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Are there really four manganese ions per centre of photosynthetic water oxidation?

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Manganese content and the amount of oxygen-evolving centres have been determined for purified PS II complexes from the cyanobacterium *Synechococcus* sp. by atomic absorption and measurements of flash-induced oxygen yields, respectively. A stoichiometry of six manganese per oxygen-evolving centre was observed. This was found not only for PS II preparations with different activities of oxygen evolution, but also when deactivating treatments by low-salt or NH_2OH incubation were carried out. Our results call into question the current thinking that four Mn ions are located in the water-oxidizing complex. A hexanuclear manganese model is discussed, instead.

Introduction

Manganese is an essential part of the membrane-bound enzyme complex named Photosystem II that catalyses the light-induced oxidation of water (see reviews, Refs. 1–3). This complex consists of several protein subunits. Two of these are the intrinsic, membrane-spanning D1 and D2 proteins, which contain the components active in the primary photoreaction [4]. This starts with the photoxidation of a special chlorophyll *a* (Chl-a_{II}, P680) [5]. The subsequent electron transfer via pheophytin (I) [6] to the stable acceptor - a special plastoquinone (Q_A) [7] – leads to a transmembrane charge separation [8]. From this, it was concluded that Q_A is located towards the outside of the membrane and Chl-a_{II} towards the inside.

Chl-a_{II}^+ oxidizes a special tyrosine of D1 [9–11], which in turn extracts an electron out of the oxygen-evolving centre (OEC). The latter contains the manganese. The OEC has not yet been located, except that it is placed on the inner side of the membrane and is protected by an extrinsic protein of 33 kDa molecular mass (see Ref. 12).

By consecutive extraction of four electrons out of the OEC by Chl-a_{II}^+ via the tyrosine, the OEC is oxidized to the redox states S_1, S_2, S_3 and S_4 [13,14]. The unstable state S_4 returns to the least oxidized state, S_0, this process being accompanied by the release of one molecule of oxygen from two H_2O. If the sample is excited by a train of short flashes of light, each oxygen-evolving centre will therefore evolve $\frac{1}{4}O_2$ per flash on the average. So, the number of centres can be calculated from the oxygen yield obtained after a certain number of flashes.

Combined with atomic absorption spectroscopy for determination of manganese, oxygen yield measurements will give the stoichiometry of manganese per oxygen evolving centre, provided that manganese is bound only to PS II complexes that are active in oxygen evolution. While this combination has been applied to intact chloroplasts [15,16], purified PS II complexes, which have been available for more than 10 years, have rarely been examined in this way. Instead, the amount of bound manganese was related to values of Chl/PS II taken from the literature, to components of electron transport or to protein subunits (for details and references see Discussion). As the number of oxygen-evolving centres was not determined, the experimental basis of the generally accepted value of 4 Mn/OEC is too limited.

Knowledge of the stoichiometry is of course essential for the elucidation of both the structure of the

Abbreviations: Chl, chlorophyll; HMCM, 20 mM Hepes-Na/0.3 M mannitol/20 mM $CaCl_2$/10 mM $MgCl_2$; OEC, oxygen-evolving centre; PS II, Photosystem II; SB12, sulfobetaine 12; SE, SB12-extracted PS II complex; SG, SE purified by sucrose density centrifugation.

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water-oxidizing centre and the mechanism of water oxidation. We have therefore addressed this problem again on an extended experimental basis. Not only was the number of oxygen-evolving centres determined from flash-induced oxygen yields, but also the total number of PS II complexes was determined from absorption changes at 320 nm due to photoreduction of Q_A. Taken together, these measurements allowed us to calculate the number of PS II complexes not capable of water oxidation. Highly purified PS II complexes from the cyanobacterium *Synechococcus* were used for these investigations. Manganese was determined by atomic absorption.

Materials and Methods

O_2-evolving PS II extracts (SE) from the cyanobacterium *Synechococcus* sp. were prepared essentially as described in Ref. 17. These were purified by a sucrose density centrifugation according to Ref. 18. For a characterization of these purified complexes (SG) see Ref. 19. They were stored at −80°C in 20 mM Mes-Na (pH 6.5), 20 mM $CaCl_2$, 10 mM $MgCl_2$, 1 M sucrose and 0.06% SB 12.

Oxygen yield was measured with a ZrO_2-oxygen sensor [20,21]. In this apparatus, oxygen evolved by a flash-illuminated sample (volume 1 ml, 10–30 μM Chl) is swept out of the cuvette by N_2 gas (< 100 ppb O_2) to a measuring cell containing the O_2-specific ZrO_2 electrode (Programmelectronic). The measured electromotive force is electronically converted into a DC-voltage proportional to the oxygen concentration in the carrier gas. This voltage is digitized and read into a computer, where the concentration–time curves can be integrated. The apparatus is calibrated by injection of 50 μl air saturated water, prepared by bubbling air through deionized water for 30 min, followed by a further 30 min strong stirring [22]. Values of the oxygen concentration in air-saturated water were taken from Ref. 23. Further details are decribed in Ref. 20.

For measurements of flash-induced oxygen yields, samples were illuminated by saturating flashes of white light from a xenon flash lamp (20 μs fwhm). Oxygen yields were measured in the presence of 2 mM $K_3[Fe(CN)_6]$ and 200 μM phenyl-*p*-benzoquinone with 40–100 flashes at 1 Hz. No systematic differences are observed between measurements with the ZrO_2-oxygen sensor and a Clark-type electrode [24].

Photoreducible Q_A was determined from the initial amplitude of the flash induced absorption change at 320 nm ($\Delta\epsilon = 13000\ M^{-1}\ cm^{-1}$). Samples contained 20–40 μM Chl, 2 mM $K_3[Fe(CN)_6]$ and 200 μM dichlorobenzoquinone. An exogenous donor was not required for complexes inactivated in oxygen evolution, as the flash frequency (1 Hz) was slow enough to allow for recombination of Q_A^- with Chl-a_{II}^+ ($t_{1/2} < 10$ ms [25]).

Chlorophyll was determined by extraction in 80% acetone/water and measurement of the absorbance at 663 nm vs. 700 nm. An extinction coefficient of $\epsilon = 73800\ M^{-1}\ cm^{-1}$ was used.

Manganese was determined with a Philips SP9 atomic absorption spectrophotometer equipped with a graphite furnace and an autosampler. Every sample was measured two or three times. For samples containing $CaCl_2/MgCl_2$, a temperature programme was used with the steps 10 s at 130°C (drying), 15 s at 800°C (ashing) and 3 s at 2400°C (atomization). If samples had been treated with cation exchange resin (see below) and therefore contained NaCl, a different temperature programme had to be used, namely 10 s at 130°C (drying), 15 s at 1400°C (ashing) and 3 s at 2200°C (atomization). The absorbance at the wavelength 279.5 nm was determined from the heights of the absorption peaks. The concentration dependence of the absorbance was linear in the range of 1 ppb to 8 ppb (0.018–0.145 μM). Standards were prepared by adding diluted $Mn(NO_3)_2$ standard solution (Fluka) to buffer of the same composition as that of the samples.

A purification of the chemicals used was found to be unnecessary. The buffer solutions contained less than 0.5 ppb (0.009 μM) Mn. Deionized water purified in a Milli-Q reagent water system (Millipore) was used in all experiments.

Salt exchange, EDTA and NH_2OH removal were achieved by gel filtration with prepacked PD 10 columns (Pharmacia) containing Sephadex G 25 M. These columns are highly efficient in the removal of small molecules [26].

Deactivation of oxygen evolution was achieved in three ways:

(1) Low-salt conditions were realized by passing SG-complexes (1.5 ml, 90 μM Chl) through a gel-filtration column equilibrated with 20 mM Hepes-Na (pH 7.5), 10 mM NaCl and 0.3 M mannitol and subsequent incubation for the time indicated. Deactivation was stopped by readdition of a 3.8-fold volume of 20 mM Hepes-Na (pH 7.5), 20 mM $CaCl_2$, 10 mM $MgCl_2$ and 0.3 M mannitol (HMCM).

(2) Deactivation by the reductant NH_2OH was realized by incubating SG complexes with the indicated concentration of NH_2OH at pH 7.5 (HMCM). The deactivation was stopped by passing the treated complexes through a gel-filtration column equilibrated with HMCM (pH 7.5).

(3) Deactivation by excess $CaCl_2$ (1 M) was performed by incubation of SG complexes under room light for 2 h. It was stopped by removal of excess $CaCl_2$ through gel filtration (HMCM, pH 7.5).

After deactivation by low salt or NH_2OH, the manganese released from the deactivated centres was com-

pletely removed by a treatment with the cation-exchange resin Chelex 100 (sodium form). The samples were shaken 15 s with 20% (w/w) of the resin. After the resin had settled (sometimes accelerated by centrifugation (Eppendorf centrifuge 5414 S) for 10 s), an aliquot of the supernatant was taken and frozen at −30°C. Before determination of manganese, these samples were diluted 5-fold with purified water. Manganese released from deactivated centres was removed by gel filtration after treatment with 1 M $CaCl_2$ and NH_2OH where indicated.

Results

Table I shows the results of manganese determinations in SE and SG preparations. These are in agreement with values of other authors obtained for PS II preparations of *Phormidium laminosum* [27] as well as with other preparations of *Synechococcus* [28–30]. Unfortunately, the ratio of Mn/OEC was not given in these publications.

A higher Mn content was found for SE compared to SG preparations, as can be seen by the higher Mn/Q_A and Mn/OEC ratios as well as the lower Chl/Mn ratio. A similar difference between unpurified and purified PS II complexes was also observed by Stewart et al. and Satoh et al. It may be due to a manganese-binding protein that is not related to water oxidation and removed upon purification. It has been reported, for instance, that CF1 of the ATPase binds manganese [31,32]. Considering that the observed difference seems to be fairly reproducible, it may be that such a manganese-binding protein is more closely associated with PS II.

The value of about 6 Mn per OEC even for the purified SG complexes is rather astonishing, as it is generally assumed that there are four manganese per OEC. A simple explanation for this discrepancy might be that our SG complexes still contain manganese from contaminations. To check this, we tried to remove Mn contaminations by various treatments as shown in Table II.

TABLE I

Results of manganese determinations with SE (4 preparations) and SG (6 preparations) complexes from cyanobacteria

Values are averages ± S.D. For comparison, the results of other authors obtained with different preparations from cyanobacteria are shown (a: Mn/Tyr(D1)).

Ref.	Prep.	Chl/Mn	Mn/Q_A	Mn/OEC
this work	SE (unpurified)	14 ± 2	4.3 ± 0.9	8.5 ± 1.4
	SG (purified)	18 ± 3	2.7 ± 0.3	5.8 ± 0.4
Stewart et al. [27]	unpurified	14	–	–
	purified	19	–	–
Satoh et al. [28,29]	unpurified	14 ± 2	3.5 ± 0.9	–
	purified	16 ± 2	3.2 ± 0.6	–
McDermott et al. [30]	–	19	3.5 ± 0.5 [a]	–

TABLE II

The effect of various treatments on the manganese content and on the relative oxygen yield of SG-complexes

The medium before gel-filtration contained 20 mM Hepes-Na (pH 7.5), 10 mM NaCl, 7 mM $CaCl_2$, 3.5 mM $MgCl_2$, 0.3 M mannitol; after gel filtration and for Chelex treatment it contained HMCM (pH 7.5).

Treatment	Mn/Q_A	Mn/OEC	Rel. O_2 yield (%)
None	2.9	6.3	100
Gel filtration (gf) alone	2.6	6.1	91
10 min 20 mM EDTA + gf	2.8	5.9	97
2 h 20 mM EDTA + gf	2.8	6.2	95
1 min 20% (w/w) Chelex	3.1	5.9	106
5 min 20 μM NH_2OH + 10 min 20 mM EDTA + gf	2.2	6.5	66
55 min 20 μM NH_2OH + gf	2.0	6.4	65
5 min 2 mM NH_2OH + gf	< 0.1		< 0.7

Gel filtration alone or in combination with a chelator (EDTA) or treatment with a cation-exchange resin (Chelex 100), all of which leave the oxygen yield practically unaffected, do not lead to a removal of manganese. EDTA was used 2-fold in excess compared to the concentration of divalent cations (Ca, Mg, Mn). The effective stability constant of the EDTA-Mn complex is $10^{13.4}$ at pH 7.5. The manganese in our SG preparations must therefore be very tightly bound.

As Mn(II) complexes are generally less stable than the complexes of higher oxidation states, we also performed a treatment with a reductant (hydroxylamine). This reagent reduces the S-states of the OEC [26] and finally leads to the release of manganese [33]. This was also observed with our SG complexes, as can be seen by the Mn/Q_A ratio. This ratio decreases with the incubation time and/or the concentration of NH_2OH to less than 0.1 Mn/Q_A. However, those centres that are still active in O_2 evolution seem to retain the manganese, i.e., about 6 Mn/OEC.

As can be seen from Table II, the ratio of Mn/Q_A is lower than the ratio of Mn/OEC. This indicates that there are more complexes that only have a photoreducible Q_A than those that are additionally capable of O_2 evolution. If those centres that do not evolve oxygen still have some manganese bound, the value of 6 Mn/OEC, calculated from the total manganese content, would not reflect the true number of manganese ions in active oxygen-evolving centres. If this assumption is correct, there should still be detectable manganese as the O_2 activity approaches zero. We therefore looked at the way the manganese content is correlated with the activity of oxygen evolution.

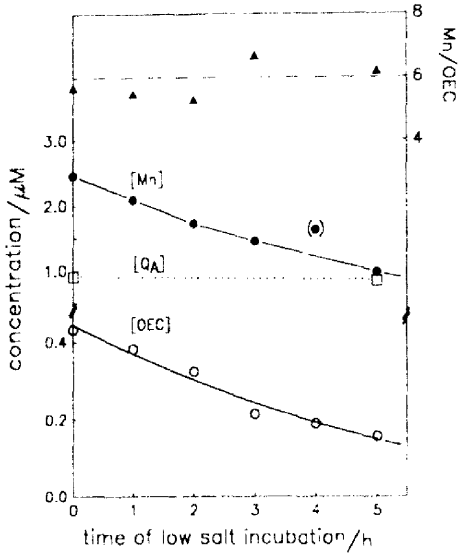

Fig. 1. Concentration of manganese, Q_A and oxygen-evolving centres and the ratio of Mn/OEC (▲) as a function of the incubation time with 10 mM NaCl, 20 mM Hepes-Na (pH 7.5), 0.3 M mannitol. For details see Materials and Methods.

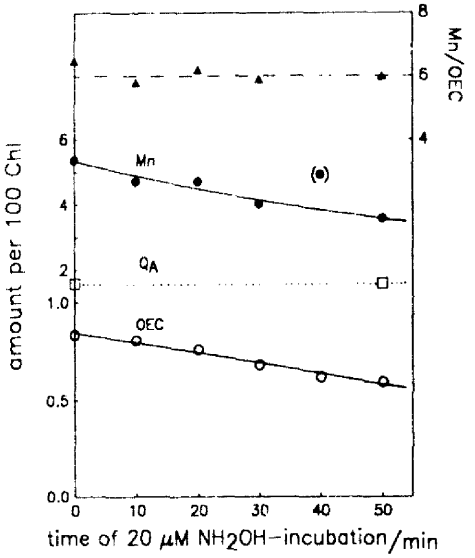

Fig. 2. Amount of manganese, Q_A and oxygen-evolving centres per 100 chlorophylls and the ratio of Mn/OEC (▲) as a function of the incubation time with 20 μM NH_2OH, HMCM (pH 7.5). Manganese was determined after Chelex treatment, the amount of OECs was determined after gel-filtration (HMCM, pH 7.5). For details see Materials and Methods.

These investigations were performed with untreated SG-complexes from preparations differing in O_2 activity (the differences are caused by unavoidable variations in the growth conditions of the cyanobacteria and/or a varying degree of deactivation in the course of preparation) but also with treated SG complexes. We used treatments where a specific deactivation of water oxidation is accompanied by a loss of manganese. This requirement was fulfilled by exposing the SG complexes to insufficient salt concentrations of 10 mM NaCl [62] (Fig. 1) or to 20 μM hydroxylamine (Fig. 2). In both cases the loss of O_2-activity was paralleled by a loss of manganese, whereas the Q_A-activity was not affected. The ratio of Mn/OEC remained constant as can be seen at the top of Figs. 1 and 2.

Fig. 3 shows the correlation between manganese per chlorophyll and oxygen evolving centres per chlorophyll. The data points are values obtained after different deactivating treatments or with untreated SG complexes. The correlation is very good (correlation coefficient = 0.98). The linear regression has a y-intercept of $0.30 \cdot 10^{-2}$ Mn/Chl. As there were about 40 to 70 Chl per Q_A in the SG complexes used, this value corresponds to maximally 0.2 Mn/Q_A.

The slope of the linear regression corresponds to the number of manganese ions released per deactivated centre. The slope is 5.5 Mn/OEC, in line with the value of about 6 Mn bound to PS II per active OEC, which was found independently in the experiments of Tables 1 and 2. The slightly lower value is explained by the y-intercept, which is not exactly zero, although it is negligible (see above). There is no correlation between the amount of Q_A per chlorophyll and the manganese per chlorophyll (not shown).

As already mentioned, the value of 6 Mn/OEC represents the true stoichiometry only if the possibility can be excluded that inactive centres contain manganese. Fig. 3 shows that there are no inactive centres containing manganese if the deactivation is complete. The possibility cannot, however, be excluded that there are inactive centres with an amount of manganese that is exactly proportional to the amount of active centres. Under these conditions the value of 6 Mn/OEC would still be an apparent ratio, and the true value would be smaller. The straight line in Fig. 3 would then be the sum of two straight lines. One of these would show the

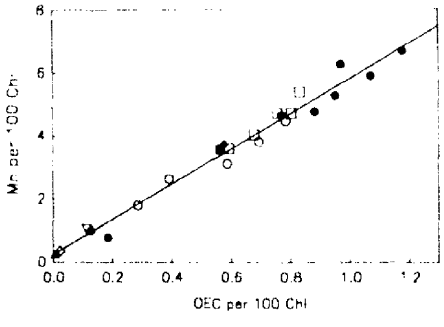

Fig. 3. Amount of manganese per 100 chlorophylls as a function of the amount of oxygen-evolving centres per 100 chlorophylls for various treated and untreated SG complexes. The symbols indicate untreated SG complexes from eight different preparations (●), low-salt treatment (○), treatment with 20 μM NH_2OH (□; ■: Mn-determination after gel filtration), 100 μM NH_2OH (△), 1 mM NH_2OH (○), 2 mM NH_2OH (▲), 20 μM NH_2OH and 20 mM EDTA (◆).

manganese content of active centres, the other that of inactive centres.

New information on the possible influence of inactive centres becomes available if the ratio of Mn/OEC is investigated in dependence on the fraction of inactive centres. Therefore, besides the number of active centres (from the oxygen yield) also the total number of PS II complexes (from the yield of photoreducible Q_A) was determined. The results obtained in this way are depicted in Fig. 4. This shows the ratio of Mn/OEC as a function of the fraction of active centres (OEC/Q_A) and thus also the fraction of inactive centres ($1-OEC/Q_A$) among the total number of PS II complexes. The data of Fig. 4 can be described by a horizontal line that corresponds to an average of 6.0 ± 0.7 Mn/OEC, i.e., this value is independent of the fraction of active and inactive centres, respectively. This is understandable only if under the conditions shown the inactive centres do not contain manganese. Therefore the value of 6 Mn/OEC does not reflect a contribution of inactive centres, but represents the true stoichiometry. If the true value were less than six, e.g., four, the value of Mn/OEC in Fig. 4 would have to decrease to four for $OEC/Q_A = 1$, because then the fraction of inactive centres is zero.

If inactive centres containing manganese are created by certain treatments, the dependence of Mn/Chl on OEC/Chl (Fig. 5) is distinctly different from that observed in Fig. 3. An example for such a treatment is a deactivation by 1 M $CaCl_2$, as has already been reported by Ono and Inoue [34]. This treatment inhibits oxygen evolution completely and reversibly in the dark. In the light, the inhibition becomes irreversible [62].

Fig. 5 shows Mn/Chl as a function of OEC/Chl for SG complexes that were treated with 1 M $CaCl_2$ for various times under illumination, followed by gel filtration. In contrast to Fig. 3, a significant y-intercept can be seen, which corresponds to about 2 Mn/Q_A. Likewise the slope of the straight line in Fig. 5 is only about 3.4 Mn/OEC. The results of Fig. 5 are explained by the additional presence of manganese in the inactive centres. The contrast between Fig. 5 and Fig. 3 supports the conclusiveness of our results in Tables 1 and 2 and Figs. 1–4. These indicate that unless the extraordinary treatment with 1 M $CaCl_2$ has been carried out, the inactive centres among our SG complexes do not contain manganese and that per oxygen-evolving centre six manganese ions are present.

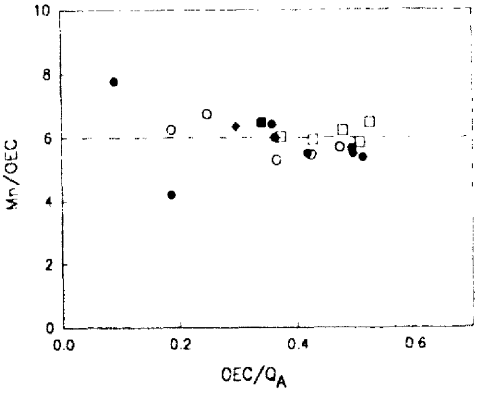

Fig. 4. Ratio of Mn per OEC as a function of the fraction of active centres, OEC/Q_A, for treated and untreated SG complexes. The symbols are the same as for Fig. 3. Note that the determination of Mn/OEC and OEC/Q_A for two untreated samples is less precise because of the low activity of these samples in respect to both oxygen evolution and Q_A photoreduction.

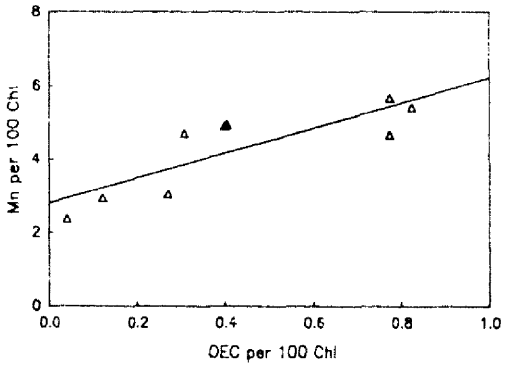

Fig. 5. Amount of manganese per 100 chlorophylls as a function of the amount of oxygen evolving centres per 100 chlorophylls for SG complexes which were deactivated by incubation with 1 M $CaCl_2$ under illumination for various times, followed by gel filtration (for details see Materials and Methods).

Discussion

As our results differ from the nowadays generally accepted stoichiometry of four Mn/OEC, we have thoroughly studied the literature of manganese determinations with PS II (reviews, Refs. 1, 2, 3, 35, and publications cited therein). Almost all of the work done in this field suffers from the omission of oxygen yield measurements to determine the amount of oxygen-evolving centres. While some authors relied on literature data for the ratio of Chl/PS II (e.g., Ref. 36), those who related the manganese content to the measured amount of Q_A [28], pheophytin I [37] or the special tyrosine of D1 [30] did not take into account the possibility that this may not be identical to the amount of oxygen-evolving centres. On the other hand, the values of manganese per electron transfer component given in these publications are in agreement with our value of 2.7 ± 0.3 Mn/Q_A (Table I).

A value of 2–4 Mn/PS II has been estimated by determining the amount of 47 kDa protein from electrophoresis densitograms [38,39]. This protein was then believed to be the reaction centre of PS II. This has been shown not to be correct [4]. Because the 47 kDa

protein is found also in PS II prepapations that do not evolve oxygen (e.g., Ref. 40), it is in any case no measure for the amount of oxygen-evolving centres.

Two groups have presented results of oxygen yield measurements in connection with manganese determinations. Abramowicz et al. [41] have reported on a ratio of 3.4–4.0 Mn/OEC for PS II particles from spinach, but no experimental details have been given in this work. In Ref. 16, Cheniae and Martin concluded that there is a total of 3 Mn/OEC, but found that oxygen evolution was abolished upon removal of only two thirds of this manganese, i.e., two manganese should be functional in oxygen evolution. The total number of Mn's per OEC was later corrected by a factor of two [33]. In the later work it was therefore concluded that four manganese are associated with oxygen evolution.

A two-step release of manganese has been reported for treatments with Tris [16,36], NH_2OH [16] or EDTA [42]. We did not, however, observe such a differential release upon the low-salt and NH_2OH treatments described (see Fig. 3). Reasons for this discrepancy are discussed in the following.

The results in Ref. 16 were obtained with whole chloroplasts. A quantitative removal of manganese liberated from the active site may have been difficult, as this manganese to some extent could have been trapped inside the thylakoids [43]. Furthermore, the conclusion in Ref. 16 that there are two pools of manganese was based on the comparison of oxygen evolution rates with the manganese content. By the treatments applied in Ref. 16, however, also the rates of electron transport in the presence of exogenous donors were affected. Therefore, under those experimental conditions the rate of oxygen evolution may have been very low, although there was still a significant amount of oxygen-evolving centres. Consequently, the residual manganese may have been due to these centres.

The results in Refs. 36 and 42 were interpreted as being due to a partial release of manganese from all oxygen-evolving centres. As the correlation between manganese content and the activity of oxygen evolution was not investigated, these results may also be explained by a complete loss of manganese from only a part of the centres.

Is the manganese content of PS II from higher plants different from that of cyanobacterial PS II? Some preparations from higher plants typically contain 40–60 Chl/Mn [44–46], but these still have the light harvesting complex and therefore a larger number of antenna chlorophylls. Detergent-solubilized and purified PS II complexes contain about 13 Chl/Mn [47,48], similar to cyanobacterial preparations (see Table I). Furthermore, comparative measurements of the EPR multiline signal [49,30] and of the manganese EXAFS spectra [30] did not show systematic differences between higher plants and cyanobacteria. This indicates that the structure of the manganese cluster of the active site is probably the same.

Although oxygen-yield measurements are essential for a determination of Mn/OEC, they are in principle affected by the misses and double hits [14] occurring with the flash-induced S-state transitions. The fraction of centres that do not make a turnover upon a certain flash (misses) escapes detection, while that fraction that perform a double turnover will be 'counted' twice. So, if the miss parameter is much higher than the double-hit parameter, the number of oxygen evolving centres determined from the average oxygen yield per flash would be systematically too small and the Mn/OEC ratio therefore too large.

Flash patterns of oxygen evolution have been measured by Meyer et al. [21] with an SE preparation and the ZrO_2-oxygen sensor used in this work. 10% misses and 7% double hits were calculated for the patterns measured under conditions very similar to those of this work. Thus, we exclude misses and double hits as a source of a systematic error in determining the number of oxygen-evolving centres from oxygen yield measurements. This is supported by the fact that no systematic differences were observed between measurements with the ZrO_2-oxygen sensor and a Clark-type electrode [24].

Though we did observe a strong effect of the salt matrix of our samples on the response of the atomic absorption spectrophotometer, which was eliminated by a suitable temperature programme (see Materials and Methods), no effect of the protein matrix was observed. The same results were obtained with both temperature programmes (see, for example, Table II, Chelex treated vs. untreated sample). These facts stand against a systematic error in the manganese determinations. As shown in Table I there is also an agreement of our results for Chl/Mn and Mn/Q_A with the results of other authors.

We do not know to which protein subunits the manganese is bound. Therefore, we cannot be sure that all the manganese is located at the same site, although we did not observe a differential release of manganese (see above). Probably, only a part of the manganese is actively participating in the mechanism of water oxidation. Most of the manganese may only have a stabilizing function. Considering the symmetry of PS II, one could also speculate that three manganese ions are bound to the D1 and D2 protein, respectively. Results in favour of manganese binding to D1 [50,51] do not rule out the possibility that D2 also binds manganese.

Complexes containing 6 manganese ions have been synthesized [52,53], which have similar Mn–Mn distances as those observed by EXAFS of the OEC, namely 2.7 Å and 3.3 Å [54–58]. Longer distances may

escape detection by EXAFS [55]. Therefore, these data do not rule out a higher nuclearity than four manganese ions per OEC. However, the coordination numbers that are obtained from the EXAFS spectra of the OEC together with the distances do not coincide with the hexamanganese complexes cited above. For this reason they may not be structural models for the OEC.

For the case of an oxygen-evolving complex with six manganese we have therefore constructed a model which is designed in accordance with the EXAFS data and the coordination chemistry of manganese [59]. Several alternative hexanuclear models are possible. We have chosen the simplest one, namely a symmetric arrangement of the manganese ions. This model is shown in Fig. 6. Two bent Mn-trimers are symmetrically connected by a μ-oxo-μ-carboxylato bridge of 3.3 Å between Mn(2) and Mn(2'). In each trimer there are two di-μ-oxo bridges of 2.7 Å between the manganese ions. Further maganese–manganese and manganese–ligand distances are presented in Table III, together with the corresponding coordination numbers (N). Terminal ligands of manganese are assumed to be oxygen or nitrogen atoms from the surrounding amino acids.

The manganese ions (1) and (1') may be the two redox active ones that are ligated with water. For the possible states of these two manganese ions and of water in the $S_0 \rightarrow S_3$ transitions, see Refs. 60, 26. Two hydroxyl ions, which are probably present as derivatives of water in the S_0 state, were therefore included as ligands of Mn(1) and Mn(1'), respectively. The oxygen atoms of these hydroxyl ions would be in a suitable distance (about 1.5 Å) for the formation of the O–O bond prior to the release of O_2. Mn(2) or Mn(2'), may be the third redox active manganese [26]. It may trigger

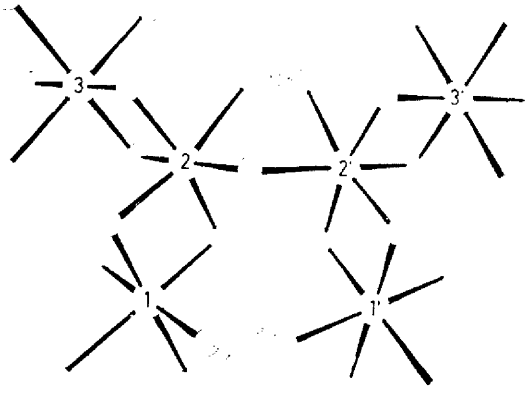

Fig. 6. A view of a hypothetical hexanuclear manganese model complex. Mn(1) and Mn(1') are suggested to be redox active in the process of water oxidation. Two hydroxyl ions are ligated to them, as proposed for the S_0 state. The carbon atom of the carboxylate bridge between Mn(2) and Mn(2') is indicated by a dot. For further details, see Discussion.

TABLE III

Distances (R) and coordination numbers (N) of the hexanuclear model complex shown in Fig. 6

Data of the hypothetical model complex (Fig. 6)			
distance between	Specifications	R (Å)	N
Mn–O	di-μ-oxo-bridges	1.8	2.7
Mn–O, N	μ-oxo-μ-carboxylato bridge and mono-bridging (terminal) ligands	2.2	2.7
Mn–Mn	Mn(1)–Mn(2), Mn(2)–Mn(3) Mn(1')–Mn(2'), Mn(2')–Mn(3')	2.7	1.3
Mn–Mn	Mn(2)–Mn(2')	3.3	0.3
Mn–Mn	Mn(1)–Mn(3), Mn(1')–Mn(3')	4.3	0.7
Mn–Mn	Mn(1')–Mn(1'), Mn(1)–Mn(2') Mn(2)–Mn(1')	4.7–4.8	1.0
Mn–Mn	Mn(3)–Mn(2'), Mn(2)–Mn(3')	5.8	0.7
Mn–Mn	Mn(1')–Mn(3'), Mn(3)–Mn(1') Mn(3)–Mn(3')	5.8–5.9	1.0

the release of O_2 by an inductive effect if it is oxidized in S_4. The increased positive charge of this Mn ion could make the oxidation potential of Mn(1) and Mn(1') sufficiently positive for an electron transfer from the oxo-atoms of water to these manganese ions. Mn(2) or Mn(2') may also be that less accessible for the reduction by NH_2OH and responsible for the creation of the S_2-state through this reagent [26,61]. According to recent results (Kretschmann and Witt, unpublished data), the remaining three Mn ions are not redox-active but are present in the S_0–S_3 state in a constant valence configuration. They may be useful as a link to the special tyrosines functional in electron transport of PS II. One could, therefore, also consider a model where a redox-active Mn trimer is bound to the D_1 protein and an inactive Mn trimer to the D_2-protein.

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