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Mercury inhibition at the donor side of photosystem II is reversed by chloride

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Mercury is an environmental contaminant that strongly inhibits photosynthetic electron transport, photosystem II being the most sensitive target. We investigated in greater detail the effect of mercury using photosystem II submembrane fractions of higher plants. Oxygen evolution was strongly inhibited and variable chlorophyll fluorescence was severely quenched by mercury. Chloride, an inorganic cofactor known to be essential for the optimal function of photosystem II, significantly reversed the inhibitory effect of mercury. However, calcium, another essential cofactor, showed no reversal capacity. It is concluded that on the donor side of PSII, mercury exerts its action by perturbing chloride binding and/or function. Considering the exceptional affinity of mercury for sulfhydryl groups of proteins, the results suggest the implication of cystein residue(s) in maintaining structural and functional integrity of photosystem II.

Photosystem II; Electron transport; Oxygen evolution; Mercury; Chloride; Calcium

1. INTRODUCTION

Mercury is an environmental contaminant that strongly inhibits photosynthetic electron transport. This heavy metal has been shown to exert its action at many sites in the photosynthetic membrane, PSII being the most sensitive target [1]. Both, the donor [1–9] and the acceptor sides [2,4,8,10] of the photosystem are affected. On the donor side, the exact site and mode of action of mercury have not been elucidated. The studies mentioned above were performed on relatively complex materials, from whole organisms to isolated thylakoid membranes. To our knowledge, no studies have been performed on PSII submembrane fractions of higher plants. In these preparations, other components of the thylakoid membranes, which are also inhibited by mercury, such as PSI [1,8] and plastocyanin [9], are depleted [11]. Moreover, both donor and acceptor sides of PSII are readily accessible to exogenous agents. Then, PSII fragments are highly suitable to explore in greater details PSII inhibition by mercury.

Besides manganese, two inorganic cofactors are closely associated to the process of water oxidation in PSII: chloride and calcium. They play structural and regulatory roles (see refs. [12–14]). In the present study, we investigated if mercury interferes with these cofactors. We show for the first time, that on the donor side of PSII, mercury exerts its action by perturbing chloride binding and/or function. Considering the exceptional affinity of mercury for sulfhydryl groups of proteins [15], our results suggest the involvement of cystein residues to maintain the structural and functional integrity of the photosystem.

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Abbreviations. Chl, chlorophyll; DCBQ, 2,5-dichlorobenzoquinone, EP, extrinsic protein; F_o, constant fluorescence; F_v max, maximum value for variable fluorescence; K_n, stability constant of metallic complexes; MES, 2-(*N*-morpholino)ethanesulfonic acid; PSII, photosystem II; TMA, tetramethyl ammonium.

2. MATERIALS AND METHODS

Photosystem II submembrane fractions were isolated from barley (*Hodeum vulgarae*) according to Ikeuchi and Inoue [16] and with modifications as described previously [17]. The PSII preparations were finally suspended in a medium containing 400 mM sucrose and 20 mM MES-TMAOH pH 6.3 and were stored in liquid nitrogen until use

Initial rates of oxygen evolution were measured at 22°C, using a Clark-type electrode [18]. Saturating continuous white light was used to illuminate the samples. The assay medium contained 400 mM sucrose, 20 mM MES-TMAOH, 0.35 mM DCBQ as PSII electron acceptor, 15 μg Chl/ml and the mentioned additives. In the absence of additives, the oxygen evolution rates (100%) varied between 370 and 420 μmol O_2/mg Chl · h

Chlorophyll fluorescence induction measurements were performed in an integrated sphere as previously described [19], with PSII preparations at a concentration of 5 μg Chl/ml.

Before measurements of oxygen evolution and chlorophyll fluorescence, the samples were incubated in the dark, at 22°C, for 5 min, the time required to obtain the maximal effect, in the presence of specified concentrations of mercury.

3. RESULTS

The oxygen evolution activity in PSII submembrane

fractions was studied at various concentrations of mercury using two salts: $HgCl_2$ and $Hg(NO_3)_2$ (Fig. 1). Both salts strongly inhibit oxygen evolution, and $Hg(NO_3)_2$ more potently. The mercury concentration required to inhibit 50% of oxygen evolution was 2.5 μM with $Hg(NO_3)_2$ compared to 10 μM with $HgCl_2$. This difference may be attributed to the dissociation properties of the two salts in solution. According to their stability constants (see Equations 1 and 2 below), $HgCl_2$ is found mainly in the complexed form in solution comparatively to $Hg(NO_3)_2$ which is almost completely dissociated [20]. Then, it is consistent that $Hg(NO_3)_2$ is a more potent inhibitor than $HgCl_2$ at a given concentration. Both salts finally caused the same maximal effect at higher concentrations and we can conclude that mercury is the inhibitory agent.

In order to determine if mercury could interfere with the two inorganic cofactors (calcium and chloride) known to be essential for the optimal functioning of the oxygen-evolving complex, various salts were added after a 5 min treatment of the PSII preparations with mercury. Table I shows that all the chloride-containing salts (TMACl, NaCl, $CaCl_2$) significantly reversed mercury inhibition (39% reversal). $Ca(NO_3)_2$ slightly reversed mercury inhibition (19%) and $CaSO_4$ did not show any effect. NO_3^- can substitute Cl^- in PSII activation but with less efficiency [18]. So, in our experimental conditions, only chloride (and a related anion NO_3^-) showed a reversal capacity to mercury inhibition. The observations that calcium as a sulfate salt ($CaSO_4$) could not reverse mercury inhibition and that reversal capacity was the same for all chloride salts including $CaCl_2$ allow to suggest that calcium sites are not affected by mercury.

The chloride effect was studied in more details using TMACl. According to [21], Na^+ can have an inhibitory effect on salt-washed PSII membranes at concentrations higher than 10 mM, while TMA^+ does not modify PSII activity. Then, in order to avoid any interfering effect caused by sodium, we used TMACl instead of NaCl in our reactivation experiments. Furthermore, the pH of the assay media was set using TMAOH instead of NaOH. Finally, in order to avoid any additive effect by the chloride contained in $HgCl_2$, the samples were treated with $Hg(NO_3)_2$.

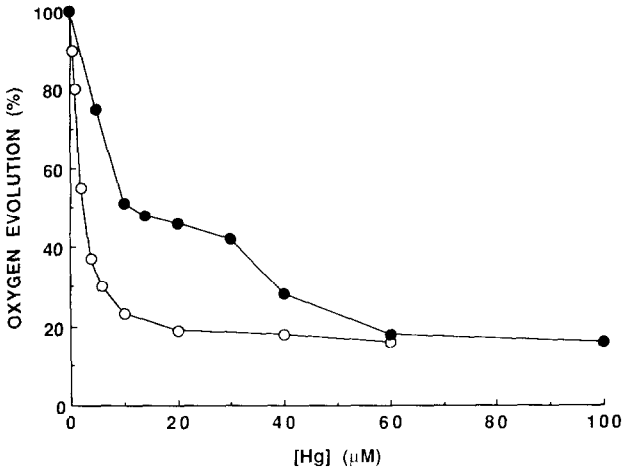


Fig. 1. Oxygen evolution as a function of mercury concentration with (●) $HgCl_2$ or with (○) $Hg(NO_3)_2$.

Fig. 2A shows the oxygen evolution rate as a function of TMACl concentration. Increasing concentrations of TMACl slightly stimulated intact PSII activity and gradually reversed the inhibition caused by mercury. The double reciprocal plot of the data presented in Fig. 2B reveals mixed-type inhibition kinetics. The reversal effect of chloride was further studied at various mercury concentrations with or without 10 mM TMACl. The inhibitory action of mercury on oxygen evolution was attenuated by chloride at any mercury concentration used (Fig. 3). This effect of chloride was also observed during fluorescence induction experiments. The gradual quenching of variable fluorescence obtained with in-

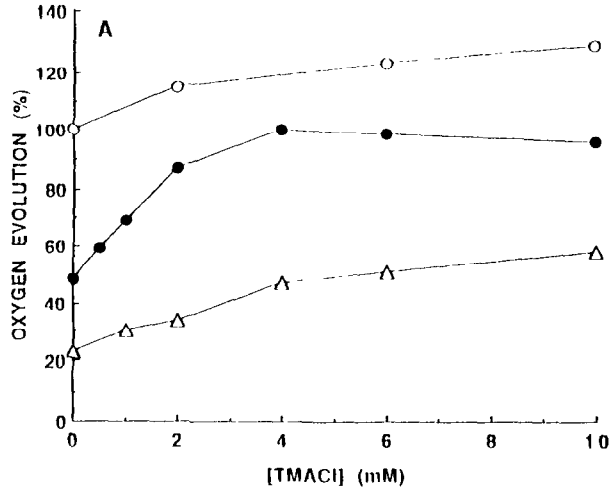
Table I

Effects of various additives on PSII activity with or without 40 μM $HgCl_2$

Salts	Concentration (mM)	Oxygen evolution (%)		Inhibition (%)	Reversal[1] (%)
		− Hg	+ Hg		
None		100	30	70	–
TMACl	20 mM	108	62	43	39
NaCl	20 mM	116	65	44	39
$CaCl_2$	10 mM	111	63	43	39
$Ca(NO_3)_2$	10 mM	69	30	57	19
$CaSO_4$	10 mM	98	27	72	0

[1] Percentage of reversal is calculated as follows.

$$\frac{\%\text{ of inhibition in absence of salt} - \%\text{ of inhibition in presence of salt}}{\%\text{ of inhibition in absence salt}} \times 100.$$



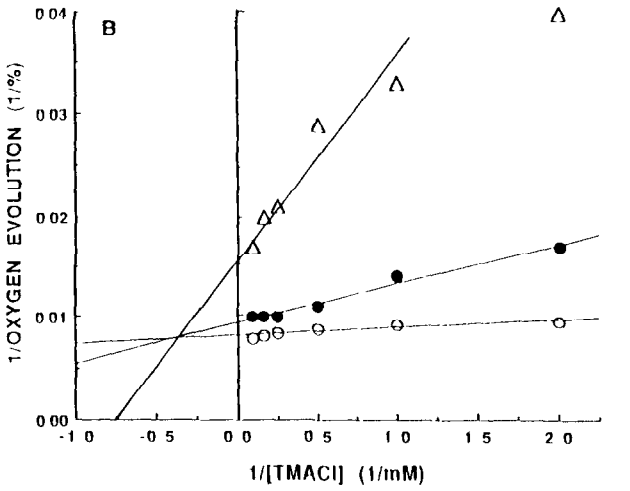


Fig. 2. (A) Oxygen evolution as a function of TMACl concentration with $Hg(NO_3)_2$: (○) 0 μM; (●) 2.5 μM; (△) 8 μM. (B) Double reciprocal plot of the data in Fig. 2A.

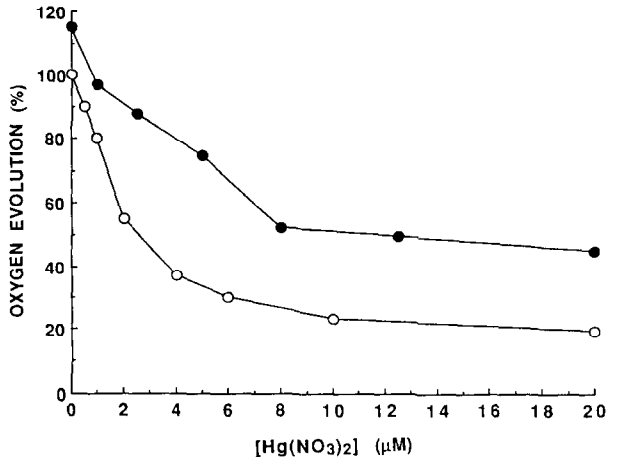


Fig. 3. Oxygen evolution as a function of $Hg(NO_3)_2$ concentration with (●) or without (○) 10 mM TMACl.

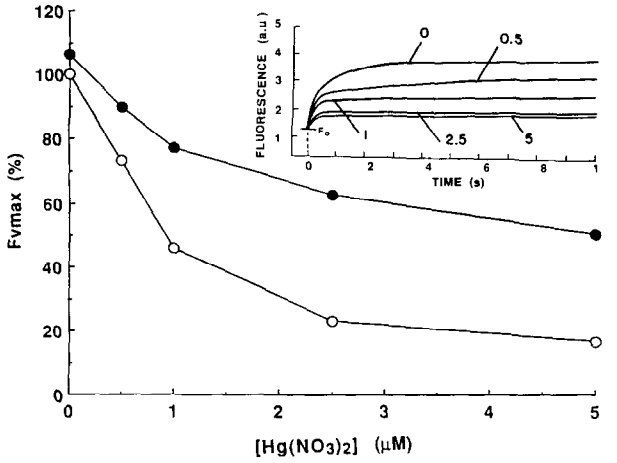


Fig. 4. Percentage of F_v max (maximum value for variable fluorescence) as a function of mercury concentration with (●) and without (○) 10 mM TMACl. F_v max was obtained by substracting the constant fluorescence (F_o) from the total fluorescence at the maximal level. Inset: Fluorescence induction kinetics for PSII preparations in the presence of mercury. Numbers show the concentration (μM) of $Hg(NO_3)_2$.

creasing concentrations of mercury (Fig. 4, inset) was significantly reversed by chloride (Fig. 4).

The above data clearly demonstrate that the action of mercury on the donor side of PSII is strongly related to chloride. However, before proposing a mechanism of action, it is important to determine whether the chloride reversal of inhibition is specifically related to PSII function or is solely due to a chemical complexation between mercury and chloride. To estimate the contribution of the complexation component, we made reversal studies using three anions: chloride, bromide and nitrate. According to their stability constant (K_n) [17], they complex with mercury in the following order: $Br^- > Cl^- \gg NO_3^-$:

$$Hg^{2+} + Br^- \overset{K_1 = 1.1\times10^9}{\rightleftharpoons} HgBr^+ + Br^- \overset{K_2 = 1.9\times10^8}{\rightleftharpoons} HgBr_2 \quad (1)$$

$$Hg^{2+} + Cl^- \overset{5.5\times10^6}{\rightleftharpoons} HgCl^+ + Cl^- \overset{3.0\times10^6}{\rightleftharpoons} HgCl_2 \quad (2)$$

$$Hg^{2+} + NO_3^- \overset{2.2}{\rightleftharpoons} HgNO_3^+ + NO_3^- \overset{0}{\rightleftharpoons} Hg(NO_3)_2 \quad (3)$$

On the other hand, those three anions also stimulate PSII activity in a characteristic sequence. Chloride, bromide and nitrate can restore PSII activity after a deleterious treatment in the following order: $Cl^- > Br^- > NO_3^-$ [22]. Thus, chloride and bromide act in a reversed order depending if they are complexing with mercury or activating the PSII function. Table II shows the reversal capacities of chloride, bromide and nitrate: they could reverse 57%, 40%, and 17% of mercury inhibition respectively, which corresponds to the sequence observed

Table II

Effects of three sodium salts on oxygen evolution activity with or without 20 μM $Hg(NO_3)_2$

Salts (10 mM)	Oxygen evolution (%)		Inhibition (%)	Reversal[1] (%)
	− Hg	+ Hg		
None	100	25	75	0
NaCl	126	89	32	57
NaBr	117	63	45	40
$NaNO_3$	87	33	62	17

[1] Percentage of reversal is calculated as in Table I.

for the activation of PSII: $Cl^- > Br^- > NO_3^-$. The possibility of complex formation cannot be totally excluded but it is not the predominant phenomenon in our experimental conditions. Those results are consistent with the kinetic data obtained in Fig. 2B. If chloride would have exclusively complexed with mercury to prevent it to bind to PSII, the reciprocal plots would have shown a pure non-competitive profile (only V_{max} modified) instead of a mixed-type inhibition as observed (V_{max} and K_m modified).

4. DISCUSSION

Chloride is a cofactor required for the optimal functioning of the oxygen evolving complex, but its exact function is still under debate, even its direct requirement in oxygen evolution is reevaluated [23]. The main functions proposed until now are that it participates directly in the catalytic process [14,24] and/or that it has a structural role maintaining an optimal organization of the oxygen evolving complex proteins [22,23]. The binding site(s) of chloride are also undetermined [12,13].

The data presented in this report clearly demonstrate that mercury specifically affects chloride binding and/or function. Important information about the mode of action of mercury and its interaction with chloride can be obtained from Fig. 2B. Secondary replots of the primary reciprocal plot data, such as slope versus mercury concentration or $1/v$ axis intercept versus mercury concentration are linear (data not shown), which implies a linear mixed-type inhibition. According to Segel [25], this system is considered a mixture of partial competitive inhibition and pure noncompetitive inhibition. The partial competitive inhibition component indicates that mercury and chloride bind to different sites on the PSII and that chloride has a lower affinity for PSII when mercury is bound to the complex (increased K_m). Further, the pure non-competitive component suggests that the binding of mercury makes the complex nonproductive of oxygen (decreased V_m). Chemical complexation between mercury and chloride could also contribute to the non-competitive component. Thus, the above discussion supports the idea that mercury exerts its action by inducing a conformational change which perturbs catalytic and/or structural function of chloride in the oxygen evolving complex.

This is the first demonstration that on the donor side of the photosystem, mercury exerts its action at a site related to chloride binding and/or function. Previous studies indicated that the oxygen evolving complex was probably affected by mercury but never pointed out any of its components [1–9]. Very few denaturating treatments perturb only the chloride function. Most of the deleterious treatments used to study structure–function relationship on PSII modify together chloride and calcium requirements for oxygen evolution. Finally, mercury presents an exceptional affinity for sulfhydryl groups of proteins, greater than for any other single ligand, and a moderate affinity for histidine residues [15]. Extrinsic and intrinsic polypeptides of PSII bear those amino acids [26]. Surely the involvement of those residues in maintaining PSII integrity and chloride function deserves further investigations.

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