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Steady-state and transient polarized absorption spectroscopy of photosytem I complexes from the cyanobacteria *Arthrospira platensis* and *Thermosynechococcus elongatus*

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Abstract

Core antenna and reaction centre of photosytem I (PS I) complexes from the cyanobacteria *Arthrospira platensis* and *Thermosynechococcus elongatus* have been characterized by steady-state polarized absorption spectroscopy, including linear dichroism (LD) and circular dichroism (CD). CD spectra and the second derivatives of measured 77 K CD spectra reveal the spectral components found in the polarized absorption spectra indicating the excitonic origin of the spectral forms of chlorophyll in the PS I complexes. The CD bands at 669–670(+), 673(+), 680(−), 683–685(−), 696–697(−), and 711(−) nm are a common feature of used PSI complexes. The 77 K CD spectra of the trimeric PS I complexes exhibit also low amplitude components around 736 nm for *A. platensis* and 720 nm for *T. elongatus* attributed to red-most chlorophylls. The LD measurements indicate that the transition dipole moments of the red-most states are oriented parallel to the membrane plane. The formation of $P700^+A_1^-$ or $^3P700$ was monitored by time-resolved difference absorbance and LD spectroscopy to elucidate the spectral properties of the PS I reaction centre. The difference spectra give strong evidence for the delocalization of the excited singlet states in the reaction centre. Therefore, P700 cannot be considered as a dimer but should be regarded as a multimer of the six nearly equally coupled reaction centre chlorophylls in accordance with structure-based calculations. On the basis of the results presented in this work and earlier work in the literature it is concluded that the triplet state is localized most likely on P_A, whereas the cation is localized most likely on P_B.

Keywords: Photosystem I; P700; Linear and circular dichroism; Excitonic coupling; Long-wavelength antenna chlorophyll; *Arthrospira platensis*; *Thermosynechococcus elongatus*

1. Introduction

Photosystem I (PS I) mediates the light-driven electron transfer from reduced plastocyanin or cytochrome c_6 on the lumenal side to ferredoxin on the stromal side of the thylakoid membrane (for a review see Refs. [1,2]). The PS I core complex is a multisubunit pigment–protein complex containing the reaction centre and a core antenna. In higher plants and algae, a peripheral light-harvesting complex I (LHC I) is closely associated with the PS I core complex. The PS I core complexes of cyanobacteria which lack the LHC I can be isolated both as monomers and as trimers [3–5].

Abbreviations: A_0, primary electron acceptor in PS I; A_1, secondary electron acceptor in PS I (a phylloquinone); Chl, chlorophyll; Cxxx, Chl absorbing at xxx nm; β-DM, n-dodecyl-β-maltoside; Fe–S, iron–sulfur-cluster; F_X, F_A and F_B, three [4Fe–4S] clusters in PS I; LWC, long-wavelength Chls; P700 ($P700^+$), primary electron donor of PS I in the reduced (oxidized) state; $^3P700$, P700 in the excited triplet state; P_A and P_B, the two Chls constituting 700 coordinated by PsaA and PsaB subunits, respectively; PMS, phenazine methosulfate; PS I, photosystem I; RT, room temperature; T-S, triplet-minus-singlet

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Though only the three-dimensional structure of trimeric PS I core complexes from *Thermosynechococcus elongatus* has been determined with high resolution [6], it is generally assumed that the PS I core complexes, and especially the cofactor arrangement in the reaction centre, are similar in all organisms. This is supported by the recent X-ray structure of plant PS I [7]. The structural model at 2.5 Å [6] includes twelve protein subunits, 96 Chls, 22 carotenoids (Car), two phylloquinones, three iron–sulfur [4Fe–4S] clusters, four lipids, about 200 water molecules and a metal ion (presumably Ca^{2+}) for each PS I monomer.

The two large subunits, PsaA and PsaB, each consisting of 11 transmembrane helices, coordinate most of the antenna pigments and the following redox cofactors involved in the electron-transfer process. P700 is a heterodimer comprised of Chl *a* (eC-B1P_B) and *a′* (eC-A1; P_A) (nomenclature of Jordan et al. [6] is used for naming cofactors). It should be noted that the two branches of cofactors related by pseudo-C_2 symmetry connect P700 and the first [4Fe–4S] iron sulfur cluster F_X. Each branch is composed of two Chls (Acc-A (eC-B2) and A_0-A (eC-A3) in the A-branch and Acc-B (eC-A2) and A_0-B (eC-B3) in the B-branch) and one phylloquinone (Q_K-A and Q_K-B, respectively). The terminal electron acceptors F_A and F_B (two [4Fe–4S] iron–sulfur-clusters) are both coordinated by subunit PsaC, one of the three extrinsic subunits located on the stromal side.

Most of the Chls are located near the stromal and lumenal membrane surface. Within these two layers a ring-shaped arrangement of antenna Chls is visible [8]. The Chls of these two rings are well connected among each other via Chls located in the membrane-integral part of the protein and with Chls located peripherally on the stromal and lumenal side, respectively. The centre-to-centre distance between any core antenna Chls and the reaction centre Chls is larger than 18 Å, except for the two so-called connecting Chls (antenna Chls A40 ands B39 that seem to provide a link between the antenna system and the reaction centre). The mean centre-to-centre distance between neighbouring Chls is 9.9 Å, which is only about 30% larger than the diameter of the chlorin ring. This dense packing indicates that excitonic interactions have to be taken into account for a description of the spectral and functional properties of PS I [8].

Strong excitonic and charge-transfer interactions of Chls with red-shifted site-energies due to their local protein environment are most probably the reason for so-called red antenna states or long-wavelength chlorophylls (LWC) which absorb at energies below that of the primary electron donor P700 (for a recent review, see [9]). Energy absorbed by LWC is transferred uphill to P700 with high efficiency at room temperature (RT), thus increasing the cross section for the absorption of red light [10,11]. Furthermore, LWC play an important role in the dissipation of excess energy absorbed by PS I [9,12]. The content and the spectral characteristics of LWC antenna states are species dependent. PS I trimers of cyanobacteria contain usually more red Chls than monomers. The 5 K absorption spectrum of PS I trimers of *T. elongatus* exhibits Chl antenna states absorbing at 708 and 720 nm [11]. The most-red Chl antenna state absorbing at 740 nm (C740) and emitting at 760 nm (77 K) is present in PS I trimers of *Arthrospira platensis* [4,12].

In this work, we used steady-state polarized absorption spectroscopy, including linear dichroism (LD) and circular dichroism (CD), to characterize the antenna and the reaction centre of monomeric and trimeric PS I complexes from the cyanobacteria *T. elongatus* and *A. platensis*. Especially, the properties of the LWC which have very pronounced effects on energy transfer and trapping in PS I are analyzed in dependence of the oligomeric structure for both organisms. Since the reaction centre cofactors and the core antenna pigments are inseparably bound to the same protein, information on the spectral properties of the reaction centre can only be obtained by polarized time resolved absorption difference spectroscopy which enables to monitor the changes in absorption, LD, and CD upon light-induced formation of transient functional states (charge separated states or ^{3}P700) within the reaction centre. Because of the difference, only the pigment that changed its electronic state and pigments which are coupled to this pigment contribute to the spectra.

2. Materials and methods

2.1. Preparation of PSI complexes

Monomeric and trimeric PS I core complexes from *A. platensis* have been prepared as described in Schlodder et al. [12]. PS I trimers from *T. elongatus* have been prepared according to [13]. PS I monomers from *T. elongatus* have been prepared essentially according to [14]. The amount of PS I monomers was increased by a 20 min preincubation of the thylakoid membranes in buffer containing 0.6 M ammonium sulfate. After extraction with 1% n-dodecyl-β-maltoside (β-DM) and ultracentrifugation the PS I monomers have been isolated by hydrophobic interaction chromatography (Poros 50 OH, Applied Biosystems, Germany) followed by ionic exchange chromatography (Poros 50 HQ/M, Applied Biosystems, Germany).

2.2. Measurement of steady-state optical spectra

Absorption and LD spectra were measured as described [12]. For LD measurements the PS I complexes were oriented by embedding the proteins in a gelatine gel, which subsequently was squeezed by a compression factor p in one direction (for details see Ref. [12]). The LD is expressed as

$$\mathrm{LD} = \mathrm{A}_{\parallel} - \mathrm{A}_{\perp} = -3 \cdot \mathrm{A}_{\mathrm{iso}} \langle 0.5 \cdot (3 \cdot \cos^2 \vartheta - 1) \rangle \cdot \Phi(p) \quad (1)$$

where ∥ and ⊥ are defined with respect to the plane of the PS I complex embedded in the membrane referred to as membrane plane in this work; $A_{\parallel}$ and $A_{\perp}$ is the absorption of light polarized parallel and perpendicular to this plane; ϑ is the angle between the transition moment and the normal to the membrane plane; $\langle\ \rangle$ denotes averaging over all values of ϑ if different transition moments are involved and $\Phi(p)$ is an orientation function that reflects how well the PS I complexes are oriented as a function of the compression factor p [15]. The isotropic absorption A_{iso} is given by $(2\ A_{\parallel} + A_{\perp})/3$.

CD spectra were recorded on a spectropolarimeter JASCO-715 (Japan). The CD is expressed as $\mathrm{CD} = \theta = 33\ (A_L - A_R)$, where θ is the ellipticity (in degrees), and A_L (A_R) is the absorption of left and right circularly polarized light. A Peltier thermostat was used to keep the temperature at 10 °C in the 1 cm rectangular quartz cell. Measurements at 77 K were performed using plexiglass cells with an optical path length of 2 mm or 1 cm. The spectral band width was 3 nm for measurements at 10 °C and 2 nm for measurements at 77 K.

CD difference spectra (P700 oxidized-minus-reduced) were obtained by subtraction of the spectrum of the sample with reduced P700 from the

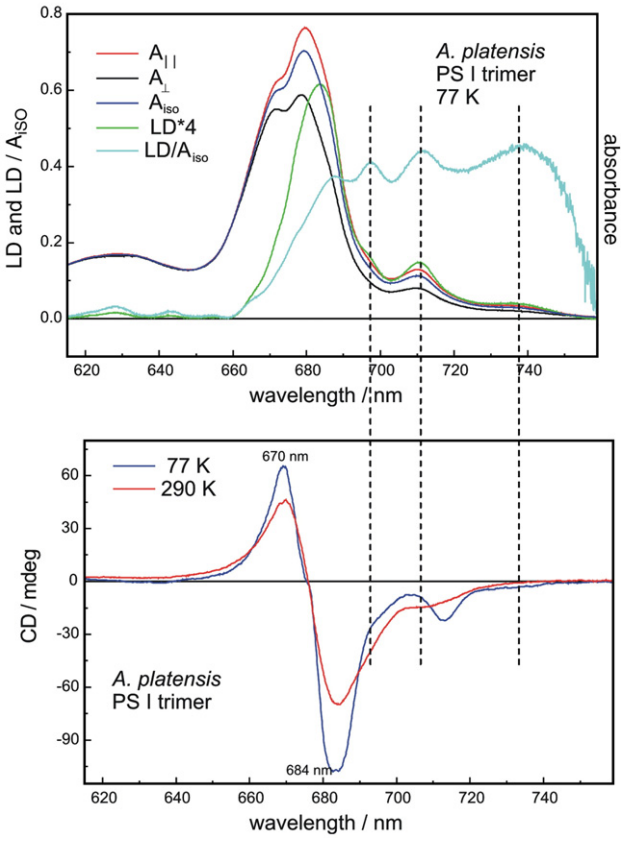

Fig. 1. Top: LD of trimeric PS I from *A. platensis*: steady-state polarized absorption spectra ($A_{\parallel}$ and $A_{\perp}$), LD spectra, $LD=A_{\parallel}-A_{\perp}$, and reduced LD spectra of trimeric PS I complexes at 77 K. The LD spectrum is multiplied by a factor of 4. Bottom: CD spectra of trimeric PS I from *A. platensis* at 77 K and 290 K.

spectrum of the sample with oxidized P700. Two approaches have been used to obtain P700 in reduced or oxidized state. (i) P700 was photooxidized by blue light illumination (10 mm LED, 470 nm, LDB13633 of company Ligitek). For keeping P700 in the reduced state in the absence of actinic illumination, 2 mM sodium ascorbate and 0.2–0.4 μM PMS were added to the sample; half-life of $P700^+$ dark reduction was 4–5 s. To diminish the influence of irreversible changes of the CD spectra the measurements under illumination were performed alternately with measurements in the dark. Difference spectra were calculated every cycle. Number of the cycles varied from 10 to 50. The calculated difference spectra have been averaged. (ii) The sample was divided in two parts. In one part P700 was oxidized by 0.1 mM ferricyanide and several CD spectra were measured immediately without changing the sample. In the second part 1 mM ascorbate and 1 μM PMS were added to the sample and several CD spectra were recorded.

Second derivatives of the spectra and Gaussian decomposition were performed using JASCO software programs and Origin 6.1.

2.3. Transient absorption spectroscopy

Flash-induced absorbance changes in the nano- to millisecond time range were performed with the set-ups described previously [16]. Flash-induced absorbance difference spectra of ($P700^+$–P700) at RT were measured with PS I complexes diluted to about 10–15 μM Chl in 20 mM Tricine (pH 7.5), 25 mM $MgCl_2$, 100 mM KCl, 0.02% β-DM, 5 mM ascorbate and 10 μM PMS.

Low temperature difference spectra of ($P700^+A_1^-$–$P700A_1$) and flash-induced triplet-minus-singlet (T-S) spectra (^{3}P700–P700) were measured as described in [17].

3. Results and discussion

3.1. Steady-state polarized absorption spectra of PS I complexes

Monomeric and trimeric PS I core complexes from the cyanobacteria *A. platensis* and *T. elongatus* were characterized by steady-state, polarized optical spectroscopy at 77 K. Fig. 1 (top) shows the absorption ($A_{\parallel}$, $A_{\perp}$, and A_{iso}), LD, and reduced LD (LD/A_{iso}) spectra of trimeric PS I from *A. platensis* at 77 K in the Q_Y region. The spectra resemble closely the previously reported spectra measured at 5 K [12]: However, in the reduced LD (LD/A_{iso}) spectrum four distinct bands around 688, 697, 711, and 737 nm are substantially better resolved due to a larger degree of orientation induced by unidirectional squeezing of the gelatine gel. In agreement with results reported for PS I from *A. platensis* and other species [12,18,19] the transitions at longer wavelengths ($\lambda>675$ nm) lie preferentially parallel to the membrane plane. The maximum of the LD spectrum is observed at 683.5 nm, i.e. about 5 nm red-shifted compared to the absorption maximum. On the short-wavelength side of the main peak, the LD is nearly zero, i.e. absorption due to transition moments with preferential orientation parallel to the membrane plane are quite well compensated by absorption due to transition moments oriented perpendicular to the membrane plane.

The reduced LD (LD/A_{iso}) is maximal for the red-most band at 740 nm. Values of 0.4–0.6 could be attained. Using a mathematical model for the orientation of disc-shaped protein complexes [15] the maximal orientation factor $\Phi(p)$ can be estimated to be about 0.40–0.45. The degree of orientation by this method is limited because rupturing of the gel occurs if the compression factor p is higher than about 2. Using Eq. (1), the angle ϑ between the 740 nm transition moment and the normal to the membrane plane can be calculated to $80°<\vartheta<100$ i.e. the transition moment of the red-most state is oriented virtually parallel to the membrane plane. For the absorbance band around 710 nm the reduced LD is only slightly lower, demonstrating that the transition moments of C710 lie also preferentially parallel to the membrane plane.

CD spectra of the trimeric PS I complexes from *A. platensis* at 77 K and at 290 K are presented in Fig. 1 (bottom). The amplitude of CD at low temperature is larger than that at RT. Both spectra display a negative band at 684 nm and a positive band at 670 nm of unequal rotational strength. Excitonically coupled Chls of PS I contribute predominantly to the CD intensity since the underlying CD due to the intrinsic chirality of monomer Chl in solution is usually much weaker. The separation in energy between the positive and negative peak would correspond to mean excitonic interaction energy of about 150 cm^{-1}. The 77 K spectrum shows in addition a distinct negative band at 713 nm and two negative CD components giving rise to shoulders around 697 and 736 nm. These features can be attributed to the low exciton band of coupled LWC. The corresponding CD from the high energy exciton band seems to be obscured by the strong CD bands at 684 and 670 nm. Negative band at 713 nm was most prominent in earlier reported

CD spectra of thylakoid membranes of *A. platensis* [4]. However, the feature due to the red-most Chls absorbing around 738 nm at 77 K has not been resolved in the former studies [20,21], whereas position and amplitude of the main bands coincide. The sensitivity of the 713 nm band in the 77 K CD spectrum to detergent treatment may indicate a location of the responsible LWC near the protein surface [20].

The absorption and LD spectra of trimeric PS I complexes from *T. elongatus* at 5 K and CD spectra at 77 K and 290 K are shown in Fig. 2. In the reduced LD spectrum distinct bands at about 688, 698, 710 nm, and about 722 nm are well resolved. The LD/A_{iso} ratio is again maximal for the red antenna states of trimeric PS I from *T. elongatus* (LD/A_{iso}=0.5–0.6) suggesting that also for this organism the transition moments of the red states are oriented virtually parallel to the membrane plane. The CD spectra resemble closely those reported previously [8,17]. The CD spectrum of the trimeric PS I complex from *T. elongatus* at 77 K also show a separate band at 711 nm with a shoulder around 720 nm which has been resolved in this work for the first time. The bands can be attributed to the LWC. The low amplitude of CD signal observed for the red most states of *A. platensis* and of *T. elongatus* might be the result of the specific organization of the excitonically coupled Chls. Calculations based on the

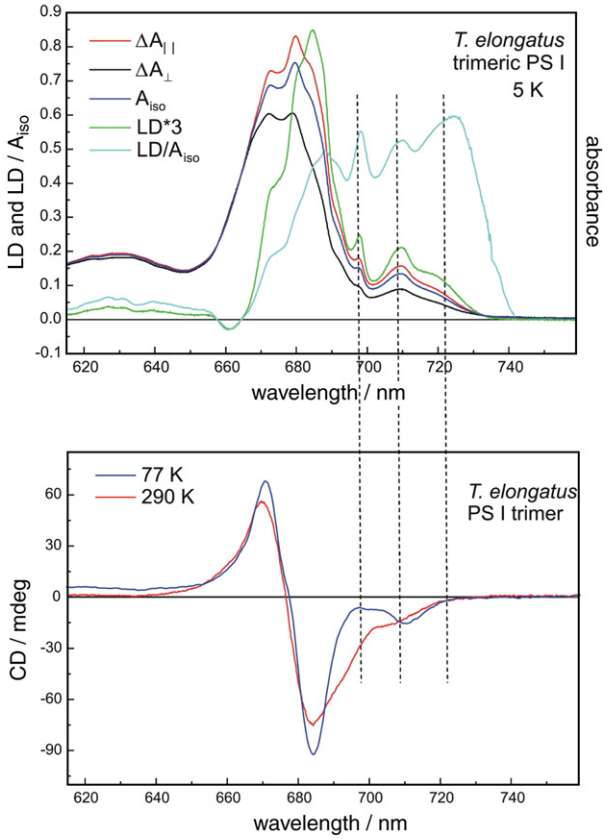

Fig. 2. Top: LD of trimeric PSI from *T. elongatus*: steady-state polarized absorption spectra ($A_{\parallel}$ and $A_{\perp}$), LD spectra, LD=$A_{\parallel}-A_{\perp}$, and reduced LD spectra of trimeric PS I complexes at 5 K. The LD spectrum is multiplied by a factor of 3. Bottom: CD spectra of trimeric PSI from *T. elongatus* at 77 K and 290 K.

Table 1
Spectral bands in low temperature absorption and CD spectra of PSI complexes of cyanobacteria (main bands marked bold)

Abs 77 K *A. platensis*	Abs 5 K *T. elongatus*	CD 77 K *A. platensis*	CD 77 K *A. platensis*	CD 77 K *T. elongatus*	CD 77 K *T. elongatus*
		Trimer	Monomer	Trimer	Monomer
661	662	664(+)		663(+)	662(+)
666	**667**	669(+)	**669**(+)		668(+)
671	**672**	**673(+)**	**673**(+)	**672**(+)	**671**(+)
677	675		677(−)		678(−)
680	**680**	**681(−)**	680(+)	681(−)	
684	**685**	**686(−)**	**684(−)**	**685(−)**	**685(−)**
687	**688**				
691	**692**			690(−)	
	695	**696(−)**	**696**(−)	(695)(−)	**697(−)**
697	**698**				
708 [a]	707				
711	**710**	**713**(−)	**713(−)**	**711**(−)	**712**(−)
	719 [a]			719(−) [a]	
735 [a]	–	735(−) [a]			

[a] Bands with large band width and Stokes shift.

2.5 Å structure of trimeric PS I complex from *T. elongatus* [6] show for example that the lowest exciton bands of the Chl trimers A31A32B7 and B31B32B33 have small rotational strengths. Another reason might be the large band width of the red antenna states of about 200 cm^{-1} [12].

For modeling energy transfer and trapping in PS I, it is of crucial importance to identify the LWC in the three-dimensional structure available for the trimeric PSI complex from *T. elongatus* at 2.5 Å resolution [6]. Taking into consideration that the transition moments of the long-wavelength antenna states are oriented almost parallel to the membrane plane, calculations on the basis of the 2.5 Å X-ray structure show that the following strongly coupled Chl aggregates remain as possible candidates for LWC: (1) A31–A32–B7 with ϑ=95.5° (ϑ is the angle between the transition moment of the lowest exciton transition and the normal to the membrane plane) and D=1.5 (D is the dipole strength of the low-energy exciton transition given as a multiple of the dipole strength of a monomeric Chl molecule); (2) B18–B19 with ϑ=85° and D=1.9; (3) A26–A27 with ϑ=90.2° and D=0.8; (4) B24–B25 with ϑ=85.5° and D=0.8; (5) B37–B38 with ϑ=91.3° and D=0.4, (6) A38–A39 with ϑ=96.1° and D=0.8; (7) A16–A17–A25 with ϑ=107.6° and D=1.6; (8) B14–B15–B23 with ϑ=107.9° and D=1.6, and (9) B31–B32–B33 with ϑ=111.5° and D=2.9. Interestingly, more than one strongly coupled aggregate is required to match the observation that the dipole strength of C720 (C710) corresponds to four (five) Chls. Different structure-based calculations have been performed to explain the spectral properties and the excitation energy transfer in PS I [8,22–27]. All of these studies agree that the strongly coupled aggregate (A31–A32–B7) located on the lumenal side close to the trimerization domain of the trimeric complexes is involved in the C720 spectral form of PSI from *T. elongatus* The assignment of the most-red antenna state to the trimer B31–B32–B33 which was suggested based on modeling fluorescence kinetics data [8,24,26,27] has been questioned in Refs.

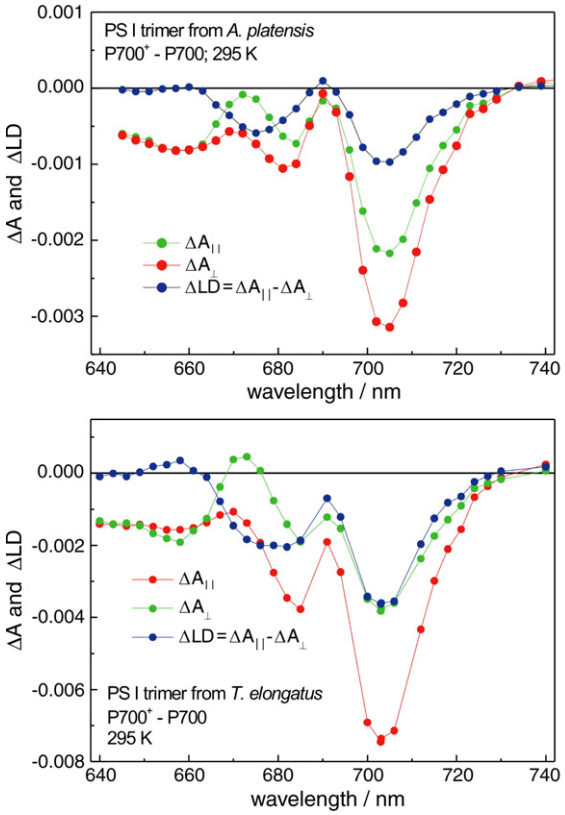

Fig. 3. ($P700^+$–P700) absorbance and LD difference spectra of trimeric PS I complexes from *A. platensis* (top) and *T. elongatus* (bottom) measured by flash-induced absorbance changes at room temperature. $\Delta A_{\parallel}$ ($P700^+$–P700), $\Delta A_{\perp}$ ($P700^+$–P700), and ΔLD ($P700^+$–P700).

[22,23,28]. There are two dimers (A38–A39 and B37–B38) where the upper exciton state carries most of the oscillator strength. The ratio between the oscillator strengths of the lower and upper exciton states is about 0.2. Therefore, the assignment of A38–A39 and B37–B38 to the long wavelength absorption [8,26–28] would explain only about 15% of the dipole strength of the red states. Damjanovic et al. [23] have been proposed the dimers B24–B25 and A26–A27 to be red Chls which would be in accordance with our LD data (see above).

Spectral components resolved in the low temperature polarized absorption spectra and the CD spectra for both organisms are summarized in Table 1. They have been identified by analyzing the spectra using Gaussian decomposition and by second derivatives. Several pronounced minima in the second derivative of the various spectra (not shown) give an indication for the wavelengths of maximal absorption of the various spectral forms. Remarkably, the second derivatives of the 77 K CD reveal most of the spectral components which has been found in the (polarized) absorption spectra at low temperature indicating the excitonic origin of the spectral forms of Chl in PS I complexes. Bands at 669–670(+), 673(+), 680(−), 683–685(−), 696–697(−), and 711–713(−) have been revealed as a common feature of all types of PS I complexes (see Table 1, (+) and (−) indicates positive and negative CD bands).

3.2. Spectral properties of the reaction centre of PS I resolved by polarized absorption difference spectra of PS I complexes at room temperature

Since the reaction centre cofactors and the core antenna pigments are inseparably bound to the same protein, information on the spectral properties of the reaction centre can be obtained by polarized time resolved absorption difference spectroscopy. Following the changes in absorption, LD, and CD upon light-induced formation of transient functional states in the reaction centre, e.g. $P700^+$, $P700^+A_0^-$, or $^3P700$, optical difference spectra of PS I core complexes are obtained. Only the pigment(s) that changed the electronic state and pigments which are coupled to this (these) pigment(s) contribute to difference spectra. Difference spectra associated with the formation of charge separated states, e.g. $P700^+A_1^-$, may additionally contain electrochromic (Stark) shifts of absorbance bands of pigments located in the vicinity of the cofactors carrying a positive or negative charge.

At RT the difference in absorption of a PS I complex in the ground state and a PS I complex with oxidized primary donor was studied. The flash-induced absorbance changes associated with the oxidation of P700 were monitored as a function of wavelength for each polarization of the measuring light. Fig. 3 shows the ($P700^+$–P700) absorbance difference spectra ($\Delta A_{\parallel}$ and $\Delta A_{\perp}$) and the LD of flash-induced absorbance changes, $\Delta LD = \Delta A_{\parallel} - \Delta A_{\perp}$, of trimeric PS I complexes from *A. platensis* (top) and *T. elongatus* (bottom) at 295 K. The isotropic absorbance difference spectra $\Delta A_{iso} = (2\Delta A_{\parallel} + \Delta A_{\perp})/3$ resemble closely those published previously [17]. The polarized ($P700^+$–P700) ΔA spectra exhibit the main bleaching band at 703 nm and a second smaller bleaching band at about 682 nm. The zero crossing in the red region is at about 730 nm. Interestingly, different signs of $\Delta A_{\parallel}$ and $\Delta A_{\perp}$ are observed around 672 nm (see Fig. 3 bottom) indicating the overlap of two bands with opposite polarization in this spectral region. The transition moment associated with the absorbance increase around 672 nm giving rise to positive $\Delta A_{\perp}$ is oriented preferentially perpendicular to the membrane plane, whereas the transition associated with the stronger bleaching around 682 nm lies preferentially parallel to the membrane plane. The broad bleaching band around 658 nm shows a small positive LD indicating an angle between the transition and the membrane normal of <54°. Using trimeric PS I complexes from *T. elongatus* the polarized absorption difference spectra have been measured for different degrees of orientation, i.e. different compression factors p. The shape of the ΔLD spectra was always the same (not shown). It shows two negative bands at 703 nm and around 676 nm. In the main bleaching band around 703 nm the maximal reduced ΔLD ($=\Delta LD/\Delta A_{iso}$) was 0.7 corresponding to a dichroic ratio $\Delta A_{\parallel}/\Delta A_{\perp}$ of 2.4. The orientation is even somewhat higher than that observed for the LWC (see above). The large dichroic ratio $\Delta A_{\parallel}/\Delta A_{\perp}$ of >2 in the main bleaching band is in agreement with the work of Breton [29] who used magnetically oriented spinach chloroplasts. The data support the conclusion [29] that the transition moment responsible for the absorption band around 703 nm,

which corresponds most probably to the transition from the ground state to the lowest excited exciton state of the reaction centre Chls, is oriented parallel to the membrane. The reduced ΔLD in the smaller bleaching band around 682 nm has the same sign and is only slightly smaller than that in the main bleaching band. It has been proposed in the literature that these two bands can be attributed to the two exciton bands of the dimer comprised of P_A and P_B [29–32]. The appearing Q_Y absorption band of the non-oxidized, monomeric Chl within $P700^+$ has been positioned around 690 nm between the two bleachings. In addition, electrochromic shifts of nearby Chls to the formation of $P700^+$ have been suggested [see ref. 1 for a more detailed discussion of earlier literature]. Based on the 2.5 Å structure it can be calculated that the angle between the membrane normal and the transition moment related with the long-wavelength exciton component is about 88°, whereas the angle related with the short-wavelength exciton component is 176°. Consequently a strong positive ΔLD at the position of the upper exciton band would be expected. The coupling between P_A and P_B has been calculated to be about 420 cm^{-1} in the point dipole approximation corresponding to a splitting between the upper and lower exciton bands of about 40 nm, i.e. the position of the short-wavelength exciton component would be around 664 nm [8]. The ratio of the oscillator strength for the transitions to the upper and lower exciton states is about 0.31. Using the extended dipole approximation or the transition monopole method, one obtains a more reliable value of about 150 cm^{-1} for the coupling corresponding to a splitting between the upper and lower exciton bands of about 15 nm, i.e. the position of the short-wavelength exciton component would be around 688 nm [8]. The ΔLD spectra do not exhibit any strong positive band which indicates that a model which takes into account only the excitonically coupled dimer comprised of P_A and P_B and minor perturbations from the surrounding chlorophylls is not sufficient to explain the ΔLD features.

In contrast to [29], the reduced LD of the absorption increase in the near infrared around 820 nm due to the formation of the cation radical was significantly smaller then that of the main bleaching band. If the angle ϑ between the transition moment of the 703 nm transition and the normal to the membrane plane is about 90° we calculate an angle of about 65° between the near infrared transition of the cation and the membrane normal. The Q_Y transition moments of P_A and P_B, the two Chls constituting P700, form angles of about 63° (for P_A) and 60° (for P_B) with respect to the membrane normal [8]. Assuming a Y polarization of the near infrared transition as predicted by ab initio quantum mechanical calculations [33] this result gives strong evidence that the cation is mainly localized on one of the two Chls constituting P700. This would be in agreement with ENDOR studies of $P700^+$ which led to the conclusion that at least 85% of the spin density is localized on P_B [34]. It should be noted that Breton and coworkers have concluded from FTIR results that the charge distribution in $P700^+$ ranges from 1:1 to 2:1 in favor of P_B [see Ref. [35] for a detailed discussion and Refs. therein). The peak-to-peak amplitude of the two differential signals attributed to the keto groups of P_A and P_B has been used as an estimate of the charge distribution. In the model of an electronically coupled dimer, the observed upshift in frequency of the carbonyl stretching frequency should depend on the charge localization. However, the quantitative relationship between charge localization and peak-to-peak amplitude or frequency upshift is not well established. Furthermore, other structural changes upon cation formation may affect the carbonyl frequency especially if there is a direct interaction between one of the keto groups and its environment via a hydrogen bond.

The ($P700^+$–P700) CD difference spectra of trimeric and monomeric PS I complexes from *A. platensis* (top) and *T. elongatus* (bottom) at RT are shown in Fig. 4. The ΔCD spectra of the trimeric PS I complexes are dominated by a band with positive rotational strength around 702 nm and a band with negative rotational strength around 678 nm. In addition, the spectra display a negative component around 692 nm, which is more pronounced in trimeric PS I from *A. platensis*, and a small positive component at 686 nm. The spectra of the monomeric PS I complexes exhibit also a positive CD around 702 nm and a negative CD around 677 nm. However, the 686(+) and 692(−) nm bands are absent in the spectrum of PS I monomers from *T. elongatus*, instead of that a positive band is observed at 693 nm. In the spectrum of PS I monomers from *A. plaensis* the amplitude of the positive band at 686 nm is significantly larger. It should be noticed that light-induced and chemically induced ΔCD spectra (see Materials and methods) resemble each other closely (not shown).

Using molar extinction difference absorption coefficient of P700 ($\Delta\varepsilon$) for *A. platensis* 83.000 M^{-1} cm^{-1} and 61.000 M^{-1} cm^{-1} for *T. elongatus* [17], it is possible to calculate the molar dichroic extinction difference coefficient ($\Delta\varepsilon_{CD}$) of the low energy exciton transition at 702 nm:

$$\Delta\varepsilon_{CD} = \varepsilon_l - \varepsilon_r = \frac{\Delta\theta}{33 \cdot c \cdot d} = \frac{\Delta\theta \cdot \Delta\varepsilon}{33 \cdot \Delta A} \quad (2)$$

ε_l and ε_r are the extinction coefficients for light with left and right circular polarization, respectively, $\Delta\theta$ is the light-induced

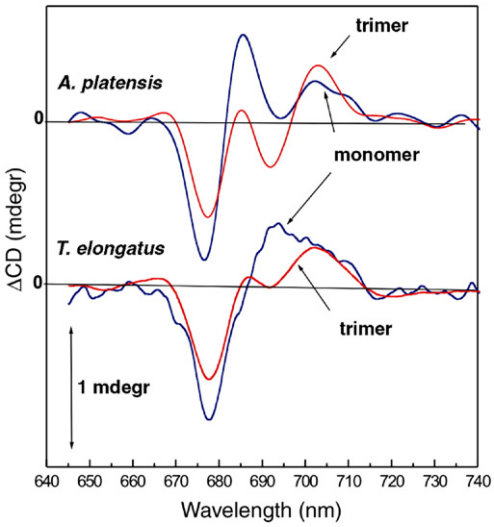

Fig. 4. Light induced ($P700^+$–P700) ΔCD spectra of PS I trimers and monomers from *A. platensis* and *T. elongatus*. Each trace is the average of 20 spectra; all spectra are normalized to $A_{680}=1$.

ellipticity change (in degrees), 33 is the transformation coefficient of ellipticity to dichroic optical density, c is the molar concentration of PS I centres, d is the optical path, ΔA is the light-induced absorption change at 702 nm.

Values between 110 and 130 M^{-1} cm^{-1} have been determined for the differential molar dichroic extinction coefficient at 702 nm. The obtained value of $\Delta\varepsilon_{CD}$ is similar to that measured earlier for PS I complexes from *A. platensis* [36]. A slightly higher value of 150 M^{-1} cm^{-1} has been reported previously for PS I trimers from *T. elongatus* [17]. The value of $\Delta\varepsilon_{CD}$ can be used for an estimation of the rotational strength R of the CD band at 702 nm by using the equation $R = 0.248 \cdot \int \frac{\Delta\varepsilon}{\nu} d\nu$. Integration was performed between ν=14493 cm^{-1} (690 nm) and ν=13986 cm^{-1} (715 nm). The estimated to be about 4 Debye–Bohr magneton.

The ($P700^+$–P700) ΔCD spectrum of trimeric PS I from *T. elongatus* resembles closely the previously published spectrum by Witt et al. [17] while the ΔCD spectra for PS I complexes from *A. platensis* differ significantly from earlier published ΔCD spectra [36]. This might be mainly due to the milder detergent β-DM used in this study, resulting in more intact PS I complexes than in case of more aggressive detergent Triton-X100 which has been used before.

The ($P700^+$–P700) ΔCD presented in this work and those from the literature [17,31,36,37] have in common a positive CD component at the position of the main bleaching band observed in the ($P700^+$–P700) absorption difference spectrum. In the literature this positive component has been attributed to the long-wavelength exciton band of the dimer comprised of P_A and P_B [31]. It has been discussed earlier [37] that this is in contradiction to the calculated CD arising from excitonic interaction between P_A and P_B. The calculation based on the 2.5 Å structure [6] results in a positive rotational strength of the low-energy exciton component while the high-energy exciton component has negative rotational strength [37]. Note that the ΔCD consisting of these components has a reversed sign, i.e. the long-wavelength component in the calculated ΔCD spectrum is negative instead of the observed positive sign. In addition, the ΔCD spectra shown in Fig. 4 and most of the ΔCD spectra in the literature show more than two components. Therefore, all the exciton interactions between the reaction centre Chls, at least those between P_A, P_B, and the accessory Chls, have to be taken into account when calculating ($P700^+$–P700) difference spectra [37–39]. This can be rationalized if the coupling strengths between neighbouring Chls in the reaction centre are of comparable magnitude. This is the case if the exciton coupling strengths are calculated by the extended-dipole-approximation or more sophisticated quantum chemical methods [8,39]. Values between 100 and 160 cm^{-1} have been obtained for the interaction energies of neighbouring Chls in the reaction centre of PS I [8,39].

Interestingly, the presented ΔCD spectra are different for different oligomeric organization (trimeric/monomeric) of the PS I complex and for different species, although it is generally assumed that the cofactor arrangement in the PS I reaction centre is always very similar. Whereas slight structural differences between different species are not implausible, our results indicate furthermore slight structural rearrangements within the reaction center as a function of the oligomeric state.

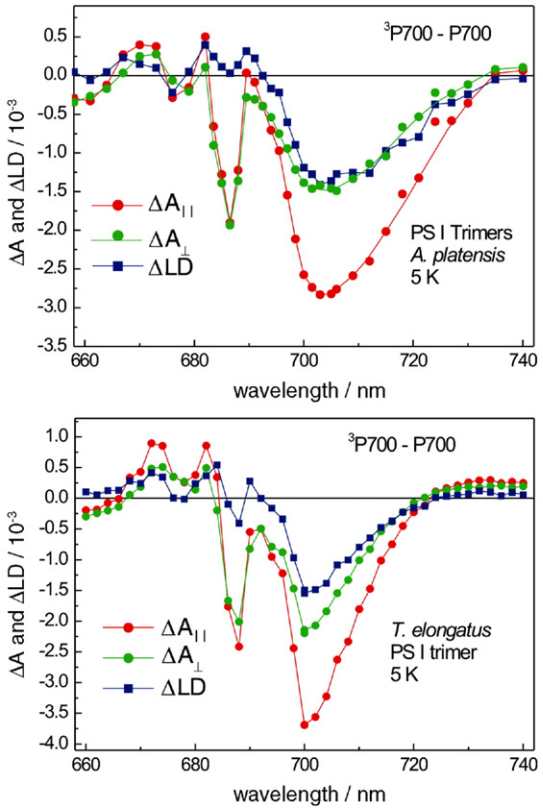

Fig. 5. (^{3}P700–P700) absorbance and LD difference spectra of trimeric PS I complexes from *A. platensis* (top) and *T. elongatus* (bottom) measured by flash-induced absorbance changes at 5 K. $\Delta A_{\parallel}$ (^{3}P700–P700), $\Delta A_{\perp}$ (^{3}P700–P700), and ΔLD (^{3}P700–P700).

3.3. Spectral properties of the reaction centre of PS I at low temperature—Comparison of difference spectra associated with the formation of the triplet and the oxidized state of P700

Fig. 5 shows (^{3}P700–P700) absorbance ($\Delta A_{\parallel}$ and $\Delta A_{\perp}$) and LD difference spectra of trimeric PS I complexes from *A. platensis* (top) and *T. elongatus* (bottom) measured by flash-induced absorbance changes at 5 K with a spectral resolution of 3 nm. The isotropic absorbance difference spectra $\Delta A_{iso} = (2\Delta A_{\parallel} + \Delta A_{\perp})/3$ are similar to those reported previously [17]. The T-S spectra were measured using PS I complexes with the secondary electron acceptor A_1 in the reduced state. Therefore, the electron transfer to A_1 is blocked and the primary radical pair, $P700^+A_0^-$, recombines to the triplet state of P700 with high yield at 5 K. The ΔA spectra of trimeric PS I complexes from *A. platensis* (see Fig. 5, top) exhibit the broad main bleaching around 704 nm and a second bleaching around 687 nm. Positive bands are located at 682 and 672 nm. The spectral features are similar for PS I from *T. elongatus* and *A. platensis*, except that the main bleaching band around 701 is significantly narrower in PS I from *T. elongatus* than that measured with PS I from *A. platensis*. The complex shape of the experimental T-S spectra

gives additional evidence for the coupling between all reaction centre Chls and hence for the delocalization of excited states. In the case of weak coupling the appearance of just one negative band is expected at the position of the site energy of the pigment that changed its electronic state. Because of the delocalization of the excited singlet states the whole spectrum of the excitonically coupled Chls is changed upon converting one pigment into the triplet state. Therefore, a multimer model, originally proposed for P680 of PS II [40] has to be used to describe the spectral properties of the PS I reaction centre. P700 should not be considered as a dimer but as a multimer of the six nearly equally coupled reaction centre Chls. This conclusion is supported by ultrafast optical spectroscopy at 10 K [41]. Excitation of PS I complexes from *Chlamydomaonas reinhardtii* at 700 nm leads to a structured initial ΔA with four positive and four negative bands spread over the range of 634 nm to 695 nm which was explained in terms of excitonic coupling between all six reaction centre Chls [41]. Recently proposed models [42,43] in which the initial charge separation starts from the excited accessory Chl in one or both branches, Acc-A and/or Acc-B, are also based upon the assumption that the lowest excitonic state, that drives the initial charge separation, is delocalized over these six Chls. However, it needs to be clarified to what extent the accessory Chls contribute to the lowest exciton state which in turn requires the knowledge of the site energies of the reaction centre Chls [see Appendix of Ref. [8] and Ref. [39]].

The ΔLD spectra of the flash-induced absorbance changes associated with the formation of the triplet state of P700 exhibit a large reduced LD of 0.47 (dichroic ratio = $\Delta A_{\parallel}/\Delta A_{\perp} = 1.74$) in the maximum of the main bleaching band at 702 nm. This confirms that the transition moment of the low energy exciton band is oriented parallel to the membrane.

The ($P700^{+}A_1^{-}$–$P700A_1$) absorbance ($\Delta A_{\parallel}$ and $\Delta A_{\perp}$) and LD difference spectra ($\Delta A_{\parallel} - \Delta A_{\perp}$) of trimeric PSI complexes from *A. platensis* and *T. elongatus* measured by flash-induced absorbance changes at 77 K are presented in Fig. 6. The low temperature spectra are again dominated by the broad negative band at 704 nm attributed to the disappearance of the low energy exciton band upon oxidation of P700, a strong narrow positive band at about 690 nm and a smaller bleaching around 682 nm. The low energy exciton band is very broad even at 5 K probably due to strong pigment–protein coupling and to mixing with charge transfer states. Remarkably, the width of this band is nearly temperature independent. A line width of 300 cm^{-1} mainly due to homogeneous broadening has been determined by hole-burning experiments [44]. The oxidized-minus-reduced spectra exhibit, in addition, contributions from electrochromic band shifts induced by the positive charge localized on the oxidized P700 and the negative charge located on A_1. Absorption changes in the Qy region induced by the electron transfer from A_1^{-} to F_X have been assigned to electrochromic shifts of absorption bands of the chlorophylls in the vicinity of the secondary electron acceptor A_1 [39,45]. The ΔLD spectra are characterized by two negative bands of about equal amplitude at 703 nm and 670 nm, and a strong positive band around 690 nm. Similar to the ($P700^{+}$–P700) difference spectra at RT, different signs of $\Delta A_{\parallel}$ and $\Delta A_{\perp}$ are observed around

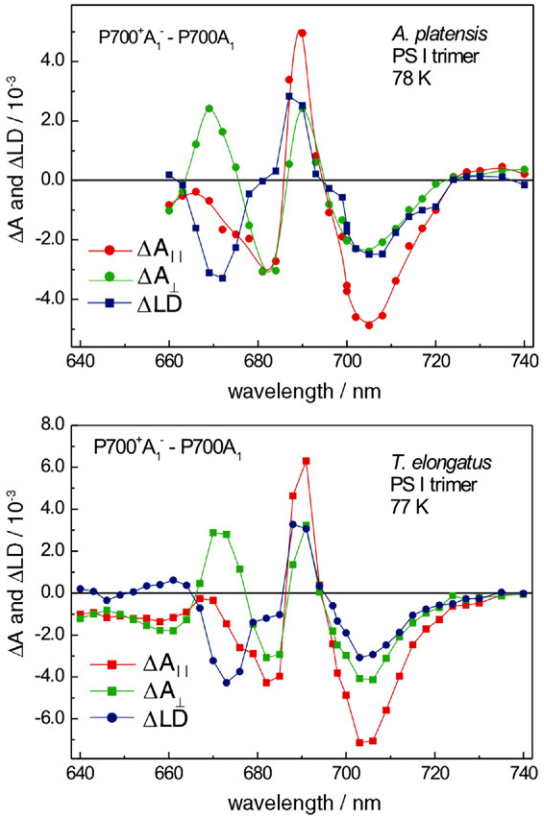

Fig. 6. ($P700^{+}A_1^{-}$–$P700A_1$) absorbance and LD difference spectra of trimeric PSI complexes from *A. platensis* (top) and *T. elongatus* (bottom) measured by flash-induced absorbance changes at 77 K. $\Delta A_{\parallel}$ ($P700^{+}A_1^{-}$–$P700A_1$), $\Delta A_{\perp}$ ($P700^{+}A_1^{-}$–$P700A_1$), and ΔLD ($P700^{+}A_1^{-}$–$P700A_1$).

671 nm (see Figs. 3 and 6) indicating the overlap of two bands with opposite polarization in this spectral region giving rise to the overall strong negative LD around 670 nm. The LD of the smaller bleaching around 682 nm is practically zero for PS I trimers of *A. platensis* and slightly negative for PS I trimers from *T. elongatus*. The broad bleaching band around 658 nm shows a small positive LD indicating an angle between the transition and the membrane normal of < 54°.

For the interpretation of the difference spectra, information on the localization of the cation radical and the triplet state is required. Virtually identical zero-field-splitting (ZFS) parameters of ^{3}P700 and of monomeric ^{3}Chl *a* or ^{3}Chl *a*′ in organic solvents at low temperature [17] were taken as evidence that the triplet state of P700 is mainly localized on one Chl. In addition, the orientation dependence of the triplet state showed that the plane of the Chl which carries the triplet state is oriented perpendicular to the membrane [46] i. e. the triplet state should be localized on P_A or P_B. The quite different shape of the oxidized-minus-reduced spectra compared to that of the T-S spectra indicates that the positive charge and the triplet state are localized on different pigments. As discussed above the cation is most probably localized mainly on P_B. FTIR experiments gave first evidence that the triplet state ^{3}P700 is located on P_A at low temperature [see Ref. [35] for a review]. The interpretation of recent ^{3}P700 EPR data would be consistent with this assignment [47]. Structure-based calculations of the ^{3}P700–

P700 and the $P700^+A_1^-$–$P700A_1$ spectra confirm the conclusion that the triplet state and the positive charge are localized on different Chls of P700 [38,39]. Using the same site energies of the reaction centre Chls for the calculations of the $^3P700$–P700 and the $P700^+A_1^-$–$P700A_1$ spectra, the quite different shapes of the spectra could only be described, if the positive charge and the triplet state are assumed to be localized on different pigments. Electrochromic band shifts contributing to the $P700^+A_1^-$–$P700A_1$ spectrum are found of minor importance. The triplet state is localized most likely on the strongly H-bonded chlorophyll P_A, whereas the cation is localized most likely P_B [38,39].

Acknowledgments

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