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Properties of iodide-activated photosynthetic water-oxidizing complexes

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Chloride, the anionic cofactor of photosynthetic water oxidation, can be replaced by iodide with only a moderate sacrifice of water oxidizing activity. Thermoluminescence studies on Photosystem II membranes with I^--activated water oxidizing complexes indicated that their S_2 and S_3 states had lower oxidation potentials than those of Cl^--activated systems, and that their S_2 state reverted to the S_1 condition more slowly. It was also determined that the functional I^- was only very slowly displaced by excess free Cl^-. The apparent immunity of activating I^- against the oxidizing power of stored oxidants in the water oxidase might suggest that the anion exerted its effects from a site quite distant from the charge-accumulating Mn-center. However, if we accept the concept that the anionic cofactor is ligated to Mn, we have to postulate that the Mn-cluster resides in an occluded hydrophobic pocket in which the oxidation of I^- by the higher S-states is prevented due to kinetic constraints. These inferences are based on the properties of known Mn(III) complexes with iodide as a ligand.

Introduction

Even though the cofactor role of Cl^- in photosynthetic electron transport of chloroplasts was recognized more than forty years ago, the function of this anion is still not understood. In the meantime the Cl^- requirement has been localized in the water oxidizing mechanism, but the precise site of action remains to be identified. Furthermore, uncertainty exists about the number of Cl^- ions that are required by each functional unit (see reviews Refs. 1, 2). For example, while most experimental results had suggested an involvement of several Cl^- ions in each water oxidizing event [3,4], some recent data point to the possibility that only a single Cl^- may be needed [5]. With regard to Cl^- binding, suggestions of a relatively unspecific electrostatic binding [6,7] were challenged by models of anion-ligation to one or more of the Mn atoms in the charge-accumulating Mn-cluster of the water oxidase [8]. A ligation of a single halide to Mn is consistent with recent X-ray absorption data collected on photosynthetic membranes after activating Cl^- had been replaced with Br^- anions which functionally are almost equally effective [9].

A unique kind of information about the possible location of the anionic cofactor in PS II has come from experiments with I^-. This substitute for Cl^- is known to be susceptible to oxidation by oxidants in the water oxidizing complex [10]. Recently it was shown that the oxidation product I_2 or I^+ specifically iodinated tyrosine-161 on the D_1-protein of the PS II reaction center [11,12]. In its electron deficient radical form, this tyrosine residue serves as an intermediate oxidant in the electron path between the charge-accumulating 'S-states' of PS II and the reaction center chlorophyll *P*-680 [13]. Since Cl^- prevented the iodination of D_1, it was concluded that e^--donation from I^- occurred while it was bound to the functional binding site for activating Cl^- [11]. Consequently, this site was postulated to be located in the vicinity of the unique tyrosine of D_1. If put into the context of the X-ray absorption data, a picture emerges in which the activating anion is ligated to a Mn that is positioned in the immediate proximity of the tyrosine and perhaps is its e-donor.

Very recently, other studies of the action of I^- [14] have cast doubt on the assumption that Cl^- competitively prevents the oxidation of I^- in PS II. These new results indicate that the photooxidants of PS II may

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Abbreviations: chl, chlorophyll; Mes, 2-(*N*-morpholino)ethanesulfonic acid; Mops, 4-morpholinepropanesulfonic acid; PS II, Photosystem II.

target I^- anions associated with sites different from those occupied by activating anions.

In our own investigations on the role of activating anions in water oxidation, we were intrigued by our finding that the ability of I^- to serve as a cofactor of water oxidation required that the extrinsic 23 and 17 kDa polypeptides were bound to the water oxidizing complexes [15]. The reason appeared to be that the extrinsic polypeptide shield of the water oxidizing site lowered the susceptibility of I^- to photooxidation in PS II. In this article we report on the properties of PS II membranes in which I^- served as a cofactor for water oxidation without being photooxidized to any significant extent. Our findings place considerable constraints on any concept that positions the activating halide ion at a site in the immediate vicinity of an oxidant in the water oxidizing complex.

Materials and Methods

For the preparation of thylakoid membranes enriched in PS II from chloroplasts of wild growing pokeweed (*Phytolacca americana*), we used a slightly modified version of the procedure developed by Yocum and his collaborators [16]. Concentrated suspensions of the preparations (approx. 6 mg chl/ml) with chl *a*/chl *b* ratios of 2.1–2.3 were stored at −85°C in a medium containing 0.5 M sucrose, 2 mM NaCl and 10 mM sodium-Mes at pH 6.2.

As will be discussed in the Results section, the effectiveness of I^- as a cofactor substitute for Cl^- required that the amount of non-functional I^- was kept at a minimum. Thus, our reconstitution procedure took advantage of the fact that activating anions are rather well retained by membranes so long as their water oxidizing complexes are associated with their 17 and 23 kDa extrinsic polypeptides [2]. Other considerations in the development of our substitution procedure (see Refs. 17, 18) were: (1) in media buffered at pH ≥ 7 and containing ≈ 100 mM Na_2SO_4 as the only inorganic salt, the 17 and 23 kDa polypeptides readily dissociate with a concomitant release of the activating anions; (2) binding of the extrinsic polypeptides in such media is promoted by the presence of millimolar amounts of salts of activating anions; (3) a pH < 6.5 favors binding and retention of the extrinsic polypeptides.

Accordingly, the following protocol was devised for the preparation of PS II membranes with I^- activated water oxidases. A suspension of PS II membranes was thawed and diluted approximately 20-fold with a medium containing 0.4 M sucrose and 10 mM sodium-Mes (pH 6.2), and centrifuged at approximately 30000 × *g*. The pellet was washed once with the same medium and finally suspended at 500 μg chl/ml in a medium containing 0.4 M sucrose, 30 mM sodium-Hepes (pH 7.2) and 75–100 mM Na_2SO_4. Following a 30 min incubation in the dark at 0°C, the suspension was distributed between two centrifuge tubes and sedimented by centrifugation. The pellet of one tube was discarded, and the pellet of the other suspended in the combined supernatants. This provided us with Cl^- depleted membranes in a medium that contained twice the amount of dissociated 17 and 23 kDa polypeptides needed for full reconstitution.

For a typical experiment, the resulting suspension was then divided into three equal portions, one of which was kept unchanged, while NaI or NaCl, respectively, were supplied to the other tubes, providing final concentrations of 10 mM in each case. After an incubation in the dark at 0°C for 30 min., sufficient unneutralized Mes from a 1 M solution was injected to change the pH to approx. 6. Following a further incubation of 30–60 min. at 0°C, the suspensions were centrifuged and the pellets suspended in 0.4 M sucrose, buffered with 25 mM sodium-Mes at pH 6.1. These preparations were usually used immediately in our experiments. Storage at −85°C always resulted in some loss of activity, but the distinct differences between Cl^-- and I^--reactivated samples on the one hand, and between these preparations and the anion-free control, were retained.

Cl^--free solutions of the 17 and 23 kDa extrinsic polypeptides were prepared from the supernatants of PS II membranes that had been washed Cl^--free, as described above, and had been incubated in a medium containing 40 mM sodium-Mops (pH 7.2), 0.4 M sucrose and 100 mM Na_2SO_4, to dissociate the two polypeptides. The supernatants were dialyzed against 25 mM sodium-Mes (pH 6.2), concentrated by ultrafiltration, and fractionated on Cl^--free DEAE-Sephacel (Sigma) columns with 10 mM sodium-Mes (pH 6.2), containing Na_2SO_4 at concentrations from 2.5 mM to 25 mM [18].

All measurements were performed in media containing 0.4 M sucrose and 25 mM sodium-Mes (pH 6.0–6.2). Taking into account the dilution steps but not inadvertent contaminations, we estimated that the concentrations of free halide ions under the assay conditions for thermoluminescence were less than 150 μM, and during the measurements of O_2 evolution activity less than approx. 10 μM. The latter were done polarographically under steady state conditions with pheryl-*p*-benzoquinone as e-acceptor. The apparatus has been described elsewhere [6]. Thermoluminescence measurements were usually performed on the membranes after a 10 min dark adaptation at room temperature and further storage in the dark at 0°C. 90 μl of a suspension with 250 μg chl/ml were applied under green safelight to a 2.5 cm × 2.5 cm piece of filter paper that was fastened to a heatable aluminum stage, of the type developed by Inoue and his collaborators [19]. All procedures and the measuring systems used

were, in fact, quite similar to what that group has described. In our setup, we detected the emitted light with a EMI 9558 photomultiplier tube, and monitored the temperature with a surface probe connected to a digital thermometer Model 650-E from Omega Engineering Corporation. Outputs from the photomultiplier tube and the thermometer drove an X-Y recorder. Flash illumination was provided from an EG&G flash tube FXP-855.

Polyacrylamide gel electrophoresis of Li-dodecylsulfate solubilized membranes was performed as described earlier [20], and chlorophyll was quantified spectrophotometrically according to McKinney [21].

Results

Data obtained in several laboratories have left no doubt that I^- belongs to those anions that can serve in place of Cl^- as activators of photosynthetic water oxidation [15,22]. It has also become clear that the effectiveness of I^- to be a cofactor of water oxidation is compromised by its ability to compete with water as an e-donor in the water oxidizing complex, especially at the high anion concentrations needed after removal of the 17 and 23 kDa extrinsic polypeptides [10,14,15]. In our studies on the dual role of I^- in PS II we suspected from the outset that an effective cofactor action would require a low concentration of free I^-. This was accomplished by trapping the activating I^- at its putative binding site behind the shield formed by the bound 23 kDa polypeptide.

In initial experiments, we followed a protocol developed earlier [23] and incubated small amounts of PS II membranes depleted of their 17 and 23 kDa polypeptides at 400 μg chl/ml in a Cl^--free medium with various concentrations of I^- or Cl^- and approx. 200 μg/ml 23 kDa polypeptide. After 10 min, the mixture was diluted 12-fold and the activity in terms of O_2 evolution was assayed, either without further anion additions, or after adjustment of the anion concentration to that used in the incubations. The data shown in Table I document that the trapping procedure worked quite well. Indeed, I^- could be shown to support considerable activity of the water oxidizing complexes so long as the concentrations of free I^- remained low. This result is consistent with recently published data from the independent studies of Papageorgiou and Lagoyanni [14].

Further experimentation required the availability of large amounts of I^--activated PS II membranes. These were prepared by the procedure described in Materials and Methods. As judged from Coomassie blue-stained electrophoresis gels, the Cl^- and I^- reconstituted membranes bore essentially normal amounts of the 23 kDa extrinsic polypeptide, but the anion-depleted control clearly remained deficient (not shown). Rebinding of the 17 kDa extrinsic polypeptide had only occurred to any significant extent during reconstitution of Cl^- as the activating anion, but even in this case the rebinding

TABLE I

Relative activities of PS II membranes depleted of their 17 and 23 kDa polypeptides and reconstituted with the 23 kDa polypeptide in the presence of an anion as indicated (see Materials and Methods)

Rates are given relative to preparation assayed in the presence of 20 mM Cl^-, i.e., 360 μmol O_2/mg chl per h. Anion concentration was either 1/12 of that in incubation due to dilution of the incubation mixture, or was adjusted to that of the incubation mixture after dilution.

Anion	mM [anion] in incubation	Relative activity of preparation with mM [anion]	
		1/12 of incubation	as in incubation
–	–	14	14
Cl^-	0.2	63	66
	0.5	72	81
	20	88	100
I^-	0.2	51	53
	0.5	60	60
	20	57	20

TABLE II

Some characteristics of PS II preparations used in this study

For assay conditions see Materials and Methods. (Insufficient number of measurements in the presence of DCMU were available to provide meaningful standard deviations.)

Property	Preparation			
	Native	Cl^--depleted	Cl^--reconstituted	I^--reconstituted
Rate of O_2 evolution (μmol O_2/mg chl per h)	470 ± 40	117 ± 32	397 ± 56	313 ± 52
Thermoluminescence emission temperature (°C) ($S_2Q_B^-$ recombination)	37 ± 3	40 ± 4	36 ± 3	47 ± 4
Thermoluminescence emission temperature (°C) ($S_2Q_A^-$ recombination)	≈ 12	≈ 20	≈ 12	≈ 26

was far from complete. The observed pattern of reacquistion of the extrinsic polypeptides was consistent with the reported dependence of the extent of binding on the presence of an activating anion and its activating effectiveness [17,18]. It also was not unexpected that the 17 kDa polypeptide would be particularly susceptible to the dissociating action of the approx. 100 mM Na_2SO_4 that remained present in the reconstitution medium [17,24].

Despite the incomplete rebinding of the extrinsic 17 kDa polypeptide, the association of the membranes with the reconstituted anion was maintained after sedimentation of the membranes and their suspension in anion-free media. Typically, the water oxidizing activity of the I^--activated membranes was about two-thirds of that measured with Cl^--activated preparations (Table II). Fig. 1 displays the light intensity dependences of oxygen evolution of Cl^- and I^- reactivated membranes in a rate vs. rate/intensity plot, to allow comparisons of the relative quantum yields (intercepts of lines with abscissa). It can be seen that the lower effectiveness of I^- as an activator was reflected in maximal rate and quantum efficiency to approx. the same extent, just as earlier studies had shown for the moderately effective activator NO_3^- and for a suboptimal activation with Cl^- [23]. The essentially linear relationship between rate and rate/intensity seen for the I^- activated preparation down to quite low light intensities suggested that no significant loss of oxidants to I^- began to occur as their turnover became slower.

Further evidence for the effectiveness of I^- as a cofactor in the water oxidizing mechanism was sought in thermoluminescence measurements. In principle, thermoluminescence is a thermally activated reversal of the light driven storage of an oxidant-reductant pair in PS II. It typically results from charge recombination involving the oxidants of the S_2 or S_3 states and the

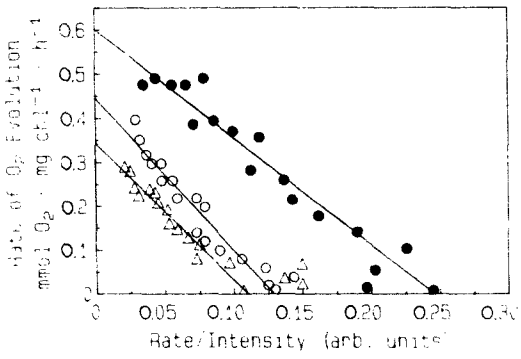

Fig. 1. Oxygen evolution activity of PS II membranes as a function of light intensity, plotted as rate vs. rate/intensity. ●, native, untreated PS II membranes; ○, PS II membranes initially depleted of an activating anion, and then reconstituted with Cl^- as a cofactor; △, same, but reconstituted with I^- as a cofactor.

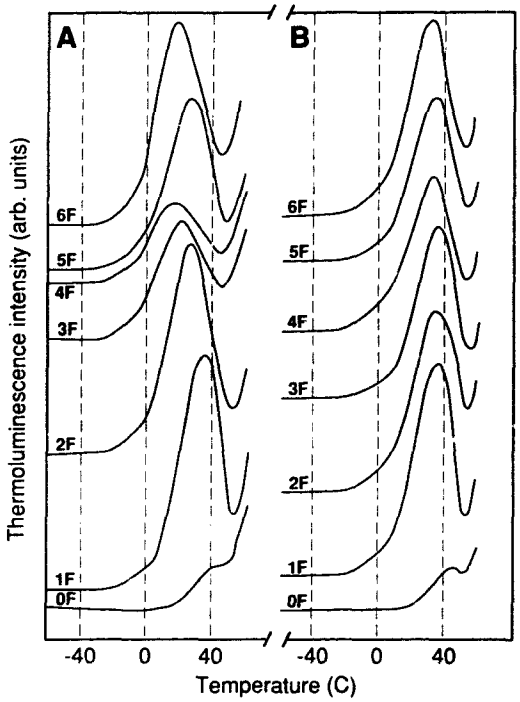

Fig. 2. Emissions of B-band thermoluminescence from PS II membranes as a function of the number of light flashes given prior to the assay (at ≈ 5C). A (left side): native, untreated PS II membranes; B (right side): PS II membranes depleted of activating Cl^- according to the procedure described in Material and Methods.

reductants Q_A^- or Q_B^- [25]. The relative intensity of the thermoluminescence emission, therefore, reflects, among other things, the abundance of oxidant-reductant pairs capable of coupling a charge recombination reaction to an excitation of the reaction center chlorophyll. The temperature at which this recombination and, hence, a light emission occurs, is a measure of the difference of the energy stored in the oxidant-reductant pair and the energy needed for a ground state–singlet transition of the reaction center chlorophyll. Maximal thermoluminescence from $S_2Q_B^-$ and $S_3Q_B^-$ usually is elicited between 25 and 35°C ('B-bands' of thermoluminescence). When Q_A^- is the reductant, e.g., in DCMU-inhibited PS II complexes, maximal thermoluminescence emission from most untreated preparations occurs around 10°C [25].

In Figs. 2 and 3, the pattern of thermoluminescence emission as a function of the number of preceding flashes is shown for PS II membranes before and after depletion of the activating anion, and for Cl^- and I^- activated complexes. Control membranes showed the normal period-four oscillation that reflects the cycling of oxidant accumulation through the unstable S_2 and S_3 states, which participate readily in recombination reactions, and then through the stable S_0 and S_1 states.

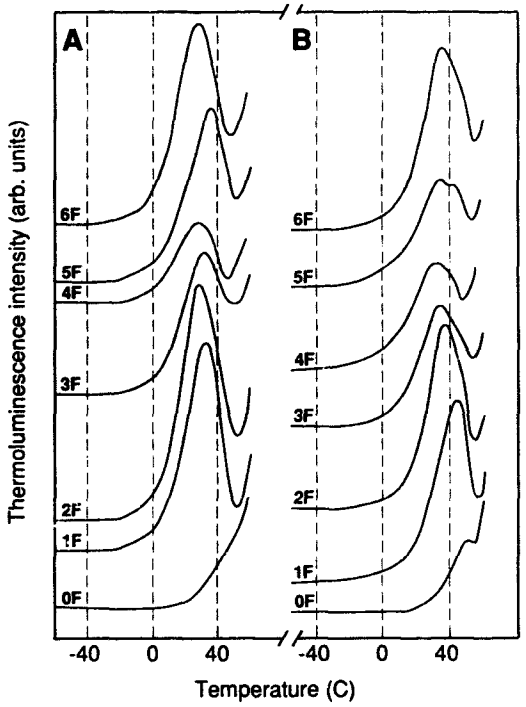

Fig. 3. Emissions of B-band thermoluminescence from Cl^- and I^- reconstituted PS II membranes as a function of the number of light flashes given prior to the assay (at ≈ 5°C). PS II membranes were depleted of their activating anion, as described in Materials and Methods, and then reconstituted either with Cl^- (left side, A) or I^- (right side, B).

As expected, and shown in previous studies [26], no such oscillations were evident in anion-depleted membranes. After the second flash the emission intensity remained essentially constant, suggesting a transition from the dark resting-state S_1 over S_2 to S_3, but no further. While this interpretation was challenged by the work of Ono et al. [27], recently published results obtained by Boussac et al. [28] reaffirm that charge accumulation in a Cl^--depleted PS II is blocked after S_3.

In both, Cl^- and I^- reconstituted membranes, the restoration of a considerable portion of the original water oxidizing activity was reflected by an oscillatory pattern of their thermoluminescence emission (Fig. 3). However, the period of the oscillation observed with I^- reconstituted membranes never was as convincingly four as it was with Cl^--activated preparations. This, probably, was a consequence of the lower quantum efficiency of I^--activated PS II complexes, which may have caused more 'misses' of the individual flashes. Another difference was that, after an illumination with a single flash, I^--reactivated membranes emitted most of their thermoluminescence at a temperature that was higher than what we measured with Cl^--activated and anion depleted preparations (see also Table II). This was unexpected because, in all instances reported thus far, the insertion of an activating anion into a Cl^--depleted PS II down-shifted the emission temperature of thermoluminescence resulting from $S_2Q_B^-$ recombinations (e.g., Refs. 26,29).

At the pH of our assays, the peak of the thermoluminescence accompanying the recombination reaction of the charge pair of $S_3Q_B^-$ typically is positioned at a lower temperature than that of the thermoluminescence from a back reaction between S_2 and Q_B^- in the same preparations [30]. This was true also for the I^--reconstituted membranes, but the temperature remained higher than that of the corresponding emission from Cl^--activated membranes. It can be concluded that less energy is stored in both the $S_2Q_B^-$ and the $S_3Q_B^-$ charge pairs of I^--activated water oxidases than in the corresponding charge pairs of Cl^--activated complexes. Since an upshifted temperature also characterized the thermoluminescence band from a $S_2Q_A^-$

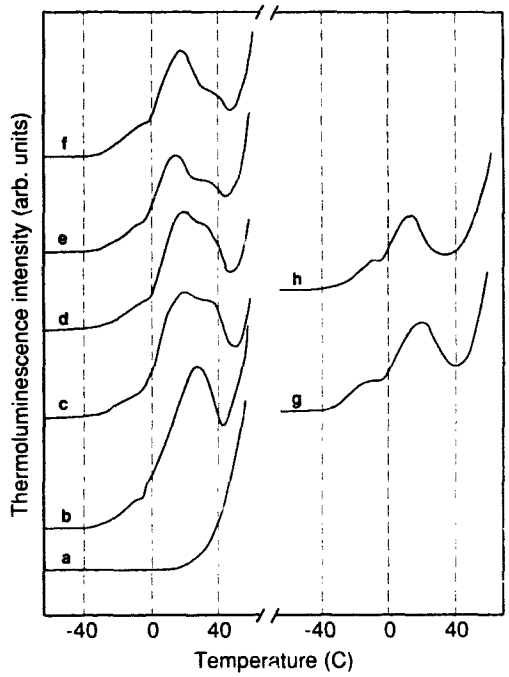

Fig. 4. Binding of Cl^- to the functional anion site as monitored by thermoluminescence, from $S_2Q_A^-$ recombinations. 20 mM NaCl were added to anion depleted or I^--reconstituted PS II membranes (250 μg Chl/ml), which were then incubated at 0°C for various periods of time. Subsequently, 20 μM DCMU was added and one light flash was given at ≈ −12°C. Traces (a)–(f) represent the thermoluminescence emission from I^--reconstituted membranes: (a), no Cl^- added, no illumination (baseline); (b), no Cl^- added; (c), (d), (e) and (f), after incubation with Cl^- for 5, 15, 60 and 90 min, respectively. Traces (g) and (h) were obtained with anion-depleted membranes in the absence of added Cl^- (g) and approx. 1 min after addition of Cl^- (h).

recombination in the presence of DCMU (Table II and Fig. 4), it is likely that the abnormal redox potentials are attributable to the S states and not to the reduced e^--acceptors Q_A^- or Q_B^-.

The elevated emission temperatures of the B-bands emitted by the I^--reconstituted preparations suggested a relatively high stability of the respective S-states. For the S_2 state, this was verified by following the declining ability of the membranes to emit a B-band when they were kept in the dark at 20°C after a single flash. At pH 6.0, the estimated stabilities were I^--activated > anion-depleted > Cl^--activated with halftimes of deactivation of approximately 270, 200 and 140 s. The range of these values was consistent with that of S_2 life-times calculated and measured by Vass et al. [31] for PS II preparations that resembled ours with respect to the temperature characteristics of their thermoluminescence emission. In order to unequivocally assign the loss of the B-band to a deactivation of S_2, while eliminating an autooxidation of Q_B^- as the predominant reason, we also measured the thermoluminescence from the dark incubated samples after they had been supplied with DCMU and illuminated for 45 s at 77 K. This protocol allows delivery of an electron to Q_A at the expense of some e-donor other than the S-complex and, therefore, provides each existing S_2 oxidant with a partner for a recombination reaction [19]. Our measurements confirmed that after a few minutes of dark incubation the abundance of S_2 had decreased considerably in all preparations, but a precise quantitative analysis was not undertaken because of a presence of a measurable portion of centers with a very stable S_2. This population of S_2 states turned out to belong to water oxidizing complexes that had lost their functional Ca^{2+} during the anion- and polypeptide-depleting treatments they had been subjected to (c.f. Ref. 32). A similar presence of such very stable S_2 states may perhaps explain the very long-lived S_2 states which previously Ono et al. [27] had encountered in PS II membranes which they had depleted of Cl^- by a procedure similar to that used in this study.

The abnormal thermoluminescence property of I^--activated PS II membranes not only made it possible to unequivocally verify successful insertions of the anion, but also to monitor its displacement by the natural cofactor Cl^-. In a relevant experiment, we chose to look at the luminescence from an $S_2Q_A^-$ recombination in DCMU-inhibited membranes rather than at the B-bands because of the more pronounced differences of emission temperatures (see Table II). As Fig. 4 shows, Cl^- binding to a Cl^--depleted water oxidizing complex was as rapid as it took us to inject NaCl into the membrane suspension and to prepare it for the thermoluminescence measurement, i.e. approx. 45 s. For I^--reactivated PS II complexes, on the other hand, an incubation for 1.5 h with 20 mM Cl^- was not long enough to convert them completely to Cl^--activated complexes. Obviously, bound I^- at the activating site was not readily exchanged for Cl^-, the natural anionic cofactor. Preliminary experiments gave no hint that the displacement of I^- was significantly faster when the preparations were incubated in room light (not shown).

Discussion

Ever since it has been recognized that iodide ions can be an anionic cofactor of the water oxidizing mechanism as well as an e^--donor, the question had to be answered how those two actions can be reconciled. An obvious answer was that the anionic activator site is sufficiently distant from any potential oxidant in the water oxidizing complex. Yet, some laboratories have provided experimental evidence for a direct ligation of the activating anion to Mn of the charge-accumulating metal center of the water oxidase. Some of this evidence was indirect, (see reviews in Refs. 13, 32) but the recent X-ray absorption data appear to provide more direct support for the view of a very close proximity of activating anions and the Mn-center [9].

Electron-donation from I^- appeared to occur to a significant extent only when a pool of excess free I^- was available in the suspension medium. In fact, our investigations reveal that an iodide-activated water oxidizing system is quite stable and is active in the light for at least a minute without any apparent loss of its anionic activator to photoxidation. This was also evident from the observation that the S_2 state of I^- activated water oxidizing complexes actually was more stable than that in complexes operating with Cl^- as anionic cofactor. The greater stability could be attributed to a lowered oxidation potential on the basis of the higher temperature that was needed to elicit thermoluminescence from the $S_2Q_B^-$ and $S_2Q_A^-$ charge pairs of I^--reconstituted membranes. From the results obtained by Inoue and his collaborators with I^- as anionic cofactor [11,29], we actually had predicted that I^--activated PS II preparations would emit their thermoluminescence at the same temperature as normal, Cl^--activated ones. A factor contributing to this difference may be the pH of the suspension media which was 7.5 in the experiments of Inoue's group, but was chosen to be around 6 in our assays, in order to assure retention of the extrinsic 23 kDa polypeptide and, hence, of I^-.

At a first glance, the immunity of activating I^- to photooxidation appears to rule out its direct ligation to a member of the water oxidizing Mn-center because during the S-cycle its oxidation potential should rise above that of I^- anions. Hence, if I^- were ligated to a Mn that becomes oxidized as light causes an advance of the S-state, an oxidation of the anion would seem inevitable. Yet, synthetic Mn(III)-complexes with an

iodide ligand are known [33,34]. They would appear to be attractive models for an anion association with the Mn-center of PS II but, unfortunately, they are stable only in a nonaqueous environment [35]. This may not be an entirely forbidding constraint however, because several authors envisage the access of substrate water to the active site of the water oxidase, i.e., the Mn-center, to be rigorously controlled (e.g., Refs. 1, 13, 32 and 36). Evidence has actually been obtained in support of a model according to which the e^--acceptor $TyrZ^+$ of the Mn-center is located in a solvent-excluding hydrophobic pocket [13]. The very slow conversion of I^--activated water oxidases to Cl^--activated centers is another piece of evidence for the occluded nature of the domain behind the 23 kDa polypeptide that holds the anionic cofactor. Our preliminary data suggest, furthermore, that the anion remains sequestered in this domain during the S-cycle. This implies that any displacement of the anion from Mn during the S-cycle by substrate water (c.f. Ref 32) would have to involve a switch from one binding site to another close-by.

Aside from the requirement of nonaqueous conditions, the suitability of the synthetic Mn(III)-complexes to serve as models for the Mn-center of the water oxidase may be compromised also by their inadequate oxidation potentials. These Mn(III)-complexes arise during an oxidation of the corresponding Mn(II)-complexes with molecular oxygen [33,34] while the Mn of PS II oxidizes water to oxygen. Again, there may be a way to reconcile the seemingly incompatible properties of the synthetic Mn-complexes and the water oxidizing apparatus. One could assume that during the early S-transitions PS II stores some free energy in a form other than redox-energy (e.g., as conformational energy) and makes it available only during one of the final steps. At that stage, electron abstraction from water may be kinetically favored over an oxidation of I^-. In fact, one can see the remarkable resistance of S_3 to reduction by added e^--donors like NH_2OH and NH_2NH_2 [37] as evidence for a kinetic barrier that protects the water oxidizing step.

All these speculations are unnecessary, of course, if one proposes that the anion related effects in PS II are consequences of structural reorganizations emanating from an anion binding site, or anion binding sites, remote from the Mn center [38]. Even so, such anion actions must still be visualized to result in unique changes of the ligand environment of the Mn-assembly of PS II. This follows from the reported competitive character of NH_3-binding to the Mn-center with respect to added Cl^- (see Refs. 13, 32).

The combined oxidizing power of four oxidizing equivalents at the active site of the photosynthetic water oxidase can be discharged against two water molecules only when the oxidizing charges are adequate and are properly distributed, and after two water molecules have become bound and can be deprotonated. Our studies leave open the question as to which particular event is targeted by the an anionic cofactor, but they place constraints on our perception of the site from which it operates.

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