspartate, glutamine, proline or threonine residues), which apparently resulted from a decreased stability of the modified α-polypeptides due to an inability to bind BChl [11]. While B880 was retained when αAla28 was changed to glycine, serine or cysteine, detailed spectroscopic analyses of these mutants were not performed. In the present report, low-temperature fluorescence excitation, emission and polarization spectra of chromatophores from these and several B880-depleted mutant strains were obtained to further characterize the optical transitions of their antenna BChl components.

The B880-depleted mutants of Bylina et al. [11], formed photochemically active reaction centers, and when constructed in a B800-850$^-$ background, have proved useful for direct in situ photochemical analyses of mutant reaction centers [12]; however, these strains exhibited reduced growth rates under photoheterotrophic conditions. Impaired photosynthetic growth was also reported for another B880$^-$ mutant [13], but the photooxidizable reaction centers formed by this strain were thought to be energetically uncoupled from both the B800-850 and cytochrome bc_1 complexes and to be assembled with an altered membrane topography. In the present study, the electrochromic carotenoid response was used to examine electron transport capabilities of the site-directed mutants of Bylina et al. [11]. This red shift in the visible carotenoid absorption spectrum is generated by charge separation and donor rereduction within the reaction center, as well as by electrogenic reactions of the cytochrome bc_1 complex [14], and thus serves as a useful probe for assessing whether reaction centers are appropriately linked to the other components of light-driven cyclic electron flow.

2. Materials and methods

The *R. capsulatus* strains with oligonucleotide-mediated, site-directed mutations in the *pufA* gene encoding the B880-α polypeptide [11], were kindly provided by Edward J. Bylina and Douglas C. Youvan of the Massachusetts Institute of Technology. They were constructed by complementing the U15g deletion background (RC$^-$ B880$^-$ B800-850$^+$) strain in *trans* with plasmid pU2922 [15] containing mutagenized copies of *pufA*. The pU2922 plasmid, which was constructed by fusion of pBR322 and pRK290 derivatives, carries tetracycline and kanamycin resistance markers and the *puf* operon containing the *pufBALMX* structural genes [15]; in addition to the B880-α polypeptide, these genes encode the B880-β and reaction center L and M subunits, as well as the *pufX* gene product [16,17]. The control strains used were U15g(pU2922) (RC$^+$ B880$^+$ B800-850$^+$) and U43(pU2922) (RC$^+$ B880$^+$ B800-850$^-$) which were constructed by complementation of the respective background strains in *trans* with pU2922. The mutant and control strains were grown semiaerobically in the dark in a mixture of RCV

and MPYE media (2:1, v/v) [18] supplemented with kanamycin (50 μg/ml). Because of the gratuitous formation of the photosynthetic apparatus under these conditions, reversion is mininized. The low levels of a B880-like component detected here in αHis32 → Gln and αAla28 → His mutants, previously reported to be depleted in the B880 complex [11], are unlikely to have arisen by reversion, since the αAla28 mutant was constructed with a GCG → CAC nucleotide change. Membrane fractions were isolated as described in [19] and stored at −80°C in the presence of 50% (v/v) glycerol.

Absorption spectra were measured on a Johnson Research Foundation DBS-3 double-beam spectrophotometer equipped with Hamamatsu R928 and R406 photomultiplier tubes for measurements in the visible and near-IR regions, respectively. Continuous saturat-

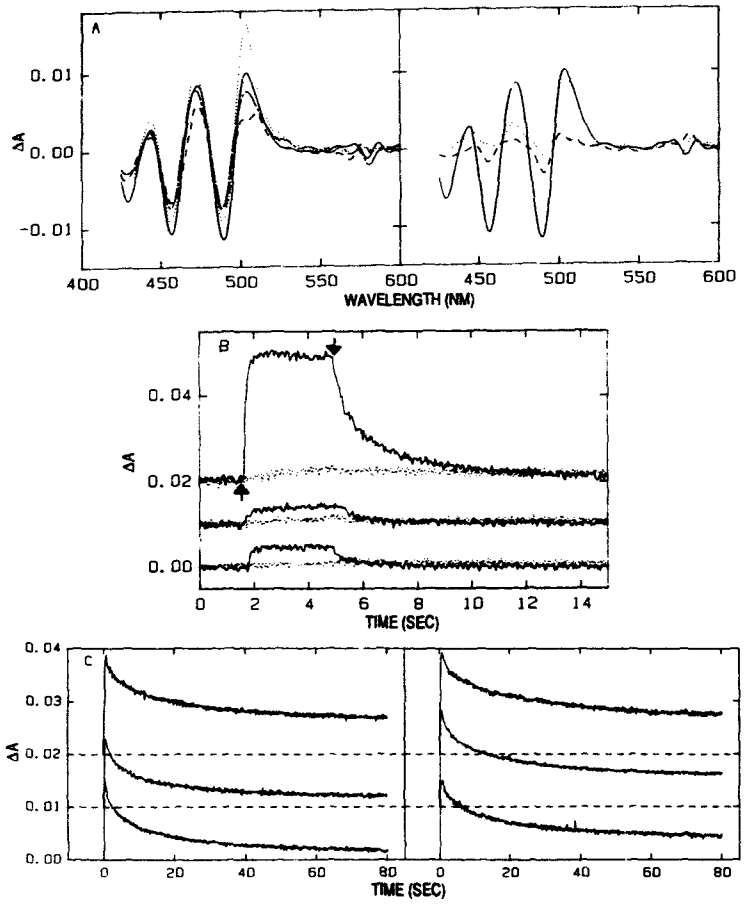

Fig. 1. Electrochromic carotenoid response of chromatophores from control and mutant strains. A. Constant illumination-minus-dark difference spectra. The chromatophore preparations were suspended at 10 mg of BChl/ml in 20 mM MOPS (pH 7.0) containing 100 mM KCl and 0.01% sodium ascorbate. Left panel, (———) control strain U15g(pU2922); (· · · · · ·) αAla28 → Ser mutant; (- - - - -) αAla28 → Cys mutant; (·–·–·) αAla28 → Gly mutant. Right panel, (———) control strain U15g(pU2922); (· · · · · ·) αHis32 → Asn mutant; (- - - - -) αHis32 → Gln mutant. These spectra, as well as the traces in panels B and C, were normalized to a B800-850 concentration of 10 μM which was determined with the extinction coefficients of Sturgis et al. [27]; the 77-K absorption spectra were used to approximate positions of the B850 and B880 bands to correct for spectral crossover. Light-minus-dark difference spectra obtained in the presence of 1 μM valinomycin were subtracted from total light-induced absorption difference spectra. B. Constant-illumination induced transients measured at 503–490 nm. Upward and downward arrows indicate light on and light off, respectively. Upper trace, control strain U15g(pU2922); middle trace, αHis32 → Asn mutant; αHis32 → Gln mutant. Dotted traces represent response in the presence of 1 μM valinomycin. C. KCl-valinomycin-induced carotenoid bandshift measured at 503–490 nm. Chromatophores were suspended at 10 μg BChl/mL in 20 mM MOPS (pH 7.0) containing 100 mM NaCl, 1 μM antimycin A and 1 μM valinomycin. Response was initiated by addition of KCl to a final concentration of 12.5 mM. The transients were corrected for dilution caused by the addition of the KCl solution. Horizontal lines indicate corrected zero ΔA points for upper traces. The extent of the slow decay phase was assessed from the ΔA remaining after 60 s. Values obtained were: Left panel, upper trace, control strain U15g(pU2922), 40%; middle trace, αHis32 → Asn mutant, 18%; lower trace αHis32 → Gln mutant, 14%. Right panel, upper trace, αAla28 → Ser mutant, 42%; middle trace; αAla28 → Gly mutant, 36%; lower trace, αAla28 → Cys mutant, 34%.

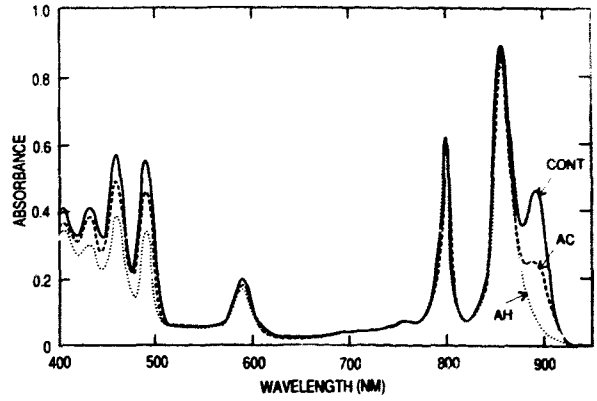

Fig. 2. Absorption spectra of chromatophores from control and mutant strains at 77 K. Chromatophore preparations at 10 μg of BChl/ml in 20 mM MOPS (pH 7.0), containing 100 mM KCl, were suspended in 50% (v/v) glycerol prior to freezing. Spectra were normalized at their B850 absorption maxima. CONT, control strain U15g(pU2922); AC, αAla28 → Cys mutant; AH, αAla28 → His mutant.

ing illumination for light-minus-dark carotenoid difference spectra was provided by a Unitron tungsten microscope lamp passed through a Wratten 88A filter; the photomultiplier was protected by a 5 mm Corning 4–96 blue-glass filter. For measurements of valinomycin-KCl-induced carotenoid band shift transients [20], samples were mixed continuously with a magnetic stirrer and the reaction was started by addition of a pulse of KCl. The instrument was modified for near-IR absorption and fluorescence spectra at 77 K as described by Westerhuis et al. [21]; fluorescence polarization measurements were made with Polaroid HR filters. Reaction center photo-bleaching was determined under saturating constant illumination at 605–540 nm as described above, using the extinction coefficient of Dutton et al. [22]. BChl was estimated as in [23].

3. Results

Bylina et al. [19] reported that while the B880 complex was lost from the ICM with all site-directed mutations in the B880 αHis32 codon, or when B880 αAla28 was changed to aspartate, glutamate, histidine or phenylalanine, the reaction centers present in these strains exhibited normal light-minus-dark difference spectra. In the present study, the ability of the αHis32 and αAla28 mutant strains to generate an electrochromic

Table 1
Absorption and fluorescence yield properties at 77 K of chromatophores from *R. capsulatus* strains with site-directed mutations in B880 αAla28 and αHis32 residues

Strain	B880 maxima		PSU [b] size	Energy transfer
	Absorbance [a] (nm)	Emission	total BChl/RC (mol/mol)	B850 → B880 efficiency (%) [c]
Control (U15g(pU2922))	893	914	119	94
B880 containing				
αAla28 → Cys	~ 891	914	97	80
αAla28 → Gly	896	917	100	100
αAla28 → Ser	899	924	92	~ 100
B880 depleted				
αAla28 → His	–	–	27	37 [d]
αHis32 → Gln	–	915 [d]	38	45 [d]

[a] These values represent approximations due to crossover by B850 background absorbance.
[b] PSU, photosynthetic unit; RC, reaction center.
[c] Energy transfer efficiencies were calculated from comparisons of relative heights of respective bands in fluorescence excitation spectra to those of the fractional absorption spectra in Fig. 4, and were taken at absorption maxima.
[d] The emission maximum (885-nm excitation) and energy transfer efficienies are for the putative 'pseudo-B880' complex described in the text.

carotenoid band shift was used to determine the extent to which these reaction centers were coupled energetically to the cytochrome bc_1 complex. Mutants constructed with the green U15g *puf* deletion strain were ideal for this purpose, since they accumulated a single spectral form of carotenoid of the neurosporene type and formed the B800-850 complex, with which the electrochromically active carotenoids are associated [24–26]. Constant illumination-minus-dark difference spectra of chromatophores from the B880-containing mutants (Fig. 1A) showed carotenoid spectral changes with maxima and minima similar to those of the control U15g(pU2922) strain, but with somewhat reduced amplitudes in the αAla28 → Gly and αAla28 → Cys mutants. In contrast, with the B880-depleted αHis32 mutants, the amplitudes of the absorbance differences were greatly diminished (Figs. 1A and 1B); Fig. 1B also demonstrates that the carotenoid changes were sensitive to valinomycin, which abolished the membrane potential. Fig. 1C shows that the decay of the electrochromic carotenoid response generated by K^+-valinomycin diffusion potentials was more rapid in the B880-depleted than in the B880-containing strains. This is reflected in the extent of the slow decay phase remaining after 60 s, which amounted to ~ 15% of the initial absorbance difference in the former strains, as compared to 40% in the control chromatophores. These data are consistent with an increased ion permeability in the membranes of the αHis32 mutants, possibly as a result of incomplete sealing during cell breakage. Therefore, the diminished light-induced carotenoid response of these preparations may result from an inability to sustain a membrane potential.

Fig. 2 shows absorption spectra obtained at 77 K with chromatophores from the various mutants. The ratio of the absorbance of the B880/B850 bands, which was approximately 0.6 in the control strain, was reduced to ~ 0.35 in the αAla28 → Cys mutant (or by nearly one-half if the B850 background absorbance is taken into account). Although the relative levels of the B880 and B850 bands in the αAla28 → Ser and αAla28 → Gly mutants were essentially the same as that of the control (see below), the absorbance maxima of the B880 band were red shifted by ~ 6 and 3 nm in the αAla28 → Ser and αAla28 → Gly mutants, respectively (Table 1). While no residual B880 was apparent in the αAla28 → His mutant, a shoulder near 880 nm was observed in the absorption spectrum of the αHis32 → Gln mutant, and excitation spectra revealed the presence of low levels of an apparent B880 complex in both of these strains (see below). The photosynthetic unit sizes of the αAla28 → His and the αHis32 → Gln strains were reduced by 3- to 3.5-fold in comparison to those of the control and the B880-containing mutants (Table 1). These differences arose from both the lower B880 levels and the higher reaction center/B800-850 ratio in the former. The positions and relative absorbance of the B800 and B850 bands were essentially unaltered in the mutants (Fig. 2), while the absorbance differences of the carotenoid peaks at 420–500 nm were a reflection of the B880 levels in these strains, since the carotenoid/BChl (mol/mol) is 1:1 for B880 and 1:2 for B800-850.

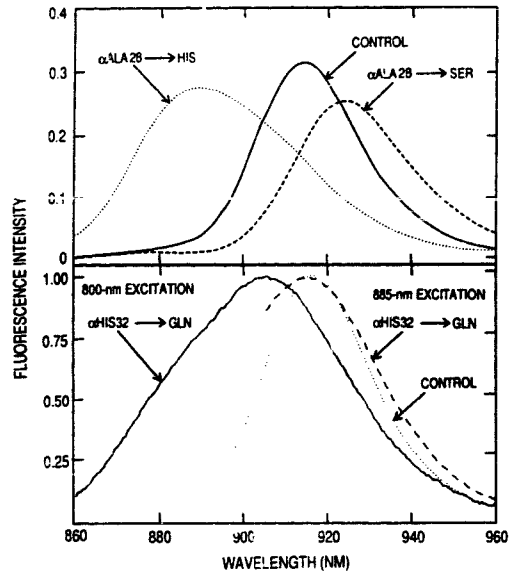

Fig. 3. Fluorescence emission spectra of chromatophores from control and mutant strains at 77 K. Chromatophores (5 μg of BChl/ml) were prepared for freezing as described in Fig. 2. Panel A, excitation was at 800 nm; spectra were normalized on the basis of the relative number of quanta absorbed, using $1 - T_{800\,nm}$. Fluorescence intensity is expressed in arbitrary units. Panel B, spectra were normalized at an arbitrary maximal fluorescence intensity of 1.0 Blue side of emission spectra resulting from excitation at 885 nm is not shown because of interference by stray light in this region. No further shifts in emission maximum were observed when the chromatophores from the αHis32 → Gln mutant were excited at either 890 or 895 nm. The broadening in the red wing of the emission spectrum of this strain (885-nm excitation) in comparison to that of the control strain is apparently due to a red tail on the emission spectrum of the B860-850 complex (Westerhuis, W.H.J. and Niederman, R.A., unpublished) which is present at elevated levels in the former.

Fluorescence emission spectra obtained at 77 K for several of the mutants are presented in Fig. 3. For both the B880-containing αAla28 → Gly and αAla28 → Cys mutants and the control strain, the shifts in the emission maxima were comparable to the respective shifts in the absorption maxima (Table 1); however, for the αAla28 → Ser mutant, the Stokes shift was somewhat greater. The presence of an apparent B880 complex in the αAla28 → His and αHis32 → Gln mutants was also revealed by their fluorescence emission spectra (Fig. 3A and 3B, respectively). Upon excitation at 800 nm, their emission maxima were red shifted by ~ 5 and 20 nm, respectively, in comparison to that reported for *R.*

capsulatus strain Y5 [28], which contains B800-850 as the sole pigment-protein complex. Furthermore, after preferential excitation at 885 nm in the red wing of the absorption band, the emission maximum of chromatophores from the αHis32 → Gln mutant coincided with that in the control preparations where an essentially pure B880 emission band is seen (Fig. 3B). These results, together with the further spectral characterization presented below, suggest that the B880-depleted strains contain low levels of a modified form of the B880 complex.

Fluorescence excitation, polarization and fractional absorption $(1 - T)$ spectra obtained at 77 K for the various strains are presented in Fig. 4. As noted above, the excitation spectra of the αAla28 → His and the αHis32 → Gln mutants showed shoulders on the red edge of their B850 peaks also apparent in the fractional absorption spectrum of the latter strain. In the αAla28 → Ser and αAla28 → Gly strains the efficiency of energy transfer from B850 to B880 was comparable to that in the control (Table 1). In the αAla28 → Cys mutant, the reduction in B880 levels was accompanied

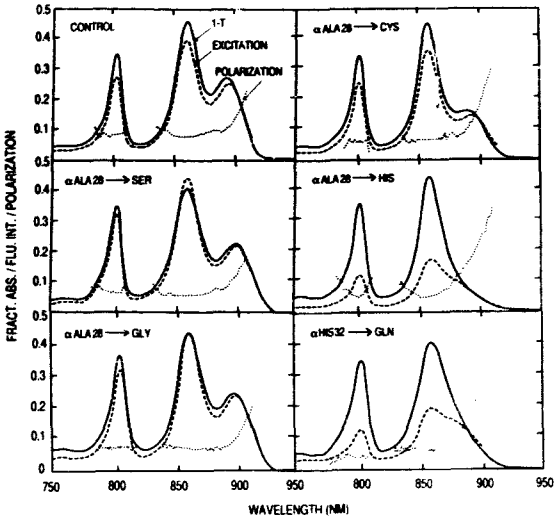

Fig. 4. Near-IR fractional absorption and fluorescence excitation and polarization spectra of chromatophores from control and mutant strains at 77 K. Chromatophores prepared for freezing as described in Fig. 2; emission was detected through a 935 nm band-pass filter for excitation and polarization spectra. Excitation spectra were normalized on the red wing rather than at the maximum of the B880 band in fractional absorption spectra due to red shifts in the excitation bands; such a lack of spectral alignment has been ascribed to structural heterogeneity within the core antenna (Westerhuis, W.H.J. and Niederman, R.A., in preparation). Polarization values are given by: $p = (I_{\parallel} - I_{\perp})/(I_{\parallel} + I_{\perp})$, where $I_{\parallel}$ and $I_{\perp}$ are the relative intensities of fluorescence with polarization either parallel or perpendicular, respectively, to the polarization direction of the excitation light. Fract. Abs., fractional absorption (1-T); Flu. Int., fluorescence intensity.

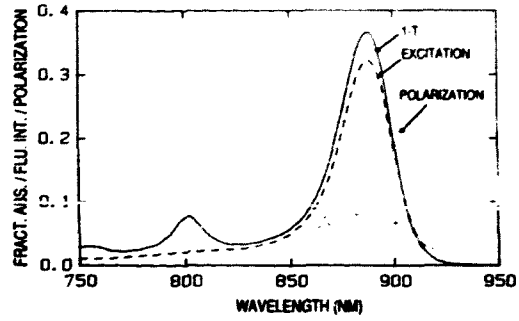

Fig. 5. Near-IR fractional absorption and fluorescence excitation and polarization spectra obtained at 77 K with membranes from strain U43(pU2922) which lacks the B800-850 complex. Excitation and polarization spectra performed as described in Fig. 4.

by a somewhat lower B850 → B880 energy transfer efficiency of ~80%. In the B880-depleted strains, a considerably lower connectivity of B850 to the apparent B880 complex was observed, where the efficiency of energy transfer to this red-wing component (Table 1) was reduced to ~40%. The latter values are likely to be overestimated due to a considerable contribution of the red tail of the B850 emission band to fluorescence detected at 935 nm (Westerhuis, W.H.J. and Niederman, R.A., unpublished data).

In an effort to elucidate the basis for the spectral properties of the B880 bands of the site-directed mutants, low-temperature spectra were obtained for the B880 complex in membranes from the B800-850⁻ strain U43(pU2922) (Fig. 5). The low and essentially uniform fluorescence polarization values over the blue side of the B880 absorption band and the steep rise over the red wing are similar to the polarization spectrum observed for the B875 complex of *R. sphaeroides* [29], and suggest that these two LHI antennae have similar structural properties. While this non-uniform polarization spectrum was originally ascribed to a distinct long-wavelength subantenna, designated as B896 [29], this phenomenom is now thought to be a manifestation of more extensive spectral heterogeneity intrinsic to LHI antenna complexes. As will be further discussed below, this has alternatively been ascribed to inhomogeneous [30] or to homogeneous band broadening (Westerhuis, W.H.J. and Niederman, R.A., unpublished data). However, both models are in qualitative agreement with the observation, in the latter study, that with a decrease in the oligomerization state of the antenna, the emission maximum undergoes a blue shift while the fluorescence polarization is enhanced upon excitation in the blue wing and center of the absorption band. Within this context, concerted red shifts in absorption and polarization spectra, observed in an *R. sphaeroides* mutant lacking *pufX*, have been inter-

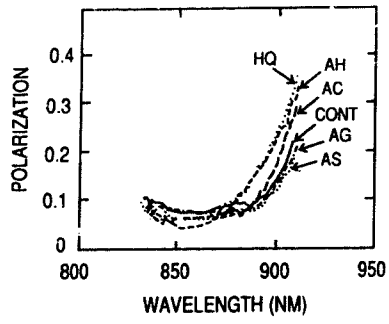

Fig. 6. Fluorescence polarization within the redmost absorption bands of chromatophores from control and mutant strains at 77 K. HQ, αHis32 → Gln; AH, αAla28 → His; AC, αAla28 → Cys; CONT, control strain U15g(pU2922); AG, αAla28 → Gly; AS, αAla28 → Ser.

preted to reflect excessive aggregation of the B875 complex in this strain [21].

In the U15g(pU2922) control strain, the fluorescence polarization of the B880 complex was seen, together with polarizations of < 0.1 characteristic of B800 and most of the B850 band [31] (Fig. 4). The rises in the fluorescence polarization spectra of the αAla28 → Ser and αAla28 → Gly strains showed slight red shifts relative to that of the control (Fig. 6), rather than the markedly diminished and red shifted polarization rise observed for the highly oligomerized B875 complex of the *R. sphaeroides pufX*⁻ mutant [21]. Thus, the B880 absorption and emission red shifts of these Ser and Gly mutants appear to arise from alterations in the protein environment that do not modify the aggregation properties of the complex. In the αAla28 → Cys mutant, the reduction in B880 levels was accompanied by an increase in polarization that was shifted about 5 nm towards the center of the absorption band (Fig. 6), indicative of a lower degree of aggregation. The polarization spectra for the B880-depleted strains, which were also blue shifted relative to that of the control, did not appear to reflect a significantly altered mode of oligomerization for the B880-like component, since the absorption band, as reflected in the corresponding excitation spectra, appeared to be blue shifted by a similar amount. A possible structural basis for the presence of this component is discussed below.

4. Discussion

The inability of chromatophores from the B880⁻ strain of Jackson et al. [13] to generate a light-induced electrochromic carotenoid response was ascribed to the absence of electron transport between active reaction centers and the cytochrome bc_1 complex; however, it was not possible to test this in the B880-depleted mutants of Bylina et al. [11], since membranes isolated from these strains were largely unable to sustain a membrane potential. Although impaired electron flow could explain the lower photosynthetic growth rate in these strains, these growth defects could also have arisen from inefficient energy transfer between the peripheral antenna and reaction centers.

While B880-depleted mutants of Bylina et al. [11] have been used to study specifically mutated reaction centers [12], no consensus exists on the ability of mutants with defects in the LHI antenna to form photochemically active reaction centers [13,32–35]. A more critical strategy for construction of antenna-depleted strains suitable for analysis of in situ reaction center photochemistry was recently developed in *R. sphaeroides* [36]. Genes encoding polypeptides of the reaction center, and both the core and peripheral antennae, were deleted from the chromosome. When reaction center genes were reintroduced on a plasmid, the resulting strains were capable of photoheterotrophic growth and assembled reaction centers that showed normal photochemical and spectroscopic properties.

With regard to the spectroscopic characterization of the B880 complex in the site-directed mutants of Bylina et al. [11], these workers previously demonstrated that B880 was only retained when αAla28 was replaced by uncharged residues of sufficiently small size. Additional steric factors were proposed to explain the observation that substitution by valine, with a side chain smaller than that of cysteine, appeared to result in a less stable B880 complex. Results presented here show that differences between control and mutant strains were least marked with the glycine exchange, where the only effect was a small spectral red shift (3 nm), while the overall levels and apparent oligomerization state of the complex were essentially unaltered. In this connection, it is noteworthy that glycine is the only naturally occurring substitution at the histidine N-4 position of the α-polypeptides of LHI complexes sequenced thus far [6]. Although the effects of the serine substitution were qualitatively the same, the magnitude of the spectral red shifts were two- to three-fold larger (6–10 nm). Taken together, replacement of αAla28 by glycine and serine apparently causes small changes in BChl environments, that will be discussed further below. In contrast, substitution of cysteine for αAla28 resulted in a nearly two-fold reduction in B880 levels, a decreased energy transfer efficiency between B850 and B880, and an apparent reduction in the oligomerization state of the B880 complex. This suggests that the cysteine exchange results in the formation of an antenna protein with reduced structural stability and a diminished capacity to form well-organized core structures. These effects may be even more pronounced when αAla28 is replaced by valine.

Recently, a number of other reports have appeared in which mutations in conserved amino acid residues of

bacterial antenna proteins were made, and alterations in structural and spectral properties were assessed [35, 37–39]. Site-directed exchanges in the conserved αTrp43, located in the C-terminal domain of the B880 protein of *R. capsulatus*, resulted in spectral blue shifts of up to 11 nm [35], and this residue was concluded to interact with BChl and contribute to the red shift of this complex [35]. Optimized combinatorial casette mutagenesis of the *R. capsulatus* B800-850-β polypeptide at residues believed to be located on the same face of the α-helix as the BChl tetrapyrrole, demonstrated that amino acids at the βHis38 N-7 position could promote an additional red shift from 850 to ~875 nm [37]. Since these changes also caused the loss of the B800 BChl, they resulted in the transformation of the modified B800-850 protein into a B880-like complex. In a site-directed mutagenesis study of the B800-850 protein of *R. sphaeroides* [38], a correlation was demonstrated between the presence of the αTyr44 and αTyr45 residues in the C-terminal domain and the position of the absorption maximum of the B850 band. When these amino acids were changed to phenylalanine and leucine, respectively, a complex was formed that absorbed at 800 and 826 nm, showing that much of the red shift of the B850 band is promoted by the proximity, and in particular the hydrogen bonding capabilities [39], of these tyrosine residues to the tetrapyrrole rings of BChl. Thus, the red shifts induced by the B880 αAla28 to glycine and serine exchanges may reflect small changes in the positions of polar groups relative to the BChl macrocycle. Moreover, the latter exchange could result in the introduction of an additional hydrogen bond with the BChl chromophore, a possibility that may be addressed by Resonance Raman spectroscopy.

Low-temperature fluorescence emission, excitation and polarization spectra presented here suggest that the B880-depleted strains form low levels of a modified form of the B880 complex. In the αHis32 → Gln mutant, this component was observed as a spectral shoulder in the red wing the B850 band, and gave rise to an emission spectrum strikingly similar to that of the B880 complex in the control strain when excited at 885–895 nm. The marked rise in fluorescence polarization in these B880-depleted mutants indicated that this apparent B880-type complex is assembled with the intrinsic spectral heterogeneity characteristic of the wild-type B880 complex; however, these putative B880 complexes have reduced capabilities as acceptors of excitations from the B800-850 complex, possibly as a result of their presence at reduced levels. Because of an inability to bind BChl, the modified B880-α polypeptide in the B880-depleted strains is presumably degraded [40,41]. Although pulse labeling studies in *R. capsulatus* demonstrated that in the absence of the B880-α subunit, the β polypeptide was inserted into the ICM at normal rates, but did not accumulate [42], the complex that was detected in the B880-depleted mutants by the sensitive spectroscopic procedures used here, may represent a 'pseudo-B880' antenna assembled solely from B880 β-polypeptides. In reconstitution studies of the *Rhodospirillum rubrum* B880 complex in the presence of detergent, a homodimer of β-subunits absorbing near 820 nm could be formed [43], but oligomerization into a fully red-shifted antenna only occurred if the α-polypeptide was also present. It is possible, however, that the membrane provides additional stability allowing the formation of larger aggregates consisting of only the β-polypeptide.

The conceivable formation of an almost fully red-shifted B880 complex from β-subunits alone, provokes further consideration of the extent to which pigment–pigment interactions within αβ-heterodimers contribute to the spectral red shift of the core antenna. The absence of such interactions has previously been suggested from the observation that the acid-induced disappearance of the LHI absorbance band in *R. sphaeroides* membranes lacking B800-850, occurred in two spectrally and kinetically distinct phases of about equal amplitude [44]. These results were interpreted as a sequential release of spectrally independent α- and β-pigment species.

On the basis of the oligomerization-state dependence of the spectral properties of B875 complexes isolated from *R. sphaeroides* [45], a structural model has been proposed in which the core antenna is thought to consist of separate pools of α- and β-BChls, arranged in two concentric curvilinear [46] arrays (Westerhuis, W.H.J. and Niederman, R.A., in preparation). Interaction among pigments within a single array would give rise to a number of excitonic components (homogeneous band broadening), in which the lowest energy species would correspond to that identified originally as 'B896.' Thus, assembly of a spectrally intact LHI antenna from β-subunits would be consistent with this model. In an alternative proposal, pigment-pigment interactions, accounting for about 15 nm of the red shift of the core antenna, are thought to occur within, but not among, αβ-heterodimers [47]; most of the remainder of the shift (~85 nm) has been attributed to specific pigment-protein associations [48]. In this case, spectral heterogeneity of LHI has been proposed to result from small random differences between the local environments of individual αβ units within an oligomer (inhomogeneous band broadening) [30]. Since the contribution of BChl–BChl interactions to the spectral shift would be relatively small, this model can be reconciled with formation of an oligomeric β-complex if the red shifts induced by oligomerization of αβ units result from α-α and β-β rather than α-β pigment–protein interactions. While the possibility that nonspecific interactions might contribute to red shifts in

such a 'pseudo B880' complex cannot be excluded, this would appear unlikely in view of the specific roles discussed above for individual amino acid residues in promoting these spectral changes in the normal LHI species.

Acknowledgements

This work was supported by the US Department of Agriculture grant 91–01640 and grants DMB85-12587 and MCB90-19570 from the US National Science Foundation. We are grateful to Edward J. Bylina and Douglas C. Youvan for providing the mutant strains and James N. Sturgis for useful discussions. The assistance of Lester S. Clowney with the computer graphics and of Huisheng Wang with membrane preparation is also appreciated.

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