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Excitonic interactions in the reaction centre of photosystem II studied by using circular dichroism

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Abstract

Changes in excitonic interactions of photosystem II (PSII) reaction centre (RC) pigments upon light-induced oxidation of primary donor (P680) or reduction of primary acceptor (pheophytin (Pheo)) were analysed using circular dichroism (CD). The CD spectrum of PSII RC shows positive bands at 417, 435 and 681 and negative bands at 447 and 664 nm. Oxidation of the primary donor by illuminating the sample in the presence of silicomolybdate resulted in nearly symmetric decrease of CD amplitudes at 664 and 684 nm. In the Soret region, the maximum bleaching of CD signal was detected at 449 and 440 nm. Accumulation of reduced Pheo in the presence of dithionite brought about much lower changes in CD amplitudes than P680 oxidation. In this case, only a small asymmetric bleaching at 680 and 668 nm in the red region and a bleaching at 445, 435 and 416 nm in the Soret region has been detected. Therefore, we suppose that the contribution of the Pheo of the primary acceptor to the total CD signal of RC is negligible. In contrast to the oxidation of primary donor, the light-induced change in the CD spectrum upon primary acceptor reduction was strongly temperature-dependent. The reversible CD bleaching was completely inhibited below 200 K, although the reduced Pheo was accumulated even at a temperature of 77 K. Since the temperature does not influence the excitonic interaction, the temperature dependence of the CD changes upon Pheo reduction does not support the model of Pheo excitonically interacting with the other chlorophylls (Chl) of the RC. We propose that Pheo should not be considered as a part of a multimer model.

Keywords: Photosynthesis; Chlorophyll; Pheophytin; Polarised spectroscopy

1. Introduction

Photosystem II (PSII) is a pigment protein complex of the thylakoid membrane of higher plants, green algae and cyanobacteria. Its role is to convert the energy of light to the electrochemical energy of a membrane potential. The primary process of this conversion is a light-induced separation of a charge, which occurs in the core of PSII, the reaction centre (RC). The RC was first isolated from spinach by Nanba and Satoh [1]. In its simplest form the RC consists of five protein subunits containing six molecules of chlorophyll (Chl), two molecules of pheophytin (Pheo), one or two molecules of β-carotene (β-Car) and one molecule of heme. Recently, a three-dimensional structure of PSII was resolved by X-ray crystallography at 3.8 Å resolution with data about the arrangement of the PSII RC protein subunits [2].

In spite of great progress in the study of PS II RC during recent years, we still do not have sufficient understanding about the structure and function of the whole RC complex. Much information can be extracted from more extended research on bacterial RCs. Based on the clear homology of the amino acid sequence of PSII RC proteins with the proteins of the purple bacterial RC a structural and functional similarity of these two RCs has been proposed [3]. In the bacterial RC a special pair of two strongly excitonically coupled bacteriochlorophyll *a* (Bchl *a*) molecules forms a dimer of a primary electron donor, one molecule of bacteriopheophytin *a* (Bpheo *a*) acts as a primary electron acceptor. Recently, the accessory Bchl *a* of the active branch was proposed to act as an alternative primary donor [4]. Similar pigment organisation was considered for the PSII

Abbreviations: Bchl *a*, bacteriochlorophyll *a*; Bpheo, bacteriopheophytin a; Car, carotene; CD, circular dichroism; Chl, chlorophyll; DM, *n*-dodecyl β-D-maltoside; P680, primary donor of Photosystem II; Pheo, pheophytin; PSII, Photosystem II; RC, reaction centre

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RC pigment structure with two Chl *a* molecules forming a special pair of primary donor P680 and one active Pheo *a* molecule acting as a primary acceptor. However, there are some considerable differences between these two RCs mainly on the pigment level.

A model of a multimer organisation of PSII RC chlorins has been proposed [5,6]. In such model the couplings between the six central PSII RC chlorins (four chlorophyll and two Pheo) are proposed to be very similar, and therefore a multimer model arises in which there is no special pair of P680 primary donor and in which the exciton may be localized on any combination of neighbouring chlorins [7].

Important information about such pigment excitonic interactions in the PS II core can be obtained from circular dichroism (CD) spectra. However, we still have only imperfect knowledge about the origin of the CD spectra of the PSII RC. Chl or Pheo monomers dissolved in organic solvents have only very weak CD signal [8]. On the other hand, in the photosynthetic pigment–protein complexes the CD signal of Chl is considerably higher [9]. In the case that a chromophore is not optically active, the rotation strength may be induced by a disorder in the symmetry of the electronic states of the molecule caused by the influence of its environment. Therefore, such a CD signal may turn up either due to a pigment–pigment or a pigment–protein interaction or both [10]. In the case of the pigment–pigment interaction the magnitude of the CD signal depends on the mutual orientation of the interacting dipoles. In a particular orientation of the interacting dipoles, no CD signal is induced even if the strong excitonic coupling exists. However, based on the structural data, such orientation was not found in the PSII RC [2,11]. At the moment, we are not yet certain about which bands of the CD signal of pigment–protein complexes result from excitonic interactions between chlorins and which are due to the interactions of chlorins with charged groups and inducible dipoles in the lipoprotein matrix.

In order to get further information on the excitonic interactions of RC pigments and the origin of the CD signal in the PSII RC, we have studied reversible bleaching of the absorbance and CD spectra upon the selective light-induced oxidation of primary donor, P680, or reduction of primary acceptor, Pheo, by using an artificial electron acceptor or donor. The temperature dependences of these spectral changes, which have a potential to distinguish between changes caused by the excitonic interaction and the temperature-dependent processes, have also been studied.

2. Materials and methods

RCs of PSII containing five Chl *a*, two Pheo and one β-Car were prepared from pea (*Pisum sativum)* using a Cu affinity chromatography method [12] with slight modification. The concentration of *n*-dodecyl β-D-maltoside (DM) during washing of the chromatography column was only 0.4 mM in contrast to a concentration of 2 mM in the original procedure.

For all measurements, samples were diluted to the final concentration of ~ 10 μg Chl ml^{-1} in a buffer containing 50 mM MES, 0.4 mM DM at pH 6.5. For low temperature spectra, glycerol was added to the sample to a final concentration of 65% (v/v).

The light-induced Pheo reduction was measured in the presence of sodium dithionite and methylviologen at concentrations of 1 mg ml^{-1} and 10 μM, respectively. Before the addition of dithionite, oxygen was removed by bubbling the sample in the cuvette with nitrogen gas. Oxidation of primary donor was measured in the presence of silicomolybdate (SiMo) at a concentration of 200 μM.

The light-induced absorbance changes were recorded by a home-built kinetic photodiode array spectrophotometer with side illumination. The white measuring light provided by a xenon flash lamp (EG&G Electro Optics, USA) was guided through the sample to the monochromator (Oriel, USA) and the spectrum was recorded by an array of diodes (Hamamatsu, Japan).

The light-induced changes in spectra of CD were recorded by using JASCO J-715 spectropolarimeter (JASCO Corporation, Tokyo, Japan) with side illumination. 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).

Side illumination of an approximate intensity of 500 μE m^{-2} s^{-1} was filtered by a Calflex heat filter and Schott RG 645 or Corning 4-96 filters, the photomultiplier was protected by Corning 4-96 or Schott RG 645 filters, respectively. Samples were frozen to 77 K and illuminated for approximately 10 s until the maximum change was achieved. After each measurement at particular wavelength,

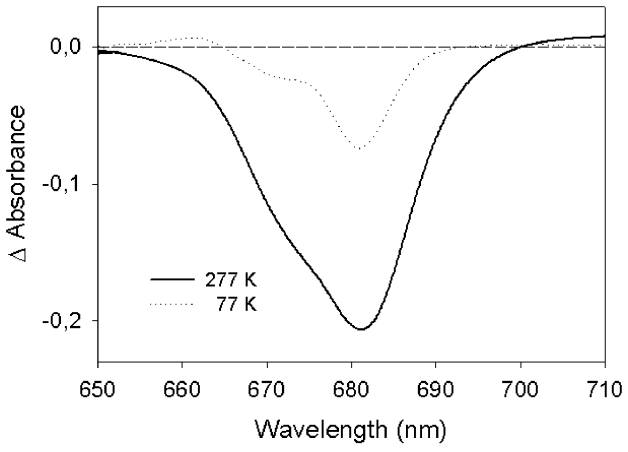

Fig. 1. Absorbance difference spectrum of PSII RCs upon light-induced primary donor oxidation in the presence of SiMo measured at 277 K (solid line) and 77 K (dotted line).

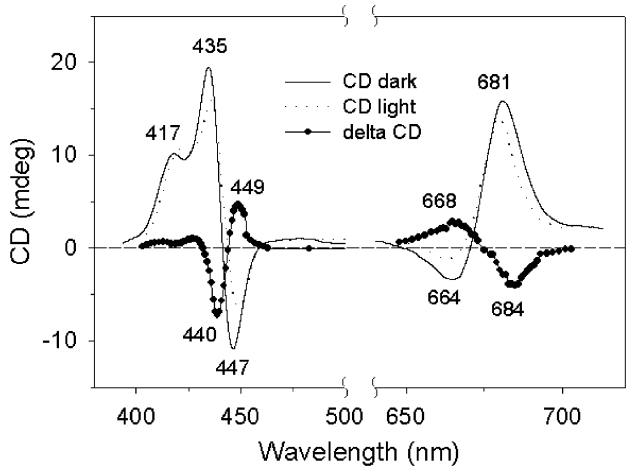

Fig. 2. CD spectra of PSII RCs measured in the presence of SiMo in dark (solid line), in light (dotted line) and the difference CD spectrum upon light-induced oxidation of the primary donor in the presence of SiMo (closed circles with solid line). All measurements were done at a temperature of 277 K.

sample was kept in the dark for at least 5 min until the signal reached the initial level.

3. Results

3.1. Oxidation of primary donor in the presence of SiMo

Illumination of PS II RCs in the presence of the electron acceptor, SiMo, resulted in an accumulation of oxidized primary donor, $P680^+$. Fig. 1 shows the reversible light-induced absorbance difference spectra of the RC upon oxidation of the primary donor at 277 K (solid line) and at 77 K (dotted line). The maximum absorbance change is at 681 nm for both 277 and 77 K temperatures as it was

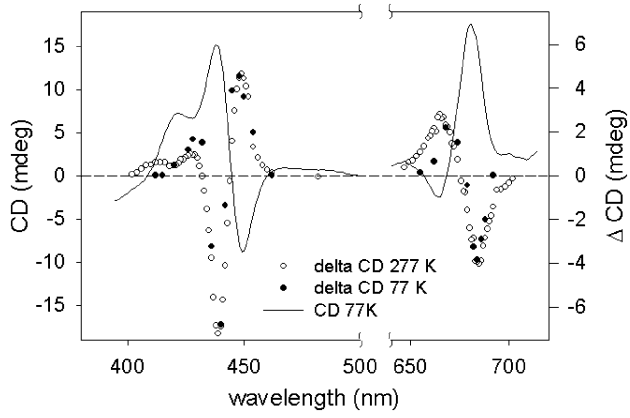

Fig. 3. CD spectrum of PSII RCs measured in the presence of SiMo in dark at a temperature of 77 K (solid line) and the light-induced changes in CD spectrum upon primary donor oxidation in the presence of SiMo at 277 K (open circles) and 77 K (closed circles).

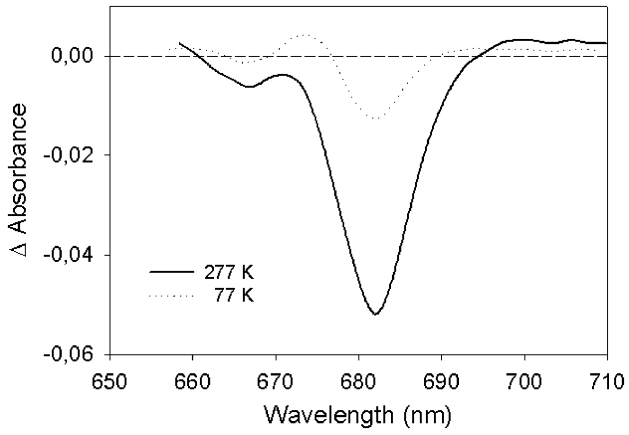

Fig. 4. Absorbance difference spectrum of PSII RCs upon light-induced primary acceptor reduction in the presence of dithionite measured at 277 K (solid line) and 77 K (dotted line).

previously reported [13]. The peak area of the light induced absorbance change at 77 K is about 20% of that measured at 277 K.

At 277 K, the CD spectrum of PSII RC in the Qy region is formed by a positive and negative band peaking at 681 and 664 nm, respectively. In the Soret region the CD spectrum shows two positive peaks at 435 and 417 nm and one negative peak at 447 nm. The control CD spectrum was not affected by addition of SiMo to the RC (Fig. 2, solid line).

The light-induced oxidation of the primary donor, P680, upon irradiation of the RC in the presence of SiMo at 277 K resulted in a decrease of the CD signal throughout the spectrum (Fig. 2, dotted line). The decrease of the CD signal was fully reversible after switching off the actinic light. The light minus dark difference spectrum is shown in

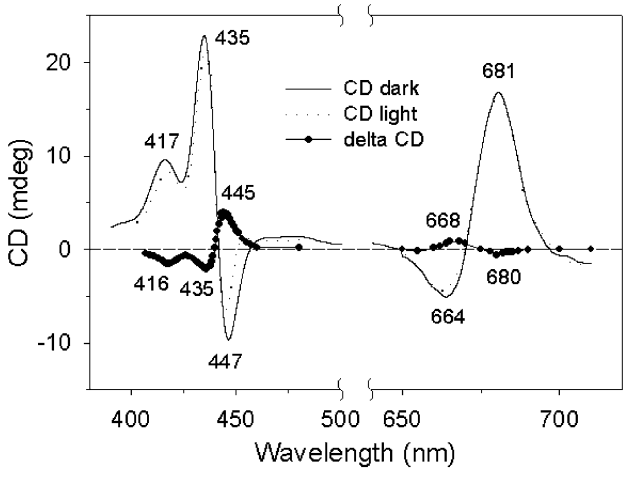

Fig. 5. CD spectra of PSII RCs measured in the presence of dithionite in dark (solid line), in light (dotted line) and the difference CD spectrum upon light-induced reduction of the primary acceptor in the presence of dithionite (closed circles with solid line). All measurements were done at a temperature of 277 K.

Fig. 2 (closed circles with solid line). In the red region the maximum changes were found at 684 nm (a decrease of about 40%) and 666 nm (an increase of about 75%). In the blue region the maximum positive change occurs at 449 nm and the negative change at 440 nm.

Fig. 3 shows the CD spectrum of the PSII RC measured at 77 K in the presence of SiMo (solid line) and compares the light-induced changes in the CD spectrum measured at temperatures of 277 K (open circles) and 77 K (closed circles). These light minus dark difference spectra are normalised at 684 nm. The amplitude of the light-induced CD change at 684 nm measured at 77 K is about one third of the change measured at 277 K at the same wavelength.

3.2. Reduction of primary acceptor

Fig. 4 shows the reversible light-induced absorbance difference spectrum of the PSII RC upon Pheo reduction in the presence of sodium dithionite and methyl viologen measured at 277 K (solid line) and at 77 K (dotted line). The maximum absorbance change is at 681 nm for both room and liquid nitrogen temperatures as previously observed [1,14]. The peak area of the light induced absorbance change measured at 77 K was about 15% of that measured at 277 K.

Fig. 5 shows the CD spectrum of PSII RC in the presence of dithionite in the dark (solid line), in the light (dotted line) and the light-induced difference CD spectrum (closed circles with solid line) measured at a temperature of 277 K. The light-induced Pheo reduction in the presence of dithionite resulted in different changes in the CD spectrum when compared to the changes after P680 Chl oxidation. Generally, the overall bleaching was significantly lower especially in the Qy region. In that region, we have observed negative change peaking at 680 nm and positive change at 665 nm. In the Soret region one positive change at 445 nm and two negative changes at 435 and 416 nm were detected. The CD amplitude decreased by only about 3.5% of the original dark signal at 680 nm and by about 17% at 665 nm.

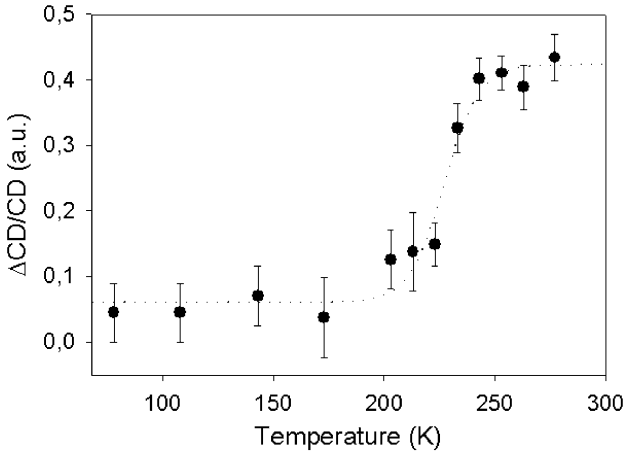

Fig. 6. The temperature dependence of the light-induced changes in the CD of PSII RCs upon the primary acceptor reduction in the presence of dithionite measured at 445 nm. Data are normalised to the absorbance change upon pheophytin reduction at given temperatures.

At 77 K, however, no reversible light-induced change in the CD spectrum was detected upon Pheo reduction. The temperature dependence of light-induced changes in the CD spectrum of PSII RC, in the presence of dithionite, measured at 445 nm is presented in Fig. 6. The wavelength of 445 nm was chosen because of the highest amplitude in the light-induced change, however, similar temperature dependence was found for other wavelengths. At a temperature of about 230 K, 50% inhibition of the light-induced difference changes was observed.

4. Discussion

The RC preparation containing 5 Chl per 2 Pheo was used for our experiments. This preparation was shown to have the same properties as preparations with 6 Chl per 2 Pheo [12,15,16] but was more useful for the spectroscopic measurements. In the 5-Chl RC the most red absorbing species at 684 nm is more visible after removing the additional peripheral Chl [15].

The dark CD spectra of the PSII RCs measured in the presence of either SiMo or dithionite (solid lines in Figs. 2 and 5, respectively) are comparable with the spectra of other 5- and 6-Chl PSII RC preparations previously published [15,17–22]. This suggests that the excitonic and pigment protein interactions within the core pigments of the RC are not affected by addition of the artificial acceptor or donor. In agreement with previous results [15], we did not see any shoulder in the CD spectrum at 674 nm when measured at 77 K, as had been previously reported [20,21]. The 674 nm absorbing species is probably an impurity that appears during isolation process [16] or a result of detergent treatment [22].

Assuming that the CD signal is mainly from the excitonic interaction of the P680 Chl dimer, then P680 Chl oxidation should result in a disappearance of the CD spectrum. If not all of the RCs were active, then only a decrease in the intensity of the CD spectrum could be expected. In the case of multimer exciton interaction, the oxidation of the primary donor breaks up the original pigment couplings and new exciton interactions are established among the rest of the RC core chlorines, leading to a new and different CD signal. Thus, we would see a shift of maxima in the resultant CD spectrum.

After primary donor P680 oxidation, the CD spectrum did not disappear and a spectral shift was observed. Maximum bleaching was detected at 684 and 666 nm, whereas the CD spectrum peaks at 681 and 664 nm. These results exclude the possibility that the total CD signal of the PSII RC comes from a Chl dimer.

A more complicated situation arises when the pigment–protein interaction also acts as a source of the CD signal. In

this case, breaking the exciton interaction between RC chlorins by oxidation of the primary donor would not diminish the whole CD signal but would result (i) in the case of dimer model, in a CD spectrum induced only by the remaining pigment–protein interactions, or (ii) in the case of multimer model, in a CD spectrum induced by a mixture of pigment–protein interactions and newly induced pigment–pigment interactions.

The assumption that the CD signal in the RC results exclusively from the excitonic interaction predicts a conservative CD spectrum, which means a null sum for the positive and negative rotational strengths of the Chl CD bands [23–27]. This is evidently not true for the dark CD spectrum, however, the light minus dark difference spectrum (Fig. 2, closed circles) is conservative, suggesting that this spectrum reflects the interruption of the original excitonic interaction within the core of the RC.

The remaining CD spectrum (Fig. 2, dotted line) is a result of contributions induced by pigment–protein interaction and remaining exciton interactions, influenced by a highly positive charge on a molecule of the primary donor Chl.

The CD difference spectra upon Pheo reduction may be discussed in the same terms as those observed upon P680 oxidation. Pheo reduction resulted in much lower bleaching of the CD spectrum than observed in the case of primary donor oxidation. The maxima of the bleaching were identical with the maxima of the original CD spectrum, suggesting little change in the distribution of the exciton interaction in the RC upon Pheo reduction.

At a temperature of 77 K, no large-scale motion of the protein is allowed. Thus, comparing the CD spectra at room temperature and 77 K can tell us about the protein dynamics induced by accumulation of reduced Pheo or oxidized P680. Under primary donor oxidation at 277 and 77 K, no variance in the light-induced difference CD spectra was observed (Fig. 3). This suggests that the accumulation of oxidised primary donor does not induce the changes in protein conformation. Therefore, the changes in the CD spectra are not caused by changes in pigment–protein interactions due to the motion of the protein surrounding of pigments but mainly by the changes in the pigment–pigment interactions.

On the other hand, we have observed dramatic temperature dependence of the light-induced difference spectrum under primary acceptor reduction (Fig. 6). At temperatures below 180 K, no reversible light-induced changes in the CD spectra were detected, even if the accumulation of reduced Pheo was detected in the absorbance difference spectrum (Fig. 4). If the molecule of Pheo is a part of the multimer interaction, its reduction would lead to a change in the exciton interaction and consequently to a change in the CD spectrum. Since the process of excitonic interaction is not dependent on temperature and the Pheo reduction does not cause any change in the low temperature CD spectrum, we suppose that the Pheo molecule is not coupled in the multimer as it was originally predicted [6].

The temperature dependence of the light-induced CD changes suggests that, at room temperature, the Pheo accumulation is likely to be accompanied by a conformational change in the protein surroundings, which affects the original CD spectrum. This conformational change is blocked at low temperature and, therefore, the Pheo reduction itself has no effect on the CD spectrum at these conditions.

In contrast to our results, Tetenkin et al. [5] observed changes in the low temperature CD spectrum after accumulation of reduced Pheo. However, this discrepancy may be explained by pre-reduction of Pheo at room temperature prior to the measurement or by irreversible changes in the spectrum.

Germano et al. [28] have measured CD spectra after a chemically exchanged Pheo and their results show dramatic changes in the CD spectra. This would suggest that relatively large part of the total CD signal originates from the Pheo molecules. Compared to our results, we conclude that such dramatic changes in the CD spectrum after Pheo exchange belongs to the pigment–protein interactions.

The charged-induced changes in the conformation of the protein surroundings were previously suggested by Konermann et al. [29] to play a role in shifting the equilibrium towards the radical pair formation, and were included in the schema of energy transfer and primary charge separation reactions in the PSII RC presented by Dekker and van Grondelle [7].

Compared to the inactive Pheo, the active Pheo is surrounded by several polar and charged amino acids such as Tyr^{126}, Tyr^{147}, Agr^{129} and Glu^{130} [11]. We suppose that these amino acids induce the protein conformational change after Pheo reduction, by a process in which the negative charge on the Pheo molecule affects the side chains of these amino acids.

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