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

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Introduction

Photosystem II (PSII) is a pigment-protein complex of a 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. The reaction centre was first isolated from spinach by Nanba and Satoh (Nanba and Satoh 1987). In its simplest form the reaction centre consists of five protein subunits containing six molecules of chlorophyll, two molecules of pheophytin, one or two molecules of β-carotene and one molecule of hem. Recently, a three-dimensional structure of PSII was solved by an X-ray crystallography at 3.8 Å resolution with a relatively good data about the arrangement of the PSII reaction centre protein subunits (Zouni et al. 2001).

A model of a multimer organization of PSII reaction centre chlorins has been proposed (Durrant et al. 1995). In such model the couplings between the six central PSII reaction centre chlorins (four chlorophyll and two pheophytin) 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 (Dekker and van Grondelle 2000).
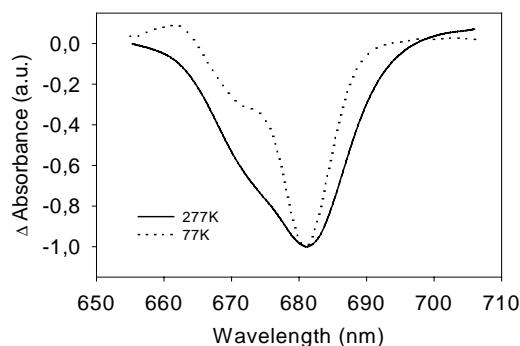
Important information about such pigment excitonic interactions in PS II core can be obtained from circular dichroism spectra. However, we still have only imperfect knowledge about the origin of the circular dichroism spectra of the PSII reaction centre.

In order to get further information on the excitonic interactions of RC pigments and the origin of the CD signal in the PSII reaction centre we have studied reversible bleaching of the absorbance and circular dichroism spectra upon the selective light induced oxidation of primary donor, P680, or reduction of primary acceptor, pheophytin, by using 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 are also a matter of the study.

Materials and Methods

RC containing 5 Chl *a*, 2 Pheo and 1 β-Car were prepared from pea (*Pisum sativum)* using Cu affinity chromatography method (Vacha et al. 1995). The light induced changes in CD were recorded by using J-715 spectropolarimeter (JASCO, Tokyo, Japan). The light induced changes of absorption spectra were recorded by a home build kinetic photodiode array spectrophotometer (SpeKin, P. Šiffel, under patent pending). Low temperature spectra were measured in Oxford
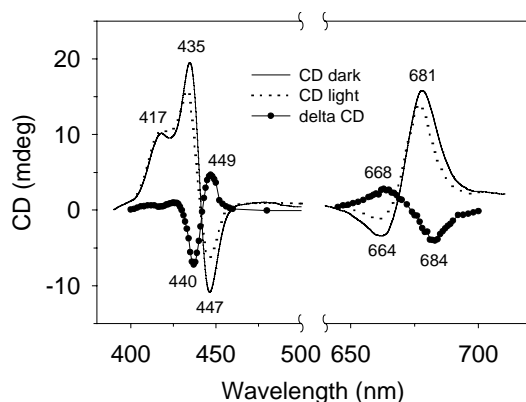
Optistat Bath cryostat (Oxford Instruments, Oxon, England). For all measurements samples were diluted to the final concentration of ~ 10 μg chl . ml^{-1} in a buffer containing 50 mM MES, 0.02% DM at pH 6.5. For low temperature spectra glycerol was added to the sample to a final concentration of 65 % (v/v). The light induced Pheo reduction was measured in presence of dithionite and methylviologen at concentrations of 1 mg . ml^{-1} and 10 μM, respectively. Oxidation of primary donor was measured in presence of silicomolybdate (SiMo) at concentration of 200 μM. Side illumination of approximate intensity of 500 μE . m^{-2} . s^{-1} was filtered by Calflex heat filter and Schott RG 645 or Corning 4-96, the photomultiplier was protected by Corning 4-96 or Schott RG 645 filters, respectively.

Results

Oxidation of primary donor in presence of SiMo

Illumination of PS II reaction centre in the presence of electron acceptor, SiMo, resulted in an accumulation of oxidized primary donor, P680$^+$. Figure 1 shows the reversible light induced absorbance difference spectrum of the reaction centre 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 temperatures. The spectra are normalised at the wavelength of 681 nm. The amplitude of the absorbance change at 77 K is about 35 % of the amplitude measured at 277 K.

At 277 K, the circular dichroism spectrum of PSII reaction centre 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 reaction centre (Figure 2, solid line).

The light induced oxidation of the primary donor, P680, upon irradiation of RC in presence of SiMo at 277 K resulted in the decrease of CD signal throughout the spectrum (Figure 2, dotted line). The decrease of the CD signal was fully reversible after switching off the actinic light. In the red region the maximum changes were found at 684 nm (decrease by about 40 %) and 666 nm (increase by about 75 %). In the blue region the maximum positive change occurs at 449 nm and negative one at 440 nm (Figure 2, closed circles with solid line).

Figure 3 shows the CD spectrum of PSII reaction centre measured at 77 K in presence of SiMo (solid line) and compares the light induced changes in CD spectrum measured at 277 K (open circles) and at 77 K (closed

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

Fig. 2. Circular dichroism spectra of PSII reaction centres measured in presence of SiMo in the dark (solid line), in the light (dotted line) and the difference CD spectrum upon light induced oxidation of the primary donor in presence of SiMo (closed circles with solid line). All measurements were done at 277 K.
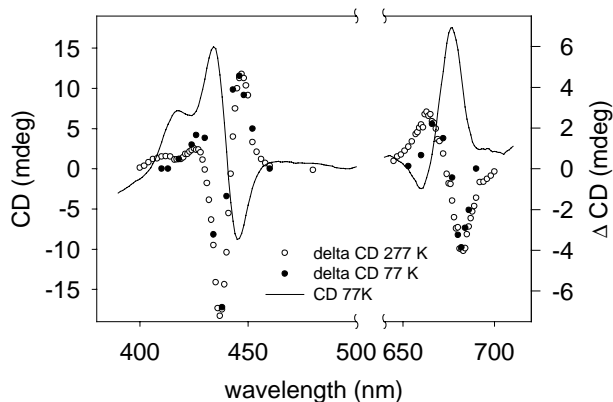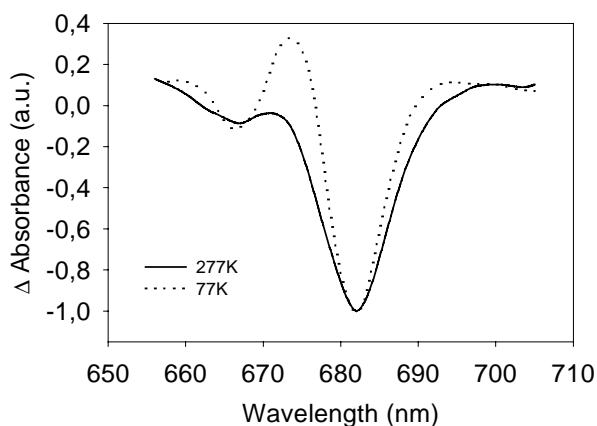
Fig. 3. Circular dichroism spectrum of PSII reaction centres measured in presence of SiMo in the dark at the temperature of 77 K (solid line) and the light induced changes in CD spectrum upon primary donor oxidation in presence of SiMo at 277 K (open circles) and 77 K (closed circles).

circles). The light minus dark difference spectra are normalised at 680 nm. The amplitude of the light induced CD change at 684 nm measured at 77K is about one third of the change measured at 277K at the same wavelength.
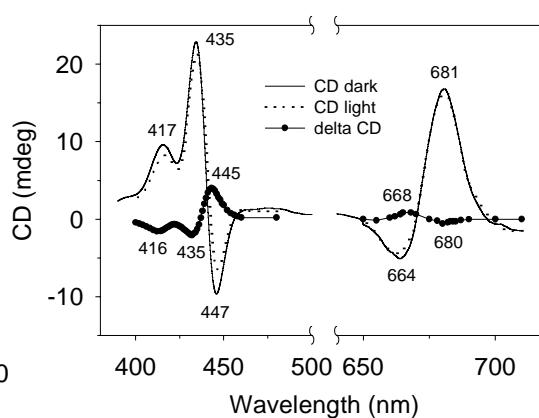
Reduction of primary acceptor

Figure 4 presents the reversible light induced absorbance difference spectrum of the PSII reaction centre upon pheophytin reduction in 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 for both the room and liquid nitrogen temperatures as it was observed previously (Nanba and Satoh 1987, Barber et al. 1987). The spectra are normalised at 681 nm, the amplitude of the absorbance change at 681 nm of the sample measured at 77 K was about 20 % of the amplitude measured at 277 K.
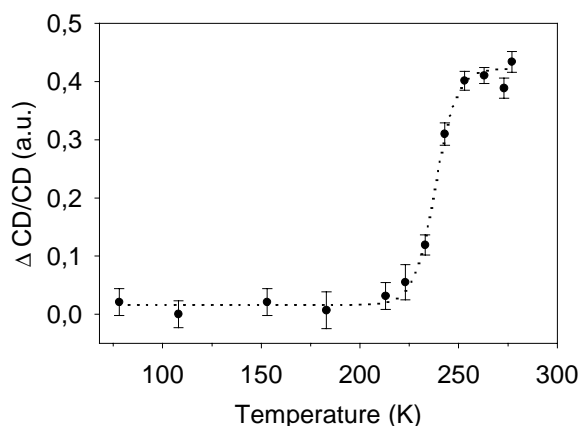
   Figure 5 shows the CD spectrum of PSII reaction centre in presence of dithionite in the dark (solid line), in the light (dotted line) and the light induced difference CD spectrum (closed circles with solid line) measured at the temperature of 277 K.
The light induced Pheo reduction in presence of dithionite induced different changes in the CD

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

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

Fig. 6. The temperature dependence of the light induced changes in the circular dichroism of PSII reaction centres upon the primary acceptor reduction in presence of dithionite measured at 445 nm.

spectrum when compare to the changes after P680 chlorophyll 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 only by about 3.5 % at 680 nm and by about 17 % at 665 nm of the original dark signal.

At 77 K, however, no reversible light induced change in the CD spectrum was detected upon Pheo reduction. The temperature dependence of the light induced changes in CD spectrum of PSII RC in presence of dithionite measured at 445 nm is presented in figure 6. The wavelength of 445 nm was chosen because of the highest amplitude in the light induced change. The decrease of the light induced difference changes has occurred within 25 K with a half inhibition at the temperature around 225 K.

Discussion

The reaction centre preparation containing 5 chlorophylls per 2 pheophytin was used for our experiment. This preparation was shown to have the same properties as the preparations with 6 chlorophylls per 2 pheophytin (Vacha et al. 1995, Eijckelhoff et al. 1997, den Hartog et al. 1998) but being more useful for the spectroscopic measurements where the most red absorbing species are not covered by the absorption of one of the peripheral chlorophyll.

The dark CD spectra of the PSII reaction centres measured in presence of either SiMo or dithionite (solid lines in figures 2 and 5, respectively) are comparable with spectra of the other 5 and 6 chlorophyll PSII reaction centre preparations previously published (Eijckelhoff et al. 1997, Newell et al. 1988, Braun et al. 1990, Newell et al. 1991, Otte et al. 1992). This suggests that the exciton interaction within the core pigments of the reaction centre is not affected by addition of the artificial acceptor or donor.

Assuming that the CD signal is mainly from the excitonic interaction of P680 chlorophyll dimer, the P680 chlorophyll oxidation should results in a disappearance of the CD spectrum. If not all of the primary donors were oxidised, only a decrease in the intensity of the CD spectrum would be expected. In the case of multimer exciton interaction oxidation of the primary donor would break up the original pigment couplings and new exciton interactions would be established among the rest of the reaction centre core chlorins leading to a new CD signal. As a result we would see a shift of maxima in the final CD spectrum.

More complicated situation arises when concerning also the pigment-protein interaction as a source of the CD signal. In this case, breaking the exciton interaction between reaction centre chlorin by oxidation of primary donor would not diminish the CD signal but it would result: i) in the case of dimer model in a CD spectrum induced only by the remaining pigment-protein interactions, ii) for the multimer model in a CD spectrum induced by a mixture of pigment-protein interaction and newly induced excitonic interactions.

The results may be even more complex when assuming the influence of a strong positive charge on the chlorophyll of the primary donor on the reaction centre environment.

P680 oxidation leads to a spectral shift in the CD spectrum. The maximum bleaching was observed at 684 and 666 nm, whereas the CD spectrum is peaking at 681 and 664 nm. The assumption that the CD signal in reaction centre 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 chlorophyll CD bands (Tinoco 1962). This is evidently not true for the dark CD spectrum, however, the light minus dark difference spectrum (fig. 2, closed circles) seems to be conservative suggesting that the P680 oxidation reflects the interruption of the original excitonic interaction within the core of the reaction centre. 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 chlorophyll. Comparing our results with simulated absorbance (Durrant et al. 1995) and CD spectra and from simulating their light induced difference changes (data not shown) we suppose that the RC core chlorophyll pigments constitute weakly coupled multimer.

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 in the case of primary donor oxidation. The maxima of the bleaching were identical with the maxima of the original CD spectrum suggesting a little change in the distribution of the exciton interaction in the reaction centre upon Pheo reduction.

At 77 K, no large-scale motion of the protein is allowed during charge separation processes. Comparing the CD spectra at room temperature and at 77 K can tell us about the protein dynamics induced by accumulation of reduced Pheo or oxidized P680. Under primary donor oxidation no variance in the light induced difference CD spectra was observed (fig. 3). It suggests that the changes in the CD spectra upon the primary donor oxidation are not caused by changes in pigment-protein interactions due to the motion of the protein surrounding of the 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 CD spectra were detected, even if the accumulation of reduced Pheo was detected in the absorbance difference spectrum (fig. 4). If the molecule of pheophytin is a part of the multimer interaction its reduction would have to lead to a change in the exciton interaction and consequently to the change in the CD spectrum. Since the process of the excitonic interaction is not dependent on temperature and the pheophytin reduction did 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 (Durrant et al. 1995).

The temperature dependence of the light induced CD changes suggests that, at room temperature, the charge separation is likely accompanied by a conformational change in the protein matrix or the amino acid side chains surrounding the RC core chlorophylls, 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. The charged-induced changes in the conformation of the protein surroundings was previously suggested by Konermann (Konermann *et al.* 1997, Dekker and van Grondelle 2000) to play a role in shifting the equilibrium towards the radical pair formation.

We suppose that the difference in the particular protein environments of the two pheophytins of the PSII RC is one of the reasons of the difference in the Pheo function. While the inactive Pheo is surrounded mainly by non-polar amino acids, the protein environment of active Pheo is formed by several amino acids with polar side chains, such as Tyr 126, Arg 129, Glu 130, Tyr 147, Ser 148 and. These side chains can be affected by the negative charge of the reduced Pheo, change their conformation and hence stabilise the charge separation. The conformation change of such amino acids can impact the whole tertiary

structure of the D1 molecule and subsequently the CD spectrum of the RC core chlorophyll molecules.

Acknowledgements

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