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Carotenoid Photoprotection in Artificial Photosynthetic Antennas

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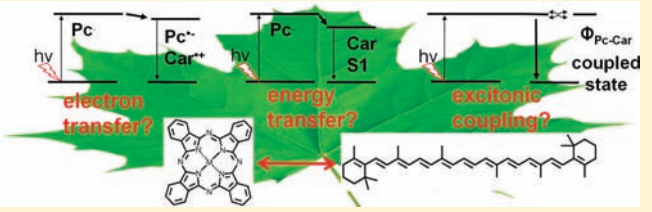
Supporting Information

ABSTRACT: A series of phthalocyanine−carotenoid dyads in which a phenylamino group links a phthalocyanine to carotenoids having 8−11 backbone double bonds were examined by visible and near-infrared femtosecond pump−probe spectroscopy combined with global fitting analysis. The series of molecules has permitted investigation of the role of carotenoids in the quenching of excited states of cyclic tetrapyrroles. The transient behavior varied dramatically with the length of the carotenoid and the solvent environment. Clear spectroscopic signatures of radical species revealed photoinduced electron transfer as the main quenching mechanism for all dyads dissolved in a polar solvent (THF), and the quenching rate was almost independent of carotenoid length. However, in a nonpolar solvent (toluene), quenching rates displayed a strong dependence on the conjugation length of the carotenoid and the mechanism did not include charge separation. The lack of any rise time components of a carotenoid S_1 signature in all experiments in toluene suggests that an excitonic coupling between the carotenoid S_1 state and phthalocyanine Q state, rather than a conventional energy transfer process, is the major mechanism of quenching. A pronounced inhomogeneity of the system was observed and attributed to the presence of a phenyl-amino linker between phthalocyanine and carotenoids. On the basis of accumulated work on various caroteno−phthalocyanine dyads and triads, we have now identified three mechanisms of tetrapyrrole singlet excited state quenching by carotenoids in artificial systems: (i) Car−Pc electron transfer and recombination; (ii)[1] Pc to Car S_1 energy transfer and fast internal conversion to the Car ground state; (iii) excitonic coupling between ^{1}Pc and Car S_1 and ensuing internal conversion to the ground state of the carotenoid. The dominant mechanism depends upon the exact molecular architecture and solvent environment. These synthetic systems are providing a deeper understanding of structural and environmental effects on the interactions between carotenoids and tetrapyrroles and thereby better defining their role in controlling natural photosynthetic systems.



INTRODUCTION

Nonphotochemical quenching (NPQ)[1] is a biological control system that allows plants and some other photosynthetic organisms to adapt to changing light levels.[2,3] It prevents photodamage by dissipating excess energy under conditions of moderate to high light levels where the nonphotochemical, downstream biochemical processes cannot keep pace with the rate of photogenerated chemical potential. In doing so, it necessarily limits photosynthetic efficiency, that is, biomass production. Anticipating that research aimed at addressing the rate-limiting downstream processes will be successful, it is essential to understand the control system in depth so that it can be "reset" to allow higher fluxes of photogenerated redox potential.

Carotenoid chromophores play a crucial role in the energy dissipation process by quenching chlorophyll singlet excited states in photosynthetic antennas. They have an additional crucial function by quenching Chl triplet excited states and singlet oxygen.[4,5] Generally, there is no single mechanism of NPQ for natural photosynthetic systems, as different classes of organisms have their own particular ways of carrying out photoprotection. Controversy persists regarding the precise molecular mechanism of the 'classical' NPQ mechanism in plant PSII. There is evidence for both energy and electron transfer quenching in NPQ of higher plants; the mechanism could depend on the type of pigment−protein complex and its position in the antenna system.[6−15] Diatoms are an important class of marine algae that show a very strong NPQ response.[16,17] Their light-harvesting complexes (LHCs) differ from those of plants and bind Chl-a and -c, fucoxanthin and diadinoxanthin. Diatoms have a diadinoxanthin cycle alongside a xanthophyll cycle,[18] which is reminiscent of but differs in an important way from the latter. In cyanobacteria, excess energy in the PSI/PSII antenna is believed to be dissipated via the IsiA protein under certain circumstances.[19,20] The orange carotenoid protein (OCP) is believed to directly

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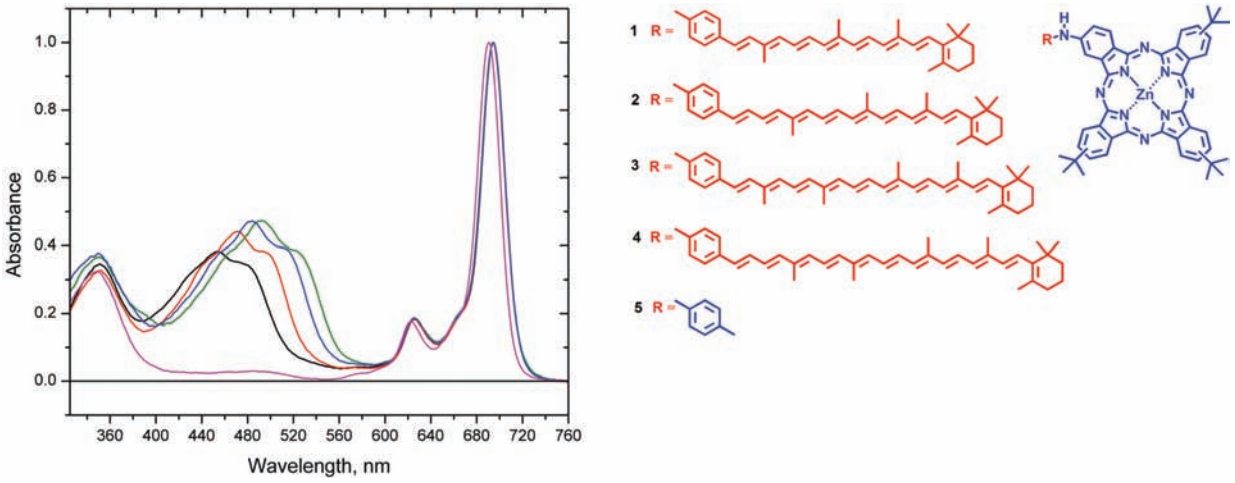

Figure 1. Chemical structures and absorption spectra of Pc−Car **1**−**4** (Dyads 8−11) and model phthalocyanine **5**. Key: black, Dyad-8; red, Dyad-9; blue, Dyad-10, green, Dyad-11; magenta, model Pc.

quench excitations in the phycobilosome antenna of cyanobacteria.[21,22]

In a synthetic model system reported earlier, it was demonstrated that the S_1 state of a cyclic tetrapyrrole (phthalocyanine, Pc) is quenched by energy transfer to the S_1 state of a carotenoid provided the π-system of the carotenoid includes at least 10 conjugated double bonds.[23] Unexpectedly for energy transfer, the quenching was much stronger in polar solvents. These results were interpreted in terms of charge-transfer states arising from carotenoid carbonyl groups strongly coupled to the carotenoid S_1 state and thereby mediating the energy transfer.[23] Indeed, the transient spectra of the S_1 state of the model carotenoids resemble those of fucoxanthin and peridinin, which also have carbonyl groups conjugated to the polyene. The S_1 states of fucoxanthin and peridinin have considerable charge-transfer character[24] that was demonstrated to be vitally important for their efficient light harvesting function.[25,26]

The present work addresses the question of whether carotenoids lacking the terminal carbonyl group, but in dyads otherwise structurally similar to the molecules studied previously, can act as quenchers of the excited states of tetrapyrroles. The study of these carotenoids is pertinent to NPQ, since the xanthophylls involved in NPQ of higher plants do not contain carbonyl groups.[27] For this purpose, a new series of dyads have been prepared which feature a secondary amine group as a linker between the carotenoid (Car) and the Pc. Dyads**1**, **2**, **3**, and **4** contain carotenoid moieties of 8, 9, 10, and 11 conjugated double bonds, respectively, in addition to a phenyl group (Figure 1). For convenience, dyads **1**, **2**, **3**, and **4** are referred to as Dyad-8, Dyad-9, Dyad-10, and Dyad-11.

MATERIALS AND METHODS

Synthesis. The synthesis of the carotenoids bearing an amino group was achieved by a Wittig reaction with the required apo-β-carotenals and [4-(*N*-acetylamino)benzyl]triphenylphosphonium bromide followed by hydrolysis of the amide as previously discussed.[28] Zinc tri*tert*-butyliodophthalocyanine was synthesized by the mixed condensation reaction of 4-iodophthalonitrile with 4-*tert*-butylphthalonitrile in a 1:3 ratio in the presence of $ZnCl_2$. From the statistical mixture of differently substituted phthalocyanines, the derivative containing one iodo group was isolated by column chromatography on silica gel in 15% yield. All tetrasubstituted phthalocyanines synthesized were obtained as mixtures of regioisomers.[29] The phthalocyanine bearing an aryl iodide was aminated with the various aminocarotenoids under conditions developed by Buchwald and co-workers.[30] Dyads were synthesized by applying these conditions to our system using a Pd-catalyst having BINAP as a ligand. Details about the synthetic procedures are described in the Supporting Information.

Spectroscopic Measurements. The spectrometer for the time-resolved experiments consisted of an integrated Ti:sapphire oscillator and regenerative amplifier laser system (Hurricane, Spectra Physics) operating at 1 kHz at a wavelength of 800 nm and producing pulses of 0.6 mJ with a duration of 96 fs.[31] A portion of the light was picked to pump a home-built noncollinear optical parametric amplifier to produce excitation pulses at 670 nm. The beam was aligned through a remotely controlled delay line to set the delay between pump and probe pulses in the region from 0 to 3 ns, and subsequently led to the sample area where it was attenuated to 80 nJ per pulse. Another portion of the light was picked and focused in a CaF_2 plate to produce a broadband continuum spanning the whole visible region. The two beams were focused by 200 and 50 mm lenses, respectively, and overlapped in the sample area with mutual polarization set to the magic angle (54.7°) to avoid photoselection effects. Changes in the absorption of the sample were recorded by focusing the probe beam into the spectrograph equipped with a 256-element diode array coupled to a phase locked chopper operating at 500 Hz in the pump beam. Samples were adjusted to an absorbance of 0.7 and placed into the focus in a 1 mm path length quartz cuvette mounted in a shaking device. The spot diameter was 200 μm.

Near-IR experiments were performed on a similar spectrometer (Mira, Rega, Coherent) operating at 40 kHz with a pulse duration of 50 fs. The principal differences in these experiments were the use of sapphire plate for generation of a broad-band continuum as a more appropriate source for near-IR experiments and a smaller pump pulse energy of 17 nJ enabled by the higher repetition rate.

Data Analysis. The data were globally analyzed with an R-based fitting package, TIMP.[32,33] The femtosecond transient absorption data were globally analyzed using a kinetic model consisting of sequentially interconverting, evolution-associated difference spectra (EADS), that is, $1 \rightarrow 2 \rightarrow 3 \rightarrow \ldots$ in which the arrows indicate successive monoexponential decays of increasing time constant. The time constant can be regarded as the lifetime of each EADS.[33] The first EADS corresponds to the time-zero difference spectrum. This procedure allows clear visualization of the evolution of the excited and intermediate states of the system. It is important to note that a sequential analysis is mathematically

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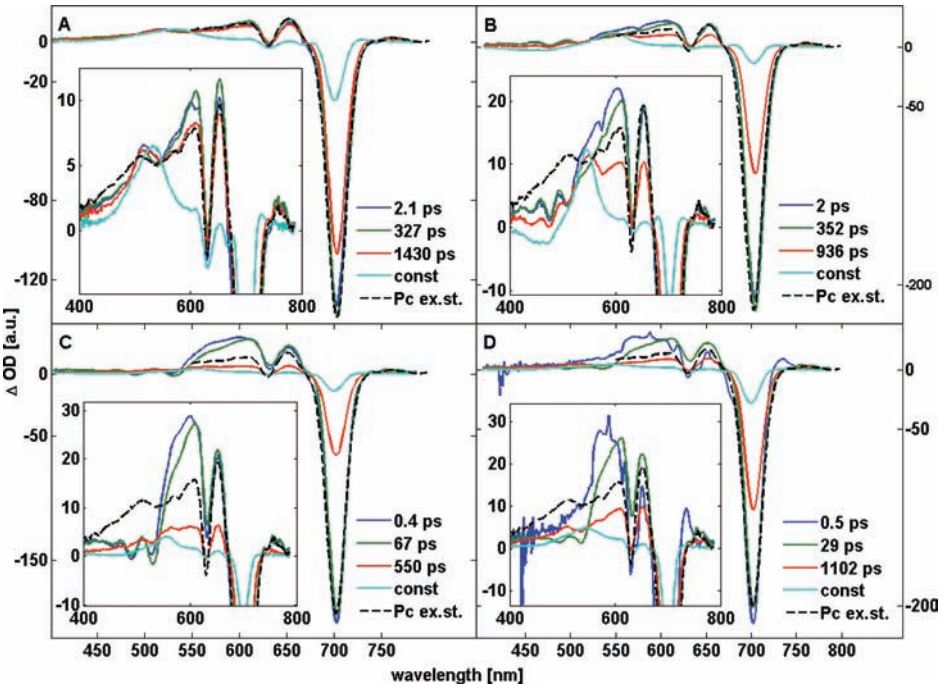

Figure 2. Evolution associated decay spectra (EADS) extracted from time-resolved spectra recorded for toluene solutions of Dyad-8 (A), Dyad-9 (B), Dyad-10 (C) and Dyad-11 (D) after 670 nm excitation. The lifetime for each EADS is indicated in the panels. The black dashed lines denote the EADS of the Pc model compound, shifted by 5 nm so as to overlap with the signal of the dyad-bound Pc moieties.

equivalent to a parallel (sum-of-exponentials) analysis. The analysis program calculates both EADS and decay-associated difference spectra (DADS) and the time constants that follow from the analysis apply to both.[33] In general, the EADS may reflect mixtures of molecular states, such as may arise, for instance, from heterogeneous ground states or branching at any point in the molecular evolution.[34−40]

RESULTS AND DISCUSSION

Dyads in Toluene. Figure 1 shows the absorption spectra of Dyads 8−11 in toluene solution. Previous studies of artificial caroteno−phthalocyanine dyads and triads have shown a strong dependence of the quenching rate of ^{1}Pc by the attached carotenoid on solvent polarity.[23,34,41] For Dyads 8−11 of the present study, differences in the steady state fluorescence yields in various solvents also suggested a significant effect of the solvent polarity on the quenching rate (results not shown). Two different solvents, tetrahydrofuran (THF) as an example of a relatively polar solvent and toluene as a low polarity solvent, were chosen to investigate this phenomenon using the time-resolved experiments.

Figure 2 shows the evolution associated decay spectra (EADS) of Dyads 8−11 dissolved in toluene with excitation at 670 nm, together with associated time constants extracted by the global fitting analysis. A time-resolved spectroscopic examination of Pc model compound **5** revealed no significant influence of the solvent polarity on the singlet excited state lifetime, which was in both cases around 4 ns. This number represents decay of ^{1}Pc by internal conversion, fluorescence, and intersystem crossing.[42] The EADS that represents the relaxed excited state of **5** is depicted in panels A−D with the dashed black line.

In toluene, quenching of the Pc excitation states in Dyads 8−11 was found to depend upon the length of the carotenoid conjugated backbone. The transient spectra required three time constants and a nondecaying component to obtain a satisfactory fit of the data to a sequential kinetic model. In Dyad-8, lifetimes of 2.1, 327, and 1430 ps were obtained (Figure 2A). The first EADS (2.1 ps, blue line) shows a negative band near 700 nm that results from Pc ground-state bleach and stimulated emission. At wavelengths shorter than 670 nm, a broad positive signal is observed that is due to excited-state absorption. The dip near 620 nm results from bleach of a vibronic band of the Pc Q transition (cf. Figure 1 for the steady-state absorption spectra).

The first EADS evolves in 2.1 ps to the second EADS, which has a lifetime of 327 ps (Figure 2A, green line). The evolution in spectral shape is very minor with a very small increase in Pc ground state bleaching at 700 nm and small rise in excited state absorption between 550 and 650 nm. The origin of this spectral evolution is difficult to assign. The first and second EADS closely resemble the excited state of the model Pc compound (Figure 2A, black dashed line), indicating that the observed excited states in Dyad-8 represent essentially singlet-excited Pc.

The second EADS evolves with a lifetime of 327 ps into the third EADS, which has a lifetime of 1430 ps (Figure 2A, red line). Their shapes are almost identical, with a 20% reduction in amplitude of the latter. The third EADS evolves in 1430 ps to the nondecaying EADS (Figure 2A, cyan line), and involves an almost 80% drop in Pc exited state transient absorption signatures. The nondecaying EADS contains a characteristic broad peak centered near 520 nm and a small amount of Pc ground state bleaching allowing an identification of this component as a mixture of Pc and carotenoid triplet states. This observation shows that the time constant for triplet−triplet energy transfer from Pc to carotenoid is smaller than (or on the order of) that for

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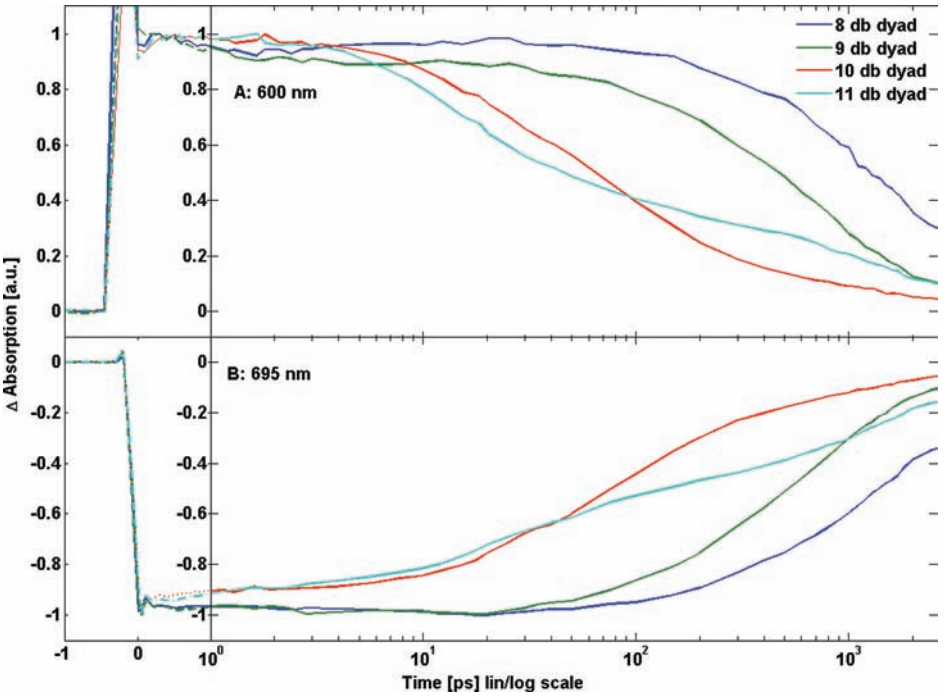

Figure 3. Normalized time traces of dyads dissolved in toluene recorded at 600 and 695 nm representing the carotenoid S_1 state absorption and Pc ground state bleach, respectively. The time axis is linear from −1 to 1 ps and logarithmic thereafter. Note that while for the first few picoseconds there are slight differences in the time evolution, at longer times the kinetics are practically identical. The kinetics in the 600 nm region are less smooth, because of relatively lower signal intensity at this wavelength. The peak around zero delay in the kinetics at 600 nm results from coherent and cross-phase modulation artifacts and are not related to the photophysics of the Dyads.

intersystem crossing in the Pc moiety. Similar phenomena have been observed in other Pc−carotenoid dyads and triads and light-harvesting antennas of oxygenic photosynthesis. In keeping with its reduced singlet excited-state lifetime as compared to Pc, the triplet quantum yield of Dyad-8 is lower (at ∼0.1−0.2 judged from its amplitude) than in Pc (∼0.6).[42,43]

These experiments show that the excited state of Dyad-8 decays multiexponentially, with a minor decay component of 327 ps and a major component of 1430 ps. We conclude that the Pc excited state is weakly to moderately quenched by the covalently attached carotenoid. In contrast to our previous work on Pc−carotenoid dyads and triads,[23,41] we do not observe any clear spectroscopic feature of a molecular state responsible for the quenching, so we cannot draw any conclusion regarding the quenching mechanism.

Dyad-9 follows an evolution similar to that of Dyad-8 with lifetimes of 2, 352, 936 ps, and a nondecaying component (Figure 2B). The 352 ps component has a larger amplitude as compared with the corresponding 327 ps component in Dyad-8. Also, the third lifetime (936 ps) is somewhat shorter than in Dyad-8. These observations imply that Dyad-9 is slightly more quenched than Dyad-8.

Figure 2C shows the results for Dyad-10. This dyad displays a clear change in its transient behavior as compared with Dyads-8 and -9. The first EADS (Figure 2C, blue line) has a lifetime of only 400 fs. The second EADS has a lifetime of 67 ps and has a shape similar to that of the 400 fs EADS. This EADS is responsible for 70% of Pc excited state decay, indicating that this component shows a 60-fold quenching of the Pc excited state (i.e., from 4 ns to 67 ps). It is followed by the third EADS with a lifetime of 550 ps. This last component is responsible for about 25% of the quenching. The nondecaying EADS again represents a mixture of Pc and carotenoid triplet states.

Dyad-10 differs significantly from Dyad-8 and the Pc model compound in the appearance of a distinct carotenoid bleaching between 400 and 500 nm and a small additional excited state absorption between 500 and 610 nm in the 400 fs and 67 ps EADS. The spectrum of the Pc model compound is flat and featureless in this spectral region (see inset of Figure 2C and Figure S1C). This observation indicates that in addition to ^{1}Pc, a carotenoid excited state is populated to some extent in these EADS. In Figures S1 and S2, we explicitly compare the signals additional to that of ^{1}Pc in Dyad-10 with the S_1 spectrum of the carotenoid as follows from direct carotenoid excitation. We conclude that the spectral shape and position of the additional bleach and induced absorption of the 67 ps component is consistent with the presence of the carotenoid S_1 state. This spectral feature does not correspond to $Car^{\bullet+}$−$Pc^{\bullet-}$ radical pair states, as no transient carotenoid cation band is observed in the near-IR (Figure S3). Likewise, the additional absorption feature between 550 and 610 nm cannot be due to Pc excited-state absorption, because if only Pc would be excited, such absorption would not be accompanied by carotenoid ground state bleaching. The implications of these observations are discussed below.

The spectral pattern in the 400 fs component may correspond to S_1 and S* states coexisting with ^{1}Pc. The S* state, controversially discussed in the literature,[37,39,44−47] exhibits a blue-shifted shoulder at the carotenoid S_1 state excited state absorption.[37,39,48,49] and disappears in 400 fs. The disappearance of the S* feature cannot be related to a vibrational cooling process, because that would be associated with absorption loss at the red side of the S_1 absorption.[50,51]

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Dyad-11 shows characteristics similar to those of Dyad-10, with time constants of 0.5, 29, and 1102 ps and a nondecaying component. The 0.5 and 29 ps EADS look essentially the same as the corresponding EADS (0.4 and 67 ps) in Dyad-10, with significant carotenoid S_1/S^* features mixed in the ^{1}Pc difference spectrum (Figure S1 and S2), although the 0.5 ps EADS is not very well resolved. The second EADS (29 ps) follows the trend of decreasing lifetime with increasing length of the carotenoid noted in the other dyads, suggesting a component that is strongly quenched. Surprisingly, the third EADS (1102 ps) does not follow such a trend at all: it is significantly longer than that of Dyad-10 (550 ps) and even slightly longer than that of Dyad-9 (936 ps). In addition, the amplitude of the 1102 ps EADS is significantly larger than that of the 550 ps EADS of Dyad-10, indicating that a larger fraction of Dyad-11 is only weakly to moderately quenched. Dyad-9 shows similar features to those of Dyad-10 and -11 in its 2 and 352 ps EADS (Figure 2B), but to a lesser extent that does not allow quantitative assessment of its molecular nature.

Berera et al. showed that a carotenoid can act as an acceptor of Pc excitation energy, thereby shortening the Pc singlet excited state lifetime.[10,23] The conjugation length of the carotenoid was critical to the quenching process and the addition of only one double bond, from 9 to 10, turned the carotenoid from a nonquencher into a strong quencher. It was shown that the quenching proceeds through energy transfer from the excited Pc to the optically forbidden S_1 state of the carotenoid, coupled to an intramolecular charge-transfer state. The excited-state energy was dissipated rapidly through internal conversion from the carotenoid S_1 state to the ground state on the picosecond time scale. An important feature of the quenching phenomenon was that it proceeded through an inverted kinetic scheme, that is, energy transfer from Pc to Car was relatively slow (on the 30−300 ps time scale), after which internal conversion from the Car S_1 state to the ground state occurred relatively fast, in ∼5 ps. As a result, the spectral evolution was characterized by a rise of Car S_1 spectral features on the 5 ps time scale at low transient concentrations on top of Pc excited-state features, giving rise to difference spectra that are similar to those reported for the (strongly quenched) 67 and 29 ps EADS in Figure 2C,D for Dyad-10 and Dyad-11, respectively.

A key difference between the present data and those of Berera et al.[10,23] is that here, no obvious rise component of the Car S_1 features associated with inverted kinetics was observed. Rather, the Car S_1 (and S^*) signatures were present in the transient spectra *immediately* after Pc excitation, within the time resolution of 100 fs. This can also be observed in Figure 3 , where the Pc kinetics are compared with the Car S_1 kinetics in Dyads 8−11: no rise component on a picosecond time scale can be discerned in the kinetics at 600 nm. This observation indicates that, in the present dyads, the quenching cannot be described with a simple sequential ^{1}Pc → Car S_1 energy transfer scheme. Rather, we suggest here that the quenching may be mediated through excitonic coupling between the ^{1}Pc and the optically forbidden Car S_1 (and/or S^*) state. In the excitonic case, the excited state is shared between the Pc and carotenoid immediately upon excitation. The lifetime of the collective state is then determined by the extent to which the carotenoid S_1 state is mixed with the Pc state and by the carotenoid S_1-S_0 internal conversion rate (which is in the order of picoseconds).[46] Such a mechanism differs significantly from that of Berera et al.,[23] where the excited state is localized on either of the cofactors and the Pc lifetime is determined by the rate of energy transfer from Pc to carotenoid. Despite the fact that excitonic coupling of the Car S_1 state with Pc excited states is not a generally established principle, especially because of the vanishingly low dipole strength of the former,[46,52] there are experimental data suggesting this mechanism in natural photosynthetic antennas.[11,53]

The question is how appreciable excitonic coupling may arise between Pc and the optically forbidden carotenoid S_1 state, as classical exciton theory predicts such coupling will be zero. First of all, it is important to note that excitonic interactions are governed by couplings essentially identical to those that govern incoherent energy transfer between closely spaced chromophores. According to traditional Förster theory, energy transfer rates that involve the carotenoid S_1 state should be rigorously zero because of the zero transition dipole moment of the latter.[54] Yet, it has been demonstrated experimentally many times that in natural and artificial light harvesting systems, Car S_1 to Chl (or for that matter, Pc) and Chl to Car S_1 can happen very rapidly on the picosecond and sub-picosecond range.[23,34,39,41,55−58] Fleming and co-workers showed that, when molecules are in close contact, the point-dipole approximation breaks down and rather the interaction of the transition densities of the two molecules has to be considered.[59] In the specific case of the carotenoid S_1 state, the molecular symmetries that govern photon absorption (which applies on the wavelength scale of the photon, 0.5−1 μm) are not the same as the molecular symmetries experienced by closely spaced chromophores. Thus, the electronic transition to a state that is optically forbidden in an isolated molecule may exhibit considerable Coulombic coupling to transitions in chromophores at close proximity.[60,61]

Additionally, the S_1 state may obtain dipole strength through mixing with the strongly allowed S_2 state, a mechanism that has been invoked to explain the fast (ps) energy transfer dynamics from Car S_1 states to Chl in natural LH antennas.[61,62] In the present dyads, such an effect may be augmented by the presence of the aminophenyl group in the conjugated backbone of the carotenoid, which introduces a symmetry-breaking element in the π-electron conjugation and may result in an increased dipole strength of the Car S_1 state.

We propose that in Dyads 10 and 11, the Coulombic coupling is so strong that it results in delocalization of the molecular wavefunctions. Classical exciton theory predicts spectral shifts and redistribution of oscillator strength in the linear absorption spectrum upon delocalization of wavefunctions.[54] The absorption of the Pc moiety in Dyads 8−11 is red-shifted by about 100 cm^{-1} with respect to the Pc model compound (Figure 1). This shift may have an origin different from excitonic interaction, because other Car−Pc dyads that do not show excitonic interactions exhibit a similar shift.[23] The effects of large Coulombic coupling on the linear absorption spectra of coupled chromophores involving optically forbidden states remains to be investigated theoretically: the point-dipole approximation, which we know is not applicable here, is used to predict extent and magnitude of spectral shifts and redistribution of oscillator strength in classical exciton theory. Hence, the absence of obvious shifts and changes of dipole moments in the linear absorption spectra of Dyads 10 and 11 does not directly address the issue of excitonic interactions.

We can safely exclude the possibility that the lack of rising Car S_1 features of Figures 2 and 3 relates to an unresolved very fast energy transfer within the time resolution of 100 fs. If inverted kinetics applies as in Berera et al.,[10,23] an unresolved rise

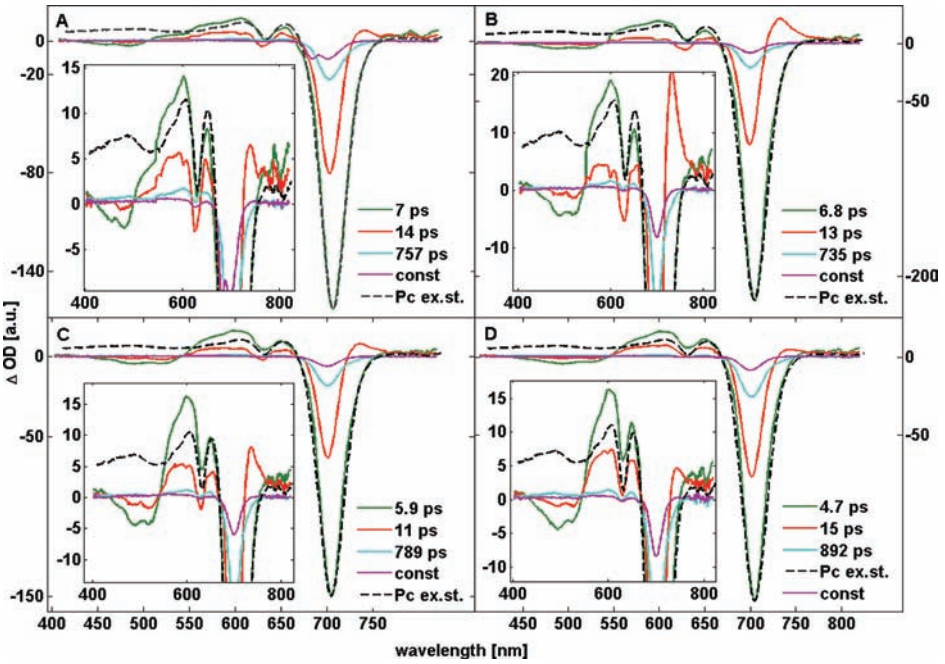

Figure 4. Evolution associated decay spectra (EADS) after 670 nm excitation extracted from time-resolved spectra recorded on Dyad-8 (A), Dyad-9 (B), Dyad-10 (C), and Dyad-11 (D) dissolved in THF. The lifetime of each EADS is indicated in the panels. The black dashed lines denote the EADS of the Pc model compound **5**, shifted by 5 nm so as to overlap with the signal of the dyad Pc moieties.

component of the Car S_1 features would actually correspond to a Car S_1 internal conversion lifetime of <100 fs, which is unrealistically short.[46] If for any reason the Car S_1 lifetime would be that short, we would not experimentally observe the carotenoid S_1 state in our spectra because with the relatively slow energy transfer from Pc (67 ps in Dyad-10, Figure 2C), its transient concentration would become undetectably low. A direct energy transfer process from Pc to the carotenoid S_1 state within 100 fs would result in a <100 fs decay of the Pc excited state, which is not observed experimentally.

The observation of multiphasic Pc lifetimes in the dyads leads to the conclusion that the dyads may adopt multiple structural conformations leading to variable Pc−Car electronic coupling. The fitted sum of exponentials may be merely an approximation of the average dynamics observed spectroscopically despite the fact that it fully fits the data within the available signal quality. Note that in other Pc−carotenoid dyads and triads, multiphasic decay of the Pc excited state was observed as well,[23,34,41,63] although it appears more pronounced in the present study. In axially linked Si−Pc−Car triads, NMR and modeling studies confirmed that multiple conformations exist.[63] Additional conformational flexibility in the present dyads may be induced by the aminophenyl groups that link the Pc and carotenoid moieties.[64,65]

Weak to moderate quenching is observed in Dyad-8 and Dyad-9, as well as the slow decay phases in Dyad-10 and Dyad-11. Interestingly, the EADS associated with these lifetime components (Figure 2) are similar to those of the model Pc compound and show little (Dyad-9) or essentially no (Dyad-8) carotenoid excited-state features or signs of inverted kinetics. The quenching mechanisms of these dyad subpopulations therefore remain unknown. They may proceed through the energy transfer or excitonic mechanisms described above, but the extent of excitonic mixing or rise of transient carotenoid S_1 population remains so low that no mechanistic conclusions may be drawn. Likewise, quenching through electron transfer and recombination[41] cannot be excluded, although this option seems unlikely given the low solvent polarity of toluene.

Dyads in THF. The evolution associated decay spectra (EADS) after 670 nm excitation of Dyads 8−11 dissolved in THF together with associated time constants extracted by the global fitting analysis from time-resolved data are depicted in Figure 4.

All dyads dissolved in THF followed a very similar and rather complex transient evolution. Four exponentials and a nondecaying component were sufficient to fit the data. The first EADS was in the 200 fs region, was poorly resolved, and hence is not shown and not further discussed. The second component (with time constant of 7, 6.8, 5.9, and 4.7 ps for Dyads-8, 9, 10 and 11, respectively, green lines in Figure 4) shows both Car and Pc excitation signatures, which may correspond to direct sharing of the excitation between Pc and Car (see Figure S4), similar to the situation with Dyads-10 and -11 dissolved in toluene. It comprises bleaching of the carotenoid ground state between 400 and 500 nm, bleaching of Pc ground state absorption near 700 and 620 nm, and broad carotenoid S_1 state absorption centering around 600 nm. The 4.7−7 ps EADS represent most of the quenching of the Pc excited state as may be concluded from the large drop in Pc ground state bleaching that is observed in the following EADS, which has lifetimes of 14, 13, 11, and 15 ps (for Dyads-8, -9, -10, and -11, respectively, red lines in Figure 4). This EADS differs from the previous one by the relative decrease of carotenoid features and the appearance of a new induced absorption shoulder near 720 nm. This EADS basically concludes the transient behavior of the dyads. A small amount of remaining excitation is contained in the fourth EADS with lifetimes 757, 735, 789, and 892 ps (for Dyads-8, -9, -10, and -11, respectively, cyan lines in Figure 4) and also in the

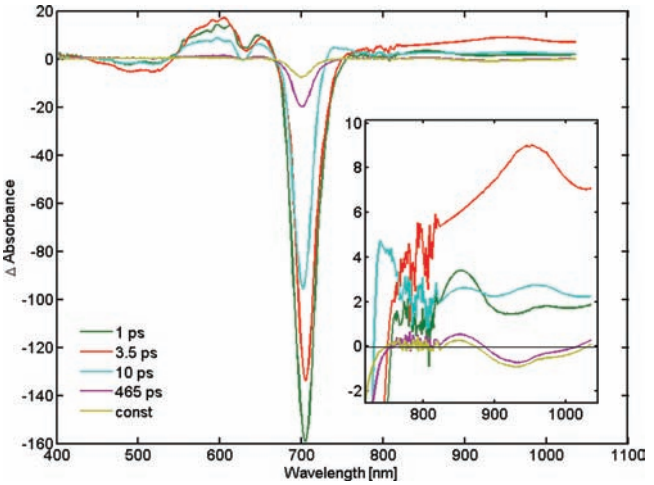

Figure 5. Evolution-associated decay spectra of Dyad-10 in the visible and near-IR regions. The excitation wavelength was 680 nm.

nondecaying component (magenta lines in Figure 4) re presenting features with lifetimes beyond the examined time window.

To investigate whether the rapid quenching proceeds through charge separation and subsequent recombination, Dyad-10 was studied in near-IR region where Pc and Car radical species absorb[66,67] (Figure 5).

Figure 5 shows the rise of a carotenoid cation radical peak around 980 nm in 1 ps, and its subsequent decay in 3.5 ps. Given the high extinction coefficient of carotenoid radical cations ($\sim 10^5$ M^{-1} cm^{-1}), the low amplitude of the 980 nm peak relative to the Pc bleach near 700 nm implies a low transient concentration. The recording of a new component (1 ps) which was not resolved in the transient absorption experiments in the visible, suggests an inverted kinetics scheme, where Pc excited state (or Pc−Car coupled excited state) is slowly (5−10 ps) depopulated by charge separation followed by rapid recombination (~1 ps), resulting in a low relative concentration of radical species during the quenching process. We conclude that all dyads dissolved in THF are strongly quenched, with the majority of the Pc excited states decaying in 5−7 ps by this process. The quenching process is not dependent on the conjugation length of the carotenoid, which is consistent with electron transfer processes observed by Kodis et al. for axially linked Pc−Car triads in polar solvent.[41] We observe a Car S_1 signature in the excited-state spectra, which suggests that excitonic mixing between Pc and the carotenoid S_1 state takes place and may contribute to quenching.

In general, the electron donating ability of the carotenoids depends on the conjugation length, with a difference in the oxidation potential of the carotenoids of ~150 mV in going from carotenoids with 8 to 11 double bonds.[68−70] The amino carotenoids of this study are better electron donors that the carotenoids of refs 68 and 70. For example, the midpoint oxidation potential of ~0.6 V versus SCE for a carboxylate terminated carotenoid of 10 double bonds is ~0.3 V versus SCE for the anilino-terminated carotenoid of the same conjugation length (not shown).[69] Thus, in every case of the systems of this study there is sufficient driving force to observe charge separation between Pc and the carotenoid, particularly in polar solvents. However, the difference in driving force between the different

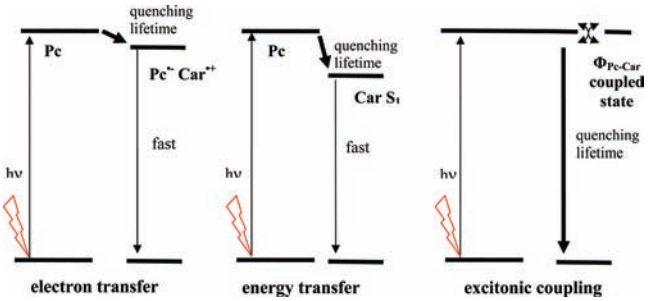

Figure 6. Quenching mechanisms described in this paper: (1) quenching via short-lived charge separated state associated with inverted kinetic scheme; (2) quenching via energy transfer to carotenoid dark S_1 state associated with inverted kinetic scheme; (3) quenching via Pc−carotenoid coupled state.

conjugation lengths of the carotenoids must not be sufficient to measure different quenching rates in the cases studied.

The 720 nm shoulder in the 11−15 ps EADS of Figure 4 and the 10 ps EADS of Figure 5 is difficult to assign. In previous work, similar signals were associated with Pc charge-transfer processes.[71,72] It may also be interpreted as a temporal broadening or a red shift of the main Q absorption peak of Pc. Such broadenings and red shifts are observed generally in steady-state absorption spectra of dyads dissolved in high polarity solvents. In the present case, transient formation of a charge-separated $Car^{\bullet+}$ $Pc^{\bullet-}$ species results in disturbing the solvent shell. Charge recombination in a few picoseconds may then result in a nonequilibrated solvent in such a way that the Pc Q-band is broadened or red-shifted showing the positive band at 720 nm, followed by solvent relaxation in 10−15 ps.

CONCLUSIONS

In both THF and toluene solvents, it is clearly demonstrated that in dyads that lack a carbonyl group in the carotenoid conjugated backbone, the Pc excited state is quenched by the attached carotenoid. The observed excited-state lifetimes are multiexponential, which may be attributed to ground-state heterogeneity, possibly associated with the existence of the molecule in a set of various conformations, which gives rise to a variety of quenching rates. Conformational heterogeneity around the phenyl−amine link where separate minima strongly influence Pc−Car coupling may be responsible for this phenomenon.

An interesting observation is that, when dissolved in THF, the quenching is, at least in part, mediated by charge separation and recombination processes, and does not show a strong dependence on the length of the carotenoid conjugated electron backbone. In toluene, on the other hand, quenching appears to be mediated by excitonic coupling between Pc and the carotenoid S_1 state and shows a clear increase in quenching rate for longer carotenoids. The transition from 9 to 10 double bond carotenoids results in an especially large increase in quenching rate, similar to observations for previously studied Pc−Car dyads.[23] In the short carotenoid dyads (Dyad-8 and Dyad-9), weak to moderate quenching by multiexponential processes is observed. This contrasts with the previously studied 9-double bond dyad where a single exponential decay identical to that of a model Pc compound was observed.[23] Thus, the present dyads do not exhibit a clear-cut 'gear-shift' mechanism, where addition of

one double bond to the carotenoid conjugated π-electron system activates the quenching.

Spectral features which could be associated with the Car S* state were observed within the first picoseconds (Figure 2C,D, evolution from blue to green EADS) but there is no direct evidence that this state plays a crucial role in the quenching process.

The cumulative work on artificial Pc−Car dyads and triads has led to the experimental observation of three distinct carotenoid-mediated tetrapyrrole singlet excited-state quenching mechanisms, as depicted in Figure 6:

(1) Electron transfer from Car to Pc followed by rapid charge recombination on the picosecond time scale (refs 41 and 63 and the dyads studied here in THF). There is evidence that similar processes between Chl and xanthophylls underlie NPQ in the plant PSII antenna.[6,8,9,12]

(2) Energy transfer from Pc to the optically forbidden S_1 state of the carotenoid.[23] Experimental evidence for such processes between Chl and carotenoids underlying NPQ in plant PSII[13] and in the cyanobacterial IsiA protein[19] has been reported.

(3) Quenching through excitonic mixing between the Pc singlet excited state and the carotenoid S_1 state. This phenomenon was directly observed here for the first time for Dyad-10 and Dyad-11 in toluene. A similar mechanism involving Chl and xanthophylls was recently proposed to account for quenching in LHCII and plant PSII.[11,53]

Thus, a considerable amount of evidence indicates that carotenoids can quench tetrapyrrole singlet excited states in a variety of ways, and relatively modest differences in the molecular architecture and environmental conditions can lead to a change in the quenching mechanism. The mechanistic flexibility exhibited by the artificial Pc−Car systems with regard to tetrapyrrole singlet excited state quenching suggests that any of the proposed mechanisms may operate in the various natural photosynthetic systems that display quenching. Further examination of such phenomena in artificial light harvesting systems will contribute to the understanding of the role of carotenoids in natural systems, where charge separation, energy transfer, excitonic coupling, and conformational changes play their roles.

■ ASSOCIATED CONTENT

Supporting Information. Synthesis of compounds; Supporting figures S1−S4. This material is available free of charge via the Internet at http://pubs.acs.org.

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(1) Abbreviations: EADS, evolution-associated difference spectrum; DADS, decayassociated difference spectrum; Pc, phthalocyanine; Car, carotenoid; NPQ, nonphotochemical quenching.

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