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Review

Calcium activation of photosynthetic water oxidation

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Contents

I. Introduction

Experiments by Joliot and Kok [1–3] on the response of photosynthetic oxygen production to short flashes of light revealed that the reaction consists of a linear, four-electron oxidation process that produces a gush of O_2 on every fourth light flash excitation of Photosystem II (PS II). The Kok model for water oxidation proposes that a set of 'S'-states (of undefined molecular composition) are associated with the reaction, in which each of the S-states represents a particular oxidation state of the oxygen-evolving system:

$$S_0^0 \xrightarrow{h\nu} S_1^{1+} \xrightarrow{h\nu} S_2^{2+} \xrightarrow{h\nu} S_3^{3+} \xrightarrow{h\nu} S_4^{4+} \xrightarrow{h\nu} S_0^0 + 4H^+ + O_2 \quad (1)$$
$$\uparrow$$
$$2H_2O$$

The version shown here implies that the S-state mechanism operates as a charge accumulating device, with H_2O undergoing oxidation only after photochemical removal of four oxidizing equivalents from the oxygen-evolving enzyme system. Data exist (see, for example, Refs. 4–6) to support such a concerted mechanism for water oxidation; although alternate models for the

Abbreviations: PS, photosystem; Mn, manganese ligated to photosystem II in unspecified oxidation states; EGTA, ethyleneglycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetate; ICaBP, intestinal calcium binding protein; Chl, chlorophyll; pheo, pheophytin; Q, plastoquinone; Y_Z, a redox active tyrosine residue located on the 32 kDa protein of the Photosystem II reaction center that serves as the electron donor to the reaction-center chlorophyll *a*; Y_D^+, a dark-stable tyrosine radical located on the 34 kDa intrinsic protein of the Photosystem II reaction center; SOD, superoxide dismutase. One-letter codes used for amino-acids are: D, aspartic; E, glutamic; F, phenylalanine; G, glycine; I, isoleucine; K, lysine; N, asparagine; Q, glutamine; P, proline; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

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reaction are certainly possible, at the present time the scheme shown in Eqn. 1 constitutes a working hypothesis that is accepted by a number of workers in the field.

A brief outline of the path of electron transfer in PS II is shown below:

$$2H_2O \rightarrow (Mn)_4 \rightarrow Y_Z \rightarrow P680 \rightarrow \text{Pheo } a \rightarrow Q_A \rightarrow Q_B \quad (2)$$
$$\downarrow$$
$$O_2 + 4H^+$$

Absorption of light by the reaction-center chlorophyll *a*, P680, promotes electron transfer to an intermediate acceptor, a pheophytin *a* molecule (denoted here as Pheo *a*). From Pheo *a*, the electron is transferred to a tightly bound molecule of plastoquinone (Q_A) and then to a second quinone, Q_B, that occupies an exchangeable binding site. The doubly reduced, protonated form of Q_B is replaced at its binding site by an oxidized plastoquinone molecule. The photooxidized reaction center, $P680^+$, oxidizes a tyrosine residue denoted Y_Z, that mediates electron transfer between P680 and the cofactors that catalyze the oxidation of water. As shown in this scheme, the actual catalyst of water oxidation in PS II is an ensemble of Mn atoms. The four atoms of the metal found in association with each reaction-center are the subject of a substantial body of literature (see Refs. 7–9 for reviews). The oxidation states of Mn in the S-state cycle are not known with certainty, although there is general agreement that the metals are probably in oxidation states of +3 or higher in the resting, dark-stable S_1 state, and that S-state advancement is accompanied by Mn oxidation.

Among potential spectroscopic probes of the Mn atoms in PS II, low-temperature EPR has been the most widely applied technique. Until recently, the S_1 state, which predominates in the dark, has been thought to be nonparamagnetic (see, however, Ref. 10); a single-turnover electron transfer event to form the S_2 state generates an EPR signal detected at cryogenic temperatures and spanning 1500 G with at least 16 lines [11]. The $s = 1/2$, $g = 2$ multiline signal is proposed, on the basis of studies on synthetic model Mn compounds, to originate from a group of 2–4 Mn atoms in a mixed valence state that contains Mn^{+3} and/or Mn^{+4} (Refs. 11–15; see also Ref. 16 for a review). Another EPR signal with a turning point at $g = 4.1$ is observed under some specific conditions that inhibit multiline signal formation (for example, illumination at low temperature) [17–19]. Optical signals have also been ascribed to oxidation state advancements in Mn [6,20]. These absorption changes, which have been the subject of some controversy [21,22], are proposed to arise from ligand-to-Mn charge transfer absorption bands. The positive absorption changes are best resolved at about 300 nm and remain stable after each single turnover flash in a series until the $S_3 \rightarrow S_4 \rightarrow S_0$ transition, which is accompanied by a disappearance of the accumulated absorbance. The pattern of the optical changes and their absorption spectrum is interpreted to indicate that each flash produces a one-electron oxidation state advancement in an individual Mn atom (from Mn^{3+} to Mn^{4+}) up to the $S_3 \rightarrow S_4 \rightarrow S_0$ transition. The rapid disappearance of the accumulated absorption changes is consistent with a reduction of higher oxidation states of Mn coincident with the four-electron oxidation of water to produce molecular oxygen.

The Mn atoms in native preparations of PS II are tightly ligated, as evidenced by the fact that they remain bound during rigorous PS II purification routines involving exposure to high concentrations of detergents or salts [23–27]. At the same time, the Mn atoms can be easily removed from PS II by relatively mild treatments, including exposure to reductants such as NH_2OH in the dark [28–30] or to high concentrations of Tris free base in the light [31,32]. It has been shown that Mn ligation and oxygen evolution activity can be restored to Mn-extracted samples under the appropriate conditions (weak-light illumination of extracted preparations in the presence of Mn^{2+}) [33,34].

In addition to Mn, Cl^- is required for oxygen evolution activity [35]. Removal of the weakly-bound halide blocks electron transfer on the oxidizing side of PS II at or very near the site of water oxidation [36–38]; Mn is not extracted from PS II by the relatively gentle treatments needed to deplete Cl^-. Under Cl-depleted conditions the $g = 2$ multiline EPR signal is not detected, but addition of Cl^- to depleted samples in the dark after a single-turnover illumination has been shown to produce an appearance of the signal [39]. In these investigations, it has been found that the EPR undetectable precursor to the multiline species exhibits an unusual stability, with a lifetime of hours. The actual number of Cl^- ions associated with each reaction center is uncertain, and attempts to define the molecular properties of the site at which Cl^- or surrogate ions act in PS II have produced conflicting hypotheses, one of which proposes that the halide interacts with amino acid residues near the site of Mn binding [40] and the other of which proposes that the halide is ligated directly to the metal [41].

As a consequence of investigations on the resolution and reconstitution of oxygen-evolution activity, Ca^{2+} has also been identified as a cofactor for the reaction. The following sections assess the data currently available on Ca^{2+} function in PS II, identify the areas where divergent results have been obtained, and compare properties of Ca^{2+} binding to well-characterized proteins with what is known about the properties of the binding and action of the metal in PS II. As will be seen, there is evidence for two types of Ca^{2+}-binding environment in PS II; one of the two sites possesses a lower Ca^{2+} affinity than the other. The principal topic

of this review is the former Ca^{2+} binding site, from which the metal is most easily removed concomitant with a strong inhibition of oxygen-evolution activity. The reader is also referred to another recent review [42] on the topic.

II. Photosystem II polypeptides

Biochemical purification of PS II activity has demonstrated that the enzyme system is comprised of both integral membrane proteins and extrinsic polypeptides (see Refs. 43–46 for reviews). The integral proteins of PS II are known to ligate chlorophyll *a*, pheophytin *a*, carotenoids, plastoquinones, the heme of cytochrome *b*-559, and a single atom of non-heme iron. Ligands for Mn and Ca^{2+} may also reside on the integral proteins. In addition, two tyrosine residues on the intrinsic reaction-center proteins denoted D1 and D2 (32 and 34 kDa species, respectively) have been shown to be redox active [47–49]; one tyrosine, Y_Z in Eqn. 2 above, transfers electrons from Mn to the reaction-center chlorophyll P680, which is also proposed to reside on the D1 or D2 polypeptide. Extrinsic 23 and 17 kDa proteins can be extracted from detergent-isolated PS II preparations with 1–2 M NaCl [50,51]. The third extrinsic protein (33 kDa) is more tenaciously bound, but can be released by exposure of the enzyme system to 1 M $CaCl_2$ [52,53] or to urea [54], procedures that allow for retention of Mn within the ensemble of integral proteins of PS II provided that the appropriate ionic conditions (elevated Cl^- concentrations) are maintained [53]. Since repeated references will be made to several PS II polypeptides, a schematic representation of key proteins of the photosystem and the oxygen-evolving reaction is presented for the reader in Fig. 1; the 17 kDa extrinsic protein is omitted from this diagram. The topological organization of the polypeptides of PS II, as well as the localization of some of the chlorophylls and quinones on these polypeptides is known with some certainty. However, the actual interactions among the intrinsic and extrinsic proteins is less well established, so this aspect of Fig. 1 should be viewed as arbitrary.

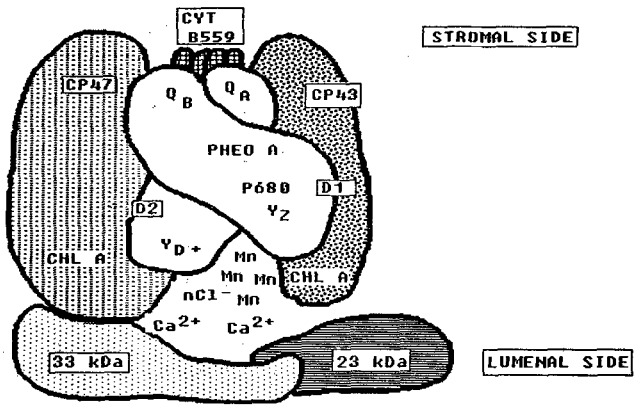

Fig. 1. Diagrammatic representation of the PS II polypeptides comprising the oxygen-evolving reaction center. The location of chromophores is approximate and the 17 kDa extrinsic protein is omitted for simplicity. The stromal side of the photoreaction faces the exterior of the chloroplast thylakoid membrane system, which in turn encloses the lumen, or interior aqueous space of the membrane system.

III. Calcium activation of oxygen evolution activity

Polypeptide extraction experiments such as those mentioned above, utilizing either everted thylakoid membrane vesicles or detergent-purified PS II preparations, showed that although Mn was retained in an EPR-silent form by PS II after the selective extraction of the 23 and 17 kDa extrinsic proteins, the resulting polypeptide-depleted PS II preparations had lost a substantial fraction (60–70%) of oxygen-evolution activity. This loss in activity could be partially restored to everted thylakoid vesicles by rebinding of either of the extrinsic polypeptides [55], with the 23 kDa species showing the greatest capacity to reconstitute activity [55,56]. Subsequent experiments with detergent-isolated PS II preparations led to the discovery that addition of Ca^{2+} to assay mixtures containing partially inactivated, salt-extracted material would also reconstitute oxygen-evolution activity [57–59] in the absence of the 23 and 17 kDa species. In the initial investigations of Ca^{2+}-activated oxygen-evolution activity, high concentrations (15 mM) of the ion were required to restore steady-state activity to maximal levels in PS II membranes lacking the extrinsic proteins [57], an observation that might be taken to suggest that Ca^{2+} was in some way acting as a non-specific agent, for example by providing positive charges normally supplied by one of the extracted polypeptides. This possibility was eliminated by experiments showing that after exposure of salt-washed PS II membranes to EGTA (to remove as much Ca^{2+} as possible), rebinding of the 23 and 17 kDa polypeptides alone would not restore oxygen-evolution activity. Addition of Ca^{2+} to assay mixtures containing the polypeptide-reconstituted material was similarly ineffective [60]. It was found, however, that oxygen-evolution activity could be restored if the Ca^{2+}-depleted, polypeptide-reconstituted samples were subjected to long incubation times (up to 60 min) with Ca^{2+} prior to assay. Using this incubation procedure, it was also observed that much lower concentrations of Ca^{2+} (0.5 mM) than were required in assays of salt-washed material (15 mM) would produce optimal reactivation [60]. These results were interpreted as evidence that the Ca^{2+} activation phenomenon in PS II was physiologically significant, and that the extrinsic polypeptides constituted part of a structural apparatus

that is responsible for concentrating and retaining Ca^{2+} at its site of action in PS II. The necessity for prolonged exposure of the Ca^{2+}-depleted, polypeptide-reconstituted samples to added Ca^{2+} for restoration of oxygen-evolution activity was proposed to arise from a diffusion barrier, interposed by the polypeptides, that prevented rapid equilibration of Ca^{2+} in the external aqueous phase with its site of action in the oxygen-evolving enzyme system. Later investigations have established that the 23 kDa protein is the salt-extractable species required for retention of Ca^{2+} at its site of action in PS II [61,62]. The 17 kDa polypeptide, on the other hand, has been proposed to play a role in the retention of Cl^- [63]. In all of the extraction experiments just described, the 33 kDa protein remains bound to the intrinsic polypeptides of PS II, and therefore roles for both the 33 kDa extrinsic protein (perhaps in concert with the 23 kDa species) and the intrinsic membrane species in the retention of Ca^{2+} must be considered possible. Progress in devising improved extraction methods has demonstrated that efficient removal of Ca^{2+} strongly suppresses more than 90–95% of oxygen-evolution activity, leaving little doubt that the metal is an essential activator of the reaction [64–69].

Although exposure of detergent-isolated PS II preparations to 1–2 M NaCl, oftentimes in the presence of a chelator such as EDTA or EGTA, remains a popular method for the release of Ca^{2+}, along with extrinsic polypeptides, other procedures have also been found which will deplete the metal or alter the Ca^{2+} demand for oxygen-evolution activity. Perhaps the most prominent alternative involves the use of lowered pH, either alone [67,69,70] or in concert with elevated ionic strength and added chelators [68]. The method described in Ref. 69 involves brief exposure (5 min) of PS II membranes to pH 3 in the presence of citrate followed immediately by a dilution step that produces a final pH of 6.5. Strong inhibition of activity is observed following this treatment, and the preparation retains both the 23 and 17 kDa extrinsic proteins. Optimal restoration of activity upon addition of Ca^{2+} is dependent on incubation with the metal for periods up to 40 min, reminiscent of the situation observed with polypeptide-reconstituted samples [60]. Exposure of PS II membranes to trypsin will also induce a requirement for addition of Ca^{2+} to oxygen-evolution assays [71]. Proteolysis produces an extensive digestion of the 17 and 23 kDa proteins, the 33 kDa protein is cleaved, and the electron transfer on the reducing side of the photosystem is also altered. However, the authors in Ref. 71 found that a 15 kDa fragment of the 33 kDa protein is retained by membranes after proteolysis, and suggest that this fragment contains critical determinants for stabilization and function of the oxygen-evolving reaction.

IV. Interactions of other metals with the calcium site in PS II

The initial examination of the ability of other metals to replace Ca^{2+} and activate oxygen-evolution showed that only Sr^{2+} could produce a measurable increase in activity in salt-washed PS II preparations [57]. Other divalent metals were either ineffective (Mg^{2+}) or slightly inhibitory (Mn^{2+}). Subsequent, more detailed investigations of the activity produced by reconstitution of Sr^{2+} into Ca^{2+}-depleted PS II preparations have shown that the oxygen-evolving enzyme turns over more slowly [72], and that the S_2 EPR multiline signal is modified, as evidenced by the appearance of more closely spaced lines (about 60 G vs. 80 G in Ca^{2+}-sufficient samples) [73]. This Sr^{2+} effect on the multiline signal is superficially similar to alterations produced in PS II samples illuminated in the presence of NH_3. However, the latter finding has been interpreted as indicating that the amine binds to a site on Mn [74], a proposal confirmed by spectroscopic characterizations of the multiline signal employing electron spin-echo envelope modulation [75]. In view of these findings, it is likely that the change in the multiline signal induced by Sr^{2+} substitution for Ca^{2+} [73] is being produced by a structural change in the arrangement of the Mn atoms in PS II caused by ligation of the larger Sr^{2+} atom (1.13 Å) at a site that normally accommodates the smaller Ca^{2+} atom (1.0 Å).

Trivalent lanthanide ions show a facile propensity for displacing Ca^{2+} from its ligand environment in biological systems [76], so it is not surprising that addition of La^{3+} to PS II preparations produces a major alteration in the structure and organization of the oxidizing side of the photosystem. Among the effects observed are an irreversible loss of activity and release of about 50% of the Mn along with the 33, 23 and 17 kDa extrinsic proteins [77]. These phenomena can be prevented by the presence of excess Ca^{2+}, in which case oxygen-evolution activity can be largely retained along with Mn and the 33 kDa protein; the 23 and 17 kDa species are nevertheless released. It has further been shown that in PS II, La^{3+} and Ca^{2+} do, in fact, compete for a common site; the La^{3+} K_I was estimated to be 0.05 mM under conditions where the Ca^{2+} K_M was estimated to be 0.5 mM [77].

The competition between La^{3+} and Ca^{2+} in PS II just described has been taken as evidence for the existence of a proteinaceous Ca^{2+} binding site [77]. Studies of the consequences of La^{3+} substitution in other Ca^{2+} binding systems, both synthetic and natural, have produced a series of general observations [76] which may be relevant to the situation observed in PS II. The ionic radii of lanthanides ($\approx$ 1.0 Å) appear to be more important for binding to Ca^{2+} sites than does the increased positive charge on the trivalent ion.

In addition, lanthanides, like Ca^{2+}, exhibit a strong preference for charged or uncharged oxygen ligands over nitrogen ligands. For both calcium and the lanthanides, the coordination numbers are similar, but not necessarily identical. Finally, a general effect of increasing the number of ionic ligands in the metal binding domain is to produce an increased stability of the La^{3+} complex as compared to the stability of the corresponding Ca^{2+} complex [76]. Such generalizations would appear, at least on the surface, to be compatible with the properties of the observed competition between La^{3+} and Ca^{2+} in PS II, and therefore suggest that the Ca^{2+} binding site in PS II may contain charged oxygen ligands.

It has also been found that monovalent metals (Na^+, K^+, and Cs^+) will act by a competitive mechanism as very weak inhibitors of Ca^{2+} activation of the oxygen-evolving reaction [65]. A similar, but stronger, competitive inhibition, has been obtained with Cd^{2+} [78], and addition of this metal to PS II membranes has been shown to inhibit formation of the $g = 2$ multiline signal [79]. As with the lanthanides, the results observed for exposure of PS II to monovalent metals and to Cd^{2+} are consistent with the results of studies with other Ca^{2+}-binding proteins, where in each case a mono-, di- or trivalent metal can interfere with the binding of Ca^{2+} (Ref. 80; see also Ref. 81 for the K_D values of a range of metals). The monovalent metals that inhibit PS II are of approximately the same (Na^+) or larger ionic radius than Ca^{2+} and are weak inhibitors (the K_I for Na^+, for example, is on the order of 5 mM). On the other hand, for the divalent metal Cd^{2+}, with an ionic radius (1.03 Å) close to that of Ca^{2+}, an estimated K_I value of 0.3 mM was obtained for the Ca^{2+} site in PS II. Although Cd^{2+} ordinarily prefers a more covalent ligand environment containing a predominance of S and N groups, interference by Cd^{2+} with Ca^{2+} binding has been reported in Ca^{2+}-binding proteins [76]. The competition by Cd^{2+} for the Ca^{2+} site in the oxygen-evolving reaction suggests that it might be possible to employ $^{113}Cd^{2+}$ NMR to probe the Ca^{2+} binding site in PS II, as has been done in other proteins [82].

It should be noted that in prokaryotic systems (the cyanobacteria), Ca^{2+} has been demonstrated to be required for oxygen-evolution activity [84–86]. However, in a series of studies on the conditions for Ca^{2+} depletion and subsequent recovery of activity in *A. nidulans*, it has been shown that under certain circumstances Na^+ ion will reactivate the oxygen-evolving reaction [87]. In *Synechocystis* PCC 6714, Na^+ depletion creates a lesion in PS II that is specifically restored by Na^+ addition; Ca^{2+} is ineffective [88]. These findings derive from experiments with whole cells, and it will be interesting to see whether similar results with regard to Na^+ activation of oxygen-evolution activity are obtained with membrane preparations from these cyanobacteria.

TABLE I

Metal ion effects on Ca^{2+}-activated O_2 evolution

Metal	Ionic radius (Å)	Coordination number	Effect on oxygen evolution activity	K_I (mM)
Ca^{2+}	0.99	6–8	(activator)	–
Sr^{2+}	1.13	6–8	(activator)	–
Ba^{2+}	1.35	6–8	weak inhibitor	–
Mg^{2+}	0.65	6	no effect	–
Mn^{2+}	0.80	6	weak inhibitor	–
Cd^{2+}	1.03	6–8	competitive inhibitor	0.30
La^{3+}	1.04	7–9	competitive inhibitor	0.05
Na^3	0.98	4–7	competitive inhibitor [a]	5
K^+	1.33	6–8	competitive inhibitor	8
Cs^+	1.65	6–8	competitive inhibitor	10

[a] Na^+ inhibition includes a small non-competitive contribution whose origin is unknown at present.

To conclude this section, Table I summarizes the available data on interactions of metals with the Ca^{2+} site in PS II preparations from spinach, derived from steady-state kinetic experiments. In accord with results from similar types of investigation on Ca^{2+}-binding proteins, the data of Table I show that those metal ions that compete for the Ca^{2+} site in PS II possess ionic radii similar to, or larger than, the physiological metal and have similar coordination numbers. Lanthanides are the most potent competitors for Ca^{2+} binding sites, while Cd^{2+} and monovalent metals bind more weakly. The value for La^{3+} shown in Table I, obtained in the presence of Na^+, could represent an overestimate of the K_I value. The failure of Mg^{2+} to compete for the Ca^{2+} site in PS II is consistent with the ligation properties of this metal, namely a lower coordination number, a smaller ionic radius, and a greater tendency than Ca^{2+} to accept nitrogen donor ligands. The K_I values so far obtained for the various metals competing with Ca^{2+} in PS II show an order of affinity that approximates the order of affinity of these same metals (determined as true K_D values) in Ca^{2+} binding proteins, where they also interfere with Ca^{2+} binding.

V. Calcium affinity and stoichiometry in PS II

The variety of methods used for Ca^{2+} depletion from PS II and the attendant differences in retention or loss of extrinsic polypeptides has produced a broad range of K_M values for the metal (0.001 mM to > 1 mM) in these preparations. Compounding this situation is the detection, in PS II membrane preparations, of substantial amounts of contaminating residual Ca^{2+} [57,89], and the observation that Na^+ can act as a weak competitive inhibitor of Ca^{2+} activation [65]. Nevertheless, a succession of observations has produced a pro-

gressive lowering of apparent K_M values, and has led to the discovery that more than a single affinity for the metal may exist in PS II. Investigations reported in Refs. 66 and 67 revealed the existence of both high- ($K_M = 0.05–0.1$ mM) and low- ($K_M = 1–2$ mM) affinity Ca^{2+} sites in salt-washed PS II material from spinach and pokeweed. Photosystem II membranes exposed to high salt conditions concomitant with the Triton X-100 step used for isolation of the membranes yielded a preparation from wheat that showed a low affinity for Ca^{2+} (about 1.5 mM) [64]. Further extraction of the membranes with high salt (2 M NaCl), EGTA and the ionophore A23187 induced both high and low-affinity Ca^{2+} sites with K_M values comparable to those observed in Refs. 66 and 67. Experiments with salt-washed membranes from spinach found a single site with an apparent K_M of about $(20–30) \cdot 10^{-6}$ M [78] when assays were conducted in the absence of Na^+. More highly purified PS II reaction-center complex preparations lacking the 23 and 17 kDa proteins have been subjected to rigorous Ca^{2+} depletion with chelators, lowered pH and the ionophore A23187 to produce material containing much less than 1 Ca^{2+} per reaction-center [68]. In this material, steady-state assays detected three discrete K_M values ($(1–4) \cdot 10^{-6}$, $1 \cdot 10^{-4}$ and $> 10^{-3}$ M). The sites of highest and lowest affinities exhibit approximately equal effects on activity, while the intermediate-affinity site appears to produce a somewhat greater activating effect. It remains to be seen whether this range of affinities for Ca^{2+} is a reflection of the presence of two separate atoms of the metal in PS II, or perhaps of a cooperative interaction between two (or more) binding sites with different K_M values for Ca^{2+} (see below).

On the basis of the results now available, it seems likely that the affinity of Ca^{2+} for PS II, measured as K_M values for the activity of the metal in steady-state assays of oxygen-evolution activity, depends on the prior history of the PS II samples utilized for assay. Photosystem II membranes exposed to high concentrations of NaCl, plus or minus chelators in the light or in darkness, can exhibit Ca^{2+} K_M values in the range of $< 10^{-4}$ to $> 10^{-3}$ M, and two affinities, one high and one low, can be detected in a single sample of membranes. More rigorous extraction procedures, accompanied by the exercise of care in eliminating contaminating Ca^{2+} from glassware, reveals three sites, the third of which possesses a K_M value in the range of $(1–4) \cdot 10^{-6}$ M, the highest affinity so far reported.

Another important factor that influences Ca^{2+} binding in PS II has been revealed in investigations of the conditions leading to reconstitution of oxygen-evolution activity in PS II preparations depleted of Mn by NH_2OH treatment. These depleted preparations show a decreased affinity for Ca^{2+}, suggesting that the presence of Mn atoms in their native oxidation states ($> Mn^{2+}$) may be required for high-affinity Ca^{2+} binding to PS II [90,91]. In addition, it has been shown that the light-driven ligation of Mn to PS II does not appear to require Ca^{2+} even though the Mn-reconstituted samples do require Ca^{2+} for steady-state oxygen evolution activity. These findings suggest that the presence of Ca^{2+} is not necessary for the Mn photooxidation process that precedes ligation of the metal, or for the binding of photooxidized Mn atoms to their native ligation sites within PS II. In experiments to photoligate Mn to PS II and restore oxygen-evolution activity, estimates have been made of the relative affinities of Ca^{2+} and Mn^{2+} for their respective binding sites in PS II in the course of photoligation of Mn; the affinities of each metal for the binding site of the other metal have also been estimated. These affinities, as computed from a model assuming that Ca^{2+} ligation follows functional assembly of 4 Mn atoms into their proper binding sites, has produced an estimate of Ca^{2+} affinity for the Ca^{2+} site of about $3 \cdot 10^{-4}$ M, a negligible affinity of Ca^{2+} for the Mn binding site (or sites), but a relatively high ($90 \cdot 10^{-6}$ M) affinity of Mn for the Ca^{2+} binding site [92]. In contrast to reports that the presence of Mn may affect Ca^{2+} binding affinity in PS II, the authors in Ref. 93 claim that Mn extraction with either Tris or NH_2OH does not diminish the amount of Ca^{2+} associated with PS II membranes.

In concert with investigations on the affinity of Ca^{2+} as an activator of oxygen-evolution activity, efforts have been made to assess the actual number of Ca^{2+} atoms associated with each PS II reaction center. Although salt-washed PS II membranes from spinach can retain large amounts of contaminating Ca^{2+}, investigations with membranes from spinach and wheat, exposed either to EDTA washing [69] or to the combination of high salt, EGTA and A23187 [64] to suppress the adventitious population of the metal have found 2 (spinach) and 3 (wheat) Ca^{2+}/reaction center. Analyses of the effects of extraction of the metal from purified spinach PS II reaction centers containing 2 Ca^{2+} per 4 Mn atoms [68] confirm that one of the atoms is much more tightly bound than the other. Comparable stoichiometries (2 Ca^{2+} per PS II reaction center) were found in less purified PS II membranes, and exposure of these preparations to acidic conditions (pH 3) with citrate releases one of the two Ca^{2+} atoms [69] concomitant with suppression of activity. Other investigations, however, have produced some contrasting observations. The data in Ref. 93 show 2 Ca^{2+}/reaction center in PS II membrane preparations, the absence of strong activity inhibition by salt-washing, and only modest reactivation by Ca^{2+} after the salt-wash step. The authors conclude that the extrinsic polypeptides of PS II are not essential for Ca^{2+} retention. The same investigators have presented data suggesting that about 1 Ca^{2+} atom is present per PS II reaction

center in purer preparations that contain fewer polypeptides [93–95].

While there is no clear explanation for differences such as those just described, at least some of the discrepancies may derive from the presence of inactive centers in certain preparations coupled with partial or complete extraction of the more labile atom of the metal in the course of purification routines. At the present time, evidence exists to indicate that 2 atoms of Ca^{2+} are present per reaction center in highly active PS II preparations from spinach, either membranes retaining the extrinsic polypeptides [69] or more purified preparations from which the 23 and 17 kDa proteins have been removed [68]; 3 atoms/reaction center have been reported in wheat preparations [64]. One of the two Ca^{2+} atoms in spinach preparations can be demonstrated to be more susceptible to extraction than the other, and release of this atom of the metal by high ionic strength and chelators or by low-pH treatment with citrate exerts a strong inhibitory effect on oxygen-evolution activity. The function of the very tightly bound Ca^{2+} atom, including the question of whether it has any direct function in oxygen evolution, remains to be determined.

VI. Calcium action in the S-state cycle

The exact nature of the lesion in the water oxidizing enzyme that is created by Ca^{2+} depletion is the subject of a number of investigations. Analyses by EPR have indicated that Ca^{2+} depletion creates a lesion in electron donation to Y_z^+ in both PS II membranes [57] and more purified preparations [68]. Investigations employing thermoluminescence [96] or delayed fluorescence [97] to monitor electron transfer in PS II after Ca^{2+} depletion have found that one or more stable oxidants are produced by illumination after Ca^{2+} depletion. These, and other data to be discussed below, suggest that the lesion produced by Ca^{2+} removal is at a site that functions prior to electron donation to Y_Z, the donor to $P680^+$. At the same time, some data exist suggesting that extraction of Ca^{2+} inhibits electron transfer from the tyrosine Y_Z to $P680^+$ in cyanobacteria [98]. As will be seen, EPR investigations with PS II preparations from higher plants place the site of Ca^{2+} action within the S-state cycle; whether the metal can also affect electron transfer events between $P680^+$ and Y_Z in cyanobacteria has not been investigated in more detail.

Substantial inhibitions of oxygen-evolution activity can be achieved by incubation of PS II preparations in high salt or at lowered pH in darkness [57–59,64,65,69,70], where the S_1 state predominates. However, it can be demonstrated that illumination accelerates the rate of the Ca^{2+}-depletion process. The rate of Y_Z^+ decay, detected by optical methods in salt-washed PS II membranes, is initially unchanged (although the Y_z^+ absorbance decay on the flash catalysing the $S_3 \rightarrow S_4 \rightarrow S_0$ transition was permanently slowed to about 5 ms) [99]. A train (> 60) of single-turnover flashes slowed the rate of Y_Z^+ decay, and subsequent addition of Ca^{2+} to flashed samples then accelerated Y_Z^+ decay. The requirement for multiple single-turnover flashes to inhibit Y_Z^+ decay was attributed to a light-dependent release of residual Ca^{2+} from salt-washed PS II membranes [99]. These results correlate with experiments demonstrating that the rate of inhibition of oxygen-evolution activity induced by exposure of intact PS II membranes to high ionic strength was accelerated when the incubation mixtures were exposed to weak continuous illumination [61].

The effect of illumination on Ca^{2+} release has also been explored utilizing short flashes of light to produce a particular S-state prior to exposure of PS II membranes to high-salt conditions [100]. The results of these experiments showed that the S_3 state was more susceptible to inhibition of oxygen evolution activity by extraction of Ca^{2+} and polypeptides than were other S states. This observation agrees with a report on the effect of Ca^{2+} depletion on S-state advancement, examined by detection of delayed fluorescence from salt-washed PS II membranes [97], which indicated that S-state advancement was blocked at the level of the $S_3 \rightarrow S_4 \rightarrow S_0$ transition. Although this result is consistent with the results on the extraction of Ca^{2+} from flashed samples, it does not explain why Ca^{2+} depletion, monitored optically by inhibition of Y_Z^+ reduction [99] appears to require multiple single turnover flashes. Thermoluminescence measurements on PS II membranes depleted of Ca^{2+} by exposure to NaCl and EDTA initially produced results indicating that the $S_3 \rightarrow S_4 \rightarrow S_0$ transition was blocked after Ca^{2+} removal (101); later experiments by the same group utilizing membranes depleted of Ca^{2+} by acidification in citrate showed that in fact the $S_2 \rightarrow S_3$ transition was affected [96]. It would be useful to determine whether the differences arising from delayed fluorescence, thermoluminescence and optical absorption change measurements reflect differences in the extents of Ca^{2+} depletion achieved by salt-washing procedures and by acidification with citrate.

Other investigations of the effects of Ca^{2+} on S-state advancement have centered on formation of the S_2 EPR multiline signal. Initially, these experiments produced a wide range of conflicting results addressing the question of whether Ca^{2+} is required for formation of the S_2 multiline signal [27,73,102–104]; the major questions that arose concerned whether Ca^{2+} was effectively removed from PS II by salt-washing, and whether chelators, for example EGTA used in concert with high concentrations of NaCl to remove Ca^{2+}, might be able to affect detection of the multiline signal by way of a

mechanism that involved an interaction of EGTA with Mn to create an EPR-silent S_2 state. Proponents of the hypothesis that Ca^{2+} is not required for formation of the multiline signal suggested that Ca^{2+} depletion treatments employing EGTA did, in fact, create an alteration to Mn [73] that produced a negative effect on the intensity of the multiline signal. An alternative view has been that salt-washed samples that produce the multiline signal retain contaminating Ca^{2+}, in some cases in substantial amounts [57,89]. Both points of view are valid. Estimates of Ca^{2+} depletion based on steady-state oxygen-evolution activity in dilute aliquots of highly concentrated samples ($\gg$ 3 mg Chl/ml) used in EPR experiments would fail to detect residual Ca^{2+}, if it were present. At the same time, although the Mn atoms in salt-washed PS II preparations are impervious to extraction by chelators such as EGTA or EDTA, it is now apparent that the carboxylate groups of these chelators may modify the conditions for multiline signal formation, and the properties of the signal itself.

Further information on the role of Ca^{2+} in S-state advancement, monitored by EPR, has recently appeared. In the first such reports, experiments were carried out that were intended to rigorously deplete Ca^{2+} (exposure of PS II membranes to 1.2 M NaCl and 20 mM EGTA, followed by dialysis in the light to rebind the extrinsic 23 and 17 kDa proteins). This treatment produced a sample which, without further illumination, exhibited an altered multiline signal (24–25 lines, 55 G spacing) [105,106]. The new signal, distinguished by an unusual stability (hours) in comparison to the normal signal from the S_2 state, was abolished by subsequent addition of Ca^{2+} (or Sr^{2+}). After Ca^{2+} or Sr^{2+} addition, the multiline signals associated with these metals could be generated by illumination. In the absence of added Ca^{2+}, subsequent illumination of samples exhibiting the modified multiline signal produced an entirely new signal, about 164 G wide [105,106], that is proposed to arise from a histidine radical [107]; the presence of the radical is assumed to mask the multiline signal by way of an exchange interaction between the two paramagnetic species.

Experiments by other groups [89, 108,109] confirm the formation of similarly altered multiline signals associated with Ca^{2+} depletion under illumination with NaCl and EDTA, or by low-pH exposure to sodium citrate in the dark, followed by illumination. The 164 G signal was also observed to form in samples exposed to citrate for Ca^{2+} depletion [108]. It was noted in the experiments of Ref. 89, but not Refs. 105, 106, 108, that addition of Ca^{2+} in the dark converted the altered signal to the normal multiline species. Citrate-induced depletion of Ca^{2+} does not extract the extrinsic proteins [69], so, with the exception of the NaCl/EDTA experiments [89], the preparations just described that produce the modified multiline signals retain, or have been reconstituted with, the 23 and 17 kDa proteins.

The use of acidification with citrate in the dark to deplete Ca^{2+} was also shown to induce a change in the temperature dependence of multiline signal formation [109]. Citrate-treated samples illuminated at $-60\,^{\circ}C$ to assure a single turnover produced no EPR signal, whereas illumination at $-5\,^{\circ}C$ generated the modified form of the S_2 signal. These data, interpreted to indicate that the temperature dependence of the $S_1 \rightarrow S_2$ transition had been shifted upward by Ca^{2+} extraction, contrast with earlier results [110] showing that Ca^{2+} depletion by high salt and EGTA substantially reduced formation of the normal multiline EPR signal across a wide range of temperatures, including those used in Ref. 109.

A resolution of some of the differences between the findings of two of the principal groups working with either citrate-acidified or NaCl/EGTA-treated PS II membranes has been presented in Ref. 111, where PS II membranes were first incubated in the light with 1.2 M NaCl and 50 μM EGTA to remove polypeptides and Ca^{2+}. These samples were then exposed to EGTA or to citrate (at pH 6.5 to avoid any further extraction of Ca^{2+}). Although no dark-stable signal was detected in these samples, if they were illuminated at $0\,^{\circ}C$ the formation of the modified multiline signal could be detected; at the same time, illumination at 198 K produced a normal signal in the presence of either chelator [111]. If, however, the NaCl/EGTA-treated membranes were allowed to dark adapt at $0\,^{\circ}C$ for 48 h, then the authors in Ref. 111 report inhibition of S_2 formation at 198 K. Mono- and dicarboxylic acids (acetic, phthalic and terephthalic acids) do not modify the multiline signal, which the authors in Ref. 111 interpret as indicating that flexible polycarboxylic acids are required to produce the modified S_2 signal; it might also be argued from the data at hand that the minimum requirement is for a tricarboxylic acid, since citrate is effective in producing the altered signal. There is now agreement [111,112] that two electrons can be withdrawn sequentially from the oxidizing side of Ca^{2+}-depleted PS II. It is also apparent from the experiments in Ref. 111 that the S_3-linked 164 G radical can be formed in samples that have not been exposed to chelators, and that plus or minus chelators, the multiline signal intensity is quenched by the appearance of the 164 G radical. However, the EPR data in Ref. 112 on samples exposed to NaCl and EDTA in the light to produce an altered multiline signal and then illuminated at 268 K show two signals (a dark stable multiline and another, very complex, signal) in the same sample as well as a new thermoluminescence component at $5\,^{\circ}C$. These findings may not contradict the data in Ref. 111; it is not at all clear that the species responsible for the second signal reported in Ref. 112 includes the 164 G radical associated with S_3.

Lastly, it is now apparent from experiments in two laboratories that the extrinsic polypeptides are not required for formation of the altered signal [111,112].

A brief summary of recent findings on formation of the S_2 multiline signal under conditions of Ca^{2+} depletion are presented in Fig. 2, with abbreviated conditions and summaries of the results of EPR analyses of the samples exposed to various treatments. It is highly probable that further publications addressing the role of Ca^{2+} in formation of the S_2 and modified S_3 state will appear in the future; a number of questions remain unresolved. If Ca^{2+} depletion is most rapidly achieved by illumination to form the S_3 state [100], then is Ca^{2+} of necessity required for S_2 formation? In other words, is Ca^{2+} depleted in the light before or after S-state advancements occur in the extraction experiments? More EPR studies on dark- and light-extracted samples would seem to be warranted, along with assessments, by chemical analyses, of the actual extents of Ca^{2+} depletion in these samples, as well as additional investigations of the effects of temperature on the light-induced formation of any signals observed in these samples. The actual mechanism of chelator-induced changes in the multiline signal also merit further investigations; the possibility that the anionic carboxyl groups of the chelators have displaced Cl^- [113] might explain in part the unusual stability of the altered multiline signal. For the present time, it is clear that Mn photooxidation can occur in the course of, or after, Ca^{2+} depletion from PS II, and that the depletion process utilizing carboxylate chelators produces alterations to the structure of the Mn atoms and/or their ligand environment in PS II.

A final point, relevant to this section, is that steady-state kinetic experiments have been carried out to determine the order of binding of Ca^{2+} and Cl^- to the oxygen-evolving enzyme system. In these experiments, the level of Na^+ was suppressed by the use of the tetramethylammonium cation as a counterion for Cl^- additions, and under these conditions it was possible to show that the activation of oxygen evolution by the two inorganic ion cofactors is a rapid equilibrium, ordered addition process in which Ca^{2+} binding precedes the binding of Cl^- [78].

VII. Where does calcium bind in PS II?

The available data from experiments on the effect of Ca^{2+} depletion and readdition using purified PS II preparations suggest that the Ca^{2+}-binding domain of the oxygen-evolving enzyme system resides within the core group of polypeptides that constitute the oxygen-evolving unit shown in Fig. 1. These species include CP47, CP43, the extrinsic 33 kDa protein, D1, D2, the 9 and 4.5 kDa polypeptides of cytochrome *b*-559, and possibly low-molecular-weight proteins (not shown in the diagram) [114,115] whose functions are as yet uncertain. Also, the extrinsic 23 kDa species cannot be conclusively eliminated as part of the native Ca^{2+}-binding domain in eukaryotes, since no data exist to assess

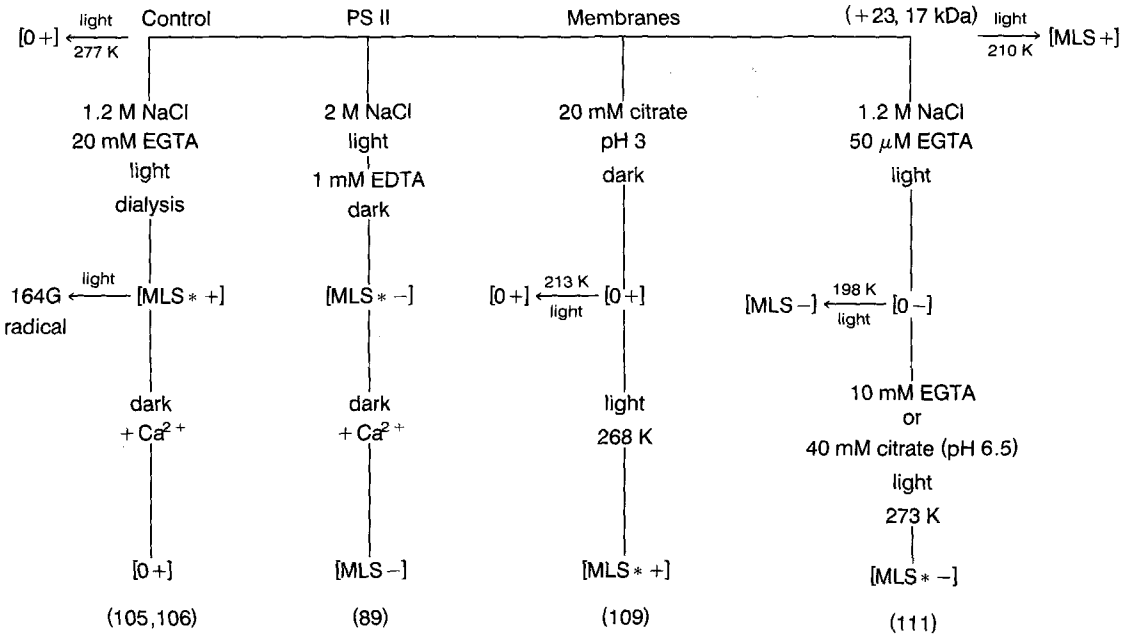

Fig. 2. Effects of Ca^{2+} extraction and illumination conditions that produce modifications to the S_2 multiline EPR signal. Unless otherwise specified, temperatures of extraction and/or illumination are 0–4 °C. The symbols used are as follows: [ ], results of attempts to detect the multiline signal; [0], no signal detected; [MLS], normal multiline; [MLS*], modified signal; +, 23 and 17 kDa polypeptides present; −, polypeptides absent. Numbers in parentheses at the bottom of the figure are for the references providing more detailed descriptions of the experiments shown.

the binding affinity of Ca^{2+} in PS II preparations that retain this protein. However, the fact that Ca^{2+} exhibits a strong activating capacity in the absence of the 23 kDa protein suggests that a major part of the Ca^{2+} site must reside within the core complex formed by the intrinsic membrane proteins and the extrinsic 33 kDa species.

The ligands that comprise the Ca^{2+} binding site or sites in PS II are widely considered to derive from amino acid residues, although it should be borne in mind that at the present time there are no data available that would conclusively eliminate polar groups on either carbohydrates or lipids as potential ligands. As noted in an earlier section, ligation of the metal by oxygen atoms would seem most likely on the basis of the known preference of Ca^{2+} for ionic carboxylate ligands, and on the basis of such data as are available from studies of competition between Ca^{2+} and other metals, such as La^{3+}, Cd^{2+} and Na^{+}, in PS II. The existence of ionic ligands is further supported by the observation that acidification of PS II to pH 3, which would facilitate the protonation of carboxyl groups, releases one of two Ca^{2+} atoms [69] concomitant with strong inhibition of oxygen evolution activity. That the acidification treatment releases only one of two Ca^{2+} atoms might suggest that the more tightly bound atom of the metal is structurally sequestered within PS II.

A ligand environment for Ca^{2+} in PS II comprised predominantly of oxygen atoms would be consistent with the general properties of Ca^{2+}-binding sites of other proteins [76,116]. The available data on PS II (Table I) suggest that this may be a reasonable hypothesis. A comparison of amino-acid sequences of Ca^{2+}-binding sites in well-characterized proteins with the sequence of the 33 kDa extrinsic protein of PS II has already occasioned proposals that this protein may in fact contain a Ca^{2+} binding site. Although an early proposal [117] that the 33 kDa protein from spinach contains a sequence homologous to the metal binding site in Mn-SOD has not been sustained by more recent sequences from the extrinsic protein from other species, other sequence data on the 33 kDa protein have been used as evidence for the possible existence of a binding domain similar to that found in an intestinal Ca^{2+}-binding protein (ICaBP). The sequence from ICaBP that was examined is DKNGNGEVSFEEFQ. For the extrinsic 33 kDa protein from pea, the proposed homologous sequence is EVSADGSVKFEEK [118], and a similar proposed homology exists for the 33 kDa protein from *Synechocystis* 6803 [119]. It has not been clearly demonstrated that the 33 kDa protein binds Ca^{2+} with a high affinity, but the possibility that the protein contributes some ligands to a metal binding site, or, perhaps, that the protein assumes a conformation that promotes Ca^{2+} ligation when bound to the intrinsic core of PS II, cannot be excluded. Further studies on the Ca^{2+}-binding capacity of the 33 kDa polypeptide are clearly warranted. It has also been suggested that the Ca^{2+}-binding site in PS II may be similar to the heteroatom binding site of concanavalin A [77], where single atoms of both Mn^{2+} and Ca^{2+} are ligated to regulate the conformation of the protein; Mn^{2+} must bind before Ca^{2+} binding can occur [120], reminiscent of the situation reported in Mn photoligation experiments [90].

A selection of sequences for Ca^{2+} binding domains (including ICaBP) is presented in Table II. Also listed are estimates of Ca^{2+}-binding affinities for the proteins shown [121]. In consulting the table, it should be borne in mind that the true affinity of PS II for Ca^{2+} in salt-washed preparations could be higher in native material retaining all extrinsic proteins, and that steady-state measurements of Ca^{2+} K_M values in PS II will detect only the lowest affinity site required for

TABLE II

Representative Ca^{2+}-binding sequences in proteins (data from Refs. 120–127)

Single-letter amino-acid codes are shown. Residues marked with * provide bidendate ligands to the metal, residues marked with # in concanavalin A and thermolysin are involved in the ligation of 2 metals (Ca^{2+} and Mn^{2+} in concanavalin A and 2 Ca^{2+} atoms in thermolysin site 1) and underlined residues contribute peptide-bond carbonyl ligands. The symbol (x) represents intervening amino-acid residues, with a subscript to indicate the number of such residues between the amino-acids that contribute ligands to Ca^{2+}

Protein		Sequence	No. of H_2O	K_d Ca^{2+} (M)
Phospholipase A_2		$\underline{Y}(x)\underline{G}(x)\underline{G}(x)_{16}E^*$	2	$2.5 \cdot 10^{-4}$
Staphylococcal nuclease		$D(x)D(x)_{17}D\underline{T}(x)E$	1	$1 \cdot 10^{-3}$
Trypsin		$E(x)\underline{N}(x)_2\underline{V}(x)_4E$	2	$4 \cdot 10^{-4}$
Concanavalin A		$D^{*\#}(x)\underline{Y}(x)\underline{N}(x)_4D^{\#}$	2	$3 \cdot 10^{-4}$
Thermolysin	(site 1)	$D(x)_{38}E^{\#}(x)_7D^{\#}(x)\underline{E}(x)_2E^{*\#}$	1	$1 \cdot 10^{-6}$
	(site 1)	$E^{\#}(x)_5\underline{N}(x)D^{\#}(x)_4E^{\#}$	2	$2 \cdot 10^{-5}$
Thermolysin	(site 2)	$D^*(x)D(x)\underline{Q}$	3	$< 10^{-6}$
Parvalbumin	(CP site)	$D(x)D(x)S(x)\underline{F}(x)E(x)_2E^*$	0	$2 \cdot 10^{-7}$
Parvalbumin	(EF site)	$D(x)D(x)D(x)\underline{K}(x)\underline{G}(x)_2E^*$	0	$2 \cdot 10^{-7}$
Calmodulin	(loop 3)	$D(x)D(x)\underline{N}(x)\underline{Y}(x)S(x)_2E^*$	0	$6 \cdot 10^{-6}$
ICaBP	(loop 2)	$D(x)\underline{N}(x)D(x)E(x)S(x)_2E^*$	0	10^{-6}

operation of the 5-state cycle; any changes to higher affinity occasioned by changes in the oxidation state of Mn will go undetected in the steady state owing to the necessity for saturating the Ca^{2+} site of lowest affinity to assure continuous operation of the S-state cycle.

The estimated K_M values ($(1-100) \cdot 10^{-6}$ M; 1–2 mM) now available for Ca^{2+} binding to site(s) in PS II, derived from measurements in the steady-state, fall within the range of K_d values exhibited by Ca^{2+} for sites in the proteins listed in Table II. As can be seen, for the parvalbumins, calmodulins, and troponin C, Ca^{2+} K_d values in the range of $(0.1-1) \cdot 10^{-6}$ M have been found. For the lectin concanavalin A the Ca^{2+} K_d is estimated to be $3 \cdot 10^{-4}$ M, while in the endopeptidase Thermolysin, two pairs of sites (which in total accommodate 4 Ca^{2+}) exist with K_d values estimated to be $1 \cdot 10^{-6}$ and $20 \cdot 10^{-6}$ M, respectively.

The sequences of the Ca^{2+}-binding domains shown in Table II illustrate the range of motifs that have evolved for binding Ca^{2+}. The EF-hand proteins (calmodulin, parvalbumin and ICaBP, for example) contain highly conserved domains for Ca^{2+} binding that are associated with the ability of these proteins to sense intracellular Ca^{2+} concentrations and trigger wide ranging changes in the cell in response to very small changes in the concentration of the metal. These proteins exhibit a very high affinity for Ca^{2+}, in keeping with their sensitivity to small changes in intracellular Ca^{2+} concentrations, and the Ca^{2+} binding sites occur in relatively short spans of amino-acid residues that exist as loops in the protein structure [121,122]. Another group of Ca^{2+} binding sites (found in trypsin [123], thermolysin [124], concanavalin A [125], staphylococcal nuclease [126] and phospholipases [127]) exist to confer stable active conformations on the proteins in which the sites reside. As Table II shows, these binding sites generally exhibit a lower affinity for Ca^{2+} than the affinities of the EF-hand domains and also exhibit a much greater sequence diversity than do the EF-hand sequences. In contrast to the well-defined EF-hand motifs, the ligating amino-acid residues in Ca^{2+} cofactor proteins are often more widely, and more randomly spaced in the primary sequence of the protein.

In spite of the range of Ca^{2+}-binding domains found in proteins and the wide variance in affinities for the metal, a common feature of Ca^{2+}-binding to the proteins listed in the table is that it produces alterations in their structure and/or stability. For the EF-hand proteins, this change in conformation alters the ability of the protein to bind to, and activate, other proteins. For the Ca^{2+}-cofactor proteins, metal binding stabilizes the protein against heat denaturation or autolysis, and may promote the catalytic activity of the protein; the most commonly found coordination numbers for the bound metal in such proteins is 6 or 7, with as many as three water molecules completing the coordination sphere [120]. Given the range, diversity and variable conservation of Ca^{2+}-binding sequences in the proteins shown in Table II, it is reasonable to ask whether any of these sequences appear in the polypeptides that make up the core of oxygen-evolving activity in PS II. The answer to this question is a qualified yes. The similarity of a sequence in the 33 kDa protein to a Ca^{2+}-binding sequence in ICaBP has already been noted [118,119]. The lumenal-facing extrinsic loops of CP47, CP43, D1 and D2 have amino-acid compositions such that potential Ca^{2+}-binding sequences might be present. Much attention is now centered on the lumenal extrinsic loops of D1 and D2 as potential sites of Mn binding, since these proteins are now known to constitute the ligation sites for reaction-center pigments, and contain redox-active tyrosine radicals. In the case of D1, a preliminary report [128] indicates that in *Synechocystis* 6803, mutagenesis of the D1 Asp residue at position 170 to a serine eliminates oxygen evolution activity. Further experiments of this nature are in progress in a number of laboratories, and may lead to the identification of some, or all, of the ligands to Mn and Ca^{2+}. In this regard, it will be interesting to see whether mutagenesis experiments can accurately identify possible bidentate carboxyl ligands that are shared between Mn and Ca^{2+}. The discovery of such ligands, if they in fact exist, would be useful in providing information on the actual polypeptide(s) involved in Ca^{2+} ligation as well as further information on the function of Ca^{2+} in water oxidation.

Table III summarizes the results of a search for Ca^{2+}-binding sequences in selected proteins of PS II. Only the spinach sequences were used as targets (the intrinsic proteins have highly conserved sequences, so the data shown are valid for a number of other species). The test sequences selected are not rigorous, and free substitutions were allowed at various positions (E for D, Q for N, etc.) to create generic, rather than specific, binding domains. In addition, incomplete Ca^{2+} binding sequences were used as probes. Restrictions were placed, however, on the number of allowed intervening amino-acids between sites of potential metal ligation. As Table III shows, several of the proteins constituting the 'core' of PS II activity contain potential sites for Ca^{2+} ligation. On the other hand, no complete sequence with strong similarity to part of a known Ca^{2+} binding site was identified in the search. This is not surprising given the unique nature of PS II and its catalytic activity. In addition, the possibility must be considered that more than one polypeptide might be involved in formation of the site of Ca^{2+} (and even Mn) ligation. For example, it is conceivable that binding of the extrinsic 33 kDa protein to one of the intrinsic species forms a Ca^{2+} site that requires ligands from both proteins. What is clear from the data of

TABLE III

Amino-acid sequence similarities between pS II proteins and partial or complete Ca^{2+}-binding sequences from the proteins shown in Table II

Single letter amino-acid codes are shown. For the intrinsic proteins, only those sequences in exposed regions on the lumenal side of the chloroplast thylakoid membrane are shown. The lumenal side sequences are derived from the model of Trebst [131] for D1 and D2 and from antibody probing experiments on the 47 and 43 kDa species (Dr. Richard Sayre, personal communication). The sequences probed are those from spinach. The sequences are shown in brackets and the number of the first amino-acid in the spinach sequence is also given

Test Sequence [a]	Present in:				
	33 kDa	47 kDa	43 kDa	D1	D2
1 D/ExN/QxxV	187 [ENNKNV]	–	–	308 [DSQGRV]	–
2 D/Ex_3N/Qx_4D/E	–	–	–	104 [EWLYNGGPYE]	–
3 Y/FxGxG	–	–	218 [FGGEG]	–	–
4 D/ExD/ExD/E	–	–	–	–	309 [DPEFE]
5 D/ExD/E	87 [EIE]	348 [DKE]	229 [DLE]	59 [DID]	309 [DPE]
	104 [EKD]	372 [DGD]			311 [EFE]
	180 [DEE]	431 [ELD]			
	181 [EEE]				
	224 [DTD]				

[a] The test sequences for parts of Ca^{2+}-binding domains are: (1) Trypsin (3 of 4 ligands, in order); (2) concanavalin A (free substitution was permitted for the tyrosine residue); (3) phospholipase A_2 (first 3 ligands); (4) and (5) generic alternating carboxyl ligand donors.

Table III is that a wide variety of potential metal binding sites are available as targets for future site-directed mutagenesis experiments.

VIII. Concluding remarks

The present state of knowledge concerning the role of Ca^{2+} in photosynthetic oxygen evolution may be summarized as follows:

(1) In PS II, Ca^{2+} is an essential cofactor of the oxygen-evolving reaction [60,64–70]. Among other metal ions so far tested, only Sr^{2+} has been shown to be able to substitute for Ca^{2+} in such a way as to produce significant oxygen evolution activity [57,72]. The lowered rate of activity observed in the Sr^{2+}-substituted system derives from a slowing of turnovers in the S-state cycle [72]. In cyanobacterial cells, Na^+ may be able to substitute for Ca^{2+} under some conditions [87,88].

(2) For the present time, it appears that intact, highly active spinach PS II preparations contain two Ca^{2+} atoms [64,68,69]; a third atom of the metal may be present in preparations from wheat [64]. In PS II membranes from spinach, extraction of the more loosely bound of the two atoms is sufficient to produce a strong inhibition of oxygen evolution activity [64,68,69]. The role of the second, more tightly-bound Ca^{2+} atom is unknown at present; this atom of the metal copurifies with the core constituents of the oxygen-evolving system, so it is not an adventitious contaminant. Since the cruder PS II membrane preparations contain substantial amounts of contaminating Ca^{2+} [57,89], assessments of the need for Ca^{2+} to stimulate oxygen evolution activity and analyses of the stoichiometry of the metal are best carried out in preparations that have been treated with chelators to suppress the adventitious population of Ca^{2+}.

(3) The affinities of Ca^{2+} for PS II in preparations lacking the 23 and 17 kDa polypeptides, measured as K_M values under steady-state illumination, are in the range of from $1 \cdot 10^{-6}$ to $50 \cdot 10^{-6}$ M, for the highest-affinity sites, and 1–2 mM for a lower-affinity site [64–68]. As many as three distinct Ca^{2+} affinities can be demonstrated, depending on the extraction methods used to deplete the ion and the purity of the PS II preparation used for the extraction routines [68]. Since all K_M measurements are made in PS II preparations lacking the extrinsic 23 kDa polypeptide, the true affinity of PS II for Ca^{2+} may still be in question, especially if the 23 kDa species were to provide some of the ligands for metal binding, or in some way regulates the structure of the actual site(s) of ligation. The origin of heterogeneity in Ca^{2+} affinities for PS II observed under some conditions has not been identified. Possible causes in some cases may be incomplete extraction of extrinsic polypeptides, incomplete extraction of Ca^{2+} or, perhaps, the existence of partial aggregation of the preparations to create diffusion barriers between added Ca^{2+} and its site of action. In addition, evidence has been presented that binding of Ca^{2+} to PS II requires the presence of a set of functional Mn atoms [90]; data also exist to indicate that Ca^{2+} affinity may be regulated by the oxidation states of the Mn atoms [61,99,100].

(4) Depending on the monitoring technique used, Ca^{2+} extraction has been claimed to block advancement to either S_3 (thermoluminescence [96]) or to S_4 (delayed chlorophyll fluorescence [97]), and examinations of the necessity for Ca^{2+} binding prior to formation of the EPR-detectable form of the S_2 state have produced a

variety of results. Rigorous extraction of the metal in darkness with high salt and chelators blocks either formation or detection of the EPR active form of the S_2 state [27,68,104]. Various modifications of the extraction conditions (light, the presence of chelators such as citrate, EGTA, or EDTA) have now been shown to cause the formation of a modified form of the EPR signal characterized by a larger number of lines with narrowed spacings between the individual lines [105,106,108,109,111,112], a modification that is attributed to the action of the chelators (citrate, EGTA, EDTA) used in Ca^{2+} extraction [111]. Another consequence of Ca^{2+} extraction with chelators is a shift in the optimum for S_2 formation to higher temperatures [109,111]. At the same time, there is now general agreement that PS II samples modified by removal of Ca^{2+} and then illuminated with or without chelators can produce two sequential one-electron oxidation events ultimately to generate a modified form of the S_3 state [111].

(5) Drawing on data assessing the comparative affinities of various metal ions for the Ca^{2+} site in PS II (Table I), it can be tentatively concluded that the Ca^{2+} site in PS II exhibits the following order of preference for various metals:

$$\sim La^{3+}, Ca^{2+} > Cd^{2+}, Sr^{2+} > Na^{+} > K^{+} > Cs^{+} \ggg Ba^{2+}, Mg^{2+}$$

Based on analogies with well-characterized Ca^{2+}-binding proteins [76], it appears likely that the predominant ligands to the metal in PS II may be oxygen atoms, which might derive from Asp or Glu carboxyl groups; based on the same analogies, -OH (from Ser) or carbonyl atoms from peptide backbones may also serve as Ca^{2+} ligands.

(6) The PS II polypeptide or polypeptides which provide Ca^{2+} ligands are unknown; a restricted group of polypeptides constituting the 'core' of oxygen evolution activity possess parts of the requisite amino-acid sequences in topologically appropriate positions to form a binding site (Table III). Although well-characterized Ca^{2+}-binding proteins contain the metal ligation environment within a single polypeptide chain, the possibility that in PS II the Ca^{2+} site is comprised of amino-acid ligands contributed by more than one polypeptide cannot, at present, be conclusively eliminated.

The variety of effects elicited by removal of Ca^{2+} from PS II or by substitutions with another metal (Sr^{2+}) at the Ca^{2+} site suggest that in the native state at least one $Ca^{2+}$is ligated in such a way as to somehow contribute to, or regulate the structure of, the ligand environment of the Mn atoms that constitute the redox mediators of water oxidation. If so, does this structural regulation bear any similarity to the effects of Ca^{2+} binding to other proteins? Possible instructive analogies to be considered in the future would continue to include Ca^{2+} cofactor proteins along with the family of EF-hand proteins. The former proteins ligate Ca^{2+} with a range of affinities that best match the estimates of the affinity of the metal in salt-washed PS II preparations determined as K_M values, and Ca^{2+} ligation to the cofactor group of proteins provides structural stability, a role that might be similar to the observed Ca^{2+}-related phenomena in PS II. A related possibility is that Ca^{2+} exerts a structural influence on the oxygen-evolving enzyme system similar to the influence of Zn^{2+} in Cu/Zn superoxide dismutase, where the Zn atom contributes to the structure of the Cu binding domain by binding to a site in close proximity to the redox active Cu, bridged by an imidazole group from His [129].

Finally, the implications of water ligation to Ca^{2+} should be considered. Since water is often found as a ligand to protein-bound Ca^{2+} the possibility exists that Ca^{2+} not only contributes a structural stability to the oxygen-evolving enzyme system, but also acts as a substrate binding site. One model showing Ca^{2+} as a water donor to the oxygen-evolving apparatus has already appeared [130]. If water oxidation by PS II is in fact a concerted, four-electron process, it is reasonable to expect that the substrate might not bind directly to Mn during oxidation of the metals prior to formation of the S_4 state. If this is so, then binding of substrate water to a site on Ca^{2+} is a distinct possibility. At the same time, if Ca^{2+} in PS II does function as the substrate binding site, then the metal would presumably have to reside in close proximity to the Mn atoms themselves. Investigations of the Mn atoms in PS II by extended X-ray absorption fine structure (EXAFS) indicate the presence of a weak scatterer at about 4.2 Å from a collection of Mn atoms, and it has been suggested that this scatterer might be Ca^{2+} [132]. This is an interesting possibility: one of many that merit attention in future research on the action of Ca^{2+} in PS II.

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References

1 Joliot, P., Barbieri, G. and Chabaud, R. (1969) Photochem. Photobiol. 10, 309–329.
2 Kok, B. Forbush, B. and McGloin, M. (1970) Photochem. Photobiol. 11, 457–475.
3 Joliot, P. and Kok, B. (1975) in Bioenergetics of Photosynthesis (Govindjee, ed.), pp. 387–411, Academic Press, New York.
4 Radmer, R. and Ollinger, O. (1986) FEBS Lett. 195, 285–289.
5 Bader, K.P., Thibault, P. and Schmid, G.H. (1987) Biochim. Biophys. Acta 893, 564–571.
6 Dekker, J.P., Van Gorkom, H.J., Brok, M. and Ouwehand, L. (1984) Biochim. Biophys. Acta 764, 301–309.
7 Amesz, J. (1983) Biochim. Biophys. Acta 726, 1–12.
8 Dismukes, G.C. (1986) Photochem. Photobiol. 43, 99–115.
9 Brudvig, G.W., Beck, W.F. and De Paula, J.C. (1989) Annu. Rev. Biophys. Biophys. Chem. 18, 25–46.
10 Dexheimer, S.L., Sauer, K. and Klein, M.P. (1990) in Current Research in Photosynthesis (Baltscheffsky, M., ed.), pp. 761–764, Kluwer, Dordrecht.
11 Dismukes, G.C. and Siderer, Y. (1981) Proc. Natl. Acad. Sci. USA, 78, 274–278.
12 Cooper, S.R., Dismukes, G.C., Klein, M.P. and Calvin, M. (1978) J. Am. Chem. Soc. 109, 7248–7252.
13 Diril, H., Chang, H.R., Zhang., X., Larson, S.K., Potenza, J.A., Schugar, H.J., Hendrickson, D.N. and Isied, S.S. (1987) J. Am. Chem. Soc. 109, 6207–6208.
14 Sheats, J.E., Czernuszewicz, R.S., Dismukes, G.C., Rheingold, A.L., Petrouleas, V., Stubbe, J., Armstrong, W.H., Beer, R.H. and Lippard, S.J. (1987) J. Am. Chem. Soc. 109, 1435–1444.
15 Brudvig, G.W. and Crabtree, R.H. (1986) Proc. Natl. Acad. Sci. USA, 83, 4586–4588.
16 Pecoraro, V.L. (1988) Photochem. Photobiol. 48, 249–264.
17 Casey, J.L. and Sauer, K. (1984) Biochim. Biophys. Acta 767, 160–167.
18 Zimmermann, J.L. and Rutherford, A.W. (1984) Biochim. Biophys. Acta 767, 160–167.
19 De Paula, J.C., Innes, J.B. and Brudvig, G.W. (1985) Biochemistry 24, 8114–8120.
20 Dekker, J.P., Van Gorkom, H.J., Wensink, J. and Ouwehand, L. (1984) Biochim. Biophys. Acta 767, 1–9.
21 Lavergne, J. (1987) Biochim. Biophys. Acta 894, 91–107.
22 Saygin, O. and Witt, H.T. (1987) Biochim. Biophys. Acta 893, 452–461.
23 Berthold, D.A., Babcock, G.T. and Yocum, C.F. (1981) FEBS Lett. 134, 231–234.
24 Kuwabara, T. and Murata, N. (1982) Plant Cell Physiol. 24, 741–747.
25 Ikeuchi, M. and Inoue, Y. (1986) Arch. Biochem. Biophys. 247, 97–107.
26 Ghanotakis, D.F. and Yocum, C.F. (1986) FEBS Lett. 197, 244–248.
27 Ghanotakis, D.F., Demetriou, D.M. and Yocum, C.F. (1987) Biochim. Biophys. Acta 891, 15–21.
28 Cheniae, G.M. and Martin, I.F. (1971) Plant Physiol. 47, 568–575.
29 Sharp, R.R. and Yocum, C.F. (1981) Biochim. Biophys. Acta 635, 90–104.
30 Beck, W.F. and Brudvig, G.W. (1987) Biochemistry 26, 8285–8295.
31 Cheniae, G.M. and Martin, I.F. (1978) Biochim. Biophys. Acta 502, 321–344.
32 Frasch, W.P. and Cheniae, G.M. (1980) Plant Physiol. 65, 735–745.
33 Callahan, F.M. and Cheniae, G.M. (1985) Plant Physiol. 79, 777–786.
34 Tamura, N. and Cheniae, G.M. (1987) Biochim. Biophys. Acta 890, 179–194.
35 Bove, J.M., Bove, C., Whatley, F.R. and Arnon, D.I. (1963) Z. Naturforsch. 18, 683–688.
36 Izawa, S., Heath, R.L. and Hind, G. (1969) Biochim. Biophys. Acta 180, 388–398.
37 Theg, S.M., Jursinic, P.M. and Homann, P.H. (1984) Biochim. Biophys. Acta 766, 636–646.
38 Itoh, S., Yerkes, C.T., Koike, H., Robinson, H.H. and Crofts, A.R. (1984) Biochim. Biophys. Acta 766, 612–622.
39 Ono, T., Zimmermann, J.L., Inoue, Y. and Rutherford, A.W. (1986) Biochim. Biophys. Acta 851, 193–201.
40 Homann, P.H. (1988) Biochim. Biophys. Acta 934, 1–13.
41 Sandusky, P.O. and Yocum, C.F. (1984) Biochim. Biophys. Acta 766, 603–611.
42 Homann, P.H. (1990) in Role of Ca^{2+} in Biological Systems (Anghileri, L.J., ed.), Vol. 5, pp. 79–96, CRC Press, Boca Raton.
43 Miyao, M. and Murata, N. (1985) Trends Biochem. Sci. 10, 122–124.
44 Babcock, G.T. (1987) in New Comprehensive Biochemistry (Amesz, J., ed.), pp. 125–158, Elsevier, Amsterdam.
45 Andreasson, L.E. and Vanngard, T. (1988) Annu. Rev. Plant Physiol. Plant Mol. Biol. 39, 379–411.
46 Ghanotakis, D.F., Yocum, C.F. (1990) Annu. Rev. Plant Physiol. Plant Mol. Biol. 41, 255–276.
47 Debus, R.J., Barry, B.A., Babcock, G.T. and McIntosh, L. (1988) Proc. Natl. Acad. Sci. USA 85, 427–430.
48 Debus, R.J., Barry, B.A., Sithole, I., Babcock, G.T. and McIntosh, L. (1988) Biochemistry 27, 9071–9074.
49 Vermaas, W.F.J., Rutherford, A.W. and Hansson, O. (1988) Proc. Natl. Acad. Sci. USA 85, 8477–8481.
50 Kuwabara, T. and Murata, N. (1982) Plant Cell Physiol. 24, 741–747.
51 Sandusky, P.O., Selvius De Roo, C., Hicks, D.B., Yocum, C.F., Ghanotakis, D.F. and Babcock, G.T. (1983) in The Oxygen Evolving System of Photosynthesis (Inoue, Y., Crofts, A.R., Govindjee, Murata, N., Renger, G. and Satoh, K.-i. eds.), pp. 189–199, Academic Press, Tokyo.
52 Ono, T. and Inoue, Y. (1984) FEBS Lett. 170, 281–286.
53 Kuwabara, T., Miyao, M. Murata, T. and Murata, N. (1985) Biochim. Biophys. Acta 806, 283–289.
54 Miyao, M and Murata, N. (1984) FEBS Lett. 170, 350–354.
55 Akerlund, H.E., Jansson, C. and Andersson, B. (1982) Biochim. Biophys. Acta 681, 1–10.
56 Akerlund, H.E. (1983) in The Oxygen Evolving System of Photosynthesis (Inoue, Y., Crofts, A.R., Govindjee, Murata, N., Renger, G., Satoh, Ki., eds.), pp. 201–208, Academic Press, Tokyo.
57 Ghanotakis, D.F., Babcock, G.T. and Yocum, C.F. (1984) FEBS Lett. 167, 127–130.
58 Miyao, M. and Murata, N. (1984) FEBS Lett. 168, 118–120.
59 Nakatani, H.Y. (1984) Biochem. Biophys. Res. Commun. 120, 299–304.
60 Ghanotakis, D.F., Topper, J.N., Babcock, G.T. and Yocum, C.F. (1984) FEBS Lett. 170, 169–173.
61 Miyao, M. and Murata, N. (1986) Photosynth. Res. 10, 489–496.
62 Waggoner, C.M. and Yocum, C.F. (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), pp. 685–688, Nijhoff, Dordrecht.
63 Miyao, M. and Murata, N. (1985) FEBS Lett. 180, 303–308.
64 Cammarata, K. and Cheniae, G.M. (1987) Plant Physiol. 84, 587–895.
65 Waggoner, C.M., Pecoraro, V. and Yocum, C.F. (1989) FEBS Lett. 244, 237–240.
66 Boussac, A. Maison-Peteri, B., Etienne, A.L. and Vernotte, C. (1985) Biochim. Biophys. Acta 808, 231–234.
67 Homann, P. (1988) Biochim. Biophys. Acta 934, 1–13.

68 Kalosaka, K., Beck, W.R., Brudvig, G. and Cheniae, G.M. (1990) in Current Research in Photosynthesis (Baltscheffsky, M., ed.), pp. 721–724, Kluwer, Dordrecht.
69 Ono, T.A. and Inoue, Y. (1988) FEBS Lett. 227, 147–152.
70 Ono, T.A. and Inoue, Y. (1988) Biochim. Biophys. Acta 973, 443–449.
71 Volker, M., Ono, T. Inoue, Y. and Renger, G. (1985) Biochim. Biophys. Acta 806, 25–34.
72 Boussac, A. and Rutherford, A.W. (1988) Chem. Scripta 28A, 123–126.
73 Boussac, A. and Rutherford, A.W. (1988) Biochemistry 27, 3476–3483.
74 Beck, W.F., De Paula, J.C. and Brudvig, G.W. (1986) J. Am. Chem. Soc. 108, 4018–4022.
75 Britt, R.D., Zimmermann, J.L., Sauer, K. and Klein, M.P. (1989) J. Am. Chem. Soc. 111, 3522–3532.
76 Martin, R.B. (1984) in Metal Ions in Biological Systems: Vol. 17, Calcium and Its Role in Biology (Sigel, H., ed.), pp. 1–49, Marcel Dekker, New York.
77 Ghanotakis, D.F., Babcock, G.T. and Yocum, C.F. (1985) Biochim. Biophys. Acta 809, 173–180.
78 Waggoner, C.M. and Yocum, C.F. (1990) in Current Research in Photosynthesis (Baltscheffsky, M., ed.), pp. 733–736, Kluwer, Dordrecht.
79 Ono, T.A. and Inoue, Y. (1989) Arch. Biochem. Biophys. 275, 440–448.
80 Levine, B.A. and Williams, R.J.P. (1982) in Calcium and Cell Function, Vol. 2 (Cheung, W.J., ed.), pp. 1–38, Academic Press, London.
81 Snyder, E.E., Buoscio, B.W. and Falke, J.W. (1990) Biochemistry 29, 3937–3943.
83 Andersson, A., Forsen, S., Thulin, E. and Vogel, H.J. (1983) Biochemistry 22, 2309–2313.
84 Piccioni, R.G. and Mauzerall, D.C. (1976) Biochim. Biophys. Acta 423, 605–609.
85 Brand, J.J. (1979) FEBS Lett. 103, 114–117.
86 England, R.R. and Evans, E.H. (1981) FEBS Lett. 103, 114–117.
87 Becker, D.W. and Brand, J.J. (1985) Plant Physiol. 79, 552–558.
88 Zhao, J. and Brand, J.J. (1988) Arch. Biochem. Biophys. 264, 657–664.
89 Ono, T.A. and Inoue, Y. (1990) in Current Research in Photosynthesis (Baltscheffsky, M., ed.), pp. 741–744, Kluwer, Dordrecht.
90 Tamura, N., Cheniae, G.M. (1988) in Light Energy Transduction in Photosynthesis: Higher Plant and Bacterial Models (Stevens, S.E. and Bryant, D., eds.), pp. 227–242, American Society of Plant Physiologists, Rockville.
91 Tamura, N., Inoue, Y. and Cheniae, G.M. (1989) Biochim. Biophys. Acta 976, 173–181.
92 Miller, A.F. and Brudvig, G.W. (1989) Biochemistry 28, 8181–8190.
93 Shen, J.R., Satoh, K. and Katoh, S. (1988) Biochim. Biophys. Acta 933, 358–364.
94 Enami, I. Kamino, K., Shen, J.R., Satoh, K. and Katoh, S. (1989) Biochim. Biophys. Acta 977, 33–39.
95 Shen, J.R., Satoh, K. and Katoh, S. (1988) Biochim. Biophys. Acta 936, 386–394.
96 Ono, T.A. and Inoue, Y. (1989) Biochim. Biophys. Acta 973, 443–449.
97 Boussac, A., Maison-Peteri, B., Vernotte, C. and Etienne, A.L. (1985) Biochim. Biophys. Acta 808, 225–230.
98 Satoh, K. and Katoh, S. (1985) FEBS Lett. 190, 199–203.
99 Dekker, J.P., Ghanotakis, P.F., Plijter, J.J., Van Gorkom, H.J. and Babcock, G.T. (1984) Biochim. Biophys. Acta 767, 515–523.
100 Boussac, A. and Rutherford, A.W. (1988) FEBS Lett. 236, 432–436.
101 Ono, T.A. and Inoue, Y. (1986) Biochim. Biophys. Acta 850, 380–389.
102 Blough, N.V. and Sauer, K. (1984) Biochim. Biophys. Acta 767, 377–381.
103 Toyoshima, Y., Akabori, K., Imaoka, A., Nakayama, H., Ohkouchi, N. and Kawamori, A. (1984) FEBS Lett. 176, 377–381.
104 De Paula, J.C., Li, P.M., Miller, A.F. Wu, B.W. and Brudvig, G.W. (1986) Biochemistry 25, 6487–6494.
105 Boussac, A., Zimmermann, J.L. and Rutherford, A.W. (1989) Biochemistry 28, 8984–8989.
106 Boussac, A., Zimmermann, J.L. and Rutherford, A.W. (1990) in Current Research in Photosynthesis (Baltscheffsky, M., ed.), pp. 713–716, Kluwer, Dordrecht.
107 Boussac, A., Zimmermann, J.L., Rutherford, A.W. and Lavergne, J. (1990) Nature 347, 303–306.
108 Sivaraja, M., Tso, J. and Dismukes, G.C. (1989) Biochemistry 28, 9459–9464.
109 Ono, T.A. and Inoue, Y. (1990) Biochim. Biophys. Acta 1015, 373–377.
110 Beck, W.F., De Paula, J.C. and Brudvig, G.W. (1986) J. Am. Chem. Soc. 108, 4018–4022.
111 Boussac, A., Zimmermann, J.L. and Rutherford, A.W. (1990) FEBS Lett. 277, 69–74.
112 Ono, T.A. and Inoue, Y. (1990) Biochim. Biophys. Acta 1020, 269–277.
113 Sandusky, P.O. and Yocum, C.F. (1986) Biochim. Biophys. Acta 849, 85–93.
114 Koike, H., Mamada, K., Ikeuchi, M. and Inoue, Y. (1989) FEBS Lett. 244, 391–396.
115 Ikeuchi, M. and Inoue, Y. (1988) Plant Cell Physiol. 29, 1233–1239.
116 Williams, R.J.P. (1986) in Calcium and the Cell, Ciba Symposium 122, (Evered, D. and Whelan, J. eds.) pp. 145–162, J. Wiley & Sons, New York.
117 Oh-oka, H., Tanaka, S., Wada, K., Kuwabara, T. and Murata, N. (1987) FEBS Lett. 197, 63–66.
118 Wales, R., Newman, B.J., Pappin, D. and Gray, J.C. (1989) Plant Mol. Biol. 12, 439–451.
119 Burnap, R., Webb, R. and Sherman, L.A. (1990) in Current Research in Photosynthesis (Baltscheffsky, M., ed.) pp. 255–258, Kluwer Dordrecht.
120 Kalb, A.J. and Levitzki, A. (1968) Biochem. J. (109) 669–676.
121 Einspahr, H. and Bugg, C.E. (1984) in Metal Ions in Biological Systems: Vol. 17, Calcium and its Role in Biology (Sigel, H., ed.), pp. 51–97, Marcel Dekker, New York.
122 Strynada, N.C.J. and James, M.N.G. (1989) Annu. Rev. Biochem. 58, 951–998.
123 Bode, W. and Schwager, P. (1975) J. Mol. Biol. 98, 693–717.
124 Holmes, B. and Matthews, B.W. (1982) J. Mol. Biol. 160, 623–639.
125 Becker, J.W., Reeke, J.N., Wang, J.L., Cunningham, B.A. and Edelman, G.M. (1975) J. Biol. Chem. 1513–1524.
126 Cotton, F.A., Hazen, E.C. and Legg, M.J. (1979) Proc. Natl. Acad. Sci. 76, 2551–2555.
127 Dijkstra, B.W. and Kalk, K.H., (1981) Nature 289, 604–606.
128 Diner, B.A., Nixon, P.J., Metz, J.G., Rogner, M. and Chisholm, D. (1990) Biophys. J. 57 (2), 405a.
129 Richardson, J.R., Thomas, K.A., Rubin, B.H. and Richardson, D.C. (1975) Proc. Natl. Acad. Sci. USA 72, 1349–1353.
130 Rutherford, A.W. (1989) Trends Biochem. Sci. 14(6), 227–232.
131 Trebst, A. (1986) Z. Naturforsch. 41C, 240–245.
132 Penner-Hahn, J.E., Fronko, R.M., Pecoraro, V.L., Yocum, C.F., Betts, S.D. and Bowlby, N.R. (1990) J. Am. Chem. Soc. (112) 2549–2557.