Photosynthesis Research 13: 199–223 (1987)

Hypothesis

A model for the mechanism of chloride activation of oxygen evolution in photosystem II

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Received 17 April 1987; accepted 19 May 1987

Key words: chloride, oxygen-evolving complex, photosystem II, D_1 and D_2 polypeptides; manganese

Abstract. A hypothesis is proposed to explain the function of Cl^- in activating the oxygen-evolving complex (OEC) of photosystem II (PS II), based on the results of recent ^{35}Cl-NMR studies. The putative mechanism involves Cl^- binding to two types of sites. An intrinsic site is suggested to be composed of three histidyl residues (His 332 and His 337 from D1 and His 337 D2). It is proposed that Cl^- binding to this site accelerates the abstraction of H^+ from water by raising the pK_a's of the histidine imidazole groups. Cl^- binding also stimulates the transfer of H^+ from this intrinsic site to a set of extrinsic sites on the 33 kD extrinsic polypeptide. The extrinsic Cl^- binding sites are suggested to involve four protein domains that are linked together by salt-bridge contacts. Chloride and H^+ donated from the intrinsic site attack these intramolecular salt-bridges in a defined sequence, thereby exposing previously inaccessible Cl^- and H^+ binding sites and stimulating the oxidation of water. This hypothesis also proposes a possible structure for the Mn active site within the D1/D2 complex. Specific amino-acid residues that are likely to participate as Mn ligands are identified on the lumenal portions of the D1 and D2 proteins that are different from those in the L and M subunits of photosynthetic bacteria; the choice of these residues is based on the metal coordination chemistry of these residues, their location within the polypeptide chain, the regularity of their spacing, and their conservation through evolution. The catalytic Mn-binding residues are suggested to be D-61, E-65, E-92, E-98, D-103; D-308, E-329, E-342 and E-333 in D1, and H-62, E-70, H-88, E-97, D-101; E-313, D-334, E-338 and E-345 in D2. Finally, this hypothesis identifies sites on both D2 and the 33 kD extrinsic polypeptide that might be involved in high- and low-affinity Ca^{2+} binding.

Introduction

Although Cl^- is a very simple anion, its influence on the function of the oxygen-evolving complex (OEC) of photosystem II (PS II) is profound (see Govindjee et al. 1983, 1985, Izawa et al. 1983, Homann 1987). The ^{35}Cl-NMR experiments described elsewhere (Coleman et al. 1987a, b, c, d) were

undertaken in order to provide direct insight into the number, location, and significance of the Cl^--binding sites in spinach PS II. The purpose of this paper is to combine the information obtained from the ^{35}Cl-NMR experiments with what is known from other sources into a simple and practical model for the mechanism of Cl^- activation.

The approach that will be used here will be to: 1) define the essential features of the Cl^- binding sites as indicated by the experimental evidence; 2) compare these features with similar, more thoroughly characterized structures in other proteins or models; and 3) identify probable sites in PS II proteins whose structure and composition would be consistent with these criteria.

There are several lines of evidence indicating that the Cl^- binding sites in the OEC are not homogeneous. Homann (1985) has found, for example, that when the pH-dependence of Cl^- binding is measured by assaying its effect on the light-driven steady-state turnover of the enzyme, Cl^- appears to bind to a group having a pK_a of about 6.0. Studies of Cl^- depletion, however, have shown that the OEC retains most of its Cl^- unless the pH of the medium is raised beyond 8.0 (Izawa et al. 1969, Theg and Homann 1982, Homann et al. 1983). Similarly, the ^{35}Cl-NMR binding studies reported in Coleman et al. (1987c, d) indicate that although the multiple line width maxima observed in measurements of the untreated membranes are eliminated by heating (Coleman et al. 1987c) or removal of the extrinsic polypeptides (Coleman et al. 1987a, d), a significant amount of Cl^- binding still occurs. Oxygen evolution is also not completely eliminated by these two treatments. A simple explanation for these apparent anomalies is that the OEC contains two different types of Cl^- binding sites, and that they are physically separate and chemically distinct. One site, located nearer to the Mn active site, will be referred to here as "intrinsic". The other site, located on the 33 kD polypeptide will be referred to as "extrinsic".

Proposed structure and properties of the intrinsic Cl^- binding site

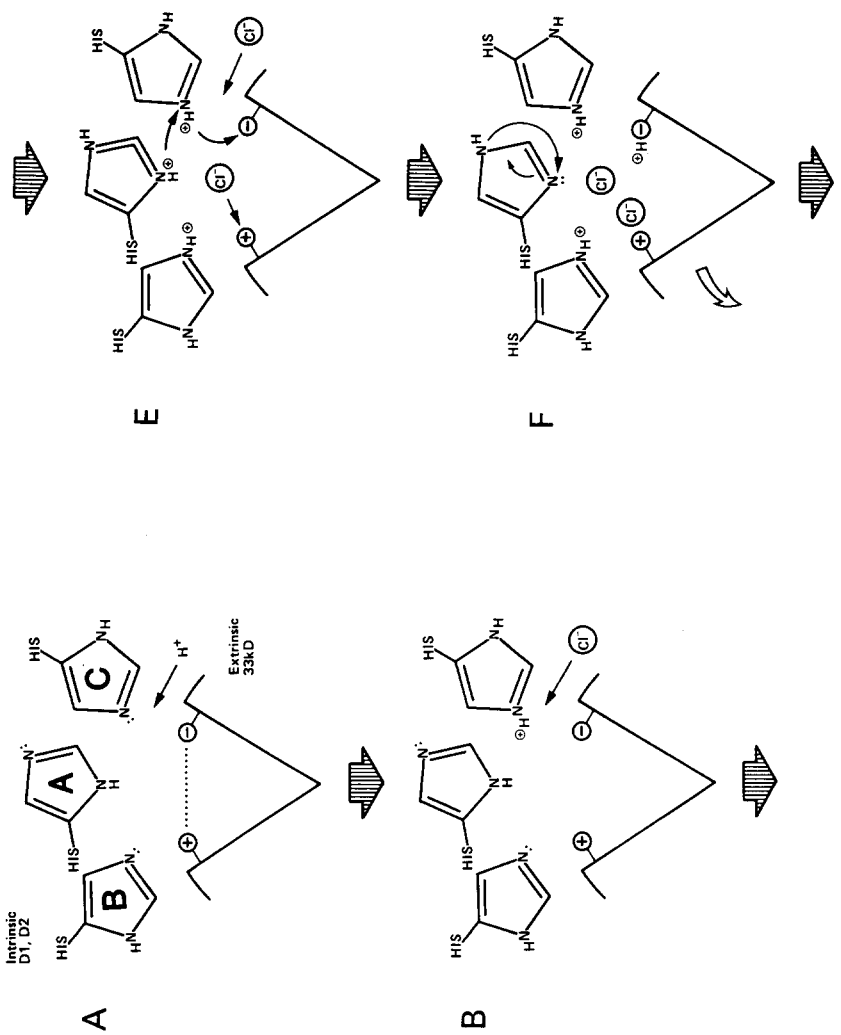
One of the major effects of Cl^- on the function of the OEC active site is a pronounced shift of the activity-pH optimum to alkaline pH (Gorham and Clendenning 1952, Critchley 1985). Massey (1953) and Alberty (1954) have described in general terms how this phenomenon can be attributed to the presence of three protonatable groups in the enzyme active site (see Coleman 1987, Coleman et al. 1987b). In applying this idea to the OEC, we make the assumption that the anion-sensitive, bell-shaped pH profile arises because one of these groups is a proton acceptor (unprotonated at the pH optimum), the second is a proton donor (protonated at the pH optimum), and the third

is capable of binding Cl^- in its protonated state. Combination of Cl^- with this third group would be expected to raise the pK_a's of the other two, producing an alkaline shift in the activity-pH curve (Coleman and Govindjee 1985). If the catalytic function of the first two groups is to remove protons from water, then the upward shift in their pK_a's will activate the enzyme by increasing the rate of O_2-evolution.

Homann's conclusion that the pK_a of the Cl^- binding group is about 6.0 places obvious constraints on the identity of the Cl^- binding residue. In addition, the pH profile for Cl^- binding, as measured by ^{35}Cl-NMR (Coleman et al. 1987b), indicates that there may be as many as three groups whose protonation states affect the binding of Cl^- in the region between pH 5.4 and 6.5. Together, these data are consistent with our suggestion that three histidyl residues contribute to the active site mechanism of the OEC.

It is not uncommon for groups of histidines to be involved in the active sites of enzymes, particularly in those which catalyze acid-base reactions. In the active site of human carbonic anhydrase, for example, there are three histidines (with pK_a's of 4.72, 6.00, and 6.14) whose pK_a's are linked (Campbell et al. 1974, Forsman et al. 1983). In pancreatic ribonuclease, there are two catalytic histidines which constitute part of a Cl^- binding site (Matthew and Richards 1982). The protonation state of these two residues is strongly influenced by the presence of bound anions (Richards and Wyckoff 1971 (see pp. 801–806) Matthew and Richards 1982). Consequently, these anions directly influence the activity of the enzyme (Kalnitsky et al. 1959). The pK_a's of the histidines also depend on the nature of the anion itself (Richards and Wyckoff 1971).

The mechanism we propose for this site (Fig. 1) involves facilitated transfer of protons (Wang 1968) from bound water at the active site Mn to protonatable groups on the 33 kD extrinsic polypeptide. The transfer pathway involves three histidine imidazole groups whose pK_a's are raised by the presence of Cl^-. Initially, Cl^- binding to this intrisic site in the Cl^- depleted system accelerates the light-driven dissociation of H^+ from water by stabilizing the H^+ on the histidine cluster. In subsequent steps, it connects the intrinsic site to the extrinsic sites by promoting the transfer of protons into previously sequestered (salt-bridged) domains of the 33 kD polypeptide. In the absence of Cl^- (Fig. 2), the intrinsic site is able to accept a single H^+ from water (depending on the pH), but cannot transfer this H^+ to the extrinsic sites. The details of the individual steps are given in Figs. 1 and 2. The kinetics of H^+ transfer between water and imidazole indicate that an imidazole group is an appropriate acceptor for H^+ donated from water (Metzler 1977; see pp. 405–406).


A
B
C
HIS
Intrinsic
D1, D2
Extrinsic
33kD
E
F

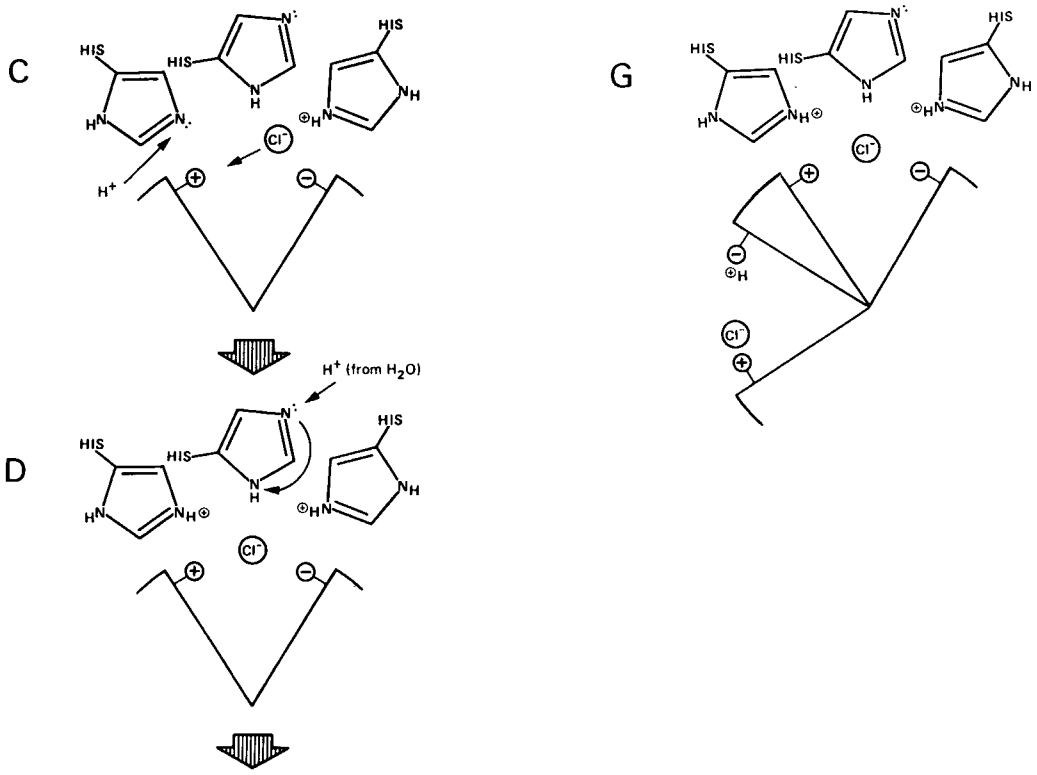


Fig. 1. A working model for the Cl^- binding mechanism at the active site of the oxygen evolving complex (OEC). As the pH of the external medium is lowered (A), His-C is protonated, enabling Cl^- to bind (B). Cl^- binding to His-C raises the pK_a of His-B, so that as the pH is lowered further to 6.0, His-B is protonated (C). Cl^- binding also raises the pK_a of His-A, which is able to accept a H^+ from the water-splitting reactions (D). When the H^+ is moved into the Cl^- binding site by a resonance shift in the imidazole group (see Metzler, 1977: 55, Ashikawa and Itoh 1979) of His-A (E), the site is destablized. Because of this instability, Cl^- and H^+ attack a salt-bridge on the 33 kD polypeptide (E). His-A then relaxes, and another Cl^- binds (F). Another extrinsic site is then open to attack (G).

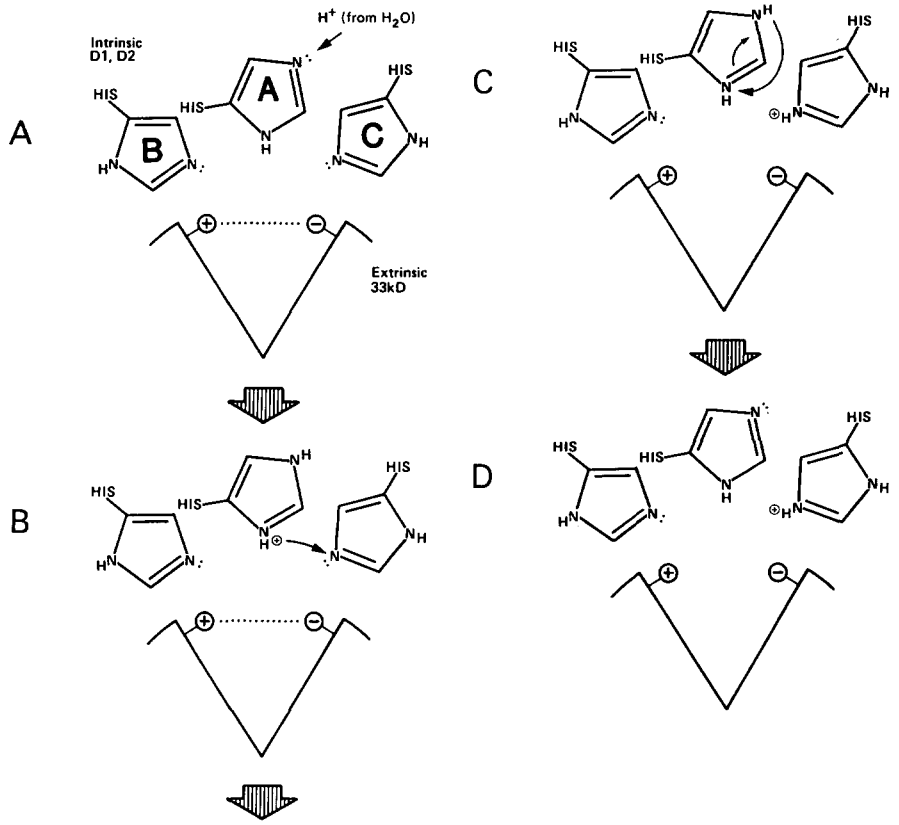


Fig. 2. A working model for proton transfer into the intrinsic Cl^- binding site in the absence of Cl^-. With no Cl^- present, His-A accepts a H^+ from water (A) and transfers it to His-C, which has a higher pK_a (B). His-A then relaxes back to its original state (C). The H^+ remains bound to His-C because there is no Cl^- present to attack the salt-bridge on the 33 kD polypeptide (D). The system cannot accept additional protons.

Justification for the mechanism at the intrinsic site

This model explains a number of experimental observations concerning the catalytic effects of Cl^- on the water-splitting reactions (Fig. 1): 1) the pK_a for optimal activation by Cl^- is 6.0 because at this pH, Cl^- is bound to a protonated His-C. His-B is also protonated at this pH, and therefore is able to donate a proton to an acceptor group on the extrinsic sites. His-A is unprotonated, and binds a H^+ from water; 2) chloride shifts the activity-pH optimum by neutralizing some of the positive charges in the cluster. By raising the pK_a of His-A, it also raises the maximum velocity of the enzyme at alkaline pH; 3) the activity of the enzyme depends on the nature of the anion because the magnitude of the pK_a shifts depends on the contribution of the anion to the electrostatic potential (Matthew and Richards 1982,

Mehler and Eichele 1984); 4) in the absence of Cl^- (Fig. 2), the OEC cannot advance beyond the S_2 state (Itoh et al. 1984, Theg et al. 1984, see also Coleman 1987) because the Cl^--free site can only accept one H^+. This H^+ is held stably and at a shorter distance from the Mn site because there is no Cl^- available to move it out onto the 33 kD polypeptide; 5) amines will compete with Cl^- (see Sandusky and Yocum 1986) at the intrinsic site by forming hydrogen bonds with the imidazole nitrogens, thereby displacing Cl^-.

Location of the intrinsic site within the OEC

Given that three histidyl residues appear to be required for the Cl^- activation mechanism at the active site, we decided to examine the published amino-acid sequences of the intrinsic PS II proteins (particularly D1 and D2), to determine whether such a cluster might exist. The proteins D1 and D2 were chosen for several reasons: 1) they have recently been implicated as the PS II reaction center core polypeptides (Nanba and Satoh 1987); 2) both proteins are believed to have long loops of charged residues (including histidine) that project into the thylakoid lumen (Trebst and Draber 1986); 3) the D1/D2 protein is believed to contain binding sites for the catalytic Mn (Metz and Bishop 1980); 4) ^{35}Cl-NMR results show that PS II membranes in which the extrinsic polypeptides have been removed are still able to bind Cl^- (see Coleman et al. 1987d); 5) the 33 kD extrinsic polypeptide contains no histidine (Oh-oka et al. 1986).

If D1 and D2 are assumed to form a heterologous dimer, there are three histidines at the carboxy-terminal ends of each protein (His 337 on D2 and His 332 and His 337 on D1) that might be close enough to each other to form a cluster (see Figs. 3 and 4). Although many of the His residues in the membrane-spanning helices of these proteins have been tentatively assigned to the binding of reaction center components (e.g., P680, Q_A, Fe, etc.; see Trebst and Draber 1986), there have been no assignments yet proposed for the 8 His residues on D1 and D2 that extend into the aqueous phase of the lumen (Trebst and Draber 1986). Since we have proposed that three of these His residues are involved in the Cl^- mechanisms, we must explain the probable function of the five remaining histidines.

Proposed structure for the Mn binding sites in PS II

Because the D1/D2 protein has been strongly implicated in Mn binding (Metz and Bishop 1980), we decided to examine the amino-acid sequence for

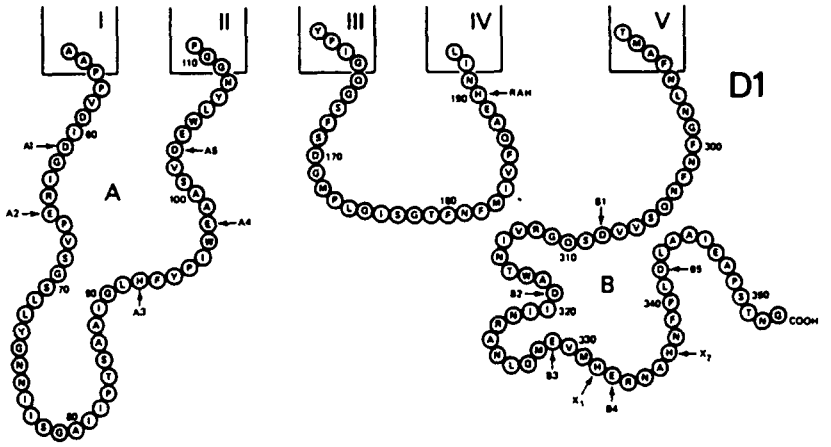


Fig. 3. A model for the arrangement of the intra-thylakoid loops of the D1 protein in spinach. Only the inner portions of the five membrane-spanning helices are shown. The amino-acid sequence and location of the loops is taken from Trebst and Draber (1986). The suggested 5 amino-acid ligands for the Mn "A" binding site are labeled A1, A2, etc. Those for the "B" site are labeled B1, B2, etc. "RAH" refers to a redox-active histidine. X1 and X2 are histidines suggested to be involved in Cl^- binding; the third histidine is on D2 (see Fig. 4).

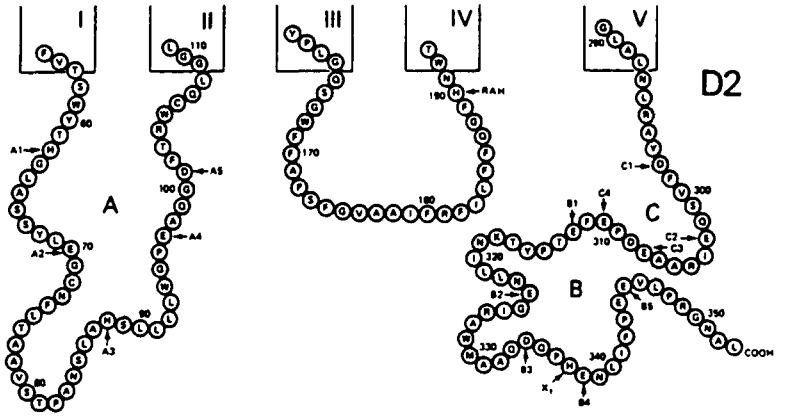


Fig. 4. A model for the arrangement of the intra-thylakoid loops of the D2 protein in spinach. Only the inner portions of the five membrane-spanning helices are shown. The amino-acid sequence is taken from Trebst and Draber (1986), but was checked against several others in order to correct discrepancies (e.g., at residues 329–332 and 347–348; see Alt et al. 1984, Holschuh et al. 1984, and also Rasmussen 1984). The suggested 5 amino-acid ligands for the Mn "A" and "B" sites are labeled as in Fig. 3. The 4 amino-acids suggested as the binding site for the Mn/Ca ("C") binding are labeled C1, C2, etc. "RAH" refers to a redox-active histidine. X1 is a histidine involved in Cl^- binding; the two others on D1 are shown in Fig. 3.

spinach D2 and D1, in order to see whether the intra-thylakoid loop regions of these proteins (described above) contain any clusters of amino-acids that are capable of forming Mn binding sites.

Studies of Mn binding sites in proteins have shown that Mn prefers to be

coordinated by the unprotonated carboxylate side chains of aspartyl or glutamyl residues and the unprotonated imidazole of histidyl residues. Either tetrahedral or octahedral geometry is theoretically possible (Hughes 1981). The X-ray crystal structure for the Mn (III) superoxide dismutase from *Thermus thermophilus* suggests that the Mn in this enzyme is part of a trigonal bipyramidal (5-coordinate) complex with water and histidine as the axial ligands and two histidines and an aspartate as the other co-planar ligands (Stallings et al. 1985). This geometry assumes that the aspartate contributes only one oxygen atom. The Mn (II) in concanavalin A is also coordinated by a combination of one histidine, two aspartates, one glutamate, and two water molecules (Hardman and Ainsworth 1972).

In searching for potential Mn ligands on D1 and D2, we assumed a simple octahedral geometry for Mn, with five protein ligands (one axial), and a sixth axial water molecule. The assumption that all of the Mn are coordinated by H_2O is based on the model of Kambara and Govindjee (1985). In order to narrow the search, we took advantage of the sequence comparison by Trebst and Draber (1986) of the spinach reaction center proteins D1 and D2 and the bacterial reaction center proteins L and M from *R. capsulatus*. Since the latter organism does not evolve O_2, we focused our search on regions of D1 and D2 that have low homology with their bacterial counterparts, or that appear to be "insertions" into the L and M sequences.

Examination of the intra-thylakoid loop regions of D1 and D2 reveals that there are four such regions, each of which contains enough potential Mn ligands to act as a Mn binding site. These regions consist of the residues between the membrane-spanning helices I and II on both proteins (labeled "A" in Figs. 3 and 4) and the COOH-terminal portions of both proteins (labeled as "B" in D2, Fig. 4). The D2-A site is consistent with possible Mn ligation, based on the presence of five potential ligands (2 His, 2 Glu, and 1 Asp). These residues are labeled A1–A5 in Figs. 3–5. Because there are more than five potential ligands at the D1-A site, we aligned the sequence of this site with the sequence of the D2-A site (Fig. 6A). The five D1 residues labeled A1–A5 (1 His, 2 Glu, 2 Asp) in Fig. 3 provide the best overall match to the D2-A site. The two sites are similar except for the substitution of D1-Asp 61 for D2-His 62. The tighter spacing in the D2-A site may explain the presence of a possible disulfide bridge.

Upon examining the regions labeled "B" in each protein (Figs. 3 and 4), we also noticed that there are two nearly identical sets of 5 carboxylate residues (4 Glu and 1 Asp on D2, and 2 Glu and 3 Asp on D1). These are labeled B1–B5 on each protein (Figs. 3 and 4). The sequence alignment (Fig. 6B) shows that the spacing between potential ligands are quite similar. Although we favor the involvement of Histidines D1-332, D1-337, and D2-337 in the binding of Cl^-, we cannot rule out their involvement in Mn binding by this kind of analysis, particularly if they participate in ligand

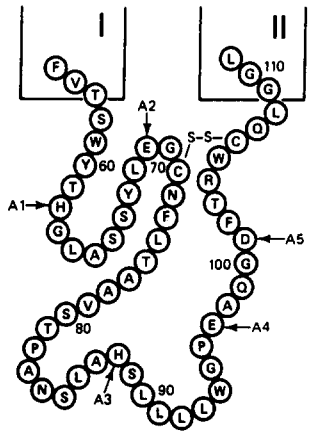


Fig. 5. A model for the arrangement of the amino acids at the Mn "A" site of the D2 protein, assuming a disulfide bridge between Cys-72 and Cys-106. A disulfide bridge between these residues is expected to create a much tighter structure for the Mn binding site.

exchange. For example, D1-His 332 and D2-His 337 (see Fig. 6B) could conceivably coordinate Mn in one conformational state of the OEC and bind Cl^- in another state by allowing D1-Glu 333 and D2-Glu 338 to replace them. Brudvig and Crabtree (1986) and Brudvig and de Paula (1987) have proposed that a conformational change in the structure of the active site Mn is necessary for water oxidation to occur. Simultaneous Mn and Cl^- binding at these two histidines is not likely, because protonation of the imidazoles would interfere with Mn coordination at the imidazole N-3 nitrogen.

In addition to the A and B sites, we also noticed that there is a dense cluster of carboxylates on D2 in the region labeled "C" (Fig. 4). The spacing

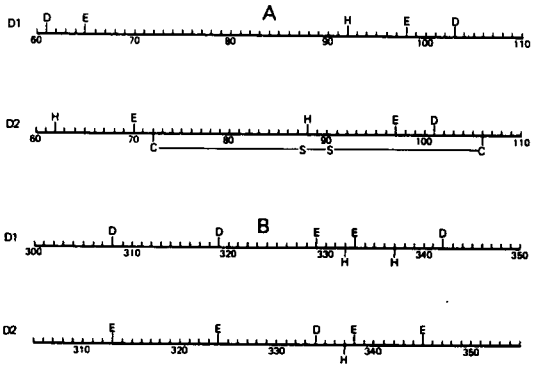


Fig. 6. Amino acid sequence alignments for the Mn binding regions in D1 and D2. (A) Alignment of the Mn "A" sites. A potential disulfide bridge is shown for D2. (B) Alignment of the Mn "B" sites. Histidines proposed to be involved in Cl^- binding are shown underneath each line.

of the non-adjacent carboxylate residues in this region (labeled as C1–C5 in Fig. 4) suggests that they might be grouped into a distorted rhombic or tetrahedral site. This kind of coordination has been suggested for Mn (II), based on the EPR properties of certain Mn (II)-substituted proteins (Haffner et al. 1974, see also Coleman 1987). Ca^{2+} might also be coordinated at this site, although the amino acid sequence does not appear to match the sequence of any well-known Ca^{2+} binding sites. Calcium binding to such a site would perhaps explain the Ca^{2+} requirement for O_2-evolution in PS II membranes depleted of the extrinsic polypeptides.

On the other hand, the existence of a site containing a transition metal in a distorted rhombic environment could partly explain the observation of a low-field ($g = 4.1$) EPR signal arising from the water-oxidizing site (for a review, see Dismukes 1986), although it is not certain whether such a site would give rise to the flash-induced component of the $g = 4.1$ signal. De Paula et al. (1986) have attributed the $g = 4.1$ signal to the tetranuclear Mn, based on measurements of its temperature dependence; however, because of the anomalous behavior of this signal compared to the S_2 multi-line EPR signal, Aasa et al. (1987) have postulated that it may arise from monomeric Mn (IV). By using Q-band EPR, Mavankal et al. (1987) have reported the presence of protein-bound Mn (II) in PS II membranes lacking the extrinsic polypeptides.

Because of their relative isolation from the other histidines and their close proximity to the proposed binding sites for the reaction center components in helices III and IV (Trebst and Draber 1986), we propose that D1-His 190 and D2-His 190 are involved in creating an electron-conducting pathway from the Mn sites in the lumen to the component Z, the donor to P680 (see Coleman 1987). This assignment is an extension of previous models that have proposed a role for histidines as a redox-active ligand (RAL) between Mn and Z (Kamabara and Govindjee 1985, Padhye et al. 1986; comparable to the A-site histidines in our model). By analogy, we have labeled each of these residues as a redox-active histidine (RAH).

The above analysis has included all of the histidines believed to reside on the intra-thylakoid domains, but has not accounted for all of the carboxylate residues. Some of these carboxylates, however, could be involved in salt-bridges with positively-charged residues at equivalent locations on the opposite protein. For example, it is possible that D1-Glu 104 pairs with D2-Arg 104 to enable the two proteins to remain linked together. Residue D1-Glu 347 was not included because it is not found in *Chlamydomonas* (Erickson et al. 1983). In deciding between two closely spaced residues, we chose the one which created the most symmetrical ligand environment or which gave the best overall match to the corresponding site on the other polypeptide.

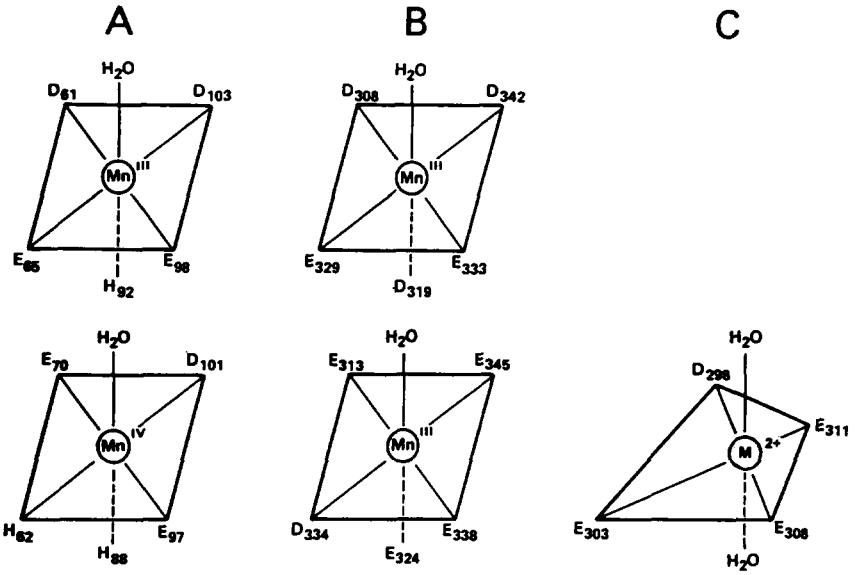


Fig. 7. A schematic representation of the possible coordination geometry of Mn in the A, B, and C sites. The Mn valence states for the A- and B-sites represent those that have been proposed for the S_2 state. See text for a discussion of the possible role of histidine at the B-site. "M^{2+}" represents a Mn or Ca ion bound to the C-site.

Comparison of D1/D2 sequences from various organisms reveals that the D1 A- and B-sites are completely conserved (residues and spacing) among *Chlamydomonas*, spinach, tobacco, and soybean (Zurawski et al. 1982, Erickson et al. 1983, Spielman and Stutz 1983, Alt et al. 1984, Holschuh et al. 1984). The D2 A-site is conserved in spinach, pea, and *Chlamydomonas* (Holschuh et al. 1984). In *Synechocystis* 6803, D2-A Asp-101 appears to be replaced by Asn (Williams et al. 1987). The D2 B-site is conserved among spinach, pea, and *Synechocystis* (Rasmussen et al. 1984, Williams et al. 1987). In *Chlamydomonas*, however, the sequence homology with spinach disappears at D2-B Glu 313, the first ligand of the B-site (Rochaix et al. 1984). The *Chlamydomonas* sequence does, however, contain a histidine in this region. The D2 C-site is conserved in spinach, pea, *Chlamydomonas* and *Synechocystis* (Alt et al. 1984, Holschuh et al. 1984, Rasmussen et al. 1984, Rochaix et al. 1984, Williams et al. 1987). Comparison with the bacterial L and M subunits indicates that these sites are not present in bacteria (see Trebst and Draber 1986).

Figure 7 provides a schematic illustration of how Mn might be coordinated at each of these sites. Based on the results of EPR studies of the S_2 state (see Coleman 1987), it has been proposed that the tetranuclear Mn active site contains 3 Mn (III) and 1 Mn (IV)(Dismukes and Siderer 1981). Studies of the Tris-sensitivity of the S_2 state also indicates that one of the four Mn atoms is uniquely sensitive to this inhibitor (Yocum et al. 1981).

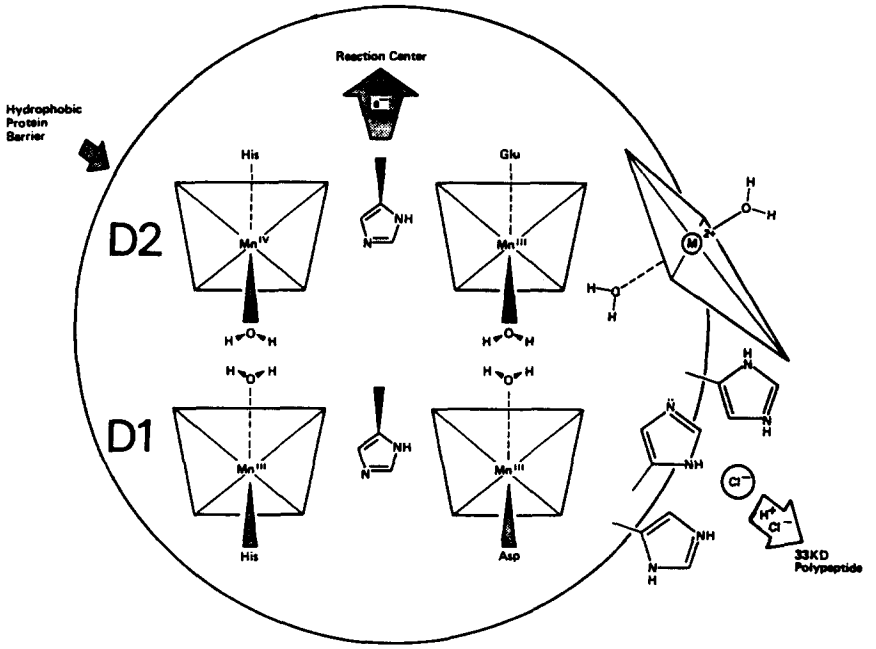


Fig. 8. A working model for the Mn active site and intrinsic Cl^- binding site of the oxygen evolving complex (OEC). The four high-valence Mn sites are depicted as a tetranuclear cluster. The hydrophobic A-sites are on the left and the hydrophilic B-sites are on the right (see text for a discussion of the possible role of histidine at the B-site). The Mn valence states represent those that have been proposed for the S_2 state of the OEC. No H_2O chemistry is shown. The monomeric Mn/Ca site (M^{2+}) connects the active site to the aqueous phase by facilitating the transfer of H_2O. The two imidazole groups in the center represent D1/D2-His 190, which are involved as redox-active groups in the transfer of electrons to Z. The three imidazole groups involved in the intrinsic Cl^- binding site are shown at the lower right.

The relatively small size of the D2-A site (consistent with the coordination of a smaller, more highly charged Mn atom), as well as its unique coordination by two imidazoles, make it an appropriate candidate for the Mn (IV) site in S_2.

We have summarized our ideas for the Mn and Cl^- sites into an overall model for the intrinsic portion of the OEC (Fig. 8). Many structural features of this model correspond to the predictions of Kambara and Govindjee (1985) that were derived to explain the function of the 0EC Mn. The A-sites, for example, reside in a domain that is more hydrophobic than that of the B-sites. In addition, the B-sites appear to be associated with the proton-withdrawing mechanism that requires Cl^-. The coordination of the tetranuclear Mn in this scheme is also not inconsistent with the model of Brudvig and Crabtree (1986) and Brudvig and de Paula (1987), since some of the oxygen/hydroxo-ligands to Mn that are proposed in their models could be supplied by the carboxylate residues we have identifieed, or by H_2O bridges

between the carboxyl groups and Mn. The function of the C-site metal atom in the present model might be to channel water molecules from the aqueous phase into the hydrophobic active site. Overall, this kind of arrangement creates an extended network of water molecules connected by hydrogen bonds (see Kambara and Govindjee 1985). As the water molecules are oxidized at the Mn sites, the electrons are channeled upward to the reaction center, and the H^+s are moved out toward the extrinsic polypeptides in the lumen. The structure is therefore consistent with the process of charge separation as it relates to the splitting of water.

Proposed structure and properties of the extrinsic Cl^- binding sites

The appearance of line width maxima in the ^{35}Cl-NMR binding curve for spinach thylakoids and PS II membranes led us to conclude that Cl^- binds to a number of sites that are sequestered from the bulk solvent in the absence of Cl^- (see Coleman et al. 1987b). The model described above for the intrinsic Cl^- binding site (Fig. 1) required that H^+ and Cl^- be transferred out of the active site region and onto the 33 kD extrinsic polypeptide. Both our ^{35}Cl-NMR studies and the work of other laboratories using O_2 evolution assays have indicated that Ca^{2+} is required for the proper functioning of the OEC in PS II membranes that have been treated with 1.0 M NaCl (see Coleman et al. 1987d). Based on the results of our search for Mn binding sites on D1/D2, we decided to examine the 33 kD polypeptide for potential divalent metal sites. By so doing, we hoped to be able to organize the structure of the protein into functional domains, in order to identify possible Cl^- binding regions.

The assumption that we used for analyzing this protein was that it possessed large sites with octahedral geometry, wherein a Ca^{2+} or other similar divalent cation could be weakly coordinated by six protein ligands. When we arbitrarily divided the protein into 4 domains, each of about 60 amino acid residues, and aligned the sequences for the carboxylate residues (which, along with carbonyl groups, typically coordinate Ca^{2+} in proteins; see Hughes 1981), we noticed that these four domains can be organized into two palindromic pairs (Fig. 9). Each member of the pair contains six carboxylate residues whose spacing is almost exactly reversed on the other domain. The two pairs are formed by residues 1–62 (Domain 1) and 187–246 (Domain 4) and by residues 62–121 (Domain 2) and 121–180 (Domain 3). This alignment implies a possible head-to-tail arrangement of the protein (see below).

Figure 10 shows how these domains might be organized into 4 octahedral sites. These structures are hypothetical, since no 3-dimensional structure is

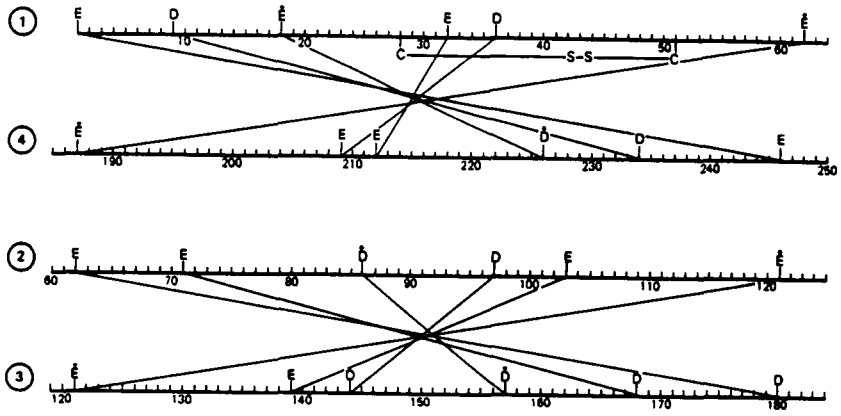


Fig. 9. Sequence alignment for the carboxylate residues on four domains of the 33 kD extrinsic polypeptide. Asterisks denote possible axial ligands in each octahedron. A possible disulfide bridge is shown for Domain 1.

known, but they nevertheless provide a useful structure for comparison with other proteins. The apparent ligand overlap at Glu-62 and Glu-121 has a parallel in the divalent metal binding sites in Concanavalin A, where the carboxyl groups of residues Asp-10 and Asp-19 are shared between Mn^{2+} and Ca^{2+} (Hardman and Ainsworth 1972).

In comparison to most of the well-characterized Ca^{2+} binding sites, the sites described here for the 33 kD polypeptides are relatively large, although thermolysin is known to have a site that spans 52 residues and coordinates a Ca^{2+} ion with five carboxylates and one water molecule (Levine and Williams 1982). The size of the putative sites on the 33 kD polypeptide may be responsible for the apparently weak binding of Ca^{2+} in the absence of the 18 kD and 24 kD polypeptides.

It is important to realize that Ca^{2+} is not likely to be bound to these four domains in the native complex. Instead, these sites are probably occupied by portions of the 18 kD and 24 kD polypeptides. In this respect, they should perhaps be considered pseudo-binding sites, because they may be able to bind Ca^{2+} only when the 18 kD and 24 kD polypeptides have been removed.

Although this four-domain arrangement accounts for most of the carboxylate residues on the polypeptides, there are two small regions between residues 87–109 and 181–187 where the spacing of the carboxylates does not fit the overall pattern. In an earlier paper (Coleman et al. 1987a), we noted that the amino-acid sequence in these two regions showed partial homology with the sequences of some of the Ca^{2+} binding sites of troponin C and calmodulin. Upon further examination, we realized that although the longer sequence is partially homologous with a calmodulin/troponin C-type Ca^{2+}

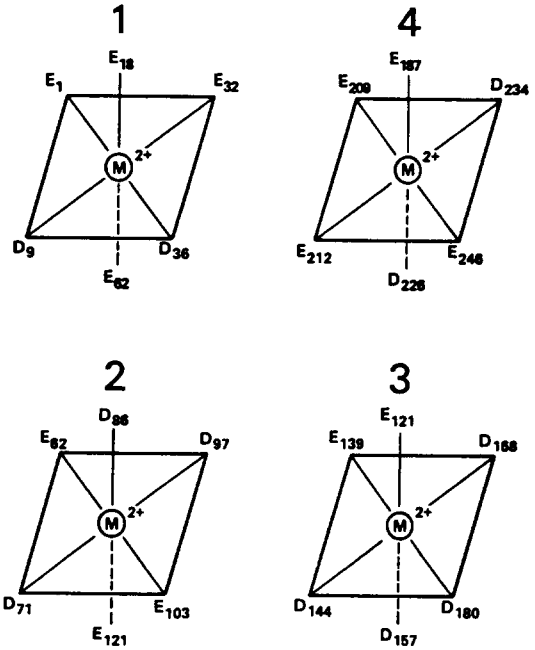


Fig. 10. A schematic representation of the possible coordination geometry for four divalent metal binding sites on the 33 kD extrinsic polypeptide. Each octahedral site corresponds to one of the four protein domains.

binding loop (Fig. 11), the shorter sequence (Fig. 12) shows better homology with the so-called acidic clusters of troponin C and calmodulin (Demaille 1982). These clusters have been suggested to be involved in binding troponin I (in troponin C; Leavis 1978) and in binding Mg^{2+} (see Milos et al. 1986, for calmodulin).

A second acidic cluster occurs in the D1 protein at residues 241–245 (Zurawski et al. 1982), which Trebst and Draber (1986) place on the outer membrane surface. This D1 cluster sequence Q-E-E-E-T shows partial homology with the "ancestral" acidic cluster sequence Q-T-E-E-E for Ca^{2+} binding proteins (Demaille 1982).

```
                                        *   *       *
      90                      100                     110
E I E G P F E V S S D G T V K F E E K D G I D Y

E E E L - K E V F - - - - K V F D E - D G - D G
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Fig. 11. Amino-acid sequence comparison between a region of the 33 kD extrinsic polypeptide (top) and a reconstructed "primordial" Ca^{2+} binding domain (bottom) for Ca^{2+} binding proteins (Demaille 1982). Asterisks denote possible Ca^{2+} ligands on the 33 kD polypeptide.

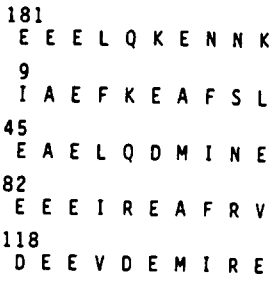


Fig. 12. Amino acid sequence comparison between an acidic cluster in the 33 kD extrinsic polypeptide (top line) and 4 similar sites in calmodulin (Milos et al. 1986).

Although Fig. 11 shows that a number of potential Ca^{2+} binding groups may be present among residues 87–94 and 100–109 of the 33 kD polypeptide, it is not certain from this analysis whether these groups would be able to constitute a relatively high-affinity Ca^{2+} binding loop, such as those in troponin C and calmodulin. It is possible that the 18 kD and 24 kD polypeptides may contribute additional ligands (see discussion in Coleman et al. 1987d) or that water molecules are involved in coordination. A better case can be made for a possible high-affinity Ca^{2+} binding loop within residues 223–232 of D2 (Fig. 13), a sequence which Trebst and Draber (1986) locate on the outer surface of the membrane. This sequence shows a greater homology with one of the Ca^{2+} sites in calmodulin (Demaille 1982, Grand 1985). The presence of, at least, two high-affinity Ca^{2+} binding sites within PS II is consistent with recent experimental evidence (Cammarata and Cheniae 1987, Katoh et al. 1987, Miller et al. 1987).

The apparent homology between regions of the D1/D2 proteins or the 33 kD polypeptide and calmodulin/troponin C may have functional significance for photosynthesis. Both calmodulin and troponin C activate Ca^{2+}/Mg^{2+} ATPases (Waisman et al. 1981, Potter and Johnson, 1982, Grand 1985), and the chloroplast ATPase belongs to this same general class (McCarty and Carmeli 1982).

Assignment of the carboxylate residues to the low-affinity and high-affinity Ca^{2+} sites and the acidic cluster leaves several of the carboxylates

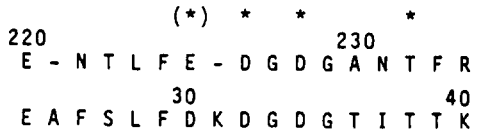


Fig. 13. Amino-acid sequence comparison between a region of the D2 protein (top) and a sequence for a Ca^{2+} binding region in calmodulin (bottom). Asterisks denote possible Ca^{2+} ligands on the D2 protein. The numbering sequence for calmodulin is taken from Grand (1980).

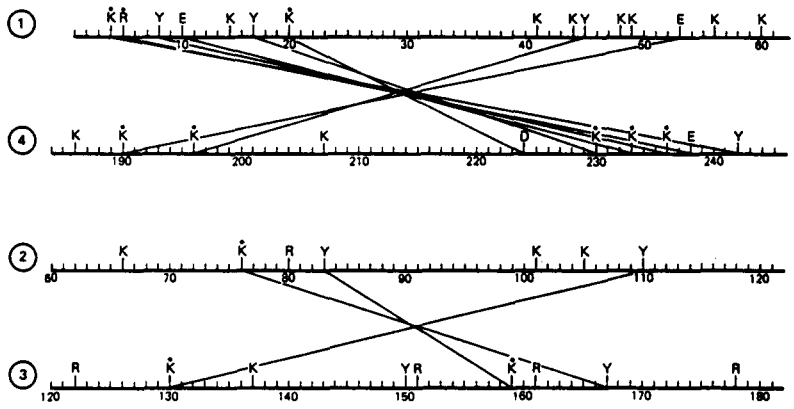


Fig. 14. Sequence alignment for the acidic amino acid residues not involved in metal binding and the positively-charged basic residues on the 33 kD extrinsic polypeptide. Asterisks denote positively-charged residues involved in salt-bridges. These residues may represent sequestered Cl^- binding sites that are distributed asymmetrically among the four domains.

unaccounted for. We have proposed (see Coleman et al. 1987b, c) that the ^{35}Cl-NMR line width maxima arise from Cl^- binding to positively charged residues (i.e., Lys or Arg) on the 33 kD polypeptide, and that these residues might be salt-bridged to negatively charged residues in the Cl^- depleted complex. With this in mind, we examined whether the remaining acidic groups (Asp, Glu, and Tyr) could be paired in an orderly way with the Lys and Arg residues. Fig. 14 shows that the (+) and (−) charges can be arranged in pairs that connect Domains 1 and 4 and Domains 2 and 3 in a palindromic manner. The positive charges in these salt-bridges are distributed as follows: 5 on Domain 4, 3 on Domain 1, 2 on Domain 3, and 1 on Domain 2. This distribution of the sequestered Cl^- binding groups among 4 Domains may not be inconsistent with the $^{35}Cl^-$-NMR results in Coleman et al. (1987b).

A possible mechanism for Cl^- binding at these sites (based on earlier models by Homann et al. 1983, Coleman and Govindjee 1985, also see Govindjee et al. 1983) is presented in Fig. 15. This mechanism proposes that Cl^- ions destabilize the salt-bridges between the domains by binding to the positive charges. This binding would be expected to raise the pK_a's of the carboxylate or phenolic groups (Mehler and Eichele 1984) and cause them to accept protons from the intrinsic Cl^- site described above (see Scheiner et al. 1985). We propose that the salt bridges rupture in a defined sequence, first exposing, say, 5 Cl^- binding sites on Domain 4, then 3 sites on Domain 1, 2 sites on Domain 3, and 1 site on Domain 2. Protons would simultaneously bind to the negative charges on the complementary domains, thereby accelerating the rate of O_2 evolution.

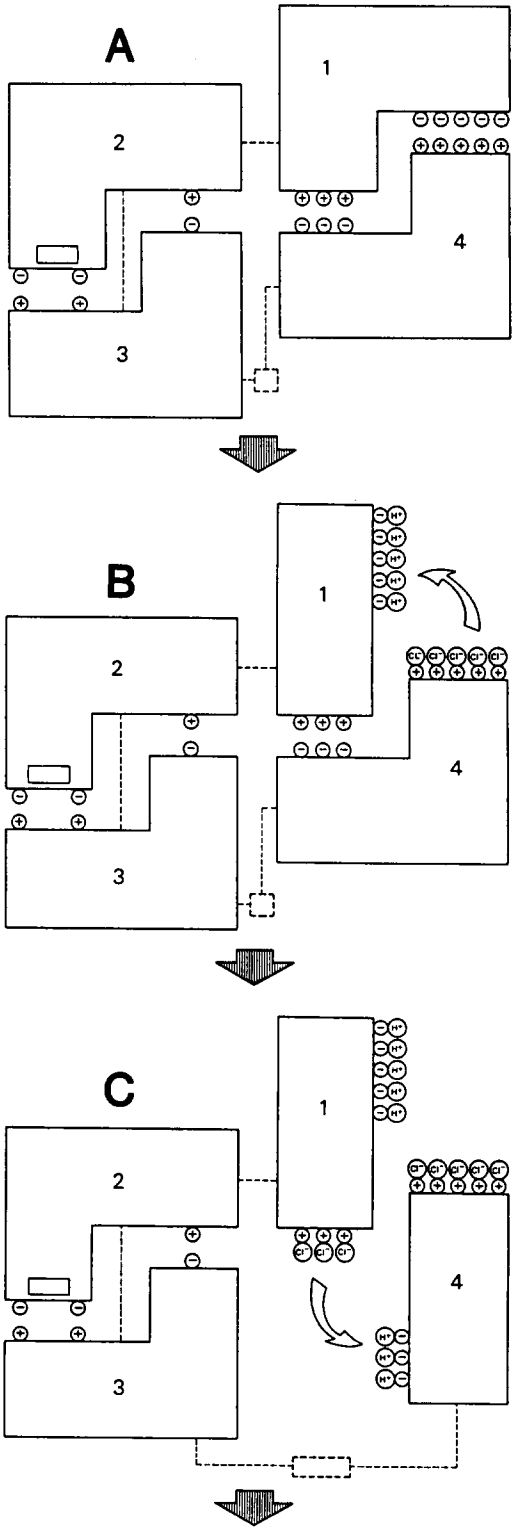
Justification for the mechanism at the extrinsic sites

This model for the extrinsic Cl^- binding sites explains the following observations: 1) The requirement of the 33 kD polypeptide for high enzyme activity; 2) the high Ca^{2+} requirement for O_2 evolution and the appearance of the ^{35}Cl-NMR line width maxima in the absence of the 18 kD and 24 kD polypeptides. The four octahedral domains are probably too large for tight Ca^{2+} binding, but neutralization of their negative charges may be necessary for the protein to attain an active conformation; 3) the presence of a high-affinity Ca^{2+} site in the reconstituted complex (see Ghanotakis et al. 1984). This site may occur in the region Glu-97 to Asp-109; 4) the observation that removal of Cl^- from the membrane requires deprotonation of strongly basic groups ($pK_a > 8$). Lys and Arg are both involved in binding Cl^- at the extrinsic sites, and both have high pK_a's; 5) the prediction that the OEC contains four sequestered H^+/Cl^- binding domains, which contribute to sharp increases in ^{35}Cl-NMR line width and O_2 evolution (at low light intensity) (Coleman et al. 1987b).

Although another intrinsic PS II protein, CP 47, contains numerous His, Asp, and Glu residues (see Morris and Herrmann (1984) for the DNA sequence), we do not believe that this protein binds the tetranuclear Mn cluster, since these residues cannot be divided into four discrete groups with the appropriate ligand composition.

Conclusion

We have presented a hypothesis for the structure and function of the Cl^- binding sites in the OEC which incorporates our recent ^{35}Cl-NMR findings and which seeks to explain the known involvement of both Ca^{2+} and several of the OEC polypeptides in the Cl^- effect. Since it is not yet feasible to predict detailed tertiary structure from the amino acid sequence, we have relied on additional information, including the physicochemical properties of these proteins and others with similar sequences, to construct a heuristic model for the architecture of the OEC. In addition to consistency with several important experimental observations, the hypothesis we have proposed predicts a number of structural and mechanistic features that can be directly tested. We view this hypothesis as a framework for developing and refining mechanistic future models of the oxygen-evolving complex.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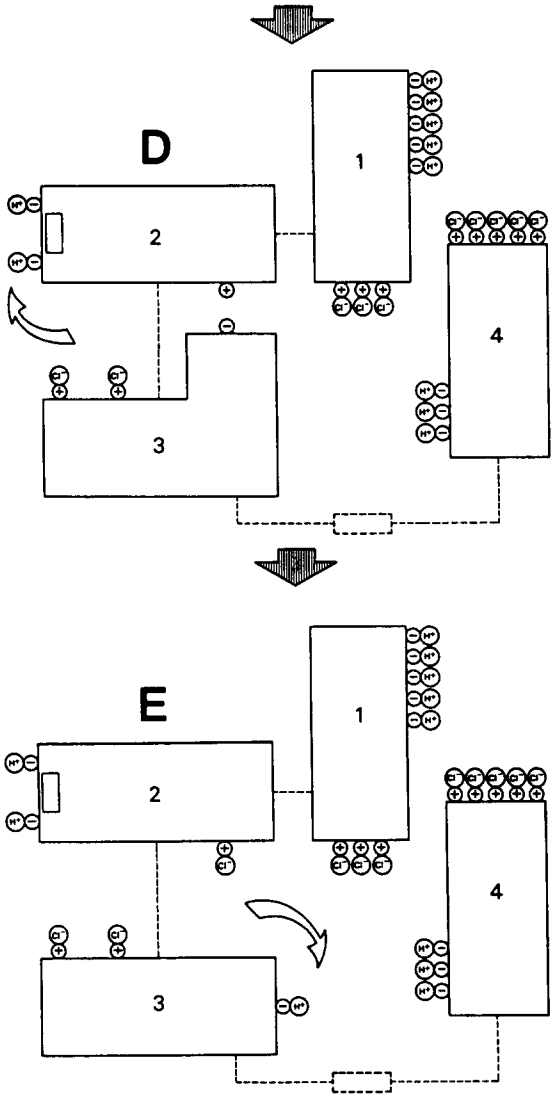

Fig. 15. A model for the mechanism of the extrinsic Cl^- binding sites on the 33 kD polypeptide. Cl^- and H^+ transferred from the intrinsic sites (Fig. 1) break the salt-bridges between the four domains of the polypeptide in an ordered sequence as the [Cl^-] increases. Cl^- binds to Lys and Arg; H^+ binds to Asp, Glu, and Tyr (see Fig. 14). The open rectangle on Domain 2 represents a possible high-affinity Ca^{2+} binding site. The open rectangle between Domains 3 and 4 represents the acidic cluster Glu 181-Glu 183. The Cl^-/H^+ binding sequence begins with all of the salt-bridges intact in the Cl^- depleted complex (A). When Cl^- and H^+ are donated to the 33 kD polypeptide from the intrinsic Cl^- binding sites (see Fig. 1), they bind first to a group of 5 paired residues at the interface between Domains 1 and 4 (B). The other groups of salt-bridges are then broken sequentially as the Cl^-/H^+ concentration rises. Thus, Cl^- binding sites open up in the following order: 5 on Domain 4 (B), 3 on Domain 1 (C), 2 on Domain 3 (D) and 1 on Domain 2 (E). Cl^- binding to these extrinsic sites may be responsible for sharp concentration-dependent increases in the ^{35}Cl-NMR line width and "bursts" in the rate of O_2 evolution.

Acknowledgements

This research was supported by grants from the University Research Board and Biomedical Research Committee to one of us (G); and from the University of Illinois Botany unrestricted funds. We thank Prof. H.S. Gutowsky for suggestions during the preparation of this manuscript.

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