THE ROLE OF CHLORIDE IN OXYGEN EVOLUTION

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INTRODUCTION

Almost exactly 40 years ago, Warburg and Lüttgens (1) published a report on the ability of various anions to stimulate photosynthetic oxygen evolution in dialyzed chloroplasts. They reported that Cl^-, Br^-, I^-, and NO_3^- were effective (although Cl^- was the most effective), whereas rhodanide, SO_4^{2-} and PO_4^{3-} were ineffective in restoring the Hill reaction. Later, Warburg and Lüttgens (2) examined further the anion specificity and noted that the effect saturated at 7 mM KCl. Several years later, amid controversy concerning the importance of Cl^- to photosynthesis *in vivo* (see refs. 3-5 for a discussion), Gorham and Clendenning (6) demonstrated that Cl^- is directly involved in stimulating the water-splitting reaction, and that it does not simply overcome the injurious effects of exposure to light. Most important, they demonstrated that adding Cl^- to depleted chloroplasts shifts the pH optimum of the Hill reaction to the alkaline side. They also noted that the stimulatory effect of Cl^- is similar to that found for dialyzed α-amylase, and that these two processes might share a common physical basis. The conclusion of Warburg and Lüttgens that Cl^- is a necessary cofactor for O_2 evolution is now the foundation for intensive study of the O_2-evolving complex (OEC) of Photosystem II (PSII) (see refs. 5, 7-9). After 40 years of research, however, the mechanism by which Cl^- activates O_2 evolution still remains a mystery.

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A MODEL FOR Cl^- ACTION IN PHOTOSYSTEM II

There have been relatively few detailed suggestions about how Cl^- functions in the OEC. Any proposed mechanism for Cl^- function must also explain the following: (1) Inactivation by Cl^- depletion is reversible (10,11); (2) Cl^--depletion is accelerated by incubating the thylakoids at high pH (7) and by adding uncouplers (12); (3) Cl^--binding is pH-dependent and reversible (13); (4) activation of the Hill reaction by added Cl^- shows hyperbolic kinetics, indicating saturation (11); (5) activation of the Hill reaction by anions is relatively specific for Cl^-, but not exclusive (2,10,11,13); and (6) the pH optimum of the Hill reaction is shifted to more alkaline pH by Cl^--binding (6,10,14). There are several possible roles for Cl^- in the mechanism of the water-splitting reactions, some of which have already been described (see 7-9,15,16). However, because of the lack of information about Cl^- binding at the molecular level, most of these suggestions must be considered as merely working hypotheses.

The model described here involves a dual role for Cl^-: (1) indirect activation of base catalysis (activation of H^+ removal from water) at the active site of the OEC (33kD-Mn-containing polypeptide(s), referred to as "proteins" hereafter) (Fig. 1A); and (2) activation of H^+ binding at other sites (e.g., at 24 and 18 kD proteins) (Fig. 1B). This kind of role is consistent with the known action of Cl^- (and other anions) in soluble enzymes. The key to this model is the ability of anions to shift the pk_a's of essential reactive groups in enzymes by suppressing adjacent positive charges on other groups. This behavior has been suggested to explain the effect of anions on the velocity-pH curves of salivary amylase and fumarate hydratase (17).

The mechanism at the active site (Fig. 1A) involves two ionized groups on the protein, which is consistent

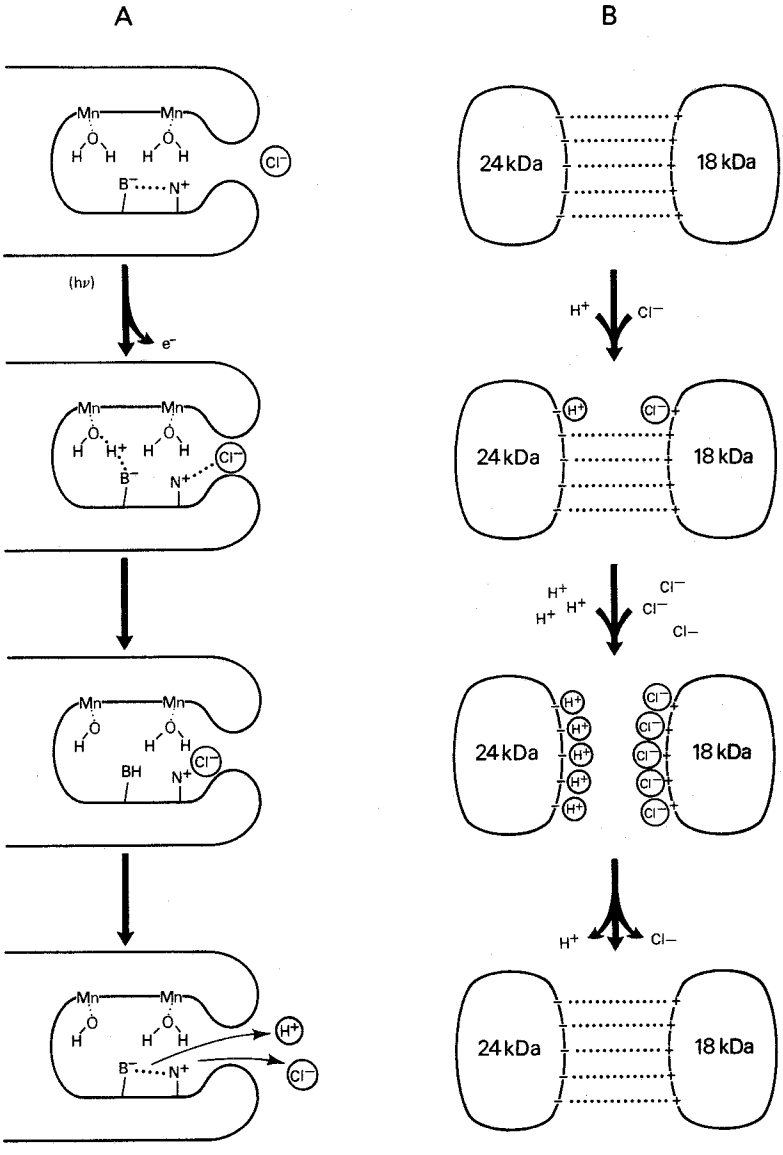

Figure 1. A Model for Cl^- Activation of O_2 Evolution in which Binding of the Anion Induces Shifts in the Apparent pk_a's of Reactive Groups on the Polypeptides of the O_2 Evolving Complex (see text for details).

with the bell-shaped velocity-pH curve for O_2 evolution. The group with the lower pK_a (the conjugate base of a weak acid) is labeled as B^-, and the group with the higher pK_a (for example, an amino group) is labeled as N^+. In its simplest form, the mechanism could operate as follows: a molecule of water is proposed to be bound to a Mn atom in the active site. The Mn serves to orient and polarize the water molecule, which facilitates removal of the protons (H^+s) (see ref. 18 for a discussion of Mn in yeast aldolase).

While the Mn (which is oxidized as a result of the light reaction) extracts an electron from water, B^- extracts a proton. We suggest that the group B^- would be able to temporarily bind this proton because the binding of Cl^- (or Br^-) to the positively charged group N^+ transiently raises the pK_a of B^-, and thus increases its affinity for the water proton. The Cl^- ion, therefore, would activate and control the rate of the water-splitting reaction by accelerating the removal of H^+s from water.

The net effect is similar to the model in ref. 8; here, Cl^- effectively stabilizes a "+" charge on the S states (preventing a back reaction) by moving it out onto the protein, even though Cl^- does not bind to the $Mn\text{-}H_2O$ complex directly. When the Cl^- unbinds from N^+ (either because its residence time is very short, as shown in 19, or because the incipient acidification of the lumen opens up new binding sites that draw the Cl^- away), the H^+ would be released from B^-, the salt bridge $B^-...^+N$ would re-form, and the cycle would be repeated.

Our model (Fig. 1A) explains the following: (1) the inactivation of O_2 evolution by Cl^--depletion is reversible (11), since the binding is electrostatic and does not require any complex architecture for the binding site; (2) Cl^- depletion is easier at high pH (7,12,20), since a pH > 8 would tend to deprotonate N^+; (3) the release of Cl^- is linked to the release of H^+s from the lumen (12,20); the coupling of Cl^- binding to H^+ binding is inherent in this model. This has also

been suggested to occur, for example, in ribonuclease (21); (4) the stimulation of O_2 evolution by added Cl^- (11) shows saturation kinetics, since Cl^- binding would accelerate the catalysis of $2H_2O \rightarrow O_2 + 4H^+$ until the binding site becomes saturated and the turnover rate of the OEC limits the reaction; (5) the effectiveness of activation by an anion depends on its charge and ionic radius (13); anions with a weak electric field (e.g., I^-) would not effect the pk_a of B^- enough (by not supressing N^+ sufficiently) to make the system reactive; those with too strong a field (e.g., F^-) would tend not to unbind as readily, and thus inhibit the turnover of the OEC; (6) Cl^- is bound more tightly in the light, presumably due to light-driven acidification of the lumen (12); the lowering of the internal pH would tend to increase the positive charge near N (cf. number 2) and thus increase its affinity for Cl^-; and (7) the pH optimum of the Hill reaction is shifted to more alkaline pH as more Cl^- is added to Cl^--depleted thylakoids (6,10,14). In our model, the alkaline shift would occur for two reasons: (a) at a slightly acid pH in the presence of excess Cl^-, the pK_a of BH is raised (relative to the Cl^- deficient case) due to the neutralization of N^+ by Cl^-; this makes B less reactive toward H_2O at slightly acid pH; and (b) at slightly alkaline pH the binding of Cl^- to N^+ creates the more reactive species B^- (compared to the unreactive $B^-...N^+$ which would prevail without Cl^-), and stimulates the water-splitting reaction. The overall effect is to shift the pH optimum for O_2 evolution to the alkaline side in the presence of Cl^-.

There are several observations that the above model alone cannot explain, and these exceptions have interesting implications: First of all, there is substantial Cl^- binding even in dark-adapted thylakoids. Secondly, this binding appears to be associated with a large, sequestered pool of bound H^+s, which remains even in the dark, and which equilibrates with the bulk phase only through the addition of uncouplers (20). Finally, a plot of the Hill reaction rate vs. the concentration of added Cl^- at low light intensity (W. Coleman, 1984 unpublished) does not give the smooth hyperbola that is

obtained at high light intensity, where it appears that a site (or sites) with a single binding affinity is saturated (11). Instead, the curve shows a "stair-step" dependence between 1 and 10 mM added Cl^-. Such a dependence has been observed for several enzymes (see e.g., refs. 17 and 22, for a discussion of this phenomenon). One hypothesis that has been proposed to explain this behavior is that these enzymes contain more than one binding site for the substrate, and these sites have different affinities (22). By analogy, therefore, we suggest that in spinach thylakoids there may be multiple Cl^- binding sites with different affinities. For this reason, we propose (Fig. 1B) that there may be a second set of Cl^- binding sites with a larger capacity and a slightly different function, namely, to assist in binding the protons released into the lumen (see ref. 12 for an earlier discussion). In this part of the model, Cl^- would promote the binding of H^+s at another site on the OEC. We suggest, as noted earlier, that this might involve binding to the 18 and 24 kD polypeptides. Here, again, Cl^- binds to amino groups and H^+ binds to negatively-charged groups (likely to be COO^- in this case).

The mechanism by which this system might operate (using, e.g., the 18 and 24 kD proteins) is as follows: At the slightly alkaline pH optimum for O_2 evolution, the two proteins would be oppositely charged based on measurements of their isoelectric points (see ref. 9 for a review). Thus, one could imagine that the excess positive charges on the 18 kD protein are at least partly neutralized by forming salt-bridges with the excess negative charges on the 24 kD protein. Discussion in (23) suggests that the 18 kD protein binds to the 24 kD protein. Light-driven H^+ pumping would tend to disturb this equilibrium by lowering the pH. The 24 kD protein would be able to bind the H^+s by breaking either some or all of the salt-bridges with the 18 kD protein, but in order to maintain charge balance, the 18 kD protein would be required to pick up a negatively charged counterion, such as Cl^-. As a result of this process, a considerable amount of

"conformational" energy could be stored. This energy might also tend to stabilize the complex against denaturation. Such protection of the thylakoid membrane by Cl^- binding has been reported (see refs. 7,11,24). Binding of the H^+s would also tend to encourage H^+ pumping into the lumen, since the free $[H^+]$ would be kept at a minimum. As described earlier, activation of the ion binding is reciprocal: Cl^- stimulates the binding of H^+s and vice versa.

If the 18 and 24 kD proteins do have the role described above, it might not be as apparent in uncoupled thylakoids, and in PSII particles or inside-out vesicles, where the two proteins would not be operating in an enclosed space. However, the role of Cl^- at the OEC (Fig. 1A) would remain the same in all cases. Further research is needed to test our model.

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