

OXYGEN EVOLUTION, MANGANESE, ESR AND NMR

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ABSTRACT

The recent application of the magnetic resonance (ESR and NMR) techniques to the investigations on the role of Mn in the O₂-evolving system of photosynthesis is reviewed here. First, a brief introduction to the kinetic steps involved in O₂ evolution, to the Mn in green plants and to the bases of the measurements on Mn by ESR and NMR is provided. Second, experimental work on both ESR and NMR measurements on thylakoids is reviewed. Available experimental results are suggestive of the following: (1) In thylakoids, Mn exists in a mixture of oxidation states (Mn²⁺ and, at least, one higher oxidation state). (2) The loosely-bound Mn, which is related to O₂ evolution, is located on the inner side of the thylakoid membrane and appears to be undetectable by ESR at room temperature. (3) Oscillatory changes in loosely-bound Mn become measurable by ESR when bound Mn is released from its native binding site by heat treatment of thylakoids after exposure to different number of light flashes; analysis of these and other experiments has led to the suggestion that during O₂ evolution, Mn undergoes redox changes although the sequential accumulation of positive charges on the water side is not accompanied by sequential oxidation of Mn in all the steps involved; in some steps, Mn seems to be reduced instead of being oxidized; and (4) Mn, related to O₂ evolution, seems to be correlated with water proton relaxation rates (PRR) when (a) Mn content is varied by incubation of thylakoids at 35°C for different times, or by treatment of thylakoids with high concentrations of MgCl₂; and (b) the state of the O₂-evolving system (S state) is varied by treatment of thylakoids with NH₂OH or with exposure of thylakoids to different number of light flashes. However, the difficulties encountered in reproducing this light flash effect, and the apparent non correlation between PRR and the loosely-bound Mn under certain experimental conditions make the use of NMR less attractive in quantitating the dynamic role of the redox changes in Mn during S state transitions in O₂ evolution without further improvement in the available instrumentation. However, measurements on both NMR and ESR of thylakoids and its "extracts" with parallel measurements on Mn content and on O₂ evolution remain the best hope for unravelling changes in Mn during O₂ evolution.

INTRODUCTION

There appear to be at least three fractions of manganese (Mn) in thylakoid membranes: a loosely bound fraction (LBMn) that may include an elusive O₂ evolving "Mn-enzyme" of Photosystem II (PSII); a tightly bound

fraction (TBMn) that may include Mn associated with the light-harvesting chlorophyll (Chl) a/Chl b complex preparation (LHC prep); and a very loosely bound fraction (VLBMn) that appears to be unrelated to the above two fractions. In addition, a fourth type of Mn may be present which is associated with superoxide dismutase activity. However, this Mn may be identified with one of the above fractions.

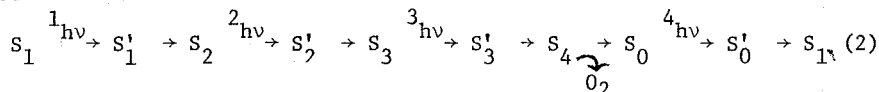
The removal of the LBMn from thylakoid membranes by various treatments (Tris, hydroxylamine and heat) results in the loss of O_2 activity without affecting electron transport through the rest of the chain. These results are generally taken to mean that the LBMn functions on the water side of PSII. It is tempting to speculate that the accumulation of charge in the O_2 evolving apparatus may somehow involve redox changes in Mn. In this paper recent attempts utilizing the techniques of electron spin resonance (ESR) and nuclear magnetic resonance (NMR) to identify the function of thylakoid Mn in relation to O_2 evolution are reviewed. A brief background on O_2 evolution, Mn, ESR and NMR methods is first presented.

O_2 Evolution [1-6]

The mechanism of oxidation of water to molecular O_2 in photosynthesis is still obscure. Four positive charges must accumulate before O_2 can be evolved (Z^+ being produced from Z as a consequence of a light reaction):



However, reaction (1) does not occur in one step. The generally accepted picture is that of Kok and coworkers:



where, S represents the state of the oxygen system, subscripts on S represent the number of positive equivalents in excess of that on S_0 , superscript primes on S represent the states created after light absorption by the reaction center of PSII, P680, and the superscript numbers on the left side of $h\nu$ represent the flash numbers (only major effects are shown).

The above picture is consistent with O_2 flash yield measurements. O_2 is evolved after the 3rd flash when a series of flashes, separated by ~1 second, is given to a dark-adapted sample; it also explains the periodicity of 4 in the pattern of O_2 /flash as a function of flash number. Other details of damping etc. are explained by occurrence of (a) "misses"; (b) double "hits" and (c) a ratio of 3:1 for $S_1:S_0$ in dark-adapted chloroplasts. The periodicity of 4 in the O_2 evolution due to the operation of the S state can affect directly or indirectly various measured parameters

(chlorophyll a fluorescence, delayed light emission, thermoluminescence peaks, conformational changes of the membrane, etc.). When one observes a periodicity of 4 in any phenomena, it is important to establish whether it indirectly reflects "S" states or monitors directly the S intermediate. In the former case, positive correlation may be converted into an absence of correlation if the "connecting link" is lost or modified.

Manganese [7,8,9]

Mn is required for plant growth as a micronutrient, occurring at about 0.07 to 0.9 mM concentration in the tissue water [10] or about 1/20 - 1/50 of the magnesium concentration [11]. The uptake of Mn is an energy linked process which is decreased by the presence of magnesium and calcium ions [12]; however, it moves freely in the cell water as the divalent ion (Mn^{2+}) [13].

The total Mn content can be analyzed by atomic absorption spectroscopy or neutron activation methods [14]. In thylakoid membranes, quantitative measurements indicate that about 4 Mn atoms per PS II trap are associated with the LBMn fraction while about 2 Mn atoms per PS II trap are associated with the TBMn fraction [3]. The amount of Mn in the VLBMn fraction can be variable, depending upon the source of plant material and method of sample preparations.

Mn is a Period IV transition metal. It occurs most commonly in the +2 oxidation state (spin = $5/2$), although it can exist in other oxidation states up to +7 [15]. Mn^{3+} is stable only in complexes containing strong electron withdrawing ligands (e.g., halides, pyrophosphate). In aqueous medium 2 Mn^{3+} ions usually disproportionate to Mn^{2+} and Mn^{4+} . In contrast, Mn^{4+} is stable only in the form of insoluble oxides such as MnO_2 , as is the case for Mn^{5+} . And, Mn^{7+} exists almost exclusively as the permanganate ion.

Magnetic Resonance Methods

ESR [16]

The unpaired d electrons in Mn confer unique magnetic properties on which magnetic resonance methods are based. ESR in particular is used to monitor unpaired electrons. When an unpaired electron is placed in a magnetic field (H_0), the magnetic moment of the electron tends to precess about H_0 . If a second alternating magnetic field (H_1) is provided at right angles to H_0 , one may disturb the motion of the dipole. If the frequency of the alternating field ($H_1 \ll H_0$) is just equal to the precessional frequency of the dipole, resonance transfer of energy occurs between the en-

ergy source of H_1 and the system of precessing dipoles; there is absorption of energy. In a conventional description, ΔE , the energy difference between the initial and the final energy levels, equals $g\beta H_0$, where β is Bohr magneton, and g is the spectroscopic splitting factor (for a free electron $g = 2.00229$). Thus, the quantum energy of the microwave field, $h\nu$ ($h = \text{Planck's constant}$, $\nu = \text{frequency}$) = $g\beta H_0$. Since g is a property of the unpaired electron in its environment, it is a characteristic of the free radical or paramagnetic species under observation. In ESR, one usually obtains spectra by plotting $\Delta A/\Delta H$ as a function of magnetic field at a fixed frequency.

Free Mn^{2+} ions in an aqueous environment give rise to a characteristic six-line ESR spectrum at room temperature. The six lines result from the hyperfine interactions between the unpaired d electrons and the nuclear magnetic moment. However, Mn in an asymmetric environment, such as in a macromolecular complex, usually does not have an ESR signal at room temperature since g tensor anisotropies lead to extreme broadening of the signal. Thus, it has usually been observed that bound Mn is ESR silent. There are reports, however, of ESR signals arising from Mn bound in macromolecular complexes [17]. Theoretically, such signals may arise from the spin quantum number $M = -1/2 \leftrightarrow +1/2$ transition providing certain symmetry requirements are met. In these cases, the ESR signal is only about 1/4 as large as the signal of the free ion.

NMR [18]

Dipolar interactions with unpaired electrons in paramagnetic ions produce strong relaxing forces on the magnetic state transition of neighboring nuclei. For the case of hydrogen nucleus (protons), Mn^{2+} is perhaps the most efficient relaxer within the second row transition ions. By comparison, high spin Fe and Mn^{3+} are relatively poor proton relaxers. The increase in the proton relaxation rates in Mn^{2+} macromolecular complexes over Mn^{2+} solutions may be as high as 10-15 fold. In the situation of bound Mn^{2+} , the electron-nuclear dipolar interactions are governed by the electron spin relaxation of the Mn^{2+} ion itself rather than by rapid translations and rotational motions of the Mn^{2+} complex. Although the paramagnetic effect is maximal on protons bound in the first coordination sphere of the Mn^{2+} ion, it is experienced by all of the protons in the sample through rapid proton exchange processes and/or energy exchange via spin flip-flop mechanisms. Thus, proton relaxation rate measurements provide an ideal way to monitor bound Mn^{2+} ions. Changes in the proton re-

laxation rates may reflect changes in the concentration of Mn^{2+} ions as well as changes in proton exchange rates or in the accessibility of the bound Mn^{2+} ions to the solvent proton.

A description of the NMR experiment for the measurement of proton relaxation rate is completely analogous to the ESR experiment. The sample is placed in a strong magnetic field \bar{H}_0 along which the nuclear dipoles tend to precess. Similar to ESR, the angular frequency of precession is proportional to the applied field ($\omega = \gamma\bar{H}_0$ where $\gamma = g\beta/\hbar$). A second field, \bar{H}_1 , at the resonance frequency is applied at right angles to \bar{H}_0 causing the nuclear dipoles to flip between spin states. However, \bar{H}_1 is applied as a brief pulse with sufficient power to cause an inversion of the nuclear spin states. After \bar{H}_1 is turned off, the system tends to relax back to its original equilibrium distribution. There are two types of magnetic relaxation rates: the longitudinal or spin lattice relaxation rate (R_1) and the transverse or spin-spin relaxation rate (R_2). R_1 refers to the rate of build-up of the nuclear magnetization vector along the direction of \bar{H}_0 while R_2 refers to the decay of the component vector orthogonal to \bar{H}_0 . It is always true that $R_2 \geq R_1$. The measurements of R_1 and R_2 for protons are most conveniently done in pulsed NMR instruments. The usual procedure for R_1 is the inversion recovery method while for R_2 the Carr-Purcell spin echo method using the Meiboom-Gill modification is usually employed [see ref. 19].

ESR MEASUREMENTS

Observations on Manganese in Thylakoids

In untreated thylakoids, a small but significant ESR signal is usually observed. Siderer et al. [20] concluded, on the basis of an analysis of the amplitude of the Mn^{2+} ESR signals between normal and acid-treated thylakoids, that the Mn^{2+} signal in untreated chloroplasts is due to bound Mn in an asymmetric environment. This Mn^{2+} signal decreased in light and reappeared in dark although the kinetics were slow. They suggested that the photooxidation of the Mn^{2+} is probably related to the Mn^{2+} involved in the water oxidation side of PSII. In contrast, Theg and Sayre [21] suggested that the Mn^{2+} ESR signal, observed by Siderer et al., arises from Mn associated with thylakoids which can be removed from the membrane by the addition of other divalent cations, or by chelation with EDTA. Khanna et al. [22,23] have shown that treatment of thylakoids with low concentrations of $MgCl_2$ produces ESR detectable Mn^{2+} ("free" Mn^{2+}) (also see ref. 24) without affecting O_2 evolution rates. Thus, it is likely that

the Mn^{2+} signal, observed by Siderer et al., is due to very loosely bound Mn^{2+} associated with thylakoids that becomes ESR detectable by preparation methods and that the photo-induced changes arise from a nonspecific oxidation of Mn^{2+} in thylakoid membranes.

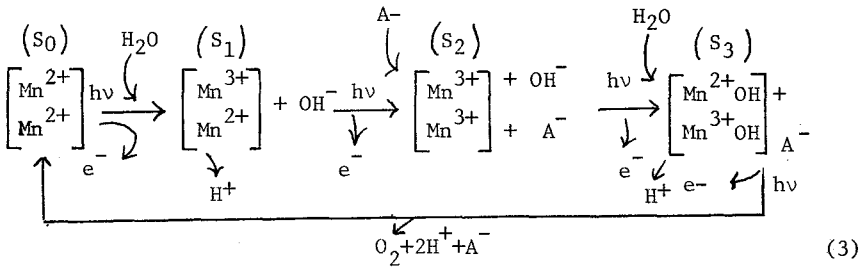
Location of LBMn Toward the Inside of the Thylakoid Vesicle

Upon inactivation of O_2 evolution by Tris, hydroxylamine, heat, etc. the Mn^{2+} ESR signal increases 4-10 fold. Blankenship et al. [25] quantitated the Mn^{2+} ESR signal with O_2 activity and confirmed the relationship between LBMn and O_2 evolution. Upon reactivation of O_2 evolution in Tris-treated thylakoids, using reduced DCIP, the Mn^{2+} ESR signal disappeared. Blankenship and Sauer [26] also found that the Mn^{2+} ESR signal cannot be washed out of the sample and that only half can be removed after sonication. The above experiments led them to conclude that the LBMn associated with O_2 evolution is located towards the inside of the thylakoid vesicle. This location of LBMn is also suggested by the following evidence: (a) the very low inhibition of O_2 evolution by an antiserum against an Mn containing protein functionally located on the O_2 evolving side of PSII [27-29]; and (b) the lack of enhancement effects on the Oxygen 17 NMR relaxation rates by thylakoids unless the vesicular structure is destroyed by detergents [14]. For a general model on the location of PSII intermediates, see Trebst [30].

Observations on Manganese After Heat Treatment of Thylakoids Following Exposure of Thylakoids to Light Flashes

Wydrzynski and Sauer [31], using ESR measurements on heat-extractable Mn^{2+} from thylakoids exposed to light flashes, have provided the best evidence to date for a dynamic role of Mn in O_2 evolution. After a series of light flashes, the amplitude of the ESR detectable Mn^{2+} released after heat treatment oscillated with a period of four with peaks after the 3rd and 7th flashes (Fig. 1). The detailed pattern was almost identical to flash-induced changes in the proton relaxation rates (R_2) observed by Wydrzynski et al. [32] and Govindjee et al. [33,34]. Analysis of these results suggested that although Mn is oxidized in steps S_0 to S_1 and S_1 to S_2 , it is reduced in the next step S_2 to S_3 , a possibility suggested earlier by Marks et al. [35] and Govindjee et al. [33].

Wydrzynski and Sauer [31] presented the following scheme.



where A⁻ may be a chloride ion.

Here, protons are released as follows 1, 0, 1, 2 during S₀→S₁, S₁→S₂, S₂→S₃, and S₃→S₀ conversions in accordance with the H⁺ release data of Fowler [36] and of Saphon and Crofts [37], but not of Junge *et al.* [38]. Since the chemistry is still completely unknown, the model can, however, be easily modified to match with Junge *et al.*'s data.

Govindjee [39] and Govindjee *et al.* [34] had earlier presented working models of Mn²⁺ that also suggested that water donates electrons to the "S" intermediates in reactions prior to the release of O₂, and that the S₃ state has more atoms of reduced Mn (Mn²⁺) than S₂ or S₁ states; this model, however, had 4 Mn atoms per O₂ evolving complex.

NMR MEASUREMENTS

Wydrzynski *et al.* [40] were the first to apply the technique of water proton relaxation rate (PRR) measurements to thylakoid membranes. Analysis of the field dispersion curves yielded relaxation parameters and correlation times characteristics for Mn²⁺ and, thus, it was concluded that the PRR is sensitive to membrane bound Mn [14,41]. Since reductants (tetraphenylboron, TPB, and NH₂OH) increased the PRR and oxidants (ferricyanide

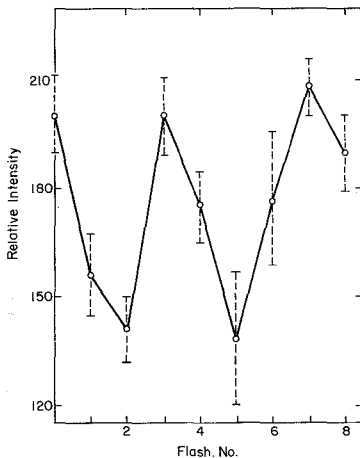


Fig. 1. Amplitudes of Mn-ESR signals from Heat Treated Thylakoids Following Exposure of Membranes to Different Number of Light Flashes. (After Wydrzynski and Sauer [31].)

and dichlorophenolindophenol) decreased it, it was concluded that Mn exists as a mixture of oxidizing states in dark-adapted chloroplasts [14,40]. The antagonistic effects of oxidants and reductants in the PRR were confirmed by Gribova *et al.* [42]; these effects occurred with PS II particles but not with PS I particles or light harvesting complexes. Recent X-ray absorption edge measurements of Mn in dark adapted chloroplast also suggest that the Mn exists in two oxidation states, probably +2 and +3 [for a brief discussion, see ref. 43].

The complexity in the interpretation of the NMR measurements was pointed out by Wydrzynski *et al.* [41]. However, three lines of experimental results were obtained to suggest that PRR monitors LBMn associated with O_2 evolution (1) in tris-acetone extracted chloroplasts (from which the LBMn is totally removed from the sample) the PRR was low, independent of the field, and insensitive to the effects of oxidants and reductant [14]; (2) parallel measurements of PRR, O_2 and Mn content using $MgCl_2$ extracted samples gave a correlation between O_2 , PRR and Mn [14]; and (3) in a series of μs flashes, the R_2 was found to oscillate with a period of four with peaks after the 3rd and 7th flashes [32-34]. As Mn is replaced by Mg both R_1 and R_2 linearly decrease up to a certain level of Mn content, R_2 extrapolates to 20% of the original value and R_1 reaches the same 20% value when Mn level reaches about 45% of the original Mn content [14]. A plot of R_1 and R_2 versus rate of O_2 evolution showed identical behavior to the Mn content to about 45% O_2 level, but, then R_2 became independent of the rate of O_2 evolution. The parallel relationship suggested that PRR is correlated with O_2 and Mn. However, the independence of R_2 on O_2 evolution at low O_2 rates suggests complexity in the relationship with O_2 evolution, although the relationship with Mn was still present. The 20% R_1 is clearly from a source other than Mn and about 50% R_2 seems also to be from a source other than Mn.

The period 4 oscillations in R_2 was observed in several experiments in pea thylakoids at pH 6.7 and pH 7.5 [33,34]. Tris-acetone extracted thylakoids and diuron-treated thylakoids showed no oscillations. However, it is important to point out that the "uncoupling" of the oscillations from O_2 evolution was also noticed. Oscillations with peaks at 2nd and 5th (instead of 3rd and 7th) flashes were observed when reductants like TPB and NH_2OH or CCCP were used; in these samples, O_2 evolution was inhibited. Thus, R_2 may monitor more than just the final O_2 evolving steps. Unfortunately, in many experiments several of our coworkers (R. Khanna, S. Rajan

and Wm. Coleman) have been unable to observe light induced oscillations in R_2 . In the original measurements, when the oscillations were observed, the effects were obvious. An example of a plot of magnetization (labeled as NMR signal here) as a function of time is shown in Fig. 2. In tris-acetone extracted thylakoids, there was no difference in R_2 (calculated from the slope) whether dark-adapted or light-exposed (1, 2, 3 or 4 flashes) membranes were in the NMR cavity. However, when untreated membranes were used, R_2 changed with flash number as shown. We do not know yet the reasons for the negative results. One possibility is that the R_2 is sensitive to Mn^{2+} ions not directly involved in O_2 evolution or to microconformational changes which are modulated by the O_2 evolving apparatus. These conditions may vary with different sample preparations. Since R_2 contains terms for nuclear dipolar interaction, it may be influenced by relaxation mechanisms other than the inner sphere paramagnetic interactions.

In an entirely different interpretation of the PRR, Robinson *et al.* [44] have suggested that the entire effect by thylakoids arises from extraneously bound Mn (or VLBMn) not related to O_2 evolution since, in EDTA

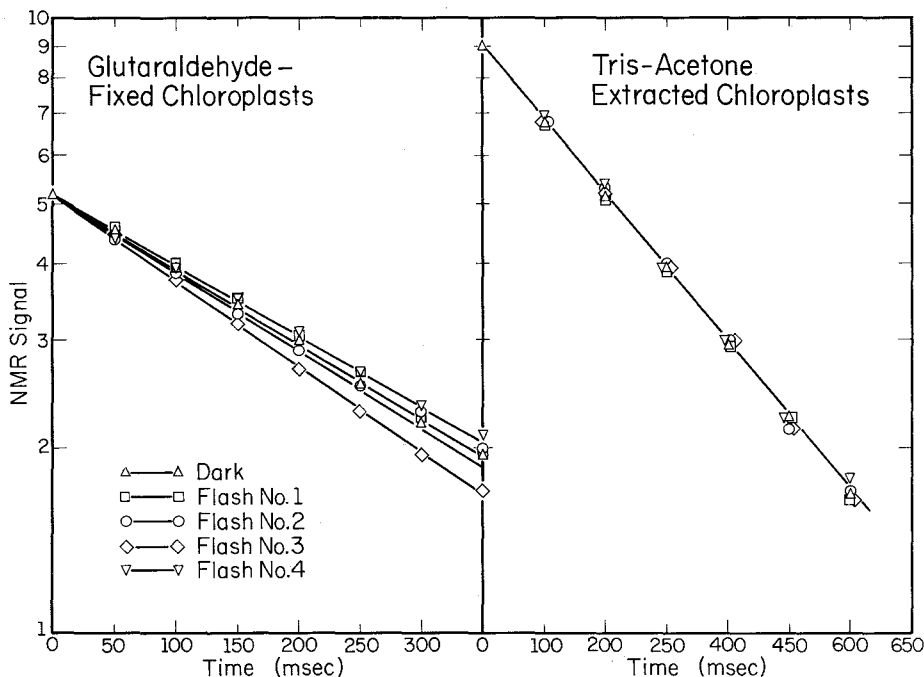


Fig. 2. Magnetization as a Function of Time from Untreated and Tris-Acetone Treated Thylakoid Membranes. Data for dark-adapted thylakoids and those exposed to 1, 2, 3, or 4 flashes are shown (after T. Wydrzynski).

treated samples, the PRR shows no field dependence or light induced changes, although O_2 activity is relatively unaffected. We have observed [45], however, that EDTA has a complex effect on the PRR. In the presence of EDTA the PRR is low, but after thorough washing of the thylakoids the PRR increases (see Table 1) and the effects of reductants and of continuous light reappear. Total Mn concentrations are unaffected whether samples are washed with EDTA present or not. It may be that the EDTA somehow affects the proton exchange rates or accessibility of bound Mn^{2+} ions to the solvent water. If the samples of Robinson *et al.* do not contain any residual EDTA then an explanation for the discrepant results is difficult.

NMR, ESR and O_2

Khanna *et al.* [22,23,46] initiated parallel measurements on NMR (membrane-bound Mn^{2+}), ESR (free Mn^{2+}) and O_2 evolution. From such studies, the following conclusions were made (for further details, see Khanna, Rajan, Govindjee and Gutowsky, this conference): (1) addition of 1-20 mM $MgCl_2$ to thylakoid membranes decreases R_2 , and increases ESR Mn^{2+} signal, without affecting O_2 evolution as already noted above: this is due to the release of VLB Mn unrelated to O_2 evolution; (2) removal of Mn by aging at 35°C, however, causes a parallel decrease in O_2 evolution and R_2 accompanied by an increase in ESR Mn^{2+} signal: this supports the conclusion that the loosely bound pool of Mn related to O_2 evolution may influence R_2 ; (3) addition of TPB increases R_2 in steps, decreases O_2 evolution monotonically, without any loss of Mn: the full interpretation of these data awaits fur-

TABLE 1. R_1^* AND R_2^* OF CONTROL AND EDTA-TREATED PEA THYLAKOIDS (AFTER WYDRZYNSKI *et al.* [45])

No. of washes	CONTROL		EDTA-TREATED	
	R_1	R_2	R_1	R_2
After homogenization**	0.976	3.772	0.244	2.392
1***	0.628 \pm 0.132	3.476 \pm 0.054	0.287 \pm 0.050	2.455 \pm 0.138
2	0.552 \pm 0.068	3.127 \pm 0.042	0.616 \pm 0.066	2.664 \pm 0.192
3	0.490 \pm 0.050	3.096 \pm 0.022	0.539 \pm 0.039	3.033 \pm 0.196

* Rates are corrected with buffer ($R_1(\text{buffer}) = 0.544$; $R_2(\text{buffer}) = 0.75$) and normalized to 3 mg Chl/ml

** For controls, thylakoids were homogenized in Sucrose-HEPES-NaCl (SHN) buffer, filtered, centrifuged and suspended in 5 ml of SHN; for EDTA-treated samples, the homogenization step included 2 mM EDTA

*** Each wash was done in a 40 ml volume

ther analysis and evaluation although it is clear that a portion of R_2 , unrelated to O_2 evolution, is also increased by TPB; and (4) addition of NH_2OH has, at least, 2 effects: (a) an increase in R_2 (reduction of bound Mn) at concentrations known to reduce "S" states and (b) release of Mn^{2+} at higher concentrations (R_2 decreases, ESR Mn^{2+} signal increases and O_2 evolution decreases). The existence of tightly bound Mn^{2+} is suggested by the continued presence of a contribution to PRR even after the loosely bound Mn^{2+} is removed by high concentrations of NH_2OH . It is quite likely that the tightly bound Mn^{2+} pool is included in the Mn^{2+} found in light harvesting Chl a/Chl b complex preparation (it is about one-third of the Mn found in washed thylakoids).

CONCLUDING REMARKS

The mechanism of the oxidation of water to molecular O_2 in photosynthesis is still obscure. It is known that four positive charges accumulate on the water side before O_2 is evolved and that manganese is somehow involved in it. Attempts, using magnetic resonance techniques, have been made in the hope to learn more about the function of manganese in O_2 evolution. From both ESR and NMR measurements, a dynamic role of manganese in O_2 evolution is suggested. Even this conclusion remains far from proven as the results are complex and sometimes discrepant. Our research group in Illinois has observed several positive correlations between the loosely bound Mn, related to O_2 evolution, with water proton relaxation rates (PRR). Under several experimental conditions, however, we have also observed non or negative correlations between PRR and O_2 evolution. In contrast, the research group in Michigan has not found any positive correlation between the Mn associated with O_2 evolution and the proton relaxation rate. Further experiments with this and other techniques are necessary to understand the functional role of Mn in O_2 evolution. Parallel measurements on NMR, ESR, Mn content and O_2 evolution still remain the best hope for unravelling redox changes in Mn associated with O_2 evolution.

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REFERENCES

- 1 Velthuys, B.R. (1980) *Annl. Rev. Pl. Physiol.* 31, 545-567
- 2 Govindjee (1980) *Pl. Biochem. J. (India) Sircar Memorial Vol.* 7-30
- 3 Radmer, R. and Cheniaie, G. (1977) *Topics in Photosynthesis* 2, 303-348, Elsevier, Amsterdam
- 4 Diner, B.A. and Joliot, P. (1977) *Photosynthesis I*, pp. 187-205, Springer, Berlin
- 5 Kok, B. and Joliot, P. (1975) *Bioenergetics of Photosynthesis*, pp. 387-412, Academic Press, New York
- 6 Mar, T. and Govindjee (1972) *J. Theoret. Biol.* 36, 427-446
- 7 Clarkson, D.T. and Hanson, J.B. (1980) *Annl. Rev. Pl. Physiol.* 31, 239-298
- 8 Lawrence, G.T. and Sawyer, T.D. (1978) *Coordination Chem. Revs.* 27, 173-193
- 9 Harriman, A. and Barber, J. (1979) *Topics in Photosynthesis* 3, 243-280, Elsevier, Amsterdam
- 10 Lonergan, J.F. (1975) *Trace Elements in Soil-Plant-Animal Systems*, pp. 109-134, Academic Press, New York
- 11 McClendon, J.H. (1976) *J. Mol. Evol.* 8, 175-195
- 12 Moore, D.P. (1972) *Micronutrients in Agriculture*, pp. 171-198, Soil Sci. Society, Madison
- 13 Triffin, L.O. (1972) See Ref. 12, pp. 199-229
- 14 Wydrzynski, T., Marks, S.B., Schmidt, P.G., Govindjee and Gutowsky, H.S. (1978) *Biochemistry* 17, 2155-2162
- 15 Cotton, F.A. and Wilkinson, C. (1966) *Advanced Inorganic Chemistry: A Comprehensive Text*, 2nd Ed., pp. 834-847, Wiley, New York
- 16 Garrett, B.B. and Morgan, L.O. (1966) *J. Chem. Phys.* 44, 890-897
- 17 Meirovitch, E. and Poupko, R. (1978) *J. Chem. Phys.* 82, 1920-1925
- 18 Dwek, R.A. (1973) *Nuclear Magnetic Resonance (NMR) in Biochemistry: Applications to Enzyme Systems*, Clarendon Press, Oxford
- 19 Farrar, T.C. and Becker, E.D. (1971) *Pulse and Fourier Transform NMR: Introduction to Theory and Methods*, Academic Press, New York
- 20 Siderer, Y., Malkin, S., Poupko, R. and Luz, Z. (1977) *Arch. Biochem. Biophys.* 179, 174-182
- 21 Theg, S.M. and Sayre, R.T. (1979) *Pl. Sci. Lett.* 16, 319-326
- 22 Khanna, R. (1980) Ph.D. Thesis in Biology, University of Illinois at Urbana-Champaign
- 23 Khanna, R., Rajan, S., Steinback, K.E., Bose, S., Govindjee and Gutowsky, H.S. (1980) Submitted for publication
- 24 Gribova, Z.P. and Tikhonov, A.N. (1977) *Biofizika* 22, 651-655
- 25 Blankenship, R.E., Babcock, G.T. and Sauer, K. (1975) *Biochim. Biophys. Acta* 387, 165-175
- 26 Blankenship, R.E. and Sauer, K. (1974) *Biochim. Biophys. Acta* 357, 252-266

- 27 Braun, B.Z. (Zilinskas) and Govindjee (1972) FEBS Lett. 25, 143-146
- 28 Zilinskas, B. and Govindjee (1974) Pl. Sci. Lett. 3, 219-227
- 29 Zilinskas, B.A. (1975) Ph.D. Thesis in Biology, University of Illinois at Urbana-Champaign
- 30 Trebst, A. (1974) Ann. Rev. Pl. Physiol. 25, 423-458
- 31 Wydrzynski, T. and Sauer, K. (1980) Biochim. Biophys. Acta 589, 56-60
- 32 Wydrzynski, T., Zumbulyadis, N., Schmidt, P.G., Gutowsky, H.S. and Govindjee (1976) Proc. Nat. Acad. Sci., U.S.A. 73, 1196-1198
- 33 Govindjee, Wydrzynski, T. and Marks, S.B. (1977) Bioenergetics of Membranes, pp. 305-316, Elsevier, Amsterdam
- 34 Govindjee, Wydrzynski, T. and Marks, S.B. (1978) Photosynthetic Oxygen Evolution, pp. 321-344, Academic Press, London
- 35 Marks, S.B., Wydrzynski, T., Govindjee, Schmidt, P.G. and Gutowsky, H.S. (1978) Biomolecular Structure and Function, pp. 95-100 Academic Press, New York
- 36 Fowler, C.F. (1977) Biochim. Biophys. Acta 462, 414-421
- 37 Saphon, S. and Crofts, A.R. (1977) Z. Naturforschg. 32c, 617-626
- 38 Junge, W., Renger, G. and Ausländer, W. (1977) FEBS Lett. 79, 155-159
- 39 Govindjee (1978) Nat. Acad. Sci. Letters (India) 1,3-6
- 40 Wydrzynski, T., Zumbulyadis, N., Schmidt, P.G. and Govindjee (1975) Biochim. Biophys. Acta 408, 349-354
- 41 Wydrzynski, T., Govindjee, Zumbulyadis, N., Schmidt, P.G. and Gutowsky, H.S. (1976) Amer. Chem. Soc. Symp. Series 34, 471-482
- 42 Gribova, A.P., Zakhorova, N.I. and Murza, L.I. (1978) Molekulyarnaya Biologia 12, 157-164
- 43 Sauer, K. (1980) Accounts of Chemical Research, in press
- 44 Robinson, H.H., Sharp, R.R. and Yocum, C.F. (1980) Biochem. Biophys. Res. Commun. 93, 755-761
- 45 Wydrzynski, T., Govindjee and Gutowsky, H.S. (1980) Abstract, this conference; paper in preparation
- 46 Khanna, R., Rajan, S., Govindjee and Gutowsky, H.S. (1980) paper in preparation