bridges in transition metal clusters are unreactive as a class, they would require special activation if they were to be involved in oxidations or O-O bond formation. We propose that the unusual electronic configuration of the MnIII cluster of the WOC is an expression of this activation.

Formation of the activated (d_5)^1(dx_2)^1 electron configuration may also be explained if the MnIII ions have only five ligand atoms, arranged so that the equatorial ligand field is weaker than the axial component. This geometry has been observed in the five-coordinate complex, H[Bpz(3,5-i-Pr)2]_2Mn(μ-O)2Mn[bpz(3,5-i-Pr)2]BH+, which also has the unusual MnIII hyperfine anisotropy as seen in the WOC. Five-coordinate MnIII ions are rarely encountered in chemistry. They are susceptible to hydrolysis and act as strong oxidizing agents. These features offer a satisfying explanation for the unique reactivity of the WOC as catalyst for water oxidation.

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OEC during the relatively slow multiple electron chemistry of water oxidation. The release of molecular oxygen by the oxidation of water is a four-electron process: \(2 \text{H}_2\text{O} \rightarrow 4\text{H}^+ + 4e^- + \text{O}_2\). During its cycle, the OEC passes through different redox states termed S-states, \(S_0-S_6\); electrons are removed from \(S_6\) to \(S_4\) and \(O_2\) is evolved at \(S_4\). A manganese cluster appears to act as both a charge accumulation device and active site. Both Cl\(^-\) and Ca\(^{2+}\) ions act as cofactors for water oxidation. (For greater detail see reviews in [2-9]).

**Results and discussion**

**Model of the D1/D2 reaction centre [1]**

P680 is assumed (see below) to be a Chl dimer, ligated by His-198 of D1 and D2, which are conserved residues analogous to the ligands found in the purple bacterial reaction centre. The D1 Chl may have two hydrogen bonds, to D1 Ile-290 and Thr-186, and the D2 Chl may have one, to D2 Ser-283. The side-chains of tyrosine residues \(Y_\alpha\) and \(Y_\beta\), are located about 35 Å apart, between the luminal ends of transmembrane helices 3 and 4 of each polypeptide. The nearest point to the special pair is about 8 Å (\(Y_\alpha\)) and 7 Å (\(Y_\beta\)). D1 Phe-182, situated between \(Y_\beta\) and the special pair, may be involved in promoting electron transfer. In D2 there needs to be a rearrangement to accommodate D2 Phe-170 which is predicted to bring D2 His-190 closer to \(Y_\alpha\) than is D2 His-190 to \(Y_\beta\). Hydrogen bonding to \(Y_\beta\) may occur with D2 His-190 and/or D2 Gln-165 (the amide oxygen of Gln-165 could serve as a hydrogen bond acceptor for the tyrosyl proton with the amide proton stabilizing the tyrosyl oxygen). In D1, interactions of \(Y_\alpha\) with D1 Gln-165 and D1 Asp-170 are favoured. The less hydrophobic environment of \(Y_\beta\) is contrasted with that of \(Y_\alpha\), which is located in a very hydrophobic pocket. The D1/D2 heterodimer probably binds the manganese cluster, mainly using D1 ligands. D1 residues in the original model that were close to \(Y_\alpha\) and capable of forming ligands to metal ions included Gln-165, Ser-167, Asp-170, Gln-189 and His-190. Asp-170 is indicated as a high-affinity binding site for manganese by site-directed mutagenesis experiments (for review, see [9,10]). It has been proposed that the C-terminus portion of the D1 protein contributes to the manganese-binding site since there is a requirement for His-332, Asp-342 and correct post-translational processing to give the terminal residue Ala-344. The C-terminus would be required to fold back under the reaction centre and this has been modelled. It is difficult to provide enough ligands to bind a tetrameric manganese (or "dimer-of-dimers") cluster using only D1 residues. Therefore, we cannot rule out that residues from D2 or other photosystem II (PSII) polypeptides provide a small number of ligands. One possible binding of a manganese cluster using D1 residues Asp-170, Gln-165, Asp-342, His-337 and Ala-344 has been modelled. D1 His-332 and Gln-189 are nearby and can interact with His-190. This gives a complex 15-20 Å from P680, the primary electron donor of PSII, 35 Å from \(Y_\gamma\) and about 7 Å from \(Y_\alpha\). A chain of conserved residues Ile-290, Phe-182, His-190, Tyr-161 and Asp-170 is found between P680 and the cluster. Of course, other arrangements are possible, some of the residues discussed may bind Ca\(^{2+}\), which may alter the position of the cluster; however, present evidence suggests that it is close to this position.

**P680**

P680 exhibits a redox potential of about 1100 mV compared with about 830 mV for Chl \(a\) in vitro. Therefore, the structure and/or environment of P680 is different to that of other types of reaction centre which have much lower redox potentials. The monomeric or dimeric nature of P680 has been a source of controversy (see [2-9] for review). It may vary depending on whether the ground state, excited state, triplet state or radical cation is analysed. The sequence homology between D1/D2 and the L/M proteins of purple bacteria plus the stoichiometry of PSII pigments argues for a 'purple bacteria' arrangement with a Chl pair bound to His-198 of D1 and D2. In contrast, e.p.r. studies of the P680 triplet state support a monomeric structure with the possibility that P680 is analogous to the accessory Chls in purple bacterial reaction centres. Absorption-detected magnetic resonance and resonance Raman data on the triplet state also favour a monomer with little or no exciton splitting. However, a Fourier transform infra-red spectroscopic study at 80 K shows bleaching of two carbonyl bands corresponding to an asymmetric dimer with an 86:14 triplet state distribution [11].

E.p.r. studies of P680\(^{2+}\) have interpreted the reduced linewidth as indicating a modified monomeric or dimeric structure. Low-temperature, time-resolved and steady-state optical techniques suggest that the ground state of P680 is dimeric, with a much weaker exciton splitting than the purple bacterial primary donors, but again indicate that formation of the triplet and radical cation states involves only one half of the dimer. The weak exciton splitting and localization of the paramagnetic states could be due to an increased separation or a differ-
ent Chl orientation relative to the purple bacterial structure. Hole-burning studies of the excited singlet state (P680') also suggest a P680 dimer but, again, one that differs significantly from purple bacteria. From a recent electron nuclear double resonance (e.n.d.o.r.) study of P680++ (S. E. J. Rigby, J. H. A. Nugent and P. J. O'Malley, unpublished work) we have developed a model for the electron spin density distribution that provides indications of both the structure and environment of P680++. The e.n.d.o.r. data for P680++ implies that this stage involves two weakly interacting Chl molecules. The spin density distribution we calculate for P680++ is about 6:1 over the two halves of the dimer (at 15 K). The concentration of electron spin density on one-half of a P680++ dimer confirms that either the relative orientation and/or the separation of the dimer components is different to that of the bacterial structure. This arrangement may be made possible by the two conserved prolines (D1-279, D2-276) in the PSII structure which are predicted to lie within helix V of each polypeptide. Proline residues are associated with helix bending. These would give a larger cavity and possible greater separation or different orientation of the Chl molecules than is observed in purple bacteria. It is unlikely that this larger pocket would accommodate one proposed dimeric structure with one Chl tilted at a steep angle to the other [11].

The consensus from all these results appears to favour a weakly interacting dimer in the ground state (and possibly P680+ and P680+++) with a temperature-dependent distribution of the triplet state. It would be appropriate for the spin density to reside mainly on the D1 Chl, supporting rapid electron transfer by Y* and slowing electron transfer by Y,.

What is responsible for the distinctive redox potential of P680? There is no obvious mechanism such as the use of a nearby positive charge unless this is provided by Y,. (see below). An unusual arrangement of the constituent pigments, such as a pheophytin/Chl heterodimer allowing D1 or D2 His-198 to be protonated could occur, although pigment stoichiometry data appear to rule this out.

Y,- Oxidation of tyrosine can release the phenoxyl proton to produce the tyrosine neutral radical. The E,, of the neutral radical is one of the lowest among those of the 20 common amino acids at 930 mV. Therefore, neutral tyrosine radicals will not easily oxidize their protein environments and make ideal constituents of high-potential biological redox systems. Y, and Y, can be observed by e.p.r. as signals of similar lineshape. Y, and Y, can be distinguished by their microwave power saturation characteristics which suggest that Y, is closer to the OEC.

The neutral tyrosine radical Y, is an important probe of structural changes to the water oxidizing system. Using e.n.d.o.r. [12] we have determined the proton hyperfine coupling constants of all four ring protons and both -methylene protons for Y, in three species covering the range of oxygenic organisms (plants, algae and cyanobacteria). Estimation of the electron spin density distribution of Y, shows that changes in -proton coupling constants in each organism arise from the slightly different orientation of the tyrosine ring, relative to the -protons. This model enables us to simulate the e.p.r. spectra and will allow the changes in the e.p.r. and e.n.d.o.r. spectra found in studies of mutationally or chemically altered Y, environments to be interpreted.

Y, is stable in the oxidized state for hours but is also able to slowly undergo redox reactions with the lower oxidation states of the OEC. During dark adaptation, the OEC relaxes to the S, state either by advancement from S, by electron donation to Y, (t, = 20–50 min at room temperature) or by deactivation from S, and S, as Y, is oxidized. Therefore, the E,, of Y,/Y, should be between that of S,/S, and S,/S,. The E,, has been estimated to be about +750 mV. The slow rate of oxidation of Y, by P680++ is difficult to reconcile with its position, as the rate of electron transfer between cofactors is strongly influenced by the distance between the reacting components [13]. A number of electron donors compete to reduce P680++ and the oxidation of Y, may be unfavourable if a series of charge equilibria exist or if, as suggested above, the charge on P680 is located on the D1 Chl, greatly increasing the distance to Y,. The function of Y, has been suggested to be in photoactivation of the OEC, the light driven process of Mn oxidation and complex assembly, or to oxidize Mn oxidation may possibly be present in S, preventing loss of manganese. However, in the light, Y, is kept in the oxidized state, perhaps allowing it to modulate the redox potential/charge distribution on the P680 dimer. A positive charge in a region of low dielectric near P680, caused by the protonation of a residue following oxidation of Y,, could significantly raise the redox potential of P680/P680++. The oxidation of Y, may also lead to structural changes which affect the OEC.
**The manganese complex: S₁ and S₃ states**

It has been proposed that a variety of treatments which affect Ca²⁺ or Cl⁻ cofactor binding and/or substrate binding cause a modification of the S₁-
state of the manganese cluster and slow the reduction of Y⁺, allowing an ‘S₃’ e.p.r. signal to be observed [13-15]. The ‘S₃’-type signals are thought to be due to an interaction, S₁X⁺, between the manganese cluster in an oxidation state equivalent to S₁ and an organic radical, either oxidized histidine or the tyrosine radical Y⁺ [13-15]. This type of inhibition suggests that Ca²⁺ and/or Cl⁻ are necessary for normal passage through the higher S-states. We have also shown that after ammonia treatment, the yield of Y⁺ and the <10 mT ‘S₃’-type e.p.r. signal are decreased by calcium addition. This indicates that these effects are probably due to calcium depletion by the ammonium cation.

The S₁X⁺ interpretation of the ‘S₃’ signal has been used to support the lack of oxidation of manganese on the S₂-to-S₁ step, although the percentage of centres having the ‘S₃’ signal may be small. A weak electron exchange interaction between an organic radical and the S’=S, S₂-state simulated the signal [13]. The weak interaction suggests that the radical is not directly liganded to the manganese cluster, but such a binding may be possible if the manganese cluster occurs as a ‘dimer-of-dimers’ and the radical is liganded to a manganese pair only weakly contributing to the S’=S, S₂ state [i.e. the radical is a manganese ligand but to the Mn₃(III, III) or Mn₃(IV, IV) pair not the Mn₃(III, IV) pair that is largely responsible for the e.p.r. properties of the S₂ state]. The ‘S₃’ signal may arise from the manganese cluster alone but the linewidth is much smaller than would be expected from a manganese complex. This lack of oxidation of manganese on the S₂-to-S₁ step has been supported by other studies [2,4-6]. A recent paper [16] has, however, indicated an oxidation of manganese on the native S₂ to S₁ step.

We have studied changes in the electron spin lattice relaxation time, T₁, of the dark-stable tyrosine radical Y₁, using pulsed e.p.r. The effects can be related to changes occurring at the manganese cluster. The T₁ relaxation times of calcium-depleted/NaCl-treated samples decreased with increasing modified S-state S₁ > S₂ > S₃, supporting the hypothesis of a manganese oxidation state change between each step. Our measurements of edge-shifts in X-ray absorption spectra [18,19] also indicate manganese oxidation on the S₁-to-S₂ step in this preparation (see [20]). This suggests that the e.p.r. data on the inhibited samples may be explained as formation of modified S₁ by manganese oxidation via Y⁺, but at a rate that allows a significant population of the S₁X⁺ intermediate to be trapped.

The most appropriate model for the manganese complex based on our extended X-ray absorption fine structure (exafs) studies [18-21] combined with that from previous reports would be a structure comprising two or more manganese oxo-bridged dimeric units. The interdimer distance would be 3.3/3.6 Å. The number of oxo-bridging ligands would vary in an S-state dependent manner. For the native state each dimer would have a Mn-Mn separation of 2.7 Å, giving rise to the S₁ state with probable oxidation state Mn(III)Mn(IV). In the calcium-depleted/NaCl-treated samples and possible ammonia-treated samples, one of the dimer Mn-Mn separations becomes 3.0 Å resulting in reduced exchange coupling.

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Study of the intermediate S-states for water oxidation in the normal and Ca-depleted photosynthetic oxygen-evolving enzyme by means of flash-induced X-ray absorption near edge structure spectroscopy

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Introduction

Photosynthetic oxygen evolution takes place at the oxygen-evolving centre (OEC) within the photosystem II (PSII) complex. A tetranuclear Mn-cluster is thought to be a chemical entity of the centre, and cycles through several redox states (corresponding to intermediate states for oxidation of water) denoted S_i-states (i = 0–4), with S_2 as the stable state after dark adaptation (see [1–3] for reviews). Absorption of a photon advances the S-state by one step to release molecular oxygen coupled with dark conversion from S_4-to-S_0 state. Ca is an indispensable cofactor for the normal cycling of the S-states, and the capability of oxygen-evolution is lost after treatments which are claimed to release a functional Ca from PSII (see [1,4] for reviews). In the Ca-depleted OEC, not only functional but also structural properties of the Mn-cluster have been assumed to be modified, as depicted by modification of line-shape of the multiline e.p.r. signal arising from the Mn-cluster in the S_2-state [5–7]. When the OECs bearing such altered S_2-state are further illuminated, an extra positive charge accumulates, exhibiting an e.p.r. signal in the \( g = 2.0 \) region [5,8,9] and a thermoluminescence band peaking at about 10°C through recombination with \( Q_A \) [9]. It has been postulated that these signals arise from an...