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ADMR study of the effect of oligomerisation on the carotenoid triplets and on triplet-triplet transfer in light harvesting complex II (LHC II) of spinach

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Abstract

Carotenoid triplets in isolated light harvesting complex (LHC) II of spinach at different concentrations were studied by absorbance-detected magnetic resonance (ADMR). Going from high to low LHC II concentrations, a change was observed in the intensity of the ADMR spectra of the $|D|+|E|$ transition recorded at 507 nm relative to that recorded at 525 nm, from approx. 0.5 to approx. 1.0. The relative intensity of the $2|E|$ transition did not change. The change in relative intensity of the ADMR signal is due to a change of the ADMR signal intensity of the $|D|+|E|$ transition that is detected at 525 nm. The effect is ascribed to an aggregation of trimeric LHC II into an oligomeric form of LHC II. Taking into account the narrowing of the zero-field resonance bands with oligomerisation, and the absence of bandshifts, the relative increase of the signal intensity of the $|D|+|E|$ transition detected at 525 nm can be explained by assuming that the oligomer consists of a multiple of trimers, between which 'inter-trimer' energy transfer occurs. This yields an increase in the number of triplets that is transferred to the carotenoid having its triplet absorption maximum at 525 nm. Our new results indicate that the carotenoids are bound to Chl *a* monomers, and not dimers as proposed earlier (Van der Vos, R., Carbonera, D. and Hoff, A.J. (1991) Appl. Magn. Res. 2, 179-202).

Keywords: Light harvesting complex II; Aggregation; Carotenoid; Absorbance-detected magnetic resonance; Triplet transfer; (Spinach)

Introduction

In the photosynthetic membrane of plants, light energy is converted into chemical energy. The first stage of this process is the collection of light energy in antenna complexes. Transfer of the captured (excitation) energy to the reaction centers of photosynthetic systems PS I and II is mediated by chlorophyll and carotenoid pigments in the antenna complexes [1]. In the reaction center, charge separation provides conversion of excitation energy into stored chemical energy. The antenna complex of photosystem II (PS II), labelled light harvesting complex (LHC) II, is a chlorophyll *a*/*b*-containing protein aggregate [2].

In LHC II three types of carotenoids are present: lutein, neoxanthin and violaxanthin [3]. The role of these carotenoids is known to be two-fold: harvesting light in the blue/green region and subsequently transferring the excitation energy to the chlorophylls of the reaction center, and photoprotection; that is preventing the formation of aggressive singlet oxygen [4,5].

One way to gain insight into the processes of light collection and excitation transfer is to study the triplet states of the carotenoid and chlorophyll pigments. To this end, optically-detected magnetic resonance (ODMR) has been used in studies of antenna complexes of bacterial systems [6,7] and higher plants [8,9]. With this technique accurate values of the triplet zero-field splitting parameters, D and E, and of the triplet sublevel decay rates can be obtained. In addition highly accurate triplet-minus-singlet (T-S) spectra can be

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Abbreviations: ADMR, absorbance-detected magnetic resonance; PS II, Photosystem II; LHC, light harvesting complex; ODMR, optically-detected magnetic resonance; T-S, triplet-minus-singlet; Chl, chlorophyll; FDMR, fluorescence-detected magnetic resonance; OD, optical density.

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monitored in an optical-microwave double resonance experiment.

In LHC II, two triplets were observed [9] with different $|D|$-values and equal $|E|$-value ($|E| = 0.00379$ cm^{-1}), which were assigned to two of the three carotenoids present in LHC II: neoxanthin ($|D| = 0.03853$ cm^{-1}) and lutein ($|D| = 0.04003$ cm^{-1}). The two triplets gave rise to a different T-S spectrum: For neoxanthin a (triplet) absorption maximum at 507 nm was reported, whereas for lutein a similar maximum was situated at 525 nm. For microwave excitation resonant with one of the carotenoid triplets, an absorption change in the region where chlorophyll *a* (Chl *a*) is known to absorb, was also observed, indicating a relatively strong carotenoid-Chl *a* interaction.

Bassi et al. [1] succeeded in separating different aggregation states of LHC II in different sedimentation bands by sucrose gradient centrifugation. When the detergent concentration of the isolated LHC II solution was increased, a second sedimentation band appeared. It was concluded that this second band consisted of trimeric LHC II (the minimal unit of the LHC II organisation, referred to as CPII), and that the dissociation product of the oligomeric LHC II complexes (referred to as CPII′) was present in the first band. For the in vivo structure of LHC II, Bassi et al. proposed an oligomeric organisation (possibly a trimer multiple), instead of a trimeric structure as proposed in [10].

Ide et al. [10] performed steady-state optical spectroscopy at room temperature on LHC II, using different chlorophyll/detergent ratios. They observed a quenching of the Chl *b* fluorescence band upon aggregation and an increase of features in the CD spectrum that were ascribed to Chl *a-b* and Chl *b-b* exciton interactions.

Carbonera et al. [8] observed a change in the fluorescence-detected magnetic resonance (FDMR) spectra of both the $|D|-|E|$ and $|D|+|E|$ transition when the LHC II concentration was varied. The FDMR spectrum of the $|D|+|E|$ transition is characterised by two maxima at about 1260 and 1310 MHz. With dilution of the sample, the relative intensity of the maximum at 1310 MHz increased, whereas the intensity of the maximum at 1260 MHz decreased. From the fits of the FDMR spectra with Gaussian bands, Carbonera et al. concluded that three bands, centered at 1263, 1292 and 1320 MHz, were needed to fit the microwave transition. In later work, Carbonera and Giacometti [11] used the results of the concentration effect obtained with FDMR for fitting the absorbance-detected magnetic resonance (ADMR) data set from Ref. 9. The zero-field splitting parameters used in Ref. 11, however, were not identical to those from Ref. 8. (The center frequencies of the $|D|+|E|$ bands of Ref. 11 were 1260, 1278 and 1315 MHz.)

The resulting uncertainty in the interpretation in Ref. 11 prompted us to study the concentration effect observed in [1,8] with ADMR. This allowed us to monitor the concentration effect on the different carotenoid triplets separately, resulting in more specific information about the aggregation effect. Such information cannot be obtained with FDMR, since the chlorophylls all fluoresce at the same wavelength, and the fluorescence signal is therefore independent of the carotenoids to which these chlorophylls transfer their energy: the carotenoids themselves hardly fluoresce.

We have found that the relative amplitudes of the $|D|+|E|$ transition of the triplets detected at 525 nm increase faster with concentration than all other ADMR transitions. Also other changes with concentration are observed in the ADMR spectra recorded at 525 nm, which are absent in the spectra detected at 507 nm. This indicates that the carotenoid triplet detected at 525 nm is located in a LHC II subunit at a site that is sensitive to oligomerisation, whereas the carotenoid giving rise to the triplet detected at 507 nm is probably located more inside the subunit, where it is not affected by the aggregation. Three zero-field transitions were needed to fit the ADMR spectra, using a Gaussian deconvolution. The results indicate that the carotenoids are bound to Chl *a* monomers, and not dimers as proposed in Ref. 9.

2. Materials and methods

Sample preparation

LHC II complexes were isolated according to the method of Hemelrijk et al. [12], with the following modifications. PS II membrane fragments were isolated from spinach thylakoids according to the method of Berthold et al. [13] and solubilized at a concentration of 1.75 mg chlorophyll/ml, with 1.25 (w/v) n-dodecyl β-D-maltoside (DM), in a BTS-400 buffer (containing 20 mM Bis-Tris (pH 6.5), 20 mM Mg Cl_2, 5 mM $CaCl_2$, 0.4 M sucrose and 0.03% (w/v) DM as described in [14]. The solubilized fraction was loaded on a Q-Sepharose anion exchange column, equilibrated with BTS-400. An LHC II enriched fraction was eluted from the column with the same buffer. After dialysis against BTS buffer without sucrose for 12 h, this LHC II fraction was loaded on a 7–28% linear sucrose gradient in BTS-400 buffer, and centrifuged at 4°C in a Beckman 70 Ti rotor at 35.000 rpm for 16 h. From this LHC II suspension, samples of different concentrations were made, each sample having a DM concentration of 0.15% DM (w/v). The samples were stored at 77 K. Before ADMR measurements glycerol was added in a ratio 2 : 1 (v/v) to ensure the formation of a clear glass.

In the present work, the final concentration of a sample is given as Optical Density (OD) per mm at 670 nm, measured at room temperature with a Shimadzu spectrophotometer. For several samples the total Chl $a+b$ concentration was determined according to [15], providing the calibration that a solution with an OD of 1 mm^{-1} at 670 nm corresponds to a total Chl $a+b$ concentration of 122 μg/ml. This relation was used throughout. The ODs of the sample varied from 0.05–0.9 mm^{-1}, resulting in a chlorophyll concentration of 6–110 μg/ml. Because the S/N ratio decreased with dilution of our sample, we could not measure below an OD of 0.05 mm^{-1}.

The low-temperature absorption spectrum of our LHC II preparation was similar to that reported in [9], with the 670 nm band somewhat more pronounced.

ADMR experiments

With ADMR spectroscopy the triplet concentration is varied by applying amplitude-modulated resonant microwaves (Fig. 4). This variation can be observed by monitoring the absorbance. Scanning the detection wavelength offers the possibility to record a T-S spectrum. Conversely, the detection wavelength can be kept constant while the microwave frequency is swept, which generates a so-called ADMR spectrum. For a more detailed discussion of the ADMR technique, we refer to Refs. [9,16]. The set-up used in this work was essentially the same as in Ref. 9. For the ADMR spectra presented in this work, the microwave transition was scanned with steps of 1–3 MHz per point, stepping once every 3 s, at a detection wavelength selected such that only one of the two carotenoid triplets from Ref. [9] was observed at each wavelength. From the T-S spectra of the two triplets [9], the wavelengths chosen were 507 and 525 nm.

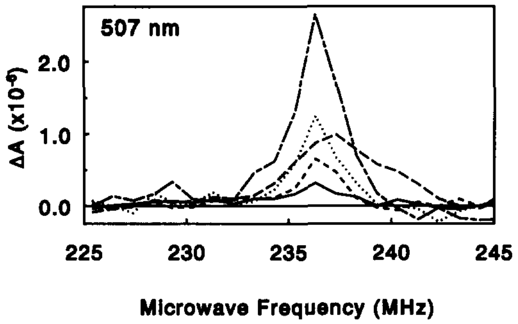

Fig. 1. ADMR spectra of the 2|E|-transition recorded at 507 nm, measured at an OD of 0.05 mm^{-1} (drawn line, average of 30 scans), 0.1 mm^{-1} (short-dashed line, 9 scans), 0.2 mm^{-1} (long-dashed line, 9 scans), 0.3 mm^{-1} (dotted line, 9 scans) and 0.7 mm^{-1} (drawn line, 4 scans).

Results

For all monitoring wavelengths the 2|E| transition was centered at 236 MHz, and the |D| + |E| transition around 1300 MHz (Figs. 1 and 2). The signal intensity of the |D| + |E| transitition was for all ODs about 30% of that of the 2|E| transition. The |D| − |E| transition intensity is one order of magnitude smaller than the |D| + |E| transition intensity (data not shown). The relative intensities of the three transitions are roughly the same as those in Refs [8,9].

The ADMR spectra of the 2|E| transition, recorded at 507 (Fig. 1) and 525 nm (not shown), are comparable in shape, and their amplitude as a function of the OD behaved similarly. As was already observed in Ref. 9,

Table 1
The zero-field splitting parameters |D| and |E| in cm^{-1} for the triplets observed in Carbonera et al. [8,11], van der Vos et al. [9] and in this work

		This work	Carbonera et al.		Van der Vos et al.	
			[8]	[11]	[9]	
2|E| transition	Centre frequency (MHz)	236	228		228	
	Resulting |E|-value (cm^{-1})	0.00394	0.00379		0.00379	
	Typical width (MHz)	3	15		15	
|D| + |E| transition	Centre frequency (MHz)	1273	1263		1270	
	Typical width (MHz)	0.0385	0.0382		0.03853	triplet I
	Resulting |D|-value (cm^{-1})	46	31		≈ 40	
		1303	1292	1278	1310	
		0.0396	0.0392	0.0388	0.04003	triplet II
		33	31	-	≈ 80	
		1322	1320	1315		
		0.0402	0.0402	0.0401	-	triplet III
		30	31			

[a] OD = 0.7 per mm.

the 2|E| transition of both triplets overlap completely. The center frequency at 236 MHz (|E| = 0.00394 cm^{-1}, see Table 1) was 8 MHz higher than reported in [8,9], and its width (full width at half maximum) considerably reduced, being 2–3 MHz vs. 10–15 MHz in [8,9]. The |D|-value and the width of the |D| + |E| transition (Fig. 2a; the |D| − |E| transition was not measured) were similar to those of Refs. [8,9] (Table 1). The observed small differences are most likely due to differences in sample preparation. The |D| and |E|-values were practically independent of the LHC II concentration. In Fig. 3, the maximal signal intensities of the 2|E| and |D| + |E| transition, each recorded at 507 and 525 nm, are displayed as a function of the OD. The increase of signal intensity for all transitions is *not* due to the mentioned aggregation effect, but to the higher concentration and thus the higher amount of LHC II present in the cuvette. It is seen, however, that the |D| + |E| transition recorded at 525 nm increases faster with LHC II concentration than all other transitions. This additional increase is most likely caused by aggregation of the complex.

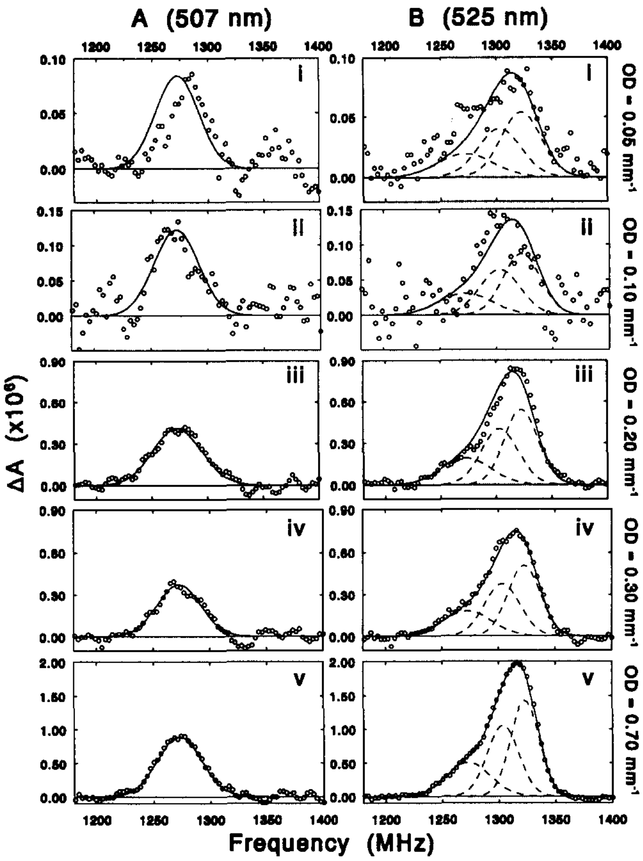

Fig. 2a. ADMR spectra of the |D| + |E| transition recorded at 507 nm (A) and 525 nm (B) with their Gaussian fits (A: center 1273 MHz, width 46 MHz; B: centers at 1273, 1303 and 1322 MHz, relative intensities 1.0:2.0:2.8, respectively, and relative widths 1.0:0.72:0.65, respectively). Dots: experimental, drawn line: fit, dashed line: constituting bands. (i) OD = 0.05 mm^{-1}, average of 64 scans. Width of the 1273 MHz band in B is 59 MHz. (ii) OD = 0.1 mm^{-1}, average of 36 scans. Width of the 1273 MHz band in B is 55 MHz. (iii) OD = 0.2 mm^{-1}, average of 36(A) and 20 (B) scans. Width of the 1273 MHz band in A is 47 MHz and in B 52 MHz. (iv) OD = 0.3 mm^{-1}, average of 30 scans. Width of the 1273 MHz band in B is 50 MHz. (v) OD = 0.7 mm^{-1}, average of 16 scans. Width of the 1273 MHz band in B is 46 MHz.

Discussion

Zero-field splitting parameters

To describe the changes induced by the concentration effect, we fitted the ADMR spectra of the |D| + |E| transition with different Gaussian bands. The central frequencies of the resonance bands observed in the ADMR spectra are not likely to depend on the state of aggregation of the complex. Changing the amplitude of the three components, with or without conservation of the relative amplitudes, did not alone result in a satisfactory fit. Therefore, we also changed their widths, while conserving the relative widths and amplitudes, with the additional constraint that at OD = 0.7 mm^{-1} the width of the 1273 MHz band observed at 525 nm was identical to that observed at 507 nm. This procedure resulted in acceptable fits over the whole concentration range. In the fits shown in Fig. 2a, the fit parameters were determined from the experiments in which solutions with an OD of 0.2, 0.3, 0.5 and 0.7 mm^{-1} were used. For the experiments with an OD of 0.05 and 0.1 mm^{-1}, the overall amplitude of the resonance bands was the only free parameter used; it was determined with an accuracy of approx. 20%. First the ADMR spectra with the highest S/N ratio were fitted, then spectra with a lower S/N ratio. For the ADMR spectra recorded at 507 nm, one Gaussian band (center, 1273 MHz; width, 46 MHz; |D| = 0.0385 cm^{-1}) was used, of which only the amplitude was allowed to change with the concentration (Fig. 2a, A). The ADMR spectra recorded at 525 nm could not be fitted with two Gaussian bands as was done in Ref. 11 without changing the (fixed) parameters of the bands rigorously for ADMR spectra taken at different concentrations. For a fit with three bands, however, only minor changes were required. The three bands are centered at 1273, 1303 and 1322 MHz, and have a width of 46, 33 and 30 MHz, respectively (at an OD of 0.7 mm^{-1}; see Fig. 2b and Table 2).

We attribute the zero-field splitting parameters of the three resonance bands resulting from the Gaussian deconvolution of the ADMR spectra to three different triplets: One with absorption maxima at 507 and 525 nm (|D| + |E| transition at 1273 MHz, |D| = 0.0385 cm^{-1}), and two other triplets that have a single absorption maximum at 525 nm (|D| + |E| transition at 1303 and 1322 MHz, corrresponding |D|-values 0.0396 and

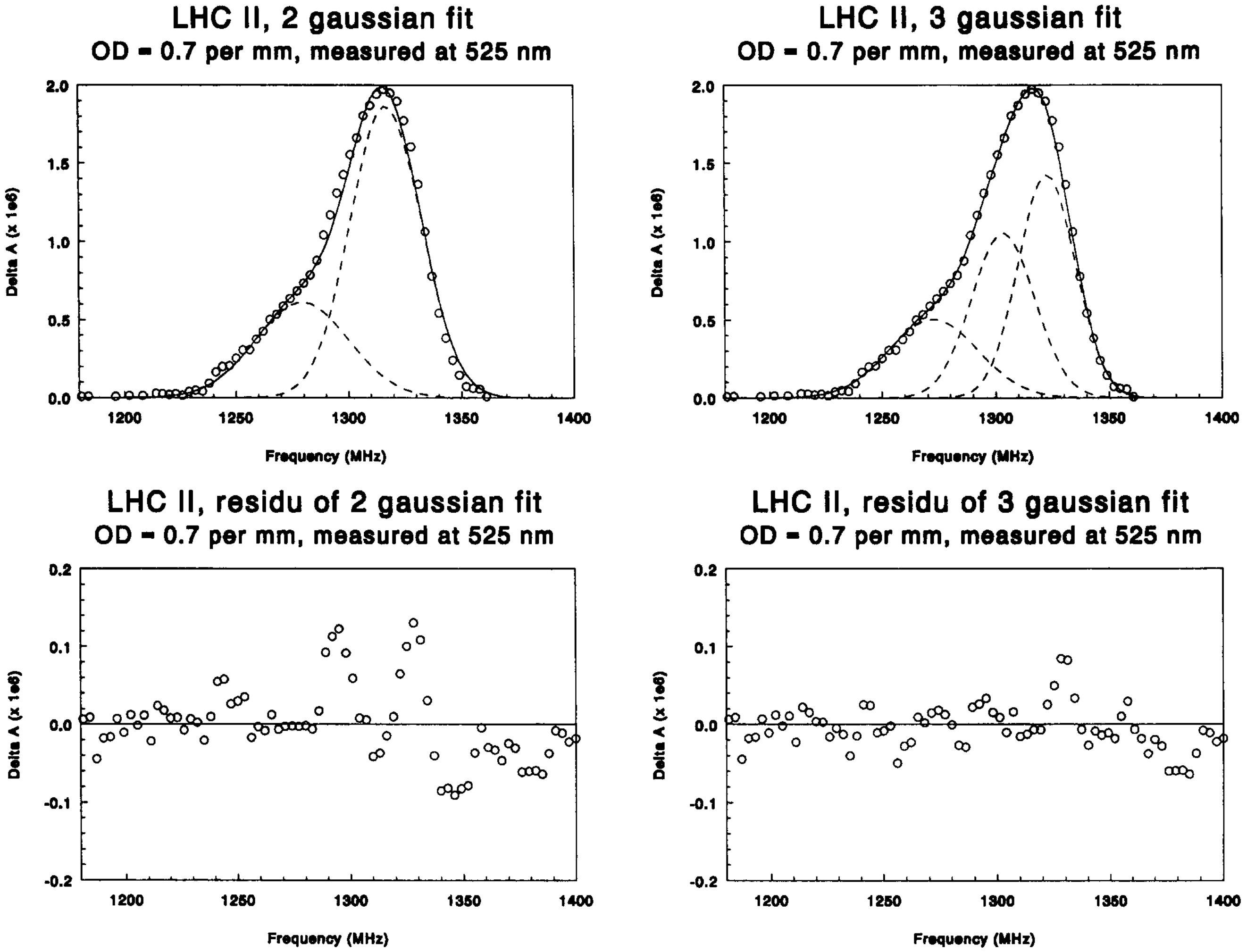

Fig. 2b. Comparison of a two-Gaussian fit and a three-Gaussian fit of the data in the right-hand panel of Fig. 2a. (v) The two-Gaussian fit has a residue significantly exceeding the signal-to-noise ratio and is therefore rejected.

0.0402 cm^{-1}, respectively). Following Carbonera et al. [8], we shall call the triplets I, II and III, in order of increasing |D|-value. When we discriminate the triplets in relation to their optical characteristics, we shall call the triplet absorbing at 507 nm the '507 nm triplet' and those observed at 525 nm the '525 nm triplets'.

From our deconvolution it is clear that the contribution of triplet I to the ADMR spectrum detected at 525 nm is appreciable (approx 20% of the total intensity). As the width of the 507 nm band is only 10 nm, the contribution of triplet I observed at 525 nm cannot

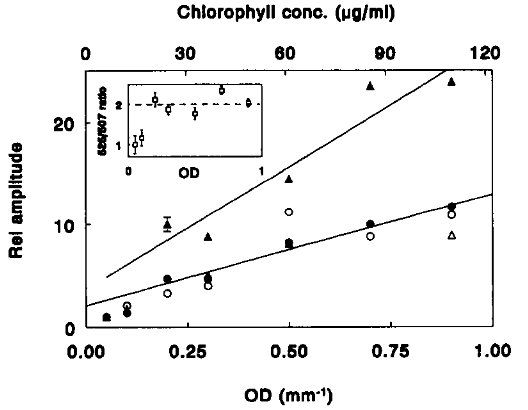

Fig. 3. Amplitude of the ADMR spectra of the 2|E| (ˆ,△) and the |D| + |E| (●,▲) transition recorded at 507 nm (ˆ,●) and 525 nm (△,▲), normalised at the intensity of the spectra recorded at an OD of 0.05 mm^{-1}. Inset: Relative intensities of the |D| + |E| ADMR-signals detected at 507 and 525 nm.

Table 2
Widths in MHz of the Gaussian bands used to fit the ADMR spectra of the |D| + |E| transition recorded at 507 and 525 nm

OD [a]	1273 MHz [b]	1273 MHz [c]	1303 MHz [c]	1323 MHz [c]
0.05	45	59	46	42
0.1	45	55	42	39
0.2	51	52	39	36
0.3	45	50	37	34
0.7	46	46	33	30

[a] Optical Density per mm.
[b] Central frequency of the resonance band observed at 507 nm.
[c] Central frequency of the resonance band observed at 525 nm.

be due to the tail of the 507 nm triplet absorption band. Nor can the additional 525 nm contribution be ascribed to the carotenoid on which triplet II is located (as in Ref. [11]), because here (and in Ref. 8) the triplet I and II fit components (centered at 1270 and 1310 MHz, respectively) show considerably less overlap than in Ref. [11]. Thus, as noted above, triplet I must also have a triplet absorption band centered at 525 nm. The two absorbance maxima of triplet I could be due to, for example, a different carotenoid conformation (for example all-*trans* or 9-*cis*), or a different environment for the carotenoid (see next section).

As we have shown that increasing the concentration has no effect above ODs of 0.2 mm^{-1}, and as the results from Ref. 9 were obtained with relatively high ODs, we may regard those results as a 'snap-shot' of an LHC II solution in the 'high'-OD limit. Therefore, assuming similar total oscillator strengths for all triplets involved, and using the result from Ref. 9 that the same relative amplitude of the $|D|+|E|$ and $2|E|$-transition was observed at 507 and 525 nm (for the higher ODs), we can recalculate the triplet abundancies of triplet I, II and III. To this end, we use the 507 and 525 nm triplet stoichiometry (1:1.8) from Ref. 9 and the relative contributions of the resonance bands observed in this work and in Ref. 8. The relative triplet abundancies then become: 1.22 (triplet I, 507 nm): 0.44 (I, 525 nm): 0.75 (II):1.0 (III) or 1.66:0.75:1.0 for triplet I:II:III. As in Ref. 8, we take into account the carotenoid stoichiometry [3] and assign triplet I to lutein, triplet II to violaxanthin and triplet III to neoxanthin. The violaxanthin contribution is somewhat larger than expected from the HPLC analysis (0.75 instead of 0.44). Possibly, the different isolation method of our preparation compared to that of Ref. 3 yields a somewhat different carotenoid stoichiometry.

Our new interpretation of the ADMR spectra facilitates greatly the interpretation of the T-S spectra reported in [9]. Since triplet I absorbs at both 507 and 525 nm, there is no need anymore to conclude to a one-to-two carotenoid-Chl relation as in Ref. 9. For the Chl region (650–700 nm), we can now conclude, therefore, that the T-S spectra of the carotenoid having a triplet absorption at 507 nm show a Chl bleaching at 677 nm, whereas carotenoids having a triplet absorption at 525 nm are related to the Chls absorbing at 670 nm. Thus, it follows that the most important argument in favour of a dimeric Chl *a* organisation in LHC II put forward in Ref. 9 is now invalidated, and that every Car is associated to just one Chl. Of course, the 1:2:3 ratio of the relative intensities of the Chl *a* bands centered at 664, 670 and 677 nm, in the absorption spectrum recorded at 4 K, remains a valid observation.

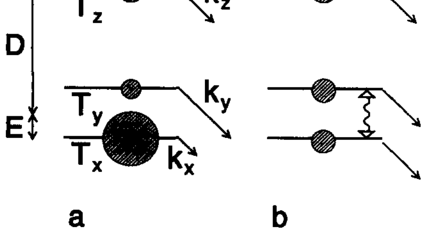

Fig. 4. (a) Qualitative polarisation pattern, from [8]. (b) The sum of the population of the T_x and T_y level is decreased by the application of resonant microwaves, which opens an additional decay channel for T_x via T_y.

Oligomerisation

The most important effect of the LHC II concentration on the ADMR spectra is the change in the relative intensity of the resonance bands of the $|D|+|E|$ transition, recorded at 507 and 525 nm, which changes from about 1 (at OD 0.05 mm^{-1}) to about 0.45 (at OD 0.9 mm^{-1}) (inset Fig. 3). Probably, this effect also gives rise to the change in lineshape of the FDMR spectra recorded by Carbonera et al. [8].

From Fig. 3 and its inset two conclusions are drawn:

(1) the change in relative amplitude with the LHC II concentration is due to a relative increase of the $|D|+|E|$ transition, detected at 525 nm, compared to that of the $|D|+|E|$ transition detected at 507 nm.

(2) the $2|E|$ transition detected at 525 nm does *not* show a concentration effect, but behaves exactly like the $2|E|$ and $|D|+|E|$ transition recorded at 507 nm.

The concentration effect observed here and by Carbonera et al. [8] is probably the same effect as that described by Bassi *et al.* [2]: At ODs lower than 0.2 mm^{-1} (see inset Fig. 3) the CPII oligomers dissociate into trimeric LHC II units. The absence of the need for a shift in the central frequencies to fit the ADMR spectra at different ODs suggests that the dissociation process does not affect the intrinsic triplet properties of the carotenoids. This supports the concept of Bassi et al. [2], because the dissociation into trimers does not change the basic unit of LHC II.

In addition to the change in relative intensity, Fig. 3 shows that the width of the resonance bands of the $|D|+|E|$ transition recorded at 525 nm changes with the LHC II concentration, whereas the width of the resonance band detected at 507 nm remains the same. This decrease of the (inhomogeneous) width of the $|D|+|E|$ transition suggests that at higher LHC II concentrations, regular, tightly bound oligomeric structures are formed, providing a more uniform environment of the carotenoid triplet state than the trimers at low OD.

Considering the polarization pattern proposed by Carbonera et al. [8] (Fig. 4), and the symmetry properties of a carotenoid molecule (with the triplet z-axis being directed along the long axis of the molecule and the triplet x and y axes perpendicular to this axis), there are two kinds of mechanisms that can give rise to the observed increase in relative intensity of the $|D|+|E|$ transition, namely mechanisms that result in an increase in the population of the T_x level and mechanisms that result in a decrease in the population of the T_z level.

The relative population of triplet sublevel i under steady-state illumination is proportional to p_i/k_i, the ratio of its population probability p_i and relaxation rate k_i. The relaxation rates are an intrinsic property of the carotenoid, and as such not likely to change with the sample concentration. Therefore, we conclude that the relative increase with the sample concentration of the ADMR signal intensity of the $|D|+|E|$ transition recorded at 525 nm, is most likely due to an increase of p_x (and p_y) and a decrease of p_z, caused by a change in sublevel selectivity of triplet-triplet transfer from a chlorophyll to the carotenoid. This selectivity may be caused by a specific arrangement of the two triplet-carrying molecules, through which the projection of the dipole tensor of ^{3}Car onto that of ^{3}Chl favours transfer to the T_x (T_y) level [17]. Because the intensity of the $2|E|$ transition detected at 525 nm does not show an increase additional to the normal increase with the concentration, $\Delta p_x/\Delta p_y \approx k_x/k_y$.

The increase of the intensity of the $|D|+|E|$ transition detected at 525 nm is probably caused by an increase in the total triplet concentration, where the extra population is located only on the triplets absorbing at 525 nm. For this, it suffices that for high LHC II concentration the oligomeric structure allows an 'inter-trimer' transfer of triplets within the oligomer, providing a mechanism by which the number of chlorophylls that can transfer a triplet to the 525 nm triplet-carotenoids increases. If the normal of the plane of the chlorophylls is more or less perpendicular to the long axis of the carotenoid, then the x and y levels will be populated predominantly (Fig. 5). Such an 'inter-trimer' energy transfer is suggested by the quenching of the Chl b fluorescence band upon aggregation as observed by Ide et al. [10]. This interpretation suggests that the 525 nm triplet-carotenoids are located at a site that plays an important role in 'inter-trimer' interactions, and that the carotenoid giving rise to the 507 nm triplet is buried inside the LHC II trimer, where it is not affected by oligomerisation effects.

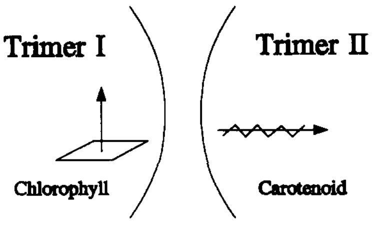

Fig. 5. Model of the mutual directions of the lutein and the chlorophyll, located on different trimers that are involved in inter-trimer triplet transfer. The arrows indicate the triplet z-axes of the chlorophyll and the carotenoid.

Conclusions

The relative intensity of the ADMR-detected $|D|+|E|$ transitions of carotenoid triplets in isolated LHC II, recorded at 507 and 525 nm, changes with concentration. This change is due to an increase with concentration of the signal amplitude of the ADMR spectrum of the $|D|+|E|$ transition detected at 525 nm relative to all other transitions. The effect is probably due to an oligomerisation process at high protein concentration in which oligomeric LHC II is formed from trimeric LHC II (the minimal unit of LHC II organisation [1]).

Taking into account the narrowing upon oligimerisation of the resonance bands constituting the spectra and the absence of bandshifts, the effects can be explained by assuming that the oligomer consists of a multiple of trimers, between which 'inter-trimer' energy transfer occurs. This leads to an increase in the number of chlorophylls that can transfer a triplet to the carotenoid. From the Gaussian deconvolution of the ADMR spectra we conclude that there is a one-to-one carotenoid-Chl a association and that the interpretation of Ref. 9, in which a dimeric Chl a organisation was derived from a one-to-two carotenoid-Chl a association, is no longer tenable.

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