Dimeric carotenoid interaction in the light-harvesting antenna of purple phototrophic bacteria

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The carotenoid content of intracytoplasmic membrane vesicles isolated from purple phototrophic bacteria was reduced to a variable extent by mild extraction with light petroleum. Using preparations obtained from *Rhodobacter capsulatus* strains that contained the Light Harvesting System I (LHI) complex as the only major photosynthetic holochrome, it was shown that the visible circular dichroism of the carotenoids increased with the square of the membrane carotenoid content, as expected from being caused by dimeric exciton interaction. No chirality resulting from twists of the individual planar chromophore was detected. Therefore the contribution to carotenoid optical activity of non-degenerate interactions with bacteriochlorophyll or the apoprotein does not appear to be significant. The broadening of the absorption band of the bound pigment, caused by the splitting of the monomer transition, was demonstrated in membrane vesicles of both *Rb. capsulatus* and *Rhodospirillum rubrum* as a decrease of the fine structure of the band. Furthermore, the dimeric organization of the carotenoid pigments in the bacterial LHI complex accounted for the observed quantitative relationship between the fine structure of the band and the carotenoid content of the membrane.

INTRODUCTION

The photosynthetic pigment protein complexes that carry out light harvesting and the initial photochemical reactions contain coloured carotenoids as usual constituents. Besides contributing to light absorption, these pigments provide protection against harmful photosensitized oxidations by quenching the metastable excited states of chlorophylls and oxygen (Cogdell & Frank, 1987; Siefermann-Harms, 1987) that are unavoidably formed as by-products of the primary photoprocesses (Ramírez & Aparicio, 1989). The protective function of carotenoids appears to be essential for the survival of phototrophic organisms in aerobic environments (Sistrom *et al.*, 1956; Frosch *et al.*, 1979).

A convenient system for the study of photosynthetic carotenoids is the intracytoplasmic membrane of purple phototrophic bacteria. In these micro-organisms the other major pigments present, bacteriochlorophyll *a* or *b* (in both the antenna and the reaction centre) and the corresponding pheophytin (in the reaction centre) have absorption bands that do not overlap to a high extent with the longest-wavelength band of carotenoids in the 400–550 nm range. This circumstance facilitates the study *in situ* of those properties of the carotenoids that are more or less directly related to their visible absorption spectrum.

The chromophore of most photosynthetic bacterial carotenoids is a planar polyene chain that shows no intrinsic chirality. However, the binding of the pigment to the other holochrome constituents goes along with the appearance of a c.d. spectrum (Dratz *et al.*, 1966). In the reaction centre, where the carotenoid adopts the mono-*cis* configuration, the chirality appears to result from the interaction with the protein, which twists some of the formal single bonds in the conjugated system (Lutz *et al.*, 1987; Deisenhofer & Michel, 1989). In contrast, the available spectroscopic data do not provide evidence for generalized distortions of the planar conformation of the all-*trans* carotenoids in the bacterial light-harvesting complexes (Iwata *et al.*, 1985; Noguchi *et al.*, 1990). In a recent publication from this

laboratory (Lozano *et al.*, 1990) it was proposed that the optical activity of the carotenoid in one of such complexes [the core, B880 or Light Harvesting System I (LHI) complex] was due to dimeric exciton interaction. The proposal was based on the observation that the shape of the c.d. band resembled the first derivative of the three-peak absorption band. This suggested a

Fig. 1. Visible absorption spectra of carotenoid-depleted membrane vesicles

Carotenoids (neurosporene and neurosporene derivatives) were partially extracted from freeze-dried membrane vesicles isolated from *Rb. capsulatus* MW4422. The spectra were normalized at the Q_x bacteriochlorophyll band near 590 nm. The spectrum showing the most intense carotenoid band in the 400–550 nm range is that of unextracted membranes.

Abbreviations used: LHI and LHII, Light-Harvesting Systems I and II.

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Fig. 2. Intensity of the carotenoid c.d. band as a function of the carotenoid content of the membrane preparation

Membranes obtained from spheroidene-containing *Rb. capsulatus* MW442 ($\blacktriangle$) and neurosporene-containing *Rb. capsulatus* MW442 ($\circ$) were used. The residual carotenoid content of the preparation was estimated from the relative intensity of peak II in the absorption spectra of the preparations. For strain MW442 (Fig. 1) an absorbance of 0.2 at 453 nm was assumed for carotenoidless membranes. The intensity of the c.d. band is also given relative to that of unextracted membranes. The straight and curved dotted lines are plots of the equations $y = x$ and $y = x^2$, respectively.

splitting that, owing to the complex shape of the band, failed to produce clear positive and negative c.d. components. In the present study we have used membrane preparations of varied carotenoid content to perform a more detailed analysis of the optical activity of the LHI carotenoid. It is shown that the intensity of the c.d. band depends on the relative carotenoid level of the membrane as expected from its being caused by a dimeric organization of the pigment. It will also be shown that the splitting of the electronic transition in the bound carotenoid can be detected by a decrease in the fine structure of the absorption band, and that in membranes with a lowered carotenoid content, the extent of such decrease is linearly related to the probability of random dimer formation.

MATERIALS AND METHODS

The bacterial strains used in this work, which contain only the LHI antenna, were: *Rhodospirillum rubrum* S1 (wild-type), *Rhodobacter capsulatus* MW442 (mutant lacking the LHII antenna; wild-type carotenoid phenotype), and *Rb. capsulatus* MW442 [which lacks also the Light-Harvesting System II (LHII) antenna and has a block in the carotenoid-biosynthetic pathway]. All the strains were grown in the light and in the absence of O_2 as described previously (Giménez-Gallego *et al.*, 1978). The cultures of *Rs. rubrum* were allowed to enter the stationary phase to favour the accumulation of spirilloxanthin (Liaaen-Jensen *et al.*, 1958, 1961), since the LHI complex of exponential cultures contain a carotenoid mixture that shows a composite visible absorption band of little fine structure (Lozano *et al.*, 1989). Particular care was taken to exclude O_2 from the cultures of *Rb. capsulatus* MW442, so that spheroidene was the only carotenoid present at significant levels. Otherwise

Fig. 3. Parameters used to quantify the fine structure of the carotenoid absorption band

The composite (broader) dimer band was drawn from the monomer band by computer manipulation, assuming that its components were of the same intensity and were oppositely shifted the same number of nanometres. While the difference, absorbance at a peak minus absorbance at a trough, decreases upon splitting ($\Delta A_m > \Delta A_d$), the average peak-trough absorbance does not change ($A_m \approx A_d$).

spheroidenone, that has a considerably less structured absorption band, would have also accumulated (Scolnik *et al.*, 1980b). Strain MW442 of *Rb. capsulatus* contains a mixture of neurosporene, hydroxynurosporene and methoxynurosporene at comparable levels (Scolnik *et al.*, 1980a,b).

Intracytoplasmic membrane vesicles were isolated by differential centrifugation of cell-free extracts that had been prepared from cells suspended in 50 mM-potassium phosphate, pH 7, with a French press. After washing with the same buffer, the vesicles were suspended in distilled water and freeze-dried. Mild extraction of carotenoids from the freeze-dried membrane preparations was made as follows: 4–10 mg of membranes in glass centrifuge tubes were dispersed at 0 °C in a variable volume (0.5–3 ml) of an organic solvent with the aid of a tube mixer and a sonifier. Then the samples were kept for a variable length of time (0–30 min) at a controlled temperature (from 0 to 30 °C), centrifuged (10 min at 2–4 °C and 12000 g), and the sediment was vacuum-dried at 40 °C. The supernatant was checked spectrophotometrically for the presence of extracted porphyrins and, if found, the dried membrane preparation was discarded. Among the solvents tried, only pure light petroleum (b.p. 50–70 °C) passed this test. The dried sediments were suspended in 50 mM-potassium phosphate, pH 7, sonified at 0 °C to reduce light-scattering and kept frozen at –30 °C for future use. In some cases the extraction with light petroleum was repeated once or twice prior to sediment drying. Changes of the extraction conditions as indicated were used to obtain sets of samples with a varied carotenoid content.

Absorption spectra were scanned with a Shimadzu spectrophotometer (model UV-3000), supplemented when required with a low-temperature attachment. The instrument

Fig. 4. Variation of the fine structure of the carotenoid absorption band with the carotenoid content of the preparation

The fine structure of the carotenoid band was estimated as $\Delta A/\bar{A}$ (Fig. 3) in membranes obtained from *Rb. capsulatus* MW4422 and *Rs. rubrum* S1, at room (●) and at liquid- N_2 temperature (○). Only preparations with > 40% of the original carotenoid level were used for the room-temperature estimations.

was interfaced to a Hewlett-Packard microcomputer (HP9826) for storage and processing of the spectra. Visible c.d. spectra were obtained with a Jobin Yvon III dichrograph. The c.d. data were digitized and transferred to the Hewlett-Packard microcomputer as described before (Lozano *et al.*, 1990).

RESULTS AND DISCUSSION

Fig. 1 shows the visible absorption spectra of several preparations of membrane vesicles from which the carotenoids had been partially removed. The vesicles had been obtained from *Rb. capsulatus* MW4422, a strain that contains neurosporene and two other nonaene carotenoids with similar absorption spectra. As with the rest of the strains used in the present study, *Rb. capsulatus* MW4422 has only the LHI type of antenna (Scolnik *et al.*, 1980a,b). In Fig. 2 the intensities of the carotenoid c.d. bands of these preparations were plotted versus the respective relative carotenoid levels (estimated from the spectra of Fig. 1). It can be observed that the experimental points are closely fitted by the equation:

$$y = x^2$$

Assuming that the residual carotenoid is randomly distributed among the available LHI binding sites, the found pattern is that expected if the optical activity of the visible transition results from the splitting caused by dimeric exciton interaction. This is so because the probability of finding a carotenoid molecule at a given binding site is equal to the residual carotenoid content of the preparation, and that of finding one carotenoid molecule at each of two given sites is the square of the same relative carotenoid level. If, on the contrary, the carotenoid c.d. were due

to chirality resulting from twists of the individual chromophore, caused by non-degenerate interactions of the pigment with either (or both) bacteriochlorophyll or (and) protein, a linear dependence of the carotenoid optical activity on the residual level of the pigment would be observed.

It was proposed before (Lozano *et al.*, 1990) that both degenerate carotenoid-carotenoid interactions and non-degenerate interactions of the pigment with other constituents contributed to the c.d. of the carotenoid in the LHI complex of *Rs. rubrum*. No evidence for the non-degenerate type of interaction is apparent in the *Rb. capsulatus* data of Fig. 2. This was more clearly seen when the plot was linearized by dividing by the abscissa values in order to adjust the data to an equation of the type:

$$y/x = ax + b$$

The first-order regression line intersected the vertical axis close to its origin ($b \approx 0$), indicating that a possible linear component of carotenoid dichroism was not significant (plot not shown). This lack of monomeric carotenoid chirality is in accordance with the weak distortions of the planar structure of the *Rb. capsulatus* antenna carotenoids that had been detected by resonance Raman spectroscopy (Iwata *et al.*, 1985; Noguchi *et al.*, 1990).

The plot of Fig. 2 includes also some additional points (▲) that correspond to vesicles isolated from a culture of *Rb. capsulatus* MW442. The culture contained spheroidene as the single major carotenoid and, since even the mildest extraction treatment removed > 60% of this pigment, it was not possible to obtain from that strain a set of membrane preparations that covered the whole range of residual carotenoid levels. Never-

theless, the available experimental points fitted also the theoretical second-order curve.

Light petroleum was considerably less effective for solubilizing spirilloxanthin, the carotenoid present in the membrane preparations of *Rs. rubrum*, since three or more extractions were required to lower the carotenoid content to < 50 % of the native level. When mixed with a small percentage of a more polar solvent to increase the extent of carotenoid extraction, significant levels of bacteriochlorophyll were solubilized. But the really serious problem with this bacterium was the low intensity of its carotenoid c.d. band (Lozano *et al.*, 1990), which did not allow to carry out a reliable quantitative analysis similar to that performed with *Rb. capsulatus*. A presumably weaker carotenoid-carotenoid interaction, the lower carotenoid-to-bacteriochlorophyll ratio of this type of LHI complex (Cogdell, 1986) and the high extent of overlapping between the bands of spirilloxanthin and bacteriochlorophyll (Q_x) in the c.d. spectrum (Dratz *et al.*, 1966) are among the factors that contribute to make difficult an accurate estimation of carotenoid dichroism in the extracted membrane preparations of *Rs. rubrum*.

As an alternative interpretation of the data of Fig. 2, it seems possible that the binding of a carotenoid molecule might elicit conformational changes in the protein that, in turn, would induce chirality in another carotenoid molecule at a neighbouring site. In this way an indirect co-operative effect between pairs of carotenoid molecules would give rise to optical activity without exciton interaction, and a pattern similar to that of Fig. 2 would still be found.

In order to test the alternative interpretation, it seemed necessary to search for other observable consequences of dimeric carotenoid interaction. One of such consequences is the broadening of the absorption band that follows the splitting of the monomer transition, since the two resulting dimer bands are oppositely shifted with respect to that of the monomer. For a complex multi-component absorption band such as those of photosynthetic carotenoids, the broadening is clearly reflected in a loss of fine structure (or sharpness of the individual peaks). This is illustrated in Fig. 3, where a composite dimeric band has been simulated by symmetrical splitting of the band of a carotenoid solution. It can be observed that the splitting causes decreases of the absorption coefficients at the peaks and rises at the troughs, with isosbestic points between contiguous peaks and troughs. Consequently, an adequate quantitative estimation of the fine structure of the band is the ratio between the difference, absorbance at a peak minus absorbance at a neighbouring trough ($\Delta A = A_p - A_t$), and the average peak-trough absorbance ($\bar{A} = (A_p + A_t)/2$; this average is an approximation to the absorbance at the isosbestic point and therefore a convenient estimation of the residual carotenoid level). It can be easily demonstrated that, for randomly dimerized pigments, the fine structure of the carotenoid band should decrease linearly from the value of the monomer (with no carotenoid content) to the value of the dimer (at maximum carotenoid content). As shown in Fig. 4, the experimental data indicate that the fine structure of the carotenoid bands in the *Rb. capsulatus* and *Rs. rubrum* LHI complexes decreases with the carotenoid level of the membrane, as predicted by the dimeric model. In the case of *Rs. rubrum* the enhancement of the negative slope of the regression line at liquid-N₂ temperature is remarkable. This may be solely due to the sharpening of the band of bound spirilloxanthin, but it may also reflect a strengthening of carotenoid exciton interaction at cryogenic temperatures.

In conclusion, the experimental approach used in the present

study to obtain evidence for the dimeric organization of the carotenoids in the LHI antenna complex is based on two major assumptions. The first of them is that carotenoid extraction with light petroleum as done here does not induce important damages or changes of the properties of the LHI holochrome apart from those directly related to carotenoid removal. The assumption is supported by the absence of detectable shifts in the location of the absorption band of the residual carotenoid (Fig. 1), by the low levels of extracted bacteriochlorophyll (see the Materials and methods section) and by the insignificant increase of the intensity of the absorption band of dissociated bacteriochlorophyll near 770 nm in the extracted membranes (not shown). The second assumption, i.e. that the residual carotenoid is randomly dimerized or, what is the same, that the probabilities of extracting monomeric and dimeric carotenoid molecules from the membrane-bound LHI complex are equal, is only supported by the close agreement observed between the spectroscopic data and the predictions of the dimeric model. This second assumption implies that the dimerization does not contribute significantly to strengthen the binding of the pigment to the complex. But whether the aggregation state of the carotenoid may have some other type of bearing on the structural and functional properties that the carotenoid confers on the antenna holochrome has to be investigated. It seems that the experimental system of partially carotenoid-depleted membranes which has been developed in this work will be useful for this aim.

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