Biochimica et Biophysica Acta 848 (1986) 8–15
Elsevier

BBA 41902

The influence of the oxidation state of the oxygen-evolving complex of Photosystem II on the spin-lattice relaxation time of Signal II as determined by electron spin-echo spectroscopy

Alex de Groot [a], Johan J. Plijter [a], Rob Evelo [a], Gerald T. Babcock [b] and Arnold J. Hoff [a]

[a] *Department of Biophysics, Huygens Laboratory of the State University, P.O. Box 9504, 2300 RA Leiden (The Netherlands) and* [b] *Department of Chemistry, Michigan State University, East Lansing, MI 48824 (U.S.A.)*

(Received June 24th, 1985)
(Revised manuscript received September 30th, 1985)

Key words: Manganese; Electron spin-echo spectroscopy; Spin-lattice relaxation time; Photosystem II; Oxygen evolution; (Spinach chloroplast)

The spin-lattice relaxation time of Signal II, which arises from two plastoquinol cation radicals, D^+ and Z^+, has been measured with electron spin-echo spectroscopy in Photosystem II preparations with inactivated and with intact oxygen-evolving complex. In Tris- and subsequently EDTA-washed Photosystem II preparations the spin-lattice relaxation times of D^+ and Z^+ are equal and remain unchanged if the pH is increased from 6.0 to 8.3. In preparations in which the oxygen-evolving complex is not inactivated by the Tris washing, the spin-lattice relaxation times of D^+ and Z^+ are affected by the redox state of the oxygen-evolving complex. At pH 6.0 the spin-lattice relaxation time decreases with higher redox state of the manganese cluster. The relaxation behavior at pH 8.3 indicates that at this pH the stability of the S_1 state is decreased such that in dark-adapted samples nearly 100% of the systems is in the S_0 state. This was confirmed by optical experiments where the period-four oscillation in the absorption at 350 nm was monitored as a function of the flash number.

Introduction

Oxygen evolution in green plants and algae takes place at the donor side of the so-called Photosystem II (PS II, for a recent review see Ref. 1). Between the oxygen evolving complex and the primary electron donor of PS II, P-680, two more donor sites have been identified, D and Z. The latter is presumably the direct electron donor to P-680, whereas D may function as an alternative donor to P-680. D^+ and Z^+ are characterized by a similar EPR signal, the so-called Signal II (SII), on which a large number of continuous wave EPR experiments have been carried out. From the latest work it was concluded that the stable radical D^+ (and also Z^+) is an oxidized plastosemiquinol cation radical [2–7]. The appearance of the EPR signal due to Z^+ is dependent on the sample preparation procedure, while a contribution of D^+ is always present. It has been shown that four turnovers of the plant photosystem are needed to release one molecule of oxygen. Each turnover changes the redox state of oxygen-evolving complex (the so-called S states [8]). In the oxygen-evolving complex a cluster of four manganese atoms is involved in the oxidation of water [9–11].

Abbreviations: PS II, Photosystem II; Tricine, *N*-2-hydroxy-1,1-bis(hydroxymethyl)ethyl glycine; DCBQ, 2,5-dichloro-*p*-benzoquinone; Mes, 4-morpholineethanesulfonic acid.

0005-2728/86/$03.50

From absorbance difference experiments, Dekker et al. [9] recently concluded that turnovers inducing S-state transitions from $S_0 \rightarrow S_1$, $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ correspond to simultaneous oxidation of Mn(III) to Mn(IV). That Mn(III) and Mn(IV) are involved in the oxygen evolution was already suggested by the observation of the so-called multiline EPR signal [12–15]. This EPR signal, which appeared to be correlated with the S_2 state, shows a certain resemblance with the EPR spectrum of an Mn(III)-Mn(IV) dimer, of which the two ions are antiferromagnetically coupled by an exchange interaction [12,16]. The fact that the $S_0 \rightarrow S_1$, the $S_1 \rightarrow S_2$ and the $S_2 \rightarrow S_3$ transitions each correspond to the additional oxidation of an Mn(III) to Mn(IV), while only the S_2 state displays an EPR signal, indicates that the geometry (i.e., the interatomic distances) of the cluster is determining the mutual interactions. Earlier reported electron spin-echo studies in spinach chloroplasts [17,18] showed that the spin-lattice relaxation time of SII depends on the illumination procedure.

In this work we present an electron spin-echo study in which the spin-lattice relaxation times (T_1) of D^+ and Z^+ are used as a probe for the relaxation behavior of the manganese cluster in its various photo-induced redox states. It is well known that the spin-lattice relaxation time of a paramagnetic species can be influenced by a strongly relaxing paramagnetic molecule in its vicinity. Hence, changes in the relaxation properties of this neighboring molecule can be monitored by determining the spin-lattice relaxation of the paramagnetic species, which in the case of PS II preparations with intact oxygen-evolving complex is the D^+ radical. From our relaxation study it is concluded that the spin-lattice relaxation time of the oxygen-evolving complex decreases with higher S state, and that the lifetime of the S_1 state is strongly dependent on the pH.

Materials and Methods

PS II particles were prepared from spinach chloroplasts according to Ref. 19 but modified as in Ref. 4. PS II particles at pH 6.0 were prepared by washing in a Mes buffer containing 50 mM Mes (pH 6.0), 5 mM $MgCl_2$, 15 mM NaCl, 0.4 M sucrose and 0.01% Triton X-100. In the following, these preparations will be referred to as PSII/6.0. For PS II preparations at pH 8.3 (hereafter labeled PSII/8.3) the Mes buffer was replaced by Tricine buffer containing 50 mM Tricine (pH 8.3), 5 mM $MgCl_2$, 15 mM NaCl, 0.4 M sucrose and 0.01% Triton X-100. Tris washing was accomplished by resuspending PS II particles in 0.8 M Tris-HCl (pH 8.3) to a chlorophyll concentration of 0.2 mg Chl/ml, incubating at 0°C for 15 min and pelleting at $40\,000 \times g$ for 20 min. Tris-washed particles at pH 6.0 (labeled Tris/6.0) were prepared by washing the Tris-washed particles in the pH 6.0 Mes buffer as mentioned above and pelleting. For Tris-washed particles at pH 8.3 (Tris/8.3) the washing was done with the pH 8.3 Tricine buffer.

Tris- and EDTA-washed particles were prepared by resuspending Tris-washed particles in 0.5 mM EDTA (pH 6.4) to a Chl concentration of 1 mg Chl/ml, incubating at 0°C for 30 min and pelleting. pH adjustment was performed as described above, yielding the pH 6.0 and pH 8.3 preparations (Tris,EDTA/6.0 and Tris,EDTA/8.3, respectively). The electron spin-echo samples, with a concentration of 5 mg chlorophyll/ml, were contained in 3 mm diameter Suprasil quartz tubes and dark-adapted at 0°C for 1 h. For all preparations, at both pH 6.0 and 8.3, two different samples were prepared that differed with respect to the illumination and subsequent freezing procedure. In the following, each sample will be denoted with the format preparation procedure/pH/illumination procedure. For example, Tris,EDTA/8.3/L denotes a sample of the Tris,EDTA-washed preparation at pH 8.3, frozen to 77 K under continuous illumination.

With the PS II particles at pH 6.0 three additional samples (PSII/6.0/140K, PS II/6.0/200K and PSII/6.0/250K) were prepared, which were illuminated at a temperature of 140, 200 and 250 K, respectively. The dark adaptation, temperature equilibration and illumination procedure was as in Ref. 14.

The electron spin-echo experiments were carried out on a home-built phase-sensitive electron spin-echo spectrometer similar to that described in Ref. 20. Electron spin-echo field-swept spectra (where the magnetic susceptibility, χ'', is displayed versus the magnetic field) were recorded using a two-pulse echo sequence with spacing, τ, between the two pulses of 500 ns (see also Fig. 1A). T_1 measurement were performed using a three-pulse sequence (as indicated in Fig. 2) in which the two-pulse echo intensity is monitored as a function of the time, T, between the first (perturbing) pulse and the subsequent two-pulse echo sequence. In these experiments a fixed τ of 500 ns was used. All electron spin-echo experiments were carried out at a repetition rate of 7 Hz.

The optical experiments were carried out in a single-beam apparatus as described in Ref. 4 with an optical pathlength of 1.2 mm. The time constant was about 0.3 ms. Illumination was provided by a series of saturating Xe flashes with 400 ms between two successive flashes. The optical samples contained 200 μg chlorophyll/ml and 100 μM DCBQ in the indicated buffer.

Results and Discussion

Electron spin-echo field-swept spectra

All samples displayed a similar electron spin echo field-swept spectrum, of which a characteristic example is depicted in Fig. 1A. To facilitate comparison with continuous wave EPR, where $d\chi''/dH$ is monitored, we numerically calculated the first-derivative spectrum (Fig. 1B) which yields the familiar Signal II lineshape. When the magnetic field is scanned over a larger interval (not shown), the SII lineshape appears to be superimposed on a broad background signal similar to that in Refs. 17 and 18. Consequently, the measured spin-lattice relaxation of SII (observed at $g = 2.0047$) is determined by that of D^+ and Z^+, as well as by that of the background. The intensity of this background was never larger than 10% of the intensity at $g = 2.0047$. Since its T_1 was independent of pH and of the illumination procedure, the contribution of the background to the measured T_1 is constant for all samples with the same preparation procedure. None of the samples showed a noticeable τ-dependence of the SII lineshape. The electron spin-echo field-swept spectra were checked for nonuniform T_1 by recording the spectra at varying repetition rates. Up to a rate of 1000 Hz no noticeable repetition rate dependence of the lineshape was observed. This was confirmed by T_1 measurements performed at different field positions (as indicated by 1, 2 and 3 in Fig. 1A). In the following we will therefore discuss only those T_1 measurements carried out on the top of the SII lineshape (position 2 in Fig. 1A) with the

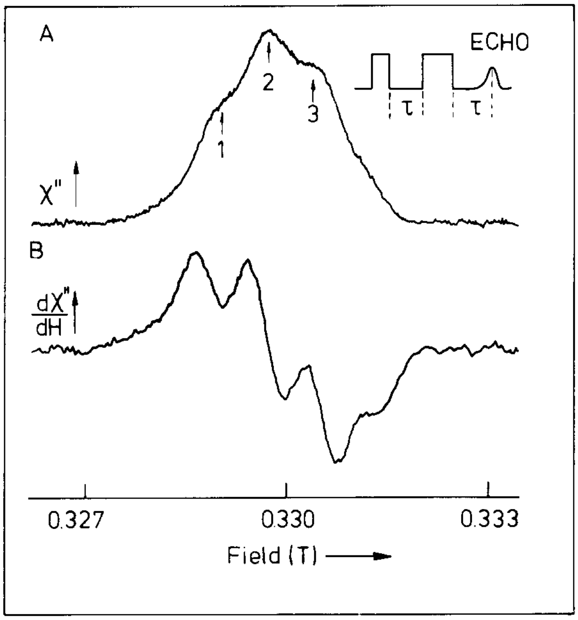

Fig. 1. (a) Electron spin-echo field-swept spectrum of D^+ in the PSII/6.0/D sample, and (b) its first derivative. Pulse sequence as indicated with $\tau = 500$ ns; repetition rate, 7 Hz. Temperature, 5 K.

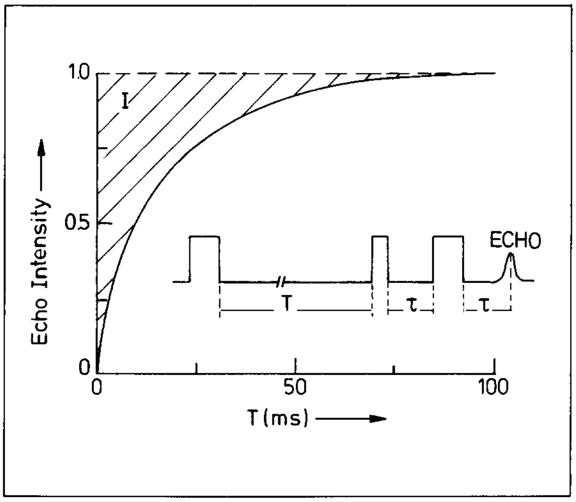

Fig. 2. Definition of the surface, *I*. The pulse sequence used to monitor the relaxed population is indicated (not on scale) in the figure.

repetition rate fixed on 7 Hz and τ fixed on 500 ns.

Spin-lattice relaxation measurements

We will first define a data analysis procedure which makes a quantification of the T_1 measurements possible. The T_1 relaxation traces obtained for SII are all poly-exponentials which cannot be defined by one single characteristic relaxation time. In our experiments we monitor the relaxed population at time T after the perturbing pulse. After normalization of the initial amplitude to unity, the surface I (see Fig. 2), obtained by numerical integration, is a measure of the average spin-lattice relaxation time: the smaller the surface, I. The faster the spin-lattice relaxation is. In fact, the saturation recovery trace of Fig. 2 can be written as a sum of exponentials:

$$f(t) = 1 - \frac{\sum_i a_i \exp(-t/\tau_{c_i})}{\sum_i a_i} \qquad (1)$$

where a_i and τ_{c_i} are the amplitude and characteristic time constant of the ith relaxation process. The surface, I, is given by:

$$I = \int_0^\infty [1 - f(t)]\mathrm{d}t = \frac{\sum_i - a_i\tau_{c_i}}{\sum_i a_i} = -\bar{\tau}_c \qquad (2)$$

Thus $|I|$ represents the average spin-lattice relaxation time, $\bar{\tau}_c$.

Some of the recorded T_1 traces are depicted in Fig. 3. The values $\bar{\tau}_c$ for all samples are listed in Table I. Note that as a consequence of the superposition of the SII and the background signals as mentioned above, the absolute value of $\bar{\tau}_c$ of samples that differ with respect to Tris and/or EDTA treatment cannot be compared.

Tris- and EDTA-washed preparations

In these preparations the oxygen evolution was inhibited by Tris washing, and a subsequent EDTA washing was carried out to remove the loosely bound manganese. Under these conditions, SII, which is still present, can arise from D^+ in dark-adapted samples and from both D^+ and Z^+ in samples illuminated during freezing to 77 K.

From the fact that for pH 6.0 as well as for pH 8.3 $\bar{\tau}_c$ is equal for dark-adapted and illuminated

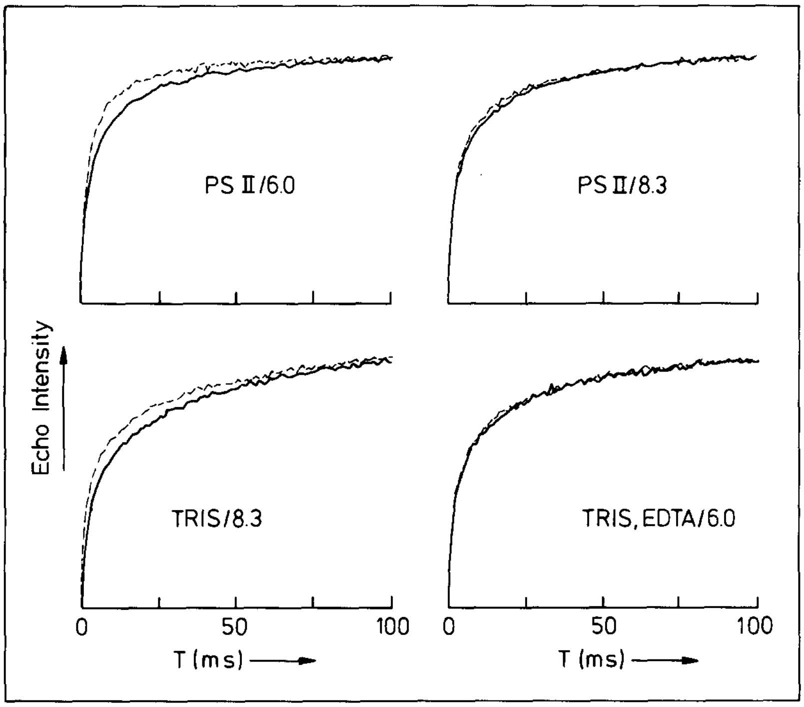

Fig. 3. Some of the recorded saturation recovery traces obtained with the pulse sequence depicted in Fig. 2 and under the same conditions as in Fig. 1. Preparation procedure and pH as indicated in the figure. ——— indicates the /D and - - - the /L samples.

TABLE I

AVERAGE SPIN-LATTICE RELAXATION TIME OF SII OBTAINED BY ELECTRON SPIN-ECHO SPECTROSCOPY

The samples are labeled (preparation)/pH/(illumination procedure). $\bar{\tau}_c$ equals the surface, $|I|$, in Fig. 2; error ±0.5 ms. Relative signal height indicates the relative SII intensity of a sample compared to that of its /D analogue.

Sample	$\bar{\tau}_c$ (ms)	Relative signal height
Tris,EDTA/6.0/D	14.2	
/L	13.4	2
Tris,EDTA/8.3/D	14.4	
/L	13.8	2
Tris/6.0/D	16.7	
/L	13.4	2
Tris/8.3/D	18.8	
/L	14.8	2
PSII/6.0/D	12.1	
/140K	12.2	1
/200K	10.6	1
/250K	6.5	1
/L	7.5	1
PSII/8.3/D	13.7	
/L	12.4	1

samples (Tris,EDTA/pH/D and Tris,EDTA/pH/L in Table I, respectively), while the signal intensity is doubled in the illuminated (/L) samples as a result of the photogeneration of Z^+, we conclude that Z^+ and D^+ have the same T_1. Furthermore, as all four samples have the same T_1, we conclude that the pH does not influence the T_1 of Z^+ or D^+ itself.

In samples where the EDTA washing is omitted (indicated as Tris/pH/L and Tris/pH/D in Table I) the oxygen-evolving complex is inactivated by the Tris washing. However, since it is not actively removed by EDTA washing, an interaction between its residues, such as the inactivated manganese, and SII is still possible. From Table I it is seen that both at pH 6.0 and 8.3 the illuminated samples have a smaller $\bar{\tau}_c$ than their dark-frozen analogues. The signal intensity of the illuminated samples is twice that of the dark-frozen samples. Presumably in the latter only D^+ is contributing to the echo signal, while in the former both D^+ and Z^+ are present in equal amounts. It was found that in these preparations D^+ is not reduced at high pH, in contrast to observations on Tris-inactivated chloroplasts in Ref. 21. We already concluded that the $\bar{\tau}_c$ values of D^+ and Z^+ are equal in samples without an active oxygen-evolving complex. It follows that the light-induced $\bar{\tau}_c$ change, as observed in preparations which are only Tris-washed, reflects an interaction between D^+ and/or Z^+ and the loosely bound residues of the oxygen-evolving complex and that this interaction is different for these two radicals. It seems likely that the difference between the relaxation properties of the Tris and the Tris,EDTA preparations is caused by the active removal (in the latter) of the strongly relaxing paramagnetic manganese ions of the oxygen-evolving complex. Apparently, one or more of these manganese ions interacts with D^+ and/or Z^+.

Illumination at low temperature

PS II preparations at pH 6.0 possess an intact oxygen-evolving complex, as they still produce O_2 [19]. The period-four oscillation pattern of the Mn absorption as measured optically confirmed that in a dark-adapted (PSII/6.0/D) sample, 75% of the systems are in the stable S_1 state, while 25% are in the S_0 state [9]. From Table I it is seen that at pH 6.0 the $\bar{\tau}_c$ of a sample illuminated at 140 K (PSII/6.0/140K in Table I) is equal to that of a dark-adapted sample (PSII/6.0/D). At 140 K no S-state transitions occur [14] and consequently the S-state distribution in the dark of 25% S_0 and 75% S_1 is preserved. The paramagnetic species of unknown origin that, according to Ref. 22, might be light-induced at 140 K, showing an EPR line at $g = 4.1$, apparently does not affect the $\bar{\tau}_c$ of D^+ and Z^+. When the illumination is carried out at 200 K (the PSII/6.0/200K sample), only the transitions $S_0 \rightarrow S_1$ and $S_1 \rightarrow S_2$ can occur [14] in addition to the formation of the $g = 4.1$ signal [15]. Since the $\bar{\tau}_c$ value of PSII/6.0/200K is somewhat smaller than those of PSII/6.0/D and PSII/6.0/140K, where only the S_0 and S_1 states are present, we conclude that the S_2 state decreases $\bar{\tau}_c$ more than the S_0 and/or the S_1 state. In the PSII/6.0/250K sample, where the illumination was carried out at 250 K, the $S_2 \rightarrow S_3$ transition can also occur, whereas the $S_3 \rightarrow S_0$ transition is still blocked or very inefficient [23] so that a relatively high percentage of the oxygen-evolving complexes will be in the S_3 state. For PSII/6.0/250K the value of $\bar{\tau}_c$ is decreased considerably with respect to the $\bar{\tau}_c$ value of PSII/6.0/200K (Table I). Hence, in the S_3 state the relaxation is much stronger than the S_2 state. The fact that the $\bar{\tau}_c$ value of PSII/6.0/L seems to be slightly larger than that of PSII/6.0/250K is probably due to a less efficient accumulation of the higher S states by illumination during cooling.

High pH

At pH 8.3 the $\bar{\tau}_c$ for dark-adapted samples is somewhat higher than at pH 6.0. From the PSII/6.0 results it was concluded that $\bar{\tau}_c$ decreases with higher S state. Thus, the somewhat higher $\bar{\tau}_c$ for PSII/8.3/D compared to PSII/6.0/D (Table I) could be explained by assuming that in the former more oxygen-evolving complexes are in the S_0 state. At pH 8.3 the difference in $\bar{\tau}_c$ between the

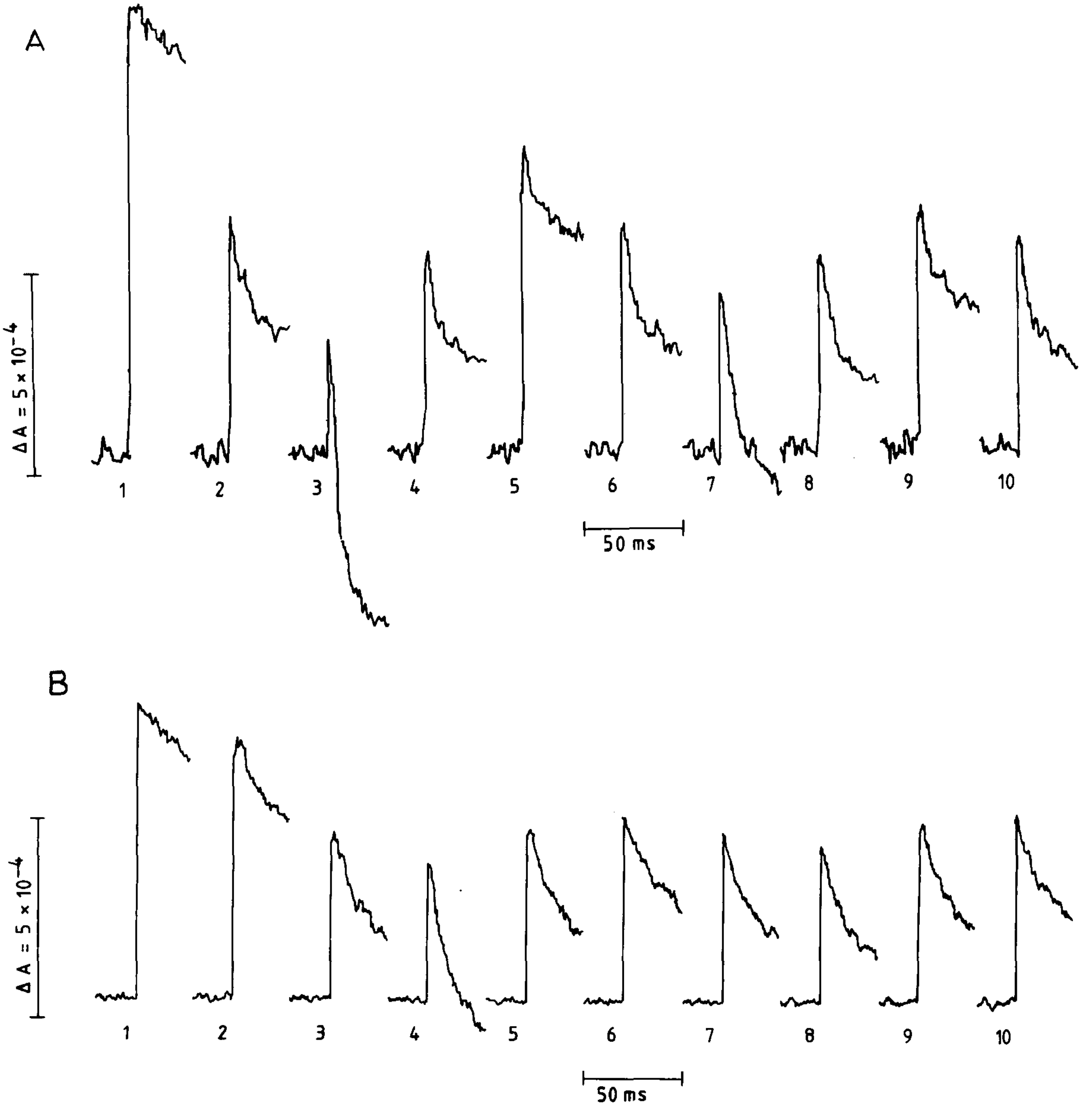

Fig. 4. The absorbance changes at 350 nm induced by ten successive saturating flashes spaced 400 ms of dark-adapted PS II preparations at (a) pH 6.0; (b) pH 8.3.

dark-adapted (PSII/8.3/D) and the illuminated samples (PSII/8.3/L) is much smaller than that at pH 6.0, with the $\bar{\tau}_c$ value for PSII/8.3/L being remarkably close to that of PSII/6.0/D. This suggests that also in the PSII/8.3/L sample most systems are in the lower S states. Two possible explanations are that high pH and high light intensity inhibit progression through the S states, so that little or no S_2 or S_3 is formed, or that the high pH promotes a fast back-reaction from the higher S states to the S_0 and S_1 state. To sort out these two possibilities the progression through the S states was verified by measuring absorbance changes at both pH 6.0 and 8.3 as a function of the flash-number, as in Ref. 9.

Absorbance changes were measured at 350 nm, where the manganese cluster causes relatively large absorbance changes superimposed on the changes of the acceptor side. The PS II preparations were dark-adapted for at least 15 min, after which the lipophilic acceptor DCBQ (100 μM) was added, and a series of saturating flashes spaced 400 ms was fired. The flash-induced absorbance changes were measured in successive 50 ms sweeps, the off-set being adjusted before each flash. Fig. 4 shows the absorbance changes as a function of the flash number of PS II preparations in 50 mM Mes NaOH (pH = 6.0), 5 mM $MgCl_2$, 15 mM NaCl, 0.01% Triton X-100 and in 50 mM Tricine NaOH (pH = 8.3), 5 mM $MgCl_2$, 15 mM NaCl, 0.01% Triton X-100, respectively. At pH 6.0 (Fig. 4A) the manganese reduction on flash numbers 3 and 7 is clearly seen. The oscillation pattern could be fitted with a dark contribution of 25% S_0 and 75% S_1 (see above). The oscillation has minima on flash numbers 4 and 8, suggesting that the initial state was S_0 rather than predominantly S_1 and could be fitted with 100% S_0, 11% double hits and 11 misses on all transitions with the amplitude of the oscillation about 70% of that at pH 6, showing that the manganese cluster is still active for at least two cycles in the majority of the reaction centers. Since the electron spin-echo data show that the SII amplitudes of PSII/8.3/D and PSII/8.3/L are equal, the first flash does not generate D^+ or a long-living Z^+. Thus it appears that the normally stable S_1 state indeed reverts to the S_0 state at pH 8.3.

Conclusions

(a) The spin-lattice relaxation times of D^+ and Z^+ are influenced by the presence of one or more strongly relaxing species in their vicinity.

(b) This strongly relaxing species is associated with the oxygen-evolving complex. At pH 6.0 the average spin-lattice relaxation time of SII is smallest when the oxygen-evolving complex is in the S_3 state at pH 8.3 (Fig. 4B) and increases with lower redox state of the oxygen-evolving complex:

$$\bar{\tau}_c(S_0) > \bar{\tau}_c(S_1) > \bar{\tau}_c(S_2) > \bar{\tau}_c(S_3)$$

The most likely candidate for a species in interaction with SII is the manganese cluster [24,25]. In Ref. 9 it was concluded that the S-state transitions $S_0 \rightarrow S_1$, $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ correspond to progressive oxidation of the manganese cluster, with each transition being associated with the oxidation of one Mn(III) to Mn(IV). Apparently, the relaxing influence of the manganese cluster increases with its oxidation state. This is not unexpected, because in general the spin-lattice relaxation of a molecule shortens with increasing spin-orbit coupling, which for the manganese free ion increases with its oxidation state [26].

(c) The pH does not affect the relaxation of D^+ and Z^+ when the interaction of D^+ and Z^+ with the oxygen-evolving complex is absent (Tris,EDTA preparations).

(d) In preparations with intact oxygen-evolving complex the pH strongly influences the stability of the normally stable S_1 state. At a pH of 8.3 dark-adapted preparations are predominantly in the S_0 state.

The conclusions summarized above show that the spin-lattice relaxation of SII is a sensitive probe for the redox states of the oxygen-evolving complex. In principle, determination of the T_1 values of SII as a function of the S state permits insight into the relative distances of the manganese ions to D^+ and Z^+. For practical application, however, one needs to know the exact T_1 values of the manganese ions themselves in their respective environment, which are presently unknown.

Acknowledgements

We thank Ms. Lenneke Nan for skillfully preparing the Photosystem II samples. We are indebted to Dr. H.J. van Gorkom for helpful discussions and a critical reading of the manuscript. This work was supported by the Netherlands Foundation for Chemical Research (SON), with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).

References

1 Van Gorkom, H.J. (1985) Photosynthesis Res. 6, 97–112

2 Ghanotakis, D.F., O'Malley, P.S., Babcock, G.T. and Yocum, C.F. (1983) in The Oxygen Evolving System of Photosynthesis (Inoue, Y. et al., eds.), pp. 91–101, Academic Press, Tokyo

3 Diner, B.A. and De Vitry, C. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. I, pp. 407–411, Martinus Nijhoff/Dr. W. Junk, Dordrecht

4 Dekker, J.P., Van Gorkom, H.J., Brok, M. and Ouwehand, L. (1984) Biochim. Biophys. Acta 764, 301–309

5 O'Malley, P.J. and Babcock, G.T. (1984) Biochim. Biophys. Acta 765, 370–379

6 O'Malley, P.J., Babcock, G.T. and Prince, R. (1984) Biochim. Biophys. Acta 766, 283–288

7 Brok, M., Ebskamp, F.C.R. and Hoff, A.J. (1985) Biochim. Biophys. Acta 809, 421–428

8 Kok, B., Forbush, B. and McGloin, M. (1970) Photochem. Photobiol. 11, 457–475

9 Dekker, J.P., Van Gorkom, H.J., Wensink, J. and Ouwenhand, L. (1984) Biochim. Biophys. Acta 767, 1–9

10 Dekker, J.P., Plijter, J.J., Ouwenhand, L. and Van Gorkom, H.J. (1984) Biochim. Biophys. Acta 767, 176–179

11 Amesz, J. (1983) Biochim. Biophys. Acta 726, 1–12

12 Dismukes, G.C. and Siderer, Y. (1981) Proc. Natl. Acad. Sci. USA 78, 274–278

13 Hansson, Ö. and Andreasson, L.E. (1982) Biochim. Biophys. Acta 679, 261–268

14 Brudvig, G.W., Casey, J.L. and Sauer, K. (1983) Biochim. Biophys. Acta 723, 366–371

15 Zimmerman, J.L. and Rutherford, A.W. (1984) Biochim. Biophys. Acta 767, 160–167

16 Mathur, P. and Dismukes, G.C. (1983) J. Am. Chem. Soc. 105, 7093–7098

17 Nishi, N., Hoff, A.J., Schmidt, J. and Van der Waals, J.H. (1978) Chem. Phys. Lett. 58, 164–170

18 Nishi, N., Hoff, A.J. and Van der Waals, J.H. (1980) Biochim. Biophys. Acta 590, 74–88

19 Berthold, D.A., Babcock, G.T. and Yocum, D.F. (1981) FEBS Lett. 134, 231–234

20 Gast, P., Mushlin, R.A. and Hoff, A.J. (1982) J. Phys. Chem. 86, 2886–2891

21 Boussac, A. and Etienne, A.-L. (1982) FEBS Lett. 148, 113–116

22 Casey, J.L. and Sauer, K. (1984) Biochim. Biophys. Acta 767, 21–28

23 Inoue, Y. and Shibata, K. (1978) FEBS Lett. 85, 193–197

24 Warden, J.T., Blankenship, R.E. and Sauer, K. (1976) Biochim. Biophys. Acta 423, 462–478

25 Yocum, C.F. and Babcock, G.T. (1981) FEBS Lett. 130, 99–102

26 Abragam, A. and Bleaney, B. (1970) Electron Paramagnetic Resonance of Transition Ions, Oxford University Press