Regular paper

Mn²⁺ reduces Y_Z^+ in manganese-depleted Photosystem II preparations

Curtis W. Hoganson,[1,2] Demetrios F. Ghanotakis,[3,4] Gerald T. Babcock[1] & Charles F. Yocum[3]
[1] *Department of Chemistry, Michigan State University, East Lansing, MI 48824-1322, U.S.A.;* [2] *Current address: Department of Biochemistry and Biophysics, Chalmers Institute of Technology, S-41296 Goteborg, Sweden;* [3] *Division of Biological Sciences, the University of Michigan, Ann Arbor, MI 48109-1055, U.S.A.;* [4] *Current address: Department of Chemistry, University of Crete, Ihraklion, Crete, Greece*

Abstract

Manganese in the oxygen-evolving complex is a physiological electron donor to Photosystem II. PS II depleted of manganese may oxidize exogenous reductants including benzidine and Mn²⁺. Using flash photolysis with electron spin resonance detection, we examined the room-temperature reaction kinetics of these reductants with Y_Z^+, the tyrosine radical formed in PS II membranes under illumination. Kinetics were measured with membranes that did or did not contain the 33 kDa extrinsic polypeptide of PS II, whose presence had no effect on the reaction kinetics with either reductant. The rate of Y_Z^+ reduction by benzidine was a linear function of benzidine concentration. The rate of Y_Z^+ reduction by Mn²⁺ at pH 6 increased linearly at low Mn²⁺ concentrations and reached a maximum at the Mn²⁺ concentrations equal to several times the reaction center concentration. The rate was inhibited by K⁺, Ca²⁺ and Mg²⁺. These data are described by a model in which negative charge on the membrane causes a local increase in the cation concentration. The rate of Y_Z^+ reduction at pH 7.5 was biphasic with a fast 400 μs phase that suggests binding of Mn²⁺ near Y_Z^+ at a site that may be one of the native manganese binding sites.

Abbreviations: PS II – Photosystem II, Y_D – tyrosine residue in Photosystem II that gives rise to the stable Signal II EPR spectrum, Y_Z – tyrosine residue in Photosystem II that mediates electron transfer between the reaction center chlorophyll and the site of water oxidation, ESR – electron spin resonance, DPC – diphenyl-carbazide, DCIP – dichlorophenolindophenol

Introduction

The oxygen-evolving complex of PS II is comprised of four manganese atoms, along with some calcium and chloride ions and three water-soluble polypeptides: the association of these components with membrane-bound polypeptides of PS II produces the complete assembly required for oxygen evolution. Knowledge of the arrangement of these components is limited. There is some information about the binding of the polypeptides to one another (for reviews, see Babcock 1987, Andréasson and Vänn-

gaård 1988, Babcock et al. 1989), there are XAFS data suggesting manganese-ligand distances in the complex (Yachandra et al. 1986, Sauer et al. 1988, George et al. 1989, Penner-Hahn et al. 1989), and a pair of ESR signals associated with the S2 state have been detected (Dismukes and Siderer 1981, Casey and Sauer 1984, Zimmerman and Rutherford 1984) and characterized in some detail (Hansson et al. 1987). In the functional assembly, oxidizing equivalents are generated by photooxidation of a specialized chlorophyll, P_{680}, and are subsequently transferred to manganese and water by way of a

redox active tyrosine, Y_Z (Barry and Babcock 1987).

When the oxygen-evolving complex is inactivated, typically the manganese is released from the complex. Under these conditions, the normal electron donor to Y_Z^+ is absent, and the lifetime of the Y_Z^+ radical is considerably lengthened. Exogenous reductants can react with Y_Z^+ and thereby decrease its lifetime. The change in the lifetime of Y_Z^+ can be measured with time-resolved ESR spectroscopy and indicates the rate of reaction of the exogenous reductant with Y_Z^+. In this paper, we report the results of our investigations on the ability of Mn^{2+} to react with Y_Z^+.

Materials and methods

PS II membranes were isolated from spinach (Berthold et al. 1981, Ghanotakis et al. 1984). Tris inhibited membranes were prepared by suspending the preparation in 0.8 M Tris-HCl, 50 mM Na_2 EDTA, pH 8.0 for 30 min under room light, followed by three washings with SMN (400 mM sucrose, 50 mM MES, pH 6.0 and 15 mM NaCl), except where noted in the text. Membranes retaining the 33 kDa extrinsic polypeptide were prepared by incubating intact PS II membranes with 2 M NaCl, pelleting and resuspending in SMN, incubating with 1 mM hydroquinone and 1 mM ascorbic acid in the dark, and rewashing with SMN. The presence or absence of the 33 kDa polypeptide was confirmed by SDS polyacrylamide gel electrophoresis. For the experiments directly comparing the membranes with and without the 33 kDa polypeptide, care was taken that the samples were washed an equal number of times.

Time-resolved ESR measurements were made by using a Bruker ER200D spectrometer as described previously (Hoganson and Babcock 1988). 17 μs xenon flashes excited the sample. 4.4 Gauss field modulation at 100 kHz and 20 mW microwave power were used. The magnetic field of the spectrometer was set to the low field peak of the Y_D^+ signal (Babcock and Sauer 1975, Hoganson and Babcock 1988). Y_D^+ is stable on the minutes time scale in these tris-washed preparations, even in the presence of Mn^{2+} at the concentration levels used in the experiments described below. Moreover, the low field peak in the Y_D^+/Y_Z^+ spectrum does not

overlap the narrower $g = 2$ signals that arise from chlorophyll radicals. Thus we attribute the kinetic traces reported here to oxidation/reduction reactions of the Y_Z species. Each measurement of the Y_Z^+ lifetime is the average of between 200 and 500 flashes, depending on the time constant used. Equimolar ferri-/ferrocyanide was present as a redox buffer to prevent net oxidation or reduction of the reactants.

Results

Flashes of light given to Tris-washed PS II membranes induce the ESR signal of Y_Z^+ with a maximal spin concentration essentially stoichiometric with the PS II concentration and a decay time of about 20 ms (Fig. 1a). Addition of 100 μM $MnCl_2$ to a suspension of PS II membranes causes a decrease in the lifetime of the Y_Z^+ radical observed after repetitive flashes (Fig. 1b) but no change in the signal
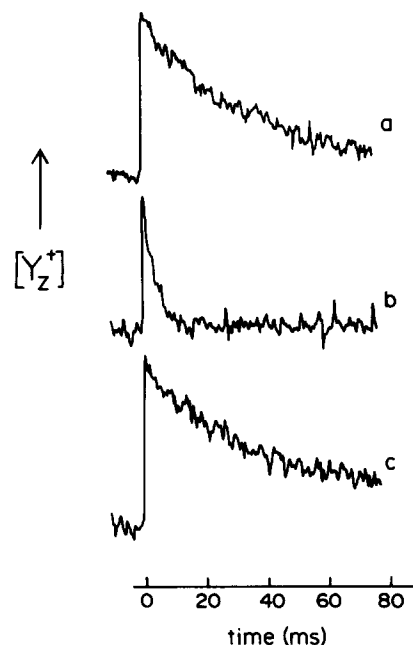

Fig. 1. Kinetic ESR traces of the signal of Y_Z^+ induced by flashes of light given to PS II membranes at pH 6.0. 200 flashes were averaged. 20 mW microwave power and 4.4 Gauss field modulation were used. All three samples contained Tris-washed PS II membranes at 2 mg Chl/ml, 1 mM $K_3Fe(CN)_6$, 1 mM $K_4Fe(CN)_6$ in SMN. (a) no further additions, (b) 100 μM $MnCl_2$, (c) 100 μM $MnCl_2$ and 1.9 mM Na_2 EDTA.

amplitude. Addition of 1.9 mM EDTA abolishes the effect of Mn^{2+} on the lifetime of Y_Z^+ (Fig. 1c). For a suspension of PS II membranes with a chlorophyll concentration of 2 mg/ml and containing 1 mM $K_3Fe(CN)_6$ and 1 mM $K_4Fe(CN)_6$ at pH 6.0, the decay of the Y_Z^+ signal approximately follows first-order kinetics (Fig. 1b). This set of experiments shows that aqueous Mn^{2+} can reduce Y_Z^+ but does not compete with Y_Z in its reaction with P_{680}^+. Mn^{2+} chelated by EDTA does not react with Y_Z^+. The similarity of kinetics in 1a and 1c also demonstrates that the membranes contain very little loosely bound manganese.

The apparent first-order rate constant is plotted against the concentration of added Mn^{2+} in Fig. 2. The graph shows a linear region for Mn^{2+} concentrations less than 50 μM, suggestive of a second-order mechanism in which the reaction rate is determined by diffusion of Mn^{2+} to Y_Z^+. The slope of this line yields an apparent second-order rate constant. At higher Mn^{2+} concentrations the graph shows a leveling off of the rate and a slight decrease in the rate between 100 and 200 μM, as if substrate inhibition were occurring (Cornish-Bowden 1979). The bend in the curve occurs at about 50 μM, which is five times the PS II reaction center concentration in the sample (ca. 10 μM). A comparable experiment performed with a salt-washed, hydroquinone-treated sample with the same chlorophyll concentration gave identical results (Table 1). Because the presence of the 33 kDa polypeptide in experi-

Table 1. Effect of the 33 kDa a polypeptide on apparent second-order rate constants for Y_Z^+ reduction*

Donor	33 kDa	Rate constant ($10^6 M^{-1} s^{-1}$)
Mn^{2+}	−	6.0
Mn^{2+}	+	6.1
Benzidine	−	3.2
Benzidine	+	3.0

* Rates were measured with 2 mg Chl/ml, 1 mM $K_3Fe(CN)_3$, 1 mM $K_4Fe(CN)_6$ in SMN, pH 6.0 with flashes at 0.25 Hz. [Mn^{2+}] from 0–20 μM and [Benzidine] from 0–40 μM were used.

ments conducted with hydroquinone-treated samples has no effect on the lifetime of Y_Z^+, we conclude that this polypeptide does not hinder access of Mn^{2+} to Y_Z^+. In a sample with 3.6 mg Chl/ml the bending region occurred at about 90 μM $MnCl_2$ (Fig. 2b), which is again about five times the reaction center concentration. That the maximum rate is achieved at a constant Mn^{2+}/PS II ratio, rather than at a constant total Mn^{2+} concentration, can be explained if most of the Mn^{2+} in these experiments is associated with the membranes. Such association is well known (Amesz 1984), is of an electrostatic nature (see below), and is substantiated by the greatly diminished amplitude of the aqueous Mn^{2+} six-line spectrum (data not shown) in these membrane suspensions. If the added Mn^{2+} is associated with the membrane surface, diffusion on that surface would determine the kinetic parameters and be determined by the ratio of moles of Mn^{2+} to mem-
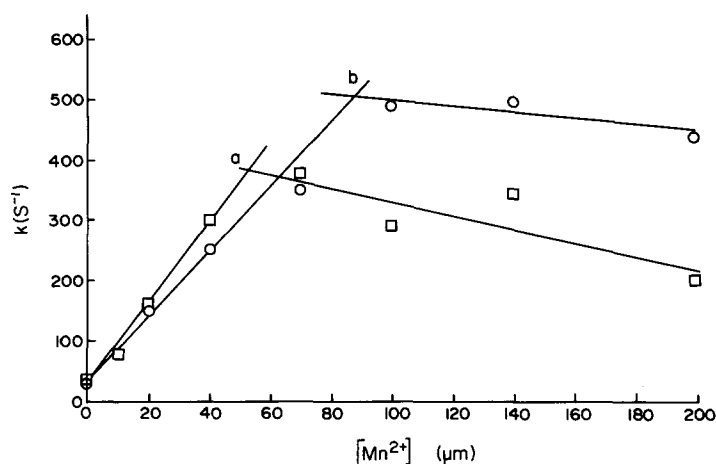

Fig. 2. Plot of pseudo-first-order decay rate constants of the Y_Z^+ signal versus Mn^{2+} concentration. (a) Tris-washed membranes were used at 2.0 mg Chl/ml; (b) NaCl/hydroquinone-treated membranes were used at 3.6 mg Chl/ml. Both samples contained 1 mM $K_3Fe(CN)_6$ and 1 mM $K_4Fe(CN)_6$ in SMN, pH 6.0.

brane surface area. The particular ratio of $Mn^{2+}/$ PS II that gave the highest reaction rate in these experiments probably has no particular significance. It is determined by the convolution of the two functions that represent the second order reaction of Mn^{2+} with Y_Z^+ and the process that results in the decreasing rate at higher Mn^{2+} concentrations, respectively. In particular, there is no reason to suppose that it is related to the fact that four manganese atoms per PS II are required for oxygen evolution.

The concentration dependence of the Mn^{2+}/Y_Z^+ reaction is in marked contrast to the concentration dependence of the reaction of benzidine with Y_Z^+ (Table 1). With benzidine, the pseudo-first-order rate is proportional to the benzidine concentration, at least up to $150\,\mu M$. Thus the reaction with benzidine follows a second-order rate law (see also Yerkes and Babcock 1980). The presence of the 33 kDa polypeptide has no effect on the $Y_Z^+/$ benzidine reaction rate.

To investigate further the mechanism of the Mn^{2+}/Y_Z^+ reaction, we varied the concentrations of $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$, keeping the proportions the same. Increased concentrations of these compounds decreased the second-order rate constant; lower concentrations had the opposite effect (Table 2). $CaCl_2$ and $MgCl_2$, in the millimolar concentration range, also cause a decrease in the slope of the initial part of the rate vs. Mn^{2+} curves (Table 3) and the maximum rate of Y_Z^+ reduction. The data of both Tables 2 and 3 can be rationalized by a simple model. It is known that thylakoid membranes bear a net negative charge at pH 6 (evidence reviewed by Barber 1980) particularly in the neighborhood of Y_Z (Yerkes and Babcock

1981, Conjeaud and Mathis 1986). This surface charge causes an increase in the local concentration of cations, especially of multivalent cations (Barber 1980) like Mn^{2+}. Addition of other cations to the solution partially displaces Mn^{2+} from the volume near Y_Z. This effect depends on cations rather than on all ions: the 'cationic strength' (that is, the contribution of the cations to the ionic strength), but not the ionic strength, allows the data of Tables 2 and 3 to be described by a single curve (Fig. 3). Plots of the reciprocal of the apparent second-order rate constant versus the concentration of $K_{3/4}Fe(CN)_6$, $CaCl_2$ or $MgCl_2$ give straight lines (not shown) and allow the following inhibition constants for K^+, Mg^{2+} and Ca^{2+} to be calculated: 1.5 mM, 0.4 mM and 0.4 mM, respectively. Competition of cations for the site on the oxidizing side of PS II has also been demonstrated by Inoue and Wada (1987) who found that manganese-catalyzed photooxidation of H_2O_2 by PS II was strongly inhibited by 1 mM Ca^{2+} or Mg^{2+} at pH 6.5.

The data from one experiment, however, do not follow this relation (Table 2, line 6). In this case, 15 mM NaCl and ca. 21 mM NaOH, which were used in preparing the buffer for experiments in Table 2, lines 1–5, were replaced by potassium salts. The Mn^{2+}/Y_Z^+ reaction was strongly affected by this replacement, as if inhibited by K^+, but not Na^+. The product of $[K^+]$ with the second-order rate constant is given to demonstrate the nearly hyperbolic nature of the relationship and to show that the data in line 6 are consistent with the 'cationic strength' model if Na^+ is not included in

Table 2. Effect of $[K^+]$ on apparent second-order rate constants for Y_Z^+ reduction*

K^+ source	$[K^+]$ (mM)	k_2 $(10^6\,M^{-1}s^{-1})$	$[K^+]*k_2^*\,10^{-4}$
$K_{3/4}Fe(CN)_6$	0.7	29	2
	3.5	18	6.3
	7	7.1	5.0
	14	4.1	5.7
	28	2.2	6.2
KCl, MES–KOH	42.7	1.36	5.8

* All samples contained Tris-washed PS II membranes at 2 mg Chl/ml, 400 mM sucrose, 50 mM MES pH 6.0. Flashes at 1 Hz were used.

Table 3. Effect of divalent cations on the apparent second-order rate constant

Cation	Concentration (mM)	k_2 $(10^6\,M^{-1}s^{-1})$
Control	0	6.6
Ca^{2+}	0.01	6.1
	0.05	6.8
	0.1	5.8
	1	4.4
	10	1.9
Mg^{2+}	0.05	6.7
	1	4.2
	2	3.6
	3	2.8

Samples contained Tris-washed PS II membranes at 2 mg Chl/ml, 1 mM $K_3Fe(CN)_6$, 1 mM $K_4Fe(CN)_6$. Flashes at 1 Hz were used. Manganese concentrations from 0–40 μM were used.

Effect of Solution Composition on the Reaction of Mn²⁺ and Y_z⁺ at pH6.0
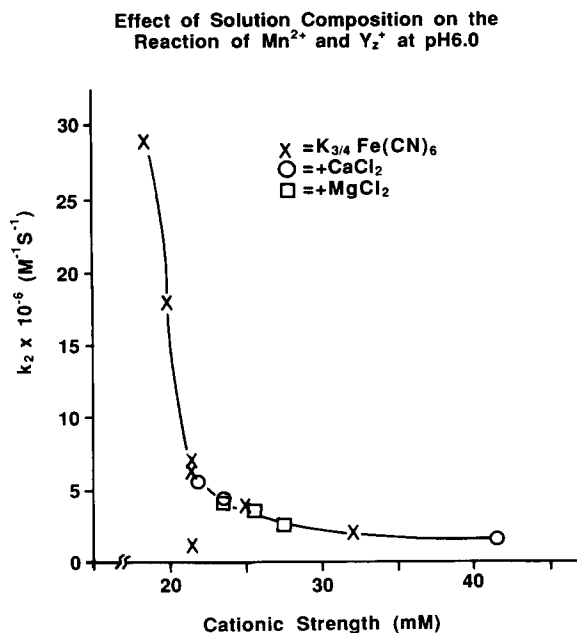

Fig. 3. Plot of apparent second-order rate constants for the reaction of Mn^{2+} with Y_Z^+ versus the 'cationic strength' of the medium. Data are from Tables 2 and 3. Cationic strength is one half the sum of the concentrations of cations in the solution weighted according to the square of the charge of the cation.

the calculation. If Na^+ and K^+ are assumed to compete with Mn^{2+} at a single site (the aqueous volume near Y_Z^+), then Na^+ and K^+ compete with each other, and the first five lines in Table 2 allow us to calculate that the inhibiton constant for Na^+ is at least 30 times that of K^+. The difference in behavior between Na^+ and K^+ is curious, but similar observations have been made previously with PS II preparations. Manganese reconstitution into PS II at pH 8, which required submicromolar Mn^{2+}, was reported to be insensitive to 350 mM Na^+ (Klimov et al. 1982). Stewart and Bendall (1981) found that during dark incubation of PS II particles from *Phormidium laminosum* in either 100 mM NaCl, 100 mM KCl or low ionic strength buffer, oxygen-evolving activity was stabilized by 100 mM NaCl, but was totally lost in 100 mM KCl. The difference in activity between Na^+ and K^+ may result from the difference in hydrated radii of the two ions, Na^+ being larger (Cotton and Wilkinson 1967). The larger size limits its approach to the charged groups on cation exchangers, so binding is weaker (Helfferich 1962). A similar model may explain the ineffectiveness of Na^+ relative to K^+ in

competing with Mn^{2+} for binding sites on the negatively charged PS II membranes.

The lifetime of Y_Z^+ as a function of added Mn^{2+} was also measured at pH 7.5, with $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ both present at 1 mM. The PS II reaction center concentration was about $12 \mu M$. The kinetics of pH 7.5 are distinctly different from those at pH 6.0. The Y_Z^+ signal decays biphasically when $MnCl_2$ concentrations are between 2 and $20 \mu M$ (Fig. 4). The fast phase has a lifetime of about 300–500 μs at all concentrations above 5 μM, while the slow phase becomes faster as the Mn^{2+} concentration increases. The fraction of the signal in the fast phase is maximal by 40 μM and is half-saturated by 10 μM. There was no decline in the rate of the fast phase at 200 μM Mn^{2+}, although at 200 μM Mn^{2+}, the amplitude of the Y_Z^+ signal was noticeably attenuated compared to the amplitude observed with lower Mn^{2+} concentrations. This diminished amplitude may possibly indicate the formation of a manganese species capable of reducing Y_Z^+ at a rate faster than the instrument time resolution.

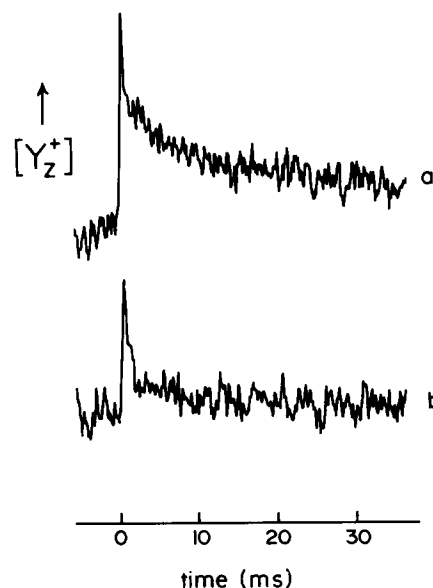These experiments showing biphasic reduction

Fig. 4. Kinetic ESR traces of the signal of Y_Z^+ induced by flashes of light given to PS II membranes at pH 7.5. 20 mW microwave power and 4.4 Gaussfield modulations were used. Both samples contained Tris-washed PS II membranes at 2 mg Chl/ml, 1 mM $K_3Fe(CN)_6$, 1 mM $K_4Fe(CN)_6$ in SMN. The $MnCl_2$ concentration was (a) 5 μM and (b) 20 μM.

kinetics of Y_Z^+ are readily interpreted by a model in which, at pH 7.5, Mn^{2+} binds to one or two sites on PS II, one of which is near Y_Z and allows bound manganese to reduce Y_Z^+. The rate of the faster phase does not depend on the amount of added Mn^{2+}, so its rate is not controlled by diffusion. It therefore indicates reaction at sites occupied by Mn^{2+} before the flash. The amplitude of this faster phase does increase with the amount of added Mn^{2+}, consistent with the above interpretation. That this rate does not change with Mn^{2+} concentration also suggests that Mn at only a single binding site accounts for most or all of the reduction of Y_Z^+. In contrast, the rate of the slower phase does increase with added Mn^{2+}, so it is controlled by diffusion. The slower phase represents reaction at sites where Mn^{2+} is not bound before the flash and its rate is similar to the rates observed at pH 6 for comparable amounts of added Mn^{2+}. The data, of course, do not rule out more complex mechanisms.

Discussion

Divalent manganese has long been known to be oxidized by PS II (Ben-Hayyim and Avron 1979, Izawa 1970, Blankenship 1975). The cofactor of PS II that oxidizes Mn^{2+}, however, has not been well established; earlier work with lower time resolution and less resolved preparations suggested that Y_Z^+ did not oxidize Mn^{2+} (Babcock and Sauer, 1975). The experiments presented here strongly support the idea that the oxidized tyrosine radical, Y_Z^+, oxidizes Mn^{2+}. Furthermore, the biphasic disappearance of Y_Z^+ at pH 7.5, shows that Mn^{2+} binds to PS II membranes at the site at which it reacts. The rapid reduction of Y_Z^+ at pH 7.5 in the presence of Mn^{2+} is most likely the reason that Babcock and Sauer (1975) concluded that Mn^{2+} did not reduce Y_Z^+, as the rapidly decaying signal would have escaped detection under the spectrometer conditions they used. Chelation of Mn^{2+} by EDTA has been shown to prevent its reaction with Y_Z^+, in agreement with the observation that this also prevents electron transfer from Mn^{2+} to DCIP (Izawa 1970).

The observation that the 33 kDa polypeptide does not alter the rates of Y_Z^+ reduction by either benzidine or exogenous Mn^{2+} (Table 1) contrasts with the situation in salt-washed PS II preparations that lack the 17 and 23 kDa polypeptides but retain the 33 kDa subunit and the endogenous manganese ensemble (Ghanotakis et al. 1984). In the latter preparations Y_Z^+ reduction by benzidine is considerably slower than we report in Table 1 for preparations that retain the 33 kDa, but not endogenous manganese. This suggests either that the 33 kDa does not play a role in providing a diffusion barrier for reductants in the solution phase to oxidizing equivalents on the water side of PS II or, more likely, that the association of the 33 kDa species to membranes that retain four bound manganese ions is required to establish this barrier.

Although Mn^{2+} reduces Y_Z^+ at pH 6 and appears to associate with PS II membranes, the monophasic disappearance of Y_Z^+ indicates that, at this pH, Mn^{2+} does not bind strongly to the site at which it reacts with Y_Z^+. This implies that the equilibrium dissociation constant is larger than at pH 7.5 or that the rate of dissociation is larger than the electron transfer rate. The Mn^{2+} concentration required to reach half-saturation of the Y_Z^+ reduction rate was $10\,\mu M$ at pH 7.5 and about $25\,\mu M$ at pH 6.0. Thus, Mn^{2+} binding appears weaker at pH 6 than at pH 7.5. Protonation of a ligand in the binding site may account for the pH dependence of the affinity of the site for Mn^{2+}. Because of additional binding sites for Mn^{2+} (Hsu et al. 1987) on Tris-treated PS II membranes and the high concentration of membranes used for ESR experiments, our data do not allow accurate determination of the dissociation constant, only that it is less than $5\,\mu M$ at pH 7.5. Several estimates of the binding constants give value(s) between 0.04 and $2\,\mu M$ (Klimov et al. 1982, Inoue and Wada 1987, Inoue et al. 1987).

Our finding that Mn^{2+} binds more strongly at pH 7.5 than at pH 6 is contrary to the conclusion reached by Hsu et al. (1987). They studied electron donation by diphenylcarbazide (DPC) to PS II with dichlorophenolindophenol (DCIP) as an acceptor. They found that at pH 6.1 Mn^{2+} competitively inhibited the reaction with an inhibition constant of $0.15\,\mu M$, but that at pH 7.7 Mn^{2+} inhibited noncompetitively and had an inhibition constant of $0.55\,\mu M$. Our results show that Mn^{2+} does not merely block access of other reductants to Y_Z^+, but that Mn^{2+} is oxidized by PS II in the light, probably to Mn^{3+}, itself a strong oxidant. The mechanistic

details of the reaction after electron transfer from Mn^{3+} are unknown. The Mn^{3+} may remain bound or dissociate. It could be reduced in our experiments by $Fe(CN)_6^{4-}$, or enter into a dismutation reaction with another Mn^{3+} ($2\ Mn^{3+} \rightarrow Mn^{2+} + Mn^{4+}$). In the experiments of Hsu et al. (1987), Mn^{3+} may oxidize $DCIPH_2$ or DPC and the rates of both of these reactions may be pH dependent. The shift from competitive to noncompetitive inhibition suggests that some aspect of the reaction mechanism changes with pH. An additional possibility is that manganese bound at more than one site may inhibit DPC photooxidation but that only one of these sites permits electron transfer from Mn^{2+} to Y_Z^+. Given these complications, the binding constants determined by Hsu et al. may need reevaluation.

The standard redox potential of aqueous Mn^{2+}/Mn^{3+} couple is about 1.5 V, but the redox potential of the Y_Z/Y_Z^+ couple is about 1.0 V (Bouges-Bocquet 1980, Yerkes et al. 1983). The two couples would not seem to be well positioned for a rapid and quantitative reduction of Y_Z^+ by Mn^{2+}. However, ligands to the bound Mn probably lower its redox potential by stabilizing the more oxidized and more highly charged Mn^{3+}. The Mn^{2+} EDTA/Mn^{3+} EDTA couple, for example, has a reduction potential of about $+1.0$ V, 0.5 V lower than that of the aqueous ion (Buckingham and Sargeson 1964). Although Mn^{2+} EDTA is a stronger reductant than aqueous Mn^{2+}, it fails to reduce Y_Z^+ in our experiments, probably because the bulkiness of the chelating ligands imposes a kinetic limitation on the electron transfer.

The finding that Mn^{2+} reduces Y_Z^+ in 300–500 μs at pH 7.5 shows that a completely assembled OEC is not required for rapid manganese oxidation and suggests that oxidation may occur with only a single Mn^{2+} present per reaction center. That the maximum electron transfer rate observed in these experiments is similar to the rate of the electron transfer from the S_2 state of the OEC to Y_Z^+ (Hoganson and Babcock 1988) suggests that the site at which Mn^{2+} reacts is one of the sites that eventually binds Mn in the mature OEC. The pH dependence of Mn binding to the site suggests the presence of a protonatable ligand with a pKa between 6 and 7.5, such as a histidine residue as suggested by Tamura et al. (1989).

Since the Mn^{2+} binding site identified here is probably one of the four sites for Mn in the active OEC, the reaction we have described is likely to be the first step in the assembly of the manganese complex in the OEC. Mn^{2+} is the stable, soluble form of manganese at ambient redox potential and has been strongly implicated in photoactivation of the OEC (Radmer and Cheniae 1977, Ono and Inoue 1983, Callahan and Cheniae 1985, Tamura and Cheniae 1986). Our results support the arguments of Tamura and Cheniae (1987) that the photooxidation of Mn^{2+} by Y_Z^+ is a necessary initial step in the photoactivation process.

The presence of ferricyanide or of reducing agents (Tamura and Cheniae 1987) inhibits the photoactivation process. Because both 1 mM ferrocyanide and ferricyanide was present in most of our experiments, photoactivation was not expected to occur. Nevertheless, there was an indication that a second step in the photoactivation process might occur with a low yield at pH 7.5 (i.e., the diminished amplitude of the Y_Z^+ signal with 200 μM Mn^{2+} present). Further experiments varying both ferrocyanide concentration and dark time between flashes should help to resolve the question.

High concentrations of Mn^{2+} can inhibit photoactivation if Ca^{2+} is deficient (Ono and Inoue 1983); this phenomenon, in which Mn^{2+} occupies a Ca^{2+} site, may be related to the mild substrate inhibition that we observe at pH 6 at Mn^{2+} concentrations between 100 and 200 μM. The stable tyrosine radical, Y_D^+, may assist the photoactivation process but is not essential to it, since a transformant, wherein that tyrosine has been replaced with phenylalanine, assembles functional oxygen-evolving complexes but grows more slowly than the control transformant (Debus et al. 1988a). Y_Z^+, however, would appear to be essential not only for electron transfer during oxygen evolution (Debus et al. 1988b), but also for photoactivation of the OEC.

References

Amesz J (1983) The role of manganese in photosynthetic oxygen evolution. Biochim Biophys Acta 726: 1–12

Andréasson LE and Vänngård (1988) Electron transport in Photosystems I and II. Ann Rev Plant Physiol Plant Mol Biol 39: 379–411

Babcock GT, Barry BA, Debus RJ, Hoganson CW, Atamian M, McIntosh L, Sithole I and Yocum CF (1989) Water oxidation in Photosystem II: from radical chemistry to multielectron chemistry. Biochemistry, submitted

Babcock GT (1987) The photosynthetic oxygen-evolving process. In: Amesz J (ed) New Comprehensive Biochemistry: Photosynthesis, pp 125–158. Amsterdam: Elsevier

Babcock GT and Sauer K (1975) Two electron donation sites for exogenous reductants in chloroplast Photosystem II. Biochim Biophys Acta 396: 48–62

Barber J (1980) Membrane surface charges and potentials in relation to photosynthesis. Biochim Biophys Acta 594: 253–308

Barry BA and Babcock GT (1987) Tyrosine radicals are involved in the photosynthetic oxygen-evolving system. Proc Natl Acad Sci USA 84: 7099–7103

Ben-Hayyim G and Avron M (1970) Mn^{2+} as electron donor in isolated chloroplasts. Biochim Biophys Acta 205: 86–94

Berthold D, Babcock GT and Yocum CF (1981) A highly resolved oxygen-evolving Photosystem II preparation from spinach thylakoid membranes. FEBS Lett 134: 231–234

Blankenship R (1975) The role of manganese in photosynthetic oxygen evolution. PhD dissertation, University of California, Berkeley

Callahan P, Ellis F and Cheniae G (1985) Studies on the photoactivation of the water-oxidizing enzyme. Plant Physiol 79: 777–786

Casey JL and Sauer K (1984) EPR detection of a cryogenically photogenerated intermediate in photosynthetic oxygen evolution. Biochim Biophys Acta 767: 21–28

Conjeaud H and Mathis P (1986) Electron transfer in the photosynthetic membrane. Influence of pH and surface potential on the P-680 reduction kinetics. Biophys J 49: 1215–1221

Cotton FA and Wilkinson G (1967) Advanced Inorganic Chemistry. New York: Wiley and Sons, New York

Debus RJ, Barry BA, Babcock GT and McIntosh L (1988) Site-directed mutagenesis identifies a tyrosine radical involved in photosynthetic oxygen-evolving system. Proc Natl Acad Sci USA 85: 427–430

Debus RJ, Barry BA, Sithole I, Babcock GT and McIntosh L (1988) Directed mutagenesis indicates that the donor to P_{680}^+ in the Photosystem II is tyrosine-161 of the D1 polypeptide. Biochem 27: 9071–9074

Dismukes GC and Siderer Y (1981) Intermediates of polynuclear manganese center involved in photosynthetic oxidation of water. Proc Natl Acad Sci USA 78: 274–278

George GN, Prince RC and Cramer SP (1989) The manganese site of the photosynthetic water-splitting enzyme. Science 243: 789–791

Ghanotakis DF, Babcock GT and Yocum CF (1984) Structural and catalytic properties of the oxygen-evolving complex. Biochim Biophys Acta 765: 388–398

Hansson O, Aasa R and Vänngård T (1987) The origin of the multiline and $g = 4.1$ electron paramagnetic resonance signal from the oxygen-evolving system of Photosystem II. Biophys J 51: 825–832

Helfferich F (1962) Ion exchange, pp 158–159. New York: McGraw-Hill

Hoganson CW and Babcock GT (1988) Electron-transfer events near the reaction center in O_2-evolving Photosystem II preparations. Biochemistry 27: 5848–5855

Hsu B-H, Lee J-Y and Pan R-L (1987) The high-affinity binding site for manganese in the oxidizing side of Photosytem II. Biochim Biophys Acta 890: 89–96

Inoue H, Akahor H and Noguchi M (1987) Activation of electron donation from hydrogen peroxide by manganese in nonoxygen evolving Photosystem II particles. Plant Cell Physiol 28: 1339–1343

Inoue H and Wada T (1987) Requirement of manganese for electron donation of hydrogen peroxide in Photosystem II reaction center complex. Plant Cell Physiol 28: 767–773

Izawa S (1970) Photoreduction of 2,6-dichlorophenolindophenol by chloroplasts with exogenous Mn^{2+} as electron donor. Biochim Biophys Acta 197: 328–331

Klimov VV, Allakhverdiev SI, Shuvalov, VA and Krasnovsky AA (1982) Effect of extraction and re-addition of manganese on light reactions of Photosystem-II preparations. FEBS Lett 148: 307–312

Ono T-A and Inoue Y (1983) Requirement of divalent cations for photoactivation of the latent water-oxidation system in intact chloroplasts from flashed leaves. Biochim Biophys Acta 723: 191–201

Penner-Hahn JE, Fronko RM, Pecoraro VL, Yocum, CF, Betts SD and Bowlby NR (1989) Structural characterization of the Mn sites in the photosynthetic oxygen-evolving complex using X-ray absorption spectroscopy. J Am Chem Soc, in press

Radmer R and Cheniae G (1977) Mechanisms of oxygen-evolution . In: Barber J (ed) Primary Processes in Photosynthesis, Vol. 2, pp 301–348. Amsterdam, New York: Elsevier

Sauer K, Guiles RD, McDermott AE, Cole JL, Yachandra VK, Zimmerman J-L, Klein MP, Dexheimer SL and Britt RD (1988) Spectroscopic studies of manganese involvement in photosynthetic oxygen evolution. Chemica Scripta 28A: 87–91

Stewart AC and Bendall DS (1981) Properties of oxygen-evolving Photosystem II particles from Phormidium laminosum, a thermophilic blue-green algae. Biochem J 194: 877–887

Tamura N and Cheniae GM (1986) Requirements for the photoligation of Mn^{2+} in Photosystem II membranes and the expression of water-oxidizing activity of the polynuclear Mn-catalyst. FEBS Lett 200: 231–236

Tamura N and Cheniae G (1987) Photoactivation of the water-oxidizing complex by Photosystem II membranes in VIIth International Congress on Photosynthesis (Biggins J, ed). Dordrecht: Martinus Nijhoff

Tamura N, Ikeuchi M and Inoue Y (1989) Assignment of histidine residues in the D1 protein as possible ligands for functional manganese in photosynthetic water oxidizing complex. Biochim Biophys Acta 973: 281–289

Yachandra VK, Guiles RD, McDermott A, Britt RD, Dexheimer SL, Sauer K and Klein MP (1986) The state of man-

ganese in the photosynthetic apparatus. Biochim Biophys Acta 850: 324–332

Yerkes CT and Babcock GT (1980) Photosystem II oxidation of charged electron donors: surface charge effects. Biochim Biophys Acta 590: 360–372

Yerkes CT and Babcock GT (1981) Surface charge asymmetry and a specific calcium ion effect in chloroplast Photosystem II. Biochim Biophys Acta 634: 19–29

Yerkes CT, Babcock GT and Crofts AR (1983) A tris-induced change in the midpoint potential of Z, the donor to Photosystem II, as determined by the kinetics of the back reaction. FEBS Lett 158: 359–363

Zimmerman JL and Rutherford AW (1984) EPR studies on the oxygen evolving enzyme of Photosystem II. Biochim Biophys Acta 767: 160–167