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The association of functional anions with the oxygen-evolving center of chloroplasts

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The association of the site of photosynthetic water oxidation with Cl^- and other activating anions was analyzed with Photosystem II-enriched thylakoid particles prepared by Triton X-100 treatment. On the basis of the experimental evidence it is proposed that, regardless of the presence of the extrinsic 18 and 23 kDa polypeptides, the reactivation of Cl^--depleted particles by added anions is contingent upon the protonation of membrane-bound buffering groups with an apparent pK_a of approx. 6. The rate of dissociation of the anion from the site of water oxidation followed the order $NO_3^- > ClO_4^- > Br^- \approx Cl^-$. A model is developed that takes into account the known requirement of more than 1 anion/center for maximum activity. It assumes a finite and anion-dependent capacity of the water-oxidizing site for the activating anions, and that the rate of photosynthetic oxygen evolution is proportional to the number of anions at the water-oxidizing enzyme. In accord with suggestions made earlier, and in agreement with their further elaboration by Coleman and Govindjee (Proc. 16th FEBS Congress, Moscow, VNU Science Press, Utrecht), it is proposed that the Cl^- requirement of Photosystem II is linked to protonation-deprotonation events associated with photosynthetic water oxidation.

Introduction

Chloride appears to be an essential cofactor of the enzyme system catalyzing photosynthetic water oxidation. This contention is based on the observation that, after an appropriate Cl^--depleting treatment of isolated thylakoids or thylakoid particles, optimal rates of photosynthetic water oxidation require the addition of Cl^-, or certain other monovalent anions, notably Br^-, I^-, NO_3^- and ClO_4^- [1–4]. Investigations in our laboratory have identified an exposure of the water-oxidizing enzyme system (water oxidase) to alkaline conditions as the single important event responsible for creating Cl^- deficiency [5,6]. However, since the site of water oxidation is located on the lumen side of the thylakoid membrane, it is in ready equilibrium with the pH of the suspension medium only when the membranes are made permeable to protons, e.g., by aging, elevated temperatures, or the action of uncoupling agents. These conditioning treatments can be avoided when PS II-enriched thylakoid particles are used in which the water-oxidizing site is directly exposed to the aqueous bulk phase.

With such purified PS II preparations, we have analyzed the pH dependence of the activation of the water oxidase by Cl^- and other anions. Our results are compatible with a mechanism according to which the association of the water oxidase with

Abbreviations: Caps, 3-(cyclohexylamino)-1-propanesulfonic acid; Chl, total chlorophyll; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Mes, 4-morpholineethanesulfonic acid; PS, Photosystem.

an activating anion is contingent upon the protonation of buffering groups with a pK_a of approx. 6. Cl^- substitutes such as NO_3^- have been found to have a lower affinity for the water oxidizing site than Cl^-, and to bind as well as dissociate more rapidly.

Materials and Methods

Throughout this study, PS II-enriched particles were used which had been prepared by Triton X-100 treatment of thylakoids isolated from leaves of pokeweed (*Phytolacca americana*) or spinach (*Spinacea oleracea*). We essentially followed the procedures of Kuwabara and Murata [7], or of Berthold et al. [8], but lately used the formers' isolation procedure and the latters' treatment protocol. The Chl *a*/Chl *b* ratio of the preparations was usually 2.1 ± 0.1, and their content of PS I-associated polypeptides was very low as judged from Coomassie blue-stained polyacrylamide gels after electrophoresis of SDS-solubilized particles. With 500 μM 2,5-dimethylbenzoquinone as e-acceptor, the maximal rate of O_2 evolution in 200 W/m^2 red light at approx. 25°C was around 0.4 mmol/mg Chl per h. Higher rates were measured when 350 μM phenyl-*p*-benzoquinone served as e-acceptor, especially in the much stronger light that was used in some experiments (up to 3 kW/m^2). The preparations (5–8 mg Chl/ml) were stored for no longer than 2 weeks at −70°C in media containing 0.7 M sorbitol, 10 mM Na-Mes (pH 6.5) and 1 mM NaCl.

Cl^--depletion was accomplished by injecting the thylakoid preparation into the assay medium buffered with 2 mM Na-Caps at pH 9.6–9.7, and incubating it for 5 s. Then 1 M Na-Mes or Na-Hepes was added to bring the pH to its desired value at 20 mM buffer concentration. When the effect of Mg^{2+} was studied ,these latter buffers were adjusted with 160 mM MgO supplemented with NaOH, and the final Mg^{2+} concentration in the assay medium was 3.2 mM. The buffer addition was followed, if desired, by an addition of salt and, usually 3 min later, of the e-acceptor. The rate without added Cl^- generally was less than 15% of the rate with optimal Cl^- added, and the latter was 80–90% of the rate measured with undepleted preparations in the presence of NaCl. In some experiments, varying amounts of Na_2SO_4 were added to control the ionic strength, or 25 mM Na_2SO_4 was present in all reaction mixtures, but this did not alter measurably the obtained results.

Removal of the extrinsic 18 kDa polypeptide, and of most of the 23 kDa polypeptide, was accomplished by treating the particles after their first or second washing with 1.5 M NaCl in 20 mM phosphate buffer (pH 6.5) in the presence of 1 mM phenylmethylsulfonyl fluoride [9]. Cl^- was then removed through repeated washings with 500 mM sorbitol buffered at pH 6.5 with 20 mM Mes-NaOH.

Dilution experiments were performed by treating with 10 mM Na-Caps in a total volume of 250 μl, neutralizing with Na-Mes, adding salt if desired, and then injecting the suspension into 4.8 ml assay medium of an appropriate composition and pH.

Results

pH-dependence of the reactivation of Cl^--depleted PS II particles by Cl^-

In their early study on the Cl^- requirement of the Hill reaction, Hind et al. [2] observed that, after Cl^- addition to a Cl^--depleted thylakoid suspension, about 30 s were required for the restoration of maximal activity. Since in thylakoid particles the water-splitting site should be quite accessible to Cl^-, one might assume that a maximal response to an addition of Cl^- can be elicited in a few seconds. Surprisingly, this is not the case. as can be seen from Fig. 1, a maximal effectiveness of low Cl^- concentrations at pH 6.1 and 6.9 required a preincubation for at least 2 min and up to 1 min was needed even with saturating amounts of Cl^-. With NO_3^- as the activating anion, the response was somewhat faster. Hence, in the reported kinetic analyses the PS II preparations were allowed to be in contact with the added anion for at least 3 min before the light was turned on to measure their activity.

The method of Cl^- depletion used in this study provided membrane samples which responded with an increase in their O_2-evolving activity even to Cl^- additions below 100 μM. This generally allowed an analysis of the Cl^- requirement without a cumbersome and somewhat arbitrary correction

for the remaining Cl^- content. Kelley and Izawa [3] have shown previously that, with respect to the Cl^- requirement of O_2 evolution, Cl^- can be treated as a substrate, or essential activator, of the water-oxidizing enzyme 'E' according to

$$E + Cl^- \rightleftarrows ECl^- \xrightarrow[h\nu]{+2\,H_2O} ECl^- + 4\,H^+ + O_2 \qquad (1)$$

Hence, a double reciprocal plot of rate vs. Cl^- concentration yields a straight line intercepting the ordinate at $1/V_{max}$ (the reciprocal of the maximal rate) and the abscissa at $-1/K_m$ (the negative reciprocal of the apparent Michaelis-Menten constant for Cl^-).

Fig. 2 shows such double reciprocal plots $1/v = f(1/[Cl^-])$ for two light intensities. It can be seen that the apparent Michaelis constant for Cl^- was essentially independent of the light intensity when the latter was varied nearly 10-fold. This independence of the Cl^- effect from the light intensity had been noted already by Gorham and Clendenning [1] and has two important consequences for our analytical approach: first, it shows that the turnover rate of the enzyme does not influence the measured binding constants for Cl^-; second, it gives general validity to our measurements which were made with nonsaturating light intensities.

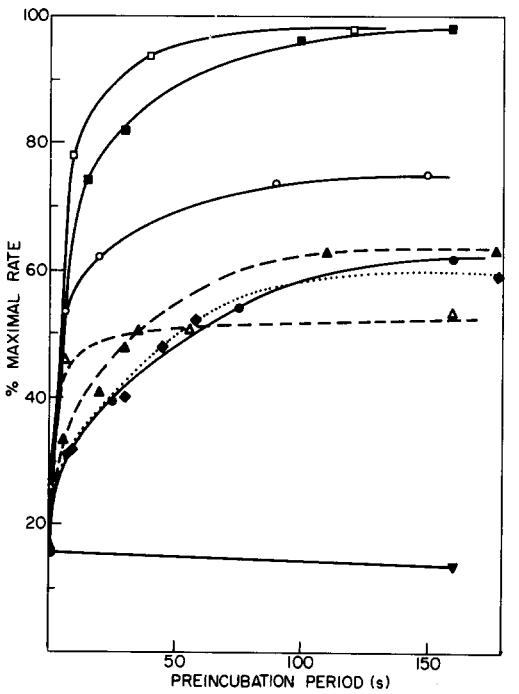

Fig. 1. Dependence of the anion-activated rate of O_2 evolution on the incubation time with the anion. PS II particles from spinach (6 μg Chl/ml) in a medium containing 500 mM sorbitol and 25 mM Na_2SO_4 buffered either at pH 6.90 with Na-Hepes (- - - - - -) or Mg-Hepes (· · · · · ·), or at pH 6.12 with Na-Mes (————). ▼, no activating anion added; ●, 0.3 mM NaCl; ▲, ◆, 0.8 mM NaCl; ■, 10 mM NaCl; ○, 0.6 mM $NaNO_3$; △, 1.0 mM $NaNO_3$; □, 10 mM $NaNO_3$. Assay in 2 kW/m² (λ < 725 nm) red light.

Fig. 3a displays double reciprocal plots for five media differing in pH within a range that assured approximately identical maximal rates at nonlimiting Cl^- concentrations [10]. The plots show that a pH dependence of the Cl^- requirement existed well into the acid region. One possible explanation of the pH effect is a competition by OH^- for the Cl^--binding site [4]. Indeed, a secondary plot of the apparent K_m values of Fig. 3a against $[OH^-]$ (Fig. 3b) yielded a straight line, in accordance with the prediction from a competitive interaction of Cl^- and OH^- at E. However, since $[OH^-] \propto 1/[H^+]$, the result can also be interpreted as a linear relationship between the apparent K_m and $1/[H^+]$, which would be compatible with an ordered sequential binding of H^+ and Cl^- [11]:

$$\begin{array}{l} E + H^+ \leftrightarrows EH^+ \\ \qquad + \\ \qquad Cl^- \\ \qquad\qquad (2) \\ \qquad \downarrow\uparrow K_{Cl^-} \\ EHCl \xrightarrow[h\nu]{+2\,H_2O} EHCl + 4\,H^+ + O_2 \end{array}$$

Regardless of the mechanism, the intercept of the straight line in Fig. 3b yields a K_m for Cl^- binding of 70 μM. From the intercept with the abscissa one can estimate the dissociation constant K_{H^+} of the critical protonatable group, or the inhibitor constant K_I for OH^-, depending on the assumed mechanism of the pH-dependence of the Cl^- action. As indicated in Fig. 3b, the obtained values are $K_{H^+} = 1$ μM, and $K_I = 10$ nM, respectively. Interestingly, with preparations from pokeweed leaves, we tended to obtain lower K_m values than with particles from spinach chloroplasts.

The analysis of the pH dependence of the Cl^- interaction with the water oxidase was applied also to media containing Mg^{2+}. We have shown earlier

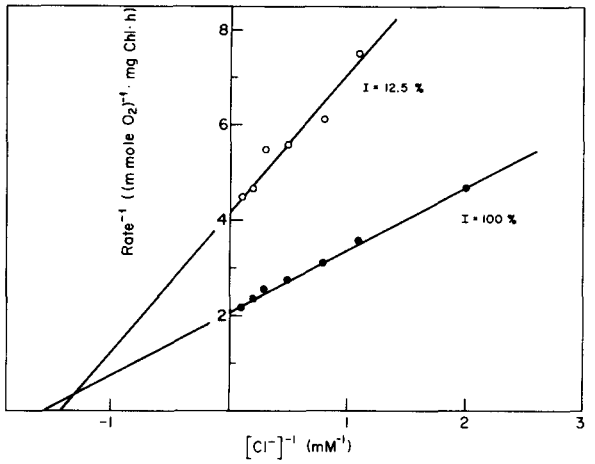

Fig. 2. Double reciprocal plots of the dependence of the O_2-evolving activity on the concentration of added Cl^- at two light intensities. PS II preparation from spinach thylakoids (8 μg Chl/ml) in 500 mM sorbitol buffered with Na-Mes at pH 6.5. 100% light intensity = 3.5 kW/m^2 (λ < 725 nm) red light.

[6,10] that the presence of divalent cations such as Mg^{2+} causes the pH profile of Cl^- release from PS II to be displaced towards the acid region by several tenths of a pH unit. At pH 7.2, we have now determined that 5 mM Mg^{2+} accelerated the Cl^- loss from PS II particles nearly 10-fold, from a half-time of several minutes to one of less than 1 min. We have explained the effect of Mg^{2+} by a pH increase over critical membrane surfaces caused by an accumulation of the divalent cations in the diffuse electrical double layer over negatively charged surface domains [6,10]. If our interpretation is correct, we should see a downward shift of the apparent pK_a of the groups governing Cl^- binding, i.e., an upward shift of K_{H^+}.

In several attempts to confirm such a relationship, we were surprised by the lack of a significant response of the measured K_{H^+} to the presence of Mg^{2+}. The result of one experiment is included in Fig. 3b. Any K_{H^+} shift seen by us was always in the expected direction, but never corresponded to a pK_a drop that was larger than 0.1. Clearly, the observed labilization of Cl^- cannot be accounted for by a change of the dissociation state of protonated groups essential for Cl^- binding. Since the K_m for Cl^- binding remained unchanged as well, one might assume that Mg^{2+} accelerates not only the release of Cl^-, but also its binding. However,

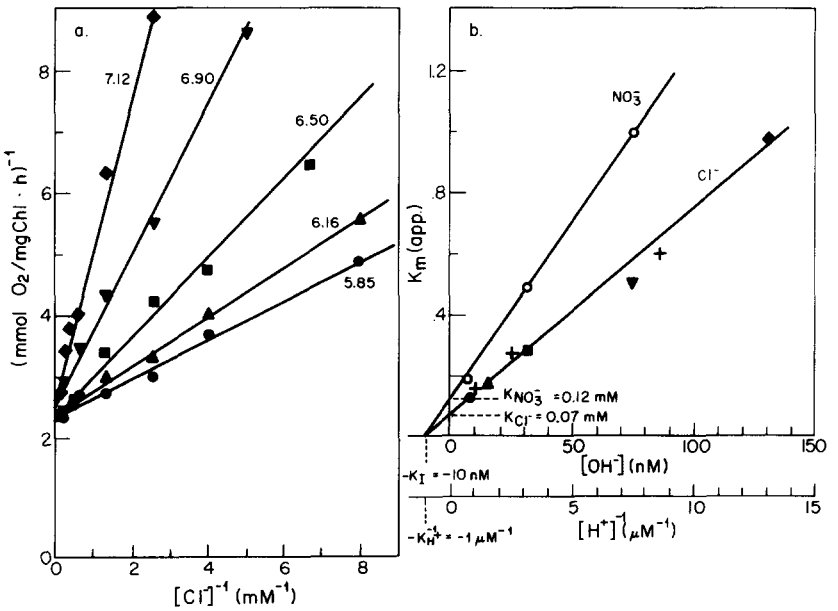

Fig. 3. (a) Double reciprocal plots of the dependence of the O_2-evolving activity on the concentration of added Cl^- in media of different pH. PS II preparations from spinach (6.5 μg Chl/ml) in media containing 500 mM sorbitol and 25 mM Na_2SO_4 and buffered with Na-Mes (pH 5.85, 6.16 and 6.50) or Na-Hepes (pH 6.9 and 7.12). Light intensity 2 kW/m^2 (λ < 725 nm) red light. (b) Secondary plots derived from double reciprocal plots (see text): filled symbols, data points determined from the experiment in panel a); +, data points determined in a separate experiment using Mg-Mes or Mg-Hepes as buffer; open symbols, data points from an experiment with NO_3^- as the activating anion.

as shown in Fig. 1, this apparently was not the case. Possible reasons for this discrepancy will be given in the discussion.

Substitution for Cl^- by other anions

Photosynthetic water oxidation in Cl^--depleted thylakoid preparations can be restored by a number of monovalent anions, but Cl^- supports the highest rate [1]. As can be seen from Fig. 4, the lower effectiveness of a substitute such as NO_3^- is due to its failure to restore optimal usage of light-generated redox energy even at low light intensities. In this respect, reactivation by saturating amounts of NO_3^- was equivalent to partial reactivation by limiting amounts of Cl^-.

Otherwise, the water oxidase responded to NO_3^- very much as it did to Cl^-, thus allowing the same analytical approach. Assuming the same mechanism of interaction, we expected to find a common K_{H^+}, but different K_m values for the anion binding. Within the limits of experimental error ($\pm 10\%$), this was found to be true. Fig. 3b contains a representative plot from an experiment with NO_3^- which intercepts the abscissa at the same $-1/K_{H^+}$ as that for Cl^-, while the K_m value for NO_3^- (intercept with the ordinate) turned out to be about twice as large.

The apparent lower affinity of the water-oxidizing site for NO_3^- and, as we found, for other Cl^- substitutes such as ClO_4^-, explained why Cl^--sufficient PS II particles responded only little to additions of these anions even in Cl^--free media. Another contributing factor to the resistance of the PS II-associated Cl^- to displacement is its very sluggish release into Cl^--free media of a pH at or slightly below neutrality. For example, even at pH 7.2 we estimated the half-time of release to be as long as about 7 min. On the other hand, anions such as NO_3^- and ClO_4^- were lost much more readily from the water-oxidizing site. This was established in the following way.

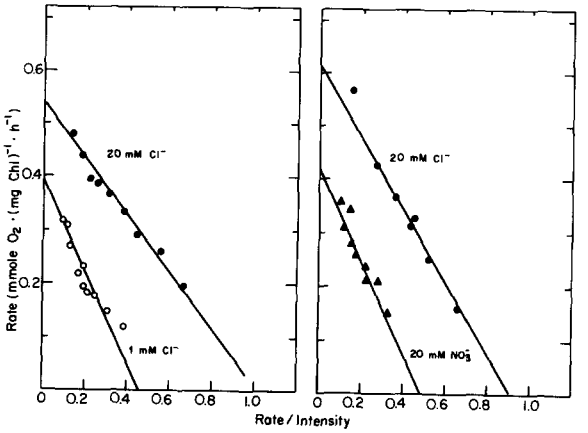

Fig. 4. Light intensity dependence of the rate of O_2 evolution in the presence of limiting amounts of Cl^-, and approximately saturating amounts of Cl^- or NO_3^-; plotted as rate = f(rate/intensity) with intensity units kW/m^2 red light. Two different preparations of PS II particles from spinach thylakoids, 8 μg Chl/ml, in 500 mM sorbitol buffered at pH 6.55 with Na-Mes.

TABLE I

RETENTION OF FUNCTIONAL ANIONS AT THE WATER-OXIDIZING SITE

Retention of a reactivating anion was measured in terms of O_2-evolving activity (μmol O_2/mg Chl per h) remaining 45 s after the reactivation mixture (250 μl plus 3 μmol = 12 mM anion) had been diluted 20-fold (see Materials and Methods); pH of reactivation and assay mixtures 6.9; amount of PS II particles equivalent to 30 μg Chl. Control: 3 μmol anion added to assay medium. Expected: 12 mM anion added to assay medium.

Anion	Anion retention		
	control	expected	found
Cl^-	130	290	260
Br^-	130	260	230
NO_3^-	130	200	140
ClO_4^-	70	120	105

Cl^--depleted PS II particles were incubated at pH 6.9 with 3 μmol of an anion in 250 μl medium (i.e., with 12 mM anion). The suspension was then diluted 20-fold and the O_2-evolving activity was compared with the rates obtained in media containing 12 mM anion ('expected rate') or 3 μmol anion/5 ml assay medium ('control rate'). As can be seen from the data in Table I, a significant retention of activity after dilution was observed only after activation with Cl^- and Br^-. Similar results were obtained with intact thylakoids.

Aspects of the Cl^- requirement after removal of the extrinsic 18 and 23 kDa polypeptides

After removal of the extrinsic 18 and 23 kDa polypeptides from the site of photosynthetic water

oxidation, an addition of Ca^{2+} is often necessary for optimal O_2-evolving activity [9,12]. It is now also well documented that the loss of the two polypeptides significantly increases the Cl^- demand for maximal activity, i.e., increases the K_m [13,14]. Our experiments confirmed these results. We also noted that the effectiveness of various monovalent anions to act as substitutes for Cl^- was dramatically changed by the polypeptide removal. Most importantly, anions such as I^-, ClO_4^- and SCN^- not only lost their activating capacity, but became inhibitory. Consequently, the relative effectiveness of various anions as substitutes for Cl^- might be expected to vary between PS II preparations of different integrity. Such variability was indeed encountered by us, and is also apparent when relevant reports from different laboratories are compared.

Divalent cations, including Ca^{2+}, become inhibitors of O_2 evolution from polypeptide-stripped PS II particles when the pH is raised above 6.5, especially at suboptimal Cl^- availability. This precluded a thorough analysis of the pH profile of the

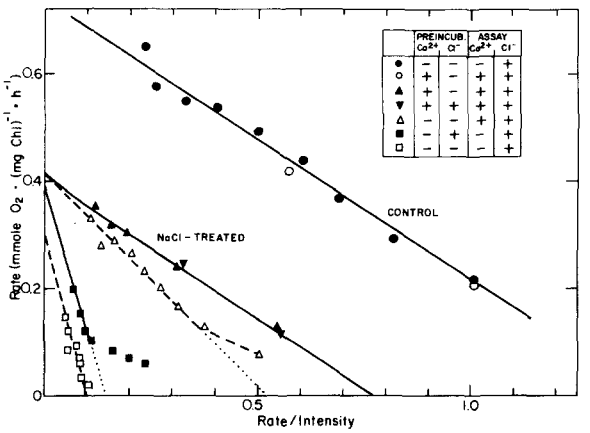

Fig. 5. Light intensity dependence of the rate of O_2 evolution of untreated, and NaCl-treated, PS II preparations, plotted as rate = f(rate/intensity) as in Fig. 4. PS II particles from spinach thylakoids, 6 μg Chl/ml of the control, 10 μg Chl/ml of the NaCl-treated preparations. All preparations were made Cl^--deficient (the control by exposure to high pH, the NaCl-treated preparations by washings with Cl^--free media) and assayed in 500 mM sorbitol buffered at pH 5.9 with Na-Mes supplemented with Ca-Mes when applicable to give a 40 mM buffer concentration and 6 mM Ca^{2+}. 40 mM NaCl was added either prior to the injection of the PS II particles, or after a 20 s preincubation of the particles in the assay medium. Further details are indicated at the top of the figure.

CCl^- requirement with such material. The available data, however, allowed the conclusion that the polypeptide removal, regardless of the presence of Ca^{2+}, did not change by more than 0.3 the pK_a which controls Cl^- binding.

In contrast to the experience of other laboratories, we never attained a 100% restoration of the O_2-evolving capacity of our polypeptide depleted PS II particles upon Ca^{2+} addition, and the action of Ca^{2+} was almost exclusively on the quantum efficiency. This is documented in Fig. 5. The irreversible activity loss did not correlate with the extent of an often observed loss during the NaCl treatment of a third polypeptide having an approximate molecular weight of 10 kDa (c.f. Ref. 15). Instead, we see as the likely reason a detrimental effect of the removal of Cl^- from our preparations prior to their storage and use. Their lability is evident from the observation that an irreversible loss of the quantum efficiency of O_2 evolution ensued when Cl^- was withheld for a short a time period as 20 s after an aliquot of our preparation had been injected into Cl^--free assay medium. Relevant data are presented in Fig. 5, which also shows that such inactivation could be prevented by a presence of Ca^{2+}.

Discussion

In this study, the interaction of functional Cl^- with the site of photosynthetic water oxidation was analyzed quantitatively using PS II-enriched membrane particles prepared by Triton X-100 action on chloroplast thylakoids. The chosen approach made use of the evidence from work of others [3] that the functional Cl^- can be treated kinetically like a substrate, or an essential activator, of the water-oxidizing enzyme. It is shown here that the experimental data can be fitted to a relationship which considers Cl^- binding to be contingent upon the protonation of a group with a $pK_a \approx 6$ (Eqn. 2). Such a situation would be strikingly similar to the conditions governing Cl^- binding of carbonic anhydrase, where the participating H^+-acceptor has a $pK_a = 5.64$ [16]. An alternative explanation of the data invoking a competitive displacement of Cl^- by OH^- is not favored because such a mechanism would imply an unrealistic 10^4-fold advantage of OH^- over Cl^- at the water-oxidizing

site, and would set its interaction apart from all other enzyme activations by Cl^- known to me. A similar conclusion was reached independently by Izawa [17] and Coleman and Govindjee [18].

However, the application of the model of an enzyme-activator interaction ignores the finding that a complete activation of a water-splitting enzyme may require an association with several [5] and perhaps as many as 40 chloride ions [17]. This shortcoming can largely be overcome by acknowledging that the Henri-Michaelis-Menten equation which underlies the double reciprocal plots of enzyme-substrate interactions is formally identical to the Langmuir adsorption isotherm:

$$N = \frac{N_{max}}{1 + K_d/c} \quad (3)$$

Here N designates the number of adsorbed molecules per unit adsorbent, K_d the desorption constant, and c the concentration of free adsorbable molecules. This equation would be applicable to the anion interaction with the O_2-evolving site if the following conditions are met. (i) The 'adsorbing' sites act independently of each other. Such a postulate may be somewhat unrealistic, but would have to be accepted as a rough approximation. (ii) The number of binding sites is finite and a function of protonation. Obviously, this condition is accommodated readily. In fact, it can be shown that a sequential adsorption of H^+ and Cl^- leads to an equation analogous to that which is derived for the enzyme model and which forms the basis of our analysis. (iii) The rate of O_2 evolution is proportional to the number of anions associated with the water oxidase, that is, $V_{O_2} = K_A N$.

The 'adsorption' or 'multiple binding' model is flexible in that the effectiveness of an anion must not be entirely attributed to its interaction with the water-oxidizing enzyme proper. Instead, its ability to support the process of O_2 evolution and suppress futile or destructive light-induced events in PS II [2] would be a function of the amount of that anion which can be accommodated at the O_2-evolving center and its effectiveness as activator (magnitude of K_A). Hence, Cl^- substitutes at saturating concentrations would be equivalent to an incomplete activation by limiting amounts of Cl^-, as is suggested by the data in Fig. 4, and by Sinclair's elegant measurements of the kinetics of O_2 evolution during illumination with light flashes [19].

Another problem with our data was the somewhat unsatisfactory quantitative correlation between the estimated rates of Cl^- binding and release, and the K_m values determined from the double reciprocal plots. This was particularly apparent in the experiments with Mg^{2+}-containing media. They suggested that Mg^{2+} did not affect the K_m of the Cl^-–PS II association even though, as discussed earlier, this cation greatly accelerated Cl^- loss while leaving the rate of Cl^- rebinding rather unaffected (Fig. 1). The apparent incompatibility of our data may indicate that their analysis on the basis of simple equilibrium conditions is not appropriate. There is also evidence [4,5,18,19] that the PS II-associated Cl^- population is heterogeneous. In Mg^{2+}-containing media an additional complication arose from the considerable degree of irreversibility of the activity loss that accompanied Cl^- dissociation from the water-splitting site [10].

Our investigations on PS II preparations lacking the extrinsic 18 and 23 kDa polypeptides suggested that neither they nor the added Ca^{2+} significantly influence the pH dependence of the Cl^- association with the water-splitting site. We must assume, therefore, that the pH profile of the interaction with Cl^- is determined by groups close to the active site of the enzyme. The stabilizing effect of Ca^{2+} under Cl^--deficient conditions, furthermore, points to a close spatial proximity of the action of these two ions. This brings to mind the Ca^{2+} dependence of the activation of pancreatic α-amylase by Cl^- [20]. Indeed, the relative effectiveness of different monovalent anions as activators of α-amylase [21] is almost identical to the relative magnitude of their capacity to activate O_2 evolution of Cl^--deficient thylakoids [2–4], or PS II particles (unpublished data). The tendency of some of the less effective anions to become inhibitory when the 18 and 23 kDa polypeptides are removed from the water oxidizing site is very likely due to an unfavorable balance under such conditions of their activating potential, and of a detrimental influence brought about by their chaotropic nature.

Sandusky and Yocum [22] have proposed that

Cl^- serves as a bridging ligand in the Mn cluster at the active site of the water oxidase. However, the many similarities between the anion requirement of photosynthetic water splitting and of other enzymatic mechanisms may point to a less unique role of monovalent anions in PS II.

In an earlier paper [23] we had proposed that the activating anions may stabilize positively charged, protonated groups close to the catalytic site of the water oxidase. Those groups were suggested to be involved in the conduction of protons into the lumen space of the thylakoids. Coleman and Govindjee [19] have considered an essential role of the protonatable groups in the protolytic steps of the process of water oxidation. In their view, Cl^- is required as activator for the catalytic step involving H^+ transfer from water. It is noteworthy that a Cl^--sensitive protonation-deprotonation event has very recently been identified also in another enzymatic reaction [24].

The nature of the protonated groups linked to the Cl^- association with PS II is unknown at this time. While the observed $pK_a \approx 6$ would be compatible with that of histidine residues, their low abundance in the polypeptides of the water-oxidizing site [25] may rule them out as likely candidates. Lysine residues, on the other hand, are quite abundant, but would be endowed with such a low pK_a only under very unusual circumstances [26]. An almost identical situation appears to puzzle researchers who try to identify the anion-binding groups of the anion translocator in the red cell membrane [27,28].

In independent studies, Itoh et al. [29] and we [30] have found that in Cl^--deficient thylakoids the water-oxidizing site is unable to store more than two oxidizing charges. Both a bridging-ligand and a protonation-linked function of Cl^- could cause such a lesion. We believe in the latter because we agree with Gorham and Clendenning [1], who proposed more than 30 years ago that a common mechanism underlies the activating role of Cl^- in different biological systems. What has been learned in the meantime about Cl^- effects strengthens their perceptive arguments.

Acknowledgements

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