Hypothesis

A manganese–chloride cluster as the functional centre of the O_2 evolving enzyme in photosynthetic systems

Christa Critchley and Alan M. Sargeson

Department of Botany, The Faculties and Research School of Chemistry, Australian National University, Canberra, ACT 2601, Australia

Received 4 June 1984

Photosynthetic O_2 evolution *O_2 evolving complex mechanism* *Manganese* *Chloride* *Metal cluster*

Many aspects of the mechanism for photosynthetic O_2 evolution remain unknown despite considerable research effort over the past decades and despite the accumulation of a large amount of biochemical and biophysical knowledge about this biological process. Several excellent reviews dealing with this topic are available [1–6] so that, for the sake of brevity, this paper will be restricted to a discussion of a hypothesis for the O_2 evolving centre with reference to these reviews and citing only a few other relevant individual publications.

The overall reaction for photosynthetic O_2 evolution is

$$2H_2O \xrightarrow{h\nu} O_2 + 4H^+ + 4e^- \quad (1)$$

In experiments with single turnover flashes of light it was shown [7] that the release of one O_2 molecule required the sequential accumulation of four charges and the individual intermediate states were designated as S-states:

$$S_0 \xrightarrow{h\nu} S_1 \xrightarrow{h\nu} S_2 \xrightarrow{h\nu} S_3 \xrightarrow{h\nu} S_4 \longrightarrow S_0 \quad (2)$$

(The subscripts indicate the number of positive charges accumulated.)

Our model is based on and accommodates 4 well-established experimental observations: (i) the photo-oxidation of water to molecular O_2 proceeds via the successive storage of four oxidising equivalents [7]; (ii) the 4 protons released for every molecule of O_2 liberated during the charge accumulation occur with a stoichiometry sequence of 1, 0, 1, 2 over the successive S-states [8]; (iii) 4 manganese atoms per active centre appear to be optimal [9,10]; (iv) chloride is a necessary cofactor [11,12].

The multiple electron transfers required, the Cl^- involvement, the charging aspect of the process and the multiplicity of Mn ions all imply a manganese cluster with bridging Cl^- between the Mn centres as a principal unit in the oxygen evolving system. The proposition that Cl^- bridges between Mn ions was put forward years ago [13] and has been 'rediscovered' in a recent publication [14]. The present authors had the same notion arising from the demand for Cl^- in the mangrove photosystem [12]. Bridging Cl^- between Mn^{2+} is well known in inorganic systems and it can lead to coupling of the metal ions in an antiferromagnetic manner and to EPR silent systems [15]. Such a combination therefore could explain the failure to observe the EPR signal due to the manganese ions in the S_0 state [16]. Subsequent oxidation to Mn(III) (S_1) leads inevitably to an EPR active cluster.

The schematic representation in fig.1 would accommodate the factors outlined earlier for the reactions through the S-states. The Cl^--coupled Mn^{2+} ions allow multiple and concerted intramolecular electron transfers through the bridges

Published by Elsevier Science Publishers B.V.

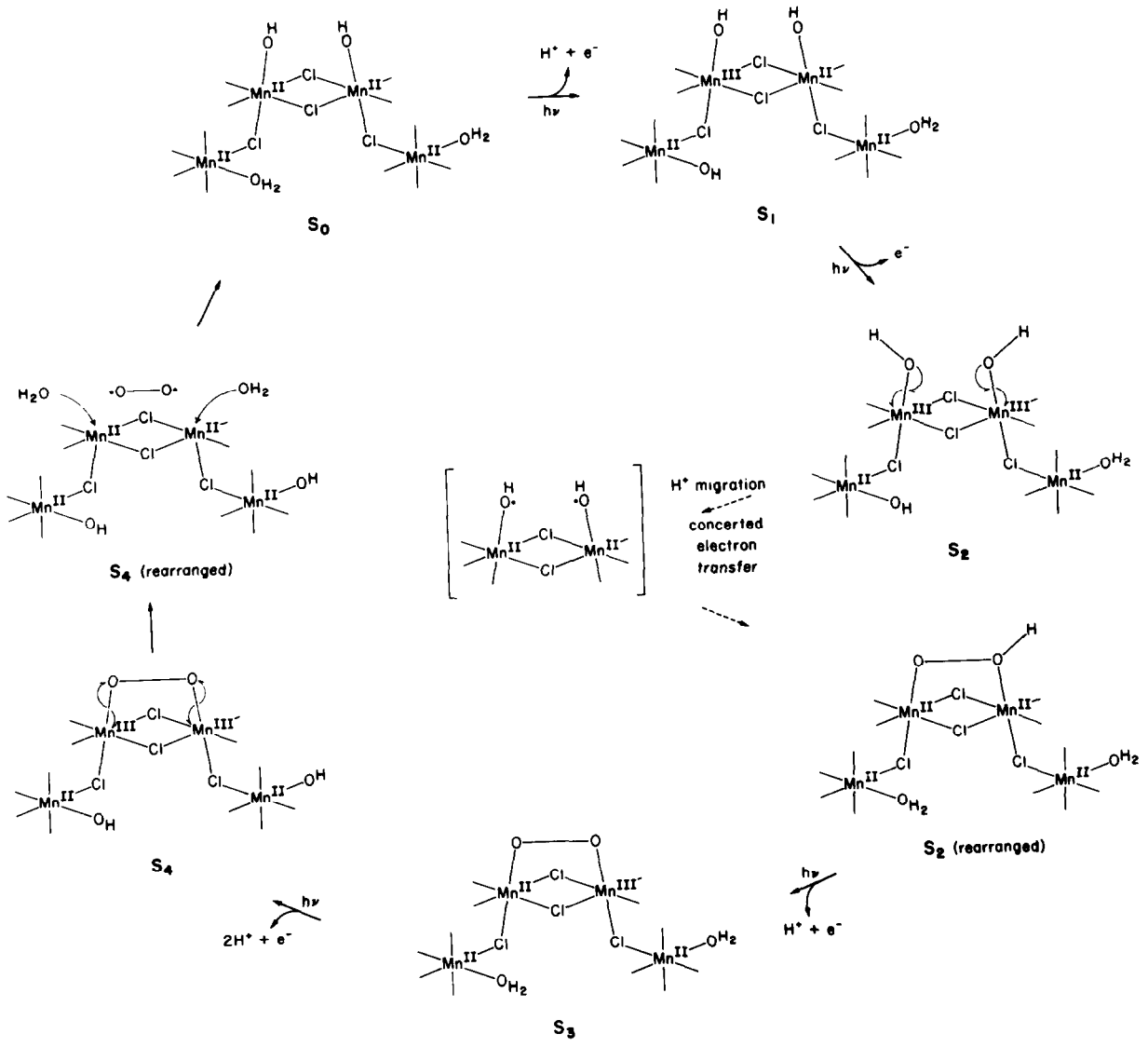

Fig.1. Proposed model for the mechanism of photosynthetic O_2 evolution indicating the sequence of reactions through the S-states.

and also help to stabilise the cluster systems [17]. At least two oxygen atoms need to be bound to two metal sites in close proximity so that the O–O bond can be generated in a facile intramolecular manner. The proton releases are probably effected through deprotonation of water molecules and/or OH^- ions bound to the metal centres. Finally, the whole cluster would need to be stabilised on the polypeptide framework by ligating atoms of the peptide to the Mn ions, as well as by the bridging Cl^-.

Polypeptides of 33, 23 and 16 kDa are functionally associated with the O_2 evolving system [18]. None of these polypeptides appear to contain Mn. However, a 33-kDa polypeptide from spinach thylakoids has recently been claimed to contain two Mn ions [19]. Also, a 13-kDa polypeptide with two Mn ions bound has been identified in thylakoids from a cyanobacterium, *Plectonema boryanum* [20]. In blue-green algae, two such polypeptides may associate to form the 4 Mn cluster which seems to be optimal for O_2 evolution, whereas in

higher plants a single 33-kDa protein may suffice.

The scheme in fig.1 shows the reaction sequence. In S_0 all 4 Mn atoms are in the Mn(II) oxidation state and as well as being bonded to the protein they are bonded to both Cl^- (bridging) and OH^- or H_2O. After the first flash the S_1 state is generated with simultaneous loss of one proton and one electron and oxidation of Mn(II) to (III). The increase in acidity of H_2O bound to Mn(III) relative to Mn(II) permits ready proton loss. S_2 is formed after the second flash with the loss of one electron and oxidation of another Mn(II) to (III). No proton is lost in this step. Electron and atom rearrangement ensues. In a concerted two-electron process, the powerful oxidants, Mn(III), remove an electron each from the bound OH^- ions reverting to the Mn(II) state. The resultant bound hydroxyl radicals dimerise to form coordinated peroxide. In the process, the newly formed peroxide loses a proton which is recaptured by the neighbouring coordinated OH^- bound to a Mn(II) centre. The cluster can flex in such a way that it adjusts the distance between the coordinated OH groups somewhat to facilitate the O–O bond formation.

The next step, $S_2 \rightarrow S_3$, arises from the third flash which generates an electron, a consequent Mn(III) ion and a proton. The proton would be lost from the coordinated hydroperoxide ion. Following the final flash, $S_3 \rightarrow S_4$, another electron is removed, Mn(III) is generated and two protons are lost from the complex. At this point, a concerted two-electron oxidation is effected by the two Mn(III) ions to remove two electrons from peroxide ion and O_2 is generated. The dioxygen molecule evolves and the two Mn(II) ions remaining capture two water molecules. Proton rearrangement in the cluster regenerates the S_0 complex. All this chemistry could be conducted in the time scale implied by the kinetic studies on the photosystem [4]. The intramolecular electron and proton transfers should be very fast and the intramolecular $HO^{\cdot}\cdots\cdots{}^{\cdot}OH$ radical dimerisation should also be rapid [21]. The final concerted two electron transfers from the O_2^{2-} ion to two Mn(III) centres would be expected to generate O_2 in its less reactive ground state (triplet).

Mn(II) in the S_0 condition seems to be the logical oxidation state involved with oxygen and nitrogen atoms the most likely coordinating atoms. Bearing in mind the $Mn(H_2O)_6^{2+/3+}$ redox potential (+1.5 V (*vs* NHE)) [22] and that anticipated for $Mn(NH_3)_6^{2+/3+}$, approx. +0.6 V (estimated from the potentials of $Mn^{2+/3+}$ hexaazacryptate complexes), a combination of the two types of ligating atom coupled with the bridging Cl^- ligands could generate a potential consistent with that assessed for the O_2 evolving cluster (+0.85 V) [4]. The pK_a for H_2O bound in $Mn(H_2O)_6^{2+}$ is 10.59 (25°C) [20] but the value for $Mn(H_2O)_6^{3+}$ is unknown. Given that there is a parallel between Mn(II)(III) and Fe(II)(III) for high-spin systems. $Mn(H_2O)_6^{3+}$ should have a $pK_a \sim 6$ [23]. Deprotonation of the H_2O bound to Mn(III) in a high-spin condition therefore should occur below pH 7. By a similar argument, H_2O_2 bound to two Mn(II) ions might be expected to lose one proton but not both in neutral conditions. H_2O_2 is a somewhat stronger acid than H_2O (pK_a 11.8) [23] and O_2^{2-} is a very strong base (pK_a $HO_2^- > 20$). It follows that after the dual concerted oxidation of two OH^- and formation of peroxide ($S_0 \rightarrow S_1 \rightarrow S_2$) one proton migrates from the peroxide centre to an adjacent coordinated OH^- ion. There is therefore no net release of H^+ after the second flash [24]. A Mn(III) and Mn(IV) cluster seems to be unlikely in view of the types of ligands and the exalted redox potentials implied. Mn(II)(III) mixtures also are implied by X-ray absorption edge spectroscopy [25]. If Mn(III) was present in the dark condition and the system was inactivated to release manganese, chlorine should also be evolved. A consequence of the present proposal is the intermediacy of peroxide ion and its presence needs to be probed further.

Clusters of the type proposed have an ability to undergo multiple electron changes and transfer electrons rapidly, provided the metal ions communicate through the bridges. The individual oxidation states of the metal centres cannot therefore be differentiated and they are only formally assigned in fig.1 to take account of the stoichiometry. The pK_a of a proton bound to any ligand therefore will depend on the charge of the cluster rather than any individual metal centre. It follows that the proposal would cope just as well with a different proton release sequence, e.g., 1, 1, 1, 1. The cluster would merely dissociate a proton from a bound water molecule or hydroperoxide ion at the appropriate point following the flash. It is even possible that the observed flash/proton release events are unrelated to deprotonation events at the

cluster since proton pools in the membrane may be involved [26].

Inhibition of water oxidation by NH_3 and OH^- could arise readily by substitution of these ligands for Cl^-, as has indeed been suggested [13]. The coordination of NH_3 would destroy the cluster arrangement and thus inhibit electron transfer. Hydroxide ion could substitute for the Cl^- in the bridges and alter the redox potential of the cluster. Inhibition by NH_2OH and NH_2NH_2 [27] is especially interesting since these molecules are isosteric with peroxide ion if lone pairs of electrons on the oxygen atoms are equated with protons on the N centres. Presumably such amine systems could bind competitively across the two Mn ions and inhibit two OH^- binding, or they could displace peroxide and prevent the system evolving O_2. They could possibly consume peroxide via regular chemical processes and obviate O_2 release but it is doubtful if the rate of such reactions would be sufficiently fast.

ACKNOWLEDGEMENTS

C.C. wishes to acknowledge the support by a Reserve Bank of Australia, Rural Credits Development Fund Grant and helpful discussions with Dr C.F. Yocum.

REFERENCES

[1] Cheniae, G.M. (1970) Annu. Rev. Plant Physiol. 21, 467–498.
[2] Radmer, R. and Cheniae, G.M. (1977) in: Topics in Photosynthesis (Barber, J. ed.) vol. 2, pp. 303–348, Elsevier, Amsterdam, New York.
[3] Govindjee, Wydrzynski, T. and Marks, S.B. (1977) in: Bioenergetics of Membranes (Packer, L. et al. eds) pp. 305–316, Elsevier, Amsterdam, New York.
[4] Govindjee (1980) Plant Biochem. J., S.M. Sircar Memorial vol., pp. 7–30.
[5] Govindjee, Wydrzynski, T. and Marks, S.B. (1978) in: Photosynthetic Oxygen Evolution (Metzner, H. ed.) pp. 321–344, Academic Press, London.
[6] Amesz, J. (1983) Biochim. Biophys. Acta 726, 1–12.
[7] Kok, B., Forbusch, B. and McGloin, M. (1970) Photochem. Photobiol. 11, 457–475.
[8] Saphon, S. and Crofts, A.R. (1977) Z. Naturforsch. 32C, 617–626.
[9] Cheniae, G.M. and Martin, I.F. (1971) Plant Physiol. 47, 568–575.
[10] At least two publications ([16] Klimov et al. and Yamamoto and Nishimura) have shown that two of the four manganese ions can be replaced by Mg^{2+} with the O_2-evolving system still functioning, albeit at a reduced level.
[11] Hind, G., Nakatani, H.Y. and Izawa, S. (1969) Biochim. Biophys. Acta 172, 277–289.
[12] Critchley, C. (1982) Nature 298, 483–485.
[13] Bove, J.M., Bove, C., Whatley, F.R. and Arnon, D.I. (1963) Z. Naturforsch. 186, 683–688.
[14] Sandusky, P.O. and Yocum, C.F. (1983) FEBS Lett. 162, 339–343.
[15] Jensen, S.J., Anderson, P. and Rasmussen, S.E. 1980 (1962) Acta Chem. Scand. 16.
[16] Klimov, V.V., Allakhverdiev, S.I., Shuvalov, V.A. and Krasnovsky, A.A. (1982) FEBS Lett. 148, 307–312.
Yamamoto, Y. and Nishimura, M. (1983) Biochim. Biophys. Acta 724, 294–297.
[17] The enhanced stability of clusters is typified by the Fe_4S_4 clusters found in ferredoxins, for example.
[18] Current research in this area is especially active and the reader is referred to many articles in 'The Oxygen Evolving System of Photosynthesis' (Inoue, Y. et al. eds) (1983) Academic Press, Tokyo.
[19] Dismukes, G.C., Abramowics, D.A., Ferris, K.F., Mathur, P., Siderer, Y., Upadrashta, B. and Watnik, P. (1984) in: Proceedings of the 6th International Congress on Photosynthesis (Sybesma, C. ed.) Martinus Nijhoff, The Hague, in press.
[20] Okada, S. and Asada, K. (1983) Plant Cell Physiol. 24, 163–172.
[21] Taube, H. (1970) Electron Transfer Reactions of Complex Ions in Solution, Academic Press.
[22] Vetter, K.J. and Manecke, G. (1950) Z. Phys. Chem. 195, 270.
[23] 'Stability Constants' Special Publications 17 and 25, The Chemical Society (London), 1964 and 1971.
[24] Peroxide bridging trivalent metal ions are usually doubly deprotonated but two Mn^{2+} should not polarise the HO_2^- sufficiently to dissociate the proton in neutral conditions. See Yorino, T., Matsushita, T., Masuda, I. and Shinra, K. (1970) J. Chem. Soc. Chem. Commun. 1317–1318. Also Fallab, S. and Mitchell, P.R. (1984) in: Advances in Inorganic and Bioinorganic Mechanisms (Sykes, A.G. ed.) vol. 3, in press.
[25] Kirby, J.A., Goodin, D.B., Wydrzynski, T., Robertson, A.S. and Klein, M.P. (1981) J. Am. Chem. Soc. 103, 5537–5542.
[26] Dilley, R.A., Baker, G.M., Bhatnagar, D., Millner, P. and Laszlo, J. (1981) in: Energy Coupling in Photosynthesis (Selman, B.R. and Selman-Reimer, S. eds) pp. 47–58, Elsevier, Amsterdam, New York.
[27] Radmer, R. and Ollinger, O. (1983) FEBS Lett. 152, 39–43.