

**LIGHT-INDUCED ABSORPTION SPECTRA OF THE  
D1-D2-CYTOCHROME *b*559 COMPLEX OF PHOTOSYSTEM II: EFFECT OF  
METHYL VIologen CONCENTRATION.**

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**Keywords:** absorption, chlorophyll, pheophytin, photosystem II, reaction centre, spectroscopy.

## ABSTRACT

The light-induced difference absorption spectra associated to the photo-accumulation of reduced pheophytin *a* were studied in the isolated D1-D2-Cyt *b559* complex in the presence of variable methyl viologen concentrations and different illumination conditions under anaerobiosis. Depending on the methyl viologen/reaction centre ratio, the relative intensities of the spectral bands at  $681.5\pm 0.5$ ,  $667.0\pm 0.5$  and  $542.5\pm 0.5$  nm were modified. The reduced pheophytin *a* located at the D1-branch of the complex absorbs at  $681.7\pm 0.5$  nm, and at least two additional pigment species contribute to the  $Q_y$  band of the difference absorption spectra with maxima at  $667.0\pm 0.5$  and  $680.5\pm 0.5$  nm. We propose the additional species correspond to a peripheral chlorophyll *a* and the pheophytin *a* located at the D2-branch of the complex, respectively. The blue absorbing chlorophyll at 667 nm is susceptible to chemical redox changes with a midpoint reduction potential of +470 mV. The  $Q_x$  absorption bands of both pheophytins localised at the D2- and D1-branch of the D1-D2-Cyt *b559* complex were at  $540.7\pm 0.5$  and  $542.9\pm 0.5$ , respectively. The results indicated that the two pheophytin molecules can be photoreduced in the D1-D2-Cyt *b559* complex in certain experimental conditions.

## INTRODUCTION

The reaction centre of photosystem II (PSII RC) is a membrane-bound pigment-protein complex which constitutes the minimum unit able to make charge separation and catalyses light-induced electron transfer from water to plastoquinone *in vivo* in oxygenic photosynthetic organisms (higher plants, algae and cyanobacteria) (for reviews see Satoh 1993; Seibert 1993; Satoh 1996). According to the present understanding this pigment-protein complex, also known as D1-D2-Cyt *b559* complex, contains in its native form six chlorophyll (Chl) *a* and two  $\beta$ -carotene molecules per two pheophytin (Pheo) *a* (Eijkelhoff and Dekker 1997; Konermann and Holzwarth 1996). The spectroscopic and functional studies on the PSII RC are complicated by the fact that the  $Q_y$  absorption and fluorescence bands of Chls and Pheos in the complex strongly overlap. Although considerable research based on absorption (Garlaschi et al. 1994; Cattaneo et al. 1995; Konermann and Holzwarth 1996), fluorescence (Kwa et al. 1994; Konermann et al. 1997) and hole-burning (Jankowiak et al. 1989; Tang et al. 1990, 1991; Chang et al. 1994) spectroscopic measurements has been done to investigate the assignment of these single pigments, there is a debate on the exact positions in the absorption spectrum, particularly in respect to the accessory Chl *a* and Pheo *a*. The  $Q_y$  absorption band of isolated D1-D2-Cyt *b559* complex at room temperature has a maxima at 675.5 nm and splits into two peaks near 670 nm and 679 nm at cryogenic temperatures (Tetenkin et al. 1989; Braun et al. 1991; Montoya et al. 1993). The former peak is generally assigned to accessory Chls and the latter mainly to P680. In addition, an absorption band of variable intensity depending on the preparations at around 683 nm in the spectra at 4K is also apparent (Otte et al. 1992; Kwa et al. 1994). The Pheo

*a* localised at the D1-branch (Pheo<sub>1</sub>) of the PSII RC complexes has been associated to bands at 672 nm (Montoya et al. 1993), 676 nm (van Kan et al. 1990; Otte et al. 1992), and 680-682 nm (Nanba and Satoh 1987; Tang et al. 1990; Braun et al. 1991; Yruela et al. 1994) while the Pheo *a* localised at the D2-branch (Pheo<sub>2</sub>) of the PSII RC complexes has been related to bands at 671 nm (Mimuro et al. 1995) and 680 nm (Shkuropatov et al. 1997).

Since the first report on the isolation and characterisation of the D1-D2-Cyt *b*559 complex from spinach (Nanba and Satoh 1987) the steady-state photoaccumulation of the reduced primary acceptor Pheo<sub>1</sub> *a*<sup>-</sup> has been considered as a probe of the charge separation activity of this complex (Nanba and Satoh 1987; Braun et al. 1991; Montoya et al. 1993; Yruela et al. 1994). At present, there is a consensus that the photoactive Pheo<sub>1</sub> *a* absorption band lies very close to P680 band (Tang et al. 1991; Kwa et al. 1994; Mimuro et al. 1995; Konermann et al. 1997; Shkuropatov et al. 1997). In this work, we present results on the effect of experimental conditions in the steady-state photoaccumulation of Pheo *a*<sup>-</sup> spectra in the isolated D1-D2-Cyt *b*559 complex.

## EXPERIMENTAL PROCEDURES

**Preparation of the D1-D2-Cyt *b559* complex.-** The D1-D2-Cyt *b559* complex was isolated from market spinach according to the method of Nanba and Satoh (1987) with some modifications mainly concerning to the detergent concentration and the presence of taurine in the buffers used during the isolation procedure. Highly purified PSII membranes (Berthold et al. 1981) at 1 mg/ml Chl were solubilized with 4% (w/v) Triton X-100 for 2 h, centrifuged at 100,000 x g for 1 h at 4 °C and the resultant supernatant purified by ion-exchange chromatography (DEAE-Toyopearl TSK 650S column) using 1% (w/v) Triton X-100 in the washing buffer (50 mM Mes-NaOH, pH 6.5, 30 mM NaCl and 1.5% (w/v) taurine). The detergent Triton X-100 was subsequently replaced by 0.1% (w/v) *n*-dodecyl- $\beta$ -*D*-maltoside before a 60-350 mM NaCl elution gradient was applied. The  $Q_y$  absorption band maximum of the complex was at 675.5-676 nm at 4 °C indicative of the high quality of the preparation. The pigment stoichiometry of the complex was six Chl *a* and one  $\beta$ -carotene per two Pheo *a*. All the steps of the preparation were done in dim green light in a cooled chamber at 4 °C. D1-D2-Cyt *b559* complex containing five Chl *a* per two Pheo *a* was also prepared as described Vacha et al. (1995). The pigment composition of the isolated D1-D2-Cyt *b559* complexes was measured as described in Eijkelhoff and Dekker (1997).

**Spectroscopy.-** To measure the photoaccumulation of Pheo *a*<sup>-</sup>, D1-D2-Cyt *b559* complex samples were diluted with a buffer containing 50 mM Mes-NaOH (pH 6.5) and 0.1% (w/v) *n*-dodecyl- $\beta$ -*D*-maltoside. The light-induced difference absorption spectra were recorded at 4 °C with a Beckman DU 640 spectrophotometer provided with a Pharmacia circulating bath using quartz cuvettes (optical pathlength of 1 cm).

The D1-D2-Cyt *b559* samples were treated with 2  $\mu\text{l}$  aliquots from a saturated solution of sodium dithionite at pH 7.5 and variable amounts of methyl viologen (for details see Figure Legends). All the measurements were carried out under anaerobic conditions attained by adding 0.23 mg/ml glucose oxidase, 80  $\mu\text{g/ml}$  catalase and 10 mM glucose to the sample (McTavish et al. 1989). Before the light-induced measurements, samples were incubated in the presence of those chemicals and enzymes for 5 min in the dark. To photoaccumulate Pheo  $a^-$  the D1-D2-Cyt *b559* samples were illuminated with a heat-filtered white light ( $550\text{-}2800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) provided with a light projector placed on top of the cuvette for 5, 10, 15 and 20 s prior to measurements (for details see Figure Legends).

Potentiometric redox titrations were carried out under argon at 12  $^{\circ}\text{C}$  using D1-D2-Cyt *b559* complex samples (5  $\mu\text{M}$  Chl) in 50 mM Mes-NaOH at pH 6.5 by following the absorbance changes at 665 nm induced by sequential addition of aliquots of 0.1 M sodium dithionite. The measurements were performed in an Aminco DW-200 UV-Vis spectrophotometer using the dual wavelength mode and 650 nm as the reference wavelength. Samples were previously oxidised with 25  $\mu\text{M}$  potassium ferricyanide. The redox potentials of the medium were simultaneously measured with a potentiometer (Methrom Herisau, Switzerland) provided with a combined Pt-Ag/AgCl microelectrode (Crison Instruments, Spain) previously calibrated against a saturated solution of quinhydrone ( $E'_m$ , pH 7 = +280 mV at 20 $^{\circ}\text{C}$ ). In addition to ferricyanide ( $E'_m$ , pH 7, +430 mV), 10  $\mu\text{M}$  1,4-benzoquinone ( $E'_m$ , pH 7, +280 mV) was used as a redox mediator.

Spectral deconvolution analysis was performed into gaussians. The resultant difference curve was fitted to three and four gaussians. The goodness of fit was evaluated by the chi-square function  $\chi^2$ . Absorption spectra at 77K were recorded

with a variable liquid nitrogen cryostat DN1704 (Oxford Instruments). For oxidised and reduced conditions, samples were preincubated with 5 mM potassium ferricyanide and 2 mM sodium dithionite, respectively, before measurements. Spectral manipulation was possible using the software GRAMS (Galactic Industries Corporation, Salem NY).

## RESULTS

The steady-state photoaccumulation of Pheo  $a^-$  was measured in the D1-D2-Cyt *b559* complex isolated in the presence of 1.5% (w/v) taurine at pH 6.5, containing six Chl per RC. The  $Q_y$  absorption band maximum was at 675.5-676 nm at 4 °C indicative of the high quality of the preparation and it was very stable during the measurements. It has been reported that taurine plays an important role in the stabilisation of membranes and it has a protective effect as antioxidant (Wright et al. 1986). Taurine has been also used in the isolation procedure of the D1-D2-Cyt *b559* complexes by others (Kwa et al. 1992). The light-induced difference absorption spectra were recorded in the presence of sodium dithionite and variable amounts of methyl viologen at 4 °C under anaerobic conditions (for details see Figure Legends). The absorption changes were reversible suggesting that no degradation processes occur during the measurements. All the light-induced difference absorption spectra (Fig 1, line 1) showed minima at  $681.5 \pm 0.5$  and  $542.2 \pm 0.5$  which correspond well with those published for Pheo *a* reduction (Braun et al. 1991; Otte et al. 1992; Yruela et al. 1994). The details of the experimental conditions modified the shape of the spectra, particularly, variations in the methyl viologen/Chl concentration ratio and light intensity during illumination affected the shape of the spectra in the  $Q_y$  absorption band region. However, the minimum of the main  $Q_y$  difference band was always at 681.5 nm indicating that no photodamage occurs. The changes are probably mainly due to modifications of the redox potential of the medium. Similar effects were also observed by Klimov et al. (1977) in more intact preparations such as PSII-enriched particles. Depending on the concentration of methyl viologen per RC in the medium and illumination conditions, an additional reversible light-induced absorption band at



around 667 nm was clearly observed (Fig. 1). Similar band at around 670 nm was also measured by others at room temperature (Barber and Melis 1990; Kaminskaya and Shuvalov 1994; Shkuropatov et al. 1997) and at 6K (Otte et al. 1992). Some authors assigned it to Chl degradation (de las Rivas et al. 1993) but in our measurements the absorption change at 667 nm was reversible indicating that it was not due to degradation processes. Figure 1 also shows the absorption difference spectra recorded after 10 min dark incubation of illuminated samples. The degree of Pheo  $a^-$  reversibility was dependent on the experimental conditions, *i.e.*, from 18% up to 70%. The recovery was more pronounced in the presence of increasing amounts of methyl viologen. The remained spectra (Fig. 1, line 2) in all the cases showed minima at  $680.5 \pm 0.5$  and  $540.7 \pm 0.5$ , indicating that Pheo  $a^-$  contribution is still present. It is interesting to point out that both the  $Q_y$  and  $Q_x$  band minima associated to Pheo  $a$  shifted 1-nm to the blue in the remained spectra after dark incubation. This finding could suggest that after illumination under our experimental conditions a partial reduction of the two different Pheo  $a$  present in the PSII RC occur. By subtracting the spectra after 10 min dark incubation (Fig. 1, line 2) from the light-induced difference spectra (Fig. 1, line 1) we obtain the reversible absorption changes. The resultant spectra are shown in Fig. 1 (line 3) with minima at  $681.7 \pm 0.5$ ,  $667.0 \pm 0.5$  and  $542.9 \pm 0.5$  nm. The data indicate that two different Pheo  $a$  with different recover life-time contribute to the photoaccumulated spectra in Fig. 1 (line 1). Shkuropatov et al. (1997) have reported that both Pheo  $a$  molecules, Pheo<sub>1</sub> and Pheo<sub>2</sub> located at the D1 and D2 branches, respectively, in the PSII RC absorb close to 680 nm. The reversible absorption changes at  $681.7 \pm 0.5$  and  $542.9 \pm 0.5$  nm would correspond to the Pheo<sub>1</sub>  $a$  and those at  $680.5 \pm 0.5$  and  $540.7 \pm 0.5$  nm to the Pheo<sub>2</sub>  $a$ . On the other hand, comparison between Fig. 1a-c (lines 2) clearly shows that the

recovery of both bands at 667.0 and 681.5 nm is not in parallel, indicating that most probably the band at 667 nm is not associated to Pheo. The  $\Delta A_{542.5-554.5}/\Delta A_{681.5}$  and  $\Delta A_{667-650}/\Delta A_{681.5}$  ratios were calculated from spectra measured in different experimental conditions. The light-induced absorption changes at 667 and 681.5 nm were not in parallel (Fig. 2) indicating again that both bands correspond to different pigments.

The gaussian deconvolution of the Pheo *a* photoaccumulation spectra provides a good fitting with three bands at  $681.5 \pm 0.5$ ,  $680.2 \pm 0.5$ , and  $667.0 \pm 0.5$  nm (Fig. 3a,b, Table I) with half-bandwidths of approximately  $8.2 \pm 0.5$ ,  $17.1 \pm 1.5$ , and  $10.0 \pm 0.5$  nm, respectively. The presence of a band with a maximum at 667 nm was the only constraint in the deconvolution analysis. Considering that a) the light-induced difference absorption spectra measured in our experimental conditions showed a minimum at 681.5 nm; b) the photoactive Pheo<sub>1</sub> *a* is the species that preferentially contributes to the reversible light-induced absorption change (Nanba and Satoh, 1987; Yruela et al. 1994; Shkuropatov et al. 1997); c) the remained band after 10 min incubation in the dark difference absorption spectra was always shifted to the blue (Fig. 1) we ascribe the three spectral contributions to Pheo<sub>1</sub> *a* (681.5 nm), Pheo<sub>2</sub> *a* (680.5 nm) and a Chl *a* other than P680 (667.0 nm). The calculated half-bandwidths were in the order reported by others (Cattaneo et al. 1995; Konermann and Holzwarth 1996). It is remarkable the difference in calculated bandwidth between the two Pheo bands being higher in the case of Pheo<sub>2</sub> than that of Pheo<sub>1</sub>. However, similar differences can be also directly observed in Fig. 1. The bandwidth of the Q<sub>y</sub> absorption band of the light-induced difference absorption spectra at 681.5 nm was narrower than that at 680.5 nm in the difference absorption spectra measured after

10 min incubation in the dark. The bandwidths measured in both spectra were  $10.1\pm 0.5$  nm and  $13.8\pm 0.4$  nm, respectively.

To determine more precisely the origin of the pigment absorbing at 667 nm we compared our photoaccumulated Pheo  $a^-$  spectra with that from a D1-D2-Cyt *b559* complex with less Chl *a* content. For that, we used the 5Chl-RC preparation isolated as Vacha et al. (1995). Reversible light-induced absorption changes similar to that in D1-D2-Cyt *b559* complex containing six Chl *a* per two Pheo *a* was measured (Fig. 4). In both spectra the 667 nm band was present indicating that the pigment absorbing at this wavelength is different to what is lost in the modified 5Chl-RC preparation. The comparison of the light-induced absorption spectra of 5Chl-RC (line 1) and 6Chl-RC (line 2) seems to indicate that the amount of total photoreduced pheophytin was smaller in the 5Chl-RC difference spectra. The data suggest that most probably Pheo<sub>2</sub> contribution decreased compared to that in the 6Chl-RC, in agreement with the spectra after 1 min in the dark after illumination (Fig. 4). Fitting analysis was consistent with this hypothesis (Table I). The magnitude of the bleach corresponding to Pheo<sub>2</sub> decreased 4.5 fold in the 5Chl-RC difference absorption spectra compared to that in the 6Chl-RC.

Figure 5A shows that a blue absorbing pigment of the D1-D2-Cyt *b559* complex is affected by chemical redox changes. Indeed, the reduced (dithionite) *minus* oxidised (ferricyanide) difference spectra at 77K presented negative bands at around  $665.0\pm 1$  nm and  $683.3\pm 0.5$  nm, and a positive band at around  $679.2\pm 0.5$  nm. The negative and positive bands at 683.3 and 679.2 nm, respectively, can be due to absorption changes due to the addition of the redox agents but a red shift of a band around 680 nm cannot be discarded. Shkuropatov et al. (1997) reported the borohydride reduction of Chl *a* at 665 nm. The anaerobic reduction titration at 667 nm

is shown in Fig. 5B. The results were described by the Nerst equation for a single electron redox step ( $n=1$ ) with a midpoint reduction potential ( $E'_m$ ) of +470 mV. This reduction potential value suggests the presence of a Chl *a* and discards the assignment of Pheo *a* to that wavelength (Jankowiak et al. 1999).

## DISCUSSION

The results presented here show that the difference absorption spectra of the photoreduction of Pheo *a* is affected by redox conditions induced both by light and chemically. Particularly the amount of methyl viologen per RC added to the medium modified the shape of the spectra in the Q<sub>y</sub> absorption band region which cannot be associated to degradation processes. It is noteworthy that both Pheo *a* (Pheo<sub>1</sub> and Pheo<sub>2</sub>) present in the isolated D1-D2-Cyt *b559* complex were reduced by light under our experimental conditions. This is the first evidence of such phenomenon in the isolated D1-D2-Cyt *b559* complex. The photoreduction of Pheo<sub>2</sub> *a* in the PSII RC has not been reported by others before and it contrasts with what occurs in the bacterial RC. The inactive bacteriopheophytin (Bpheo) can be selectively trapped in a reduced state (Robert et al. 1985). The yield of reduced Bpheo was found to depend on methyl viologen/RC ratio suggesting that this reaction it is most likely a product of a secondary photochemical pathway of low yield involving methyl viologen (Bruno et al. 1985). Similar mechanism seems also occur in the D1-D2-Cyt *b559* under appropriate redox conditions. The photoreduction of two Pheo pigments was dependent of methyl viologen/RC ratio being the reduction of Pheo<sub>2</sub> smaller when the presence of methyl viologen was higher. This fact can be explained by a partial reduction of Pheo<sub>2</sub> in the dark prior illumination that decreased the amount of this chromophore available to be reduced. The chemical reduction in the dark of a Pheo *a* molecule with a  $E'_m$  of  $-450$  mV has been reported by Shuvalov et al. (1989) which differs from that of the photoreducible Pheo<sub>1</sub> *a* molecule ( $E'_m = -610$  mV). The authors assigned the highest potential to the Pheo<sub>2</sub> *a* located in the inactive D2 branch of the PSII RC and they proposed that this cofactor contributes to the band absorbing close

to 670 nm. Later, on the basis of the modification or replacement of native chromophores in the isolated D1-D2-Cyt *b559* complex, this group have described that both Pheo *a* molecules absorb close to 680 nm (Shkuropatov et al. 1997; 1999). These findings are in contradiction to those published by Konermann and Holzwarth (1996) based on spectral decomposition but are in agreement with the results presented in the present work. Shkuropatov et al. (1997) reported that the two Pheo molecules in the D1-D2-Cyt *b559* complex absorbed at 542 nm in contrast to our results that clearly showed two  $Q_x$  absorption band maxima at  $542.9 \pm 0.5$  and  $540.7 \pm 0.5$  nm assigned to Pheo<sub>1</sub> *a* and Pheo<sub>2</sub> *a*, respectively. Different  $Q_x$  transitions of both Pheo *a* located in the D1 (active) and D2 (inactive) branches of the D1-D2-Cyt *b559* complex were also identified by others (Tomo et al. 1997; Jankowiak et al. 1999).

On the other hand, we found that the photoreduction of Pheo *a* molecules is accompanied by a reversible light-induced change of a chlorophyll absorbing at 667 nm. Our data indicated that the origin of this band is not due to a non-specific photodamage in contrast to what was observed by others at 670 nm (de las Rivas et al. 1993). The analyses of the spectra and the  $E'_m$  +470 mV indicated that the species absorbing at this wavelength corresponds to a Chl *a* other than P680. On the basis of the analogy between the RCs of PSII and the photosynthetic bacteria (Trebst 1986; Michel and Deisenhofer 1988) it was suggested that there are two monomeric Chl *a* molecules in the PSII RC complex located close to P680. With the addition of two more Chls, called “antenna Chls” (Konermann and Holzwarth 1996) or “peripheral Chls” by others (Vacha et al. 1995), the minimum stable unit of the PSII RC complex is completed. It was reported that the two “antenna Chls” have their spectral maxima at 667.7 and 677.9 nm at 10K (Konermann and Holzwarth) or near

670 nm (Vacha et al. 1995; Eijkelhoff et al. 1997). Recently, we demonstrated that the blue absorbing Chl(s) could be selectively distinguished modifying the pH of the sample medium conditions, suggesting that it (they) would be located on the surface of the PSII RC complex, in contact with the medium (Yruela et al. 1999). Assuming that the “antenna Chls” are located on the surface of the protein, they could be removed by further purification steps, yielding PSII RC complexes with lower pigment content. However, the modified 5Chl-RC preparation conserved the blue absorbing pigment at 667 nm as shown in Fig. 4, indicating that our redox-active Chl *a* is different to that is lost in the 5Chl-RC and it is supposed to have its absorption maximum towards the red at around 670 nm (Eijkelhoff et al. 1997). According to that, our redox-active Chl *a* could be identified with the highest energy pigment in the D1-D2-Cyt *b*559 complex.

## **ACKNOWLEDGEMENTS**

The authors are grateful to Maria V. Ramiro for skilful technical assistance and Dr. M. Seibert for helpful discussion. This work was supported by the Dirección General de Investigación Científica y Técnica (Grant PB98-1632).



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**ABBREVIATIONS:** Bpheo, bacteriopheophytin; Chl, chlorophyll; Cyt, cytochrome; D1 and D2, core polypeptides of the photosystem II reaction center; Mes, 2-(N-morpholino) ethanesulphonic acid; MV, methyl viologen; P680, primary chlorophyll donor of the photosystem II reaction center; Pheo<sub>1</sub>, primary electron acceptor pheophytin molecule located at the D1-branch of the photosystem II reaction centre; Pheo<sub>2</sub>, pheophytin molecule located at the D2-branch of the photosystem II reaction centre; PSII, photosystem II; RC, reaction centre.

**TABLE I**

Fitting parameters obtained from the gaussian deconvolution of the Q<sub>y</sub> absorption band of the PSII RC light-induced absorption spectra.

Assignment	Spectral maxima (nm)	FWHM (nm)	Amplitude (a.u.)
<b>6Chl-RC</b> <i>Spectra Fig. 3a</i>			
Pheo <sub>1</sub>	681.6±0.5	7.9±0.2	0.33±0.05
Pheo <sub>2</sub>	680.2±0.6	16.4±0.5	0.27±0.05
Chl	667.0	10.1±0.5	0.09±0.05
<i>Spectra Fig. 3b</i>			
Pheo <sub>1</sub>	681.7±0.5	8.5±0.5	0.46±0.05
Pheo <sub>2</sub>	680.1±0.5	18.6±0.5	0.13±0.05
Chl	667.0	10.2±0.5	0.10±0.05
<b>5Chl-RC</b> <i>Spectra Fig. 4</i>			
Pheo <sub>1</sub>	681.5±0.5	9.5±0.5	0.38±0.05
Pheo <sub>2</sub>	681.0±0.7	12.6±0.5	0.06±0.05
Chl	667.0	11.0±0.6	0.19±0.05



## FIGURES LEGENDS

**Figure 1.** Light-induced difference absorption spectra in the isolated D1-D2-Cyt *b559* complex (line 1) and subsequently 10 min incubation in the dark (line 2) in the presence of 0.42  $\mu\text{M}$  (MV/RC = 0.11) (a,c) and 0.83  $\mu\text{M}$  (MV/RC = 0.20) (b) methyl viologen at 4 °C under anaerobic conditions. Double difference spectra (line 3) by subtracting the 10-min incubation in the dark difference absorption spectra (line 2) from that of light-induced (line 1). *Insets:*  $Q_x$  absorption band region of the spectra. Samples were illuminated with white actinic light ( $2,800 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) for 20 s (a,b) and 10 s (c).

**Figure 2.** Dependence of the  $\Delta A_{667-650}/\Delta A_{681.5}$  ( $\lambda$ ) and  $\Delta A_{542.5-554.5}/\Delta A_{681.5}$  ( $\sigma$ ) ratios on the methyl viologen concentration per PSIIRC in the medium.

**Figure 3.** Gaussian spectral deconvolution of the light-induced difference absorption spectra of Figs.1a (a) and 1b (b), respectively. Experimental conditions were as described in Fig. 1.

**Figure 4.** Light-induced difference absorption spectra in the isolated D1-D2-Cyt *b559* complex containing five Chl *a* (line 1) and six Chl *a* (line 2) per two Pheo *a* after 10 s illumination. Difference absorption spectra of the 5Chl-RC after 1 min incubation in the dark (line 3). Measurements were done in the presence of 0.42  $\mu\text{M}$  methyl viologen (MV/RC = 0.11) at 4 °C. Experimental conditions were as in Fig. 1.

**Figure 5.** (A) Absorption spectra of the D1-D2-Cyt *b*559 complex at 77K in the presence of 2 mM sodium dithionite (solid line) and 5 mM ferricyanide (dashed line). Reduced (dithionite) *minus* oxidised (ferricyanide) difference spectrum. Samples were diluted three times with glycerol. (B) Reduction potential titration of the chlorophyll absorbing at 667 nm. Redox mediators are indicated in Materials and Methods.

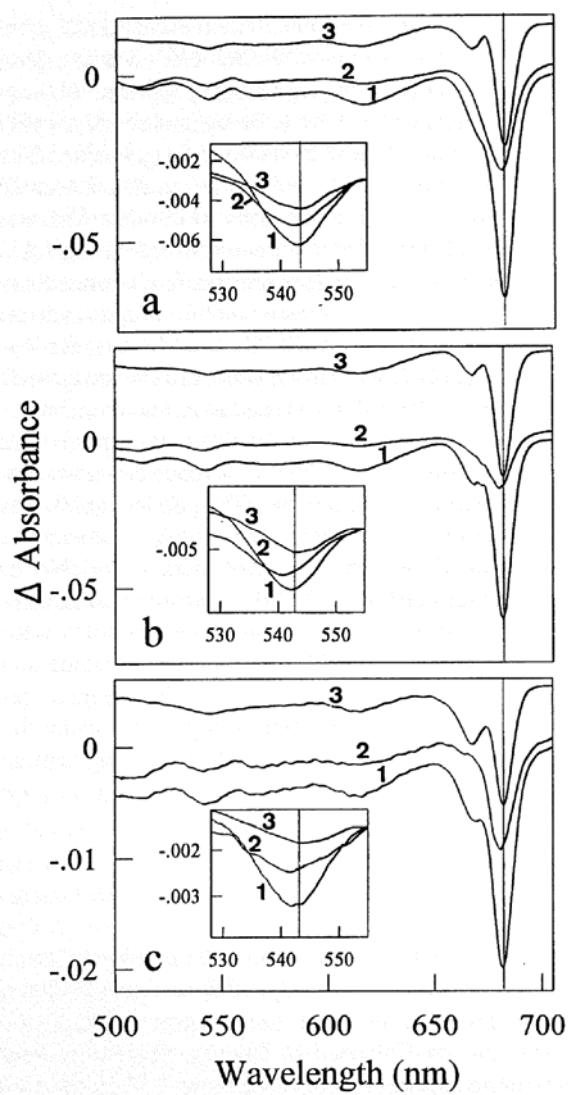


Figure 1

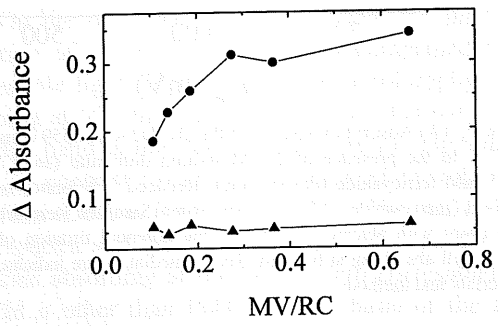


Figure 2

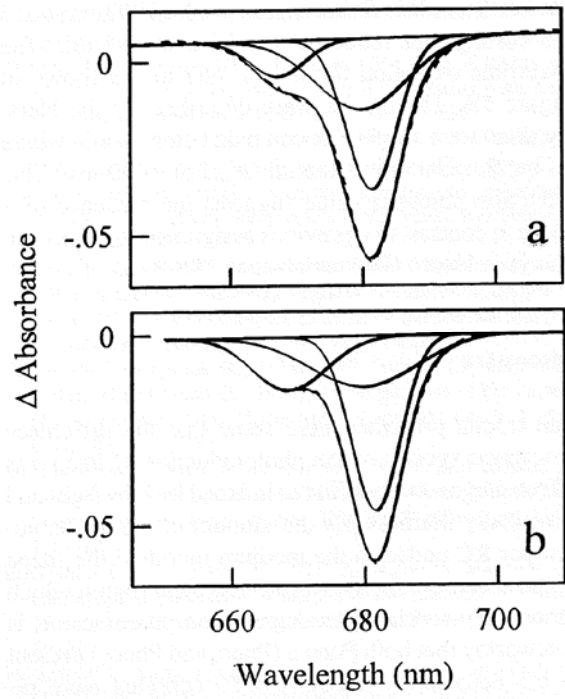


Figure 3

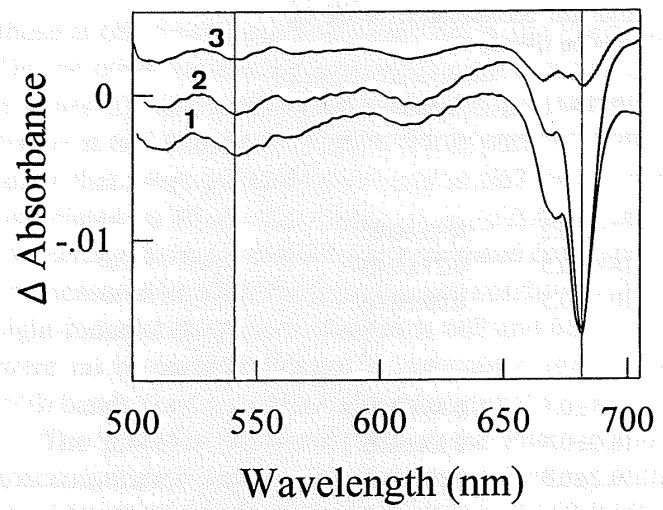


Figure 4

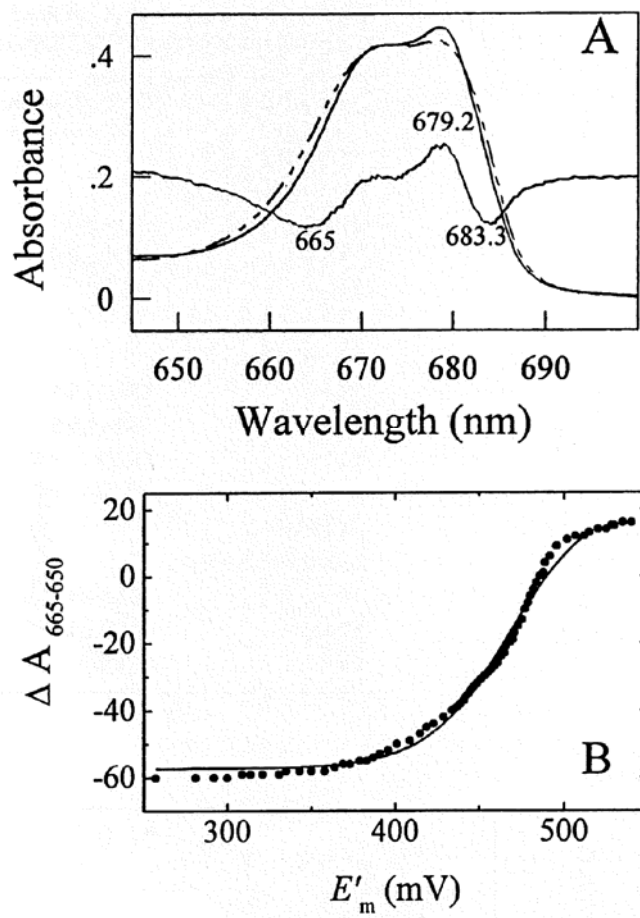


Figure 5