S22-026

Low temperature optical - MCD and EPR studies on a fully active plant PS II complex containing 32 Chl per reaction centre.

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Introduction

We describe (see S22-027, Smith et al.) a new PS-II Core Complex preparation prepared from spinach, which is fully active in O_2 evolution and contains only CP43, CP 47, Reaction Centre (RC) and Cyt. b_{559} . The total chl a content is ~32 per RC. Illumination of dark adapted (S1) samples at 200-250 K generates the S2 multiline (ML) EPR visible state of the Mn cluster in high yield. Near infra-red (NIR) illumination at 140 K efficiently converts this ML state to the g = 4.1 EPR signal form of S2 (yield ~ 60%, see S22-027). The Mn content is 4.5 per PS II. The Core Complex material forms high quality optical glasses in ethylene glycol/glycerol cryo-protected buffers, in which functional turnover to the S2 state EPR forms is retained. The optical spectrum at 4 K exhibits well resolved pigment absorption features. The exciton split components of the P680 reaction centre chl a dimer in functional plant PS II are identified from absorption, circular dichroism (CD) and magnetic CD (MCD) spectroscopies. The prominent, low energy $Q_{y\text{-}}$ band of the P680 pair is resolved in the absorption spectra (683.5 nm). In addition, the active and inactive branch pheophytins of the RC are resolved in the Q_x region around 550 nm.
The cyanobacterial PS II structure (Zouni 2001) locates the centres of density for the pigment and redox active components of the RC precisely, although molecular orientation and atomic detail is generally absent. Assuming a generally equivalent structure for the pigment containing regions of plant and cyanobacterial PS II, the latter provides a geometric framework within which to interpret optical band shifts due to charge displacements within the plant RC. Thus charge movent during S1 to S2 turnover may be followed, using P680, the RC pheophytins and CP 43 chls as electrochromic reporters. In higher plants *Two* Mn cluster spin states are observed in S2 (ML and g4.1), whereas in cyanobacteria, only the ML form is unambiguously known to arise from Mn. Here we examine the extent of spatial charge redistribution that occurs between the spin states of the Mn catalytic cluster in the ML and g 4.1 forms, and the 'minimum' size of the cluster in higher plants implied by this.

Materials and methods

The preparative procedures, EPR and biochemical characterization of our PS II core complex have been recently reported (Smith, 2001). Optical samples (~ 0.3 mg chl./ml) were diluted to 40% 1:1 ethylene glycol/glycerol, in a 1mm path-length cell, then cooled to 4 K over ~40 s in the MCD cryostat. (Oxford SM4). Absorption and (M)CD were collected, *simultaneously* . Sample illumination was performed in situ: S1 $\rightarrow$ S2(ML), 15 s green light (255 K); S2 (ML)$\rightarrow$ S2(g 4.1), 15 min 720 < λ < 1000 nm (140 K). This optimises Mn centre turnover and minimises chl photo-oxidation. EPR characterisation of the turnover state was determined on

parallel samples made under closely similar conditions in the same buffer/cryo-protectant media (see S22-027).

Results and Discussion

Fig. 1a) shows the absorption, CD and MCD of the PS II complexes in the chl $Q_y(0,0)$ region (1.5 K). Features assigned to *functionally intact* P680 are indicated. The asymmetry of the + and − CD bands (Fig.1 b) suggests that the P680 chl. ring planes are *not* parallel, as in cyanobacterial PS II (Zouni,2001), and further separated (11.5 Å versus 10.0 Å) (Fig. 2). Partially overlapping Q_x absortion bands from the active and inactive branch pheo a of the RC region are seen around 18200 cm^{-1} (~550 nm, Fig. 1d)

The MCD intensity of chl is dominated by the Faraday B-term. For an isolated chl a chromophore, the Q_y MCD band shape is very similar to the absorption band shape, with a characteristic $\Delta\varepsilon_M$ /ε ratio (molar elipticity/extinction coefficient) of 5.53×10^{-4} T^{-1} (Kobayashi, 1996). The "MCD Deficit" curve in Fig. 1a) indicates the extent to which the MCD intensity in the PS II complexes is suppressed below the monomer value. This suppression is associated with chromophore coupling and we identify the absorption structured, MCD deficit region with the RC chromophores. The deficit at the 683.5 nm band, ~ 50%, is consistent with essentially pair-wise coupling (Kobayashi, 1996). The broad ~gaussian component centred around 14950 cm^{-1}, with little MCD deficit and ~70% of the total Q_y intensity, we associate with the ~26 chl a in CP43 and CP 47.

Turnovers from the S1 state to the ML and g 4.1 forms of S2 induce distinct band shifts, mainly in the RC pigments (low energy chl a Q_y and pheo a Q_x, Figs. 1c), 1e) respectively). We interpret these as *electrochromic shifts*, due to charge separation from the donor side region to Q_A^- (Fig. 2). In addition to the pheo a Q_x shifts, we are able at present to assign Q_y band shifts for the P680 components, the active branch pheo a and possibly 1-2 antenna chl a, presumably in CP 43 (Fig 1c). The overlap corrected data are listed in Table 1.

Table 1

	P680 Q_y		Pheo$_1$ Q_y		Pheo$_1$ Q_x		CP43 chl a	
Band Centre, Shift[a] (cm^{-1})	ν_o	$\delta\nu_o$	ν_o	$\delta\nu_o$	ν_o	$\delta\nu_o$	ν_o	$\delta\nu_o$
S1	14630		14590		18320		14930	
S1→S2(ML)		0.3		4.4		30		~0
S2(ML)→S2(4.1)		1.0		0.9		6±2		2±1

a): Uncertainty ~ 10 % unless otherwise indicated. Positive value $\Rightarrow$ blue shift

For a unit positive charge located at vector **r** from the chromophore centre, the band shift, $\delta\nu_o$, is:

$$\delta\nu_o \ (\text{cm}^{-1}) = \ 2.42{\times}10^4 \ \Delta\boldsymbol{\mu}.\mathbf{r} \ /\varepsilon_{\text{eff}} \ r^3(\text{Å}^3) \qquad 1)$$

where $\Delta\boldsymbol{\mu}$ is the transition dipole moment difference vector (in Debyes) and ε_{eff} the local dielectric constant (Mulkidjanian 1996). The S1→S2 turnover is dominated by the electrostatic interaction of Q_A^- and pheo$_1$ (Fig. 2), which are separated by ~13 Å (Zouni, 2001). The pheo$_1$ Q_x shift is **8 times** larger than the Q_y shift, implying that $\Delta\boldsymbol{\mu}_x$, $\Delta\boldsymbol{\mu}_y$ must be oriented approximately as shown. Assuming that $\Delta\mu_x$~$\Delta\mu_y$~1D (Mulkidjanian 1996) and r~13 Å, eqn. 1) suggests ε_{eff}=3-4.

The P680 pair geometry is such that the $\Delta\mu_y$ dipoles are expected to nearly cancel (Fig. 2). Only small Q_y transition shifts are seen on both ± components. However the S2(ML)→S2(4.1) transition shifts on P680 and pheo$_1$ are consistent with a significant *net movement* of positive charge *towards* the trans-membrane region.
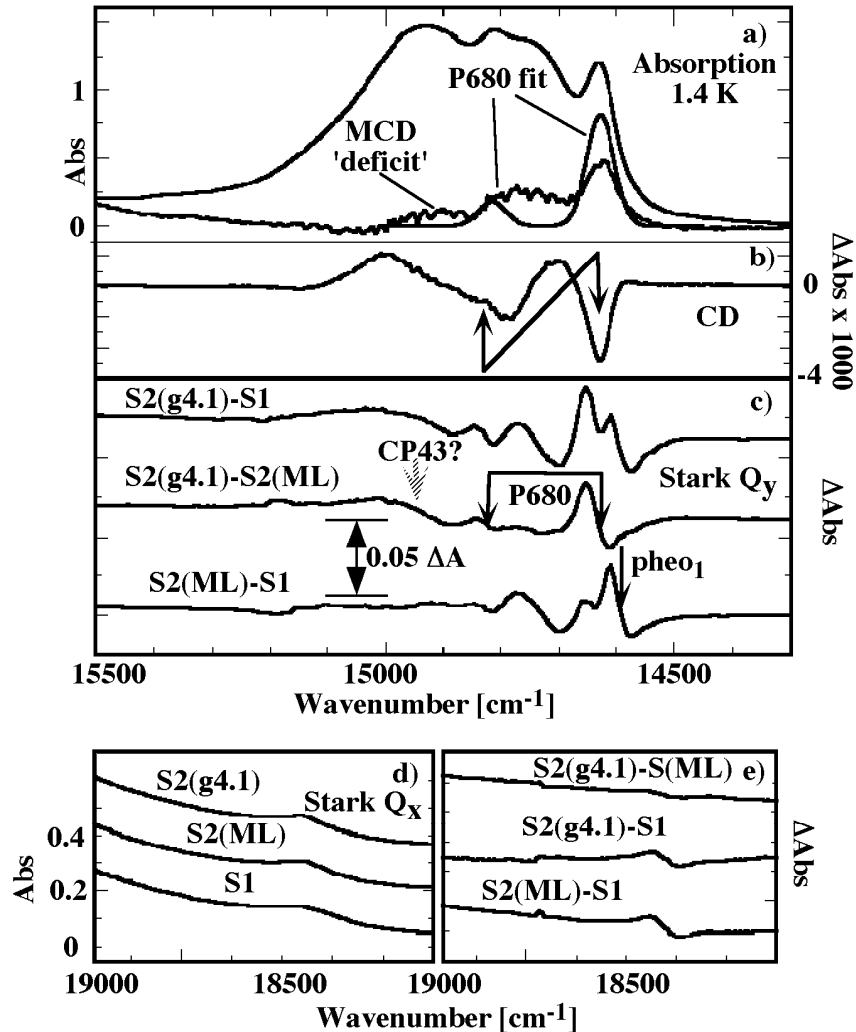

Fig. 1 a) Absorption and MCD (1.5 K, field, 5.0T) of chl. Q_y region of PS II core complexes. Gaussian fits to the features assigned to the Q_y ± components of the P680 coupled pair are indicated. MCD is displayed as a Deficit Spectrum as described in text.

b) CD spectrum of region in a). The P680 components are marked. The − band (negative) is ~5 times the intensity of the + band (positive), suggesting that the two chromophore planes of the coupled pair are significantly non-parallel.

c) Q_y region turnover difference spectra, as indicated. The band centres of relevant components are marked. Shifts in Table 1 were determined by gaussian analysis of partially overlapping derivative shaped spectral features.

d),e) Pheophytin region absorption and turnover difference spectra respectively. The difference spectra are strongly dominated by changes in the pheo$_1$ band position (lower energy). Both pheo bands are significantly asymmetric. Shifts were determined by direct simulation of the turnover differences generated by energy displacement of the S1 spectrum. Step features around 18700 cm^{-1} are instrumental artefacts.

If charge moves with an approximately constant orientation with respect to the chromophore, (and $_{eff}$~constant), then $\Delta\mu \propto 1/r^2$. Since the Mn cluster, pheo$_1$ and Q_A are nearly co-linear and several distances are known (Fig 2), the data from Table 1 may be used to estimate, Δ, the *net*

displacement of unit positive charge *towards* $pheo_1$ on the S2(ML) $\rightarrow$ S2(4.1) transition. This gives (from $pheo_1$ Q_x and Q_y shifts), $\Delta = 10 \pm 2$ Å, assuming the hole is first located at the position of the Mn cluster in the cyanobacerial PS II structure. Similarly, the P680 shift data
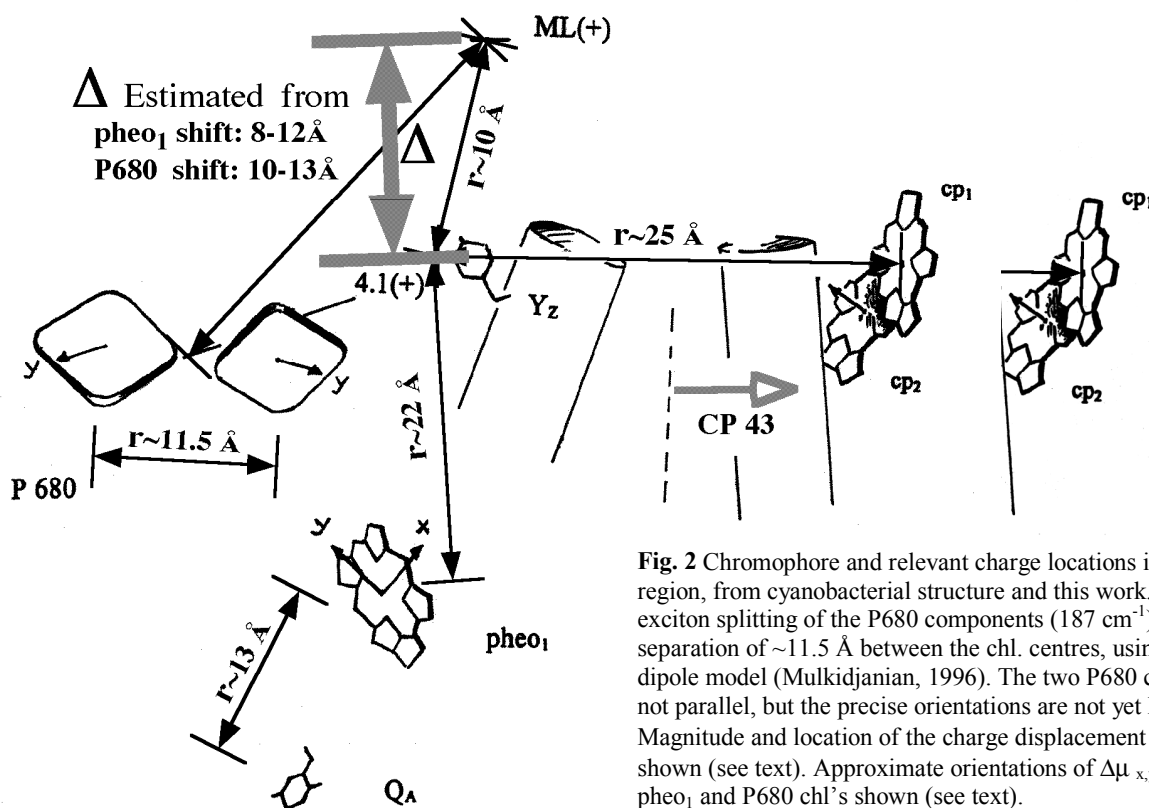

Fig. 2 Chromophore and relevant charge locations in *plant* PS II RC region, from cyanobacterial structure and this work. The inferred exciton splitting of the P680 components (187 cm^{-1}), gives a separation of ~11.5 Å between the chl. centres, using the transition dipole model (Mulkidjanian, 1996). The two P680 chl planes are not parallel, but the precise orientations are not yet known. Magnitude and location of the charge displacement vector, Δ, is shown (see text). Approximate orientations of $\Delta\mu_{x,y}$ components on $pheo_1$ and P680 chl's shown (see text).

give $\Delta = 10$-13 Å, although the constant orientation assumption is only an approximation here. The effect on the CP 43 chl(s) (nearest around 25 Å distant) suggests $\Delta > 5$ Å. The most probable final location of the hole is around the level of the redox active tyrosine Y_z. (Fig. 2). At least two possibilities suggest themselves for the charge movement:-

1) A *proton* relocates ~10 Å *towards* Y_z on the ML$\rightarrow$g 4.1 transition. This would need to occur in the protein matrix at 140 K due to NIR illumination. No obvious mechanism exists for this, but some form of 'proton wire' is conceivable.

2) The Mn cluster in higher plants is more *extended* than in cyanobacteria (Kuzek, 2001) and the shift is due to electron transfer (from Y_z *towards* the ML Mn centre). In this model, the g 4.1 signal arises from a Mn$-Y_z^+$ interaction, which could not occur in cyanobacteria.

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