Characteristics of Iodide Activation and Inhibition of Oxygen Evolution by Photosystem II†

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ABSTRACT: Oxygen evolution by photosystem II (PSII) is activated by chloride and other monovalent anions. In this study, the effects of iodide on oxygen evolution activity were investigated using PSII-enriched membrane fragments from spinach. In the absence of Cl^-, the dependence of oxygen evolution activity on I^- concentration showed activation followed by inhibition in both intact PSII and NaCl-washed PSII, which lacked the PsbP and PsbQ subunits. Using a substrate inhibition model, the range of values of the Michaelis constant K_M in intact PSII (0.5−1.5 mM) was smaller than that in NaCl-washed PSII (1.5−5 mM), whereas values of the inhibition constant K_I in intact PSII (9−17 mM) were larger than those in NaCl-washed PSII (1−4 mM). Studies of I^- inhibition of Cl^--activated oxygen evolution in intact PSII revealed that I^- was primarily an uncompetitive inhibitor, with uncompetitive constant $K_i' = 37$ mM and Cl^--competitive constant $K_i > 200$ mM. This result indicated that the activating Cl^- must be bound for inhibition to take place, which is consistent with the substrate inhibition model for I^- activation. The S_2 state multiline and $g = 4.1$ EPR signals in NaCl-washed PSII were examined in the presence of 3 and 25 mM NaI, corresponding to I^--activated and I^--inhibited conditions, respectively. The two S_2 state signals were observed at both I^- concentrations, indicating that I^- substitutes for Cl^- in formation of the signals and that advancement to the S_2 state was not prevented by high I^- concentrations. A model is presented that incorporates the results of this study, including the action of both chloride and iodide.

Photosystem II (PSII)[1] catalyzes the reduction of plastoquinone via electron transfer from water as a result of light absorption in higher plants and cyanobacteria. The concurrent production of O_2 is an important result since it provides nearly all oxygen used by respiring creatures. The oxygen evolving complex (OEC) of PSII includes a manganese cluster that cycles through a series of oxidation states, designated S_0 through S_4, with oxygen evolution taking place from the highest oxidation state of S_4 (for reviews see refs *1*−*3*).

Chloride has long been recognized as a cofactor required for oxygen evolution by photosystem II (PSII) (*1*, *2*, *4*−*6*). Activating Cl^- can be replaced by several other monovalent anions, including Br^-, NO_3^-, I^-, and NO_2^-, although with varying efficiency (*7*−*12*). Chloride has been found to bind with high affinity to one site on PSII (*7*, *13*, *14*).

The availability of Cl^- and Ca^{2+} at the oxygen evolving complex (OEC) is evidently regulated by two extrinsic subunits, PsbP and PsbQ, with apparent molecular masses of 23 and 17 kDa, respectively (*15*). Their presence has a significant impact on the ease with which activation by Cl^- can be investigated. Using dialysis (*7*) or short-term high-pH treatment (*16*), it is possible to deplete PSII of Cl^- without disturbing the protein composition, but there remains significant residual oxygen evolution activity (25−35%) in the absence of Cl^-. On the other hand, removal of PsbP and PsbQ by treatment with high NaCl concentration appears to cause spontaneous release of Cl^- (*17*). In this case the residual activity is near zero in the absence of added Cl^-, and oxygen evolution activity requires addition of Ca^{2+} (*18*, *19*).

Iodide has been a focus of study because of its properties as an electron donor in PSII. I^- can reduce Tyr Z^+ of the D1 polypeptide when manganese is removed (*20*−*22*) or reduce the Mn cluster in PSII membranes lacking PsbP and PsbQ (*22*). The D1 polypeptide is iodinated during turnover, but the D2 polypeptide is also labeled in the dark (*20*). When PsbP and PsbQ are removed, I^- can activate O_2 evolution in the absence of Cl^- but is inhibitory at all concentrations in the presence of Cl^- (*23*). Activating I^- can also be reconstituted into the Cl^- site, with subsequent rebinding of the PsbP and PsbQ subunits (*24*). The lifetimes of the S_2 and S_3 states were investigated using UV absorption difference spectroscopy in the presence of several anions that can replace activating Cl^-, including Br^-, NO_3^-, I^-, and NO_2^- (*10*). This study revealed the presence of a second anion site from which the higher S states could be reduced by NO_2^- and I^- (*10*).

In this study, enzyme kinetics were used to characterize the activation and inhibition properties of I^- in intact PSII and in PSII lacking PsbP and PsbQ. We found that the behavior of iodide in the absence of Cl^- fits a model of sequential binding of activating and inhibiting ions. In the

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[1] Abbreviations: EPR, electron paramagnetic resonance; Mes, 2-(*N*-morpholino)ethanesulfonic acid; OEC, oxygen evolving complex; PSII, photosystem II.

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presence of Cl^-, I^- was primarily an uncompetitive inhibitor of Cl^- activation. Both lines of evidence indicate that an anion, either Cl^- or I^-, must be bound for inhibition due to I^- to take place in PSII. EPR spectroscopy of the S_2 state showed in addition that the inhibitory I^- did not interfere with S_2 state formation.

MATERIALS AND METHODS

Preparation of PSII-Enriched Membrane Fragments. PSII-enriched thylakoid membrane fragments were prepared from fresh market spinach by extraction with Triton X-100 as described by Yocum and colleagues (*25*) and modified by others (*26*, *27*). PSII preparations were suspended in buffer containing 20 mM Mes−NaOH, pH 6.3, 0.4 M sucrose, and 15 mM NaCl and stored in liquid N_2. In some cases (Figure 3), 5 mM $MgCl_2$ was present in the last buffer but was diluted to less than 150 μM during the experiment. Chemicals were obtained from Fisher Scientific (Fair Lawn, NJ) except where otherwise stated.

O_2 evolution assays were measured at 25 °C using a Clark-type O_2 electrode (Yellow Springs Instruments, model 5331) in the presence of 1 mM phenyl-*p*-benzoquinone (Aldrich, Milwaukee, WI) as described previously (*28*). O_2 evolution rates of control samples were usually 500−600 μmol of O_2 (mg of Chl)$^{-1}$ h^{-1}. Rates given represent the averages of three or more separate measurements. Error bars are based on 10% error in activity measurements; although the standard deviations of the repeat measurements were generally much lower (around 5%), we felt that the standard deviation did not properly take into account likely systematic error. Data were analyzed in terms of kinetic models using the program Sigma Plot, version 8.0 (SPSS, Inc.); errors quoted for the kinetic parameters were based on the statistical fits.

Chloride Depletion Methods. Chloride depletion of intact PSII-enriched membrane fragments was carried out by dialysis using a procedure similar to that described by Lindberg and colleagues (*7*, *13*), using a buffer containing 20 mM Mes−NaOH, pH 6.3, and 0.40 M sucrose. We estimate that the residual chloride concentration was 30−50 μM based on measurements using a Cl^--sensitive electrode. PSII-enriched membrane fragments were thawed from storage and suspended in the buffer to a concentration of 0.5−0.8 mg of Chl mL^{-1}. The samples were then centrifuged at 20000*g* for 8 min to pellet. The pellets were resuspended in the same buffer and centrifuged again to remove Cl^- from the buffer. The samples were then resuspended to a concentration of ∼2.3 mg of Chl mL^{-1} and placed in dialysis tubing (Spectra/Por 2.1, 15 kDa cutoff; Sigma, St. Louis, MO). The PSII samples were dialyzed against the Cl^--depletion buffer at 4 °C for 21−22 h in the dark.

NaCl-washed PSII was prepared essentially as described previously (*29*). Intact PSII was incubated in buffer containing 1.5 M NaCl in 20 mM Mes−NaOH, pH 6.3, and 0.40 M sucrose for 1 h. The PsbP and PsbQ extrinsic subunits and Cl^- were removed by two successive washes by centrifugation in the same buffer without NaCl. A final wash was carried out using 20 mM Mes−$Ca(OH)_2$, pH 6.3, and 0.40 M sucrose buffer (∼5 mM Ca^{2+}).

EPR Spectroscopy. For EPR sample preparation, NaCl-washed PSII membrane fragments were split into four parts and diluted to 0.3−0.4 mg of Chl mL^{-1} in the Cl^--depletion buffer. To each part was added either NaCl or NaI from a 1.0 M stock solution to a final concentration of 3 or 25 mM, as specified, or no addition was made for samples with no anion. After incubation on ice in the dark for ∼30 min, the membrane fragments were centrifuged to pellet and resuspended to 5−6 mg of Chl mL^{-1} in medium with the same anion. PSII samples were transferred to 4 mm outer diameter precision-bored, clear fused quartz EPR tubes (Wilmad Glass, Buena, NJ) and dark adapted on ice for 35−45 min before being frozen in liquid nitrogen.

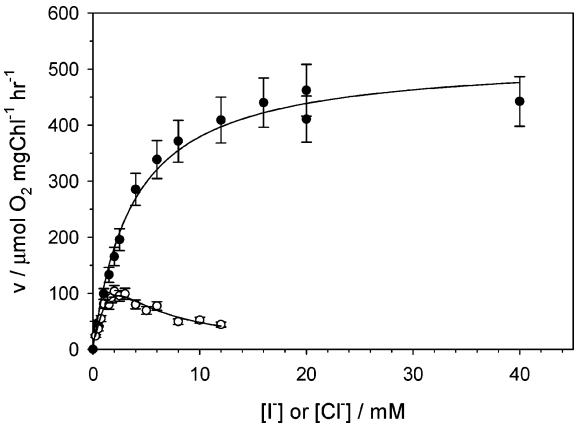


FIGURE 1: Activation of O_2 evolution by Cl^- (closed circles) or I^- (open circles) in NaCl-washed PSII. Assays were carried out in 20 mM Mes−$Ca(OH)_2$, pH 6.3, 0.4 M sucrose, and the indicated amounts of NaCl or NaI. The solid lines show fits to the data using K_M = 3.7 mM and V_{max} = 521 μmol of O_2 (mg of Chl)$^{-1}$ h^{-1} for activation by Cl^- and K_M = 5.3 mM and K_I = 1.1 mM, setting V_{max} = 521 μmol of O_2 (mg of Chl)$^{-1}$ h^{-1}, for activation by I^-.

EPR spectra were taken of each sample in the dark-adapted state and the illuminated state for calculation of the difference spectrum. The S_2 state was achieved by illuminating in a dry ice/methanol bath (195 K) for 8 min using a pair of Dolan-Jenner model 190 Fiber-lite illuminators. The dual light beams were each passed through ∼6 cm of 5 mM $CuCl_2$ solution in cylindrical glass containers before reaching the sample.

X-band EPR spectroscopy was carried out using a Bruker Instruments (Billerica, MA) EMX 6/1 EPR spectrometer equipped with a standard ER4102ST cavity (9.48 GHz). Temperature was controlled with an Oxford Instruments ESR 900 liquid He cryostat. Signals were observed at 10.0 K using a microwave power of 20 mW, modulation frequency of 100 kHz, and modulation amplitude of 18 G.

RESULTS

Activation/Inhibition by Iodide in the Absence of Chloride. The activation of oxygen evolution by I^- was studied in NaCl-washed photosystem II preparations from spinach in the absence of added Cl^-. Removal of PsbP and PsbQ resulted in a preparation that showed essentially zero O_2 evolution activity in the absence of added Cl^- or other anion. With the addition of NaI, the activity increased until it reached a maximum at about 3 mM I^-, after which further addition of NaI reduced the activity (Figure 1). The activation by NaI was compared with the activation by NaCl, which at high concentrations produced a maximum activity that was about 5 times that produced by I^-. This plot closely resembles data published previously by Papageorgiou and

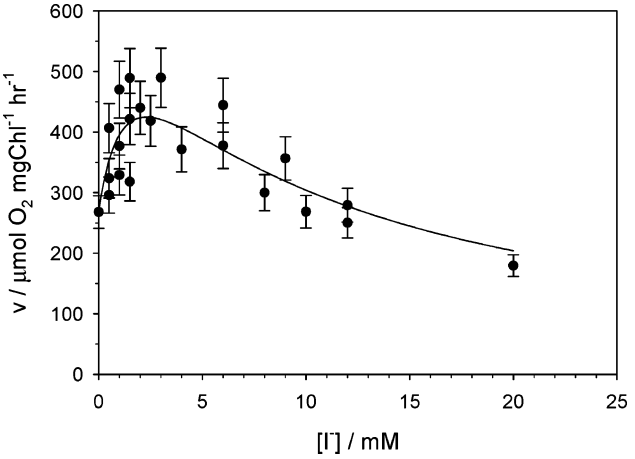


FIGURE 2: Activation of O_2 evolution by I^- in Cl^--depleted intact PSII. Assays were carried out in 20 mM Mes−NaOH, pH 6.3, 0.4 M sucrose, and the indicated amounts of NaI. The solid line shows a fit to the data using $K_M = 1.5$ mM, $K_I = 8.8$ mM, $V_0 = 268$ μmol of O_2 (mg of Chl)$^{-1}$ h^{-1}, and $V_{max} = 407$ μmol of O_2 (mg of Chl)$^{-1}$ h^{-1}.

Lagoyanni (*23*), who used tetrabutylammonium as the counterion rather than sodium, and by Hasagawa and coworkers (*30*), who found a higher maximum rate of O_2 evolution relative to that for Cl^- at low light intensities.

To determine whether the extrinsic subunits PsbP and PsbQ restrict access of the relatively large I^- ion to the site of activation, the activation of O_2 evolution by I^- was studied in intact PSII that had been depleted of Cl^- by dialysis (Figure 2). Both activation and inhibition phases were also observed for intact PSII, although the maximum activity occurred at about 2 mM NaI, a slightly lower concentration than for NaCl-washed PSII. The maximum activity was much higher in intact PSII than in NaCl-washed PSII; however, that can be mostly explained by the large background activity in the absence of added anion. These experiments were carried out in the absence of added calcium. It was found that calcium improved the activity by only about 10% in the presence of 20 mM Cl^-, indicating that the Cl^- depletion procedure produced a PSII preparation that contained mostly intact PSII.

Although chloride (and iodide in some cases) is an activator in the reaction carried out by photosystem II to produce O_2, its activation kinetics can be treated using models developed for substrates. This is because the reaction is promoted as a consequence of chloride binding, by analogy with substrate binding, while the true substrate H_2O is present in vast abundance. Activity curves that show both activation and inhibition phases can be interpreted in terms of a substrate inhibition model (*31*, *32*) that involves sequential binding of two substrate molecules, with the first substrate molecule activating and the second inhibiting. The reaction velocity in this model is given by

$$v = \frac{V_{max}[S]}{K_M + [S] + [S]^2/K_I} \qquad (1)$$

where [S] is the concentration of the activator (I^- in this case), K_M is the Michaelis constant for activation, K_I is the dissociation constant for inhibition, and V_{max} is the maximum reaction velocity promoted by activator I^-. This equation adequately accounts for the activation/inhibition properties observed for iodide in NaCl-washed PSII (Figure 1).

Equation 1 was not able to fully account for the activation behavior in intact PSII of Figure 2, which showed a high level of residual activity after Cl^- depletion. This residual activity, presumably due to Cl^- that remained bound, was subject to inhibition by I^- as seen by the decrease in activity below the initial activity level. A second term was introduced into the equation to fully account for the observations. The term was derived by considering only binding of inhibitory I^- to PSII that was already activated with initial velocity V_0.

$$v = \frac{V_{max}[S]}{K_M + [S] + [S]^2/K_I} + \frac{V_0}{1 + [S]/K_I} \qquad (2)$$

For intact Cl^--depleted PSII (Figure 2), V_0 was set to 268 μmol of O_2 (mg of Chl)$^{-1}$ h^{-1}, the average level of activity observed before addition of I^-.

One problem with fitting the data of Figures 1 and 2 was that the three parameters (K_M, K_I, and V_{max}) were generally highly interdependent because of the overlap of activation and inhibition regions of the curve. This prevented unambiguous determination of the parameters. However, a range could be determined for the parameters assuming that the value of V_{max} (or $V_0 + V_{max}$) for I^- could not be greater than that for Cl^- nor less than the observed maximum velocity for I^-. Using this approach for the NaCl-washed PSII, it was found that $K_M = 1.5-5.3$ mM and $K_I = 1.1-3.9$ mM by assuming that V_{max} was between 521 and 200 μmol of O_2 (mg of Chl)$^{-1}$ h^{-1}. [Assuming $V_{max} = 100$ μmol of O_2 (mg of Chl)$^{-1}$ h^{-1}, the observed maximum, gave an unacceptably poor fit.] The best fit according to r^2 values (where r is the correlation coefficient) was found using the highest allowed V_{max} of 521 μmol of O_2 (mg of Chl)$^{-1}$ h^{-1}. For intact Cl^--depleted PSII, it was found that $K_M = 0.6-1.5$ mM and $K_I = 8.8-17$ mM by assuming that $V_0 + V_{max}$ was between 500 and 675 μmol of O_2 (mg of Chl)$^{-1}$ h^{-1}. Again, the best fit according to r^2 values was found using the highest allowed V_{max} of 675 μmol of O_2 (mg of Chl)$^{-1}$ h^{-1}.

Uncompetitive Inhibition by Iodide in the Presence of Chloride. Experiments were carried out in which I^- was treated as an inhibitor of Cl^--activated O_2 evolution in intact PSII. By varying the concentrations of NaCl and NaI, dissociation constants for I^- as a competitive inhibitor (K_i) and as an uncompetitive inhibitor (K_i') were determined as has been described previously for ammonia (*33*, *34*), azide (*35*), and acetate (*36*). The data were analyzed in terms of Dixon and Cornish-Bowden plots (*37*, *38*), which are convenient for representation of competitive and uncompetitive inhibition constants, respectively (Figure 3). The data revealed that iodide showed essentially no Cl^--competitive inhibition (Figure 3A), so it was estimated that $K_i > 200$ mM. The data showed uncompetitive inhibition with a constant of $K_i' = 37$ mM (Figure 3B). This result implies that the Cl^- ion must bind before the inhibitory I^- ion can bind. The small amount of curvature of the points observed in Figure 3B, although close to the error level, is probably real since it is consistent with some activation by I^-.

EPR Spectroscopy. It has been previously observed that the S_2 state multiline EPR signal does not form in the absence

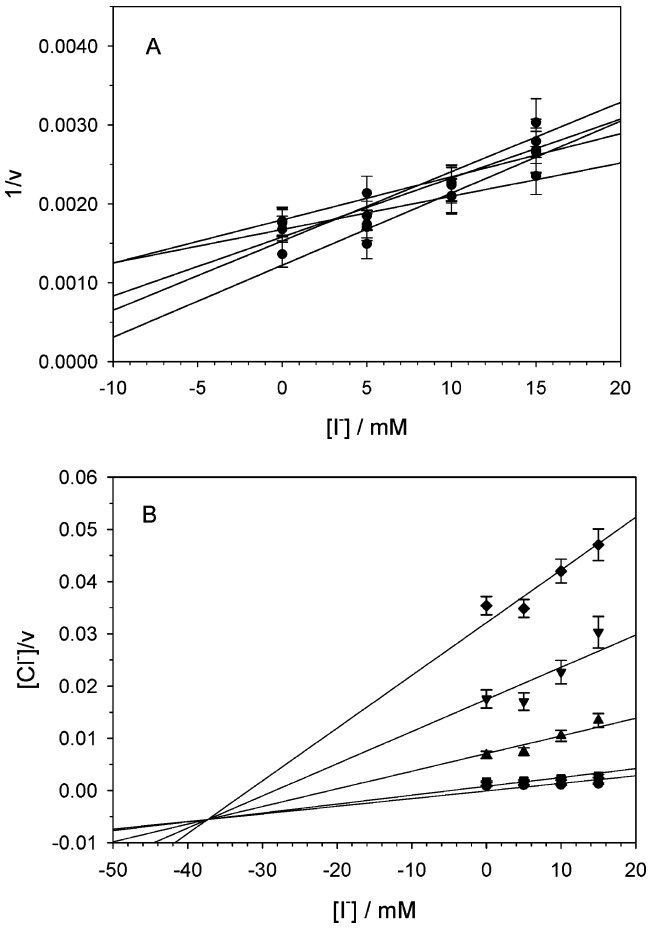


FIGURE 3: Dixon plot (A) and Cornish-Bowden plot (B) of I^- inhibition of Cl^--activated O_2 evolution in intact PSII. Assays were carried out in 20 mM Mes−NaOH, pH 6.3, 0.4 M sucrose, and the indicated amounts of NaCl and NaI. Chloride concentrations were 0.5 mM (circles), 1.0 mM (squares), 5.0 mM (triangles), 10 mM (inverted triangles), and 20 mM (diamonds). Solid lines for panel A show separate linear fits to the data for each Cl^- concentration. Solid lines for panel B show a combined fit of all the data, giving $K_i' = 37$ mM.

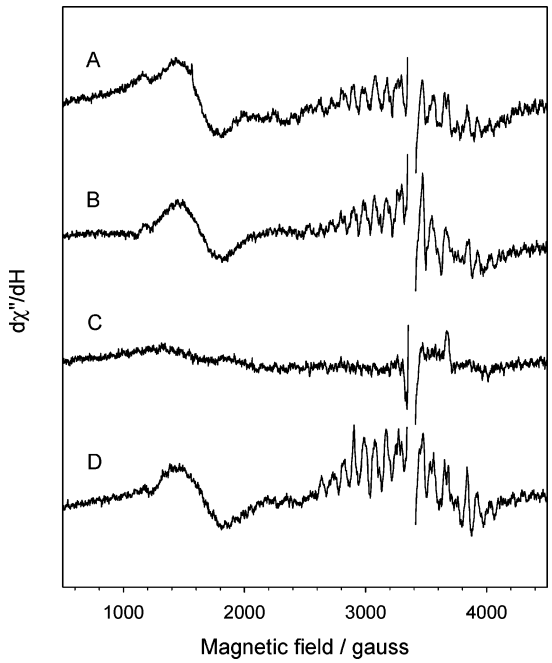


FIGURE 4: EPR spectra of the S_2 state multiline and $g = 4.1$ signals in NaCl-washed PSII treated with (A) 3 mM NaI, (B) 25 mM NaI, (C) no anion, and (D) 25 mM NaCl. Difference spectra of the illuminated minus dark-adapted sample are shown. Sample preparation and EPR spectroscopy were carried out as described in Materials and Methods.

of added Cl^- (*7*, *39*, *40*). To investigate the effects of I^- on the S_2 state signals, NaCl-washed PSII samples depleted of Cl^- were prepared with 3 and 25 mM NaI. Samples were also prepared with 25 mM NaCl and no added anion as positive and negative controls, respectively.

In the absence of added anion, neither the multiline signal nor the $g = 4.1$ signal was observed in illuminated samples, whereas in the presence of Cl^- both signals were observed (Figure 4). Iodide was found to promote formation of the multiline and $g = 4.1$ EPR signals at both high and low concentrations. Although not quite as intense, the signals from the I^--containing samples were comparable to those of the Cl^--containing sample. Similar results were found using intact PSII that had been Cl^- depleted (not shown), except that the $g = 4.1$ signal was present in the absence of Cl^-. This indicates that inhibitory concentrations of I^- did not prevent advancement to the S_2 state as judged by formation of the S_2 state signals.

DISCUSSION

In this study, the characteristics of I^- as an activator at low concentrations and an inhibitor at high concentrations were investigated. Iodide shows activation of O_2 evolution in both Cl^--depleted intact PSII and in PSII lacking PsbP and PsbQ. This indicates that the two extrinsic subunits do not prevent access to the site, even though I^- has an ionic diameter of 4.40 Å, much larger than that of Cl^- at 3.63 Å (*41*). The K_M for activation by I^- in intact PSII was found to be 0.6−1.5 mM, which was lower than in PSII lacking PsbP and PsbQ, where it was found to be 1.5−5.3 mM. This result can be seen as consistent with the idea that the extrinsic subunits function to retain the activating I^- anion, as for Cl^- (*15*, *17*). The values found for the inhibition constant K_I in intact PSII (8.8−17 mM) and PSII lacking PsbP and PsbQ (1.1−3.9 mM) were significantly different, which strongly suggests that the site of inhibition is located near the OEC. The higher K_I found for intact PSII compared to PSII lacking the extrinsic subunits means that the effect of the subunits is to protect the OEC from inhibition at this site.

In the presence of Cl^-, I^- was found to act as an uncompetitive inhibitor ($K_i' = 37$ mM), a characterization of I^- inhibition not previously reported. This result implies that Cl^- must be bound before inhibition by I^- can take place. Although we employed a simple enzyme kinetics model for activation involving only one enzyme−activator complex, there are in fact many intermediate states corresponding to the oxidation states of the OEC. The finding of uncompetitive inhibition probably indicates the activating Cl^- binds during an earlier S state than does the inhibiting I^-. Previous studies from the literature indicate that Cl^- binds before or during the S_2 state, while inhibiting I^- probably binds or acts at the S_2 state or later. The earlier binding of Cl^- is indicated by its requirement for formation of the S_2 state multiline EPR signal (*7*, *9*, *39*, *40*), an observation also made in the study presented here. The inhibitory action of I^- (and NO_2^-) was found in a study using UV absorption difference spectroscopy to be due to reduction of the S_2 and S_3 oxidation states of

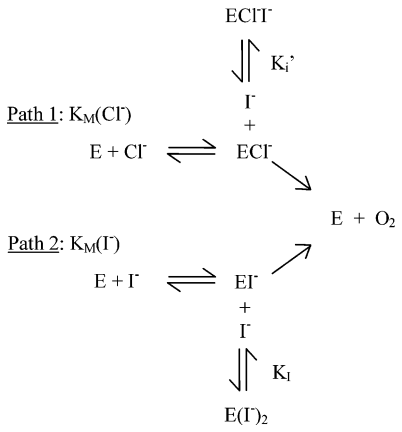
the OEC, an effect that was not related to binding to the activating Cl^- site (*10*). Our observation that inhibitory amounts of I^- did not prevent formation of the S_2 state EPR signals strongly suggests the inhibition must occur at a higher state than S_2 (or perhaps in the S_2 state at temperatures above 200 K.)

The EPR spectra examined here showed that I^- at both high and low concentrations supported the formation of the S_2 state EPR signals, probably by substituting for Cl^- at the site of activation. Olesen and Andréasson also found that 10 mM I^- promoted formation of the S_2 state EPR signals in intact PSII after 20 s or 2.5 h incubation with I^- (*8*). Our results and those of the latter researchers are contrary to an earlier study in which I^--treated PSII was unable to form the S_2 state multiline signal using similar illumination conditions (*9*). The most notable difference between that study and the one presented here is that the anion treatment was carried out at pH 7.5, which may have had an effect on the reactivity of the inhibitory site. It is also noteworthy that in our study neither the multiline signal nor the $g = 4.1$ signal was able to form in the absence of anion in NaCl-washed PSII. It has been found in other studies using intact PSII that the $g = 4.1$ signal is able to form in Cl^--depleted conditions (*7*, *39*, *40*), although the multiline signal does not. The absence of the $g = 4.1$ signal in the study presented here must therefore be related to the removal of the PsbP and PsbQ subunits.

The results of this study imply that a Cl^-- or I^--bound active species of PSII must be present before inhibition by I^- can take place. One possibility to consider is that only one anion site is involved in the observed behavior. It could be speculated that the inhibition by I^- takes place from the same site as Cl^- or I^- activation after the Mn cluster reaches a higher oxidation state; in this case, the activity observed would be from PSII centers that make it past the point where inhibition can take place. However, this suggestion is difficult to reconcile with the results of Figure 1, since the data would then require that higher concentrations of I^- (a macroscopic property) decrease the likelihood that individual centers could make it past the point of inhibition (a microscopic event). It might instead be postulated that a change in the site could occur through an ionic strength effect on the protein, but such an effect would be expected for other monovalent anions as well. We conclude that the only reasonable interpretation of the data is that I^- has two sites on PSII, one from which I^- or Cl^- activates and another from which I^- either inhibits directly or indirectly through its effect on the first site.

As an inhibitor in the absence of Cl^-, the behavior of iodide can be described by the substrate inhibition model, in which inhibition is due to the binding of a second iodide anion. As an inhibitor in the presence of Cl^-, I^- acts as an uncompetitive inhibitor; i.e., inhibition requires binding of an activating Cl^- ion. These observations can be summarized by the reaction path in Scheme 1. In this scheme, two activators, Cl^- and I^-, can bind to enzyme E and promote the formation of product O_2. Each activator promotes steady-state activity with its own K_M, $K_M(Cl^-)$ or $K_M(I^-)$. Activator I^- can also act as an inhibitor when it binds to a second site on each enzyme−activator complex, ECl^- or EI^-, forming ECl^-I^- or $E(I^-)_2$. The inhibitor dissociation constant when binding to ECl^- corresponds to the uncompetitive inhibition

Scheme 1: Activation of Enzyme E by Two Activators, Cl^- and I^-, Where I^- Can Also Act as an Inhibitor[a]



[a] Binding of the true substrate, H_2O, is not shown.

constant K_i', while the inhibitor dissociation constant when binding to EI^- corresponds to the inhibition constant K_I, which was derived from the substrate inhibition model.

The above model for the action of iodide on PSII is a new model that takes into account the data presented here and is consistent with the results of other researchers. Also of significance, we have determined or estimated kinetic parameters for each of the activation or inhibition steps involving iodide for the first time. Rachid and Homann examined iodide activation of PSII using PSII prepared by reconstitution of the PsbP and PsbQ extrinsic subunits in the presence of I^-, thereby reducing access of inhibitory I^- (*24*). No kinetic constants were determined, although the authors estimated that V_{max} and the quantum efficiency in I^--reconstituted PSII were about two-thirds those in Cl^--reconstituted PSII. Papageorgiou and Lagoyanni observed activation at low concentrations of I^- followed by inhibition at higher concentrations in PSII lacking PsbP and PsbQ (*23*), similar to the data presented in Figure 1. Kinetic constants were again not reported, but the authors noted that the inhibition was reversible and occurred from a site preceding electron donation to Tyr Z, separate from the Cl^- activation site. They characterized the inhibition by iodide as noncompetitive with respect to Cl^-. Wincencjusz and co-workers, in addition to showing activation by I^-, characterized the inhibition of Na_2SO_4-treated PSII by I^- in terms of S state lifetimes using UV absorption difference spectroscopy (*10*). They found rapid deactivation of the S_2 and S_3 states by I^- (and NO_2^-) and interpreted this as resulting from damage by oxidation products of I^- leading to iodination of Tyr Z or other sites on D1, as described by Ikeuchi and co-workers (*22*).

The possible iodination of Tyr Z has significance here regarding the mechanism of inhibition. We have employed kinetic models that assume reversibility of binding steps, but irreversible inhibition would be expected if an iodination reaction took place. Most of the studies of PSII labeling by iodide involved Mn-depleted PSII, in which iodination was most obvious, but iodination was also observed in Mn-retaining PSII (*22*). The conditions of iodination included illumination of PSII samples for 15 min using red light (>600 nm), which are considerably different from those used here. We have minimized the effect of possible irreversibility using initial rates for kinetic studies. Also, normal formation of

the S_2 state EPR signals indicates that for the most part normal electron transfer from the Mn cluster to Tyr Z took place, at least for the S_1-to-S_2 transition. Nevertheless, irreversible inhibition may have affected the values of the inhibition constants reported here, perhaps by decreasing them somewhat.

The study of the effect of iodide on PSII activity has made it possible to characterize two distinct anion binding sites near the site of O_2 evolution. Of the two I^- binding sites reported here, one has clear functional significance since it is associated with activation of O_2 evolution, a role usually filled by Cl^-. The functional role is less clear for the second I^- binding site, which is associated with inhibition. This site is evidently located in close proximity to Tyr Z of the OEC, if it is the same site as that from which I^- can donate electrons and label D1. The recent 3.5 Å resolution crystal structure of PSII (*42*) revealed an anion binding site at the Mn_4Ca metal cluster (occupied by bicarbonate in the crystal structure). The authors suggested that this site is normally occupied by water taking part in catalysis and that Cl^- is coordinated to the Ca^{2+} ion at another location. It thus appears that the catalytic region of the OEC has the capacity for multiple binding sites for water and ions. The inhibitory I^- binding site observed here could be one that is normally occupied by water or hydroxyl ion, perhaps participating in the hydrogen bond network required for function of Tyr Z. This possibility is reminiscent of the two ammonia binding sites (*33*, *34*, *43*−*45*), one of which is associated with Cl^- binding and the other with water binding. Another possibility is that the inhibitory I^- site is an anion site that usually facilitates the binding and exchange of Cl^- at its site of activation. The exchange of Cl^- was found in a previous study to be promoted by the presence of NO_3^- in the medium (*46*), leading to a proposal that more than one anion can be present near a sequestered domain containing the Cl^- binding site. It could also be related to the previously reported observation of a shift in the affinity of PSII for Cl^- between high-affinity (closed) and low-affinity (open) forms (*7*, *8*). The question of the functional role of a second anion site at the OEC is of interest for further investigation.

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