The mechanism of amine inhibition of the photosynthetic oxygen evolving complex

Amines displace functional chloride from a ligand site on manganese

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Received 29 July 1983

Amines inhibit photosynthetic oxygen evolution by binding to manganese in the oxygen evolving complex. This inhibition is more effective if chloride is absent. Steady-state kinetic analyses show that ammonia and chloride compete for the same site in the oxygen evolving complex; inhibition of oxygen evolution by either chloride depletion or by ammonia addition induces Signal II_f, which saturates at high microwave power. These data indicate that chloride is bound at the manganese site in the oxygen evolving complex. We propose that the anion functions as a bridging ligand mediating electron transfer between manganese atoms and perhaps also between manganese and Z, the primary donor to P680.

Photosystem II *Oxygen evolution* *Amine inhibition* *Chloride* *Bridging ligand* *Signal II_f*

1. INTRODUCTION

Certain amines (Tris buffer, NH_2OH) inactivate photosynthetic oxygen evolution while releasing manganese from the oxygen evolving center (OEC) [1]. Other amines (ammonia and methylamine, for example) produce a freely reversible inhibition characterized by the induction of the EPR free radical signal II_f [2]. Extensive studies on ammonia inhibition of the OEC [2–5] have led to the conclusion that the amine free base binds to OEC manganese in the S_2 and S_3 states. Effects of several amine inhibitors have been characterized [2]; inactivation of oxygen evolution by these compounds follows a pattern characteristic of a Lewis acid–Lewis base interaction where manganese is the Lewis acid. Increasing bulk on the amine lowers the microwave power necessary to saturate signal II_f. These results have been interpreted to indicate that amines bind to a site which perturbs a manganese interaction with the primary donor to $P680^+$.

Aside from manganese, chloride is the chemical species most widely implicated in photosynthetic oxygen evolution [6–9]. The presence of chloride, or one of a limited group of other anions (including Br^-, I^-, NO_3^-, formate and bicarbonate) is required for oxygen evolution [6]. Conflicting speculations exist as to the function of chloride in oxygen evolution activity. Chloride might be a ligand to manganese, where it would stabilize higher oxidation states of the metal [10]. Chloride could be involved in electron transport via binding at or near the OEC [11], however, the role of chloride may be to neutralize charge and thus stabilize a supermolecular conformation necessary for oxygen evolution [9].

Here we show that ammonia inhibition of oxygen

Abbreviations: OEC, oxygen evolving center; PS, photosystem; Chl, chlorophyll; DCBQ, 2,5-dichloro-*p*-benzoquinone

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Published by Elsevier Science Publishers B.V.
00145793/83/$3.00 © 1983 Federation of European Biochemical Societies

evolution is impeded by the presence of chloride. Ammonia and chloride compete for the same binding site and we therefore conclude that chloride is a ligand to OEC manganese.

2. MATERIALS AND METHODS

Thylakoid membranes were isolated from market spinach as in [12]. Where indicated, these membranes were chloride depleted by incubating a dilute suspension (200 μg chl/ml) in a medium containing 50 mM Na_2SO_4, 5 mM $MgSO_4$, 2 mM$(HN_4)_2SO_4$, 3 μg gramicidin/ml and 50 mM Tricine (pH 8). After 30 min at 4°C in the dark, the membranes were recovered by centrifugation and stored at −80°C in a medium containing 20 mM HEPES (pH 7.5) and 400 mM sucrose. Such preparations showed a 10-fold increase in oxygen evolution activity when assayed in the presence, rather than absence, of chloride. Assays of oxygen evolution were done as in [13] in a medium containing 50 mM HEPES (pH 7.5), 2.5 mM $Fe(CN)_6^{3-}$ and 250 μM DCBQ. Salt concentrations were varied as described in the tables and figures. Measurements of signal II_f were carried out on a Bruker ER 200D X-band spectrometer fitted with a TM cavity to accomodate a large aqueous flatcell (Scanco S-813). Instrument settings are given in table 2.

Table 1

Effects of salts on oxygen evolution in control and amine-inhibited thylakoid membranes

Addition to assay	O_2 Evolution activity (μmol . h^{-1} . mg chl^{-1})
100 mM NaCl	200
50 mM Na_2SO_4	179
60 mM $(NH_4)_2SO_4$	30
60 mM $(NH_4)_2SO_4$ + 100 mM NaCl	68
60 mM $(NH_4)_2SO_4$ + 50 mM Na_2SO_4	26

Activity was assayed as in section 2. Thylakoid membranes (30 μg chl/ml) were incubated for 1 min with either NaCl or Na_2SO_4; the amine, ferricyanide and DCBQ were added immediately before illumination

3. RESULTS

Fig.1 compares the inhibitory effects of the chloride and sulfate salts of ammonia when oxygen

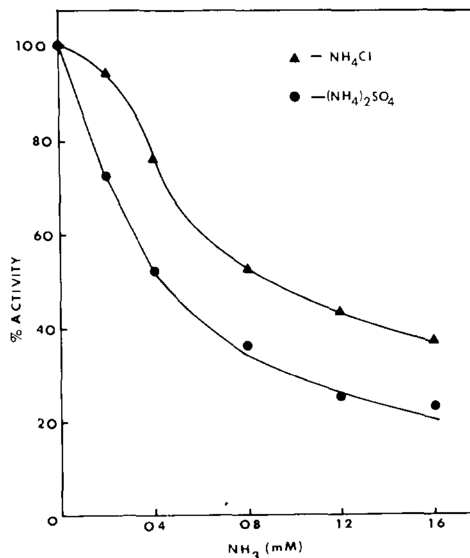

Fig.1. Effects of chloride and sulfate on the inhibition of oxygen evolution by ammonia. The assay procedure is described in section 2. Thylakoid membranes (30 μg chl) were incubated for 1 min in the assay medium prior to addition of the amine salt, ferricyanide and DCBQ.

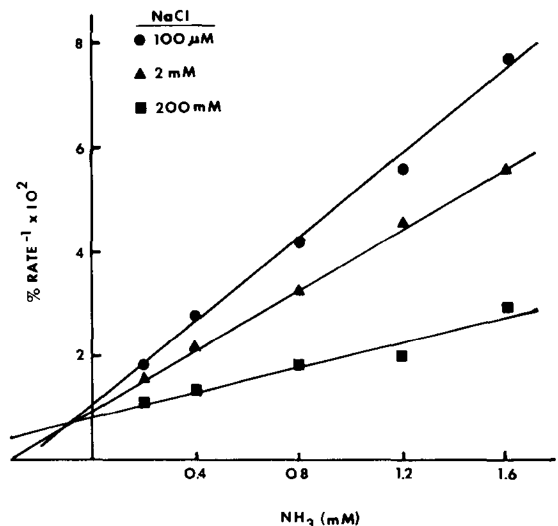

Fig.2. Effect of ammonia concentration on oxygen evolution activity assayed at 3 chloride concentrations. The assay procedure was as given in the legend to fig.1; the ammonia was added as ammonium sulfate. Rates are presented as percent of control to adjust for the differences observed at various chloride concentrations minus the amine. The control rates (μmol . h^{-1} . mg chl^{-1}) were: 100 μM NaCl, 301; 2 mM NaCl, 290; 200 mM NaCl, 196.

Table 2

Dark-(II_s) and light-induced (II_f) signal II amplitudes in inhibited and chloride-depleted thylakoid membranes: measurements at two microwave powers

	Signal amplitude (arbitrary units)						II_f (214 mW) / II_f (21 mW)
	21 mW			214 mW			
	II_s	$II_s + II_f$	II_f	II_s	$II_s + II_f$	II_f	
NH_3-Inhibited	39	50	11	41	57	16	1.45
Cl^--Depleted	22	36	14	20	41	21	1.50

Amine-inhibited membranes (3 mg chl/ml) in 20 mM HEPES, 15 mM NaCl and 400 mM sucrose were placed in the flat cell along with 50 mM $(NH_4)_2SO_4$ and 100 μM FMN; Chloride-depleted membranes (1.8 mg chl/ml) suspended in the same medium minus chloride were placed in the flatcell with the same concentration of FMN. Each sample was scanned in the dark, and again in the light to measure the II_f amplitude. Instrument settings were: frequency, 9.7 GHz; center field, 3495 G; modulation amplitude, 8 Gpp; gain, 2.6×10^5; sweep width, 100 G

evolution activity was assayed in the absence of added chloride (100 μM chloride was carried over to the assay from the thylakoid suspension). It is clear from these data that the sulfate salt produces a stronger inhibition of oxygen evolution than does the chloride salt. Table 1 presents data showing that sulfate by itself (as Na_2SO_4) is only weakly inhibitory; therefore, we consider that the effects seen in fig.1 are due to chloride protection of the OEC against ammonia inhibition.

Fig.2 shows the results of experiments where the amine concentration (introduced as $(NH_4)_2SO_4$) was varied at 3 fixed concentrations of chloride. The Dixon plot shows that chloride can be treated as a substrate for oxygen evolution, and that chloride and ammonia compete for the chloride binding site. If ammonia inhibits oxygen evolution by displacing chloride from an essential binding site in the OEC (fig.2), then we would expect that chloride depletion of thylakoid membranes would produce an induction of signal II_f similar to that for ammonia inhibited thylakoid membranes [5]. This is observed in table 2. Both NH_3-poisoned and chloride-depleted thylakoid membranes show the presence of signal II_f with a high microwave power dependence.

4. DISCUSSION

Our results (fig.2) show that the mechanism of amine inhibition of oxygen evolution activity involves the competitive displacement of chloride from an essential binding site in the OEC. Extensive characterization of the mechanism of amine attack on the OEC has strongly implicated manganese as the amine binding site [2–5], and we therefore think it likely that the site of chloride binding in the active OEC is as a ligand to manganese. If our hypothesis, that ammonia inhibition is equivalent to chloride depletion, is correct, then it might be expected that S-state behavior in ammonia-poisoned and chloride-depleted thylakoid membranes would be similar. In fact, it has been observed in independent investigations [4,7] that the S_2 and S_3 states have enhanced lifetimes in both ammonia-poisoned and chloride-depleted preparations. Our hypothesis would also predict that under appropriate conditions, low-temperature EPR signals ascribed to the S_2 state [14] might exhibit hyperfine structure arising from an interaction with the chloride nucleus ($I = 3/2$).

In considering the possible function of a halide, nitrate or carboxylate ligand to manganese in the OEC, it is of interest to note that all of these anions can act as bridging ligands in polynuclear transition metal complexes [15]. Furthermore, halides, nitrate and carboxylates, by acting as bridging ligands, can mediate electron transfer between transition metals [16,17]; ammonia is an ineffective mediator in these systems and can be used as a blocking ligand. A similar mechanism involving electron-transfer mediation by bridging

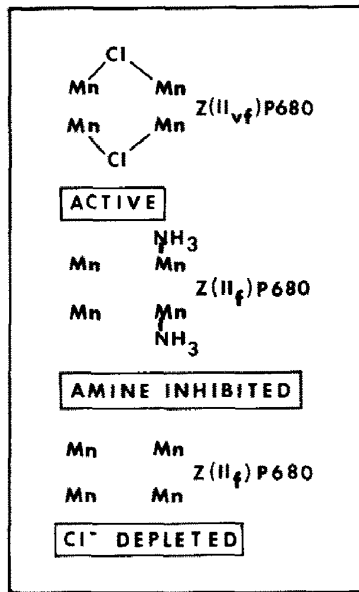

Fig.3. Model for manganese ligand function of chloride in the OEC: Amine inhibition and chloride depletion.

ligands may occur between transition metals and organic radicals [18]. We thus consider it possible that chloride or a chloride surrogate anion functions in the OEC by mediating electron transfer either between manganese atoms, between manganese and Z, or both. Our conclusions and conjectures are summarized in fig.3. Although we have left the chloride ligand sites vacant in depicting the chloride-depleted OEC of our model, we feel it likely that at high pH this site could be occupied by hydroxide ion, which might explain the inactivation of oxygen evolution at elevated concentrations of this anion [19], and which would agree with [11].

We wish to emphasize that our conclusions, as shown in fig.3, are preliminary; we have observed that the sulfate salt of Tris is a more effective inhibitor of the OEC than is the chloride salt and that ammonia is a competitive inhibitor of chloride-induced reactivation of the OEC, findings consistent with our conclusions. However, by the considerations outlined above, we cannot, explain why fluoride, an excellent bridging ligand, is ineffective as a replacement for chloride. We would note that others [6] have shown fluoride to be a complex inhibitor of oxygen evolution, and we are currently conducting further investigations on this question ourselves.

ACKNOWLEDGEMENTS

We thank Drs G.T. Babcock and D.F. Ghanotakis for stimulating discussions. This work was supported by grants to C.F.Y. from the USDA/SEA Competitive Reserach Grants Office (82-CRCR-1-1127) and from the National Science Foundation (PCM-8214240).

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