DETERGENT EFFECT ON CYTOCHROME b_{559} ELECTRON PARAMAGNETIC RESONANCE SIGNALS IN THE PHOTOSYSTEM II REACTION CENTRE.

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ABSTRACT

Detergent effect on Cytochrome b_{559} from spinach photosystem II was studied by electron paramagnetic resonance (EPR) spectroscopy in D1-D2-Cyt b_{559} complex preparations. Various *n*-dodecyl-β-D-maltoside concentrations from 0 to 0.2% (w/v) were used to stabilise the D1-D2-Cyt b_{559} complexes. Low spin heme EPR spectra were obtained but g_z feature positions changed depending on detergent conditions. Redox potentiometric titrations showed a unique redox potential cytochrome b_{559} form (E'_m = +123-150 mV) in all the D1-D2-Cyt b_{559} complex preparations indicating that detergent does not affect this property of the protein in those conditions. Similar effect on Cytochrome b_{559} EPR spectrum was observed in more intact photosystem II preparations independently of their aggregation state. This finding indicates that changes due to detergent could be a common phenomenon in photosystem II complexes. Results are discussed in terms of the environment each detergent provides to the protein.

1. INTRODUCTION

Cytochrome (Cyt) b_{559} is an integral component of photosystem (PS) II reaction centre (RC). Its presence is critical for the biogenesis and stable assembly of the PSII RC, but it is not involved in the primary electron transport in PSII. D1-D2-Cyt b_{559} complexes with the minimum polypeptide composition that are able to perform efficient light-induced primary charge separation still bound Cyt b_{559}.[1] Harsh treatments are required to separate Cyt b_{559} from this complex [2,3] and the tight relationship with the D1/D2 heterodimer that binds the essential cofactors for PSII primary photochemistry suggests an essential role of Cyt b_{559}. However, despite many studies performed during the last decades[4-6] the exact function(s) of this hemoprotein is still unclear. Cytochrome b_{559} consists of two small polypeptides, the α (9 kDa) and β (4.5 kDa) subunits with a transmembrane α-helical domain and heterodimeric structure.[6,7] Two histidine residues within the hydrophobic domain of each polypeptide act as ligands of heme iron.

One of the most intriguing properties of Cyt b_{559} is the remarkable variability of the midpoint redox potential (E'_m) of the heme group. In its natural membrane environment it exhibits a labile high-potential (HP, $E'_m \approx +380$ mV) and a stable low-potential (LP, $E'_m \approx +20 - +200$ mV) form. An intermediate potential (IP) was also reported in chloroplasts, thylakoids and intact PSII membranes.[6,8-12]

Heme iron of Cyt b_{559} in PSII RC is known to display the typical low-spin electron paramagnetic resonance (EPR) signals $g_x \approx 1.5$, $g_y \approx 2.3$ and $g_z \approx 3.0$. However, slightly different g_z values for Cyt b_{559} have been observed depending on the preparation and extent of purification of samples.[6] These changes in EPR spectra were connected in the past with changes between different redox forms of Cyt b_{559}. Indeed, it has been suggested that HP and LP forms of Cyt b_{559} can be distinguished by their EPR spectra. The HP form of Cyt b_{559} was associated to a g_z around 3.01-3.08 based on EPR measurements in chloroplasts and PSII membranes with high content of this form. On the other hand, LP Cyt b_{559} form was related to a g_z in the 2.93-3.04 range.

The g_z value of 2.93 was measured in the isolated Cyt b_{559}.[6,13-17] Nevertheless, a straightforward relationship between redox potential and EPR signal is not clear. Other factors that were suggested to influence the EPR g_z position were the purification degree of the sample and the hydrophobicity of heme environment.[6,14]

In order to elucidate factors controlling EPR signal, we study here in detail EPR spectra of oxidised LP Cyt b_{559} in the D1-D2-Cyt b_{559} complex and in more intact PSII preparations. Our results clearly demonstrate that detergents modify the EPR spectrum of Cyt b_{559} and suggest that EPR signal variations and midpoint redox potential could not be directly related. The way detergents affect EPR parameters is discussed in terms of a more general concept of hydrophobicity referred in.[14]

2. MATERIALS AND METHODS

Preparation of PSII membranes.- Highly enriched-PSII membranes were isolated from market spinach according to Berthold et al.[18] Samples were suspended in 0.4 M sucrose, 15 mM NaCl, 5 mM $MgCl_2$ and 50 mM 2-(N-morpholino) ethanesulfonic acid (Mes)-NaOH, pH 6.0, frozen in liquid nitrogen and stored at –80ºC until use. PSII membranes exhibited oxygen evolution rates of 520 ± 30 μmol of O_2 mg Chl^{-1} h^{-1} using DCBQ as artificial electron acceptor.

Preparation of D1-D2-Cyt b_{559} complexes.- A standard D1-D2-Cyt b_{559} complex preparation containing six chlorophyll (Chl) molecules per RC was isolated from highly purified oxygen-evolving PSII membranes from market spinach[18] according to the procedure of Nanba and Satoh[1] and modified by Montoya et al.[19] This method makes use of a Toyopearl TSK-DEAE column. Samples loaded in the column were washed with 0.05% (w/v) Triton X-100 until absorbance at 417 nm was higher than that at 435 nm. Detergent was subsequently exchanged by *n*-dodecyl-β-D-maltoside (β-DM) at different concentrations ranging from 0 to 0.2% (w/v) or 0.15% (w/v) sucrose monocaprate. Detergent replacement was done until Triton X-100 absorbance at 280 nm was lower than 0.01. Then, D1-D2-Cyt b_{559} complexes were eluted with a linear salt gradient in the same buffer and fractions were collected at 1 ml/min.

This method was also used with three different modifications described in the literature.[20-22] The variations, concerning basically the column and washing buffer conditions are the following: *i*) Toyopearl TSK-DEAE column, 1% (w/v) Triton X-100; *ii*) Toyopearl TSK-DEAE column, 1% (w/v) Triton X-100 and 1.5% (w/v) taurine ; *iii)* Q-Sepharose Fast-Flow (Pharmacia) column and 0.15% (w/v) Triton X-100. The β-DM concentration in the elution buffer was 0.1% (w/v) for these latter samples and detergent replacement was done as explained above. All isolated D1-D2-Cyt b_{559} complex preparations contained six Chl per RC.

D1-D2-Cyt b_{559} complexes were isolated at different pH in the 5.5-7.7 range by changing the pH of the washing buffer. For pH 5.5-6.5 and pH 7.0-7.7 Mes-NaOH and tris(hydroxymethyl)aminomethane (Tris)-HCl buffers, respectively, were used. Desalted D1-D2-Cyt b_{559} complex preparations were prepared by a 2 h dialysis against the salt free elution buffer free of salt using a 30,000 kDa cut-off dialysis tube (Spectrapor).

All isolation procedures were done in darkness in a cooled chamber at 4 ºC. Samples were then frozen in liquid N_2 and stored at –80 ºC. Pigment composition of isolated D1-D2-Cyt b_{559} complex preparations was determined as described in Eijckelhoff and Dekker.[23] Cytochrome b_{559} content was calculated from the dithionite-reduced *minus* ferricyanide-oxidised absorption difference spectra using an extinction coefficient of 21.0 $mM^{-1}cm^{-1}$ at 559 nm.[1]

Preparation of PSII core complexes.- PSII core complex samples were prepared following the method described in[24] with some modifications. An ion exchange Toyopearl TSK-DEAE column was used. The column was washed at 2 ml/min for 2 h and subsequently the core complexes were eluted with a linear salt gradient in 50 mM Mes-NaOH, pH 6.5 with 0.03% (w/v) Triton X-100. Additionally, PSII core complexes were eluted from the column after detergent exchange with 0.1% (w/v) β-DM in the same buffer. Detergent exchange was done until Triton X-100 absorbance at 280 nm was lower than 0.01. Pigmented fractions were concentrated in Centripep (Amicon) tubes.

Isolation of monomeric and dimeric PSII RC and core complexes.- . Monomeric and dimeric complexes were isolated by sucrose density gradient centrifugation. To do that, PSII RC were suspended in 50 mM Mes-NaOH, pH 6.5 or 50 mM Tris-HCl, pH 7.2 and 0.1% β-DM (w/v).[25] PSII cores were suspended in 25 mM Mes-NaOH, pH 6.5, 10 mM NaCl, 5 mM $CaCl_2$ and 10 mM $NaHCO_3$ and incubated with β-DM to a final concentration of 1.25% (w/v).[26] The solubilised PSII RC and PSII core samples were homogenised, loaded onto a freshly prepared 0.1-1.0 M sucrose gradient and centrifuged at 90,000xg in a Beckman SW41 swing-out rotor overnight and 75,000xg in a Beckman SW28 swing-out rotor for 22 h, respectively. The sucrose gradient buffer composition was 10 mM NaCl, 50 mM Mes-NaOH, pH 6.5 or 50 mM Tris-HCl, pH 7.2 and 0.1% (w/v) β-DM for PSII RC samples and 10 mM NaCl, 5 mM $CaCl_2$, 25 mM Mes-NaOH, pH 6.5, and 0.03% (w/v) β-DM for PSII cores. The chlorophyll-rich fractions were then removed from the sucrose gradients, frozen in liquid nitrogen and stored at -80 ºC.

Potentiometric redox titrations.- Potentiometric redox titrations were carried out under argon at 12 ºC using D1-D2-Cyt b_{559} complex samples (5 μM Chl) in 50 mM Mes-NaOH, pH 6.5, by following the absorbance changes at 559 *minus* 570 nm induced by sequential addition of aliquots of 0.1 M sodium dithionite. The measurements were performed in an Aminco DW-2000 UV-Vis spectrophotometer using the dual wavelength mode. Samples were previously oxidised with 25 μM potassium ferricyanide. The redox potential in the reaction cell were simultaneously measured with a potentiometer (Methrom Herisau, Switzerland) provided with a combined Pt-Ag/AgCl microelectrode (Crison Instruments, Spain) previously calibrated against a saturated solution of quinhydrone (E'_m, pH 7, +280 mV at 20 ºC). In addition to ferricyanide (E'_m, pH 7, +430 mV) the following redox mediators were used: 10 μM 1,4-benzoquinone (E'_m, pH 7, +280 mV), 20 μM 2,3,5,6-tetramethyl-*p*-phenylendiamine (E'_m, pH 7, +240 mV), 20 μM 1,2-naphthoquinone (E'_m, pH 7, +145 mV), 2.5 μM N-methyl-phenazonium methosulfate (E'_m, pH 7, +80 mV), 10 μM N-methyl-phenazonium ethosulfate (E'_m, pH 7, +55 mV) and 20 μM tetramethyl-*p*-benzoquinone (E'_m, pH 7, +5 mV).

EPR measurements.- Samples were concentrated (0.5 – 1.2 mM Chl) in Centripep-30 and Centricon-30 (Amicon) tubes for EPR measurements. Continuous wave EPR spectra were recorded with a Bruker ESP380E spectrometer working at the X-band (frequency about 9.6 GHz). Typical measurements were achieved at 8 K with 1.46 μW microwave power (which ensures no saturation effects on the signal) and 1 mT of modulation amplitude.

3. RESULTS

Effect of n-dodecyl-β-D-maltoside on Cyt b_{559} EPR signal.

The influence of β-DM on Cyt b_{559} EPR spectrum was observed in a standard D1-D2-Cyt b_{559} complex preparation containing six Chl per RC. D1-D2-Cyt b_{559} complexes were isolated in the presence of various β-DM concentrations ranging from 0 to 0.2% (w/v) (for details see Materials and Methods). No pigment and polypeptide composition varied among samples after detergent treatments. All samples displayed a typical low spin heme EPR signal, with principal values of the g tensor being around $g_z \approx 3.0$, $g_y \approx 2.3$ and $g_x \approx 1.5$.[6] Although all spectra were similar, some differences were detected in their g_z features, with no changes at g_x and g_y positions. Following Taylor's model[27] it is expected that g_y value remains nearly unaffected when a small shift in g_z value occurs. Besides, considering that the g_x feature is very broad, it is not possible to detect small changes in it as those observed for g_z. Intermediate field feature in the EPR spectra stay at $g_y = 2.26$ for all samples and, when the high field feature is detected, a g_x value of 1.53 is obtained. Figure 1 shows the detergent effect on the low field feature in EPR spectra of Cyt $b_{559.}$ The D1-D2-Cyt b_{559} complex sample suspended in the presence of 0.1% (w/v) β-DM displays a g_z feature with a maximum at 2.98 and a full width at half maximum (FWHM) of 13 mT (Fig. 1A,a). On the other hand, D1-D2-Cyt b_{559} complex samples in the presence of lower β-DM concentration (0.03% (w/v) β-DM) display a g_z feature with a maximum at 2.93 that seems to be asymmetric showing a smoother decrease towards lower fields and FWHM of 15 mT (Fig. 1A,b). An EPR signal with similar g_z maximum value has been reported in the literature for D1-D2-Cyt b_{559} complexes isolated following the same procedure.[28]

The dependence of g_z position on β-DM concentration is shown in Fig. 1B. The concentration at which g_z peak shifts (0.03-0.06% (w/v)) is above the critical micelle concentration (c.m.c.) of β-DM.[29] At c.m.c. detergent monomer molecules selfassociated form structures called micelles and the complete and stable solubilization of membrane proteins generally occurs.

In order to find out whether the observed effect is just due to detergent micelle formation, we measured EPR spectrum of Cyt b_{559} in two D1-D2-Cyt b_{559} complex preparations. One eluted in the presence of 0.05% (w/v) Triton X-100 (Fig. 1A,c) and the other in the presence of 0.15% (w/v) sucrose monocaprate (Fig. 1A,d). Both detergent concentrations are higher than their c.m.c. (0.02% and 0.12% (w/v), respectively[29]), and the corresponding spectra display the asymmetric g_z signal at 2.93 similar than that observed in the presence of a β-DM concentration equal or lower than its c.m.c (0.03% (w/v)). The results indicate that EPR g_z-signal variation is dependent on the nature of the detergent. Since influence of sucrose monocaprate on stabilisation of D1-D2-Cyt b_{559} was reported to be close to that of Triton X-100 and much lower than that of β-DM[29], EPR g_z feature position could be related to sample stability provided by detergents.

Figure 2 illustrates that the β-DM influence on EPR spectrum of Cyt b_{559} is a reversible phenomenon. Cytochrome b_{559} EPR spectrum in D1-D2-Cyt b_{559} complexes, eluted from the chromatographic column in the presence of 0.03% (w/v) Triton X-100, presents an asymmetric g_z signal at 2.93 (Fig. 2,a). The EPR signal is similar to those of Fig. 1A,b-d. The g_z position shifts to lower magnetic field (g_z = 2.98) when the same D1-D2-Cyt b_{559} complex sample is reloaded on a new chromatographic column and washed with 0.1% (w/v) β-DM in order to replace Triton X-100 (Fig. 2,b). The shape of this EPR spectrum is coincident with that of a new D1-D2-Cyt b_{559} preparation directly eluted from the column in the presence of 0.1% (w/v) β-DM after detergent exchange (Fig. 2,c). Cytochrome b_{559} EPR spectrum shows again g_z at 2.93 after removing β-DM of the sample buffer in the latter sample (Fig. 2,d). These findings clearly show that EPR spectrum changes are reversible. It is worth noting that detergent replacement is not detected by EPR unless it occurs through a chromatographic column. This fact suggests detergent-protein interactions are strong and therefore detergent replacement without column is very slow.

Cytochrome b_{559} was also characterised by EPR spectroscopy in various standard D1-D2-Cyt b_{559} complex preparations isolated following other procedures reported in the literature (for details see Materials and Methods). The same EPR spectrum and detergent behaviour were observed (data not shown). The influence of pH on the D1-D2-Cyt b_{559} preparations was also investigated but no effects on EPR signals were observed in the 5.5 to 7.7 range (data not shown). Additionally, no spectral changes were detected upon changing salt concentration conditions in the sample buffer (data not shown). For that, samples collected after elution with a linear salt gradient and subsequently dialysed against buffer free of salt were compared.

The results obtained so far indicate that EPR spectrum of Cyt b_{559} in D1-D2-Cyt b_{559} complexes is affected by the kind of detergent and its concentration in the sample buffer.

Cytochrome b_{559} redox potentials in D1-D2-Cyt b_{559} preparations.

Since g_z-values in Cyt b_{559} EPR spectra have previously been associated to redox potentials of the cytochrome[6], it is interesting to check whether the observed g_z variations are related to any redox potential changes. To do that we carried out redox titration measurements of Cyt b_{559} in our D1-D2-Cyt b_{559} preparations obtained with different detergent conditions. Anaerobic reduction titrations at 559 *minus* 570 nm are shown in Fig. 3. The same data points were found in the oxidative titration. HP form was not found in any of our D1-D2-Cyt b_{559} complex samples. All titration curves are described by the Nernst equation for a single electron step (n=1) with a midpoint redox potential (E'_m) between 123±15 and 150±15 mV at pH 6.5. Similar E'_m value was earlier observed in the D1-D2-Cyt b_{559} complex at pH 7.2[28], being assigned to a LP form, although this value is between those reported in the literature for IP and LP forms.[6] The results indicate that detergent have no effect on Cyt b_{559} midpoint redox potential although they influence EPR Cyt b_{559} spectral features.

Cytochrome b_{559} EPR spectra in more intact PSII preparations.

To gain further insight on whether the detergent effect on Cyt b_{559} occur in more intact PSII preparations we measured EPR spectra in PS II core complex samples. The HP Cyt b_{559} form was not detected in any of PSII core complex samples studied here, the same has been reported in similar PSII preparation.[10] Figure 4 shows the low field feature in EPR spectra of PSII core complex samples in the absence and the presence of β-DM. Cytochrome b_{559} spectrum of the PSII core complexes obtained with 0.03% (w/v) Triton X-100 shows an asymmetric g_z signal at 2.93. On the other hand, a g_z signal at 2.98 was measured when this detergent was exchanged by 0.1% (w/v) β-DM through a chromatographic column. Similar detergent effect was observed in other PSII core complex preparations obtained following other methods described in the literature[26,30] (data not shown). Accordingly, the data suggest that the same detergent effects observed in isolated D1-D2-Cyt b_{559} complexes also occur for more intact PSII preparations. Although Cyt b_{559} is known to be altered by loss of Mn and extrinsic polypeptide subunits bound to PSII complexes, our data indicate that EPR spectrum is just influenced by the concentration of β-DM in the sample buffer at this stage of purification.

The methods used to prepare PSII core complexes as well as D1-D2-Cyt b_{559} complexes yield preparations with different content in dimeric and monomeric forms.[25,26] We examined the contribution of dimeric and monomeric PSII fractions to the EPR spectra. To do that, we separated both fractions in each preparation through a sucrose gradient (for details see Materials and Methods). No differences between both components in each PSII complexes were observed indicating that such variations in the aggregation state do not affect EPR signal (data not shown).

4. DISCUSSION

The study presented here provides new and detailed information on EPR spectra of Cyt b_{559} in the PSII RC. Our data clearly indicate that Cyt b_{559} can attain different g_z values between 2.93 and 2.98 depending on the presence of β-DM detergent in the sample buffer and irrespective of PSII integrity degree and aggregation state. The g_z position shifts to lower magnetic field when the β-DM concentration exceeds its c.m.c. whereas it remains at 2.93 below that concentration. The same 2.93 value is found in the presence of Triton X-100 or sucrose monocaprate.

The use of detergents is essential in the purification of membrane proteins. It is well known that detergents are highly effective for the initial dispersion of lipids and membrane fragmentation. However, they are not necessarily optimal for maintaining the isolated membrane fragments or proteins in their native state in solution.[31,32] Stability of membrane proteins, in particular that of D1-D2-Cyt b_{559} complexes strongly depend on nature of detergent(s) used for solubilisation and storage, and their c.m.c..[29] The positive effect of β-DM on the stabilisation of the isolated D1-D2-Cyt b_{559} complex has widely been recognised.[29,33,34] However, to our knowledge no detailed studies on the influence of detergents on Cyt b_{559} have been done yet.

In general, detergents can act as artificial lipids and envelop the hydrophobic domain of integral membrane proteins instead of the lipid bilayer.[31,32] The behaviour of a specific detergent depends on its structure and the stoichiometry of the head group and tail. β-DM due to its long hydrophobic tail bound to the carbohydrate group could interact with the heme group surroundings increasing the hydrophobicity of the heme protein domain. This fact would be expected since Cyt b_{559} is located on the outermost region of the PSII RC complex and is exposed to the medium.[7] This is in agreement with previous results that found small shifts in g_z values of Cyt b_{559} of intact PSII membranes that were attributed to a more hydrophilic heme environment.[6,14]

The g_z feature of purified Cyt b_{559} was measured at 2.93[13-17], the same value is found in purified PSII RC complexes in the presence of Triton X-100 whereas EPR g_z

values of intact systems appear at lower magnetic fields. Accordingly, our results suggest that the g_z value shift to lower magnetic fields should be interpreted as a specific effect due to β-DM and its properties in terms of being able to stabilise and maintain the PSII RC integrity and photochemical activity.[29,33,34] In contrast, detergents that destabilise the PSII RC complexes as Triton X-100 should be expected to give EPR g_z signals at higher magnetic fields.

From the measured g values, the following crystal field parameters, Δ/λ and V/λ, can be estimated using the Taylor's model[27], where Δ and V are the axial and rhombic crystal field parameters, respectively, and λ is the spin-orbit coupling parameter. We obtained $\Delta/\lambda = 3.2 \pm 0.3$ and $V/\lambda = 1.87 \pm 0.07$ for PSII samples with β-DM concentration lower than its c.m.c. and $\Delta/\lambda = 3.5 \pm 0.2$ and $V/\lambda = 1.85 \pm 0.04$ for the opposite case. The V/λ value was the same in both cases meanwhile a small change was detected in the axial parameter value. Nevertheless, this change is within the error margin and no direct conclusions about the modifications in the heme group can be obtained. These parameters are typical of bis-imidazol co-ordinated heme complexes having parallel imidazol rings.[35] Protein-detergent interaction is a complex phenomenon and, therefore, the perturbations that β-DM causes at the heme site affecting its EPR spectrum are difficult to establish at a molecular level. Subtle changes in g values of several model heme compounds have been found associated to modifications in heme substituents, solvents, hydrogen bounds or other factors affecting the electronic density[35-37] Interaction between detergent and protein surface near heme group may cause similar effects through a change in the polarity of the heme environment. A conformational change of the whole protein is the explanation given in[38-40] for the differences (much greater than in our case) found in EPR spectra of cytochrome samples with and without detergent. Nevertheless, changes observed in our spectra are small and may rather suggest a change in the electronic density of iron ligands driven by detergent-protein interactions near the heme centre. However, the possibility of a subtle conformational change cannot be excluded. It is worth of noting that these changes cause measurable modifications in the EPR spectra but they are not able to alter midpoint redox potentials.

Other interesting feature observed in samples suspended in buffer containing Triton X-100, sucrose monocaprate or low β-DM concentration is the asymmetric g_z EPR signal instead of the symmetric signal measured in samples with high concentration of β-DM. The asymmetry can be attributed to several inhomogeneous broadening effects, for instance g-strain or a distribution of Cyt b_{559} centres with different g_z values. This signal can be simulated taking into account the superposition of two components with $g_z = 3.02$ and $g_z = 2.92$, being the latter slightly narrower than the former (not shown). This latter possibility could mean that interaction with non-stabiliser detergents as Triton X-100 or the absence of detergent generates different populations of Cyt b_{559}. These two EPR signals could not be distinguished by their behaviour upon saturation since the g_z feature shape does not change when the microwave power is increased (data not shown). Since D1-D2-Cyt b_{559} complex preparations display only a redox potential form, this potential heterogeneity cannot be evaluated by midpoint redox potential measurements.

In summary, data presented in this work indicate that detergents affect EPR g_z signal of Cyt b_{559} but they do not modify its midpoint redox potential. Besides, g_z position of Cyt b_{559} can also vary due to β-DM concentration irrespective of the integrity of PSII preparations and their aggregation state. Variations detected at g_z seem to be associated with hydrophobic ambient around heme group which is provided by detergents and suggest that β-DM reconstitutes a hydrophobic environment around Cyt b_{559} closer to that of native PSII membranes.

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ABBREVIATIONS

Chl, chlorophyll; c.m.c, critical micelle concentration; Cyt, cytochrome; D1 and D2, core polypeptides of the photosystem II reaction centre; DCBQ, 2,6-dichlorobenzoquinone; E'_m, midpoint potential; EPR, electron paramagnetic resonance; FWHM, full width at half maximum; HP, high potential; IP, intermediate potential; LP, low potential; Mes, 2-(*N*-morpholino)ethanesulfonic acid; PS, photosystem; RC, reaction centre; Tris, tris(hydroxymethyl)aminomethane.

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FIGURE LEGENDS

Figure 1. (A) Low field region of EPR spectra of oxidised Cyt b_{559} heme in D1-D2-Cyt b_{559} complex samples eluted from the column in the presence of a) 0.1% (w/v) of β-DM; b) 0.03% (w/v) of β-DM; c) 0.05% (w/v) of Triton X-100; d) 0.15% (w/v) sucrose monocaprate. (B) Dependence of the Cyt b_{559} EPR g_z value on β-DM concentration. EPR conditions: temperature, 8K; microwave power, 1.46 μW. Other experimental conditions are described in Materials and Methods.

Figure 2. Low field region of EPR spectra of oxidised Cyt b_{559} heme in D1-D2-Cyt b_{559} complexes: a) sample eluted from the column in the presence of 0.03% (w/v) Triton X-100; b) the same RC sample in (a) subsequently treated with 0.1% (w/v) β-DM through a DEAE-Toyopearl TSK-650S column to replace Triton X-100; c) sample eluted from the column in the presence of 0.1% (w/v) β-DM after detergent exchange; d) the same RC sample in (c) after removing β-DM detergent. EPR conditions: temperature, 8K; microwave power, 1.46 μW. Other experimental conditions are described in Materials and Methods.

Figure 3. Potentiometric redox titrations of Cyt b_{559} heme in D1-D2-Cyt b_{559} complex preparations at pH 6.5 in the presence of 0.03% (w/v) β-DM concentration (μ); 0.1% (w/v) β-DM (□); 0.15% (w/v) sucrose monocaprate (△). Experimental conditions are described in Materials and Methods.

Figure 4. EPR spectra at the g_z region of the oxidised Cyt b_{559} heme in PSII core complex sample in the presence of a) 0.03% (w/v) Triton X-100; b) 0.1% (w/v) β-DM EPR conditions: temperature, 8K; microwave power, 1.46 μW. Other experimental conditions are described in Materials and Methods.

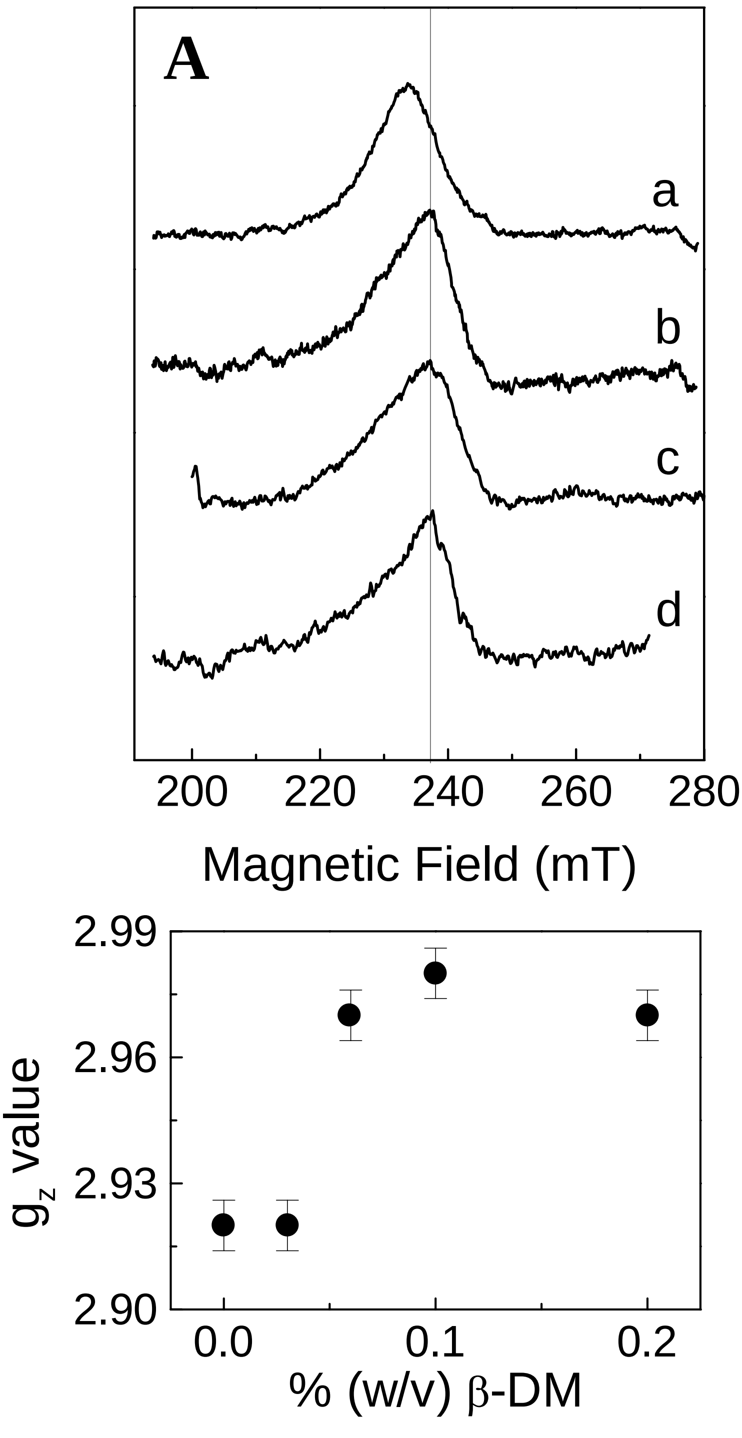

A
a
b
c
d
200
220
240
260
280
Magnetic Field (mT)
2.99
2.96
2.93
2.90
gz value
0.0
0.1
0.2
% (w/v) β-DM

Fig. 1

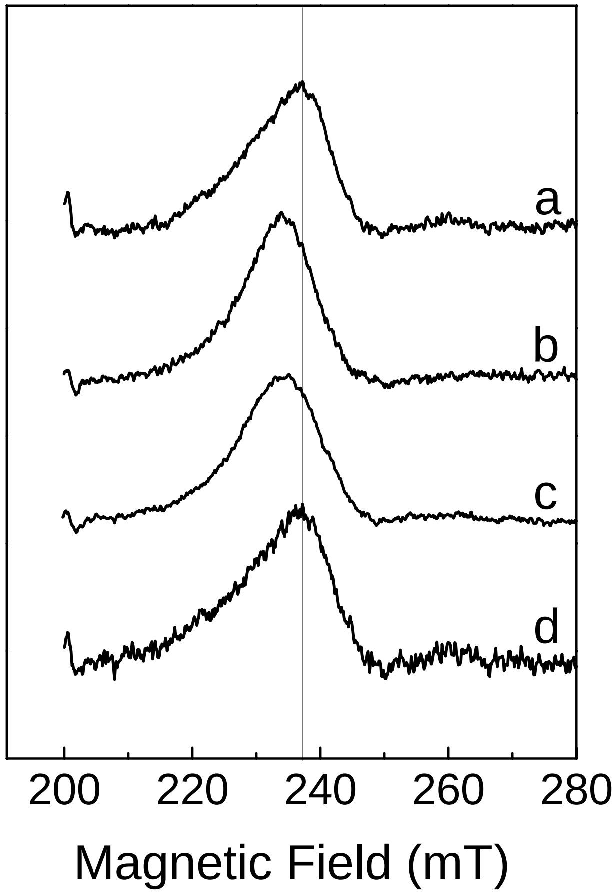
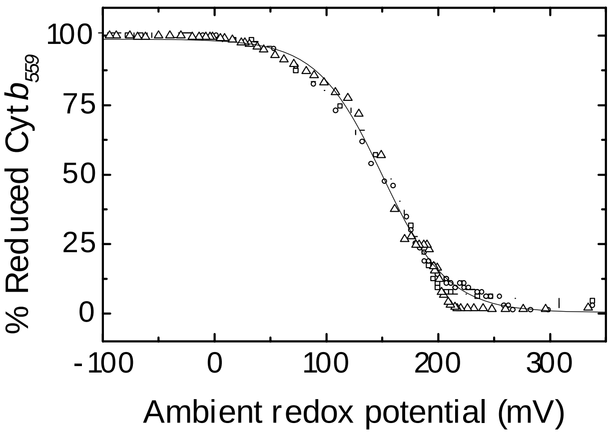


Fig. 2



100
75
50
25
0
-100
0
100
200
300
% Reduced Cyt b559
Ambient redox potential (mV)

Fig. 3

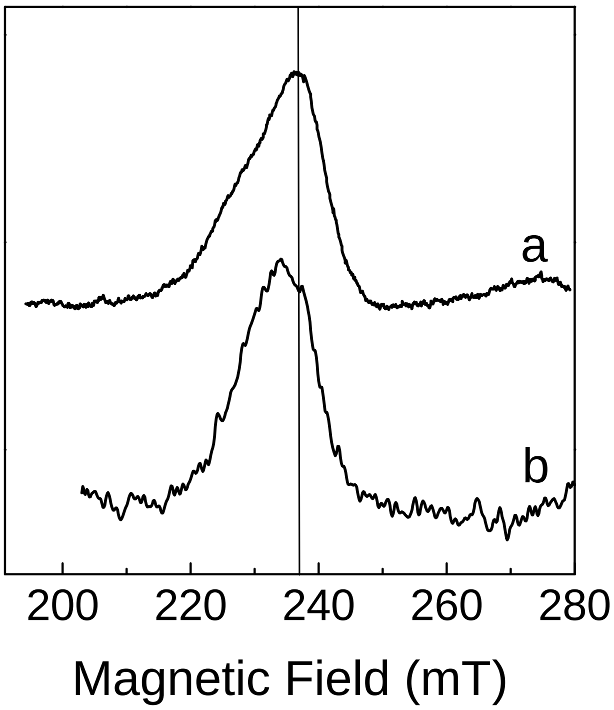


Fig. 4