Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochimica et Biophysica Acta 1709 (2005) 119 – 126
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
http://www.elsevier.com/locate/bba

Circular dichroism of the peripheral chlorophylls in photosystem II reaction centers revealed by electrochemical oxidation

Tatyana N. Kropacheva[a,*], Marta Germano[b], Giuseppe Zucchelli[c], Robert C. Jennings[c], Hans J. van Gorkom[b]

[a]*Chemistry Department, Udmurt State University, Universitetskaya 1, Izhevsk, 426037, Russia*
[b]*Biophysics Department, Huygens Laboratory, Leiden University, P.O. Box 9504, 2300 RA Leiden, The Netherlands*
[c]*Istituto di Biofisica del Consiglio Nazionale delle Ricerche, Sezione di Milano, Dipartimento di Biologia, Università di Milano, Via Celoria 26, Milano, Italy*

Received 7 March 2005; received in revised form 14 April 2005; accepted 19 April 2005
Available online 18 July 2005

Abstract

Visible absorption spectra and circular dichroism (CD) of the red absorption band of isolated photosystem II reaction centers were measured at room temperature during progressive bleaching by electrochemical oxidation, in comparison with aerobic photochemical destruction, and with anaerobic photooxidation in the presence of the artificial electron acceptor silicomolybdate. Initially, selective bleaching of peripheral chlorophylls absorbing at 672 nm was obtained by electrochemical oxidation at +0.9 V, whereas little selectivity was observed at higher potentials. Illumination in the presence of silicomolybdate did not cause a bleaching but a spectral broadening of the 672-nm band was observed, apparently in response to the oxidation of carotene. The 672-nm absorption band is shown to exhibit a positive CD, which accounts for the 674-nm shoulder in CD spectra at low temperature. The origin of this CD is discussed in view of the observation that all CD disappears with the 680-nm absorption band during aerobic photodestruction.

Keywords: Photosystem II; Reaction center; Electrochemical oxidation; Circular dichroism

1. Introduction

The reaction center of Photosystem II (PSII RC) can be isolated as the so-called D1D2Cytb_{559} complex [1]. The D1 and D2 polypeptides form an approximately C_2-symmetrical structure that binds 6 chlorophyll *a* (Chl) and 2 pheophytin *a* (Pheo) molecules. Two of the Chls occupy a peripheral position, while the other pigments are placed centrally in a U-shaped arrangement much like that found in the reaction centers of other photosynthetic systems [2,3]. The complex also contains 2 β-carotene (Car) molecules, both of which may be placed on the D2 side [4].

Unlike the situation in other photosystems, the central pigments in the PSII RC are almost iso-energetic and appear to be collectively responsible for a 'multimeric' absorption band around 680 nm [5,6], which is so little red-shifted relative to that of the 'monomeric' peripheral Chls around 672 nm that these bands can be only partially resolved at cryogenic temperatures. Nevertheless, even at room temperature, where the Q_Y absorption of all chlorins seems to merge in a single unresolved band peaking at 675–676 nm, absorption difference spectra readily distinguish changes that selectively affect the peripheral or the central pigments. The Q_Y absorption of the PSII RC exhibits a remarkably strong circular dichroism (CD) with a large positive band near 681 nm and a smaller negative one near 664 nm [5,7]. Because Chl monomers in solution have a small negative intrinsic rotary strength [8], excitonic interactions between

Abbreviations: PSII, Photosystem II; RC, reaction centers; Chl, chlorophyll *a*; Pheo, pheophytin *a*; Car, β-carotene; NHE, normal hydrogen electrode; P_{680}, primary donor of PSII; CD, circular dichroism; E_m, midpoint potential

* Corresponding author. Tel.: +7 3412 526085; fax: +7 3412 755866.
E-mail address: krop@uni.udm.ru (T.N. Kropacheva).

0005-2728/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2005.04.005

some or all of the central pigments are believed to be responsible for part of the CD, but that would produce only a 'conservative' CD spectrum, with equal positive and negative lobes. The observed net positive chirality must be imposed by the protein structure and is not yet understood. Of special interest in this respect is the appearance of a partially resolved positive CD band at 674 nm at low temperature [9,10]. This band appeared to be unaffected when the Pheos were replaced by 13^1-OH-Pheo, which caused a large decrease of conservative CD [11]. Significantly, the 674-nm CD feature was reported to be missing in PSII RC preparations that lack one of the peripheral Chls [12].

In order to investigate the contribution of the peripheral Chls to the CD of the Q_Y absorption at room temperature, we have compared the effects on the CD spectrum of irreversible oxidative pigment bleaching at room temperature by three different methods:

(1) *Illumination in the presence of oxygen.* Since the secondary electron donors and acceptors involved in water oxidation are lost during isolation of the PSII RC, illumination produces only the primary charge separation, in the active branch (D1-side) of the central pigment group. The radical pair $P_{680}^+Pheo^-$ is rapidly lost by recombination, in part to the triplet state $^3P_{680}$. In the presence of oxygen, $^3P_{680}$ is converted to the ground state by producing highly reactive singlet oxygen, $^1O_2$, which can lead to oxidative destruction of the same or a nearby pigment. This causes an irreversible bleaching of its Q_Y absorption at 680 nm and a blue shift of the remaining central pigments due to their decreased exciton interactions, which is indicated by disappearance of the CD [5,7].

(2) *Anaerobic illumination in the presence of silicomolybdate.* Damage to the central pigments can be suppressed or at least delayed by the absence of oxygen and the presence of an electron acceptor like silicomolybdate (SiMo) to oxidize $Pheo^-$ before charge recombination occurs [13,14]. In the latter case P_{680}^+ lives long enough to oxidize Car and peripheral Chls, resulting in an initially selective bleaching around 672 nm [15,16]. The reversible photo-oxidation of P_{680} in the presence of SiMo decreases the conservative CD [12], but the effect on CD of the 672 nm bleaching has not been reported.

(3) *Electrochemical oxidation in the dark.* This method was expected to allow selective oxidation of the peripheral Chls because the oxidation midpoint potential of the monomeric Chl^+/Chl redox couple in organic solvents is around +0.8 V (vs. NHE) [17], whereas that of P_{680}^+/P_{680} is presumably larger than +1.2 V [18] and those of the other central pigments must be higher than that. Recent calculations based on the PSII crystal structure also showed a large difference in midpoint potential of core Chls (+1.1 V to +1.3V) and the peripheral species (+0.9V) [19]. In spite of irreversible secondary reactions, initially selective oxidation of the peripheral Chls was indeed obtained.

2. Materials and methods

The isolation of spinach PSII RC was performed essentially as described in [20]. Tris-washed 'BBY' PSII membrane fragments [21] were resuspended in BTS-400 buffer (20 mM bis-Tris, pH 6.5, 20 mM $MgCl_2$, 5 mM $CaCl_2$, 10 mM $MgSO_4$, 400 mM sucrose, 0.03% (w/v) n-dodecyl-β-D-maltoside (DM)) and solubilized during 30 min using 1.25% DM in the dark at room temperature, followed by centrifugation at 40,000×g. The supernatant was loaded at 4 °C on a Q-Sepharose column equilibrated with BTS-200 (as BTS-400, but with 200 mM sucrose) and washed with BTS-200 to elute the bulk of the light-harvesting complex. The PSII core particles bound on the column were incubated for 20 min with 10% Triton X-100 in BTS-200 at 4 °C. Subsequent washing with BTS-200 removes most of the core antenna proteins; a second incubation was performed to complete the separation. Excess Triton X-100 was removed by washing the column with BTS-200, after which the RCs were eluted by increasing the concentration of $MgCl_2$ up to 100 mM. The RCs were concentrated to OD_{675} of about 50 cm^{-1} using an Amicon ultrafiltration device with 30 kDa membrane filter.

The Chl, Pheo and Car content of the D1D2Cytb_{559} preparation was determined using an absorption spectrum of 80% aqueous acetone extract according to [22] by their absorbances at 412, 431, 460, and 480 nm. The observed ratios (normalized to 2.0 Pheo) were: Chl/Pheo/Car = 5.8:2.0:1.8. The ratio observed for the absorbances at 412 nm and 431 nm was $A_{412}/A_{431}=1.04$, compared to calculated values 1.01 for 6:2:2, 1.06 for 6:2:1 and 1.10 for 5:2:1 preparations [23]. Therefore, the PSII RC preparation used in the present study contained 6 Chls and 1–2 Car per 2 Pheos molecules.

The electrochemical titrations were performed with a Princeton Applied Research model 173 potentiostat at room temperature under aerobic conditions in the dark using an optically transparent thin-layer cell with optical path length of 0.1–0.2 mm described in [24]. The concentrated stock solution of RC was diluted ~10 fold in a 50 mM phosphate buffer (pH 6.8) containing 100 mM KCl, 0.1% DM and a mixture of mediators. The redox mediators (*tris*(1,10-phenantroline)iron(II) sulfate, $[Fe(phen)_3]SO_4$, dicyano*bis*(1,10-phenantroline)iron(II), $[Fe(phen)_2(CN)_2]$, and potassium tetracyanomono(1,10-phenantroline)ferrate(II), $K_2[Fe(phen)(CN)_4]$, were synthesized according to [25]. In the visible region the reduced form of all mediators absorbs in the 400–550 nm range (with extinction coefficients ϵ of 5–10 mM^{-1} cm^{-1} [26]), becoming practically colorless upon oxidation. Redox titrations of the

mediators performed in the spectro-electrochemical cell showed that equilibration of the sample at each potential step of 10 mV takes place within 2–5 min and the reversibility of oxidation is fairly good. The redox titration curves were approximated with one-electron Nernst equation with E_m=+0.59 V ($[Fe(phen)(CN)_4]^-/[Fe(phen)(CN)_4]^{2-}$), E_m=+0.80 V ($[Fe(phen)_2(CN)_2]^+/[Fe(phen)_2(CN)_2]$). The E_m value for the ($[Fe(phen)_3]^{3+}/[Fe(phen)_3]^{2+}$) redox couple was determined by cyclic voltammetry in a regular electrochemical cell and gave E_m=+1.12 V. These values differ by 10–20 mV from those reported in the literature under different conditions [26]. The typical concentrations of mediators during electrochemical titrations were 50–100 μM ($[Fe(phen)_3]SO_4$), 20 μM ($[Fe(phen)_2(CN)_2]$), 50–100 μM ($K_2[Fe(phen)(CN)_4]$). Reaction center preparations were used at concentrations corresponding to OD_{675}=5–10 cm^{-1} for absorption measurements and to $OD_{675} \approx 30$ cm^{-1} for CD measurements.

Reaction center photooxidation was performed by continuous illumination with white light at room temperature for different time intervals. Reaction centers (OD_{675}=0.5–0.7 cm^{-1}) were illuminated in a 1 cm quartz cuvette in the same detergent/buffer/electrolyte solution as in the case of electrochemical studies (except for the redox mediators). A stock solution (10 mg/ml) of silicon molybdenic acid (ICN Pharmaceutical) was freshly prepared and used at about 0.2 mM concentration for photooxidation experiments. To remove the dissolved oxygen, the samples were carefully bubbled with argon and handled in a nitrogen box.

Optical spectra were recorded at room temperature with a Perkin-Elmer Lambda 900 UV/VIS/NIR spectrometer. Room temperature CD spectra were measured with a Jasco-600 spectropolarimeter or with a CD6 Jobin Yvon Spex. CD spectra were recorded at 0.2 nm interval, bandwidth 2 nm, recording time 5 min, number of accumulations: 1 (photochemical oxidation) or 4 (electrochemical oxidation).

3. Results

The initial room temperature absorption and CD spectra of the PSII RC used (solid lines in Fig. 1, upper frames) correspond to those reported in the literature. The large absorbance below 450 nm is due to the Soret bands of Chl at 435 nm, of Pheo at 417 nm and to oxidized Cytb_{559}. The small bands around 485, 507 and 545 nm are due to the β-carotenes and the Pheo Q_X absorption, respectively. The Chl

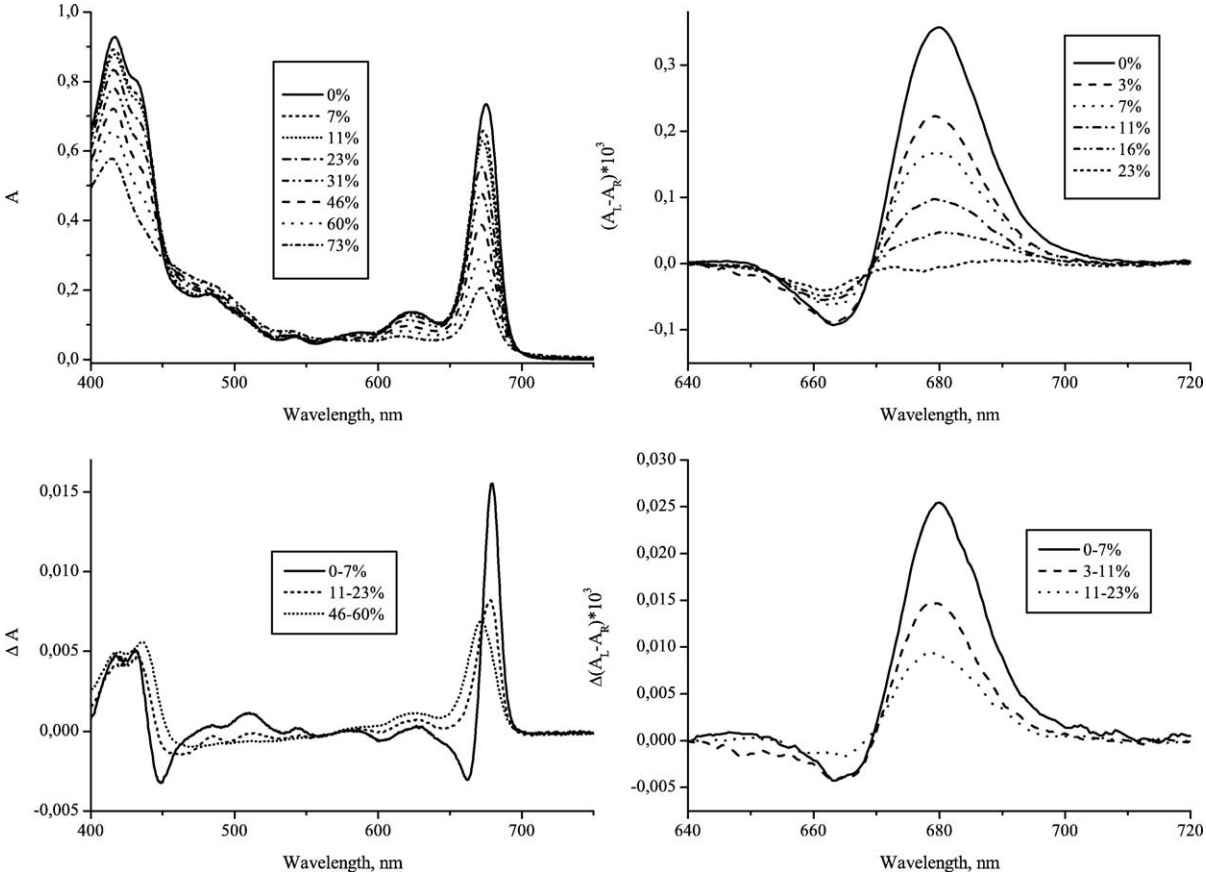

Fig. 1. Absorption spectra (left) and CD of the Q_Y band (right) of PSII RC at successive stages of irreversible photo-bleaching in the presence of oxygen at room temperature. For each spectrum, the % decrease of Q_Y absorption, integrated from 650 to 700 nm, is given in the inset. The lower frames show representative difference spectra for selected phases of the bleaching process, normalized to the associated fraction of Q_Y bleaching.

and Pheo Q_Y absorbance forms a single band peaking at 675 nm and is associated with an asymmetric CD spectrum showing large positive chirality at 680 nm and a smaller negative lobe at 663 nm.

Illumination at room temperature, especially in the presence of oxygen, causes a rapid inactivation and eventually almost complete bleaching of the isolated PSII RC, as illustrated in Fig. 1 by absorption and CD spectra taken after increasing illumination times. The percentage decrease of the Q_Y absorbance, integrated from 650 to 700 nm, relative to that of the initial spectrum is indicated for each curve. The Q_Y band bleaches on the long wavelength side first and the CD is already gone when only 20% of the Q_Y absorbance has disappeared. Because one might expect each Chl to contribute about 14% and each Pheo 7% to the initial Q_Y absorbance [11], the bleaching of one or two pigments apparently suffices to obliterate the total CD signal. The lower frames show difference spectra of pigment bleaching for selected phases of the process, normalized to the extent of Q_Y bleaching during that phase. The pronounced blue shift that accompanies the initial phase of Q_Y bleaching confirms the disruption of excitonic interaction, as indicated also by the disappearance of CD. The CD signals of the different fractions differ in amplitude, but not significantly in spectral shape.

Fig. 2 shows the corresponding data for anaerobic illumination in the presence of silicomolybdate (SiMo) as an electron acceptor. SiMo absorbance complicates the analysis below 450 nm. At higher wavelengths, the efficient bleaching of both carotenes is obvious, and comparison to Fig. 1 shows in fact that they must have been partially lost before illumination, presumably by stray light during SiMo addition. The Q_Y band bleaches on the short wavelength side first, but the absorbance decrease centered at 672 nm is accompanied by a significant increase on both sides of the 672 nm decrease, suggesting a broadening of the 672 nm band rather than a bleaching. The loss of integrated Q_Y absorption associated with this process amounts to much less than one Chl per RC. (The amplitude normalization of the difference spectra labeled 0–2% and 2–6% in the figure was based arbitrarily on the amplitude of the 672 nm bleach, because the overall loss of Q_Y absorbance was negligible, especially in the former). Remarkably, the CD spectrum initially shows a red shift rather than a decrease in amplitude and the CD difference spectrum associated with the first few percent of Q_Y bleaching is actually similar in shape to the corresponding absorption difference spectrum. This sug-

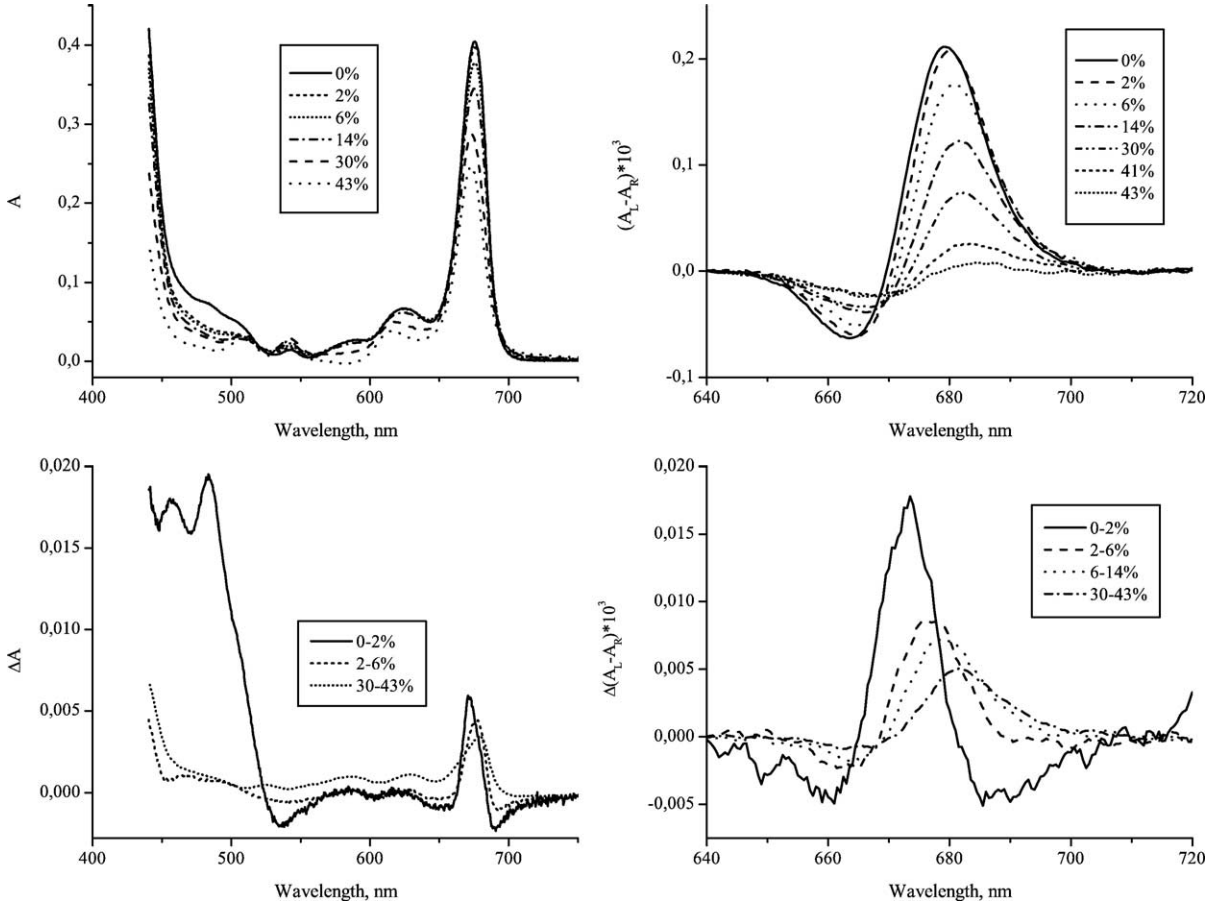

Fig. 2. Measurements as in Fig. 1, but for anaerobic photo-bleaching in the presence of silicomolybdate.

gests that the 672-nm absorption band is associated with a positive CD of the same spectral shape, before as well as after its broadening. At later stages the CD amplitude decreases, although less rapidly than in Fig. 1, and the resulting difference spectra are similar to those observed in the absence of SiMo.

Electrochemical oxidation of PSII RCs in the dark was performed as described in Methods. Rapid irreversible oxidations effectively prevent reversible equilibrium titrations of the PSII RC pigments, but we nevertheless succeeded in obtaining partial selectivity in oxidizing the different pigments by studying these irreversible oxidations as a function of time after imposing a fixed potential. The oxidation was performed in two steps. First, a potential of +0.9 V vs. NHE was applied and the absorption or CD spectrum was measured at regular intervals until the changes became very slow. Bleaching of nearly half of the initial Q_Y absorbance was obtained in a reasonable time (20–30 min). Then a potential of +1.0 V was applied for 10–20 min, resulting in 'complete' RC oxidation. This left about 10–15% of the initial Q_Y absorbance, which seems typical for Chl and Pheo oxidation products in organic solvents [27–29]. Reversibility was checked at successive stages of the oxidation process by applying a voltage of 0 V. Up to a bleaching of about half of the Q_Y band, this failed to restore any absorption. After more extensive oxidation, an increasing fraction of the bleaching was reversible by reduction, up to 20–25% after complete oxidation. However, the recovered Q_Y absorption was blue shifted to 672 nm (data not shown).

Fig. 3 illustrates the spectral changes caused by the electrochemical oxidation process. The extra absorption seen up to about 560 nm in the initial spectrum, as compared to that in Fig. 1, is due to the added redox mediators. Absorption changes due to mediator oxidation accompany the initial phase of oxidation, but the difference spectrum of the pigments oxidized during the first 10% of Q_Y bleaching is obviously dominated by the bleaching of both carotenes and of a Chl absorbing at 672 nm. The concomitant change of the CD spectrum shows that the Q_Y absorption of this Chl had a large positive chirality and could well be the same absorption band that was seen to broaden upon photochemical oxidation of carotene in the presence of SiMo. Unfortunately, no distinction could be made in potential or time between the electrochemical oxidation of Car and of this Chl. After about 20% Q_Y bleaching, the absorbance and CD difference spectra of the pigments being oxidized have become similar to those obtained above with other methods

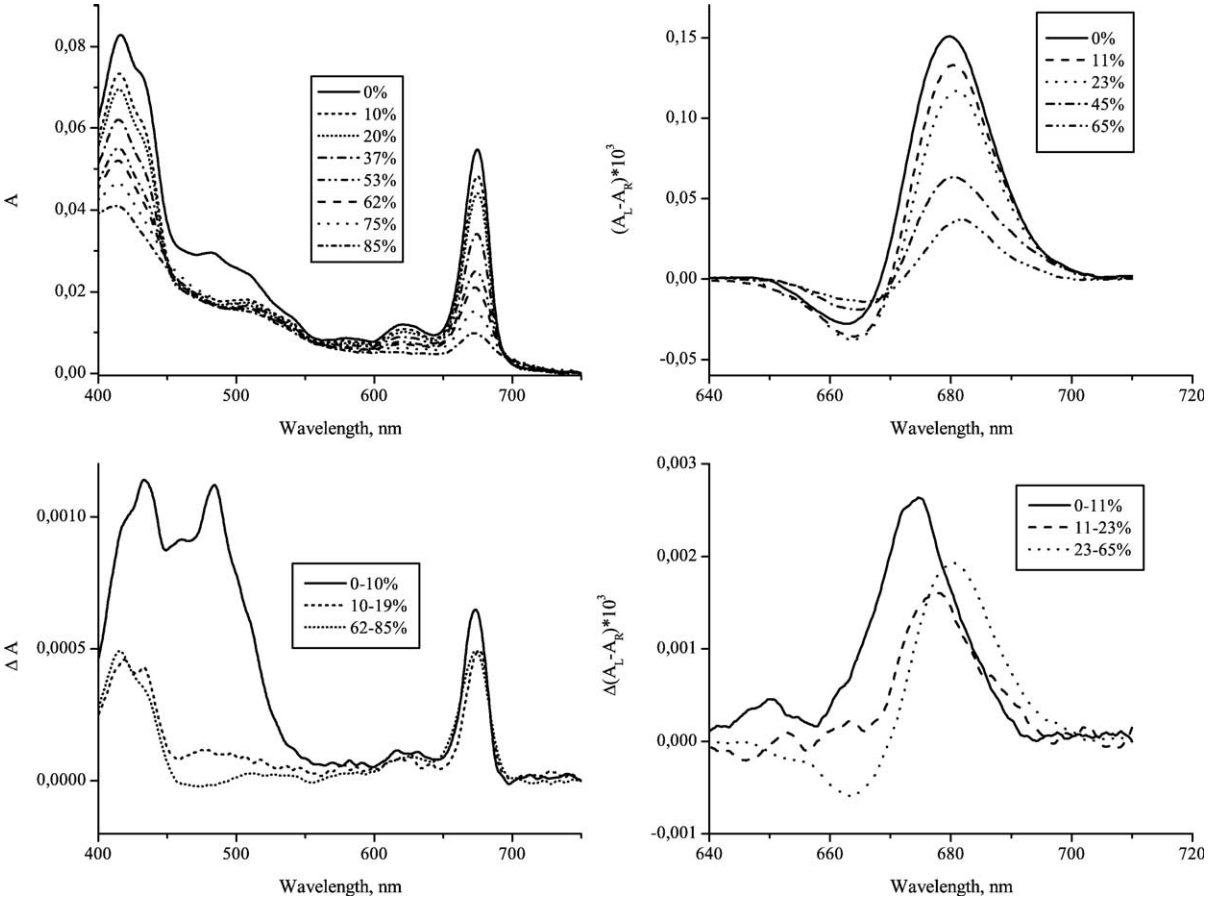

Fig. 3. Spectra as in Figs. 1 and 2, measured at increasing times during electrochemical oxidation at +0.9 V in the dark, reaching about 50% Q_Y bleaching after 20 min, followed by oxidation at +1.0 V for another 20 min.

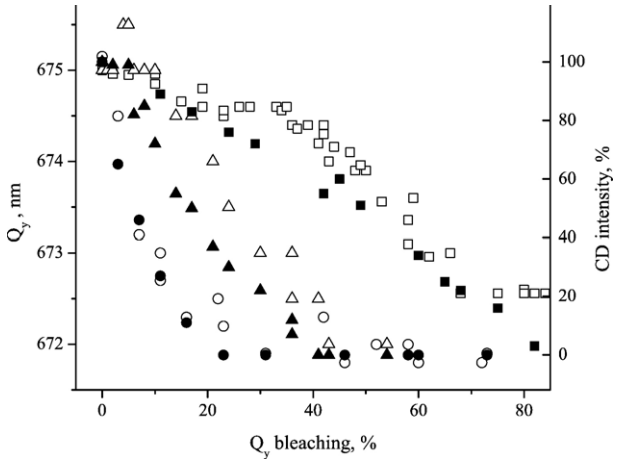

Fig. 4. Wavelength position of the Q_Y absorption maximum (open symbols) and amplitude of the CD maximum (solid symbols) as a function of the extent of Q_Y bleaching during aerobic photo-bleaching (circles), anaerobic photo-bleaching with silicomolybdate (triangles), and electrochemical oxidation in the dark (squares).

of oxidative bleaching, except that the decrease of CD in this case is as slow as, or even slightly slower than, that of Q_Y absorbance. A gradual increase of the 418/432 nm ratio and of the 545 nm Pheo Q_X band in the absorption difference spectra indicates some priority for Chl over Pheo oxidation, but the distinction is much smaller than the midpoint potentials of oxidation of these species to their cation radicals (E_m=+0.8 V and +1.2 V, respectively) might predict.

The very different decay rates, relative to that of integrated Q_Y absorption, of the CD amplitude obtained during oxidative bleaching of the Q_Y band by the three different methods are compared in Fig. 4, solid symbols. The decrease of the CD is correlated with a blue shift of the Q_Y absorption maximum (open symbols), which is expected because both the long wavelength absorbance and the CD are attributed to excitonic interaction. The blue shift lags behind the CD decrease during photo-oxidation with SiMo and during electrochemical oxidation because of the opposing effect of the initial bleaching at 672 nm under these conditions, which must cause a red-shifted peak position of remaining absorbance. In the case of photo-oxidation with SiMo, the net result is an initial red shift before the integrated Q_Y absorption decreases.

4. Discussion

These results confirm the expected initial selectivity of different pigments for the three different methods of oxidative bleaching. The notorious instability of isolated PSII RC under illumination at room temperature is due to singlet oxygen formed by the reaction of oxygen with the triplet state resulting from charge recombination. At low temperature, this triplet state is localized on the accessory Chl [30] in the active branch [11], but at room temperature, it must be distributed over more pigments [31] and singlet oxygen may not always react with the same pigment where it was formed. The selective decrease and blue shift of 680 nm Q_Y absorption, and the inactivation of charge separation, may be explained by oxidation of any of the core pigments in the active branch.

The absence of oxygen and the presence of the electron acceptor SiMo protect the RC from this damage. When the oxidation of $Pheo^-$ by SiMo prevents charge recombination, P^+_{680} oxidizes Car instead, with a concomitant absorption decrease at 672 nm that has been attributed to oxidation of a peripheral Chl [15,16]. At low-temperature photooxidation of Car and peripheral Chl by P^+_{680} has been shown to occur in more intact PSII preparations, where a secondary electron acceptor is still available to stabilize the charge separation [32,33]. However, the first absorption difference spectrum of the irreversible changes measured after illumination in Fig. 2 shows that the strong decrease of Car absorbance is accompanied by a broadening, rather than a bleaching, of a Q_Y absorption band. This finding does not rule out reversible oxidation of a peripheral Chl by P^+_{680} during illumination, but shows that its irreversible oxidation does not occur before the carotenes are irreversibly bleached. Therefore, it seems that a peripheral Chl, characterized by a 672-nm Q_Y absorption with positive chirality, responds spectrally to the oxidation of Car.

The electrochemical pigment oxidation described here presumably does involve their π-cation radical as an intermediate, but is followed by secondary reactions that make the bleaching irreversible. This explains why a potential of +1 V eventually bleached all central pigments, although the midpoint potential of oxidation to the cation radical must be much higher for all of them. The relative oxidation rates of different pigments at a given potential will depend not only on the population of their cation radical state at the imposed potential, but also on the rates at which these radicals are irreversibly converted to secondary products. If the Pheo cation is much less stable than the Chl cation [34], this may explain at least in part why we see so little selectivity for Chl oxidation over that of Pheo, in spite of their large midpoint potential difference. In the purple bacterial RC, accessory BChl and BPheo were also found to be oxidized in the same potential range [24]. Perhaps more surprising is the fact that any 'reversibility' was observed at all, but the 672 nm absorbance that could be recovered may well have been due to a chemically modified pigment [27].

The key result of our spectro-electrochemical approach is that initially selective oxidation of at least one of the peripheral Chls does occur at somewhat lower potentials, +0.8 to +0.9 V vs. NHE, which are close to the in vitro E_m of Chl oxidation to the cation radical. Unlike those in Fig. 2, the difference spectra in Fig. 3 clearly show a net bleaching of the 672-nm absorption band rather than a broadening, in both the absorbance and the CD spectra. One of the peripheral Chls can be removed by a Cu affinity chroma-

tography method [35]. Circular dichroism spectra at 77 K of the resulting '5-Chl preparation' lack the 674 nm shoulder [36,12]. Zehetner et al. reported reconstitution of the missing Chl and show CD spectra at 4 °C that suggest an increase of CD in the 670–675 nm range [37]. While these findings are clearly in agreement with our conclusions, the reported total absence of the 674-nm shoulder at 77 K remains puzzling. If, in spite of the RC symmetry, the positive CD band were due to only one of the two peripheral Chls, we would have to assign it to that on the D2 side because of its spectral shift upon Car oxidation (Fig. 2), if both Cars are located on the D2 side [4]. However, the peripheral Chl on the D1 side seems more likely to be removed by Cu affinity chromatography, because the D2 side is shielded by the Cytb_{559} and *PsbI* proteins [37]. The CD spectrum of the 5-Chl preparation at much lower temperature, where the 674 nm shoulder in 6-Chl preparations becomes a partially resolved peak [9], may yet reveal the CD of the remaining peripheral Chl.

The origin of the positive chirality of the 672 nm absorption band is not clear. If it is imposed by the local protein structure around the peripheral Chls, the observation that all CD disappears when one or two central pigments are bleached by singlet oxygen (Fig. 1) seems hard to understand, unless gross denaturation or specific long-distance conformational effects are postulated to occur simultaneously. Alternatively, the CD of the peripheral Chls might depend on excitonic interactions between the central Chls, if the net positive chirality of Q_Y absorbance of the PSII RC is due to an overall left-handed helicity in the arrangement of the excitonic Q_Y transition moments of all Chls in the C_2-symmetric structure. Such 'psi-type' CD [38,39] is not expected to be significant in particles whose size is only 1% of the wavelength, but we are not sure that it can be ruled out. The peripheral Chls could contribute substantially to the effective size of the helical arrangement, and thereby selectively increase the psi-type component of the CD signal. An opposite situation may apply to both Pheos, whose chemical substitution by 13^1-OH-Pheo was found to decrease only the conservative CD component [11]. The remaining CD spectrum then is positive throughout the Q_Y region of the Chls and is dominated by the 672-nm band that we now attribute to the peripheral Chls.

Acknowledgements

This work was initiated by Prof. Arnold Hoff and supported by the Netherlands Organization for Scientific Research (NWO) for Russian-Dutch scientific collaboration (project 047.009.008) and by INTAS (project 00-404). We thank Dr. Jan Raap (Leiden University) for help with the synthesis of redox mediators, Willem Versluys for constructing the thin-layer spectro-electrochemical cell, and Dr. Thijs Aartsma for advice.

References

[1] O. Nanba, K. Satoh, Isolation of a photosystem II reaction center consisting of D-1 and D-2 polypeptides and cytochrome b-559, Proc. Natl. Acad. Sci. U. S. A. 84 (1987) 109–112.
[2] B. Svensson, C. Etchebest, P. Tuffery, P. van Kan, J. Smith, S. Styring, A model for the photosystem II reaction center core including the structure of the primary donor P680, Biochemistry 35 (1996) 14486–14502.
[3] A. Zouni, H.-T. Witt, J. Kern, P. Fromme, N. Krauss, W. Saenger, P. Orth, Crystal structure of photosystem II from *Synechococcus elongatus* at 3.8 Å resolution, Nature 409 (2001) 739–743.
[4] N. Kamiya, J.-R. Shen, Crystal structure of oxygen-evolving photosystem II from *Thermosynechococcus vulcanus* at 3.7-Å resolution, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 98–103.
[5] V.L. Tetenkin, B.A. Gulyaev, M. Seibert, A.B. Rubin, Spectral properties of stabilized D1/D2/cytochrome b-559 photosystem II reaction center complex, FEBS Lett. 250 (1989) 459–463.
[6] J.R. Durrant, D.R. Klug, S.L.S. Kwa, R. van Grondelle, G. Porter, J.P. Dekker, A multimer model for P680, the primary donor of photosystem II, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 4798–4802.
[7] P. Braun, B.M. Greenberg, A. Scherz, D1–D2-cytochrome b559 complex from aquatic plant *Spirodela oligorrhiza*: correlation between complex integrity, spectroscopic properties, photochemical activity, and pigment composition, Biochemistry 29 (1990) 10376–10387.
[8] C. Houssier, K. Sauer, Circular dichroism and magnetic circular dichroism of the chlorophyll and protochlorophyll pigments, J. Am. Chem. Soc. 92 (1970) 779–791.
[9] S.C.M. Otte, R. van der Vos, H.J. van Gorkom, Steady state spectroscopy at 6 K of the isolated photosystem II reaction centre: analysis of the red absorption band, J. Photochem. Photobiol., B Biol. 15 (1992) 5–14.
[10] L. Finzi, G. Elli, G. Zucchelli, F. Garlaschi, R.C. Jennings, Long wavelength absorption transitions in the D1/D2/cytochrome b-559 complex as revealed by selective pigment photobleaching and circular dichroism measurements, Biochim. Biophys. Acta 1366 (1998) 256–264.
[11] M. Germano, A.V. Shkuropatov, H. Permentier, R. de Wijn, A.J. Hoff, V.A. Shuvalov, H.J van Gorkom, Pigment organization and their interactions in reaction centers of photosystem II: optical spectroscopy at 6 K of reaction centers with modified pheophytin composition, Biochemistry 40 (2001) 11472–11482.
[12] F. Vacha, M. Durchan, P. Siffel, Excitonic interactions in the reaction centre of photosystem II studied by using circular dichroism, Biochim. Biophys. Acta 1554 (2002) 147–152.
[13] A. Telfer, J. Barber, Evidence for the photo-induced oxidation of the primary electron donor P680 in the isolated photosystem II reaction centre, FEBS Lett. 246 (1989) 223–228.
[14] Y. Takahashi, K. Satoh, S. Itoh, Silicomolybdate substitutes for the function of a primary electron acceptor and stabilizes charge separation in the photosystem II reaction center complex, FEBS Lett. 255 (1989) 133–138.
[15] A. Telfer, W.-Z. He, J. Barber, Spectral resolution of more than one chlorophyll electron donor in the isolated photosystem II reaction centre complex, Biochim. Biophys. Acta 1017 (1990) 143–151.
[16] A. Telfer, J. De Las Rivas, J. Barber, β-Carotene within the isolated photosystem II reaction center: photooxidation and irreversible bleaching of this chromophore by oxidized P680, Biochim. Biophys. Acta 1060 (1991) 106–114.
[17] T. Watanabe, M. Kobayashi, Electrochemistry of chlorophylls, in: H. Sheer (Ed.), Chlorophylls, CRC Press, Boca Raton, 1991, pp. 287–315.
[18] F. Rappaport, M. Guergova-Kuras, P.J. Nixon, B.A. Diner, J. Lavergne, Kinetics and pathways of charge recombination in photosystem II, Biochemistry 41 (2002) 8518–8527.

[19] H. Ishikita, B. Loll, J. Biesiadka, W. Saenger, E.-W. Knapp, Redox potentials of chlorophylls in the photosystem II reaction center, Biochemistry 44 (2005) 4118–4124.
[20] P.J. van Leeuwen, M.C. Nieveen, E.J. van de Meent, J.P. Dekker, H.J. van Gorkom, Rapid and simple isolation of pure photosystem II core and reaction center particles from spinach, Photosynth. Res. 28 (1991) 149–153.
[21] D.A. Berthold, G.T. Babcock, C.F. Yocum, A highly resolved, oxygen-evolving photosystem II preparations from spinach thylakoid membranes, FEBS Lett. 134 (1981) 231–234.
[22] C. Eijckelhoff, J.P. Dekker, A routine method to determine the chlorophyll *a*, pheophytin *a* and *β*-carotene contents of isolated photosystem II reaction center complexes, Photosynth. Res. 52 (1997) 69–73.
[23] C. Eijckelhoff, J.P. Dekker, Determination of the pigment stoichiometry of the photochemical reaction center of photosystem II, Biochim. Biophys. Acta 1231 (1995) 21–28.
[24] T.N. Kropacheva, A.J. Hoff, Electrochemical oxidation of bacteriochlorophyll *a* in reaction centers and antenna complexes of photosynthetic bacteria, J. Phys. Chem., B 105 (2001) 5536–5545.
[25] A.A. Shilt, Mixed ligand complexes of iron (II) and (III) with cyanide and aromatic di-imines, J. Am. Chem. Soc. 82 (1960) 3000–3005.
[26] E. Bishop, Oxidation-reduction indicators of high formal potential, in: E. Bishop (Ed.), Indicators, Pergamon Press, Oxford, 1972, pp. 531–684.
[27] J.-H. Fuhrhop, D. Mauzerall, One-electron oxidation of metalloporphyrins, J. Am. Chem. Soc. 91 (1969) 4174–4181.
[28] M.S. Davis, A. Forman, J. Fajer, Ligated chlorophyll cation radicals: their function in photosystem II of plant photosynthesis, Proc. Natl. Acad. Sci. U. S. A. 76 (1979) 4170–4174.
[29] J.-P. Chauvet, R. Viovy, One-electron oxidation of photosynthetic pigments in micelles. bacteriochlorophyll *a*, chlorophyll *a*, chlorophyll *b*, and pheophytin *a*, J. Phys. Chem. 85 (1981) 3449–3456.
[30] F.J.E. van Mieghem, K. Satoh, A.W. Rutherford, A chlorophyll tilted 30 degrees relative to the membrane in the photosystem II reaction center, Biochim. Biophys. Acta 1058 (1991) 379–385.
[31] S.V. Pashenko, I.I. Proskuryakov, M. Germano, H.J. van Gorkom, P. Gast, Triplet state in photosystem II reaction centers studied by 130 GHz EPR, Chem. Phys. 294 (2003) 439–449.
[32] J. Hanley, Y. Deligiannakis, A. Pascal, P. Faller, A.W. Rutherford, Carotenoid oxidation in photosystem II, Biochemistry 38 (1999) 8189–8195.
[33] C.A. Tracewell, A. Cua, D.H. Stewart, D.F. Bocian, C.W. Brudvig, Characterization of carotenoid and chlorophyll photooxidation in photosystem II, Biochemistry 40 (2001) 193–203.
[34] J. Fajer, D.C. Borg, A. Forman, R.H. Felton, D. Dolphin, L. Vegh, The cation radicals of free base and zinc bacteriochlorin, bacteriochlorophyll, and bacteriopheophytin, Proc. Natl. Acad. Sci. U. S. A. 71 (1974) 994–998.
[35] F. Vacha, D.M. Joseph, J.R. Durrant, A. Telfer, D.R. Klug, G. Porter, J. Barber, Photochemistry and spectroscopy of a five-chlorophyll reaction center of photosystem II isolated by using a Cu affinity column, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 2929–2933.
[36] C. Eijckelhoff, F. Vacha, R. van Grondelle, J.P. Dekker, J. Barber, Spectral characterization of a 5 Chl *a* photosystem II reaction center complex, Biochim. Biophys. Acta 1318 (1997) 266–274.
[37] A. Zehetner, H. Scheer, P. Siffel, F. Vacha, Photosystem II reaction center with altered pigment-composition: reconstitution of a complex containing five chlorophyll *a* per two pheophytin *a* with modified chlorophylls, Biochim. Biophys. Acta 1556 (2002) 21–28.
[38] D. Keller, C. Bustamante, Theory of the interaction of light with large inhomogeneous molecular aggregates: II. Psi-type circular dichroism, J. Chem. Phys. 84 (1986) 2972–2980.
[39] G. Garab, Linear and circular dichroism, in: J. Amesz, A.J. Hoff (Eds.), Biophysical Techniques in Photosynthesis, Kluwer Academic Publishers, Dordrecht, 1996, pp. 11–40.