A structural role of the carotenoid in the light-harvesting II protein of *Rhodobacter capsulatus*

Jesús ZURDO, Concepción FERNANDEZ-CABRERA and Juan M. RAMIREZ*
Centro de Investigaciones Biológicas del CSIC, Velázquez 144, 28006 Madrid, Spain

The membrane-linked light-harvesting II protein (LHII) of *Rhodobacter capsulatus* was partly depleted of carotenoids by selective extraction with light petroleum. Carotenoid removal was accompanied by bleaching of the $Q_y(S_1 \leftarrow S_0)$ absorption band of bacteriochlorophyll (Bchl) *a* near 800 nm, by a bathochromic shift and a broadening of the other Bchl Q_y band at 850 nm, and by the formation of a weak Q_y band of dissociated Bchl near 770 nm. The changes in the 800 and 850 nm bands seemed to reflect alterations in only those Bchl molecules that had lost their associated carotenoids, firstly, because the extent of the changes was closely correlated to the degree of carotenoid extraction, and, secondly, because the residual fraction of carotenoid-containing LHII, which could be almost quantitatively recovered from the membrane after detergent solubilization and ion-exchange chromatography, showed an unmodified LHII absorption spectrum. The Bchl responsible for the shifted 850 nm band remained bound to protein, since its visible (Q_x) transition seemed to retain the induced optical activity of the native bound pigment. Besides, the shifted Bchl could act as an efficient acceptor of singlet excitation energy from the pigments of the intact LHII fraction. The close similarity between the spectroscopic Bchl changes that accompany carotenoid extraction and the differential spectral features of carotenoidless LHII of *Rhodobacter* mutants, previously reported, strongly suggests that the direct cause of the spectral modifications is the absence of carotenoid and not any independent effect of the experimental manipulation of the membrane. Several interpretations of the structural changes that underlie the observed spectral changes are possible. The simplest one is to assume that carotenoid removal elicits an alteration in the angle between the Q_y transition moments of two strongly interacting Bchl molecules.

INTRODUCTION

The photosynthetic pigmented proteins, reaction centres and light-harvesting antenna complexes very often contain coloured carotenoids in association with chlorophylls. Although the carotenoids do not appear to participate in the main pathway of light-elicited electron transfer, they help to collect light energy and also play an essential role as protectors of the photosynthetic cell from harmful photo-oxidations (Cogdell and Frank, 1987; Siefermann-Harms, 1987; Truscott, 1990; Koyama, 1991). Both carotenoid functions require processes of intermolecular energy transfer between carotenoids and chlorophylls that are favoured by short donor-to-acceptor distances and by adequate relative orientation of the pigments. The arrangement of the chromophore cluster, which usually consists of several chlorophyll and carotenoid molecules, appears to be largely sustained by the apoprotein through specific non-covalent bonds (Zuber, 1986). However, it is possible that molecular interactions among the pigments themselves may also contribute to stabilization of the structure of the pigment–protein complex.

Many of the strains of purple phototrophic bacteria that have been described contain two types of light-harvesting antenna proteins (Zuber, 1986). The core or light-harvesting I protein (LHI) is thought to be directly associated with the photochemical reaction centre and is frequently designated B875 or B880 because its absorption spectrum shows a single Q_y band ($S_1 \leftarrow S_0$) of bacteriochlorophyll (Bchl) *a* near 880 nm. In the other light-harvesting protein, the peripheral or LHII complex, Bchl shows two well-resolved Q_y transitions near 800 and 850 nm which seem to correspond to two distinct fractions of the pigment in a ratio of 1:2 respectively (Clayton and Clayton, 1981). Thus LHII is also called B800–850 or B800–B850. As has been known for some time, LHII does not appear to be present in *Rhodobacter* mutants unable to synthesize coloured carotenoids (Marrs, 1978). In contrast, a double mutant of *Rhodobacter capsulatus* (reaction-centre defective, carotenoidless) and some carotenoidless strains of *Rhodobacter sphaeroides* contain LHII with an atypical electronic spectrum that lacks the Q_y transition at 800 nm (Davidson and Cogdell, 1981a; Tadros et al., 1989). These observations suggest that the carotenoid is involved in establishing and/or maintaining the native conformation of LHII. In order to ascertain whether such an involvement occurs at LHII itself (i.e. carotenoid interactions are essential to stabilize the structure of the wild-type pigment cluster) and/or during the synthesis and assembly of the protein, we have investigated some of the changes that carotenoid removal elicits in LHII of *R. capsulatus*. To this aim we have used a selective procedure of carotenoid extraction that was originally developed to study degenerate carotenoid interactions in LHI of *Rhodospirillum rubrum* and *R. capsulatus* (Zurdo et al., 1991, 1992). The results of the present work suggest that the carotenoid is required to stabilize the wild-type structure of the Bchl cluster in LHII. The structural role of the carotenoid is also reflected in the decreased stability of carotenoidless LHII when solubilized with some detergents. The effects of carotenoid extraction account for most of the anomalies observed in the light-harvesting antenna of carotenoidless mutants of *R. capsulatus* and *R. sphaeroides* without having to assume additional effects at the levels of gene expression and assembly of LHII.

MATERIALS AND METHODS

R. capsulatus strain BY1424 (Scolnik et al., 1980) was used

Abbreviations used: Bchl, bacteriochlorophyll; LHI and LHII, the bacterial light-harvesting proteins I and II.

* To whom correspondence should be addressed.

throughout the work. This strain carries a mutation in the *puf* operon that prevents the synthesis of both the photochemical reaction centre and LHI. Thus it contains LHII as the only major chromoprotein, a circumstance that allows the use of isolated intracytoplasmic membrane vesicles as suitable preparations for the study of many properties of LHII. The carotenoids of this bacterial strain are neurosporene and two close derivatives of almost identical absorption spectra (Scolnik et al., 1980). Since strain BY1424 lacks the reaction centre, it is non-phototrophic and has to be cultured heterotrophically in the dark. Cells were grown using the medium of Lascelles (1956) supplemented with 6 mg/ml glucose and 50 mM dimethyl sulphoxide, which was included as a terminal acceptor of anaerobic respiration (Scolnik et al., 1980). Aerobic cultures were not used because their LHII showed a decreased A_{800} to A_{850} ratio.

Cells harvested at the end of the exponential phase of growth were used for the isolation of intracytoplasmic membrane vesicles by a previously described method (Lozano et al., 1990). Freeze-dried vesicles were partially depleted of carotenoids by selective extraction with light petroleum, essentially following the procedure used before for membrane vesicles isolated from other bacterial strains (Zurdo et al., 1991). A minor modification was that the residues of light petroleum were removed from the preparations of extracted vesicles by evaporation under vacuum at room temperature.

LHII was solubilized from suspensions of isolated freeze-dried vesicles ($A_{588} = 10\ cm^{-1}$) in 10 mM Tris/HCl, pH 7.5, by the addition of 45 mM dodecyldimethylamine *N*-oxide, a non-ionic detergent. The mixture was left in an ice bath and in the dark for 1 h, then diluted tenfold with the same buffer and centrifuged at 120000 *g* for 1 h. A similar procedure was followed when other detergents were used. The resulting sediment was discarded, and the supernatant subjected to anion-exchange chromatography using an f.p.l.c. system (Pharmacia). Samples of 2–2.5 ml of the detergent extract, containing 7.5 nmol of Bchl and 0.4 mg/ml dodecyldimethylamine *N*-oxide, were loaded on to the column (Mono Q, Pharmacia). Protein was eluted using a linear gradient of NaCl (0–0.5 M) in a solution containing 20 mM Tris/HCl and 0.4 mg/ml dodecyldimethylamine *N*-oxide, pH 8.0. Bchl concentration in the extracts was determined after dilution of a sample with 4 vol. of an acetone/methanol mixture (7:2, v/v). A molar absorption coefficient of $76\ mM^{-1} \cdot cm^{-1}$ at 770 nm was used (Clayton, 1966).

Absorption spectra of vesicle suspensions and detergent extracts were scanned in the secondary sample compartment of a double-wavelength spectrophotometer (Shimadzu, UV-3000). The instrument was interfaced to a Hewlett–Packard microcomputer (HP9816) and its standard S20 photomultiplier was replaced by an S1 detector (Hamamatsu R473) to improve the signal to noise ratio in the near-i.r. spectral range. The aqueous suspensions of vesicles that had undergone the process of freeze-drying showed a flattened absorption spectrum, possibly due to an intensification of the sieve effect resulting from an increase in the average size of the pigmented particles (Duysens, 1956). In order to compensate for the flattening, the spectral data used as reference were always obtained from unextracted vesicles that had been subjected to freeze-drying.

Fluorescence measurements were done in a home-made filter fluorimeter equipped with an S1 end-window photomultiplier (EMI 9684B). A concentrated solution of Rhodamine B in ethylene glycol (3 mg/ml) was used as a photon counter to correct differences in excitation intensity. The samples were suspensions of membrane vesicles of absorbance lower than $0.1\ cm^{-1}$ at 588 nm, so that Bchl fluorescence emission was a linear function of concentration. Visible c.d. spectra were obtained with a Jobin Yvon Dicrograph III, using cells of 1 cm optical path.

RESULTS

The nonaene carotenoids of freeze-dried intracytoplasmic membranes isolated from *R. capsulatus* BY1424, a mutant strain that contains LHII and lacks other photosynthetic chromoproteins (Scolnik et al., 1980), were relatively resistant to extraction with light petroleum. Thus three successive exposures to the organic solvent were required to lower the carotenoid content of the freeze-dried membrane preparations to about 40 % of the original value. This contrasts with the ease with which the same carotenoids were removed from the membranes of *R. capsulatus* MW4422 (Zurdo et al., 1991), a strain that contained only the LHI antenna type (Scolnik et al., 1980). The levels of Bchl in the light-petroleum extracts were insignificant (Figure 1), but since the absorption spectrum of the extracted vesicle preparation (Figure 2) showed a new weak band near 770 nm that was probably due to dissociated Bchl resulting from partial LHII denaturation, further carotenoid extraction was not attempted.

The decrease in the carotenoid level in the membrane preparation was accompanied by modifications in the LHII absorption spectrum, as illustrated in Figure 2. Apart from the expected reduction in intensity of the three-peaked carotenoid band in the 420–500 nm range and the already mentioned weak Q_y band near 770 nm, the most noticeable modification was a bleaching of the Bchl Q_y transition at 800 nm. There was also an area increase and an asymmetric broadening of the Q_y band at 850 nm. In contrast with the near-i.r. bands, the $Q_x(S_2 \leftarrow S_0)$ transition of Bchl near 590 nm (which was not resolved for the fractions absorbing at 770, 800 and 850 nm) was only slightly shifted by the treatment used to remove the carotenoids (Figure 2). For this reason we used the Q_x band as an intrinsic reference when comparing the spectra of preparations with different carotenoid contents.

Before trying to draw any conclusion from the spectroscopic changes undergone by LHII Bchl during carotenoid extraction, it seemed necessary to establish whether they were directly

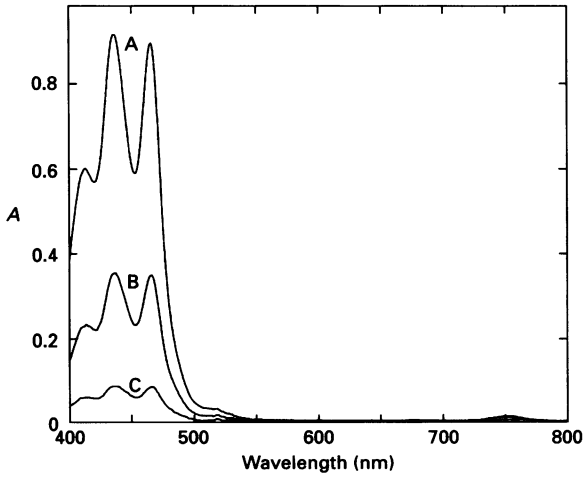

Figure 1 Absorption spectra of light-petroleum extracts from freeze-dried *R. capsulatus* vesicles

The spectra of three successive extracts (A–C) are shown. The three-peaked band in the 400–500 nm range is due to the nonaene carotenoids present in the bacterial vesicles. The spectra are extended to the near-i.r. to show that only insignificant amounts of porphyrins, absorbing in the 740–770 nm range, were simultaneously extracted.

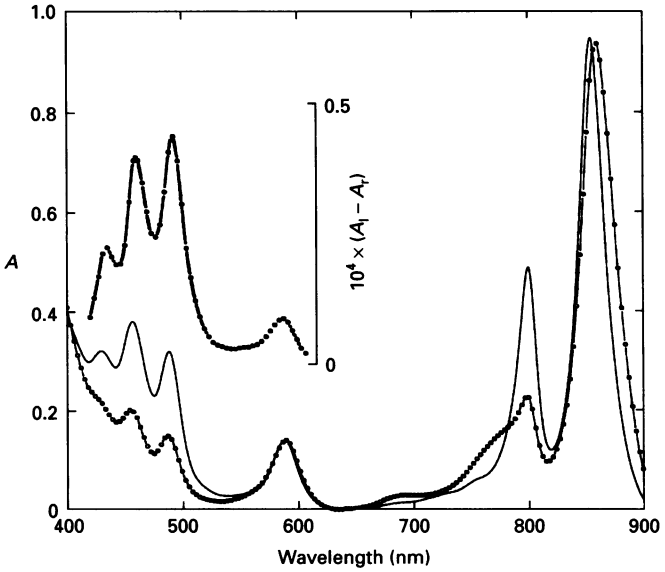

Figure 2 Absorption and c.d. spectra of membrane-linked LHII preparations

The absorption spectra of isolated intracytoplasmic vesicles, containing all (——) or only about 40% (○) of their native carotenoid complement, are shown in the visible and near-i.r. ranges. Both spectra were normalized at the Q_x band of Bchl at 588 nm after assuming that the absorbance at 640 nm was 0, to minimize differences in light scattering among the samples. The visible c.d. spectrum of the unextracted preparation is shown in the inset (●). A_l and A_r are absorbances of left and right circularly polarized light.

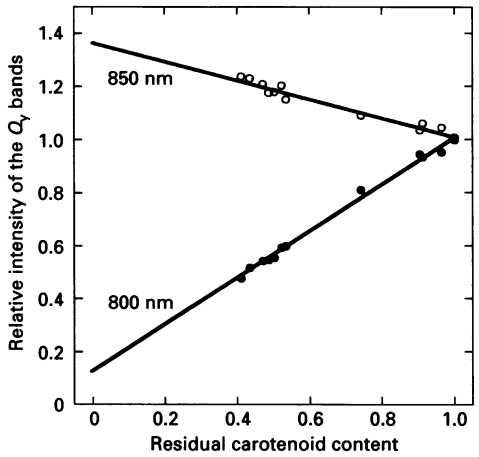

Figure 3 Effect of carotenoid content on the intensities of the Bchl Q_y bands of membrane-linked LHII

The residual carotenoid content of each preparation was estimated from the intensity of the carotenoid band in the absorption spectra after normalization at the Q_x band of Bchl at 588 nm (Figure 2). The published spectrum of *R. capsulatus* carotenoidless strain Ala^+ (Feick et al., 1980) was used as the baseline. The intensity of the 850 nm band (○) was measured by its area, estimated from the product of the absorbance at its maximum by its half bandwidth in cm^{-1}, in order to take into account both the red shift and the broadening effects of carotenoid depletion. The intensity of the 800 nm band (●) was estimated from the absorbance at its peak.

related to the removal of the carotenoid from its binding site at the protein or were just an independent effect of the experimental treatment used to extract the carotenoid. The first alternative implies a well-defined correlation between the carotenoid extraction and the spectroscopic modifications of LHII Bchl. Such a correlation was actually observed when the extents of the

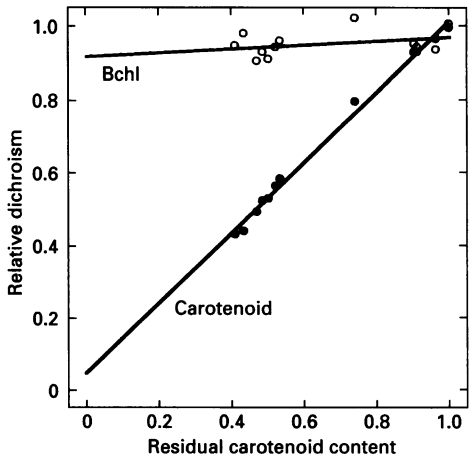

Figure 4 Effect of carotenoid content on the visible c.d. of carotenoids and Bchl in membrane-linked LHII

The residual optical activity of the carotenoid (●) in each preparation was estimated from the peak-minus-trough amplitude of the less energetic component of the carotenoid c.d. band (Figure 2), and that of Bchl (○) from the intensity of the c.d. band near 590 nm.

bleaching of the 800 nm transition and the increase in the area under the 850 nm band were plotted versus the residual carotenoid fractions of preparations from which the carotenoid had been extracted to a diverse degree, as shown in Figure 3.

More conclusive evidence for the direct relation between carotenoid loss and the changes in the Bchl spectrum would be provided by demonstrating that both took place on the same fraction of the LHII preparation. If the pigment clusters of the proteins from which the carotenoids had not been removed retained their native structure, the unmodified spectral features of the carotenoid-containing fraction should be present in the membrane preparation partly depleted of the carotenoid. Thus the observation that the residual absorption bands of Bchl at 800 nm and of carotenoids in the 420–500 nm range did not show any significant shifts or modifications, apart from the bleachings themselves (Figure 2), is in accordance with the absence of modifications from the carotenoid-containing LHII fraction of the preparation. Besides, a linear correlation was observed between the intensities of the c.d. and the absorption carotenoid bands (Figure 4), clearly indicating that the specific optical activity of the residual pigment remained constant. Since the chirality of the LHII carotenoid is induced by its binding to the protein (Scolnik et al., 1980), it would be expected to change if the environment of the residual pigment were significantly altered by the extraction treatment.

In order to obtain further evidence for the direct relation between carotenoid removal and modification of the LHII spectroscopic properties, we attempted the separation by biochemical methods of the LHII populations present in membranes partially depleted of carotenoids. Protein was extracted from the membrane with dodecyldimethylamine *N*-oxide, a non-ionic detergent that has been widely used to solubilize bacterial photosynthetic proteins. The extracts had higher levels of free Bchl than the corresponding carotenoid-depleted membrane preparations from which they were obtained, as indicated by the increased absorption in the 700–800 nm and 400–430 nm ranges (Figure 5a). Other detergents tested yielded similar results. In contrast, the presence of free porphyrins was not apparent in detergent extracts of the membrane preparations that had not been previously subjected to carotenoid extraction (Figure 5a), which exhibited the characteristic spectrum of solubilized LHII

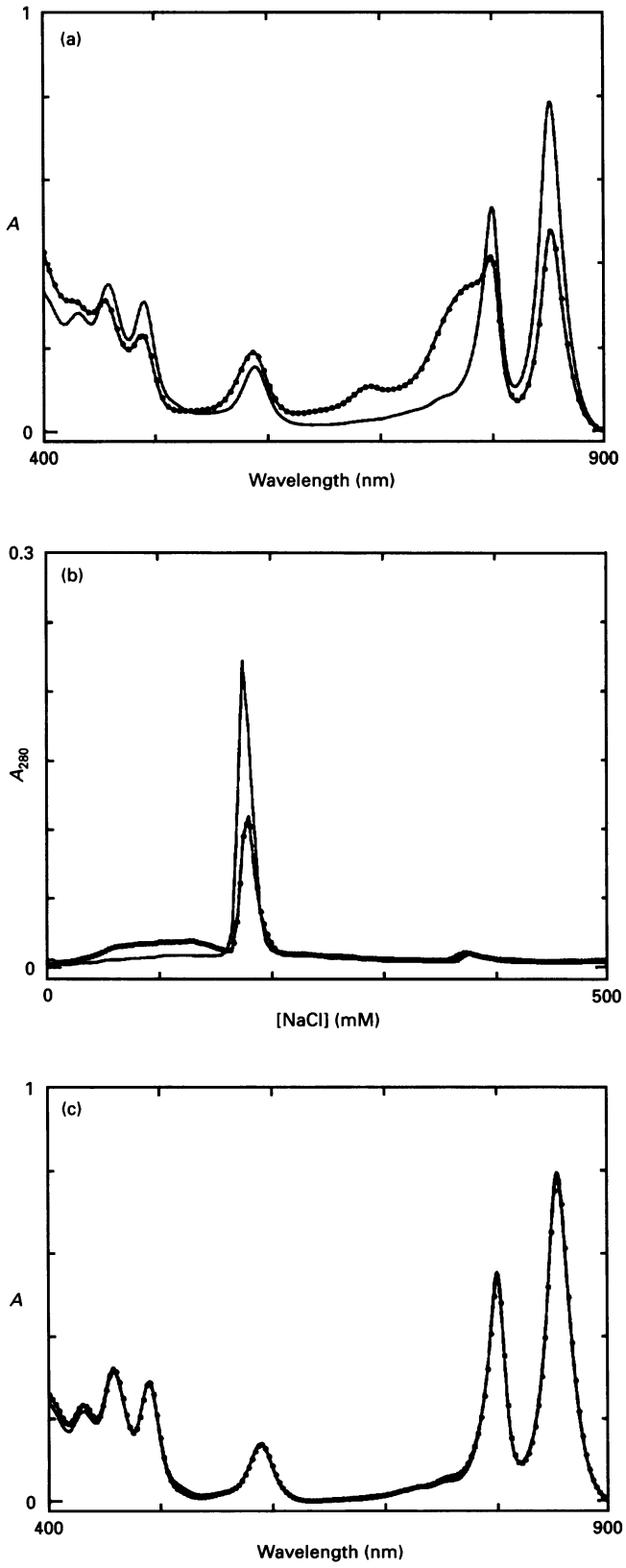

Figure 5 Solubilization and partial purification of LHII from membrane vesicles

LHII was extracted with the aid of dodecyldimethylamine *N*-oxide from membrane preparations that had been depleted previously of 44% of their native carotenoid content, and the extract subjected to anion-exchange chromatography as described in the Materials and methods section. The absorption spectrum of the detergent extract (**a**), the elution profile of material absorbing at 280 nm (**b**), and the spectrum of the pooled fractions corresponding to the peak in the eluate (**c**) are shown for the carotenoid-depleted preparation (●) and also for a preparation with an intact carotenoid content (——). Absorption spectra were normalized at the Q_x band to facilitate comparison between (**a**) and (**c**). The chromatograms in (**b**) were drawn for the same amount of Bchl in the sample loaded on to the column.

(Clayton and Clayton, 1981; Shiozawa et al., 1982). These observations suggest that the main or only source of free Bchl in the detergent extracts was the LHII fraction that had previously lost carotenoid on exposure of the vesicles to light petroleum and which was denatured by the detergent. In accord with such a conclusion, the only Bchl fraction that was found to be associated with protein in the detergent extract of carotenoid-depleted membranes showed the same chromatographic behaviour as LHII obtained from membranes not depleted of carotenoids (Figure 5b), and had the absorption spectrum of unmodified LHII (Figure 5c). On the other hand, there was close agreement between the residual content of carotenoid in the membrane preparation and the amount of unaltered LHII recovered in the chromatographic step. Thus the vesicle preparation partly depleted of carotenoids that was used to obtain the detergent extract analysed in Figure 5 had a residual carotenoid level of 0.56, relative to that of unextracted membranes (not shown). This value compares well with the measured ratio of 0.52 between the respective protein peaks in the chromatographs of the solubilized preparations (Figure 5b).

The preceding results clearly show that the LHII fraction that retained its carotenoid complement did not undergo any significant change in its absorption spectrum. Therefore the modifications of the Bchl Q_y transitions observed in the membrane preparations that were partially depleted of carotenoids (Figure 2) must correspond to the structural changes of the LHII fraction that had lost the carotenoid. The trend of such changes (Figure 3) indicates that the spectrum of a preparation fully depleted of carotenoids would almost completely lack the 800 nm transition, and would show both a weak band near 770 nm and a major broadened and red-shifted band near 860 nm. It appears that the weak band near 770 nm was due to dissociated Bchl resulting from denaturation of some of the LHII, because the Q_y transition of monomeric Bchl *a* in organic solvents lies close to 770 nm, and denaturation of Bchl *a* proteins by detergents, acids or other reagents shifts the wavelength of the band of bound Bchl to the same spectral range (see Figure 5 as an example). In contrast, it seems that the Bchl fraction responsible for the major Q_y band near 860 nm was specifically bound to the protein, as suggested by the following data.

The Q_x transition of LHII Bchl near 590 nm shows a positive c.d. band (Figure 2), which is induced by the binding of Bchl to the protein (Bolt et al., 1981). Although such optical activity is very weak and estimation of its intensity cannot be very accurate, extrapolation of the experimental data of Figure 4 indicates that its decrease on full carotenoid extraction would be no more than 10%. This decrease could be accounted for by the dissociation of Bchl that resulted from denaturation of a small fraction of LHII, as indicated also by the appearance in the preparation of the weak 770 nm band. The permanence of most of the induced Bchl dichroism when the carotenoid was extracted is consistent with the view that most of the Bchl was not released from the protein. The slight effect of carotenoid extraction on the c.d. of LHII Q_x contrasts with the behaviour of a similar c.d. band of LHI, which disappeared when the carotenoid was removed by either mutation (Lozano et al., 1990) or solvent extraction (Zurdo et al., 1992).

As light-harvesting pigments, the photosynthetic carotenoids absorb light and transfer singlet excitation energy to Bchl. Significant efficiency of carotenoid-to-Bchl energy transfer requires a very short distance (electronic overlap) between the

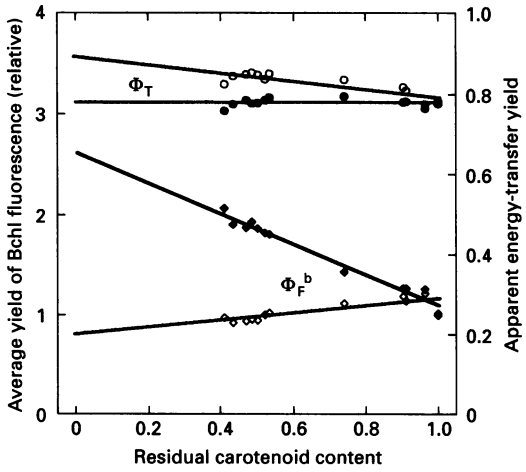

Figure 6 Effect of carotenoid content on the relative average yield of fluorescence emission (Φ_F^b) by Bchl in membrane-linked LHII and on the apparent quantum yield of carotenoid-to-Bchl singlet-energy transfer (Φ_T)

Interference filters of about 13 nm half bandwith, centred at 590 or 480 nm, were used in combination with an i.r.-absorbing glass to select excitation at Bchl or at the carotenoids respectively. Emitted light was measured after filtration through a red cutoff glass (RG715) and either an 862 nm (○, ◇) or a 912 nm (●, ◆) narrow-band interference filter. In the plots of Φ_F^b (◇, ◆), the fluorescence intensities at each wavelength are given relative to that of the respective unextracted preparation but, since it was observed that light petroleum induced a 15–25% increase in the emission, these reference values were not used to draw the regression lines. The values of Φ_T (○, ●) are the ratios between the emissions excited at 480 and 590 nm, after correction for differences in exciting light intensity and in light absorption. The contribution of non-carotenoids to light absorption and fluorescence excitation at 470 nm was assumed to be insignificant. Other conditions were as in Figure 3 and Materials and methods.

donor and the acceptor, as has been shown for model systems of covalently linked pigments (Wasielewski et al., 1986). Thus, in the photosynthetic proteins, direct carotenoid-to-Bchl singlet-energy transfer only occurs between pigments of a single cluster, and changes in the structure of the cluster may considerably decrease the efficiency of the process. Subsequent Bchl-to-Bchl singlet-energy transfer, which may take place efficiently at longer distances, is responsible for exciton migration among different pigment units (Borisov, 1989). Since the adequate relative orientation of the donors and acceptors of excitation energy is maintained by specific pigment–protein interactions (Zuber, 1986), it appears that only specifically bound pigment molecules may participate with high efficiency in singlet-energy transfer. Therefore estimation of the yields of the energy-transfer processes in LHII preparations may provide additional information on whether the major Bchl fraction of carotenoidless LHII is specifically bound to the protein.

The bathochromic shift that carotenoid extraction elicited in the 850 nm-absorptive transition of membrane-linked LHII Bchl was accompanied by a parallel shift in the fluorescence emitted by the porphyrin. This is illustrated by the opposite effects that carotenoid removal had on the relative fluorescence yields of Bchl (Φ_F^b) measured at the high- and low-energy sides of the emission band: whereas the yield of the fluorescence emitted at 862 nm was decreased by carotenoid extraction, that emitted at 912 nm was increased (Figure 6). When Φ_F^b was plotted versus the residual carotenoid content of the membrane vesicles, a close fit to linear regressions was again observed (Figure 6), as expected from carotenoid loss being directly related to the red shift of Bchl fluorescence emission.

If the Bchl present in the preparation is homogeneous or if excitation densities reach thermal equilibrium among the types of Bchl present, the quantum yield of carotenoid-to-Bchl energy transfer, Φ_T, is equal to the quotient between the yields of carotenoid- and Bchl-sensitized fluorescence of Bchl (Φ_F^c/Φ_F^b). Usually, this apparent yield is obtained from the ratio between the intensities of the carotenoid bands in the excitation spectrum of Bchl fluorescence and in the fractional absorption spectrum, after normalization of both spectra at a Bchl band, since

$$\Phi_T = \Phi_F^c/\Phi_F^b = (f^c/a^c)/(f^b/a^b) = (f^c/f^b)/(a^c/a^b)$$

where f is the intensity of Bchl emission, a is absorption at the excitation wavelengths and the superscripts refer to excitation or absorption at the carotenoid (^{c}) or Bchl (^{b}) bands. In the intact LHII antenna, where all the Bchl molecules seem to be directly associated with carotenoids and excitation densities reach thermal equilibrium (Van Grondelle, 1985), the apparent Φ_T coincides with the actual yield of carotenoid-to-Bchl energy transfer.

According to our interpretation of the spectroscopic Bchl changes, the loss of the carotenoid from a given pigment cluster is accompanied by spectroscopic alteration of the Bchl of that cluster. If such a modification causes 'uncoupling' of the porphyrin, in the sense that it becomes unable to accept excitation energy from unmodified Bchl in other clusters, average Bchl emission will no longer reflect the emission of the Bchl associated with the carotenoid, and the apparent value of Φ_T, as estimated above, will no longer coincide with that of the real transfer efficiency, which will remain unmodified if the residual carotenoid remains bound to unmodified LHII. In fact, *total* uncoupling of the Bchl that has lost the associated carotenoid would be expected to affect inversely, but to an identical extent, the average value of Φ_F^b and the apparent value of Φ_T. Partial uncoupling of the modified Bchl (or a moderate decrease in the efficiency of energy transfer from intact to modified Bchl) will affect the apparent Φ_T less than the average Φ_F^b. Therefore, the observation that the apparent Φ_T changed considerably less than the average Φ_F^b on carotenoid extraction (Figure 6) demonstrates that the Bchl fraction that lacked the associated carotenoid was still an efficient acceptor of excitation energy from Bchl of intact LHII units. The small apparent increase in Φ_T elicited by carotenoid removal when the fluorescence was measured at 862 nm (Figure 6) can be explained by the accumulation of low levels of dissociated Bchl (Figure 2), which contributed to light absorption at 590 nm but did not emit at 862 nm. When fluorescence intensity was measured at 912 nm, where emission by modified bound Bchl was predominant, a similar increase was not observed (Figure 6), possibly because it was compensated for by a small decrease in the efficiency of energy transfer between intact and modified Bchl.

DISCUSSION

The trend of the specroscopic changes resulting from carotenoid extraction (Figures 1 and 2) suggest that the spectrum of the LHII preparation from which the carotenoid was eventually completely extracted would lack most of the 800 nm Q_y transition and would exhibit a major Q_y band near 860 nm. Although it seems clear from the present data that these spectroscopic alterations in Bchl were unequivocally related to carotenoid extraction, it is not apparent from our results alone whether they were a direct consequence of carotenoid removal or just happened simultaneously (as an additional effect of the organic solvent on those proteins that had lost the carotenoid). However, little doubt remains when it is considered that the LHII detected in

carotenoidless *Rhodobacter* mutants also shows a single Q_y transition near 860 nm (see below). Since, in those systems, LHII did not undergo any physical or chemical treatment, it is reasonable to conclude that carotenoid depletion elicits the spectroscopic changes in LHII Bchl, independently of whether the absence of the pigment is due to physical or genetic manipulation.

As mentioned above, LHII is absent from primary *Rhodobacter* mutants that are unable to synthesize coloured carotenoids (Marrs, 1978). This phenotype can be explained by the structural modifications elicited by the absence of the carotenoid (detected in this report by both the spectroscopic changes in bound Bchl and the reduced stability of LHII in detergent extracts), since it is quite commonly observed that proteins with a modified structure show an increased susceptibility to endogenous proteolytic attack. Therefore the presence of carotenoidless LHII in the living cell at significant levels would require compensatory mutations to enhance its proteolytic stability. This may be the reason why LHII-containing carotenoidless strains have been obtained only as variants of the primary mutants (Davidson and Cogdell, 1981a; Tadros et al., 1989). In one such strain, *R. sphaeroides* R26.1, a single-residue change (Val-24 → Phe) was detected in the α-subunit of LHII (Theiler et al., 1984), as expected from the requirement of compensatory mutations to stabilize the protein. The failure to restore the 800 nm Q_y transition on carotenoid reconstitution of the anomalous LHII of strain R26.1 (Davidson and Cogdell, 1981b) may be argued as evidence against the absence of carotenoid being the only reason for the spectral LHII alterations. However, as discussed by Theiler et al. (1984), it is often difficult to reach reliable conclusions from a negative result. In that particular case, the failure to restore the 800 nm band was possibly due to the use of dodecyl sulphate to solubilize the reconstituted protein (Davidson and Cogdell, 1981b), since other authors simultaneously showed that such detergent caused bleaching of the band (Clayton and Clayton, 1981). Besides, the change in the α-subunit of the mutated protein of strain R26.1 (Theiler et al., 1984) could also have interfered with the restoration of the 800 nm transition of bound Bchl on carotenoid reconstitution. Therefore it appears that additional effects of carotenoid depletion are not required to explain the carotenoidless phenotypes, although they cannot be ruled out by the present data.

It is not possible to offer an unambiguous interpretation of the structural changes that underlie the spectroscopic effects of carotenoid depletion, although it seems obvious that carotenoid removal alters the composition and/or the arrangement of the LHII Bchl cluster, probably by inducing conformational changes in the apoprotein. A current structural model of LHII (Kramer et al., 1984) assigns the 800 and 850 nm Q_y transitions to two distinct sets of Bchl molecules, which would be in a 1:2 ratio within the pigment cluster, in agreement with the conclusions of previous work (Clayton and Clayton, 1981). If it is assumed that the model is essentially correct, bleaching of the 800 nm transition could be interpreted as a reflection of changes at the binding site of the minor Bchl population, which in a fraction of carotenoid-depleted LHII would be released and in the rest would shift its Q_y transition beyond 850 nm. This would explain why the 800 nm bleaching was accompanied by the appearance of a band of dissociated Bchl near 770 nm and by an apparent red shift and broadening of the native 850 nm band. However, the large red shift (about 60 nm) undergone by the 800 nm Bchl that remained bound to the protein is difficult to understand. A different interpretation of the spectroscopic Bchl changes, also based on the model of Kramer et al. (1984), would assign the bleaching of the 800 nm transition to dissociation of the minor Bchl population and the bathochromic shift of the 850 nm band to a structural change in the major LHII Bchl fraction. A weak point of this interpretation is that the intensity of the 770 nm band of dissociated Bchl seems too low to account for the whole bleaching of the 800 nm band. This apparent inconsistency could be explained, however, by a marked decrease in the Q_y absorption coefficient on dissociation.

If the model of Kramer et al. (1984) were ignored and the spectral resemblance between the Bchls of LHII and those of the bacterial reaction centre were assumed to reflect profound structural similarities, both the 800 and the 850 nm Q_y transitions should be assigned to a single pair of interacting Bchl molecules (Friesner and Won, 1989). In the dimer, the intensity of each of the split transitions depends on the relative orientation of the transition moments of the interacting monomers (Tinoco, 1963). Thus, both split transitions have the same intensity when the angle between the monomeric transition moments is 90°, but only the split transition of lower energy is allowed when the angle is 0°. Then, if the angle between the Q_y transition moments were close to 74° in the native protein (in accordance with the observed relative intensities of the 800 and 850 nm bands), the spectral changes in the Bchl that remains bound to LHII after carotenoid extraction could simply be explained by a reorientation of the two interacting molecules, the Q_y transition moments of which would become parallel. Strong support for this interpretation would be the demonstration that LHII contains 2 molecules per $\alpha\beta$ polypeptide heterodimer, and that this composition remains the same on carotenoid extraction. In carotenoidless LHII of *R. sphaeroides* R26.1, such a Bchl/polypeptide ratio was actually found, and the absorption and c.d. spectra of Bchl in that protein were explained on the basis of dimeric exciton interaction (Braun and Scherz, 1991). However, analysis of the pigment composition of the protein of wild-type strains yielded conflicting results (Shiozawa et al., 1982; Evans et al., 1988; Picorel and Gingras, 1988). The Bchl/polypeptide ratio of carotenoid-containing and carotenoid-depleted LHIII preparations should therefore be reinvestigated using similar analytical procedures. The increased lability of the solubilized protein after carotenoid extraction, which interfered with our initial attempts of purification, did not allow us to perform such comparative analyses in the present study.

We thank Dr. Julián Gómez and Mr. Javier Varela for their kind help with c.d. measurements and f.p.l.c. respectively. This work was supported by grant PB87-0204 from the Dirección General de Investigación Científica y Técnica, Spain.

REFERENCES

Bolt, J. D., Sauer, K., Shiozawa, J. and Drews, G. (1981) Biochim. Biophys. Acta **635**, 535–541
Borisov, A. Y. (1989) Photosynth. Res. **20**, 35–58
Braun, P. and Scherz, A. (1991) Biochemistry **30**, 5177–5184
Clayton, R. K. (1966) Photochem. Photobiol. **5**, 669–677
Clayton, R. K. and Clayton, B. J. (1981) Proc. Natl. Acad. Sci. U.S.A. **78**, 5583–5587
Cogdell, R. J. and Frank, H. A. (1987) Biochim. Biophys. Acta **895**, 63–79
Davidson, E. and Cogdell, R. J. (1981a) FEBS Lett. **132**, 81–84
Davidson, E. and Cogdell, R. J. (1981b) Biochim. Biophys. Acta **635**, 295–303
Duysens, L. N. M. (1956) Biochim. Biophys. Acta **19**, 1–12
Evans, M. B., Cogdell, R. J. and Britton, G. (1988) Biochim. Biophys. Acta **935**, 292–298
Feick, R. and Drews, G. (1980) Biochim. Biophys. Acta **501**, 499–513
Friesner, R. A. and Won, Y. (1989) Biochim. Biophys. Acta **977**, 99–122
Koyama, Y. (1991) J. Photochem. Photobiol. B. **9**, 265–280
Kramer, H. J. M., Van Grondelle, R., Hunter, C. N., Westerhuis, W. H. J. and Amesz, J. (1984) Biochim. Biophys. Acta **765**, 156–165
Lascelles, J. (1956) Biochem. J. **62**, 78–93
Lozano, R. M., Fernández-Cabrera, C. and Ramírez, J. M. (1990) Biochem. J. **270**, 469–472
Marrs, B. L. (1978) Curr. Top. Bioenerg. **8**, 261–294
Picorel, R. and Gingras, G. (1988) Biochem. Cell Biol. **66**, 442–448

Scolnik, P. A., Zannoni, D. and Marrs, B. L. (1980) Biochim. Biophys. Acta **593**, 230–240
Shiozawa, J. A., Welte, W., Hodapp, N. and Drews, G. (1982) Arch. Biochem. Biophys. **213**, 473–485
Siefermann-Harms, D. (1987) Physiol. Plant. **69**, 561–568
Tadros, M. H., García, A. F., Gad'on, N. and Drews, G. (1989) Biochim. Biophys. Acta **976**, 161–167
Theiler, R., Suter, F., Zuber, H. and Cogdell, R. J. (1984) FEBS Lett. **175**, 231–237
Tinoco, I. (1963) Radiat. Res. **20**, 133–139
Truscott, T. G. (1990) J. Photochem. Photobiol. B. **6**, 359–371
Van Grondelle, R. (1985) Biochim. Biophys. Acta **811**, 147–195
Wasielewski, M. R., Lidell, P. A., Barrett, D., Moore, T. A. and Gust, D. (1986) Nature (London) **322**, 570–572
Zuber, H. (1986) Trends Biochem. Sci. **11**, 414–419
Zurdo, J., Lozano, R. M., Fernández-Cabrera, C. and Ramírez, J. M. (1991) Biochem. J. **274**, 881–884
Zurdo, J., Fernández-Cabrera, C. and Ramírez, J. M. (1992) Biophys. J. **61**, 1462–1469

Received 10 July 1992/28 August 1992; accepted 11 September 1992