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Effects of cholate on Photosystem II: selective extraction of a 22 kDa polypeptide and modification of Q_B -site activity

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A quinone-mediated two-electron gate is shared by Photosystem II (PS II) and the photosystem of purple bacteria. In the bacterial reaction center, electron transfer from the reduced primary quinone acceptor, Q_A^- , to the secondary quinone, Q_B , as well as the sensitivity of this electron transfer step to inhibition by terbutryn, are regulated by the H subunit of the reaction center. Sequential removal of three polypeptides (10 and 22, followed by 28 kDa) from O_2 evolving PS II reaction center complex preparations impairs Q_B activity. Removal of the 22 kDa protein does not abolish the herbicide sensitivity of electron transfer mediated by an added *p*-benzoquinone, but another of these proteins, a species of 28 kDa that binds chlorophyll, appears to regulate the ability of the herbicide DCMU to interfere with PS II electron transfer (Bowlby, N.R. et al. (1990) *Curr. Res. Photosynth.* I, 539–542). In this communication, we show that exposure of PS II to Na-cholate yields a preparation in which substantial depletion of a 22 kDa intrinsic protein has occurred. In the depleted preparations, a hydrophilic oxidant ($Fe(CN)_6^{3-}$), rather than a lipophilic *p*-benzoquinone acceptor, supports the highest rates of O_2 evolution in a reaction that shows little sensitivity to the Q_B inhibitor DCMU. Although this observation might indicate a disruption of Q_B function that is associated with removal of the 22 kDa protein, this is not the case. It is shown here that under some conditions, Na-cholate will modify the response of PS II to $Fe(CN)_6^{3-}$ without release of the 22 kDa protein.

Introduction

Primary charge separation in Photosystem II (PS II) is stabilized by secondary electron transfer events; the oxidized primary donor P_{680}^+ is reduced by Y_Z , a tyrosine residue on one of the constituent RC polypeptides [1,2] and the reduced primary acceptor pheophytin (I^-) is oxidized by a tightly bound quinone denoted Q_A [3]. The state $Y_Z^+P_{680}IQ_A^-$ subsequently decays; the manganese cluster of the O_2 -evolving complex is oxidized by Y_Z^+ , and an exchangeable quinone, Q_B is reduced

by Q_A^+ . A similar quinone-mediated electron transfer reaction occurs in photosynthetic bacteria and the crystal structures of the RCs of *Rhodospseudomonas viridis* [4,5] and *Rhodobacter sphaeroides* [6,7] are cited as tentative models for the structure of the quinone-binding domain of the acceptor side of PS II [8]. In addition to Q_A and Q_B , an atom of non-heme iron is present in both PS II and bacterial RCs. Although the iron atom is not essential for electron transfer between the quinones in purple bacteria [9], it is normally coupled to the quinones through magnetic interactions that are revealed by EPR spectroscopy.

Traditional inhibitors of electron transfer from Q_A^- to Q_B (DCMU in PS II or terbutryn in bacteria) compete with quinones at the Q_B site [10,11]. In bacteria, Terbutryn sensitivity is decreased upon removal of the H subunit, and then increased upon subsequent rebinding of this protein [12], suggesting that electron transfer on the reducing side of the photoreaction is influenced by this polypeptide. In PS II the Q_A and the Q_B sites are buried within the protein complex; the hydrophilic, anionic oxidant $Fe(CN)_6^{3-}$ is ineffective in

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Abbreviations: Chl, chlorophyll; cyt, cytochrome; DCBQ, 2,6-dichloro-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl) 1,1'-dimethylurea; DM, dodecyl- β -D-maltoside; LHClI, light harvesting complex of Photosystem II; Mes, 2-(*N*-morpholino)ethanesulfonic acid; PQ, plastoquinone; PS II, Photosystem II; RC, reaction center; TBS, 25 mM Tris-HCl (pH 7.5), 150 mM NaCl; Tris, Tris[hydroxymethyl]aminomethane.

supporting high rates of activity. Native Q_B -site activity in PS II, consisting of DCMU-sensitive quinone-dependent electron transfer, is affected by proteolysis of thylakoid membranes with trypsin. After trypsin treatment, decreased sensitivity to DCMU inhibition is accompanied by stimulation of $Fe(CN)_6^{3-}$ -dependent electron transfer at the expense of *p*-benzoquinone-supported activity [13]. Purification procedures also produce alterations to herbicide-sensitive electron transfer activity. Highly-resolved PS II preparations incapable of O_2 evolution activity retain one herbicide binding site per RC [14] but the herbicide inhibition constant (I_{50}) in such preparations is greatly reduced. Likewise, in highly-purified PS II preparations retaining the capacity for O_2 evolution activity, Yamada et al. [15] found that DCMU-insensitive electron transfer was present, and that the highest rates of such activity were obtained with $Fe(CN)_6^{3-}$, rather than a *p*-benzoquinone, as the electron acceptor.

Stepwise removal of intrinsic proteins from PS II has identified species that may function in regulation of PS II reducing side activity. Ghanotakis et al. [16] showed that a purified O_2 evolving PS II preparation depleted of LHCII and 23 and 17 kDa extrinsic polypeptides retained DCMU sensitive, quinone-catalyzed activity. Subsequent removal of 28, 22 and 10 kDa subunits from this preparation by gel filtration [17] created a preparation exhibiting DCMU-insensitive electron transfer. Sequential removal of these same subunits by ion-exchange chromatography (Ref. 18 and Bowlby, N.R. et al., data not shown) in detergent yielded a preparation with subunit composition and DCMU-insensitive activity similar to that reported by Yamada et al. One of the polypeptides (a 28 kDa Chl-binding species) removed by purification appears to have a function in PS II analogous to that of the bacterial H-subunit; selective removal of this species abolishes the ability of DCMU to interfere with DCBQ reduction [18]. While the 28 kDa subunit in PS II binds Chl *a*, and perhaps Chl *b* [19,20], the 22 and 10 kDa subunits appear to have no tightly-bound chromophores; the 10 kDa subunit has been suggested by Packham [21] to be the H-subunit analog in PS II based on similarities between the primary sequence of the plastid encoded *psbH* gene product [22] and two bacterial H-subunits.

Here, we describe a method for the selective depletion of the 22 kDa PS II subunit by treatment of the O_2 -evolving PS II RC complex with Na-cholate. Other investigators have shown that cholate affects the oxidizing side of PS II by extracting extrinsic polypeptides [30]. The results presented in this communication show that in addition to 22 kDa protein removal, Na-cholate can provoke a DCMU-insensitive $Fe(CN)_6^{3-}$ reduction reaction in PS II. At the same time electron transfer catalyzed by a *p*-benzoquinone acceptor, which is more

closely related to Q_B site integrity, remains sensitive to inhibition by DCMU after removal of the 22 kDa species.

Materials and Methods

PS II membranes and O_2 -evolving PS II RC-complexes were prepared as described in Ref. 16 to yield material with high rates of O_2 evolution ($> 1600 \mu\text{mol } O_2/\text{mg Chl per h}$). Cholic acid was recrystallized twice from ethanol. A 10% (w/v) solution at pH 7.0 was prepared as the sodium salt and was filtered through a $0.2 \mu\text{M}$ filter before use. Cholate treatment of RC complexes (0.25 mg Chl/ml) or PS II membranes (1 mg Chl/ml) was carried out at 4°C in the dark for thirty min. with occasional gentle mixing. Preliminary experiments were conducted with the RC complex to optimize the detergent and salt conditions which led to the greatest change in quinone-dependent activity with minimal inhibition of O_2 evolution activity. For the experiments reported here, PS II preparations in SMC (0.4 M sucrose, 50 mM Mes-NaOH (pH 6.0), 20 mM $CaCl_2$) were exposed to 2.5% Na-cholate and 250 mM NaCl by additions from 10% and 4 M stock solutions respectively. Incubation mixtures were centrifuged at $40000 \times g$ for 30 min, washed once in SMC at 0.25 mg Chl/ml (RC preparations) or 1.0 mg Chl/ml (PS II membranes) and resuspended in SMC to 1 mg Chl/ml (RC preparations) or 3 mg Chl/ml (PS II membranes) with a paintbrush before being frozen and stored at -60°C . The cholate extract was dialyzed overnight at 10°C against 200 volumes of 20 mM Mes-NaOH (pH 6.0). Precipitated material was then recovered by centrifugation at $40000 \times g$ for 30 min. and resuspended in a small amount of SMC.

Antibodies were raised in a rabbit following standard procedures by using electrophoretically pure 22 kDa protein as antigen prepared as follows. After separation of SDS-denatured RC complex proteins on a 15% acrylamide gel as described in Ref. 16, the band containing the 22 kDa protein was excised from the gel, frozen in liquid N_2 and pulverized with a mortar and pestle under liquid N_2 . Protein was extracted into 25 mM Tris-HCl (pH 7.5) with 0.1% SDS by several rounds of resuspension of the frozen gel powder in excess buffer followed by incubation at room temperature for 15 min and centrifugation to compact the swollen gel particles. The extracts were combined and concentrated by vacuum dialysis. A second preparative gel with 12.5% acrylamide and 6 M urea resolved additional proteins, and the extraction of pure 22 kDa protein was repeated with liquid N_2 as just described. Pre-immune serum was collected and a portion of the protein solution (200–500 μg) was emulsified with complete Freund's adjuvant in a 1:1 ratio and injected (intramuscular) at four sites (250 μl each) on the upper thighs of a young white rabbit. After six weeks, whole

blood was collected and the animal was given another set of injections by using the purified protein in an emulsion with Freund's incomplete adjuvant. Portions of whole serum were stored at -60°C .

For analysis of PS II subunit composition before and after cholate treatment, SDS-PAGE was carried out by using 13.5% acrylamide and 4 M urea. Protein bands were visualized by staining with Coomassie brilliant blue or Western blotting to nitrocellulose followed by immune decoration. Primary antibodies were allowed to bind to Western blots overnight by shaking in TTBS (TBS with 0.05% Tween-20) at a typical dilution of 1:1000 for whole serum. The blot was washed (3×10 min) in TTBS and secondary antibody (Goat anti-rabbit/HRP conjugate, Bio-Rad) was allowed to bind at room-temperature in TTBS for 1–2 h, followed by 3-times 10-min washes in TBS to remove Tween-20 and unbound secondary antibody. Antigen-antibody complexes were visualized by development with HRP Color Development Reagent (Bio-Rad) according to the manufacturer's instructions.

For cyt b_{559} determinations, O_2 -evolving PS II RC complex or cholate-treated RC complex preparations (0.5 mg Chl/ml) were solubilized with DM added from a 10% stock solution. For the RC complex, the final DM concentration was 0.5%; for the cholate-treated RC complex, the final concentration of DM was 1.0%. After addition of DM, samples were diluted with 50–100 vols. SMC for spectrophotometry. The amount of cyt b_{559} was estimated from the dithionite-reduced minus $\text{Fe}(\text{CN})_6^{3-}$ -oxidized difference spectrum by using $17.5 \text{ mM}^{-1} \text{ cm}^{-1}$ as the differential extinction coefficient at 559 nm [23]. The Chl concentration was determined following dilution of 0.5 ml of sample with 2 ml acetone by using the extinction coefficients of Arnon [24].

Steady-state rates of O_2 evolution were measured in a Clark type electrode at 25°C . The assay mixture (1.6 ml) contained 50 mM Mes-NaOH (pH 6.0), 20 mM CaCl_2 and electron acceptors as described in the figure legends; DCMU was added from a stock solution in methanol to the final concentrations indicated. Estimates of V_{max} for O_2 evolution and the K_m for $\text{Fe}(\text{CN})_6^{3-}$ were made by a weighted regression analysis [25] modified to run under BASIC on an IBM-XT compatible desktop computer. Plastoquinone quantification was performed as in Ref. 26. Room-temperature EPR spectroscopy was performed by using a Bruker ER-200D spectrometer fitted with a TM cavity. Instrument settings and experimental conditions are described in Results and the figure legends.

Results

Fig. 1 presents an electrophoretic analysis of the constituent polypeptides in native RC complex and

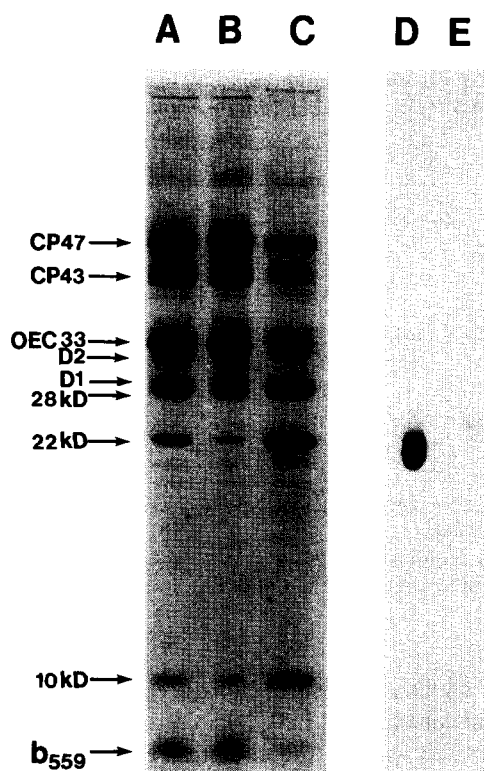


Fig. 1. Removal of 22 kDa protein by cholate treatment. A Coomassie-stained gel (left) and Western blot challenged with anti-22-kDa serum (right) are shown. Lanes A and D contain O_2 -evolving PS II RC complex preparations before and after (Lanes B and E) treatment with Na-cholate and the supernatant fluid following dialysis as described in Materials and Methods (Lane C).

cholate-treated material, as well as proteins recovered from the dialyzed supernatant produced by cholate treatment. The major polypeptides released by cholate treatment include the 22 kDa intrinsic protein and, to substantially lesser extents, 47, 28 and 10 kDa proteins (Lane C). Lanes D and E show a Western blot probed with polyclonal antiserum directed against the 22 kDa species. The RC complex (Lane D) shows a strong signal corresponding to the 22 kDa protein; little reactivity is seen in the cholate-treated preparation (Lane E). Optical absorption spectra of the cholate-treated RC complex and an untreated sample are nearly identical (not shown) confirming that little pigment was extracted. Table I presents data on analyses of chromophore content before and after cholate extraction. Hydroquinone-reducible, high-potential b_{559} was not detected in either preparation, although about 30% and 10% of the total b_{559} was reduced by ascorbate before and after cholate treatment (not shown). The data of Table I and Fig. 1 confirm that negligible amounts of pigment-binding proteins are lost from PS II as a result of cholate treatment.

A comparison of the effect of cholate treatment on electron transport in the RC complex and PS II membranes is also summarized by data presented in Table

TABLE I

Effects of cholate treatment on component stoichiometries and activities of the PS II RC complex and BBY membranes

	RC complex		Membrane sheets	
	-Cholate	+Cholate	-Cholate	+Cholate
Chl/2 b_{559}	65 ± 3	65 ± 3	275 ± 25	275 ± 25
Mn/RC ^{a,b}	4	4	4	n.d. ^c
PQ-9/RC	2.2 ± 0.1	1.9 ± 0.1	3.2 ± 0.01	n.d.
V_{max} DCBQ ^d	2464 ± 144	1082 ± 39	920 ± 68	382 ± 17
K_m (μ M)	92 ± 9	53 ± 4	80 ± 10	30 ± 10
V_{max}				
Fe(CN) $_6^{3-}$	732 ± 27	1298 ± 28	56 ± 4	203 ± 3
K_m (μ M)	125 ± 16	181 ± 12	34 ± 15	113 ± 4

^a Assuming two cytochromes b_{559} per O₂-evolving RC.

^b Nearest integer.

^c n.d., not determined.

^d Expressed as μ mol O₂/mg Chl per h.

I. After cholate exposure, both preparations show an enhanced capacity for Fe(CN) $_6^{3-}$ reduction. In contrast to the activity seen in the cholate-treated RC complex, however, cholate-treated PS II membranes still reduce DCBQ at higher rates than can be obtained with Fe(CN) $_6^{3-}$. The interaction between DCBQ and Fe(CN) $_6^{3-}$ in supporting O₂ evolution activity can be seen in Fig. 2 for untreated (top) and cholate-treated (bottom) RC complexes. After cholate treatment, two differences are observed with respect to the effects of DCBQ addition on Fe(CN) $_6^{3-}$ reduction. First, the increase in activity with DCBQ occurs only at low concentrations of the quinone; higher DCBQ concentrations are inhibitory. Second, the V_{max} for Fe(CN) $_6^{3-}$ reduction (after correcting for DCBQ-dependent activity) is significantly increased at all DCBQ concentrations when compared to the V_{max} for Fe(CN) $_6^{3-}$ reduction in the native RC complex.

Effects of cholate treatment on the kinetics of Fe(CN) $_6^{3-}$ -dependent O₂ evolution at increasing concentrations of DCBQ are presented as double-reciprocal plots in Fig. 3. Activity due to DCBQ alone was subtracted from the observed rate in order to reveal the kinetics for Fe(CN) $_6^{3-}$ -dependent activity. In the untreated RC complex Fig. 2A) DCBQ inhibition of Fe(CN) $_6^{3-}$ -supported activity is competitive, indicating that the Q_B site provides the major pathway for Fe(CN) $_6^{3-}$ reduction. In addition, lower concentrations of DCBQ (< 50 μ M) decrease the K_m for Fe(CN) $_6^{3-}$ reduction while higher concentrations have the opposite effect.

Fig. 3B shows a similar analysis of the cholate-treated RC complex. These data show the synergistic effect of the electron acceptors only at concentrations lower than 50 μ M DCBQ. At concentrations lower than 50 μ M the effect of added DCBQ is to decrease

K_m and V_{max} , whereas in the untreated RC complex, the effect is to decrease K_m and increase V_{max} . Furthermore, the type of DCBQ inhibition of Fe(CN) $_6^{3-}$ reduction is changed, becoming noncompetitive as the concentration of the quinone is increased. These results indicate that cholate treatment has produced a significant modification to the Q_A-Fe-Q_B domain on the reducing side of PS II; these changes are reflected in a 30% reduction in steady-state O₂ evolving activity (Table I).

To analyze other alterations in the Q_B site, experiments were carried out in the presence of DCMU. The insets in Fig. 3 show the effect of 6.25 μ M DCMU on the kinetics of Fe(CN) $_6^{3-}$ reduction in the absence (solid line) and presence (dashed line) of 125 μ M DCBQ. In control preparations, DCMU is a competitive inhibitor at the Q_B site regardless of whether Fe(CN) $_6^{3-}$ or DCBQ is the electron acceptor. Addition of DCMU also changes the type of inhibition by DCBQ, becoming uncompetitive with respect to Fe(CN) $_6^{3-}$ reduction. Following cholate treatment, 6.25 μ M DCMU is a less potent inhibitor of Fe(CN) $_6^{3-}$ -dependent activity, but the inhibition of this activity by DCBQ remains noncompetitive. These cholate-induced changes in acceptor reduction kinetics in the presence of DCMU provide strong evidence for a substantial alteration of

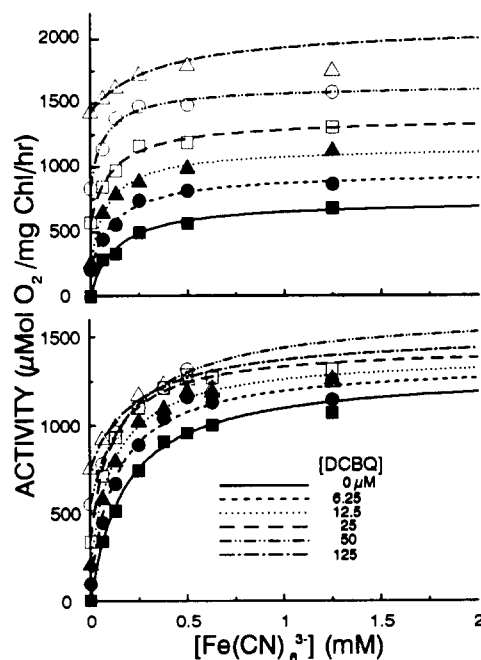


Fig. 2. Oxygen-evolution activity before and after cholate treatment. Velocity vs. [Fe(CN) $_6^{3-}$] plots are presented for the PS II RC complex (top) and cholate-treated RC complex (bottom) at various DCBQ concentrations. Activity is expressed in μ mol O₂/mg Chl per h. Symbols show experimental data (average of three experiments) and solid curves show model equations determined by the HYPERO program [25]. Symbols represent data at DCBQ concentrations of (μ M): 0, \blacksquare ; 6.25, \bullet ; 12.5, \blacktriangle ; 25, \square ; 50, \circ ; 125, \triangle . The Chl concentration was 5 μ g/ml for all assays.

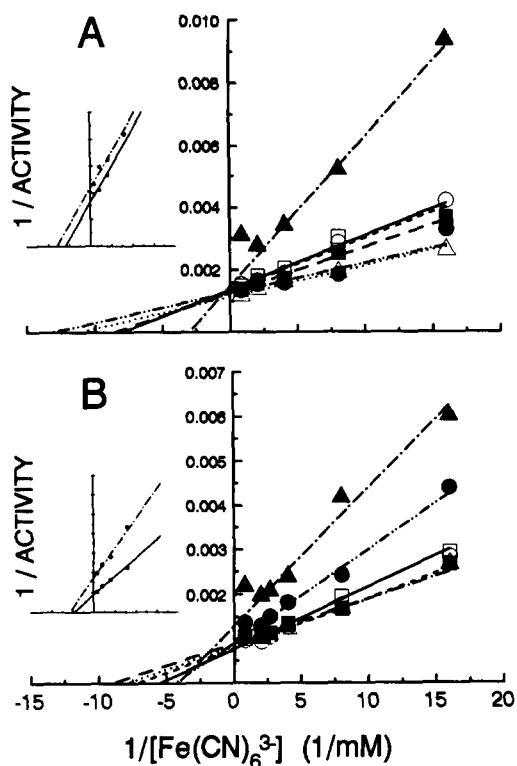


Fig. 3. Inhibition of ferricyanide-dependent O_2 -evolution activity by DCBQ. The figure shows double-reciprocal plots of $Fe(CN)_6^{3-}$ -dependent O_2 -evolution activity at different concentrations of DCBQ in untreated PS II RC complex (A) and following cholate treatment (B). Symbols as in Fig. 2. The insets show double-reciprocal plots of $Fe(CN)_6^{3-}$ -dependent O_2 -evolving activity without (squares) and with (circles) $125 \mu M$ DCBQ in the presence of $6.25 \mu M$ DCMU (activities in the absence of $Fe(CN)_6^{3-}$ were 185 and $266 \mu mol O_2 / mg Chl$ per h for the untreated and treated RC complex, respectively). Other experimental conditions as in Fig. 2.

the quinone-iron acceptor complex which is characterized by a diminished interaction of acceptors with the Q_B site. The results are consistent with the cholate-induced appearance of a new site of acceptor reduction prior to the Q_B site.

To investigate possible damage by cholate to donor side components of PS II, room-temperature EPR spectroscopy was carried out to examine the properties of Y_D^+ and Y_Z^+ . Measurement of the intensity of the low field peak in the derivative spectrum (denoted by the arrow in Fig. 4) provides a convenient means of quantifying the abundance of tyrosine radicals in PS II provided a standard with a known concentration of spins is available. The PS II membrane preparation has been well-characterized with respect to tyrosine radicals [27], and was used as the spin standard in these studies. Fig. 4 shows spectra in the $g = 2$ region for the RC complex and cholate-treated complex. The Chl concentration in each sample was adjusted to provide nearly equivalent RC concentrations based on cyt b_{559} determinations and the spectra have been normalized with respect to spectrometer gain. This allows a direct

comparison of the dark and light-induced signals shown in the figure. In the untreated RC complex, very little signal increase is observed under continuous illumination, showing that reduction of Y_Z^+ is rapid. Following cholate treatment, about half of the signal attributed to Y_D^+ is observed, when compared to the untreated control. Under continuous illumination, the signal intensity nearly doubles even though higher rates of $Fe(CN)_6^{3-}$ -supported O_2 -evolution activity are observed. This indicates that the dark stability of Y_D^+ has been affected; the radical is restored to full intensity on illumination. Another signal is present following long-term dark adaptation in the cholate-treated RC complex that is not seen in the untreated RC complex. This signal persists during illumination, but decays in the dark. The spectrum taken after 15 min of incubation in the dark following illumination shows a well resolved Y_D^+ spectrum with little evidence of the other $g = 2$ radical, which could be regenerated by a second illumination (not shown). These results suggest that after exposure to cholate, subtle changes have occurred on the donor side of PS II that influence the stability or EPR properties of Y_D^+ . The treatment also leads to the appearance of an as yet unidentified EPR signal that disappears following illumination.

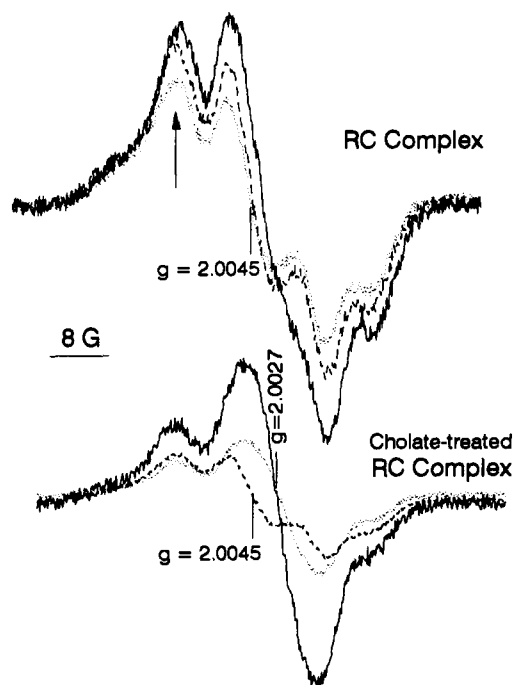


Fig. 4. Room temperature EPR spectra of the PS II RC complex before and after treatment with cholate. The dotted spectra were recorded following several hours of dark incubation of the sample on ice and solid spectra were acquired under continuous illumination of the sample in the EPR cavity. The dashed spectra were recorded following a 15-min incubation in the dark after illumination was terminated. Chlorophyll concentration was $2.1 mg/ml$ for both samples and $1 mM Fe(CN)_6^{3-}$ was present. Spectrometer conditions as follows: Microwave frequency, $9.8 GHz$; power, $10 mW$; modulation amplitude, $8 G_{pp}$.

Discussion

Previous investigations of the effect of cholate on PS II activity have emphasized the ability of the detergent to extract polypeptides that influence electron transfer reactions on the oxidizing side of PS II [28–31]. As we show here, cholate treatment also alters electron transfer through the iron-quinone complex of PS II; minimal effects on steady-state electron transfer events at the site of O₂ evolution are observed when assays are conducted in the presence of a saturating amount of CaCl₂. Cholate treatment under our conditions induces a selective extraction of a 22 kDa protein, but produces little change in chromophore and manganese abundance (Table I). It should be noted that the amount of cholate used in the experiments with the RC complex presented here was 75-fold greater on a RC basis than was used in a previous study with thylakoid membranes [30]. Nevertheless, the O₂-evolving complex remains intact as evidenced by retention of the 33 kDa protein, high rates of activity, and EPR-silent manganese. Based on two cyt *b*₅₅₉ per O₂-evolving center, our quantification of other species reveal the presence of about four EPR-silent manganese, 65–75 Chl, and two plastoquinones in cholate-extracted material. These data would suggest that the decrease in O₂-evolution activity most likely arises from alterations in the rate constants for acceptor reduction following detergent exposure.

Our data show that the interaction between DCBQ and Fe(CN)₆³⁻ in the native RC complex is consistent with a model in which the quinone is the preferred oxidant of the Q_B site; in the presence of both acceptors the preferred path of electron transfer is from the Q_B site to DCBQ. Ferricyanide acts primarily as an electron sink that maintains a nearly constant supply of the oxidized lipophilic acceptor, and only secondarily as a direct oxidant of the Q_B site. These effects of Fe(CN)₆³⁻ are apparent in the experiments of Fig. 2, where addition of small amounts of DCBQ affect the apparent *K*_m of Fe(CN)₆³⁻, and in our observations that in all experiments with DCBQ, addition of Fe(CN)₆³⁻ increased the rate of electron transfer supported by the quinone acceptor.

Steady-state kinetic analyses of DCBQ and Fe(CN)₆³⁻ reduction in the untreated RC complex, with and without the inhibitor DCMU, show that the dominant pathway for reduction of both acceptors includes the Q_B site, although another site of much lower activity is also present. Similar kinetic analyses following cholate treatment show that the interactions of Fe(CN)₆³⁻ and DCBQ with the reducing side of PS II have been altered which suggests that an electron transfer pathway has been created on the reducing side of the photoreaction. The newly-created site of acceptor reduction is characterized by DCMU insensitivity,

more efficient reduction of Fe(CN)₆³⁻, and by a diminished capacity for DCBQ reduction (Figs. 2 and 3). Cholate produces similar, but less dramatic changes in PS II membrane preparations (Table I). On account of the substantial loss of DCMU sensitivity and the increase in Fe(CN)₆³⁻-catalyzed activity, we are led to conclude that cholate binding and/or loss of the 22 kDa protein modify the structure and redox activity of the iron-quinone complex in PS II.

We suggest that the former is the case based on reconstitution experiments with the 22 kDa protein and cholate-treated RC complexes that were unsuccessful in restoring native Q_B activity. Although reconstitution conditions were chosen to minimize loss of O₂ evolution, the 22 kDa protein did not rebind at all, probably the result of irreversible aggregation of the protein following removal and dialysis. The hydrophathy profile of the 22 kDa protein predicted from the *psbS* gene sequence [32] suggests up to four membrane spanning helices, which could account for the difficulty in achieving successful reconstitution. In addition, this sequence shows no similarities with bacterial H-subunits [32,33]. Results presented in Ref. 18 also argue against the involvement of the 22 kDa protein in the regulation of electron transfer. Finally, experiments with PS II membranes indicate that alterations to the reducing side are also seen following cholate treatment. These changes are not accompanied by loss of the 22 kDa protein.

In summary, we would conclude that cholate treatment of PS II induces more substantial changes in electron transfer than previously suspected. In contrast to the previously reported release of extrinsic polypeptides [28,30], we show here that in both PS II membranes and in RC complex preparations, the detergent can alter acceptor reduction, herbicide sensitivity, and extract substantial amounts of the 22 kDa protein. Although treatment of purified PS II complexes with cholate alters acceptor reduction, sensitivity of electron transfer to DCMU is only attenuated, but not abolished. From the data now available, it is clear that the 22 kDa protein is a tightly-bound component of PS II, but further experimentation is required to define its function in relation to other detergent extractable species.

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