# Enhancement of carotenoid-to-chlorophyll singlet energy transfer by carotenoid–carotenoid interaction

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ABSTRACT The apparent quantum yield of singlet–singlet spirilloxanthin-to-bacteriochlorophyll *a* energy transfer increases linearly with the residual spirilloxanthin content in *Rhodospirillum rubrum* membrane vesicles from which this carotenoid has been partially removed. Since it has been previously shown that carotenoid–carotenoid interaction is a linear function of the residual spirilloxanthin level in the major pigment-protein complex of those vesicles (Zurdo, J., R. M. Lozano, C. Fernandez-Cabrera, and J. M. Ramirez. 1991. *Biochem. J.* 274:881-884), it appears that such degenerate interaction enhances singlet energy transfer. Part of the enhancement may be explained if the energy donor is the spirilloxanthin $1B_u \rightarrow 1A_g$ ($S_2 \rightarrow S_0$) transition, because exciton coupling probably brings its energy closer to that of the $Q_x$ ($S_2 \leftarrow S_0$) transition of bacteriochlorophyll. In contrast, it seems that the possible stabilization of the spirilloxanthin $2A_g$ ($S_1$) state would hardly improve energy transfer, because this hidden state probably lies below the $S_1$ bacteriochlorophyll state. In any case, the stabilizing effects of carotenoid–carotenoid interactions seem insufficient to explain the enhancement of energy transfer. Direct or indirect effects of carotenoid dimerization on the three-dimensional structure of the pigment cluster appear to be required to account for such enhancement.

## INTRODUCTION

The colored carotenoids of photosynthetic membranes are constituents of the intrinsic pigmented proteins or holochromes that carry out the primary photophysical and photochemical processes (Cogdell and Frank, 1987; Siefermann-Harms, 1987). Basically, these photosynthetic pigments are hydrocarbons of forty carbon atoms, mostly arranged in a linear chain that includes a conjugated system of seven or more double bonds (Liaaen-Jensen, 1978). Although the carotenoids do not participate in the main pathway of photosynthetic electron transfer, they are involved in at least two physiological processes, light harvesting and protection against photooxidative damage. While as light-harvesting pigments they are donors of singlet excitation energy to ground-state chlorophylls, in their protective role they appear to act as quenchers of the triplet state of chlorophylls (Cogdell and Frank, 1987; Koyama, 1991; Siefermann-Harms, 1987; Truscott, 1990).

The noncovalent interactions between polypeptides and cofactors in the photosynthetic holochromes determine not only the arrangement (distance and relative orientation) of the cofactors (Zuber, 1986); they may also stabilize particular conformational or configurational forms of the non-protein constituents and/or favor degenerate or nondegenerate interactions among them (Noguchi et al., 1990; Ramirez, 1992). Each of these effects influences to some extent the processes of electron and/or excitation energy transfer which underlie the physiological functions of the holochromes. In the core or light-harvesting complex I (LHI) of purple phototrophic bacteria, the specific molecular interactions among the constituents of the holochrome induce optical activity in the visible absorption band of the all-*trans* carotenoid. This suggests, either or both, an asymmetric environment or/and weak to moderate exciton coupling (Bolt et al., 1981; Kramer et al., 1984; Lozano et al., 1990). A recent analysis of the fine structure and the optical activity of the visible carotenoid transition in LHI preparations with a decreased carotenoid content showed that the pigment was randomly dimerized (Zurdo et al., 1991). The availability of such LHI preparations, in which the extent of carotenoid–carotenoid interaction is a linear function of the carotenoid content, has allowed to investigate here whether such a kind of interaction has any influence on the apparent quantum yield of carotenoid-to-bacteriochlorophyll singlet energy transfer. Using *Rhodospirillum rubrum* preparations, in which the energy of the $2A_g$ ($S_1$) state of the carotenoid (spirilloxanthin) seems to lie below the $S_1$ state of bacteriochlorophyll *a*, we have found that carotenoid aggregation causes an almost threefold enhancement of the efficiency of transfer. The contribution of the energy effects of carotenoid–carotenoid interaction to the enhancement seems to be small, and very unlikely if the singlet energy donor is the spirilloxanthin $2A_g \rightarrow 1A_g$ ($S_1 \rightarrow S_0$) transition. Additional effects of carotenoid interactions on the structure

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of the pigment cluster are required to account for the stimulation of energy transfer.

## MATERIALS AND METHODS

Three different strains of *R. rubrum* were used in this work: S1, wild-type; G9, a carotenoidless mutant (Sistrom, 1978); and another mutant that lacks the reaction center, T102 (Gomez et al., 1982). The phototrophic strains, S1 and G9, were grown in the light and in the absence of air, and the reaction-centerless mutant, T102, in the dark under limited oxygenation (Gimenez-Gallego et al., 1978). Growth was continued to the stationary phase to favor spirilloxanthin accumulation in the holochromes of the carotenoid-containing strains, S1 and T102 (Lozano et al., 1989).

Since *R. rubrum* contains only one type (LHI) of light-harvesting holochrome and the reaction center of phototrophic strains is significantly less abundant than LHI, the main features of the absorption and fluorescence spectra of cells and isolated membranes are due solely to LHI. Thus, isolated membrane vesicles were used throughout this work as preparations of the LHI holochrome. Membrane vesicles were prepared by differential centrifugation from cell-free extracts that had been obtained with a French press, and the carotenoid content of freeze-dried membrane vesicles was reduced to a variable extent by extraction with light petroleum under carefully controlled conditions. The preparative procedures have been described before (Zurdo et al., 1991). A minor modification was that residual light petroleum was removed from the extracted vesicle pellet by evaporation under vacuum at room temperature.

Absorption spectra were scanned with a Shimadzu spectrophotometer (UV-3000; Columbia, MD) interfaced to Hewlett Packard microcomputer (HP9816; Palo Alto, CA). The standard S20 photomultiplier was replaced by an S1 detector (Hamamatsu R473; Middlesex, NJ) to improve the signal to noise ratio in the near infrared spectral range. The relative carotenoid content of preparations partly depleted of spirilloxanthin was estimated from the mean of the absorbances at 512 nm (peak) and at 535 nm (trough; see Fig. 2 below), in order to compensate for changes in the fine structure of the band (Zurdo et al., 1991). The absorption spectrum of membrane vesicles isolated from the carotenoidless mutant, strain G9, was used as the baseline. It appears that the fraction of unbound spirilloxanthin in the native photosynthetic membrane of *R. rubrum* is not significant (Lozano et al., 1989). This seems true also for preparations partly depleted of carotenoids, because it will be shown here (Figs. 3 and 4) that the bacteriochlorophyll spectral changes that are elicited by carotenoid extraction exhibit a simple linear dependence on the residual carotenoid level. Thus, the probability of finding a carotenoid molecule at a given binding site is equal to the relative carotenoid content ($c$). In addition, it has been shown that interacting pairs of carotenoid are randomly distributed (Zurdo et al., 1991). Thus, the level of dimers is $c^2$, which is the probability of finding two carotenoids at two neighboring binding sites, and the fraction of carotenoids that appear as dimers is $c^2/c = c$, the residual carotenoid content of the preparation.

Fluorescence measurements were done in a home-made filter fluorimeter equipped with an extended-S20 end-window photomultiplier (EMI 9659B). A solution of Rhodamine B in ethylene glycol (3 mg/cm$^3$) was used as a photon counter to correct differences in the excitation intensity. The samples were suspensions of membrane vesicles of absorbance lower than 0.1 cm$^{-1}$ at 590 nm, so that bacteriochlorophyll fluorescence emission was a linear function of concentration. Strain G9 membranes were used to estimate non-carotenoid absorption and fluorescence excitation at 500 nm as required for the determination of the apparent quantum yield of singlet energy transfer from spirilloxanthin to bacteriochlorophyll (see more details under Results). Visible circular dichroism spectra were obtained with a Jobin Yvon dicrograph III (Paris, France), using cells of 1-cm optical path.

## RESULTS

The antenna carotenoids of purple bacteria transfer singlet excitation energy to bacteriochlorophyll with reported quantum yields of 0.3 to 0.9 (Goedheer, 1959; Scolnik et al., 1980; Kramer et al., 1984), the exact value depending on the carotenoid species and on several geometric and structural factors that are mainly controlled by the apoprotein (Zuber, 1986; Noguchi et al., 1990; Ramirez, 1992). Consequently, such factors change with the particular bacterial strain and with the holochrome type. For the spirilloxanthin-containing LHI holochrome of *R. rubrum,* quantum yields of energy transfer close to 0.3 have been reported (Goedheer, 1959).

The efficiency of singlet energy transfer may be appropriately evaluated from the ability of the carotenoid to sensitize the fluorescence of holochrome-bound bacteriochlorophyll. Thus, the apparent quantum yield of transfer is equal to the ratio between the amplitudes of the carotenoid bands in the excitation spectrum of bacteriochlorophyll fluorescence and in the fractional absorption spectrum, after normalizing both spectra at a bacteriochlorophyll band. In a previous study (Zurdo et al., 1991), it was concluded that the residual carotenoid of partially depleted LHI preparations was randomly dimerized. This means that the fraction of residual carotenoid that is in dimeric form is equal to the fraction of total carotenoid that remains in the preparation (see Materials and Methods). Then, when plotted versus the residual carotenoid level, the apparent quantum yield of energy transfer is expected to change linearly from the value of the dimer (in the unextracted preparations) to that of the monomer (at zero carotenoid level), and a horizontal line would indicate that carotenoid aggregation has no influence on the energy transfer process. As shown in Fig. 1, this type of plot suggests that the spirilloxanthin dimers of the *R. rubrum* LHI complex exhibit an apparent yield of singlet energy transfer to bacteriochlorophyll almost three times higher than that of the monomers. Since the other (minor) holochrome present in the preparations, the photochemical reaction center, is a quencher of LHI bacteriochlorophyll fluorescence, and the extent of quenching is different for open and closed centers (Godik and Borisov, 1977), the determinations of the efficiency of energy transfer were also done in LHI preparations obtained from a reaction-centerless strain. The experimental values obtained using the mutant

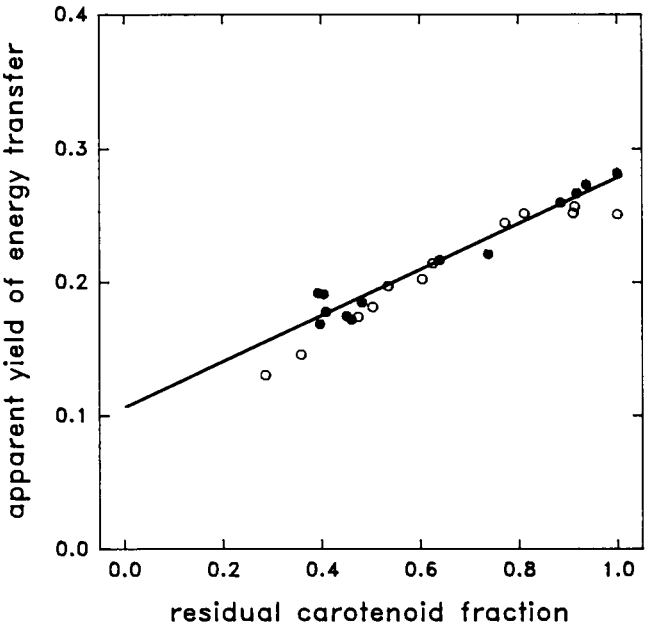


FIGURE 1 Dependence of the apparent quantum yield of singlet–singlet spirilloxanthin-to-bacteriochlorophyll energy transfer on the residual carotenoid fraction. Isolated membrane preparations, obtained from wild-type strain S1 (●) were partially depleted of spirilloxanthin by mild extraction with light petroleum and used to estimate the apparent quantum yield of transfer from the relative efficiencies of spirilloxanthin and bacteriochlorophyll to sensitize fluorescence emission from the latter. Interference filters of ~13 nm half-bandwidth, centered at 500 and 590 nm, were used in combination with an infrared absorbing glass to select spirilloxanthin and bacteriochlorophyll excitation, respectively. The detector was shielded from excitation light by both a cut-off (RG715) and a narrow-band interference filter centered at 862 nm. The yield of the emission at this wavelength, when excited at 590 nm, did not change significantly with carotenoid extraction. The regression line has been extended to the origin of the $x$-axis to facilitate an estimation of the hypothetical energy transfer yield for spirilloxanthin monomers (see text for more details). The square root of the coefficient of determination ($R$) was 0.974. Experimental values corresponding to preparations of strain T102, a reaction-centerless mutant, are also shown (○).

preparations appear to follow a pattern similar to that of the wild-type preparations (Fig. 1).

Before considering the implications of these results, it seems convenient to discuss whether the actual quantum yield of singlet–singlet carotenoid-to-bacteriochlorophyll energy transfer may be reliably estimated from our data, because although the ratio between the intensities of the carotenoid bands in the fluorescence and absorption spectra is usually taken as an accurate estimation of such yield, some sources of error may become important, particularly in preparations with reduced carotenoid levels such as those used in this present work. Thus, the contribution of non-carotenoids to light absorption (and fluorescence excitation) in the blue spectral range becomes significant at low carotenoid content, as illustrated by the comparison of the spectra of carotenoid-containing and carotenoidless preparations (Fig. 2). To compensate for this effect, we used the preparation obtained from carotenoidless strain *R. rubrum* G9 (Fig. 2) to correct both absorption and fluorescence data before plotting Fig. 1.

In the absorption spectra of the *R. rubrum* preparations, the visible bands of spirilloxanthin and bacteriochlorophyll overlap to some extent (Fig. 2). A significant contribution of spirilloxanthin to absorption at 590 nm, which is the wavelength taken as a reference for bacteriochlorophyll excitation, would lead to an overestimation of the yield of energy transfer that would be higher in the samples of higher carotenoid content and, consequently, would increase the slope of the correlation line in Fig. 1. But even assuming that spirilloxanthin were responsible for 20% of the absorption of 590-nm light in the spectrum of *R. rubrum*, obviously a generous estimate, the corrected slope would still be ~86% of that shown in Fig. 1. Thus, the enhancement of the yield of singlet energy transfer at high carotenoid levels would also be obvious after such correction.

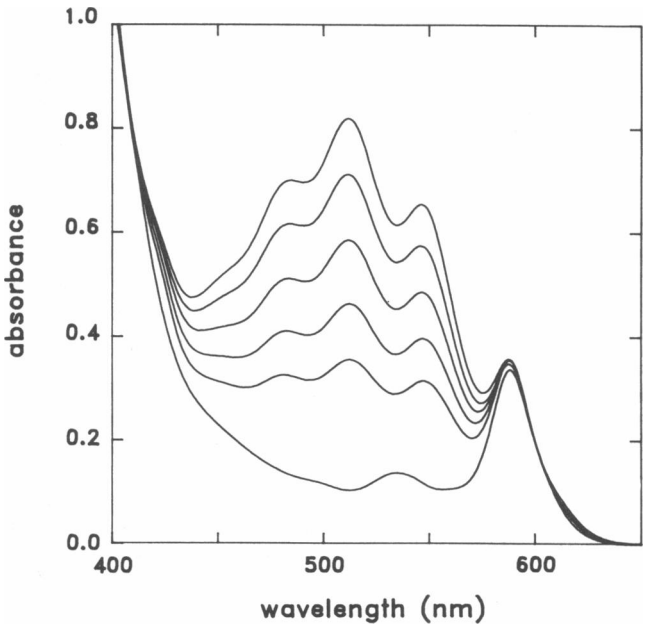


FIGURE 2 Visible absorption spectra of preparations of *R. rubrum* membranes with diverse spirilloxanthin content. The samples showing the carotenoid three-peaked absorption band in the 450–550 nm range were obtained from wild-type strain, S1, and the one lacking that band from carotenoidless mutant strain G9. The spectra were normalized to the same absorbance at 600 nm, in the long wavelength side of the bacteriochlorophyll $Q_x$ band, after adjusting absorbance at 650 nm to zero in order to minimize the effect of differences in light scattering among the samples.

Errors may also be caused by the presence of free bacteriochlorophyll and/or carotenoids in the samples treated with the organic solvent. While the unbound porphyrin absorbs also the light (590 nm) used as a reference for fluorescence excitation, its emission is blue-shifted by more than 100 nm with respect to that of the bound pigment and cannot be sensitized by the carotenoid. However, free bacteriochlorophyll was easily discarded by the absence of the characteristic absorption band near 770 nm in the spectra of the extracted LHI preparations. A more difficult error source to detect is the possible presence of dissociated carotenoid. Since only holochrome-bound carotenoids can transfer energy to bacteriochlorophyll, the free pigment would contribute to light absorption but not to bacteriochlorophyll fluorescence, and would cause a decrease of the apparent yield of energy transfer. In many solvents, the carotenoid absorption bands are located at higher energies than in the photosynthetic holochromes (Britton, 1985). Therefore, the observation that the residual carotenoid band is not blue-shifted (Fig. 2; see also Zurdo et al., 1991) suggests that the levels of free carotenoid in the LHI preparations are not significant. However, large red shifts have been reported upon carotenoid incorporation to liposomes or upon unspecific binding of the pigment to serum albumin (Davidson and Cogdell, 1981). Then, it seems that more stringent tests should be used to check whether most of the residual carotenoid remained bound at its specific holochrome site in the partially depleted preparations. A practical way of performing such tests is by measuring any property of the holochrome (or of any of its constituents) that may be reliably and quantitatively related to the level of bound carotenoid.

Early observations on the effect of carotenoid removal by mutation showed that carotenoids induce a red shift of several nanometers in the $Q_y$ ($S_1 \leftarrow S_0$) transition of LHI bacteriochlorophyll (Aagaard and Sistrom, 1972; Scolnik et al., 1980) and although, to our knowledge, the type of dependence of the bandshift on the carotenoid level has not been studied, the demonstration of any unequivocal quantitative relationship between both parameters in our preparations would provide strong evidence for the bound state of the unextracted carotenoid. As shown in Fig. 3, such a relationship was observed for *R. rubrum* LHI, suggesting that the levels of free spirilloxanthin were not significant. Another effect of the *R. rubrum* carotenoid is the induction of optical activity in the $Q_x$ ($S_2 \leftarrow S_0$) transition of LHI bacteriochlorophyll (Lozano et al., 1990). Fig. 4 shows that the visible circular dichroism of bacteriochlorophyll in the LHI preparations also exhibited a linear dependence on the spirilloxanthin level. Again, the observation that the visible bacteriochlorophyll dichroism correlates with the carotenoid content of the LHI preparations supports the conclusion that most of the residual carotenoid remained bound at its specific holochrome site.

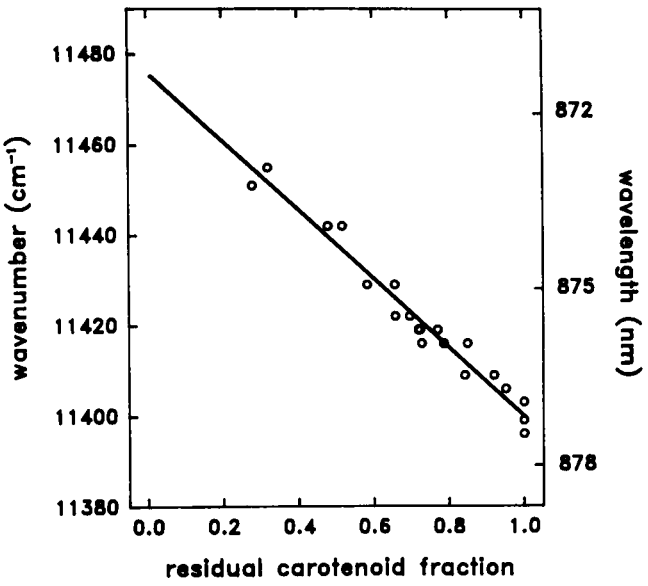


FIGURE 3 Dependence of the energy of the bacteriochlorophyll $Q_y$ transition on the spirilloxanthin content of the *R. rubrum* LHI holochrome. The wavelength reproducibility of the instrument was ±0.2 nm, and its accuracy ±0.4 nm. Three sets of preparations obtained from wild-type strain S1 were used for the measurements ($R = 0.981$).

## DISCUSSION

The preceding results suggest that carotenoid–carotenoid interaction has a marked stimulatory effect on the transfer of singlet excitation energy from spirilloxanthin to bacteriochlorophyll in the LHI complex of *R. rubrum*. Before discussing how such kind of interaction may influence some of the factors that govern singlet energy transfer, it should be mentioned here that there is also evidence for carotenoid–carotenoid interactions in other photosynthetic proteins. Thus, the first-derivative shape of the carotenoid band in the circular dichroism spectrum of the other bacterial light-harvesting complex, LHII (Scolnik et al., 1980), suggests excitonic splitting. A more recent report also claims the detection of β-carotene excitons in solubilized preparations of Photosystem II reaction centers (Newell et al., 1991). To our knowledge, the relations of the degenerate interactions of carotenoids with the processes involved in their physiological functions have not been investigated.

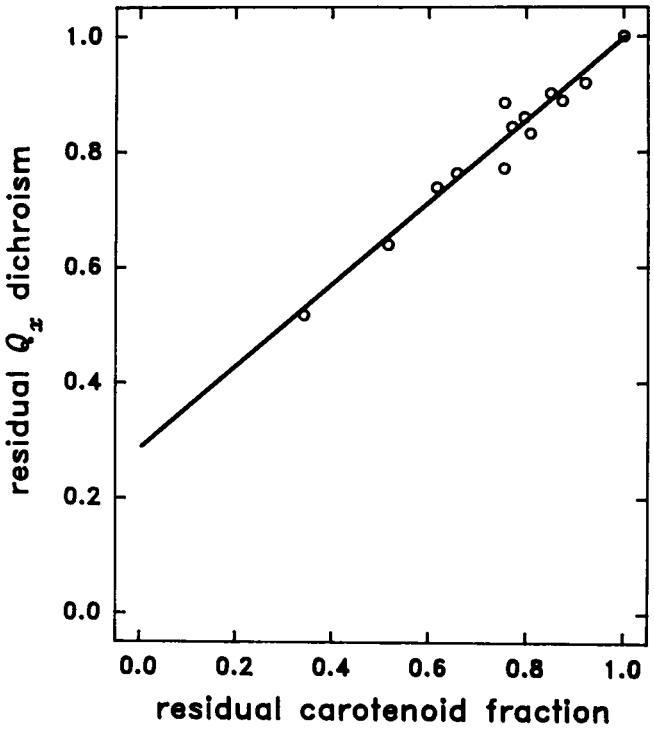


FIGURE 4 Dependence of the optical activity of the bacteriochlorophyll $Q_x$ transition on the spirilloxanthin content of the *R. rubrum* LHI holochrome. Two sets of preparations obtained from wild-type strain S1 were used ($R = 0.98$).

Due to the peculiarities of the excited singlet states of long polyenes, in general and of carotenoids in particular, it is not clear at this time whether the donor of singlet excitation energy to chlorophyll is $S_1$, $S_2$, or both. Therefore, a brief description of the electronic transitions which may be involved in the process will be included. The longest wavelength absorption band of carotenoids in the visible spectral range has been assigned to the $1B_u \leftarrow 1A_g$ ($S_2 \leftarrow S_0$) transition, because the less energetic $2A_g \leftarrow 1A_g$ ($S_1 \leftarrow S_0$) transition is symmetry forbidden. Relaxation of $1B_u$ to the ground state appears to proceed predominantly through the low-lying $2A_g$ state by a nonradiative pathway. As expected, the $2A_g$ state is considerably longer lived than $1B_u$ (Wasielewski and Kispert, 1986; Bondarev et al., 1989), and it has been proposed as the singlet energy donor to chlorophyll (Thrash et al., 1979). The low oscillator strength of the $2A_g \rightarrow 1A_g$ transition suggests for the transfer process an electron exchange mechanism of the type described by Dexter, that does not require Coulombic interaction between the transition dipoles of the donor and the acceptor (Razi Naqvi, 1980). However, recent data indicate that singlet energy transfer from the $1B_u$ carotenoid state by a dipolar mechanism is also possible (Trautman et al., 1990; Shreve et al., 1991).

Among the expected effects of carotenoid–carotenoid interaction is the stabilization of the interacting electronic states. In the case of the $1B_u$ state, such stabilization results from the splitting of the allowed $1B_u \leftarrow 1A_g$ transition, that is excitonically coupled in the LHI holochrome (Zurdo et al., 1991). In contrast, stabilization resulting from exciton coupling is not possible for the $2A_g$ state, due to the null oscillator strength of the $2A_g \leftarrow 1A_g$ transition. However, the state might be stabilized by interactions between two carotenoid molecules if $1A_g/2A_g$ excimers were formed after light absorption and $1B_u \rightarrow 2A_g$ decay. The stabilization of either excited state ($1B_u$ or $2A_g$) would favor energy transfer to bacteriochlorophyll if it caused a reduction of the energy gap between the downward donor transition (carotenoid) and the upward acceptor transition (bacteriochlorophyll) because, in such a case, the resonance condition of the energy transfer process would be better fulfilled.

The energy levels of the electronic transitions that may participate in carotenoid-to-bacteriochlorophyll singlet energy transfer are shown in Fig. 5. The transitions are depicted as horizontal bars of lengths proportional to their half bandwidths in energy units. In the scheme, optimal energy matching between a donor and an acceptor transition would be indicated by the two bars being centered at the same wavenumber. While the locations and widths of the upward transitions of bacte-

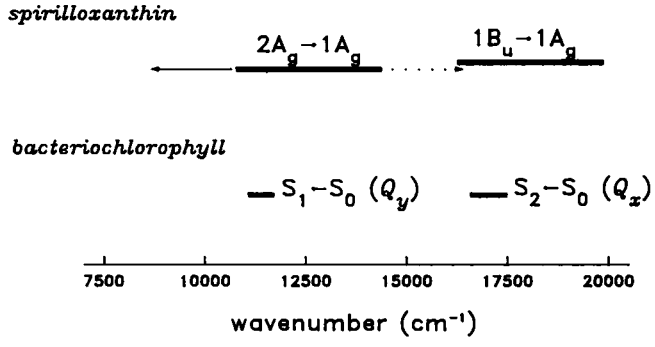


FIGURE 5 Energies and half bandwidths of the transitions possibly involved in the transfer of singlet excitation energy from spirilloxanthin to bacteriochlorophyll *a*. The data of the absorptive bacteriochlorophyll transitions have been taken from the spectrum of the *R. rubrum* LHI holochrome. The bands assigned to the downward spirilloxanthin transitions are based on the same absorption spectrum and on data from polyenes and related carotenoids (see text for details).

riochlorophyll ($Q_y$ and $Q_x$) can be directly obtained from the absorption spectrum of the LHI holochrome, those of the downward spirilloxanthin transitions are estimations based on data reported for other compounds. Thus, the $1B_u \rightarrow 1A_g$ spirilloxanthin transition has been assumed to be of the same bandwidth as the inverse transition in the absorption spectrum of the LHI holochrome, and 1,500 $cm^{-1}$ less energetic. Both assumptions are based on the relationship observed between the absorption and the emission of related carotenoids in $CS_2$ solution (Gillbro and Cogdell, 1989). Fig. 5 shows that a moderate lowering of the energy level of the transition would improve energy transfer to the $Q_x$ bacteriochlorophyll transition. From the splitting suggested by the circular dichroism spectrum of the LHI holochrome ($\leq 640$ $cm^{-1}$; Lozano et al., 1990) and the data assumed to draw Fig. 5, it can be estimated that the integral overlap of the carotenoid and the bacteriochlorophyll transitions would increase by 10–15% upon carotenoid dimerization. Even within the limitations imposed by the lack of more direct experimental data, it seems clear that this value does not explain the marked enhancement of the efficiency of energy transfer that is elicited by carotenoid–carotenoid interaction (Fig. 1).

The width and energy assigned in Fig. 5 to the $2A_g \rightarrow 1A_g$ spirilloxanthin transition are still more uncertain. Since in polyenes with eight or less conjugated double bonds, which exhibit fluorescence from the $2A_g$ state, the width of the emission band is about the same as that of the absorption band (Cosgrove et al., 1990), a similar situation has been assumed here for LHI spirilloxanthin. The difference between the energy levels of the $1B_u$ and the $2A_g$ states of β-carotene was estimated to be $\sim 3{,}500$ $cm^{-1}$ (Thrash et al., 1977), and lower values have been deduced from transient absorption changes (Bondarev et al., 1989). However, the interpretation of those results has been seriously questioned (Watanabe et al., 1987; Cosgrove et al., 1990). For this reason the spirilloxanthin transition has been drawn in Fig. 5 between two arrows that extend from a value based on the interstate gap as estimated by Thrash et al. (1977), on the high energy side, to another value based on the trend exhibited by the energies of the $2A_g \rightarrow 1A_g$ emissions, observed for polyenes with less than 9 conjugated double bonds (Cosgrove et al., 1990). Although a system of 13 double bonds is too far to make reliable the extension of those $2A_g$-fluorescence data, it still seems likely that the spirilloxanthin $2A_g$ state lies in the low energy side of the range covered by the arrows in Fig. 5. If such is the case, further lowering of its level by carotenoid–carotenoid interaction may even increase the energy gap between the $2A_g \rightarrow 1A_g$ and the corresponding ($Q_y$) bacteriochlorophyll transition, thus reducing the efficiency of energy transfer from the $2A_g$ state.

From the preceding considerations, it seems that the stabilizing effects of carotenoid–carotenoid interaction might only contribute to the enhancement of the efficiency of energy transfer if the donor were $1B_u$ spirilloxanthin. But even in that case they would account only for a small part of the enhancement. However, the existence of additional $A_g$ states with energy levels lower than $1B_u$ cannot be discarded. In fact, some experimental observations could be better understood if such states existed (Cosgrove et al., 1990). That would provide new possibilities for energy transfer and would leave open the question of whether the stabilizing effects of degenerate carotenoid interactions could account for the enhancement reported here. Besides, it also should be noted that the effects of carotenoid–carotenoid interaction are not restricted to lowering the energies of the electronic transitions. Thus, the magnitudes and orientations of the split transition moments in the dimer are different from those of the monomers (Kasha, 1963; Tinoco, 1963), and both magnitude and orientation are relevant to energy transfer. Since in the dipolar mechanism (that would presumably operate during transfer from the $1B_u$ state) the orientation factor may take values from 0 to 4, that cannot be averaged for the photosynthetic complexes because their pigment clusters have well defined three-dimensional structures (Borisov, 1989), a change of orientation of the donor transition moment, caused by disruption of dimeric interaction, could have a marked influence on the efficiency of energy transfer. In transfer taking place from the $2A_g$ state by an electron exchange mechanism, delocalization of excitation energy over two molecules in an excimer might also have a significant influence on the extent of electronic overlap required for the process.

In addition to the direct effects of carotenoid–carotenoid interaction, such as those that have been mentioned here, indirect effects should also be considered. In the photosynthetic holochromes the apoprotein provides, through specific noncovalent bonding, the three-dimensional structure and the local environment of the pigment cluster (Zuber, 1986). The removal of the carotenoid from a given binding site in the LHI complex elicits alterations of the associated bacteriochlorophyll (Figs. 3 and 4) and protein (Brunisholz et al., 1986). Since there exist interactions of diverse type among the porphyrins and among the polypeptides of different binding sites (Zuber, 1986), secondary effects of carotenoid extraction on neighboring pigment clusters should not be surprising. With complications such as these and those intrinsic to the carotenoids, a definitive

interpretation of the effect of spirilloxanthin dimerization on the efficiency of singlet energy transfer to bacteriochlorophyll should not be expected in the near future.

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