# Carotenoid Triplet States Associated with the Long-Wavelength-Emitting Chlorophyll Forms of Photosystem I in Isolated Thylakoid Membranes

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*Received: July 2, 2004; In Final Form: October 20, 2004*

The carotenoid triplet populations associated with the long-wavelength-emitting chlorophyll forms of photosystem I (PS I)[†] have been investigated in isolated spinach thylakoids by means of fluorescence-detected magnetic resonance in zero field. The spectra collected in the 730−800 nm emission range can be globally fitted assuming the presence of four different carotenoid triplet states coupled to long-wavelength-emitting forms of PS I, having zero-field-splitting parameters $|D| = 0.0359$ cm$^{-1}$ and $|E| = 0.00371$ cm$^{-1}$, $|D| = 0.0382$ cm$^{-1}$ and $|E| = 0.00388$ cm$^{-1}$, $|D| = 0.0395$ cm$^{-1}$ and $|E| = 0.00397$ cm$^{-1}$, and $|D| = 0.0405$ cm$^{-1}$ and $|E| = 0.00411$ cm$^{-1}$. On the basis of the triplet-associated fluorescence emission profile, it is suggested that those triplets are associated with light-harvesting complex I, the peripheral antenna complex of PS I.

## Introduction

Thylakoid membranes are the site of photosynthetic electron transport, which is mediated by two photosystems, namely, photosystem II (PS II) and photosystem I (PS I), operating in series in the photosynthetic apparatus of higher plants. The photosystems are large macromolecular complexes, composed of several protein subunits. Some can bind chromophores and are organized into a core that functions as an inner antenna in the proximity of the reaction center cofactors, where the primary photochemical reactions take place, and an outer antenna, whose polypeptides are often referred to as the light-harvesting complex (LHC). There are essentially two classes of pigments that are associated with both PS II and PS I of higher plants, namely, chlorophylls (Chl's) and carotenoids (Car's). Chl *a* is present in all Chl-binding polypeptides, while Chl *b* is found only in the outer antenna components. Similarly, two main classes of carotenoids are found: oxygenated carotenes, including lutein, violaxantin, neoxantin, and zeaxantin, also called the xanthophylls, which are found in both LHC I and LHC II outer antenna complexes, and a nonoxygenated carotene, $\beta$-carotene, which is present in the inner antenna and the reaction center binding complexes of both PS II and PS I (see refs 1 and 2 for reviews).

Carotenoids have multiple functions in photosynthetic systems. They are thought to play a significant role in the stabilization of folding of Chl binding polypeptides, at least for outer antenna components,[3,4] as evinced by the structures of LHC II[5,6] and LHC I.[7] Carotenoids are effective in collecting and transferring absorbed energy and hence contribute to increasing the optical cross-section of both the photosystems,[8−10] and play a central role in the quenching of the potentially harmful triplet excited state of chlorophyll (reviewed in ref 11). In fact, while the direct population of the carotenoid triplet state by intersystem crossing is a low-yield process, a significant carotenoid triplet population has been observed in several experimental conditions in thylakoids,[12−14] as well as in isolated photosystems[13] and in Chl binding complexes, e.g., see refs 15−19, due to triplet−triplet energy transfer from the excited state of chlorophyll.[10−19] The (unquenched) triplet yield of chlorophyll is estimated to be about 30% in organic solvent, indicating that the intersystem crossing rate is comparable with those of the other excited-state decay processes, i.e., internal conversion and fluorescence.[20,21] A comparable intersystem crossing rate has been estimated for Chl bound to photosynthetic complexes.[22,23] The efficiency of Chl triplet quenching by protein-bound carotenoids has been estimated to be between 0.8 and unity, depending on the system and on the temperature.[17,24] As a result, the triplet-excited-state population of chlorophyll in photosynthetic systems is kept at extremely low levels. Naturally occurring carotenes play a central physiological role avoiding the formation of singlet oxygen, which can be generated from the interaction of the excited state of chlorophyll and the triplet oxygen ground state (for reviews see refs 11 and 25), in this way protecting the system from oxidation. The xanthophylls are also involved in the nonphotochemical quenching of chlorophyll fluorescence. This has been proposed to be a protective strategy against photoinactivation (reviewed in ref 26) by lowering the singlet-excited-state population, although some recent studies regarding the lack of correlation between the singlet-excited-state population and photoinhibition have questioned the efficiency of nonphotochemical quenching as a photoprotective mechanism.[27−31]

The carotenoid triplet states associated with the inner, e.g., see refs 18, 19, and 32, and, especially, the main outer antenna complex of PS II, LHC II, have been investigated in detail by

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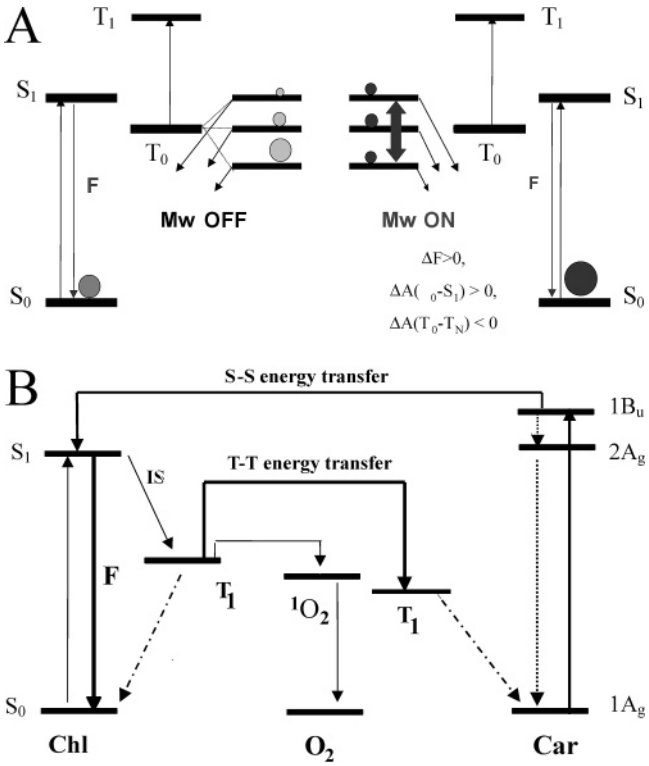
[†] Abbreviations: PS II, photosystem II; PS I, photosystem I; FDMR, fluorescence-detected magnetic resonance; ODMR, optically detected magnetic resonance; Chl, chlorophyll; Car, carotenoid; ZFS, zero-field splitting.

10.1021/jp047077k CCC: $30.25 
Published on Web 12/23/2004

**SCHEME 1: (A) Principle of ODMR Spectroscopy[a,c] and (B) Possible Pathways of Singlet and Triplet Energy Transfer among Photosystem Antenna Pigments[b,c]**




[a] The energy scale is arbitrary and serves to elucidate the triplet manifold resonance only. [b]The Car triplet state is essentially populated from the Chl triplet excited state, because of triplet−triplet energy transfer. Singlet oxygen production by the interaction of the excited Chl triplet state and ground-state oxygen is also shown. Because of singlet−singlet and triplet−triplet energy transfer, it is possible to monitor the ODMR spectra of low fluorescing carotenoids on the Chl emission. [c]Key: $S_0$, ground state; $S_1$ first singlet excited state; $T_1$, first triplet excited state; A, absorption; F, fluorescence; ISC, intersystem crossing; IC, internal convention; Mw, microwave.

means of transient optical, e.g., see refs 17, 24, and 34, and magnetic resonance, e.g., see refs 5, 16, and 33, spectroscopy. However, the carotenoid triplets associated with PS I of higher plants are poorly characterized, and only very limited data are available in the literature. To our knowledge, there are no reports related to the carotenoid triplets of PS I monitored in their natural environment, the thylakoid membranes.

In the present study we have analyzed the carotenoid triplet states associated with the red-emitting forms of PS I studied by means of the fluorescence-detected magnetic resonance (FDMR) technique, in thylakoids isolated from spinach. Because the carotenoid triplet state in photosynthetic systems is mainly populated by triplet−triplet energy transfer from the chlorophyll triplet state,[11−19] the change in the carotenoid triplet-state population induced by resonant microwaves between a pair of triplet sublevels can be monitored by means of the chlorophyll fluorescence emission. It is then possible to perform FDMR of carotenoid triplet states even though the fluorescence yield of carotenoids is extremely low (Scheme 1). At least four carotenoid triplet populations have been characterized, which are assigned to the outer PS I antenna complexes, LHC I, on the basis of the associated fluorescence emission profiles.

## Materials and Methods

Thylakoids have been prepared from freshly harvested spinach leaves as previously described.[35] The samples have been diluted to a Chl concentration equivalent to 100 $\mu$g/mL, in a 60% v/v glycerol containing buffer (30 mM Tricine, pH 8, 10 mM NaCl, 5 mM $MgCl_2$) to obtain a transparent glass matrix upon cooling.

FDMR experiments were performed in a home-built apparatus which has been previously described in detail.[16] In brief, the amplitude-modulated microwave field was irradiated on the sample through a broad-band resonator consisting of a slow-pass helix with a pitch of about 2 mm. The fluorescence was excited by a combination of broad-band filters, consisting of a solution of 1 M $CuSO_4$ (path length 5 cm), and a cutoff filter ($\lambda_{T1/2}$ = 476 nm), and collected at 45° geometry through a combination of cuton and interference filters (∼10 nm fwhm) by a photodiode and the signal demodulated and amplified by a EG&G 5220 lock-in amplifier. The modulation frequency (325 Hz) was chosen to obtain the maximum of the carotenoid triplet signals, while the microwave power was set to 600 mW. The temperature of the experiments was 1.7 K.

The global Gaussian deconvolution of the FDMR spectra was performed by software written in the laboratory and previously described in detail,[36] which allows simultaneous fitting of the spectra in different microwave regions, recorded at multiple wavelengths. We want to underline the fact that the global fitting over all three transitions of the triplet manifold is necessary to obtain physically meaningful parameters. In fact, the fitting of a single transition, even by a "global" routine as a function of the emission wavelength, or of two of them, i.e., the $2|E|$ and $|D| + |E|$ transitions, that exhibit a higher signal-to-noise ratio, gave results that were not able to describe the $|D| - |E|$ transition, as expected from the relation of the zero-field-splitting parameters. Therefore, not only were the $2|E|$, $|D| - |E|$, and $|D| + |E|$ transitions fitted simultaneously, but the peak positions of the Gaussian function used to describe the FDMR spectra were constrained to physically meaningful parameters. This was done by calculation of the peak positions of the Gaussian subbands in one selected transition as a result of the free fit parameters in the other two; different attempts were made, yielding substantially the same set of solutions. Due to the higher signal-to-noise ratio in the final fitting procedure, the Gaussian peak positions in $2|E|$ and $|D| + |E|$ were considered as free fit parameters, and the positions of the $|D| - |E|$ transitions were then derived. This not only diminishes the number of fit parameters, but limits the errors in the estimate of the $|D| - |E|$ transition to the propagation of the errors of the $2|E|$ and $|D| + |E|$ fit parameters. To discriminate between the different possible sets of solutions, associated with the initial model functions (i.e., the number of different triplet states needed to globally fit the data sets), three completely independent sets of measurements were fitted, using different initial models (i.e., a sum of three, four, or more than four Gaussian functions). The results of this analysis indicate that, to obtain consistency between the errors estimated in the single measurements by means of the Hessian (variance−covariance matrix) and the interexperimental distribution, as estimated by the Student's $t$ distribution, at least four carotenoid triplet populations should be considered. The data presented are the weighted average of the independent measurements.

## Results

The emission spectrum of thylakoid membrane at 4.2 K is presented in Figure 1. The fluorescence emission of thylakoids, at low temperature, is dominated by the PS II contribution at wavelength shorter than 690 nm and by the PS I contribution at wavelength longer than 700 nm.[37,38] The fluorescence emission spectra of the isolated proximal antenna complexes

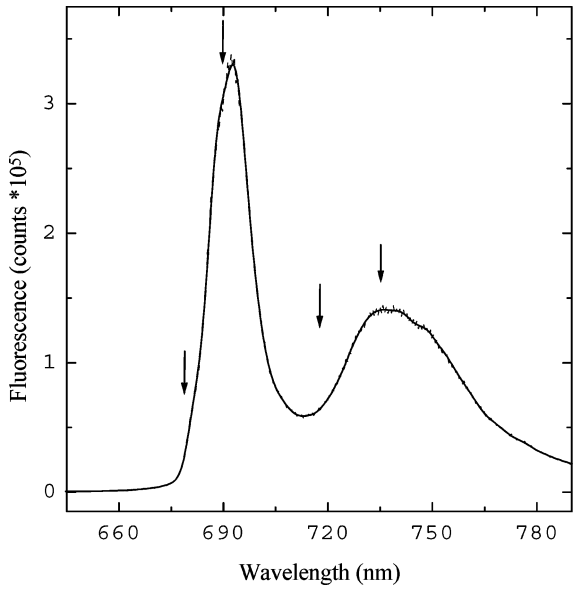



**Figure 1.** Emission spectrum of spinach thylakoids at 4.2 K (chlorophyll concentration, 4 $\mu$g/mL; excitation wavelength, 435 nm). Both the smoothed emission spectrum (solid line) and the original data (dashed−dotted line), corrected for the response of the detector, are presented. Also indicated are the emission wavelengths at which the FDMR spectra presented in Figure 2 have been collected.

of PSII, CP43, and CP47, and the most abundant outer antenna component, LHC II, show a clear emission vibrational contribution at about 740 nm, e.g., see refs 19, 32, 39, and 40, which is overlapped with the main PS I emission band. Considering the intensity of the emission associated with PS II in the thylakoids, and the ratio between the emission maximum and the vibrational band at 740 nm in the isolated complexes, the overall contribution of the latter at wavelength longer than 730 nm can be estimated to be at maximum, 10%. Therefore, at low temperature, the fluorescence emission from PS I and PS II can be essentially discriminated, and the FDMR spectra of the triplet states associated with the red-emitting forms of PS I can be detected without any substantial interference from PS II associated signals, at emission wavelength longer than 730 nm.

The FDMR spectra collected under experimental conditions which are selective for the carotenoid triplet state, that is, the microwave frequency range, amplitude modulation frequencies, and microwave power,[15,16,33] and collected at the characteristic emission wavelengths associated with the outer and core antennae of PS II and PS I (indicated by an arrow in Figure 1) are presented in Figure 2. All the triplet manifold transitions, $2|E|$, $|D| - |E|$, and $|D| + |E|$, are shown. The observed polarization pattern, $2|E| \gg |D| + |E| > |D| - |E|$, is in agreement with most of the reported ODMR spectra assigned to carotenoids.[15,16,33]

It can be appreciated that FDMR signals presented in Figure 2 peak at different microwave frequencies, and present an asymmetric band shape, which is more evident in the $|D| - |E|$ and the $|D| + |E|$ transitions. This is an indication of the presence of multiple underlying triplet populations, which might possibly be associated with different photosystem compartments, as indicated by the fluorescence emission dependency. It would be, in principle, possible to gain information about any single carotenoid triplet pool, by globally analyzing the FDMR spectra taken at different wavelengths. Unfortunately, in the absence of detailed information about the number of triplet populations present in the inner and outer antenna complexes, the analysis would be hampered. However, it is still possible to obtain some

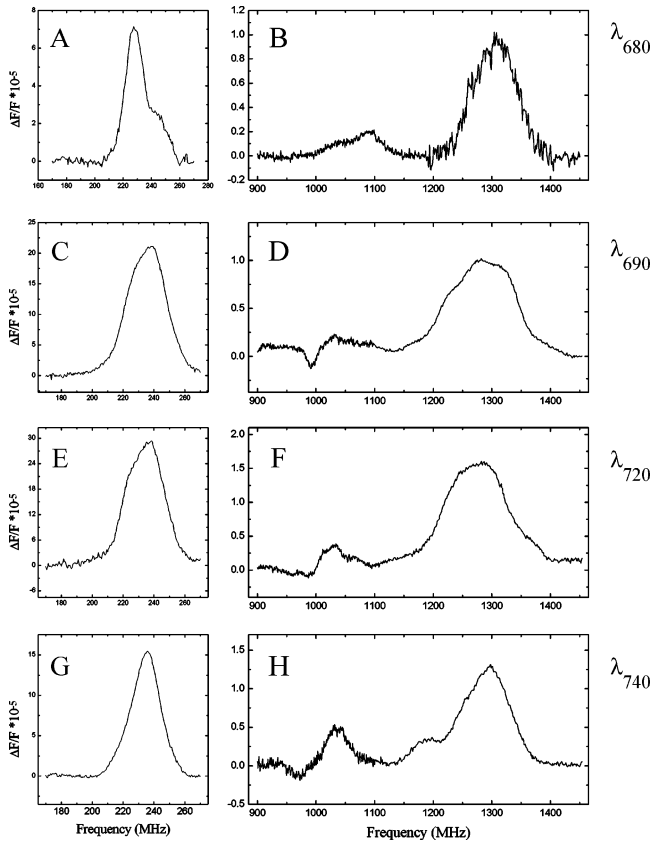



**Figure 2.** FDMR spectra of the carotenoid triplet state in isolated thylakoids: (A, C, E, G) $2|E|$ transition, (B, D, F, H) $|D| - |E|$ and $|D| + |E|$ transitions. Experimental conditions: Chl concentration, 100 $\mu$g/mL; temperature, 1.7 K; microwave frequency modulation, 330 Hz; microwave power, 0.6 mW; number of scans, between 15 and 40. The spectra presented are the average of three independent series of experiments.

information by analyzing in more detail the FDMR signals originating from the red-emitting forms of PS I, detecting the microwave-induced change in fluorescence yield in the 730−800 nm emission wavelength range, where the contribution from PS II is expected to be negligible (Figure 1).

The dataset has been fitted by a global analysis routine, in terms of Gaussian subbands. This procedure has recently been successfully applied to resolve different chlorophyll triplet populations in thylakoids.[36,41] In the present case the three FDMR signals, recorded in the $2|E|$, the $|D| - |E|$, and the $|D| + |E|$ frequency ranges and at different fluorescence emission wavelengths, are simultaneously fitted. This means that the microwave transitions associated with the same triplet state are constrained to have the same fluorescence emission profile and are scaled by an intensity factor, which is a fit parameter. A similar scaling factor is also introduced to account for the different bandwidths in the triplet transitions. As a result of the global analysis it has been found that at least four carotenoid populations are needed to obtain a satisfactory description of the FDMR spectra (see the Materials and Methods for further details regarding the data analysis). The results of this analysis are presented in Figures 3 and 4, where the fits of the $2|E|$ and the $|D| + |E|$ transitions are shown. The complete set of fit parameters used to describe the data are reported in Table 1. Some of these assignments are further supported by electron−electron double-resonance experiments (data not presented). Also presented in the table is the fit obtained of the FDMR spectrum detected at 680 nm in thylakoids, an emission wavelength at which PS II outer antenna emission is predomi-

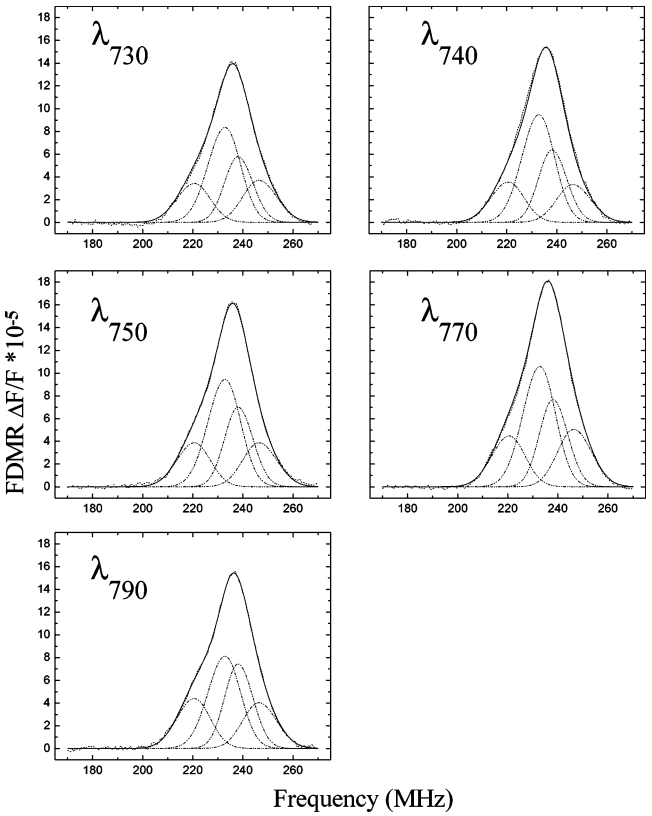



**Figure 3.** Global fitting analysis of the 2|*E*| transition of the FDMR spectra collected in the 730−790 nm emission window: solid line, experimental results; thick solid line, fit function; dashed−dotted lines, Gaussian components. Experimental conditions are as in the caption of Figure 2.

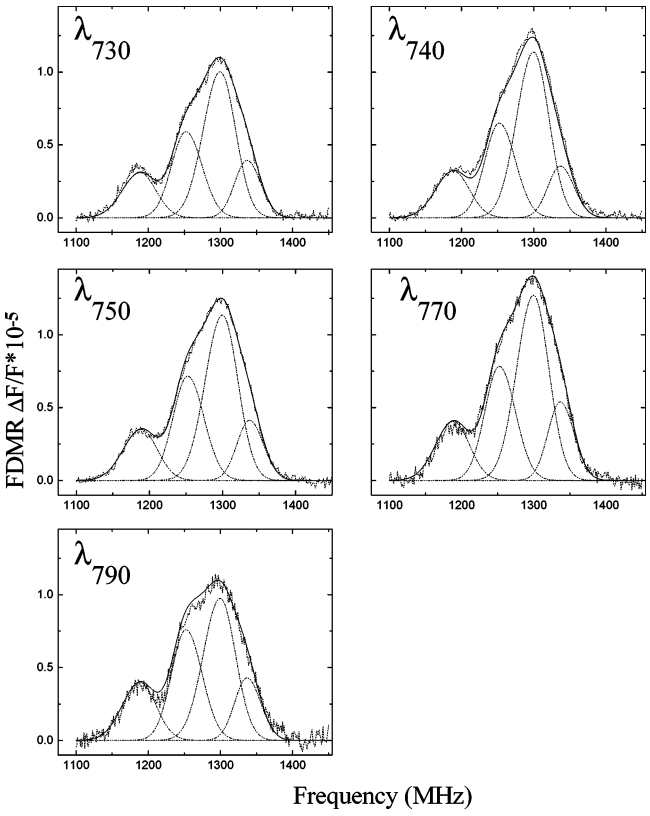



**Figure 4.** Global fitting analysis of the |*D*| + |*E*| transition of the FDMR spectra collected in the 730−790 nm emission window: solid line, experimental results; thick solid line, fit function; dashed−dotted lines, Gaussian components. Note that the tail of the |*D*| − |*E*| transition has been subtracted from the data for clarity of presentation. Experimental conditions are as in the caption of Figure 2.

nant,[37,38] and some previous estimates taken from the literature for the isolated LHC II.[16,33]

The microwave-induced fluorescence emission profiles derived from the global deconvolution analysis of the carotenoid triplet states associated with the red forms of PS I are presented in Figure 5.

## Discussion

The carotenoid triplet state in photosynthetic antennae is essentially populated by triplet−triplet energy transfer from the chlorophyll triplet state[11−19] even though population by singlet fission has also been reported.[42] Triplet−triplet transfer is thought to proceed via the so-called Dexter mechanism,[43] which requires electronic orbital overlap and close proximity between donor and acceptor molecules. Therefore, in principle, the localization of the triplet state ensures the possibility of gaining information about the chlorophyll on which the triplet state is originally populated (see ref 44 for a review). On the other hand, due to singlet−singlet energy transfer, the interpretation of ODMR results is less straightforward when fluorescence instead of absorption is monitored. This is because the modulation of the fluorescence yield induced by the resonant microwave transition can be observed on the population which carries most of the singlet-state population, which, at low temperature, is the longer wavelength emitting (absorbing) Chl pool rather than the Chl on which the triplet is originally formed. Unfortunately, in thylakoid membranes, in the absorbance-detected magnetic resonance (ADMR) mode, PS I and PS II associated triplets cannot be monitored as selectively as is possible by means of fluorescence detection, due to the more pronounced overlap of the carotenoid absorption spectra and of the resonance frequency precluding a selective microwave field excitation.

We have taken advantage of the sensitivity and selectivity of the fluorescence-detected magnetic resonance technique to monitor carotenoid triplet states associated with the long-wavelength-emitting (absorbing) chlorophyll forms of PS I in isolated spinach thylakoid membranes, which represent a fairly unperturbed environment. To satisfactorily describe the spectra collected at emission wavelength longer than 730 nm, at least four carotenoid populations are needed and are characterized by the zero-field-splitting parameters |*D*| and |*E*| reported in Table 1. It should be noted that, in a recent reinvestigation of carotenoid triplet states in thylakoids at room temperature, Jaforvi and co-workers[14] could only detect signals which can be reasonably assigned to PS II, on the basis of the Chl bleaching band at about 675−680 nm. This is probably due the fact that, at room temperature, the chlorophyll singlet lifetime of PS II, at closed reaction centers, is about 2 ns, e.g., see ref 45, while for PS I it is only about 100 ps at both open and closed traps (e.g., see 46, reviewed in ref 49). Therefore, since the bulk of the Chl excited-state population resides on PS II in these conditions, it is also expected that the carotenoid triplet population associated with PS I will be at a relatively low level. The yield of PS I fluorescence is strongly dependent on the temperature; hence, the overall probability of triplet population in PS I is expected to increase in these circumstances.

The fluorescence emission profile, associated with each carotenoid triplet pool, shows a rather flat maximum at about 760−770 nm (Figure 5), which is on the tail of the broad emission band of PS I in thylakoids that peaks at about 740 nm at the temperature of the FDMR experiments reported here (1.7

**TABLE 1: Parameters of the Global Gaussian Deconvolution of the Carotenoid FDMR Spectra[a]**

| | $2\|E\|$ | | $\|D\| - \|E\|$ | | $\|D\| + \|E\|$ | | | |
|---|---|---|---|---|---|---|---|---|
| triplet | peak (MHz) | fwhm (MHz) | peak (MHz) | fwhm (MHz) | peak (MHz) | fwhm (MHz) | $\|D\|$ ($cm^{-1}$) | $\|E\|$ ($cm^{-1}$) |
| | | | | Carotenoid Triplet Associated with PS I Red Forms | | | | |
| $T_{C1}$ | 222.6 ± 0.5 | 15.7 ± 0.8 | 968 ± 6 | 53 ± 5 | 1189 ± 5 | 55 ± 3 | 0.0359 ± 0.0002 | 0.00371 ± 0.00002 |
| $T_{C2}$ | 232.9 ± 0.8 | 15.3 ± 0.7 | 1029 ± 7 | 51 ± 4 | 1261.8 ± 6 | 52 ± 3 | 0.0382 ± 0.0002 | 0.00388 ± 0.00003 |
| $T_{C3}$ | 238.0 ± 0.6 | 13.3 ± 0.7 | 1064 ± 6 | 46 ± 4 | 1302.3 ± 7 | 50 ± 3 | 0.0395 ± 0.0002 | 0.00397 ± 0.00002 |
| $T_{C4}$ | 246.3 ± 0.9 | 16.0 ± 0.8 | 1090 ± 7 | 51 ± 4 | 1336.9 ± 6 | 43 ± 2 | 0.0405 ± 0.0002 | 0.00411 ± 0.00003 |
| | | | | Carotenoid Triplet Associated with the PS II Outer Antenna ($\lambda$ = 680 nm) | | | | |
| $T_{C1}$ | 220.0 ± 0.9 | 13 ± 1 | 1040 ± 11 | 46 ± 5 | 1260 ± 12 | 52 ± 5 | 0.0384 ± 0.0004 | 0.00367 ± 0.00003 |
| $T_{C2}$ | 228.1 ± 0.8 | 14 ± 1 | 1092 ± 13 | 47 ± 5 | 1321 ± 15 | 55 ± 7 | 0.0403 ± 0.0005 | 0.00380 ± 0.00003 |
| $T_{C3}$ | 245 ± 1.4 | 16 ± 1 | 1034 ± 16 | 49 ± 6 | 1278 ± 16 | 60 ± 8 | 0.0386 ± 0.0005 | 0.00408 ± 0.00005 |
| | | | | LHC II (From van der Vos et al.[33]) | | | | |
| $T_{C1}$ | 236 | 13 | | | 1273 | 46 | 0.0385 | 0.0394 |
| $T_{C2}$ | 236 | 13 | | | 1302 | 33 | 0.0396 | 0.0394 |
| $T_{C3}$ | 236 | 13 | | | 1322 | 30 | 0.0402 | 0.0394 |
| | | | | LHC II (From Carbonera et al.[16]) | | | | |
| $T_{C1}$ | 228 | 15 | | | 1263 | 31 | 0.0382 | 0.00379 |
| $T_{C2}$ | 228 | 15 | | | 1278 | 31 | 0.0388 | 0.00379 |
| $T_{C3}$ | 228 | 15 | | | 1315 | 31 | 0.0401 | 0.00379 |

[a] Values of the fit parameters employed in the global deconvolution analysis of the carotenoid FDMR spectra monitored either at the red emission wing of PS I or at emission wavelength (680 nm) when the emission is dominated by the PS II outer antenna. Also reported are some of the values presented in the literature for the carotenoids associated with the main PS II outer antenna complex LHC II.

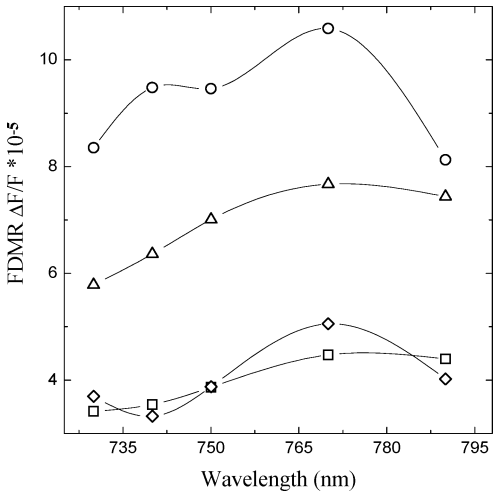



**Figure 5.** Triplet-associated fluorescence emission spectra obtained by plotting the amplitude of the Gaussian functions used to globally fit the datasets in the 730−790 nm interval: squares, $|D| = 0.0359$, $|E| = 0.00371$ $cm^{-1}$; tilted squares, $|D| = 0.0382$, $|E| = 0.00388$ $cm^{-1}$; triangles, $|D| = 0.0395$, $|E| = 0.00397$ $cm^{-1}$; circles, $|D| = 0.0405$, $|E| = 0.00411$ $cm^{-1}$.

K). It has been previously shown that the long-wavelength-emitting forms, also called the "red" forms, are, in higher plants, mainly associated with the outer antenna of PS I, the LHC I family.[47,48] It should be mentioned that a relatively small stoichiometric amount of long-wavelength-emitting forms has been detected in higher plant PS I core particles as well, even though these higher plant core-bound long-wavelength-absorbing/emitting chlorophylls have less red-shifted characteristics compared to LHC I bound forms.[48] Taken together, these observations suggest that the carotenoid triplet detected at wavelength longer then 730 nm can be reasonably attributed to the LHC I pool.

In Table 1 the zero-field-splitting parameters determined for the LHC I associated triplets are compared with the results obtained for the outer PS II antenna, detected in the same thylakoid preparation at 680 nm, as well as several datasets taken from the literature.[33−34] It can be seen that the ZFS parameters extracted for the carotenoid triplets associated with the red forms of PSI fall in the range of values previously reported for LHC II. These Chl-binding complexes are well characterized from a biochemical and spectroscopic point of view, e.g., see refs 50−53, and are thought to contain only xanthophylls.[1,51,53] As a result it is reasonable to propose that the carotenoid populations observed here are mainly associated with these carotenoids, even though a minor contribution from the $\beta$-carotene molecules coupled with the red-emitting form associated with the PS I core cannot be, in principle, excluded. The location of the carotenoids in LHC I is at present uncertain, as they have not been resolved in the crystal structure of the PS I−LHC I supercomplex.[7] An arrangement similar to that determined in the structure of LHC II can be argued on the basis of sequence homology and biochemical characterization.[50−53] In the LHC II structure, two lutein molecules are shown to be in contact mainly with Chl *a* molecules, while the neoxantin molecule is shown to be in proximity to the Chl *b* molecules. Due to the difference in absorption between Chl *a* and Chl *b* forms, and the presence of long-wavelength-emitting (absorbing) Chl forms in PS I, the excited-state population on Chl *b* is expected to be negligible, and the triplet population of this carotene is, in turn, expected to be proportionally low. These observations agree well with the suggestion of Lampoura et al.[34] that the Car triplets observed in LHC II are associated with the two lutein molecules which are bound to the transmembrane α-helices that constitute the central scaffold of the complex. On the other hand, some contribution from the more peripheral violaxantin molecule cannot be excluded on the basis of the assignment and calculated energy coupling derived from the X-ray crystal structure.[6]

The LHC I pool is a family of at least four polypeptides, named LHC1a−d, (Lhca1−4) which have been extensively characterized in recent years, and shown to have different absorption/emission characteristics.[50−53] The crystal structure of the PS I−LHC I holocomplex has shown a 1:1:1:1 stoichiometry for the LHC I polypeptides.[7] According to the spectroscopic properties reported for the reconstituted Lhca1−4 complexes, the more red-shifted chlorophyll forms belong to the Lhca1 and the Lhca4 components.[50−53] The different carotenoid triplet populations observed in the 740−800 nm fluorescence emission range are likely to be associated with

one, or both, of these antenna polypeptides. It should also be kept in mind that the data can also be fitted by a number of Gaussian functions larger than four, and the solution has been chosen on the basis of restricting the fit number parameters to a minimum. Also, the other polypeptides, namely, Lhca2 and Lhca3, may be involved in triplet quenching. However, all the triplets observed appear to have rather similar emission associated spectra, while the emission/absorption characteristics of the reconstituted complexes differ significantly[51−53] and may well fall in the region below 730 nm. Further measurements on isolated LHC I complexes and isolated PS I particles would be needed to further address this issue. Preliminary measurements on reconstituted Lhca4 complex suggest that at least three different carotenoid pools are necessary to describe the FDMR spectra, and are in close agreement with the result presented here in a more intact environment (unpublished results).

On the basis of the similarity of the emission spectra associated with the triplet states, it cannot be discounted that the slight variation in the ZFS parameters, which can be detected due to the high sensitivity of ODMR to these parameters, might be associated with different conformational states of the carotenoids embedded in the protein, trapped by freezing at low temperature. In fact, a similar suggestion has been advanced regarding the splitting of the $P_{700}$ recombination triplet ODMR spectrum.[54,55]

**Acknowledgment.** This work was supported by grants from the U.K. Biotechnology and Biological Sciences Research Council (BBSRC) (CO0350, CO7809, and B18658), the Italian Ministry for University and Research (MURST) under the project FIRB RBAU01E3CX "Meccanismi molecolari della Fotosintesi", and the European Union TMR program (Contract No. FMRX-CT98-0214) to S.S. We thank Dr. C. Syme (Queen Mary University of London) for critical reading of the manuscript.

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