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NMR paramagnetic relaxation enhancements due to manganese in the S_0 and S_2 states of Photosystem II-enriched membrane fragments and in the detergent-solubilized Photosystem II complex

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Abstract

The NMR paramagnetic relaxation enhancement (NMR-PRE) produced in the solvent proton resonance by manganese in the S_0 and S_2 states of the oxygen evolving center (OEC) has been recorded for three Photosystem II (PS II)-enriched preparations: (1) PS II-enriched thylakoid membrane fragments (TMF-2 particles); (2) salt-washed (2M NaCl) TMF-2 particles; and (3) the octylglucopyranoside (OGP)-solubilized PS II complex. The second and third preparations, but not the first, are depleted of the peripheral 17 and 23 kD polypeptides associated with the OEC. It has been proposed that depletion of these polypeptides increases the exposure of OEC manganese to the aqueous phase. The NMR-PRE response measures the quantity $(T_{1m}+\tau_m)^{-1}$, where T_{1m} is the spin relaxation time and τ_m is the mean residence time with respect to chemical exchange reactions of solvent protons in the manganese coordination sphere, and, thus, the NMR-PRE provides a direct measure of the solvent proton chemical exchange rate constant τ_m^{-1}. This study tested whether the 17 and 23 kD polypeptides shield the OEC from the solvent phase and whether their depletion enhances the S_2 and S_0 NMR-PRE signals by removing a kinetic barrier to the solvent proton chemical exchange reaction. The amplitude of the S_2 NMR-PRE signal, measured in its chemical exchange-limited regime ($\tau_m > T_{1m}$), is slightly decreased, rather than increased, in preparations (2) and (3) relative to (1), indicating that removal of the 17 and 23 kD polypeptides slightly slows, rather than accelerates, the rate-limiting steps of the solvent proton chemical exchange reactions. In addition, the lifetime of the S_2 state was shortened several-fold in the solubilized PS II complex and in salt-washed TMF-2 membranes relative to untreated TMF-2 control samples. The S_0 NMR-PRE signal, which is present in TMF-2 suspensions, was not detected in suspensions of the solubilized PS II complex, even though these samples contained high concentrations of active manganese centers (approximately double those of the TMF-2 control) and exhibited an S_2 NMR-PRE signal of comparable amplitude to that of the TMF-2 preparation. These results suggest that the 17 and 23 kD extrinsic polypeptides do not shield the NMR-visible water binding site in the OEC from the aqueous phase, although their removal substantially alters the proton relaxation efficiency by shortening T_{1m}.

Abbreviations: ADRY – acceleration of the deactivation reactions of the water splitting enzyme Y; BBY – Photosystem II-enriched membrane fragments prepared by the method of Berthold et al. (1981); CCCP – carbonyl cyanide *m*-chlorophenyl hydrazone; Chl – chlorophyll; DCBQ – 2,5-dichlorobenzoquinone; MES – morpholinoethanesulfonate; NMR – nuclear magnetic resonance; OEC – oxygen evolving complex; OGP – octylglucopyranoside; PRE – paramagnetic relaxation enhancement; PS II – Photosystem II;

SDS-PAGE – sodium dodecyl sulfate polyacrylamide gel electrophoresis; TMF-2 – Photosystem II-enriched thylakoid membrane fragments prepared by the method of Radmer et al. (1986); T_1, T_2 – longitudinal and transverse nuclear spin relaxation times

Introduction

Oxygen evolving complex (OEC) manganese, following its release from binding sites in the water oxidizing center of Photosystem II by treatment with NH_2OH or with 0.8 M Tris buffer, produces large enhancements in the NMR T_1 and T_2 relaxation rates of solvent protons. This experimental approach to the study of OEC manganese was first utilized by Wydrzynski et al. (1975, 1978) and subsequently by Robinson et al. (1980, 1981) and Sharp and Yocum (1980). In later studies Srinivasan and Sharp (1986a,b) found that redox-active manganese ions associated functionally with the S-state cycle give rise to very small NMR relaxation enhancements. These enhancements cycle with period 4 in flash experiments, are suppressed by extractants of OEC manganese, and exhibit kinetic behavior which correlates with the known decay properties of the S_2 and S_3 states. The phenomenon in which paramagnetic metal ions enhance the spin relaxation rates of solvent nuclei is termed the NMR-paramagnetic relaxation enhancement or NMR-PRE. The temperature and magnetic field dependence of NMR-PRE signals due to manganese centers in the S_0, S_2 and S_3 states has been measured and analyzed in terms of the probable oxidation states of the manganese ion(s) which bind water, as well as of other chemical and magnetic properties of the OEC (Sharp 1992, Bovet 1993). Debus (1992) has reviewed comprehensively the known spectroscopic properties of the metal centers in the OEC.

This communication reports the results of studies comparing the properties of the S_0 and S_2 NMR-PRE signals of three Photosystem II preparations, specifically, of (1) TMF-2 (Radmer et al. 1986) suspensions, which consist of Photosystem II-enriched membrane fragments, (2) of the octylglucoside (OGP)-solubilized PS II complex, prepared following Ghanotakis and Yocum (1986), and (3) extrinsic polypeptide-depleted PS II membranes prepared by exposure of TMF-2 membrane fragments to 2 M NaCl. TMF-2 preparations retain the 17, 23 and 33 kD extrinsic polypeptides that have been associated with photosynthetic water oxidation. The OGP-solubilized PS II complex lacks the 17 and 23 kD polypeptides but retains the 33 kD polypeptide, and in the presence of 10 mM Ca^{2+} the complex exhibits high rates of O_2 evolution activity. NaCl-washed membranes are likewise depleted of the 17 and 23 kD polypeptides (but not the 33 kD), and in the presence of 10 mM $CaCl_2$ they exhibit levels of O_2 evolution activity which are about 50% of the TMF-2 control.

It has been proposed that removal of the 17 and 23 kD polypeptides increases the exposure of the manganese center to the aqueous phase (Debus 1992). The magnitude of the NMR-PRE signal depends critically on the kinetics of the chemical exchange reactions which transfer solvent protons between the aqueous phase and coordination sites on OEC manganese, and it seemed that treatments which deplete these extrinsic polypeptides might, by removing a kinetic barrier to these chemical exchange reactions, enhance the NMR-PRE response. In addition, detergent solubilization of the PS II complex and exposure to high salt concentrations has been reported to affect the stability of individual S-states (Van Leeuwen 1992). This study probes the nature of these changes in the S_2 and S_0 states through their influence on the amplitudes and decay kinetics of the NMR-PRE.

Materials and methods

NMR relaxation measurements were conducted at a proton frequency of 20.5 MHz, using a home-built pulsed NMR spectrometer described by Bovet (1993). $R_1(\equiv 1/T_1)$ measurements utilized the phase-shifted triplet sequence, with quadrature phase detection, an intra-triplet spacing of 300 μs, and an inter-triplet spacing of typically 67 ms.

PS II-enriched membrane fragments (TMF-2 particles) were prepared following Radmer et al. (1986) and suspended in 0.4 M sucrose, 20 mM MES, and 15 mM NaCl at pH 6.0 (SMN buffer) for NMR studies. The OGP-solubilized PS II complex was prepared by the method of Ghanotakis and Yocum (1986) as modified by Bowlby (1989). Salt-

washed TMF-2 suspensions were prepared by mixing equal volumes of TMF-2 particles (1.5 mg Chl·mL^{-1}) and 4 M NaCl, incubating this suspension on ice for 1 h in room light, then pelleting the membranes and re-suspending in SMN buffer. Polypeptide compositions of the TMF-2, salt-washed TMF-2, and PS II complex preparations were checked by SDS-PAGE gel electrophoresis. In agreement with previous reports (Radmer et al. 1986, Ghanotakis and Yocum 1986), it was found that the 17 and 23 kD, but not the 33 kD, extrinsic polypeptides were depleted in the salt-washed and OGP-treated preparations (Fig. 1). Depletion was nearly complete after salt washing, while traces of the 17 and 23 kD polypeptides remained in the octylglucoside preparation. Oxygen evolution activity in the TMF-2 control suspensions, in salt-washed TMF-2 particles, and in the OGP-solubilized PS II complex were typically 400, 200 and 1000 μmol O_2·(mg Chl)$^{-1}$·h^{-1}, respectively, using 300 μM DCBQ as the acceptor system. All samples used for NMR study contained 2 mM EDTA, which was added to chelate non-functional Mn^{2+} which equilibrates with the aqueous phase (Robinson et al. 1980). EDTA at this concentration has little if any effect on oxygen evolution activity in our samples (Robinson et al. 1980), although a small decrease (about 20 % of the control activity) of unknown origin has been reported by Wydrzynski and Renger (1986).

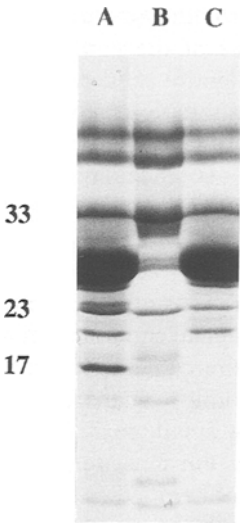

Fig. 1. SDS-PAGE gel electrophoresis of the TMF-2 PS II-enriched membrane fragments (A), the OGP-solubilized PS II complex (B), and NaCl-washed TMF-2 membrane fragments (C). The 17, 23 and 33 kD extrinsic polypeptides are indicated.

Results

The $S_1 \rightarrow S_2$ NMR-PRE transients that arise from dark-adapted TMF-2 suspensions following application of a single actinic flash are shown in Figs. 2A (18 °C) and 2B (2 °C). As Srinivasan and Sharp (1986a) observed previously in studies using BBY preparations, one flash produced a positive relaxation enhancement, the amplitude of which, for [Chl] ≅ 2 mg·mL^{-1}, was about 1% of the R_1 baseline of dark-adapted samples. This enhancement decayed back to the dark baseline over a time-scale of several tens of seconds due to the spontaneous $S_2 \rightarrow S_1$ decay. The amplitude at 2 °C is smaller than that at 18 °C due to a slowing in the kinetics of the chemical exchange reactions which transfer protons between the manganese coordination sphere and the bulk aqueous phase (Sharp 1992). The $S_2 \rightarrow S_1$ decay half-time shortened about 4-fold with increasing temperature across the 2–19 °C range (unpublished results).

The corresponding NMR-PRE signals produced by suspensions of the OGP-solubilized PS II complex are shown in Figs. 2C (18 °C) and 2D (2 °C). The $S_2 \rightarrow S_1$ decay is markedly accelerated in solubilized PS II complex relative to TMF-2 or BBY membranes. A similar acceleration of the $S_2 \rightarrow S_1$ decay was observed in suspensions of 2 M NaCl-washed TMF-2 membranes (data not shown). The amplitude of the NMR-PRE transient at 18 °C (Fig. 2C) appears to be less than that at 2 °C (Fig. 2D), contrary to the corresponding data for TMF-2 membrane fragments (Figs. 2A and B). This difference is due to the relatively rapid decay of S_2 in the OGP-solubilized PS II complex, which acts to decrease the apparent amplitude of the NMR-PRE response in these samples. The measured amplitudes of the NMR-PRE transients in the OGP-solubilized PS II complex is about 30% greater at 2 °C than that of the TMF-2 control membranes. When expressed on a basis of constant manganese, however, the NMR-PRE amplitude of the OGP-solubilized complex is about half that of the TMF-2 membranes. (In this calculation we have used Ghanotakis and Yocum's (1986) estimate that the OGP treatment leads to a 3.7-fold enrichment in OEC manganese at constant chlorophyll over PS II-enriched membranes.) Thus the NMR-PRE response of the PS II complex is significantly smaller than that of TMF-2 membranes when the data are normalized with respect to [Mn].

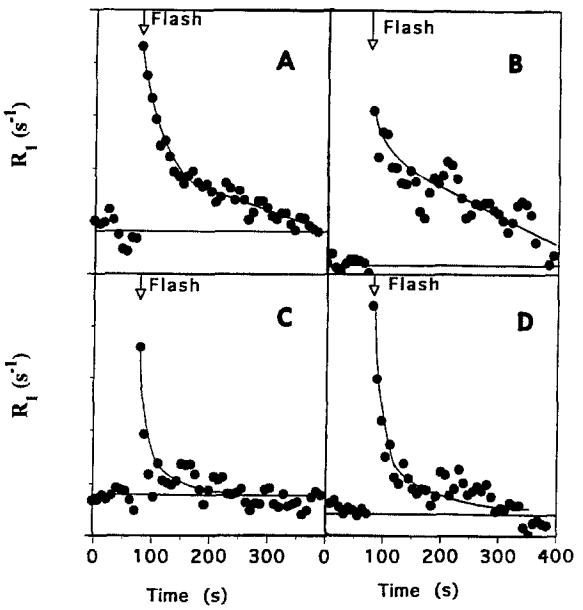

Fig. 2. The 1 flash ($S_1 \rightarrow S_2$) NMR-PRE transient produced by TMF-2 membrane fragments (A, B) and by the octylglucoside-isolated PS II complex (C, D). Data at two temperatures are shown: 18 °C (A, C) and 2 °C (B, D). A single flash was applied as indicated to suspensions which were pre-set to the S_1 state by a single flash 6 min prior to the start of measurements. Tick marks on the vertical scale denote R_1 increments of 0.002 s^{-1}. Samples of TMF-2 membranes were suspended in SMN buffer and contained 2 mM EDTA, 250 μM DCBQ, and a chlorophyll concentration of 2.3 mg·mL^{-1}. Samples of the solubilized PS II complex were suspended in SMN buffer and contained 1.3 mg·mL^{-1} chlorophyll, 2 mM EDTA, 10 mM $CaCl_2$, and 250 μM DCBQ.

Following three saturating flashes, the S-state system is predominantly in S_0. Assuming double-hit and miss probabilities of 0.05 and 0.1, respectively, we estimate that the three flash sequence of Fig. 3 results in S-state populations of about 3% S_2, 27% S_3, 64% S_0, with the remainder (6%) of centers in S_1. These assumptions gave reasonable agreement with our previous NMR-PRE flash cycle data (Srinivasan and Sharp 1986b). Figure 3 shows the NMR-PRE transient of the 3-flash sequence. In these experiments, CCCP (an ADRY reagent) was used to accelerate the decay of the S_2 and S_3 responses. The CCCP concentration was kept at least 40-fold below the concentration of OEC manganese to preclude the possibility that this reagent might interact directly, in an unknown way, with the OEC, in a manner which alters the accessibility of the water oxidizing center to the solvent and thereby influences the amplitudes of the NMR-PRE signals. The samples were incubated for 2 h on ice in total darkness and then synchronized to S_1 by a single flash, followed by a 6 min dark period. The R_1 of this sample was measured 10 times to establish the background level of the S_1 state; these measurements define the time window 0–80 s in Fig. 3. Three actinic flashes were then applied, advancing the S-state system predominantly to S_0, and the time-course of R_1 was monitored for 400 s in total darkness. S_2 and S_3 responses were present immediately after the 3-flash train, but these decayed to a new, more slowly decaying R_1 level within about 100 s. We attribute the long-lived R_1 enhancement that persists between 200 s and 450 s to a signal that originates in the S_0 population. This signal decayed slowly over a period of several minutes. Based on an estimated fractional S_0 population of 0.64, the amplitude of the S_0 NMR-PRE transient, extrapolated back to the time of the 3-flash train, is approximately 0.0013 s^{-1}·mg Chl^{-1}.

The S_0 R_1 enhancement was reversed to the dark S_1 level by the action of a single flash applied at 450 s (see Fig. 3), followed by a suitable dark period to allow decay of the S_2 and S_3 states. Finally, a second 1-flash transient was recorded at the end of the measurement cycle. This final flash at 600 s provided a measurement of the $S_1 \rightarrow S_2$ transient with the sample in an essentially pure S_1 state before the flash. The flash at 600 s also ensures that all centers are returned to S_1 at the end of the flash train, thereby providing a third measurement of the dark S_1 baseline at the end of the measurement sequence. This entire sequence was repeated 3–4 times on a given sample after which the sample was changed. The flash train shown in Fig. 3 represents an average of 16 measurement sequences, as described above, collected using five changes of sample. The S_0 NMR-PRE response shown in Fig. 3 is very small but has been observed reproducibly in different preparations of TMF-2 membranes under various experimental conditions of temperature and magnetic field strength (unpublished results). These observations confirm an earlier report from this laboratory of a small positive NMR-PRE signal associated with the S_0 state of BBY membranes (Srinivasan and Sharp 1986).

Figure 4 shows the results of corresponding 3-flash ($S_1 \rightarrow S_0$) experiments using OGP-solubilized PS II complex (no ADRY reagent was present in these experiments since the normal S_2 state decay

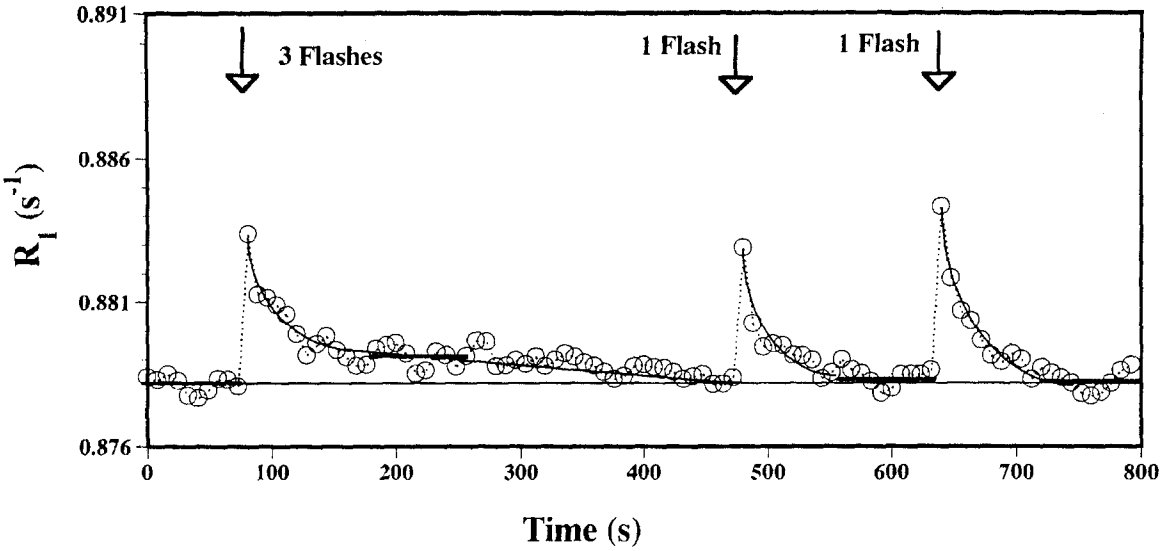

Fig. 3. The 3-flash ($S_1 \rightarrow S_0$) NMR-PRE transient produced by TMF-2 membrane fragments ([Chl]=1.8 mg·mL^{-1}) at 14 °C. This is followed by two 1-flash measurements. The sample was suspended in SMN buffer and contained 2 mM EDTA, 250 μM DCBQ, 10 mM $CaCl_2$, 0.25 μM CCCP (an ADRY reagent). The experimental protocol is described in the text.

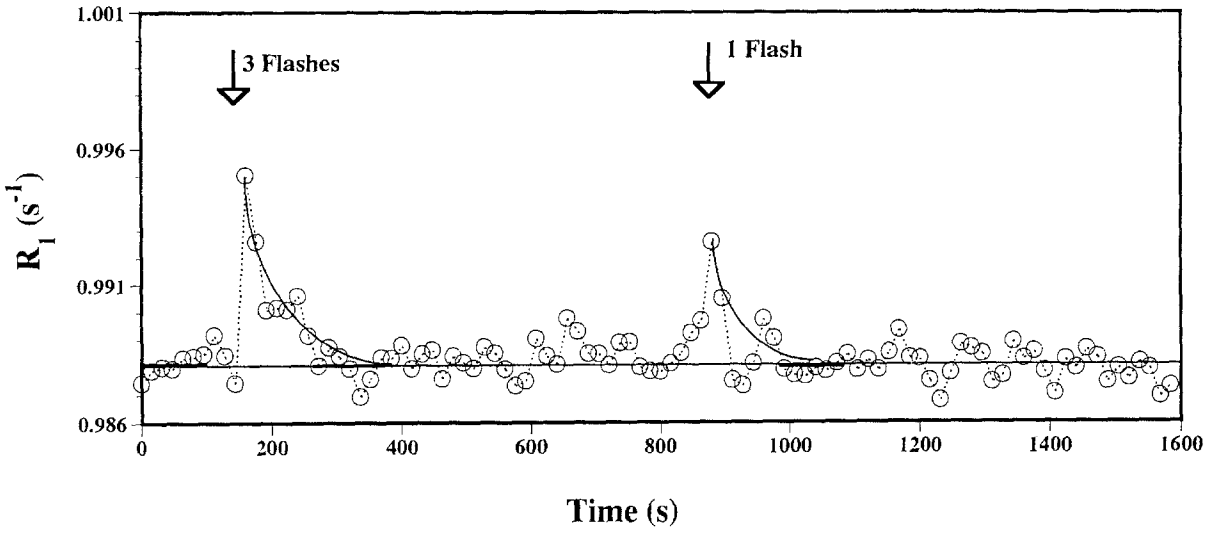

Fig. 4. The 3-flash ($S_1 \rightarrow S_0$) NMR-PRE transient produced by the octylglucoside-isolated PS II complex ([Chl]=1.3 mg·mL^{-1}) at 14 °C. This is followed by one 1-flash measurement. The sample was suspended in SMN buffer and contained 2 mM EDTA, 10 mM $CaCl_2$, plus 250 μM DCBQ. The experimental protocol is described in the text.

was relatively rapid). No long-lived relaxation enhancement due to S_0 was evident in these experiments. Figure 4 shows representative data; several experiments of this type failed to reveal a detectable long-lived S_0 signal even though the S_2 NMR-PRE response was observed in the same preparations of OGP-solubilized PS II complex and in fact had an amplitude somewhat greater than that of TMF-2 suspensions at comparable chlorophyll concentrations (see above). The concentration of OEC manganese in NMR samples of the PS II complex was about 2.5-fold higher than in the TMF-2 samples. Initially we had expected that removal of the 17 and 23 kD polypeptides from the solubilized complex might enhance the NMR-PRE signals due to increased exposure of the OEC to the aqueous

phase. That the anticipated enhancement of the signal did not occur, but rather the S_0 signal disappeared (even though the S_2 signal was observed), was surprising.

Discussion

The NMR-PRE response is described mathematically by the Luz-Meiboom (1965) relation,

$$\Delta R_{1p} = \frac{q([M]/[S])}{T_{1m} + \tau_m} \quad (1)$$

where q is the number of exchangeable solvent molecules at the water-bound metal ion, and the molar concentrations of metal centers and solvent are [M] and [S]. T_{1m} is the proton relaxation time and τ_m is the mean residence time of solvent protons in the metal coordination sphere. Equation (1) assumes that [M]<<[S], which is amply fulfilled in our studies, and that relaxation results from inner-sphere magnetic couplings between the electron spin and solvent protons. Outer sphere relaxation is relatively small for hexaaquocations ($Me[H_2O]_6^{n+}$); for metal sites that are sequestered in a protein matrix, it can readily be shown to be negligible (Koenig et al. 1971). From Eq. (1) it is evident that the amplitude of the NMR-PRE measures the quantity $(T_{1m} + \tau_m)^{-1}$ and is not simply proportional to the concentration of OEC manganese. Quantitative measurements of T_{1m} and τ_m in the S_0, S_2 and S_3 states have previously been obtained from the temperature- and magnetic field-dependent NMR-PRE data and used to assign probable oxidation states of the water-bound OEC manganese ions (Sharp 1992, Bovet et al. 1993).

From the data of Fig. 2, we estimate that the amplitude of the 1 flash NMR-PRE transient, expressed on a basis of constant [Mn], is about 2-fold smaller in the OGP-solubilized PS II preparations than in the TMF-2 suspensions. Taking the coordination number q to be fixed, this implies that $(T_{1m}+\tau_m)$ is about 2-fold longer in the solubilized PS II complex than in TMF-2 membranes. In previous studies of the magnetic field and temperature dependence of the S_2 NMR-PRE signal (Sharp 1992) we have shown that the NMR-PRE response is in the chemical exchange-limited regime ($\tau_m >> T_{1m}$) at 2 °C. Thus τ_m is lengthened (i.e., the chemical exchange kinetics are slowed) approximately 2-fold in the OGP-solubilized PS II complex relative to the TMF-2 membrane fragments.

TMF-2 membrane fragments retain the 17, 23 and 33 kD extrinsic polypeptides while the NaCl-washed and OGP-treated samples are depleted of the 17 and 23, but not the 33 kD polypeptides. It has been proposed that both the 17 and 23 kD polypeptides shield the Mn and Ca binding sites of the OEC from the aqueous phase (see review by Debus 1992). Thus it is somewhat surprising that removal of the 17 and 23 kD polypeptides slightly slows, rather than accelerates, the solvent proton chemical exchange reaction. In the S_2 state, the water-coordinated manganese ion that gives rise to the NMR-PRE is likely to be in a +3 or +4 oxidation state, and in this situation (i.e., an oxidation state > +2) the proton chemical exchange reaction very probably involves protolysis, rather than the transfer of whole water molecules (Sharp 1992). The observed proton chemical exchange rate in the S_2 state is similar to the protolysis rate observed for the hexaquo Fe^{3+} and Cr^{3+} ions. This suggests that the rate of proton chemical exchange in S_2 is determined by the protolysis rate of water in the metal coordination sphere, rather than by the reactions which transfer protons through the protein matrix to the aqueous phase. The observation that the removal of the 17 and 23 kD polypeptides does not remove a kinetic barrier to the chemical exchange reaction (i.e., this treatment does not shorten τ_m) is consistent with this hypothesis.

It should be noted in this regard that the solvent proton chemical exchange reaction which mediates the NMR-PRE response differs from the proton release reactions which accompany certain S-state transitions, specifically $S_0 \rightarrow S_1$, $S_2 \rightarrow S_3$, and $S_3 \rightarrow S_4 \rightarrow S_0$ (Debus 1992). The latter involve the deprotonation of one or more acidic functional groups and very likely do not involve solvent protons at all. Indeed, no deprotonation reaction occurs on the $S_1 \rightarrow S_2$ transition, whereas the NMR-PRE response is relatively large, indicating that the solvent proton chemical exchange reaction is distinct from the deprotonation reactions which accompany S-state turnover.

The lifetime of the S_2 state markedly decreases after both salt washing and OGP treatment (Fig. 2). Consistent with this finding, Van Leeuwen et al. (1992) observed, using UV difference spectroscopy,

a dramatic shortening of the lifetimes of the $S_{2,3}$ states ($t_{1/2}$ = 2–3 s) in a highly chlorophyll-depleted PS II core preparation. These results support the hypothesis that removal of the 17 and 23 kD extrinsic polypeptides deshields the OEC, making it more accessible to aqueous reductants (e.g., reduced DCBQ). In PS II-enriched membrane fragments which retain the 17 and 23 kD polypeptides, S_2 decays principally via back reaction between the positive charge in S_2 and mobile electrons on the acceptor side of PS II. Acceleration of these back reactions provides an alternate hypothesis for the observed acceleration of the $S_2 \rightarrow S_1$ decay in the salt washed and OGP-treated samples.

In an earlier study, Srinivasan and Sharp (1986b) reported that the S_0 state of PS II-enriched membrane fragments prepared by the method of Berthold et al. (1981) (BBY particles) gives rise to a small positive proton relaxation enhancement relative to the dark background R_1 level of the S_1 state. The results of Fig. 3 confirm this finding using the TMF-2 preparation. These experiments indicate that solvent water protons are coordinated in a labile manner to a paramagnetic center in S_0, presumably a manganese ion, that is redox-active on the $S_1 \rightarrow S_0$ transition. Our conclusion that a manganese-centered oxidation occurs on $S_0 \rightarrow S_1$ is consistent with recent studies of the Mn K_a absorption edge energy changes during flash experiments by Ono et al. (1992), who observed an edge shift of 1 eV to high energy at the $S_0 \rightarrow S_1$ transition.

However, the S_0 NMR-PRE signal that is present in TMF-2 and BBY preparations was not observed in suspensions of the OGP-solubilized PS-II complex. This result was unexpected in view of the fact that OGP treatment removes the 17 and 23 kD extrinsic polypeptides and is expected generally to increase the accessibility of the manganese center to the aqueous phase. We expected that the extrinsic polypeptides might constitute an important barrier to chemical exchange reactions in S_0, more so than in the S_2 state. The oxidation state of the water-bound manganese ion that is responsible for the S_0 NMR-PRE signal is not known at present. However it appears from this and our previous NMR-PRE studies that this ion is involved in manganese-centered oxidations on both the $S_0 \rightarrow S_1$ and $S_1 \rightarrow S_2$ steps (but not on $S_2 \rightarrow S_3$). On this basis we have proposed that a water-bound Mn(II) exists in S_0 (Sharp 1992). For divalent cations, solvent proton exchange in model systems involves the transfer of whole water molecules rather than protolysis. If the water-bound Mn ion of the S_0 state is indeed Mn(II), it seemed likely that removal of the extrinsic polypeptides might eliminate an important kinetic barrier to the chemical exchange of water molecules (even if not to mobile protons) and, if so, that OGP treatment would result in an increase, possibly quite large, in the NMR-PRE response. To the contrary, the 3-flash NMR-PRE response of the OGP-solubilized PS II complex was smaller than for TMF-2 and BBY membranes and was in fact undetectable in our experiments. Since the O_2 evolution activity of the OGP-solubilized PS II complex was quite high, we assume that the S_0 state was produced after 3 flashes with a yield at least comparable to that of the TMF-2 sample. To explain the disappearance of the S_0 NMR-PRE signal, it appears that either (1) the quantity ($\tau_m + T_{1m}$) must be substantially longer in the OGP-solubilized PS II complex than in the TMF-2 preparation or (2) the $S_0 \rightarrow S_1$ decay is more rapid in the PS II complex than in TMF-2 membranes.

Of these two possibilities, the second does not provide a very plausible explanation of our results, even though it has been reported that detergent solubilization of the PS II complex markedly destabilizes the S_0 state (Van Leeuwen et al. 1992). However, it seems very unlikely that the lifetime of the S_0 state in the OGP-treated samples is so short that the S_0 signal in Fig. 4 would be obscured by the $S_{2,3}$ responses. Thus we conclude that $T_{1m} + \tau_m$ is lengthened substantially (>2-fold) in the S_0 state and that τ_m is lengthened about 2-fold in the S_2 state relative to the TMF-2 control. The 17 and 23 kD extrinsic polypeptides appear to have little influence on the solvent accessibility of OEC manganese.

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