The action of mercury on the binding of the extrinsic polypeptides associated with the water oxidizing complex of photosystem II

Michelle Bernier, Robert Carpentier*

Centre de recherche en photobiophysique, Université du Québec à Trois-Rivières, 3351, boul. des Forges, CP 500, Trois-Rivières, Québec G9A 5H7, Canada

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Abstract **Mercury (Hg^{2+}), a sulfhydryl group reactant, was used to probe structure–function relationships in photosystem II (PSII). In the present work, we investigated the impact of mercury on the polypeptide composition of PSII submembrane preparations. Electrophoretic analysis revealed that the incubation of the membranes in the presence of mercury produces the depletion of a polypeptide of molecular weight of 33 kDa. This polypeptide corresponds to the extrinsic protein EP33 of the oxygen evolving complex removed following urea treatment. However, the two closely related extrinsic polypeptides of 16 and 23 kDa, usually removed concomitantly after urea treatment, remained unaffected after the mercury treatment. These data demonstrated the existence of an intrinsic binding site for EP23. The molecular mode of action of mercury in the oxygen evolving complex of PSII is discussed.**

Key words: Photosystem II; Oxygen evolving complex; Extrinsic polypeptide (16 kDa, 23 kDa, 33 kDa); Mercury; Sulfhydryl group

1. Introduction

Photosystem II is the thylakoid membrane bound complex where the unique process of water oxidation takes place. Among the constituents of this supramolecular complex, three extrinsic polypeptides (EP) of 16, 23, and 33 kDa respectively, and two inorganic cofactors, chloride and calcium, are thought to play structural and regulatory roles in the process (for a review see [1]). Their structural and functional organization are still under current investigation and the use of specific inhibitors constitutes a valuable approach to elucidate this question.

In a recent study [2], we have demonstrated that mercury treatment of PSII submembrane fractions leads to a strong inhibition of oxygen evolution. Furthermore, this inhibition could be prevented by the addition of moderate concentrations (10 mM) of chloride, an essential cofactor of PSII. It is known that removal of EP 16, 23, and 33 leads to the inhibition of oxygen evolution and that the activity could be recovered, at least partially, by the addition of chloride and/or calcium cofactors [3,4]. Considering these observations, it is of interest to study the impact of mercury treatment on the polypeptide composition of the PSII submembrane fractions to verify if this metal ion exerts some action on the release of the extrinsic polypeptides. Electrophoresis analysis revealed that the EP33 was removed in the presence of mercury, but not the closely related polypeptides of 16 and 23 kDa. This result discloses new information on the structural organization of PSII.

*Corresponding author. Fax: (1) (819) 376-5057.
E-mail: Robert_Carpentier@uqtr.uquebec.ca

Abbreviations: Chl, chlorophyll; DCBQ, 2,5-dichlorobenzoquinone; EP, extrinsic protein; MES, 2-(*N*-morpholino)ethanesulfonic acid; PSII, photosystem II.

2. Materials and methods

PSII submembrane fractions were isolated from barley (*Hordeum vulgarae*) according to [5] and from spinach (*Spinacia oleracea* L.) according to [6] with the modifications described previously [7]. The PSII preparations were finally resuspended in 400 mM sucrose and 20 mM MES-TMAOH pH 6.3 and were stored in liquid nitrogen until use. In one case, EP33, EP23 and EP16 were removed by urea treatment according to Myao and Murata [8] or EP23 and EP16 were removed by 1 M NaCl treatment as described by Nakatani [9].

Mercury treatment (5-min incubation) was performed in the dark, at 22°C as described previously [2]. The assay media contained: 400 mM sucrose, 20 mM MES-NaOH (pH 6.3), 15 μg Chl/ml and the indicated concentrations of $HgCl_2$. To determine the polypeptide(s) released by mercury, the PSII preparations were harvested immediately after treatment by a 8-min centrifugation in an Eppendorf microcentrifuge. The pellets were washed once in 400 mM sucrose, 20 mM MES-NaOH (pH 6.3) and were used for polypeptide analysis. The first supernatants were further centrifuged (40 min) to remove remaining membrane fragments. The supernatant was then concentrated against a sucrose gradient and dialysed against the above buffer before analysis by polyacrylamide gel electrophoresis. The latter was performed at room temperature in the buffer system of Laemmli [10] using miniature slab gels (Hoefer Scientific Instruments) containing 15% acrylamide. The gels were stained with Coomassie brilliant blue and scanned with an LKB Ultro Scan XL laser densitometer. The polypeptide content of each band was evaluated by integration of the peaks and the lines were normalized with each other using the intensity of the band of a polypeptide that is not influenced by mercury.

Oxygen evolution was measured at 22°C, using a Clark type oxygen electrode [11]. 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 concentrations of mercury. In the absence of mercury, the oxygen evolution rate in the barley preparations used was 395 ± 10 μmol O_2/mg Chl·h.

3. Results

The polypeptide profile of PSII submembrane fractions after various treatments is shown in Fig. 1. In lane 3, it is shown that a 30-min incubation of the PSII submembrane fragments in the presence of 10 μM $HgCl_2$ produces a 40% depletion of a polypeptide of molecular weight of 33 kDa compared to the control (lane 2). The 33 kDa polypeptide was recovered in the supernatant of the treated samples as can be seen in lane 4; no other polypeptides were found in the supernatant. In lane 6, the polypeptide profile following urea treatment is presented. This treatment is known to remove EP16, EP23 and EP33 of the oxygen evolving complex. The 33 kDa protein removed follow-

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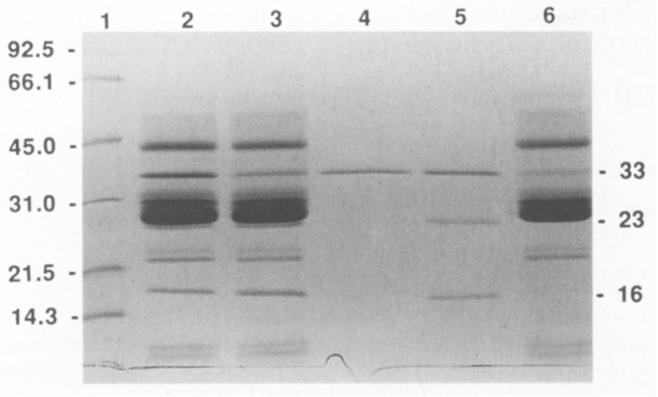

Fig. 1. Gel electrophoresis pattern of various PSII preparations illustrating the depletion of EP33 by Hg^{2+}. Lane 1, molecular weight standards for phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor and lysozyme. Lane 2, control PSII; lane 3, $HgCl_2$-treated PSII; lane 4, supernatant of $HgCl_2$-treated PSII; lane 5, supernatant of urea-treated PSII; lane 6, urea-treated PSII. Numbers on the left and right side indicate apparent molecular masses (kDa) of proteins in resolved bands. For conditions, see section 2.

ing mercury treatment (lane 4) corresponds to EP33 removed following urea treatment as shown in lane 5 corresponding to the supernatant. However, we can see that the 16 and 23 kDa polypeptides removed by urea treatment remained unaffected after mercury treatment.

The effect of mercury was studied in more detail as a function of concentration. The polypeptide content for EP33, EP23 and EP16 following an incubation of 30 min, with various concentrations of mercury is shown in Fig. 2 for PSII preparations isolated either from barley (Fig. 2A) or from spinach (Fig. 2B). At any mercury concentration, the polypeptide content in EP16 and EP23 remained unaffected. However, EP33 is gradually released with increasing concentrations of mercury up to 10 μM. Then, beyond these concentrations, the amount of polypeptide released is less significant. There is no immediate explanation to this peculiar effect. A solubility problem would be doubtful considering that we are working in the μM range, which is far below the solubility point of $HgCl_2$ (s = 6.9 g/100 ml) [12]. Thus, there seem to be two contradictory effects of mercury at higher concentrations; one to remove EP33, another to maintain the polypeptide bound to the membrane. This could explain why we could not achieve the complete removal of EP33. The maximal removal was of 50% in PSII preparations isolated from barley. In spinach preparations, up to 60% of EP33 was removed. There is a more tenacious association of the EP33 in barley than in spinach. We observed the same phenomenon after urea treatment where 60% of EP33 was removed in barley, and 100% in spinach (results not shown). In Fig. 3, the depletion of EP33 is studied in PSII preparations already depleted in EP16 and EP24. It is shown that the absence of these two polypeptides did not significantly modify the extent of depletion of EP33 nor the required Hg^{2+} concentration.

In Fig. 4, the oxygen evolution activity is compared to the release of EP33 as a function of increasing concentrations of $Hg(NO_3)_2$ following a 5 min incubation. The decrease in activity seems to take place at much lower mercury concentrations than the release of the polypeptide. Moreover, at higher concentrations, EP33 is less removed while the activity still decreases. The same behavior was observed using $HgCl_2$ (data not shown). Thus, the effect of mercury on the polypeptide removal and on oxygen evolution does not seem to be directly related. However, there is probably a primary effect of mercury which makes the oxygen evolving complex non-functional and provokes the depletion of EP33 and a secondary effect that prevents the release of the polypeptide from the membrane at Hg^{2+} concen-

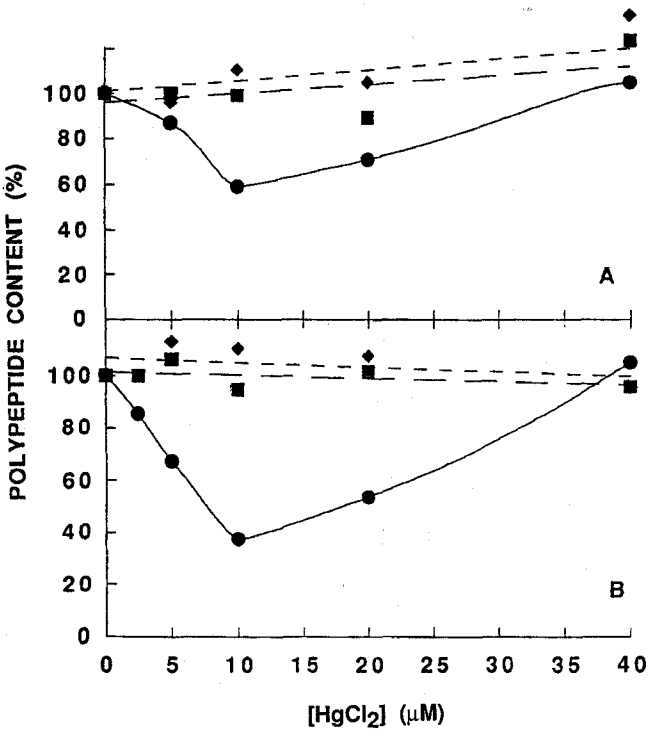

Fig. 2. Polypeptide content of PSII preparations from barley (A) and spinach (B) as a function of mercury concentration: (●) EP33, (■) EP23, and (◆) EP16.

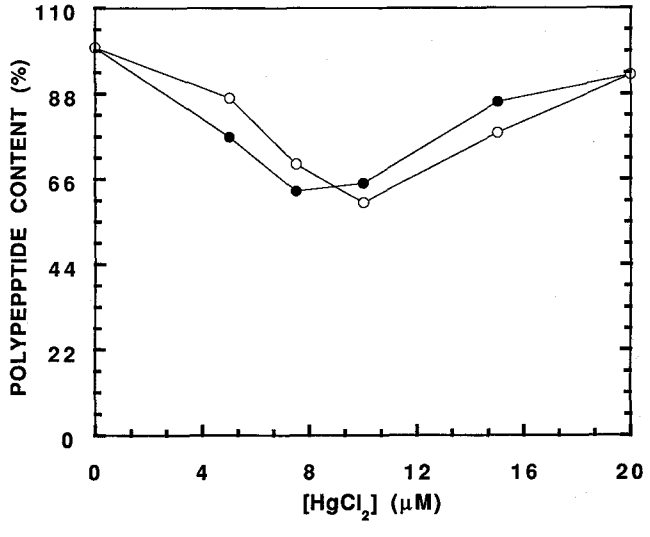

Fig. 3. EP33 content of PSII preparations used either in their native form (○) or depleted in EP16 and EP23 before mercury treatments (●) as a function of mercury concentration. The maximal depletion (40%) is reached at 10 and 7.5 μM, respectively.

trations above 12 μM and delays this release at lower Hg^{2+} concentrations even though oxygen evolution is inhibited. A relationship between inhibition and polypeptide depletion is supported by the action of Cl^- on the depletion of EP33 by Hg^{2+} (Fig. 5). The depletion is strongly reduced in the presence of Cl^- during the incubation period. This anion is known to specifically decrease the inhibitory effect of Hg^{2+} on oxygen evolution [2].

4. Discussion

The data presented above clearly demonstrate that EP33 can be removed by micromolar concentrations of Hg^{2+} without the removal of the two closely related extrinsic polypeptides of 16 and 23 kDa and that EP16 and EP23 do not even retard the depletion of EP33 (Figs. 1–3). Usual treatments that cause the release of EP33 produce the concomitant depletion of the EP16 and EP23 (see [3,4] for reviews). This is mostly interpreted as an indication that EP33 is in closer proximity of the intrinsic protein components of PSII than EP23 and EP16. In fact, reconstitution experiments demonstrated that the presence of EP33 is essential for a high-affinity and stoichiometric binding of the EP23 polypeptide, and that EP33 and EP23 are essential for the proper binding of EP16 [13–17]. Immunological studies [18] and cross-linking studies [19] have also suggested that the EP23 polypeptide binds to EP33. These results have been used to suggest that the extrinsic proteins bind to PSII in a particular sequence, EP33 binding to intrinsic protein component(s), EP23 binding to EP33 and EP16 to EP23. The present data seems to contradict the above interpretation, suggesting that the binding of EP23 and EP16 to PSII is independent from EP33. Along these lines, Yamamoto [20] suggested from phase partioning studies, that the three extrinsic proteins possess binding sites on some intrinsic components of PSII. Furthermore, Larsson et al. [21] have also shown, on the basis of immunological studies, that EP23 binds to an intrinsic protein of PSII.

Even though the reconstitution studies quoted above indicated an interaction between EP33, EP23 and EP16, Miyao and Murata [15] observed from sedimentation experiments that in solution the three polypeptides cannot bind to each other. They proposed that the binding of EP33 to some intrinsic component(s) induces a conformational change which allows subsequent binding of EP23 to EP33 or to an intrinsic site on the membrane, and so on for 16 kDa. In the same way, Kavelaki and Ghanotakis [17] proposed that a direct interaction between 33, 23, and 16 kDa polypeptides is the simplest model, but that a model where the EP33 is interacting with another protein which provides the binding sites for EP16 and EP23 cannot be excluded. Our results clearly support the second model: the fact that EP33 can be removed without affecting EP23 and EP16 (Figs. 1–3) is a direct demonstration of the existence of an intrinsic binding site for EP23. However, the possibility of interaction between EP23 and EP33 cannot be excluded, and probably this interaction is essential for optimal functioning of the oxygen evolving complex.

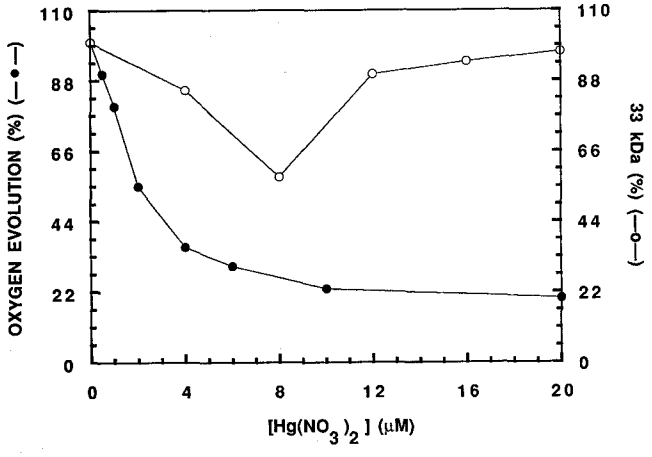

Fig. 4. Oxygen evolution (●) and EP33 content (○) of PSII preparations as a function of mercury concentration.

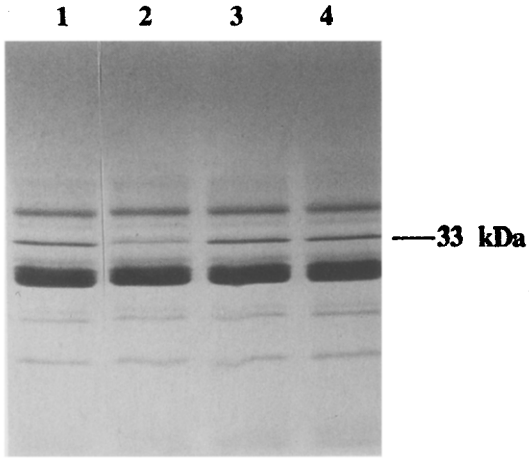

Fig. 5. Gel electrophoresis pattern of PSII preparations showing the effect of Cl^- on EP33 depletion. Lane 1, control PSII; lane 2, $HgCl_2$-treated PSII; lane 3, control PSII incubated with Cl^- (5 mM TMACL); lane 4, $HgCl_2$-treated PSII incubated with Cl^-.

It was previously shown that Cl^- prevents the inhibitory action of mercury [2]. The present study also demonstrates that this anion reduces the extent of EP33 depletion (Fig. 5). Thus, a possible mode of action that may be attributed to mercury inhibition is an interaction of Hg^{2+} (micromolar concentration) at a site that modifies Cl^- binding [2] and produces the release of EP33. Mercury is known for its high affinity for sulfhydryl groups of proteins. EP33, EP23, and many intrinsic polypeptides bear cysteins [22]. It will be important to evaluate if EP33 itself or another protein is modified by mercury. Labelling assays should clarify this question and should bring important information on the nature of the binding site(s) of EP33.

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