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Evidence for localisation of accumulated chlorophyll cation on the D1-accessory chlorophyll in the reaction centre of Photosystem II

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Abstract

Absorption and circular dichroism spectra of Photosystem II (PS II) reaction centres (RC) were studied and compared with spectra calculated on the basis of point-dipole approximation. Chlorophyll cation was accumulated during a light treatment of PS II RC in the presence of artificial electron acceptor silicomolybdate. Light-induced difference spectra and their calculated counterparts revealed the location of accumulated cation at the accessory chlorophyll of the D1 protein subunit.

Abbreviations: CD – circular dichroism; Chl – chlorophyll; D1 – *psbA* gene product; D2 – *psbD* gene product; DM – *n*-dodecyl-β-D-maltoside; FWHM – full-width at half maximum; MES – morpholinethanesulfonic acid; Pheo – pheophytin; PS II – Photosystem II; RC – reaction centre; SiMo – silicomolibdic acid

Introduction

Photosystem II (PS II) is a pigment-protein complex located in the thylakoid membrane of cyanobacteria, algae and higher plants. It performs a series of light-driven reactions, which result in separation of charge and subsequently in reduction of electron-transport chain and water oxidation. The primary site of the light energy conversion is located in the so-called reaction centre (RC). In its simplest form, RC consists of a heterodimer of D1 and D2 proteins with two subunits of cytochrome b559 α and β and psbI protein (Barber et al. 1987; Nanba and Satoh 1987). Isolated RC binds 6 molecules of chlorophyll *a*, 2 pheophytin *a*, 2 molecules of β-carotene and an atom of non-heme iron (Eijckelhoff and Dekker 1995; Zheleva et al. 1996). The structural and functional data have been originally obtained by comparing the PS II RC with very similar RCs of purple bacteria. It led to several structural models which are based on homology between RC of purple bacteria and PS II RC (Svensson et al. 1996; Xiong et al. 1998). Recently, structure of the PS II complex isolated from cyanobacteria *Synechococcus elongatus* has been presented at a resolution of 3.8 Å (Zouni et al. 2001), from *Synechococcus vulkanus* at 3.7 Å (Kamiya and Shen 2003) and from *Thermosynechococcus elongatus* at 3.5 Å (Ferreira et al. 2004). Such

resolutions can indicate protein orientation, position of most of the pigments and other cofactors, and overall shape of the system. However, the precise orientation of pigment dipoles is still under the resolution threshold.

Despite the large homology between purple bacteria RC and PS II RC, important differences exist between both types of RCs. First, bacterial RCs contain bacteriochlorophylls and bacteriopheophytins instead of chlorophyll and pheophytin in PS II RC. Further, in RCs of purple bacteria two peripheral bacteriochlorophylls are missing. Another difference is in the level of the exciton coupling between the pigment molecules. In contrast to the bacterial RCs, where the interaction between two bacteriochlorophylls of special pair is dominating, there is comparable exciton interaction among core pigments in the PS II RC. This suggests that the core pigments behave as a multimer (Durrant et al. 1995). Such multimer model was successfully applied to explain different aspects of PS II RC using calculations based on point-dipole approximation (Durrant et al. 1995; Prokhorenko and Holzwarth 2000; Jankowiak et al. 2002; Barter et al. 2003). In this report, we have combined the structural model proposed by Svensson et al. (1996) and the structure presented by Kamiya and Shen (2003). Using this model, we have calculated the absorption and circular dichroism spectra and compared them with the experimental results.

Materials and methods

All PS II RC were isolated from 14-day-old pea plants (*Pisum sativum*). PS II RCs containing six molecules of chlorophyll per two pheophytins were isolated according to the original method of Nanba and Satoh (1987).

For all spectroscopic measurements, samples were diluted to the final concentration of $\sim$10 μg Chl.ml^{-1} in a buffer containing 50 mM MES, 0.02% DM at pH 6.5. For low temperature spectra, glycerol was added to the sample to a final concentration of 65% (v/v). Absorption spectra were measured using Unicam 500 spectrophotometer (Spectronic Unicam, Cambridge, UK), spectra of circular dichroism (CD) were recorded using JASCO J-715 spectropolarimeter (JASCO Corporation, Tokyo, Japan). The light-induced oxidation of the RC chlorophyll was measured in the presence of 200 μM silicomolybdate (SiMo). The spectra of light-induced absorption and circular dichroism changes were recorded according to Vacha et al. (2002).

Optical spectra were calculated as described in Psencik et al. (2003). Frenkel Hamiltonian was used taking only Q_y transitions into account. Interaction energies were calculated in a point-dipole approximation (Pearlstein 1991). Transition energies of all pigments were assumed to be equal and set to 14920 cm^{-1} ($\sim$670 nm) which represents the transition energy of chlorophyll molecules still bound to the RC proteins, but without exciton coupling (Vacha et al. 1995; den Hartog et al. 1998). Inhomogeneous broadening was introduced by assuming a Gaussian distribution of the transition energies with maximum at 14920 cm^{-1} and a full-width at half-maximum (FWHM) of 210 cm^{-1} (Durrant et al. 1995; Prokhorenko and Holzwarth 2000; Jankowiak et al. 2002). The presented curves were obtained by averaging 4000 RC spectra. The transition dipole strengths of 23 and 14 Debye^2 for chlorophyll *a* and pheophytin *a*, respectively, were used (Durrant et al. 1995; Jankowiak et al. 2002). The exciton components of absorption and CD spectra were calculated using formulas according to Pearlstein (1991). The stick spectra were dressed with Gaussian envelopes. Each of the envelopes has the FWHM of 150 cm^{-1}, which roughly corresponds to the homogenous line-width of chlorophyll *a* at room temperature (Konermann and Holzwarth 1996).

Results and discussion

In this report, we combine the experimental and modelling approach in order to study the properties and function of the PS II RC pigments. We compare the measured absorption and CD spectra with those calculated in the point-dipole approximation. As the ground for our calculations we have used the PS II RC model of Svensson et al. (1996), similar to the other authors (Prokhorenko and Holzwarth 2000; Jankowiak et al. 2002). Since this model is based on the analogy between purple bacteria and PS II RC, it lacks the peripheral chlorophyll molecules. To compare the calculated spectra with our experimental data, peripheral chlorophylls have

to be included in calculations. Although their coupling to remaining pigments is rather weak (0.1–10 cm^{-1}), their contribution is important for the overall shape of the spectra. We have appended the peripheral chlorophylls from the PS II RC structural data (pdb file ID – 1IZL) reported by Kamiya and Shen (2003) to the PS II RC core pigments of the theoretical model of Svensson et al. (1996) (pdb file ID – 1DOP). The pigment nomenclature is depicted in Figure 1.

Figure 2a shows the absorption spectra of the PS II RC measured at 4.2 K (circles) and 273 K (triangles) together with the calculated spectra. The spectrum calculated without inclusion of the inhomogeneous broadening (dotted line) exhibits very similar spectral features as the low temperature experimental spectrum. The absorption maximum of the PS II RC measured at 4.2 K is at 678.5 nm, maximum of the calculated spectrum is at 677.5 nm. Both spectra exhibit blue shoulders at 672 and 671 nm for the experimental and the calculated data, respectively. Figure 2a shows also the calculated absorption spectrum with inclusion of the diagonal disorder (solid line). The diagonal disorder leads to broadening and smoothing of the calculated spectrum and the overall shape, and spectral distribution of the oscillator strength is similar to the experimental curve measured at 273 K. The 273 K experimental spectrum peaks at 675 nm which is 2 nm blue-shifted compared to the calculated spectrum.

The experimental CD spectrum of the PS II RC measured at 273 K (triangles) and 77 K (circles) is compared with the calculated CD spectra in Figure 2b. The maximum of the positive peak both in the experimental and calculated CD spectrum without inhomogeneous broadening (dotted line), is at 681 nm, and for the inhomogenously broadened spectrum (solid line) it is at 682 nm. The minimum of the negative peak is at 664 nm for the experimental spectra and at 667 nm for both calculated spectra. The exciton calculations always provide conservative CD spectrum, while the presented experimental CD spectra are clearly not conservative. This may be explained by the pigment–protein interaction or coupling of the Q_y to higher excited states which were not considered in the calculations.

It is believed that during irradiance of PS II RC in the presence of artificial electron acceptor, SiMo, the positive charge is accumulated on the

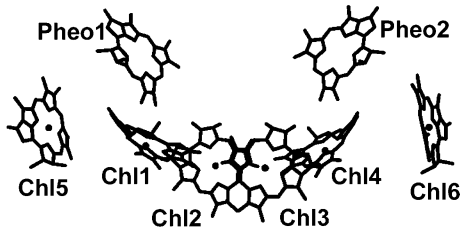

Figure 1. Nomenclature and schematic arrangement of the cofactors present in PSII RC. Six chlorophyll *a* molecules of PSII RC are labelled as follows: Chl1 – accessory chlorophyll on the D1 protein; Chl2 – chlorophyll ligated to the His198 on the D1 protein; Chl3 – chlorophyll ligated to the His198 on the D2 protein; Chl4 – accessory chlorophyll on the D2 protein; Chl5 – peripheral chlorophyll on the D1 protein ligated to the His118; Chl6 – peripheral chlorophyll on the D2 protein ligated to the His1118. Two molecules of pheophytin *a* are labelled as Pheo1 – pheophytin on the D1 protein; Pheo2 – pheophytin on the D2 protein.

chlorophyll of primary donor (Nanba and Satoh 1987; Barber et al. 1987). We have used this experimental approach, and compared the light-induced difference spectra measured in the

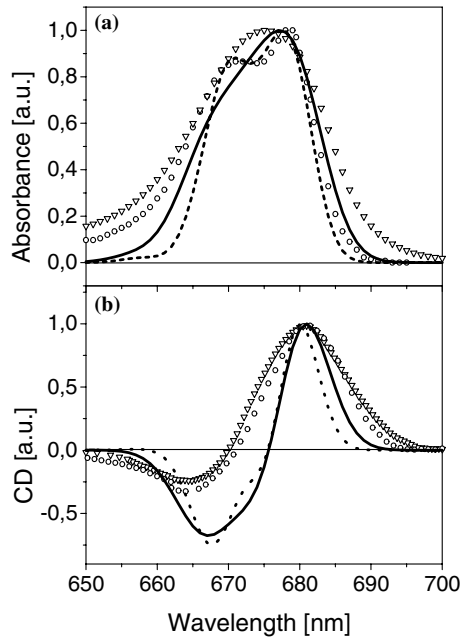

Figure 2. Comparison of measured and calculated absorption and CD spectra of PSII RC. (a) Absorption spectra measured at 273 K (triangles) and 4.2 K (circles) compared with the spectra calculated without (dotted line) and with (solid line) inclusion of inhomogeneous broadening. (b) CD spectra measured at 273 K (triangles) and 77 K (circles) compared with the spectra calculated without (dotted line) and with (solid line) inclusion of inhomogeneous broadening. All spectra are normalised to their maxima.

presence of accumulated chlorophyll cation with the calculated difference spectra. The calculated difference spectra were obtained by subtracting the original full pigment spectrum from spectrum where one particular chlorophyll molecule was omitted from the calculation. This simulates the presence of a localised cation upon the omitted pigments. The effect of the localised charge on the calculated spectra was not included in calculations, since the experimental spectra do not exhibit any electrochromic shift and are similar to the triplet-minus-singlet spectra where no charge is present (Eijckelhoof et al. 1997).

Figure 3a shows the light-induced absorbance difference spectra of the PS II RC in the presence of SiMo measured at temperatures of 273 K (triangles) and 77 K (circles) together with the calculated absorbance difference spectra for the PS II RC where one particular chlorophyll molecule is omitted from the calculation. The course of the omitted chlorophyll is inscribed in the title of each particular plot. Figure 3a indicates that the experimental and calculated data are most similar in the case when the Chl1 molecule is missing. The calculated spectra for the missing Chl1 exhibits all spectral features of the experimental spectra, including two negative minima and positive absorption in the very blue part of the spectrum. In addition, spectral position of these features does not differ more than 10 nm from the measured ones. If any other chlorophyll was omitted from the calculations, the shape and positions of maxima and shoulders of the difference spectrum clearly deviate from the experimental spectrum. This suggests that during the charge separation in the presence of SiMo, chlorophyll cation is accumulated on the accessory chlorophyll on the D1 protein. Both spectra calculated with (solid line) and without (dotted line) inclusion of the inhomogeneous broadening show the same results. In

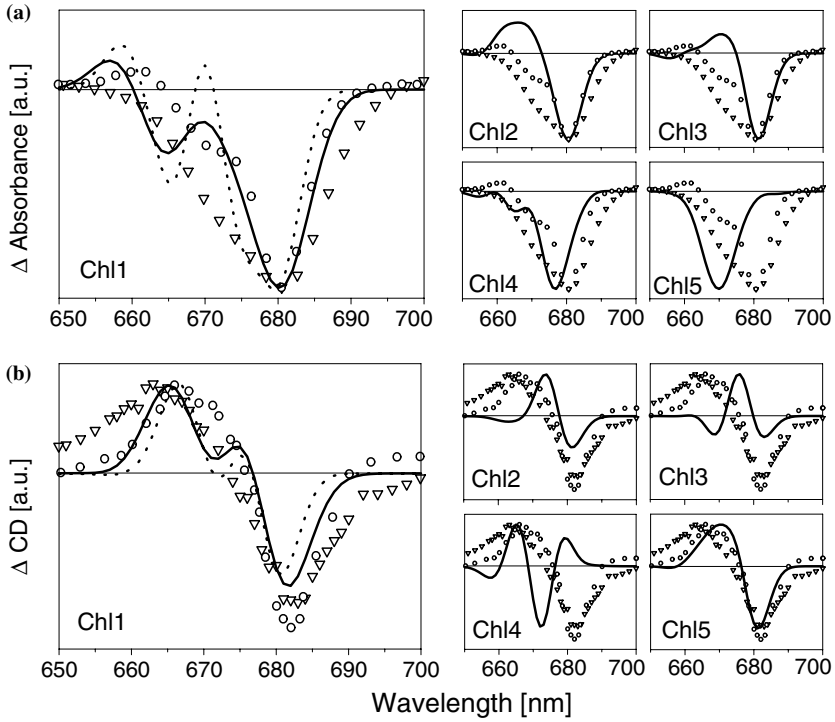

Figure 3. Light-induced (light minus dark) difference spectra of PSII RC measured at 273 K (triangles) and 77 K (circles) compared with calculated spectra without (dotted line) and with (solid line) inclusion of inhomogeneous broadening. (a) Light induced absorbance difference spectra; (b) Light induced CD difference spectra in presence of artificial electron acceptor. The individual plots are labelled by the chlorophyll molecule which was omitted from the calculation: Chl1 – accessory chlorophyll on the D1 protein; Chl2 – chlorophyll ligated to the His198 on the D1 protein; Chl3 – chlorophyll ligated to the His198 on the D2 protein; Chl4 – accessory chlorophyll on the D2 protein; Chl5 – peripheral chlorophyll on the D1 protein ligated to the His118; Chl6 – peripheral chlorophyll on the D2 protein ligated to the His1118.

the case of omitted Chl1, the implementation of the inhomogeneous broadening into the calculations leads to improved agreement with the experiment.

The same conclusions can be independently drawn also from the comparison of the recorded and calculated difference CD spectra, as it is represented in Figure 3b Similar to the case of absorption spectra, the experimental CD spectrum matches the calculated one, only for the omitted D1-accessory chlorophyll Chl1 from the calculation. Inclusion of the diagonal disorder also leads to improved agreement. For the missing Chl1, the calculated difference spectra not only represent the best match with the experiment, but also exhibit all the observed spectral features, including two positive maxima in the blue part and one negative maximum in the red part of the CD difference spectrum.

For better readability, plots Chl2 to Chl5 in Figure 3a and b, compare the experimental spectra only with the calculated inhomogeneously broadened spectra. Plots for the omitted peripheral Chl6 are not shown since the spectra are very similar to the spectra of Chl5.

For RCs it was generally assumed that the charge separation starts at a dimer of two chlorophyll molecules Chl2 and Chl3. The chlorophyll cation has than been proposed to be localised at the chlorophyll Chl2 (Diner et al. 2001; Diner and Rappaport 2002), or delocalised over two chlorophyll molecules Chl2 and Chl3 (Noguchi et al. 1998). Recently, an alternative pathway for primary charge separation process has been suggested in purple bacteria. It includes the accessory chlorophyll Chl1 as the primary electron donor (van Brederode and van Grondelle 1999). Such pathway has been proposed also for PS II RC (Prokhorenko and Holzwarth 2000; Barter et al. 2003) based on the calculations on the multimer model (Durrant et al. 1995). This idea is further supported by our conclusions concerning the site of the accumulated chlorophyll cation.

The calculations of both absorbance and CD difference spectra independently indicate that the accumulated chlorophyll cation is in PS II RC localised on the position of the accessory chlorophyll molecule Chl1. Our results support the recent evidences of the possible presence of positive charge on the accessory chlorophyll during the initial charge separation (van Brederode and van Grondelle 1999; Prokhorenko and Holzwarth 2000; Barter et al. 2003). As a conclusion, we suggest that the accessory chlorophyll on the D1 protein is the initial primary donor of PS II.

Acknowledgements

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