LOCALISATION OF ACCUMULATED CHLOROPHYLL CATION IN REACTION CENTRE OF PHOTOSYSTEM II

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Abstract
Photosystem II reaction centre absorbance and circular dichroism spectra were calculated using point-dipole approximation and compared with experimental data. Light induced difference spectra and their calculated counterparts revealed the location of accumulated chlorophyll cation at the position Chl1 - the accessory chlorophyll at D1 subunit.

Introduction

Photosystem II is a pigment-protein complex of thylakoid membrane of cyanobacteria, algae and higher plants. It performs series of light driven reactions, which result in a separation of charge and subsequently in a reduction of an electron-transport chain and water oxidation. Primary site of the energy conversion is located in a so-called reaction centre. In its simplest form the reaction centre consists of a heterodimer of D1 and D2 proteins with two subunits of cytochrome b559 and ß [1, 2]. Isolated RC binds 6 molecules of chlorophyll, 2 pheophytins, 1 or 2 molecules of ß-carotene and an atom of non-heme iron [3, 4]. In spite of a great effort there have been little success resolving the atomic structure of PSII RC. Therefore, many of the structural and functional data have been obtained by comparing the PSII RC with very similar reaction centres of purple bacteria. It led to several structural models, which are based on a homology between purple bacteria and PSII RC [5, 6]. Recently, structure of the PSII complex isolated from cyanobacteria *Synechococcus elongatus* has been presented at the resolution of 3.8 A [7]. Such resolution gives the idea of orientation of proteins, position of most of the pigments and other cofactors and overall shape of the system. However, the orientation of pigment dipoles is still unknown.

In this report we have combined the structural model proposed by Svensson et al. [5] and the structure presented by Zouni et al. [7]. Using this model we have calculated the absorbance and circular dichroism spectra and compared them with the experimental results.

Materials and Methods

All PSII RC were isolated from 14 days old pea plants (*Pisum sativum*). PSII RCs containing 5 chlorophyll molecules per two pheophytins were isolated according to Vacha et al. [8]. PSII RCs containing 6 molecules of chlorophyll per two pheophytins were isolated from PSII core particles by short Triton X-100 treatment [9] with slight modification. As a starting material we have used PSII core particles isolated according to Ghanotakis et al. [10]. PSII core particles were incubated with 1% Triton X-100 for 20 minutes in dark at 4 C and than separated on a Q Sepharose column. After washing the unbound material from column with a washing buffer 50 mM MES, 0.02% DM, pH 6.5 the PSII RC were eluted with a gradient of 0 - 200 mM MgSO4 in the washing buffer. PSII RC was detected according to their characteristic absorbance spectra.

For all spectroscopic measurements samples were diluted to the final concentration of ~ 10 g 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).

Absorbance spectra were measured on Unicam 500 spectrophotometer (Spectronic Unicam, Cambridge, UK), spectra of circular dichroism were recorded using JASCO J-715 spectropolarimeter (JASCO Corporation, Tokyo, Japan).

The light-induced oxidation of chlorophyll of the RC primary donor was measured in the presence of silicomolybdate (SiMo) at a concentration of 200 mM. The spectra of light-induced absorbance and circular dichroism changes were recorded according to Vacha et al. [11].

Low temperature spectra were measured in an Oxford Optistat Bath cryostat (Oxford Instruments, Oxon, England), temperature in the cryostat was controlled by an Oxford Temperature Controller ITC 503 (Oxford Instruments, Oxon, England).

For the pigment modelling and alignment we have used Swiss PDB Viewer program ver. 3.7 [12]. The files of structural data were downloaded from the Protein Data Bank (http://www.rcsb.org/pdb/) in the .pdb format.

Absorbance and CD spectra were calculated by point-dipole approximation [13].

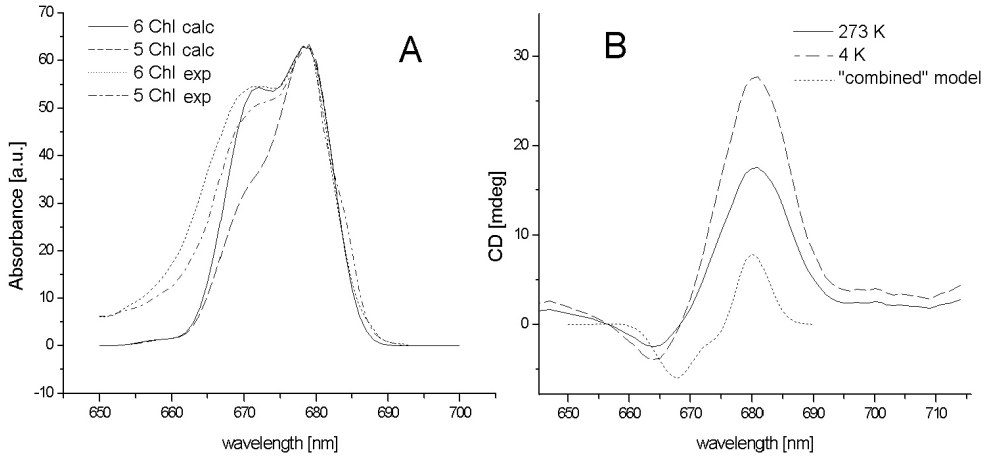

Fig. 1. Comparison of measured and calculated absorbance and circular dichroism spectra. A - absorbance spectra measured on 6 chlorophyll (dotted line) and 5 chlorophyll (dash-dotted line) reaction centres compared with calculated spectra of 6 chlorophyll (solid line) and 5 chlorophyll (dashed line) reaction centres. B - circular dichroism spectra of 6 chlorophyll reaction centres measured at 273 K (solid line) and 4 K (dashed line) compared with calculated spectra (dotted line).

Results and Discussion

In this report we combine the experimental and theoretical approach in order to study the properties and function of the PSII RC pigments. We compare the data of absorbance and circular dichroism spectroscopy with those calculated on the basis dipole-dipole interaction from a "combined" model. As the ground for our calculations we have used the PSII RC model of Svensson et al. [5]. This model is based on the analogy between purple bacteria and PSII reaction centres and it was optimised to fit the experimental spectroscopic data. However, it lacks the peripheral chlorophyll molecules (Chl_z) since they are also not presented in the purple bacteria RC. Using Swiss Pdb Viewer program [12] we have aligned the position of the PSII RC pigments of the theoretical [5] model (file ID - 1DOP) with the positions of pigments of the PSII RC from the structural data (file ID - 1FE1) reported by Zouni et al. [7]. Combination of these two structures gives us a new "combined" model. For such "combined" model we have used the position and orientation of the core pigments from the 1DOP model, however, supplemented with the position and orientation of the peripheral chlorophylls from the structural data 1FE1. Even if in 1FE1 file the resolution of the X-ray structural data is not sufficient to resolve the orientation of the dipole moments of the pigments the pyrrol rings of the chlorines are labelled according to IUPAC standard there and we have used this labels to determine the orientation of the pigment dipoles. It must be stated, however, that in the case of the peripheral chlorophyll molecules neither the orientation nor the position further from the PSII RC, than it is in the 1FE1 structure, has an influence on the calculated absorbance or CD spectra.

Figure 1A shows the absorbance spectra of the PSII RC containing 6 and 5 chlorophyll molecules together with the calculated spectra. The calculated spectra represents the PSII RC based on the "combined" model with all 6 chlorophyll molecules and with the PSII RC where one of the peripheral chlorophyll is missing. The shape of the calculated spectrum of the 5 chlorophyll PSII RC is independent on the position (either Chl_z - D1 or Chl_z - D2) from which the peripheral chlorophyll was disposed. The calculated spectrum for the 6 chlorophyll PSII RC fits well the experimental data. It the case of 5 chlorophyll PSII RC the calculated spectrum has less intensive shoulder at 670 nm compare the experiment. In the case of the experimental data the 670 nm shoulder of the 5 chlorophyll PSII RC is about one third less intensive compare to the 6 chlorophyll PSII RC [14], in the case of the calculated data the 5 chlorophyll PSII RC has the 670 nm shoulder about half intensive compare to the calculate 6 chlorophyll PSII RC.

Figure 1B shows the CD spectra of the 6 chlorophyll PSII RC compared with the calculated CD spectra of the "combined" model. The calculated CD spectra of the "combined" model are almost identical whether calculated with 6 chlorophyll or with the 5 chlorophyll PSII RC where any of the peripheral chlorophyll Chl_z depleted. The maximum of the positive peak in both experimental and calculated CD spectra is at 681 nm, the minimum of the negative peak is 664 nm for the experimental and 668 nm for the calculated spectrum. The ratio of the amplitude of the positive to negative peak is for the experimental data 6.95, for the calculated spectra 1.33.

Figure 2 shows the light induced absorbance difference spectra of the PSII RC in the presence of artificial electron acceptor SiMo measured at temperatures of 273 K and 77 K together with the calculated absorbance difference spectra for the PSII RC where one particular chlorophyll molecule is omitted from the calculation. The number of the omitted chlorophyll is inscribed in the title of particular plot. The nomenclature of chlorophylls in the PSII RC is as follows. Chl1 - accessory chlorophyll on the D1; Chl2 - chlorophyll ligated to the His198 on D1; Chl3 - chlorophyll

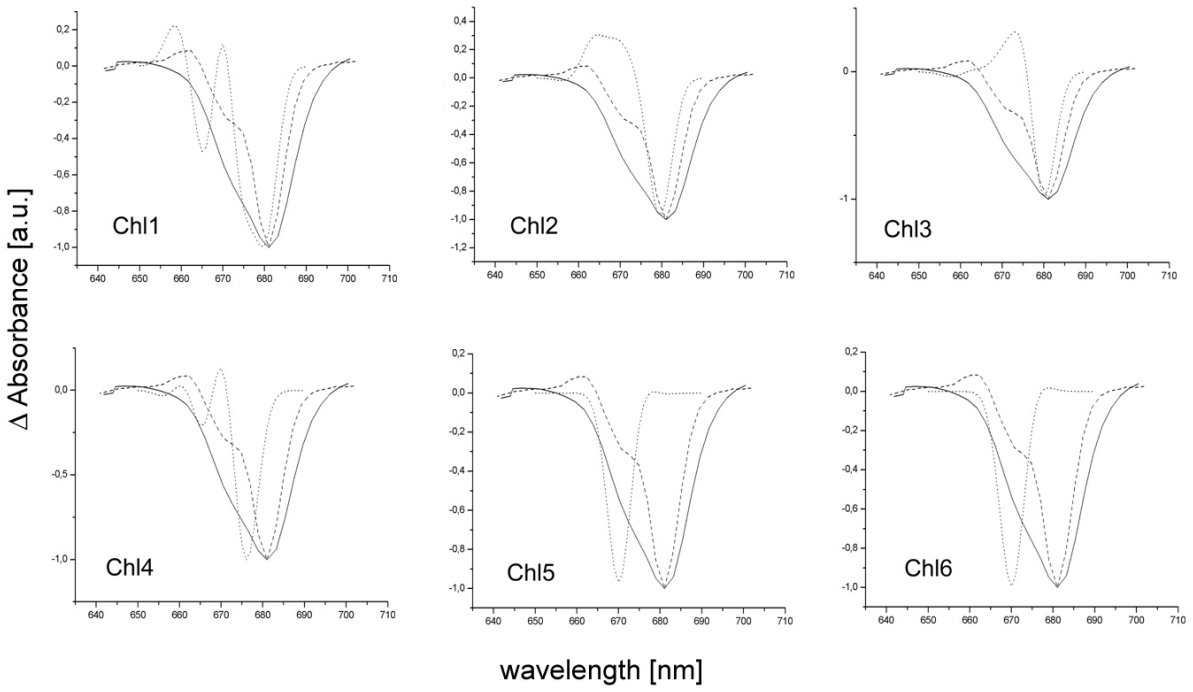

Fig. 2. Light induced (light minus dark) absorbance difference spectra of reaction centre of photosystem II measured at 273 K (solid lines) and 77 K (dashed lines) compared with calculated absorbance difference spectra (dotted lines). The calculated spectra were obtained by subtracting the original full pigment spectra from spectra where one particular chlorophyll pigment (numbered in the individual figures as Chl1-6) was omitted from the calculation.

ligated the teh His198 on D2; Chl4 - accessory chlorophyll on the D2; Chl5 - peripheral chlorophyll Chlz on the D1; Chl6 - peripheral chlorophyll Chl_z on the D2. Figure 2A indicates that the experimental and calculated data are most similar in the case when the Ch1 chlorophyll molecule is missing. This would reflect the fact that chlorophyll cation is accumulated on the accessory chlorophyll on D1 protein during the charge separation in presence of SiMo.

This is also supported by the comparison of the recorded and calculated difference CD spectra as it is represented at figure 3. Alike in the case of absorbance spectra the experimental CD spectrum matches the calculated one only in the case when the D1 accessory chlorophyll (Chl1) is omitted from the calculation.

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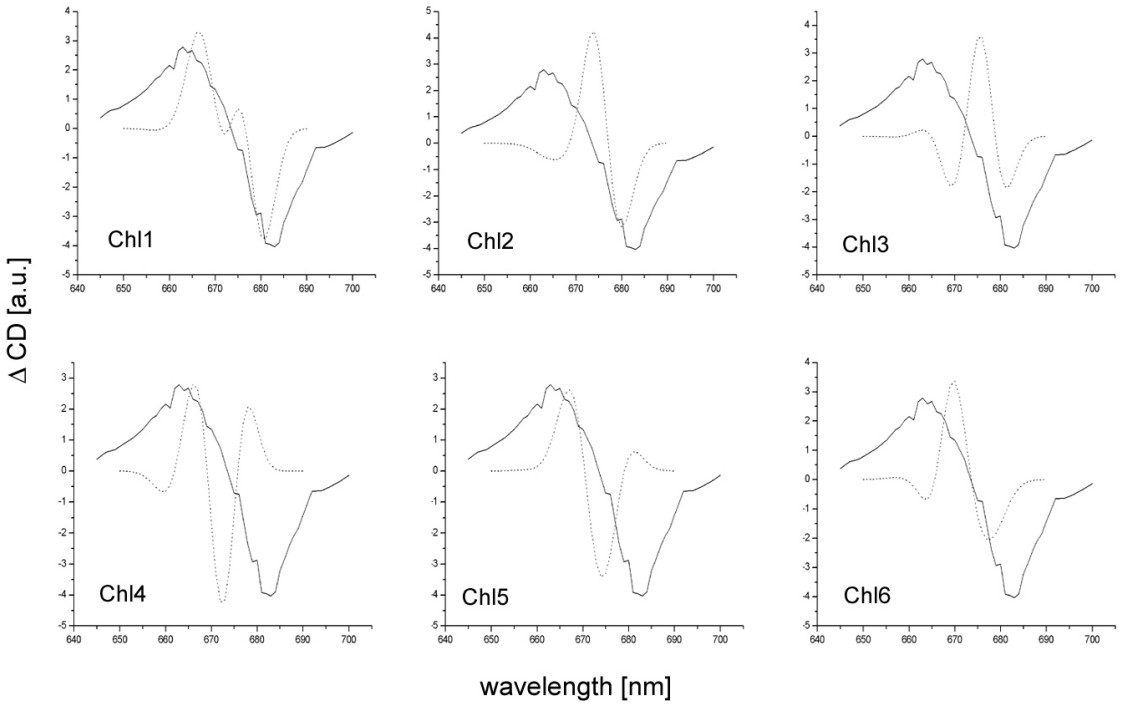

Fig. 3. Light induced (light minus dark) circular dichroism difference spectra of reaction centre of photosystem II measured at 273 K (solid lines) compared with calculated circular dichroism difference spectra (dotted lines). The calculated spectra were obtained by subtracting the original full pigment spectra from spectra where one particular chlorophyll pigment (numbered in the individual figures as Chl1-6) was omitted from the calculation.

FUNCTIONAL RECONSTITUTION OF PHOTOSYSTEM 2 INTO LIPOSOMES

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Abstract
Most recent structural data on photosystem 2 (PS2), the first membrane protein complex in the photosynthetic electron transport chain, confirm that this complex exists as a functional dimer in the thylakoid membrane of cyanobacteria [1, 2]. Besides the membrane embedded part of this dimer with dimensions of 190 Å x 100 Å x 40 Å, this complex also extends about 10 Å out of the membrane in the stromal region and 55 Å in the lumen; the latter is referred to as the oxygen evolving complex, harbouring the water-splitting site. Developing an approriate method to reconstitute dimeric PS2 into liposomes should finally help to answer the fundamental question concerning its structure-based function: Is a dimeric structure a prerequisite for optimal water-splitting activity (monomeric complexes, solubilized by detergent, are active, too, although at a lower level) and which is the impact of the lipid-phase-composition on the water-splitting activity? Here we present data on the orientation of reconstituted dimeric PS2 from the cyanobacterium *Thermosynechococcus elongatus* and also give indications for its activity within the liposomes and monomer-dimer distribution (by EM analysis).

Material and Methods

Liposomes were prepared according to [3]. PS2 core complexes, prepared essentially as in [4], were reconstituted