THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 286, NO. 8, pp. 5985–5994, February 25, 2011
© 2011 by The American Society for Biochemistry and Molecular Biology, Inc. Printed in the U.S.A.

A High Redox Potential Form of Cytochrome c_{550} in Photosystem II from *Thermosynechococcus elongatus*[*] [S]

Received for publication, July 30, 2010, and in revised form, December 3, 2010 Published, JBC Papers in Press, December 6, 2010, DOI 10.1074/jbc.M110.170126

Fernando Guerrero[‡§], Arezki Sedoud[§¶], Diana Kirilovsky[§¶], A. William Rutherford[§¶], José M. Ortega[‡], and Mercedes Roncel[‡1]

From the [‡]Instituto de Bioquímica Vegetal y Fotosíntesis, Universidad de Sevilla-CSIC, Américo Vespucio 49, 41092 Sevilla, Spain, the [§]Commissariat à l'Énergie Atomique, Institut de Biologie et Technologies de Saclay (iBiTec-S), 91191 Gif-sur-Yvette, France, and the [¶]Centre National de la Recherche Scientifique, URA 2096, 91191 Gif-sur-Yvette, France

Cytochrome c_{550} (cyt c_{550}) is a component of photosystem II (PSII) from cyanobacteria, red algae, and some other eukaryotic algae. Its physiological role remains unclear. In the present work, measurements of the midpoint redox potential (E_m) were performed using intact PSII core complexes preparations from a histidine-tagged PSII mutant strain of the thermophilic cyanobacterium *Thermosynechococcus* (*T.*) *elongatus.* When redox titrations were done in the absence of redox mediators, an E_m value of +200 mV was obtained for cyt c_{550}. This value is ~300 mV more positive than that previously measured in the presence of mediators (E_m = −80 mV). The shift from the high potential form (E_m = +200 mV) to the low potential form (E_m = −80 mV) of cyt c_{550} is attributed to conformational changes, triggered by the reduction of a component of PSII that is sequestered and out of equilibrium with the medium, most likely the Mn_4Ca cluster. This reduction can occur when reduced low potential redox mediators are present or under highly reducing conditions even in the absence of mediators. Based on these observations, it is suggested that the E_m of +200 mV obtained without mediators could be the physiological redox potential of the cyt c_{550} in PSII. This value opens the possibility of a redox function for cyt c_{550} in PSII.

In all photosynthetic oxygen-evolving organisms, the primary steps of light conversion take place in a large pigment-protein complex named PSII,[2] which drives light-induced electron transfer from water to plastoquinone with the concomitant production of molecular oxygen (for review, see Ref. 1). The reaction center of PSII is made up of two membrane-spanning polypeptides, D1 and D2, which bind four chlorophylls, two pheophytins, two quinones, Q_A and Q_B (the primary and secondary quinone acceptors of the reaction centre of PSII), a non-heme iron atom, and a cluster made up of four manganese ions and one calcium ion. In green algae and higher plants, three extrinsic proteins are associated to reaction center in water-splitting active PSII complexes: 23–24, 16–18, and 33 kDa proteins, whereas in cyanobacteria, red algae and some other eukaryotic algae, cyt c_{550}, 12 kDa and 33 kDa proteins are found. The three-dimensional structure of PSII confirmed that cyt c_{550} binds on the lumenal membrane surface in the vicinity of the D1 and CP43 (2–6).

Cyt c_{550}, encoded by the *psbV* gene, is a monoheme protein with a molecular mass of ≈ 15 kDa and an isoelectric point between 3.8 and 5.0 (7, 8). The recent resolution of the three-dimensional structure of the soluble form of cyt c_{550} from three cyanobacteria, *Synechocystis* sp. PCC 6803 (9), *Arthrospira maxima* (10), and *Thermosynechococcus elongatus* (11) has confirmed a previously proposed bis-histidine coordinated heme that is very unusual for monoheme *c*-type cytochromes (8, 11, 12). Crystal structures of both isolated and PSII-bound forms of cyt c_{550} show that the protein presents a hydrophobic inner core typical of monoheme cytochromes *c*, with three helices forming a nest for the prosthetic group and a fourth helical segment in the N-terminal domain protecting the heme from solvent, indicating that the heme structure is not very different from most *c*-type cytochromes (13).

The exact physiological role of cyt c_{550} is unclear. Extensive research has established that it does not participate in the main photosynthetic reactions despite its close proximity (22 Å) to the water oxidation complex. Cyt c_{550} is thus suggested to play the same role as the other (albeit cofactor-less) extrinsic proteins. By stabilizing the neighboring proteins and protecting the manganese cluster from external reductants, it stabilizes the oxygen-evolving complex (14, 15). Studies of phenotype of the cyt c_{550}-less mutant (ΔPsbV) of *Synechocystis* sp. PCC 6803 have shown that both the cyt c_{550} and the 12-kDa protein stabilize the binding of the Ca^{2+} and Cl^- ions, which are essential for the oxygen-evolving activity of PSII, in a manner analogous to the extrinsic 17 and 24 kDa polypeptides of higher plants (14, 16, 17). The fact that cyt c_{550} can be isolated as a soluble protein (7, 8, 18–20) suggests that other functions not directly related to PSII are possible for this protein. Several nonphotosynthetic roles have been suggested for cyt c_{550}. In fact, a function related to anaerobic disposal of electrons from carbohydrates reserves or fermentation to sustain an organism during prolonged dark and anaerobic conditions have been proposed (19, 21, 22). According to Shen and Inoue (23), cyt c_{550} can accept electron from ferredoxin II in

* This work was supported by grants from the Ministry of Education and Culture of Spain (BFU2007-68107-C02-01/BMC, cofinanced by FEDER), and Andalusia Government (PAI CVI-261) and the EU/Energy Network project SOLAR-H2 (FP7 Contract 212508).

[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Fig. S1.

[1] To whom correspondence should be addressed: Instituto de Bioquímica Vegetal y Fotosíntesis, Universidad de Sevilla-Consejo Superior de Investigaciones Científicas, Américo Vespucio 49, 41092 Sevilla, Spain. Tel.: 34-954-489525; Fax: 34-954-460065; E-mail: mroncel@us.es.

[2] The abbreviations used are: PSII, photosystem II; cyt b_{559}, cytochrome b_{559}; cyt c_{550}, cytochrome c_{550}; Cm, chloramphenicol; Chl, chlorophyll.

Downloaded from http://www.jbc.org/ at FAC BIOLOGIA/BIBLIOTECA on April 22, 2016

the presence of sodium dithionite and is proposed to remove excess electrons in anaerobically grown cells.

The E_m of cyt c_{550} is one of the key parameters for elucidating the biological role of this cytochrome. An E_m value at pH 7.0 (E_{m7}) of −260 mV was the first to be reported for purified cyt c_{550} from *Anacystis nidulans* (20). Cyt c_{550} from *Microcystis aeruginosa* and *Aphanizomenon flos-aquae* were found to be reducible by sodium dithionite ($E_{m7} = -420$ mV), but not by sodium ascorbate ($E_{m7} = +58$ mV) (7). Later, E_{m7} values from −280 to −314 mV were obtained for purified cyt c_{550} from the same species (12) and an E_{m7} of −250 mV from *Synechocystis* sp. PCC 6803 (8). In previous work, we determined an E_{m6} value of −240 mV for the soluble form of cyt c_{550} from the thermophilic cyanobacterium *T. elongatus* after its extraction from PSII (24). Such low redox potentials are well below the range normally expected for a mono-heme *c*-type cytochrome and seem incompatible with a redox function in PSII electron transfer. Using an electrochemical technique, a value 150 mV more positive ($E_{m7} \approx -100$ mV) was measured for E_m of cyt c_{550} from *Synechocystis* sp. PCC 6803 adsorbed to an electrode surface (13). This higher value was attributed to the exclusion of water from the site due to the protein binding to the electrode (13). The E_m for cyt c_{550} associated with PSII was not established until our group was able to measure it using intact PSII core complexes preparations from *T. elongatus.* Using potentiometric redox titrations, a significantly higher E_m value was obtained for cyt c_{550} when bound to PSII ($E_{m6} = -80$ mV) compared with its soluble form after its extraction from PSII ($E_{m6} = -240$ mV) (24). Moreover, although the E_m of the bound form is pH-independent, the E_m of the soluble form varies from −50 mV at pH 4.5 to −350 mV at pH 9–10 (24). The difference of E_m between the isolated and the PSII-bound forms of cyt c_{550} has been confirmed by theoretical calculations based on crystal structures of the isolated and PSII-bound forms (25). Some authors (13, 24, 26) have proposed that in conditions more native than isolated PSII core complexes, it is possible that the E_m of cyt c_{550} may be even higher than −80 mV, and thus a redox function in the water oxidation complex could be conceivable. Therefore, the precise determination of the redox potential of this protein is of fundamental importance to the understanding its function.

One of the most standard techniques for determining redox potentials of proteins is the redox potentiometry. It involves measuring the ambient redox potential (E_h) while simultaneously determining the concentration of the oxidized and reduced forms of the protein using a spectroscopic technique. Meaningful results will be obtained only if chemical equilibrium is achieved between the various species in solution and electrochemical equilibrium is established at the electrode solution interface (27, 28). Unlike many small inorganic and organic redox couples, most redox proteins do not establish stable potentials because the heterogeneous charge-transfer (electrochemical) rates are low. A predominant reason for this is that the redox center is often shielded by protein and so does not gain proper contact with the electrode surface. Redox mediators are required to act as go-betweens between the measuring electrode and the biological redox couple and thus to get rates of the electron transfer between electrode, mediator and biological component rapid enough to achieve a true equilibrium (*i.e.* one where all redox complexes in the biological electron transfer system are at the same E_h) (29). Therefore, in most redox titrations of proteins, equilibrium is ensured by the addition of a mixture of redox mediators that establishes rapid (heterogeneous) electro-chemical equilibrium with the electrode and rapid (homogeneous) electron transfer with the protein without chemically modifying it in any way.

It has been reported that the E_m of Q_A in PSII-enriched membranes was affected by the presence of redox mediators at low ambient potentials. As consequence of this, a change in the redox potential from −80 mV (active form) to +65 mV (inactive form) has been determined in the potentiometric titrations performed on PSII membranes. This effect was attributed to the loss of the very high potential Mn_4Ca cluster due to reductive attack by the mediators and the sodium dithionite itself under some conditions (30). This was confirmed by the observation that the low potential, active form of the Q_A/Q_A^- couple could be regenerated when the manganese cluster was reconstituted (31). The binding and debinding of the Mn_4Ca cluster and even of the Ca^{2+} ion is considered to be associated with conformational changes that are manifest far from the binding site itself (30, 31).

Based on these observations and taking account that in most of redox titrations of cyt c_{550} bound to PSII, equilibration was ensured by the addition of a mixture of redox mediators (24, 26), it seemed possible that E_m of cyt c_{550} when bound to PSII could suffer from this unexpected technical difficulty. The presence of these mediators could have led to the reduction of the manganese cluster, the consequent loss of the Ca^{2+} and Mn^{2+} ions and associated conformation changes in the protein. The E_m value obtained for cyt c_{550} may not reflect the fully intact form of the PSII-bound cytochrome.

The main objective of this work has been to re-evaluate the redox potential of cyt c_{550} associated with PSII considering the effect of redox mediators. To check for the latter possibility, redox titration experiments were performed using highly active and intact core complexes preparations of PSII from *T. elongatus* testing the presence and absence of redox mediators and different redox mediators.

EXPERIMENTAL PROCEDURES

Strain and Standard Culture Conditions—WT and His-tag CP43 mutant *T. elongatus* cells were grown in a DTN medium (32). Cultures were carried out in 3-liter flasks in a rotary shaker (120 rpm) at 45 °C under continuous illumination from fluorescent white lamps (100 microeinsteins m^{-2} s^{-1}) and CO_2-enriched atmosphere. For maintenance, the His-tag CP43 mutant cells were grown in the presence of chloramphenicol (Cm) (5 μg ml^{-1}) at 45 °C under continuous illumination from fluorescent white lamps (40 microeinsteins m^{-2} s^{-1}).

Construction of Plasmid for His-Tag CP43 T. elongatus Mutant—For constructing a plasmid for expression of His-tagged *psbC* in *T. elongatus*, the genome region containing the *psbD1*, *psbC* and *tlr1632* genes was amplified by PCR.

Downloaded from http://www.jbc.org/ at FAC BIOLOGIA/BIBLIOTECA on April 22, 2016

Genomic DNA of *T. elongatus* as template and the primers *CP43a* (5′-ATGACGATCGCGATTGGACGA-3′) and *CP43b* (5′-GCAATCCAATGATGGACTTAG-3′) were used. The amplified region was digested by KpnI and BamHI, and it was cloned in a pBluescript KS+ plasmid also previously digested by the same restriction enzymes. Then, by site-directed mutagenesis, the bases coding for six histidines were added in the 3′ terminal of the *psbC* gene using the synthetic oligonucleotides *CP43* His-tag a (5′-CCTCTCGATGCCCA-GCCTTGATCACCATCACCATCACCATTAGGGTTACT-GAGTCAACTTAA-3′) and *CP43* His-tag b (5′-TTAAGTT-GACTCAGTAACCCTAATGGTGATGGTGATGGTGATC-AAGGCTGGGCATCGAGAGG-3′). An SmaI site was created between the *psbC* and *tlr1632* genes using the oligonucleotides *CP43SmaIa* (5′-ACCATTAGGGTTCCCGGGT-CAACTTAACTC-3′) and *CP43SmaIb* (5′-GAGTTAAGTT-GACCCGGGAACCCTAATGGT-3′). Finally, a Cm-resistance cassette was introduced in the SmaI site. The Cm resistance cassette was obtained by amplification by PCR of a 1.1-kb fragment of the plasmid pBC SK+ Cm^R using the synthetic oligonucleotides *Cam1.1a* (5′-GCTGTGACGGAA-GATCACTTCGC-3′) and *Cam1.1b* (5′-GCTCCACGGGGA-GAGCCTGAGCA-3′). The construction obtained, pCH-Cm, was a plasmid of 7 kb. To increase the chances for *T. elongatus* transformation, this plasmid was digested by the restriction enzyme EcoRI deleting the *psbD* gene and the beginning of the *psbC* gene. The 5-kb plasmid obtained, named pCH-5.1, was used to transform WT *T. elongatus* cells.

Transformation of T. elongatus Cells and Genetic Analysis of Mutants—The pCH-5.1 plasmid containing the His-tagged psbC gene and the Cm-resistance cassette was introduced into WT *T. elongatus* cells by electroporation according to (32) with slight modifications as described in Ref. 26, creating the His-tag CP43 strain (WT′). After electroporation, cells were rapidly transferred to 2 ml of DTN medium and incubated for 48 h in a rotary shaker at 45 °C under low light conditions. Then, the cells in 0.1–0.2-ml aliquots were spread on agar plates containing Cm (2 μg ml^{-1}) and incubated at 45 °C under dim light and humidified atmosphere. After 2–3 weeks, transformants emerged as green colonies; then, these colonies were spread at least twice on agar plates containing 5 μg ml^{-1} Cm. Genomic DNA was isolated from *T. elongatus* cells essentially as described by Cai and Wolk (33). Total segregation of the mutation was checked by PCR amplification of the genome region containing the *psbC* gene and the gene for Cm resistance. The PCR gave a fragment of 3.6 kb containing the *psbC* and the Cm resistance gene, instead of a fragment of 2.5 kb observed in the WT therefore demonstrating the complete segregation of the mutant. Confirmation of the presence of the His-tag was done by sequencing of the amplified DNA fragment.

PSII Core Complex Preparation—PSII core complexes were prepared from cells of *T. elongatus* as described by Kirilovsky *et al.* (26). The PSII core complexes preparations were resuspended in 40 mM MES, pH 6.5, 15 mM $CaCl_2$, 15 mM $MgCl_2$, 10% glycerol, and 1 M glycinebetaine at ~2–3 mg of Chl ml^{-1} and stored in liquid N_2. The preparations used in this work had an oxygen evolution activity of 2700–3200 μmol O_2 mg Chl^{-1} h^{-1}.

Redox Potential Measurements—Potentiometric redox titrations were carried out basically as described in Roncel *et al.* (24). For titrations, samples contained PSII core complexes (30–50 μg Chl ml^{-1}) were suspended in 2.5-ml buffer containing 40 mM MES, pH 6.5. When indicated, a set of the following eight redox mediators was added: 10 μM *p*-benzoquinone (E_{m7} = +280 mV), 20 μM 2,3,5,6-tetramethyl-*p*-phenylendiamine (also called diaminodurol) (E_{m7} = +220 mV), 10 μM 2,5-dimethyl-*p*-benzoquinone (E_{m7} = +180 mV), 20 μM *o*-naphthoquinone (E_{m7} = +145 mV), 2.5 μM *N*-methylphenazonium methosulfate (E_{m7} = +80 mV), 10 μM *N*-methylphenazonium ethosulfate (E_{m7} = +55 mV), 20 μM duroquinone (E_{m7} = +10 mV) and 30 μM 2-methyl-*p*-naphthoquinone (E_{m7} = 0 mV). Some redox titrations were carried out in the absence of these redox mediators or in the presence of diaminodurol only. Experiments were done at 20 °C under argon atmosphere and continuous stirring.

Reductive titrations were performed by first oxidizing the samples to $E_h \approx +450$ mV with potassium ferricyanide and then reducing it stepwise with sodium dithionite. For oxidative titrations, the samples were first reduced to $E_h \approx -350$ mV with sodium dithionite and then oxidized it stepwise with potassium ferricyanide. In both cases, after the additions of potassium ferricyanide or sodium dithionite, the absorption spectrum between 500 and 600 nm and the redox potential of the solution were simultaneously recorded by using, respectively, an Aminco DW2000 UV-vis spectrophotometer and a Metrohm Herisau potentiometer provided with a combined Pt-Ag/AgCl microelectrode (Crison Instruments, Spain). Differential spectra of cyt b_{559} and cyt c_{550} in PSII core complexes were obtained by subtracting the absolute spectra recorded at each E_h during titrations from the spectra of the fully oxidized state of each cytochrome (reductive titrations) or from the spectra of the fully reduced state of each cytochrome (oxidative titrations). The absorbance differences at 559–570 nm for cyt b_{559} and 549–538 nm for cyt c_{550} obtained from these spectra were normally converted into percentages of reduced cytochrome and plotted *versus* solution redox potentials. The E_m values were then determined by fitting the plots to the Nernst equation for one-electron carrier (n = 1) with 1 or 2 components as needed and using a nonlinear curve-fitting program (Origin version 6.0, Microcal Software).

EPR Measurements—EPR spectra were recorded using a Bruker Elexsys 500 X-band spectrometer equipped with a standard ER 4102 resonator and an Oxford Instruments ESR 900 cryostat. Instrument settings were as follows: microwave frequency, 9.4 GHz; modulation frequency, 100 kHz. All other settings were as indicated in the legend of Fig. 6. 120-μl aliquots of PSII cores (~ 1 mg Chl ml^{-1}) in the same buffer used for storage were loaded into 4-mm outer diameter quartz EPR tubes. The EPR samples were frozen in a dry ice/ethanol bath at 200 K. Samples were degassed by pumping at 200 K and then filled with helium gas. EPR tubes were then transferred to liquid nitrogen prior to the EPR measurements being made. Samples were handled in darkness. Reduction was per-

Downloaded from http://www.jbc.org/ at FAC BIOLOGIA/BIBLIOTECA on April 22, 2016

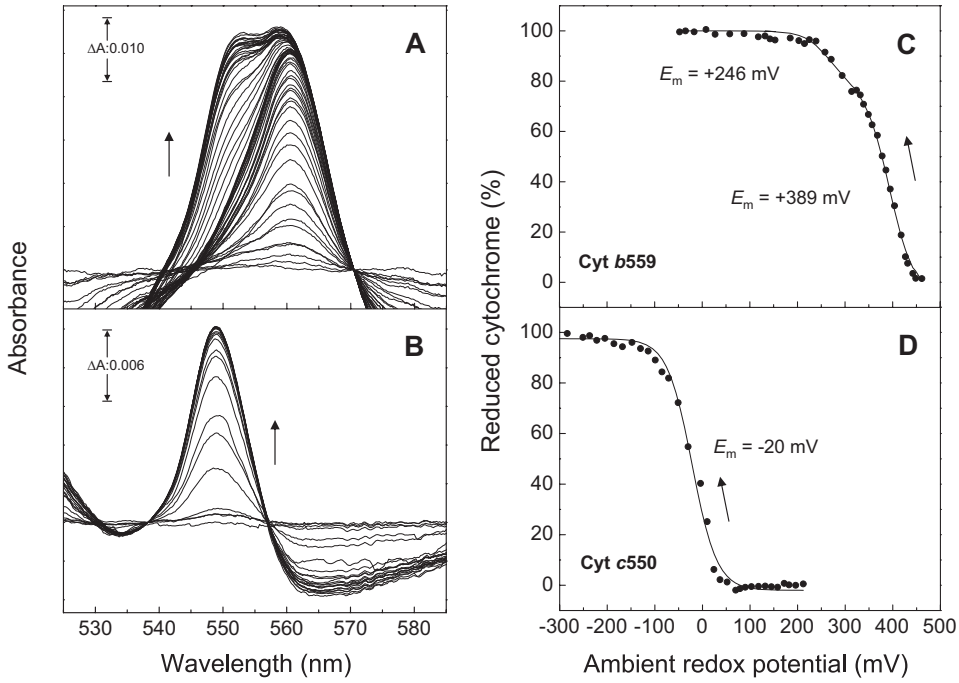

FIGURE 1. **Reductive potentiometric titrations of cyt b_{559} and c_{550} in PSII core complexes in the presence of a mixture of eight redox mediators covering the potential range between +430 and 0 mV.** *A* and *B*, difference absorption spectra in the α-band region of cyt b_{559} and cyt c_{550}. The spectra were obtained by subtracting absolute spectra recorded during the course of the redox titration between +455 and −80 mV minus the spectrum recorded at +455 mV (*A*) and the spectra recorded between +210 mV and −300 mV minus the absolute spectrum recorded at +210 mV (*B*). For simplification, only a set of selected spectra are included in *A* and *B*. *C* and *D*, plot of the percentages of reduced cyt b_{559} and reduced cyt c_{550} obtained from the absorbance differences at 559–570 nm and 549–538 nm *versus* ambient redox potentials, respectively. The *solid curves* represent the best fit of the experimental data to the Nernst equation in accordance with one-electron processes ($n = 1$) for two components (*C*) with an E_m of +246 mV (20%) and +389 mV (80%) and for one component (*D*) with an E_m of −20 mV.

formed by addition of sodium dithionite to the sample in the EPR tube to give a final concentration of 2 mM using a 30 mM stock solution made up in degassed storage buffer. Oxidation was done by addition of potassium ferricyanide to give a final concentration of 5 mM using a 25 mM stock solution. All additions were done in anaerobic conditions.

Spectroscopic Measurements of Cyt c_{550} Binding—To determine the degree of association of cyt c_{550} with PSII after treatment with sodium dithionite, PSII core complexes preparations at a concentration of 25 μg Chl ml^{-1} were incubated in 40 mM MES (pH 6.5) for 30 min in the presence ($E_h \approx -400$ mV) and absence ($E_h \approx +295$ mV) of sodium dithionite (2 mM). The solutions were kept anaerobic in the dark at 20 °C. Samples were then centrifuged in the presence of PEG 8000. Difference spectra of the resuspended precipitate and the supernatant were taken.

RESULTS

Effect of Redox Mediators—Initially potentiometric redox titrations of the isolated PSII core complexes preparations in the presence of a mixture of eight redox mediators (see "Experimental Procedures"), covering the potential range between +430 and 0 mV, were performed. This mixture excluded five mediators with negative redox potential (anthraquinone-2-sulfonate, anthraquinone-2,6-disulfonate, anthraquinone-1,5-disulfonate, 2-hydroxy-*p*-naphthoquinone, and anthraquinone), which were used in our previous work (24). Fig. 1 shows a representative potentiometric titration of PSII core complexes from *T. elongatus* at pH 6.5 under these conditions. Differential absorption spectra in the α-band region of the cytochromes were obtained by subtracting the absolute spectrum recorded at +455 mV from those recorded during the course of the redox titration (Fig. 1*A*). This figure clearly shows that PSII core complexes contain two different components with absorption maxima in the α-band at 559 and 549 nm, which are progressively reduced during the course of titration. The component with an absorption maximum in the α-band at 559 nm that appeared between +455 mV and +210 mV can be assigned to cyt b_{559}, whereas the component appearing between +210 mV and −295 mV can be assigned to cyt c_{550} as has been already described (24). Differential spectra in Fig. 1*A* reveals that both cytochromes can be sequentially titrated observing the change of the α-band of both cyt b_{559} and cyt c_{550}. Consequently, it was first possible to determine the redox potential of cyt b_{559} by measuring the relative content of cyt b_{559} from the absorbance difference between 559 and 570 nm (Fig. 1*A*). A plot of the percentages of reduced cyt b_{559}, obtained from these difference spectra, *versus* E_h could be fitted to a Nernst equation for two $n = 1$ components (Fig. 1*C*). It clearly indicated the existence of two different cyt b_{559} components with E_m values of +389 mV (accounting for ~85% of the total amount of protein) corresponding to the high potential form and +246 mV (~15% of the total amount of protein) corresponding to the intermediated potential form. These values are similar to those obtained in measurements on PSII core complexes of *T. elongatus* where reductive titrations were carried out in the

Downloaded from http://www.jbc.org/ at FAC BIOLOGIA/BIBLIOTECA on April 22, 2016

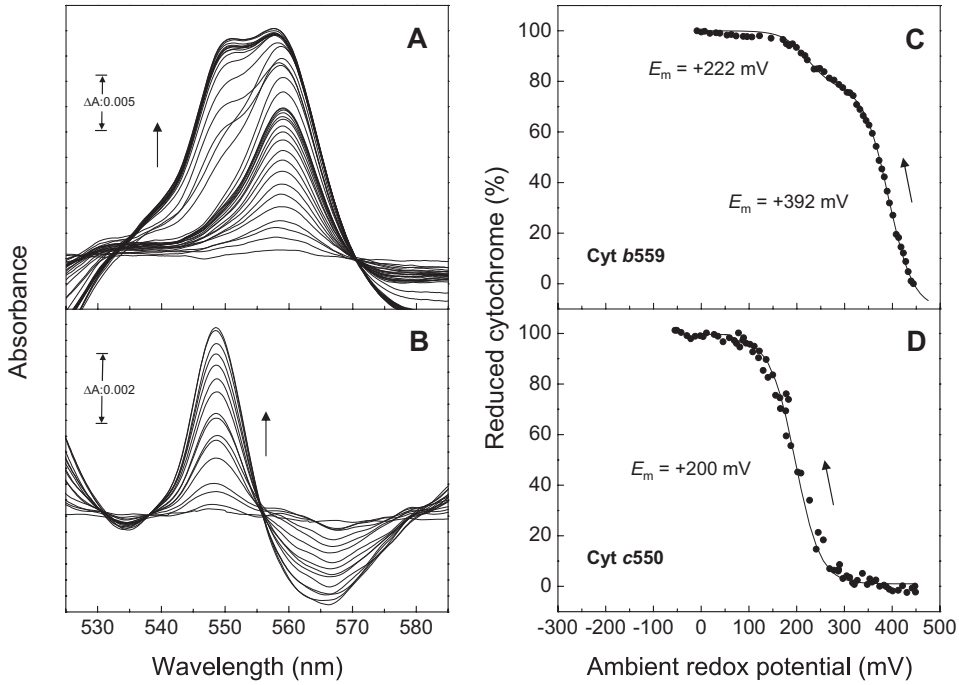

FIGURE 2. **Reductive potentiometric titrations of cyt b_{559} and c_{550} in PSII core complexes without redox mediators in the presence of 25 μM potassium ferricyanide.** *A* and *B*, difference absorption spectra in the α-band region of cyt b_{559} and cyt c_{550}. The spectra were obtained by subtracting absolute spectra recorded during the course of the redox titration between +430 and −80 mV minus the spectrum recorded at +430 mV (*A*) and the spectra recorded between +220 mV and −80 mV minus the absolute spectrum recorded at +230 mV (*B*). For simplification, only a set of selected spectra are included in *A* and *B*. *C* and *D*, plot of the percentages of reduced cyt b_{559} and reduced cyt c_{550} obtained from the absorbance differences at 559–570 nm and 549–538 nm *versus* ambient redox potentials, respectively. The *solid curves* represent the best fit of the experimental data to the Nernst equation in accordance with one-electron processes ($n = 1$) for two components (*C*) with E_m of +222 mV (20%) and +392 mV (80%) and for one component (*D*) with E_m of +200 mV.

presence of low potential mediators (24). When most cyt b_{559} was reduced at E_h of +210 mV, changes in the α-band of cyt c_{550} could be cleanly observed and consequently its E_m determined without any interference from cyt b_{559}. Differential spectra of cyt c_{550} (Fig. 1*B*) were obtained by subtracting the spectrum recorded at +210 mV (cyt c_{550} almost fully oxidized and cyt b_{559} fully reduced) from each spectrum performed at different ambient redox potential (between +210 and −295 mV). The relative content of cyt c_{550} was calculated from the absorbance difference between 549 and 538 nm. Then, the percentages of reduced cyt c_{550} *versus* E_h were plotted and an E_m value of −20 mV was calculated by fitting the experimental points to the Nernst equation for one $n = 1$ component (Fig. 1*D*). This E_m value was significantly higher than those described to date for cyt c_{550} associated with PSII (24, 26).

The E_m of the cyt b_{559} and cyt c_{550} was measured in the absence of redox mediators other than sodium dithionite and potassium ferricyanide. Complete reductive potentiometric titration at pH 6.5 of cyt b_{559} and cyt c_{550} in the PSII core complex was performed with the sample previously oxidized with potassium ferricyanide to an initial redox potential of approximately +450 mV (Fig. 2). Difference absorption spectra in the α-band region of cytochromes obtained during the course of the redox titration between +450 mV and −45 mV are shown in Fig. 2*A*. Fig. 2*C* shows a plot of the percentages of reduced cyt b_{559} *versus* ambient redox potential indicating the presence of two different forms of cyt b_{559} with E_m values of +392 mV (high potential form) and +222 mV (intermediated potential form), each representing ~85 and 15% of the total amount of protein, respectively. This result was similar to those found in the titrations carried out both in the absence (see Fig. 1*C*) and presence of low potential redox mediators (24). However, the plot of percentages of reduced cyt c_{550} obtained from the difference absorption spectra of the cyt c_{550} during the course of the redox titration (Fig. 2*B*) *versus* ambient redox potential clearly showed that cyt c_{550} had a significant higher E_m value (+200 mV) (Fig. 2*D*) than that obtained in the presence of low potential redox mediators (−80 mV) (24) and in the presence of other somewhat higher potential redox mediators (−20 mV) (see Fig. 1*D*). A similar E_m value for cyt c_{550} was obtained if the reductive potentiometric titration of PSII core complex was started from ambient redox potential of the reaction mixture without previous addition of potassium ferricyanide (data not shown).

It has been observed that to get a well defined E_m, most proteins need the presence of mediators with redox potentials within ±30–60 mV of the redox center E_m value and that for a single redox center, one mediator is ordinarily sufficient (27, 28). To verify the new value of E_m obtained for cyt c_{550} in the absence of mediators (Fig. 2*D*), reductive potentiometric titrations were also performed in the presence of a single redox mediator (Fig. 3). We selected the mediator diaminodurol (see "Experimental Procedures") with an E_{m7} of +220 mV, which is very close to the new value obtained for cyt c_{550} bound to PSII (Fig. 2). In these conditions, a value of $E_m = +215$ mV for cyt c_{550} was measured very similar to that obtained in the absence of redox mediators (Fig. 2*D*). Thus, these results show that cyt c_{550} in the absence of redox mediators (other

Downloaded from http://www.jbc.org/ at FAC BIOLOGIA/BIBLIOTECA on April 22, 2016

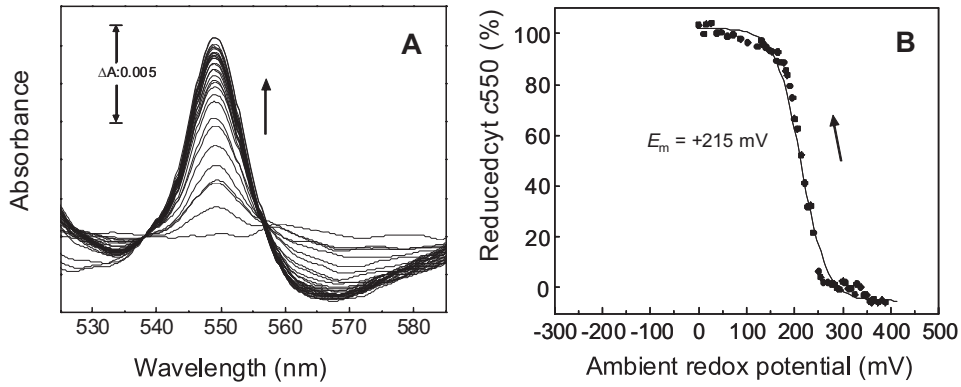

FIGURE 3. **Reductive potentiometric titration of cyt c_{550} in PSII core complexes with 20 μM diaminodurol and 25 μM of potassium ferricyanide.** *A*, difference absorption spectra in the α-band region of cyt c_{550}. The spectra were obtained by subtracting absolute spectra recorded during the course of titration minus the absolute spectrum recorded at +240 mV. For simplification, only a set of selected spectra are included. *B*, plots of the percentages of reduced cyt c_{550} obtained from the absorbance differences at 549–538 nm *versus* ambient redox potentials. The *solid curve* represents the best fits of the experimental data to the Nernst equation in accordance with one-electron processes ($n = 1$) for one component with E_m of +215 mV.

than potassium ferricyanide and sodium dithionite) or with only diaminodurol has an E_m value of ~200 mV higher than that obtained with the mixture of the eight mediators (see "Experimental Procedures") (Fig. 1*D*). These results are consistent with those obtained in redox titrations of Q_A in spinach PSII membranes where it was observed that the addition of redox mediators at low ambient potentials led to a shift of the E_m for Q_A from −80 mV to +65 mV (30).

Hysteresis—To increase confidence that titrations have been successfully performed at equilibrium, it is a common practice to perform redox titrations in both oxidative and reductive sequences, and identical results should be obtained. Oxidative potentiometric titrations were carried out in the same conditions as Figs. 1 and 2. Fig. 4*A* shows the result of an oxidative potentiometric titration of cyt c_{550} in PSII core complexes preparations in the absence of redox mediators. In this case, cyt c_{550} was previously reduced by adding excess sodium dithionite, and once it reached an E_h near −370 mV, the oxidative titration was performed by adding small amounts of potassium ferricyanide. Difference absorption spectra in the α-band region of cyt c_{550} recorded during oxidative titrations in these conditions are shown (Fig. 4*A*, *inset*). From plots of percentage of reduced cyt c_{550} *versus* ambient redox potential obtained from these spectra, it was possible to adjust the oxidative titration curve with a Nernst equation with $n = 1$ (Fig. 4*A*). Fig. 4*A* shows the presence of one component with E_m value of −220 mV, a value very similar to that described for the soluble form of cyt c_{550} (8, 12, 20, 24, 26). One striking feature of this result is that the oxidative titration curve showed considerable differences from those performed in the reductive direction (see Fig. 2*D*). The E_m determined by a reductive or oxidative potentiometric titration is usually identical in most of the biological and non-biological systems. However, cyt c_{550} bound to PSII exhibited anomalous redox chemistry, *i.e.* hysteresis was observed in the reductive and oxidative redox titrations in which an E_m of +200 mV and −220 mV were obtained, respectively.

To test whether the absence of redox mediators was responsible for the differences between reductive and oxidative titrations, an oxidative titration in the presence of eight redox mediators spanning the range between 0 to +300 mV (see "Experimental Procedures") was done. Fig. 4*B* shows difference spectra obtained during the course of this titration (*inset*) and a plot of percentage of reduced cyt c_{550} *versus* ambient redox potential obtained from these spectra. A very similar low redox potential ($E_m = -215$ mV) for cyt c_{550} could be calculated.

The above results suggest the possible existence of at least two states (A and B) corresponding to cyt c_{550} with substantially different E_m. An E_m of +200 mV can be determined by reductive titrations in PSII preparations after oxidation with potassium ferricyanide (state A). But after adding sodium dithionite, the E_m obtained by oxidative titration is −220 mV and correspond to state B. The experiments described below attempt to determine whether these two states could be interconvertible. Fig. 5 shows a cycle of two reductive and one oxidative potentiometric titrations performed on the same sample of PSII core complexes in the absence of redox mediators. The preparation was initially oxidized with potassium ferricyanide to $E_h \approx +450$ mV and a reductive titration was performed, obtaining a value of $E_m \approx +200$ mV for cyt c_{550} (Fig. 5, *curve 1*). After reducing completely the sample and reaching to an $E_h \approx -400$ mV, oxidative titration was performed by reoxidation of the sample with potassium ferricyanide (Fig. 5, *curve 2*) and a value of $E_m \approx -300$ mV was obtained. After complete reoxidation of the sample ($E_h \approx +410$ mV), a second reductive titration was held showing a similar reductive titration curve to the first one and a slightly lower value of $E_m \approx +100$ mV (Fig. 5, *curve 3*). It seems therefore that exist two extremes states for cyt c_{550} in PSII core complexes preparations that could be interconvertible.

Effect of Incubation with Sodium Dithionite—The experiments described above have shown that after addition of excess sodium dithionite and without mediators, the E_m obtained in the oxidative titration of cyt c_{550} is significantly more negative than the E_m from the reductive titration. To clarify the origin of this phenomenon, the effect of incubation with sodium dithionite on PSII and on the association of cyt c_{550} to PSII were studied.

PSII complexes were reduced with an excess of sodium dithionite (2 mM) and incubated for 10 min or 1 min and then reoxidized with potassium ferricyanide. Illumination of such

Downloaded from http://www.jbc.org/ at FAC BIOLOGIA/BIBLIOTECA on April 22, 2016

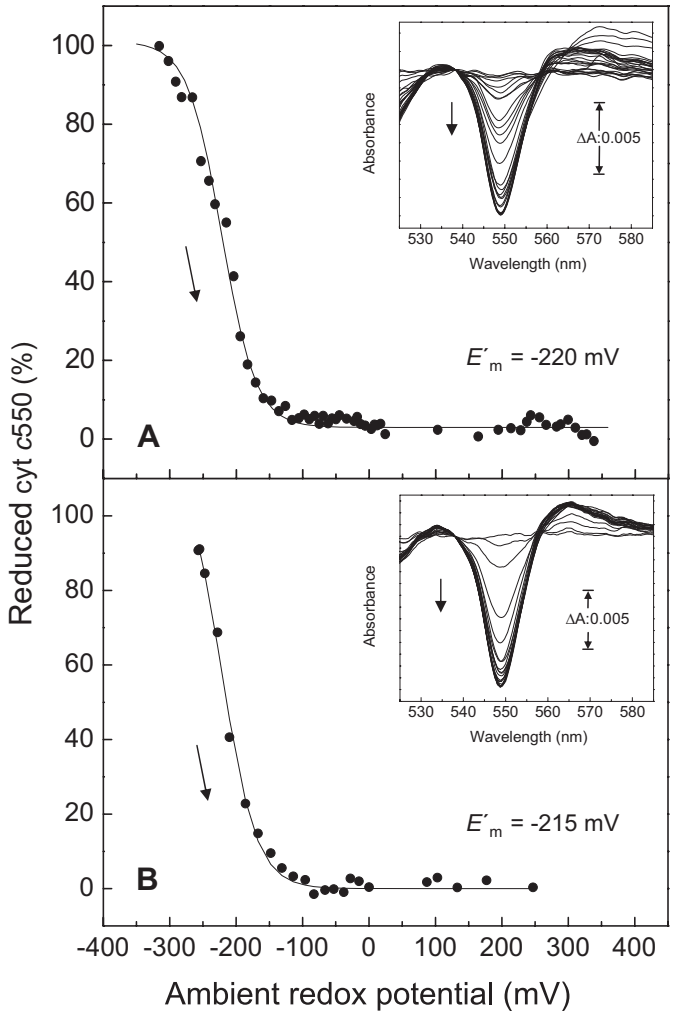

FIGURE 4. **Oxidative potentiometric titrations of cyt c_{550} in PSII core complexes in the absence and presence of redox mediators.** *A* and *B*, plots of the percentages of reduced cyt c_{550} obtained from the absorbance differences at 549–538 nm *versus* ambient redox potentials in PSII core complexes in the absence and in the presence of the mixture of eight redox mediators covering the potential range between +430 and 0 mV, respectively (see "Experimental Procedures"). The *solid curve* represents the best fits of the experimental data to the Nernst equation in accordance with one-electron processes ($n = 1$) for one component with E_m of −220 mV and −215 mV, respectively. *Insets*, difference absorption spectra in the α-band region of cyt c_{550} obtained by subtracting the absolute spectrum recorded at −330 mV from those recorded during the course of the redox titration with potassium ferricyanide in the absence or in the presence of redox mediators, respectively. For simplification, only a set of selected spectra are included.

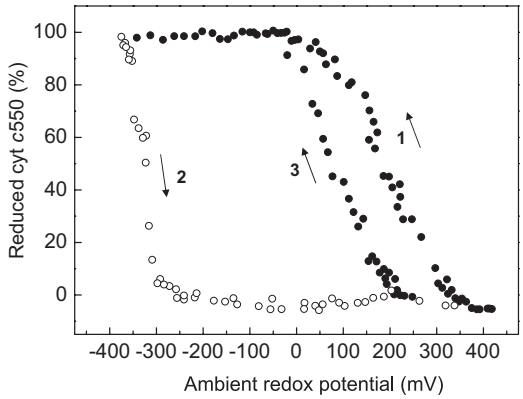

FIGURE 5. **Reversibility of the potentiometric titrations of cyt c_{550} in PSII core complexes in the absence of redox mediators.** The plot represents titration curves corresponding to further cycles of reduction and oxidation (up to three) in the same PSII core complexes preparations. The percentages of reduced cyt c_{550} were plotted *versus* ambient redox potentials in the first reductive titration (*curve 1*), in the first oxidative titration (*curve 2*) and in the second reductive titration (*curve 3*). Each curve was fitted to the Nernst equation in accordance with one-electron processes ($n = 1$) for one component.

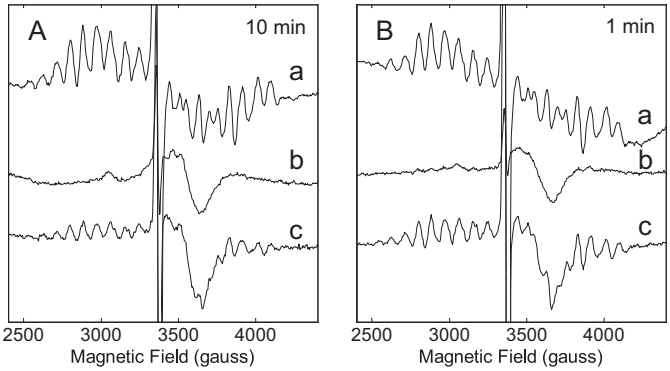

FIGURE 6. **Effect of sodium dithionite on the formation of the S_2 multiline EPR signal.** PSII complexes were incubated 10 min (*A*) or 1 min (*B*) in the absence (*a*) and in the presence of 2 mM of sodium dithionite (*b* and *c*). All spectra were difference spectra after 200 K illumination (light minus dark). *a*, untreated PSII; *b*, PSII complexes reduced by sodium dithionite and reoxidized by potassium ferricyanide; c, sample b was thawed and then was dark adapted at room temperature for 30 min, illuminated by a series of three flashes and finally dark adapted for 30 min. Instrument settings were as follows: microwave power, 20 milliwatt; modulation amplitude, 25 gauss; temperature, 8.5 K.

samples at 200 K did not result in formation of the S_2 manganese multiline signal (Fig. 6, *A*, *b*, and 6, *B*, *b*) indicating that sodium dithionite reduced the manganese cluster. Illumination of the samples at room temperature followed by dark adaptation generated a state that gave rise to a S_2 multiline signal upon illumination at 200 K (Fig. 6, *A*, *c* and 6, *B*, *c*). This indicates that the reduced state formed by sodium dithionite reduction can be reoxidized by light to form the usual S_1 and S_2 states. In the sample incubated with an excess of sodium dithionite for 10 min (Fig. 6, *A*, *c*), the extent of the multiline seen is ~30% of that seen in the unreduced control sample (Fig. 6, *A*, *a*).

The illumination treatment (three flashes) means that the S_0, S_{-1} and S_{-2} states sum up to ~30% of centers. Other experiments directly monitoring S_0 (data not shown) indicate that S_0 makes only a small contribution at this incubation time, *i.e.* the 30% giving rise to the S_2 signal arise from S_{-1} and S_{-2} (or its formal equivalent S_{-1} Tyr-D). The other centers are presumably in more reduced forms of the cluster. Measurements of O_2 evolution showed that in this sample, the O_2 evolution activity was 50% of that in untreated samples. The difference between the activity and the centers giving rise to the S_2 signal presumably reflects centers that were further reduced than S_{-2} but remained rapidly oxidizable and functional. The remaining centers are presumably either irreversibly damaged or require the low quantum yield assembly processes characteristic of photoactivation. We observed that reduction by dithionite also generates the typical Mn^{2+} signals in a small fraction of centers (supplemental Fig. S1*A*). This Mn^{2+} signal does not diminish when sample was illuminated after reoxidation with potassium ferricyanide. Ferricya-

Downloaded from http://www.jbc.org/ at FAC BIOLOGIA/BIBLIOTECA on April 22, 2016

nide is known to precipitate Mn^{2+} ions preventing them from undergoing oxidation by the reaction centers (34). The small Mn^{2+} signal thus presumably represents a small fraction of centers where the cluster is destroyed by the dithionite treatment. The Mn^{2+} signal was larger in the presence of a mediator (indigodisulfonate, $E_m = -125$ mV) indicating greater PSII damage (supplemental Fig. S1*B*).

Shorter incubation times (1 min) with sodium dithionite also showed no S_2 formation upon reoxidation with potassium ferricyanide. However nearly 80% of S_2 formation was seen after three flashes and dark adaptation (Fig. 6, *B*, *c*). This indicates that all the centers were in the S_0, S_{-1} or S_{-2} state after the dithionite treatment. O_2 evolution in such a sample was ~70%.

The EPR experiments show that treatment with excess sodium dithionite results in over-reduction of the manganese cluster and that this can be photo-oxidized again when the ambient potential is returned to a range that allows reaction center photochemistry to occur. Future experiments of this kind should allow us to define which states are formed at given times of incubation and to correlate this more precisely with the binding and redox state of cyt c_{550}.

To test for the release of cyt c_{550} by sodium dithionite treatment, reduced samples were precipitated by PEG, and difference spectra were taken of the supernatant and the pellet (Fig. 7). These were compared with supernatants and pellet from unreduced samples. In the unreduced samples, cyt c_{550} was entirely associated with the PSII (the pellet) (Fig. 7*A*, *spectrum 1*), with no cyt c_{550} present in the supernatant (Fig. 7*A*, *spectrum 2*). However, in the sample incubated with sodium dithionite, the appearance of a typical spectrum cyt c_{550} in the supernatant (Fig. 7*B*, *spectrum 2*) and the corresponding decrease of this cytochrome in the spectrum of the precipitate (Fig. 7*B*, *spectrum 1*), showed that incubation with sodium dithionite had caused the dissociation of a significant fraction of the cyt c_{550} from PSII.

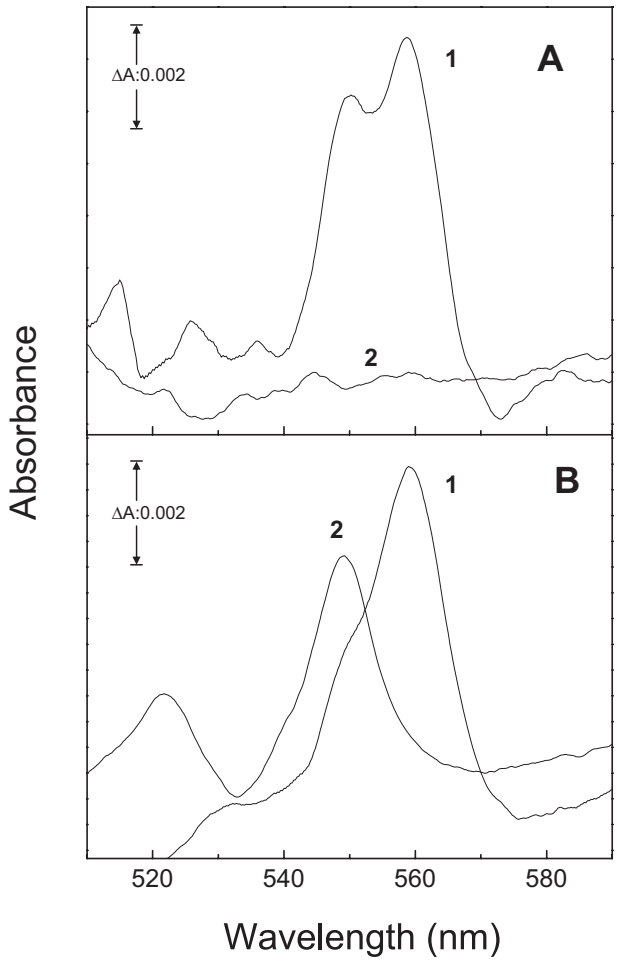

FIGURE 7. **Effect of sodium dithionite on the association of cyt c_{550} to PSII.** Difference absorption spectra of cyt b_{559} and cyt c_{550} were recorded from the pellet (*spectrum 1*) and the supernatant (*spectrum 2*) obtained by precipitation of PSII core complex preparations. The spectra were obtained by subtracting the absolute spectrum at −430 mV (the spectrum of reduced cyt b_{559} and cyt c_{550}) minus at +430 mV (the spectrum of oxidized cyt b_{559} and cyt c_{550}) in PSII core complex preparation untreated (*A*) and treated (*B*) with 2 mM of sodium dithionite during 30 min.

DISCUSSION

Redox titrations of cyt c_{550} performed on PSII core complexes from *T. elongatus* in the absence of low potential redox mediators showed an E_m value for this heme protein that is higher than was obtained previously. This E_m value of +200 mV is ~300 mV more positive than the previously determined when mediators were present ($E_m = -80$ mV) (24). A similar value was obtained in titrations carried out with diaminodurol as a mediator, the potential of which ($E_m = +220$ mV) is quite similar to that determined for cyt c_{550}. The redox potential of the sample during the titration was shown to be reliable under these conditions as demonstrated by (i) the similar E_m values obtained for the cyt b_{559} in the presence or absence of mediators and (ii) the correspondence of the E_m values for cyt b_{559} with those reported in the literature. The good fits of the data sets to one-electron Nernst curves and the large number of measurements allow further confidence in the E_m value obtained for cyt c_{550}.

When titrations were begun at low potentials the values for the E_m were shifted to low potentials, typical of the cyt c_{550} free in solution (26). This effect was specific to the cyt c_{550} and did not affect the cyt b_{559} in the same sample (data not shown). Thus, there was no technical problem in terms of establishing and measuring correct potentials.

The redox potential shift induced by low potentials is reminiscent of earlier reports on the redox potential of Q_A that were reported by Krieger *et al.* (30) and Johnson *et al.* (31). The Mn_4Ca cluster has a very high potential even in the most reduced form of the enzyme cycle. It is protected from reductive attack from the medium by being buried inside a large protein complex with access channels for substrate and products. However, reductants have access to the cluster when highly reducing conditions are used, when mediators are used or when extrinsic polypetides are removed. The reduction of the cluster leads to the weaker binding of the metal ions of the cluster to the site and eventually to their release (35, 36). Reduction of the cluster reverses the assembly process known as photoactivation, which is considered to involve protein conformational changes (37–40). We suggest that these conformational changes are responsible for the increased solvent access and weaker binding of the cyt c_{550} in the presence of

Downloaded from http://www.jbc.org/ at FAC BIOLOGIA/BIBLIOTECA on April 22, 2016

mediators giving E_m values of −80 or −20 mV. Under very reducing conditions, the cyt c_{550} even completely detaches from the PSII (Fig. 7).

Intriguingly when an over-reduced PSII preparation is allowed to become slowly oxidized during the course of a redox titration and then the reductive titration is repeated, the high potential E_m value is recovered. In the context of the explanation given above for the low potential shift, this indicates that the cyt c_{550} rebinds tightly, the protein must have returned to its original conformation and hence the Mn_4Ca cluster also must have returned to its functional state. Reassembly of the Mn_4Ca cluster by binding of free Mn^{2+} ions into the manganese-less PSII, photoactivation (37–40), is a complex process by which Mn^{2+} ions are bound and oxidized one at time by successive turnovers of reaction center photochemistry. A Ca^{2+} ion is also incorporated into the cluster and conformation changes in the protein seem to occur. This process takes place with a relatively low quantum yield. When this is done *in vitro*, high concentrations of Mn^{2+} ions, very high concentrations of Ca^{2+} ions, and the presence of an artificial electron acceptor are required for photoactivation to occur efficiently. Under the conditions of the titrations used here, the medium conditions are clearly not appropriate for this type of photoactivation to occur. Nevertheless, it is still possible to entertain the idea that the cluster "debinds and rebinds" if we propose that the reduced Mn_4Ca cluster is not released from the site upon reduction. The manganese would have to be reduced to a level where the natural geometry of the functional site is lost, and hence the changes in the protein would take place, but the Mn^{2+} and Ca^{2+} ions would not be released into the medium. This is similar to the situation encountered by Mei and Yocum (41) in which reductants were allowed access to the manganese by removal of the 23-kDa polypeptide. Some Mn^{2+} was seen by EPR but was not available to chelators, and the enzyme was rapidly activated by illumination. In the present work, the weak measuring beam used for measuring the spectra during the course of the titration could have been sufficient to reoxidize the manganese and reimpose the protein conformation required to induce the high potential form of the cyt c_{550}.

To test the feasibility of this idea, we did an EPR study that showed (*a*) the Mn_4Ca cluster was indeed reduced by sodium dithionite and upon reoxidation of the electron acceptors in the dark the Mn_4Ca remained in an over-reduced state and (*b*) the over-reduced state could be efficiently reoxidized by flash illumination. The reoxidized PSII showed water oxidation activity in a large proportion of centers. With longer times of incubation in sodium dithionite, the proportion of centers that could be reactivated by illumination diminished. Whether this effect is due to loss of manganese or to the requirement for low quantum yield photoactivation will be the subject of future work. Nevertheless, the EPR experiments are consistent with the proposition that the cyt c_{550} environment reflects conformational changes that are controlled by the redox state of the Mn_4Ca cluster. Further experimentation should allow this to be tested more directly.

The change in the PSII structure associated with the reduction of the Mn_4Ca cluster could lead to a greater accessibility of the heme to the aqueous medium and consequently to a total or partial release of cyt c_{550} from PSII. It seems likely that the increase in solvation energy that occurs when moving the heme out of the low dielectric of the protein environment into the high dielectric of water stabilizes the oxidized state more than the reduced state making the midpoint potential more negative (42–44).

These results lead us to suggest that the E_m of cyt c_{550} in PSII "*in vivo*" may be +200 mV, at least under certain conditions. This opens the possibility of a redox function for this protein in electron transfer in PSII. The nearest redox cofactor is the Mn_4Ca cluster (22 Å) (6). This long distance means that electron transfer would be slow (ms-s time scale) relative to the charge separation events in the reaction center. However, this rate remains potentially significant relative to the lifetime of the reversible charge accumulation states in the enzyme (tens of seconds to minutes) (45). Some kind of protective cycle involving a soluble redox component in the lumen may be envisioned. Before we enter into speculation, an experimental verification that cyt c_{550} does indeed donate electrons to the S_2 and or S_3 states is required.

CONCLUSIONS

We conclude that earlier redox titrations of cyt c_{550} in PSII probably reflected the situation in which the Mn_4Ca cluster is chemically reduced. Conformational changes associated with this resulted in the downshift of the E_m due to solvent access to the heme. Thus, the E_m of +200–215 mV obtained for cyt c_{550} without mediators and with only diaminodurol as a mediator is probably relevant to the most functional form of the enzyme. This E_m value of about +200 mV opens the possibility of a redox function for cyt c_{550} in the PSII as an electron donor to the Mn_4Ca cluster perhaps in some sort of protective cycle. This proposed role has yet to be demonstrated experimentally.

REFERENCES

1. Barber, J. (2009) *Chem. Soc. Rev.* **38,** 185–196
2. Zouni, A., Witt, H. T., Kern, J., Fromme, P., Krauss, N., Saenger, W., and Orth, P. (2001) *Nature* **409,** 739–743
3. Kamiya, N., and Shen, J. R. (2003) *Proc. Natl. Acad. Sci. U.S.A.* **100,** 98–103
4. Ferreira, K. N., Iverson, T. M., Maghlaoui, K., Barber, J., and Iwata, S. (2004) *Science* **303,** 1831–1838
5. Biesiadka, J., Loll, B., Kern, J., Irrgang, K. D., and Zouni, A. (2004) *Phys. Chem. Chem. Phys.* **6,** 4733–4736
6. Guskov, A., Kern, J., Gabdulkhakov, A., Broser, M., Zouni, A., and Saenger, W. (2009) *Nat. Struct. Mol. Biol.* **16,** 334–342
7. Alam, J., Sprinkle, J., Hermodson, M. A., and Krogmann, D. W. (1984) *Biochim. Biophys. Acta.* **766,** 317–321
8. Navarro, J. A., Hervás, M., De la Cerda, B., and De la Rosa, M. A. (1995) *Arch. Biochem. Biophys.* **318,** 46–52
9. Frazão, C., Enguita, F. J., Coelho, R., Sheldrick, G. M., Navarro, J. A., Hervás, M., De la Rosa, M. A., and Carrondo, M. A. (2001) *J. Biol. Inorg. Chem.* **6,** 324–332
10. Sawaya, M. R., Krogmann, D. W., Serag, A., Ho, K. K., Yeates, T. O., and Kerfeld, C. A. (2001) *Biochemistry* **40,** 9215–9225
11. Kerfeld, C. A., Sawaya, M. R., Bottin, H., Tran, K. T., Sugiura, M., Cascio, D., Desbois, A., Yeates, T. O., Kirilovsky, D., and Boussac, A. (2003) *Plant Cell Physiol.* **44,** 697–706
12. Hoganson, C. W., Lagenfelt, G., and Andréasson, L. E. (1990) *Biochim. Biophys Acta* **1016,** 203–206

Downloaded from http://www.jbc.org/ at FAC BIOLOGIA/BIBLIOTECA on April 22, 2016

13. Vrettos, J. S., Reifler, M. J., Kievit, O., Lakshmi, K. V., de Paula, J. C., and Brudvig, G. W. (2001) *J. Biol. Inorg. Chem.* **6,** 708–716
14. Shen, J. R., Qian, M., Inoue, Y., and Burnap, R. L. (1998) *Biochemistry* **37,** 1551–1558
15. Kerfeld, C. A., and Krogmann, D. W. (1998) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **49,** 397–425
16. Katoh, H., Itoh, S., Shen, J. R., and Ikeuchi, M. (2001) *Plant Cell Physiol.* **42,** 599–607
17. Nishiyama, Y., Hayashi, H., Watanabe, T., and Murata, N. (1994) *Plant Physiol.* **105,** 1313–1319
18. Kienzl, P. F., and Peschek, G. A. (1983) *FEBS Lett.* **162,** 76–80
19. Morand, L. Z., Cheng, R. H., Krogmann, D. W., and Ho, K. K. (1994) in *The Molecular Biology of Cyanobacteria* (Bryant, D. A., ed) pp. 381–407, Kluwer Academic Publishers, Dordrecht, The Netherlands
20. Holton, R. W., and Myers, J. (1963) *Science* **142,** 234–235
21. Krogmann, D. W. (1991) *Biochim. Biophys. Acta* **1058,** 35–37
22. Kang, C., Chitnis, P. R., Smith, S., and Krogmann, D. W. (1994) *FEBS Lett.* **344,** 5–9
23. Shen, J. R., and Inoue, Y. (1993) *Biochemistry* **32,** 1825–1832
24. Roncel, M., Boussac, A., Zurita, J. L., Bottin, H., Sugiura, M., Kirilovsky, D., and Ortega, J. M. (2003) *J. Biol. Inorg. Chem.* **8,** 206–216
25. Ishikita, H., and Knapp, E. W. (2005) *FEBS Lett.* **579,** 3190–3194
26. Kirilovsky, D., Roncel, M., Boussac, A., Wilson, A., Zurita, J. L., Ducruet, J. M., Bottin, H., Sugiura, M., Ortega, J. M., and Rutherford, A. W. (2004) *J. Biol. Chem.* **279,** 52869–52880
27. Clark, W. M. (1960) in *Oxidation–Reduction Potentials of Organic Systems*, Williams and Wilkins, Baltimore
28. Wilson, G. S. (1978) *Methods Enzymol.* **54,** 396–410
29. Dutton, P. L. (1978) *Methods Enzymol.* **54,** 411–435
30. Krieger, A., Rutherford, A. W., and Johnson, G. N. (1995) *Biochim. Biophys. Acta* **1229,** 193–201
31. Johnson, G. N., Rutherford, A. W., and Krieger, A. (1995) *Biochim. Biophys. Acta* **1229,** 202–207
32. Mühlenhoff, U., and Chauvat, F. (1996) *Mol. Gen. Genet.* **252,** 93–100
33. Cai, Y. P., and Wolk, C. P. (1990) *J. Bacteriol.* **172,** 3138–3145
34. Hoganson, C. W., Casey, P. A., and Hansson, O. (1991) *Biochim. Biophys. Acta* **1057,** 399–406
35. Tamura, N., and Cheniae, G. (1985) *Biochim. Biophys. Acta* **809,** 245–259
36. Debus, R. J. (1992) *Biochim. Biophys. Acta* **1102,** 269–352
37. Miyao, M., and Inoue, Y. (1991) *Biochemistry* **30,** 5379–5387
38. Dasgupta, J., Ananyev, G. M., and Dismukes, G. C. (2008) *Coord. Chem. Rev.* **252,** 347–360
39. Cheniae, G. M., and Martin, I. F. (1972) *Plant Physiol.* **50,** 87–94
40. Burnap, R. L. (2004) *Phys. Chem. Chem. Phys.* **6,** 4803–4809
41. Mei, R., and Yocum, C. F. (1991) *Biochemistry* **30,** 7836–7842
42. Mao, J., Hauser, K., and Gunner, M. R. (2003) *Biochemistry* **42,** 9829–9840
43. Wirtz, M., Oganesyanes, V., Zhang, X., Studer, J., and Rivera, M. (2000) *Faraday Discuss.* **116,** 221–234
44. Kassner, R. J. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **69,** 2263–2267
45. Moser, C. C., Page, C. C., and Dutton, P. L. (2005) *Photochem. Phobiol. Sci.* **4,** 933–939

Downloaded from http://www.jbc.org/ at FAC BIOLOGIA/BIBLIOTECA on April 22, 2016

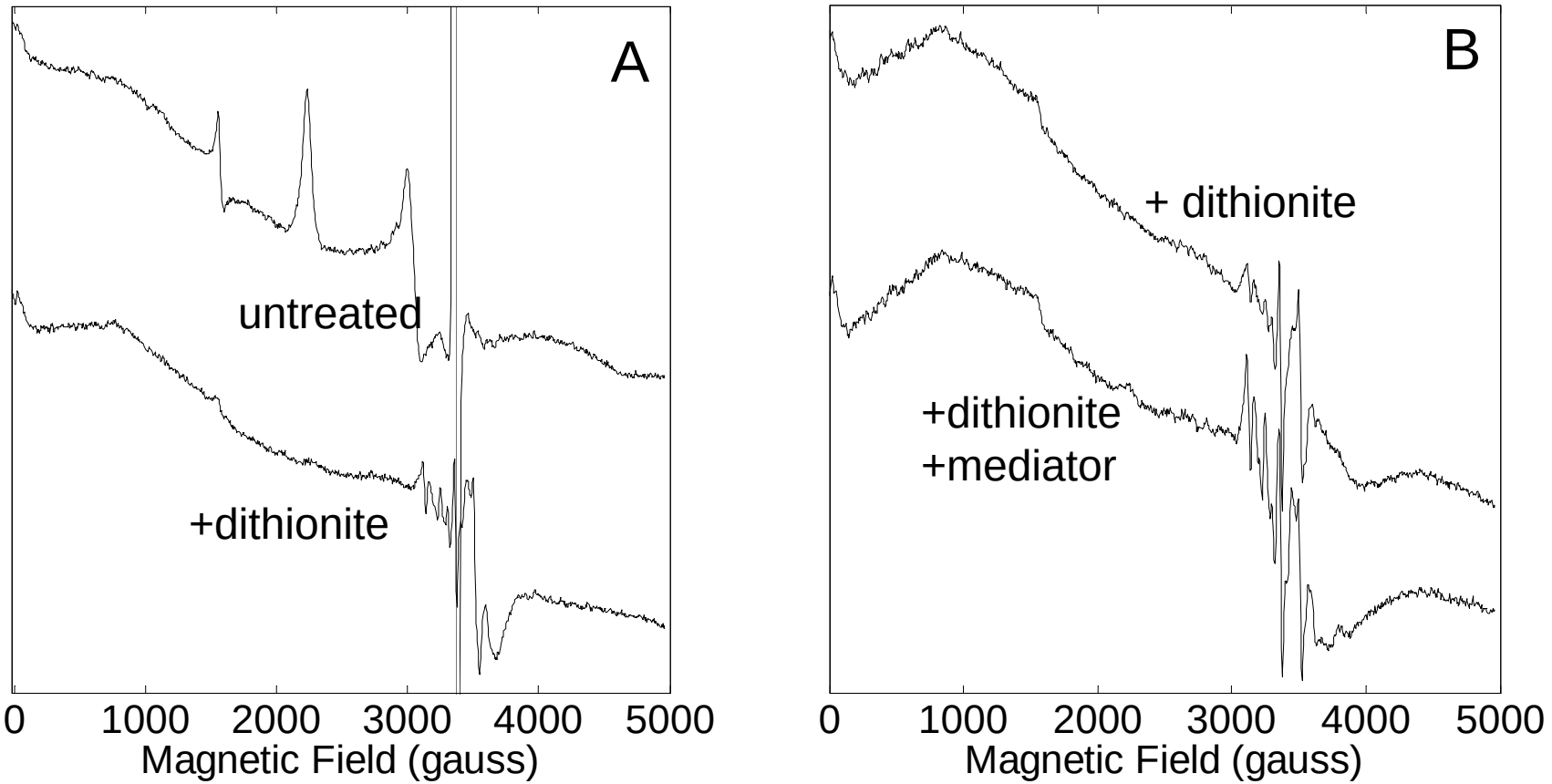

Fig S1. Effect of sodium dithionite reduction and mediator addition on Mn release in PSII.
Panel A shows the effect on PSII of sodium dithionite incubation during 10 min. The upper spectrum showed the PSII before reduction, the lower spectrum showed the reduced PSII. Panel B shows the effect of a mediator (Indigodisulfonate, 20 µM as a final concentration, incubated for 10 min) on the release of Mn^{2+}.The upper spectrum showed the reduced PSII with sodium dithionite for 30 min, while the lower spectrum showed the effect of the mediator addition. Instrument settings: microwave power, 5.6 mW, modulation amplitude, 25 gauss, temperature, 15 K. The signal observed in the presence of mediator is 80% of total signal (maximum released Mn) and in the absence is about 25%.

A High Redox Potential Form of Cytochrome c_{550} in Photosystem II from *Thermosynechococcus elongatus*

Fernando Guerrero, Arezki Sedoud, Diana Kirilovsky, A. William Rutherford, José M. Ortega and Mercedes Roncel

J. Biol. Chem. 2011, 286:5985-5994.
doi: 10.1074/jbc.M110.170126 originally published online December 6, 2010

Access the most updated version of this article at doi: 10.1074/jbc.M110.170126

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2010/12/06/M110.170126.DC1.html

This article cites 43 references, 10 of which can be accessed free at
http://www.jbc.org/content/286/8/5985.full.html#ref-list-1

Downloaded from http://www.jbc.org/ at FAC BIOLOGIA/BIBLIOTECA on April 22, 2016