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# The donor side of Photosystem II as the copper-inhibitory binding site. Fluorescence and polarografic studies*

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*Key words:* copper toxicity, Photosystem II, Spinacia oleracea

**Abstract**

We have measured, under Cu (II) toxicity conditions, the oxygen-evolving capacity of spinach PS II particles in the Hill reactions $H_2O \rightarrow$ SiMo (in the presence and absence of DCMU) and $H_20 \rightarrow$ PPBQ, as well as the fluorescence induction curve of Tris-washed spinach PS II particles. Cu (II) inhibits both Hill reactions and, in the first case, the DCMU-insensitive $H_20 \rightarrow$ SiMo activity. In addition, the variable fluorescence is lowered by Cu (II). We have interpreted our results in terms of a donor side inhibition close to the reaction center. The same polarographic and fluorescence measurements carried out at different pHs indicate that Cu (II) could bind to amino acid residues that can be protonated and deprotonated. In order to reverse the Cu (II) inhibition by a posterior EDTA treatment, in experiments of preincubation of PS II particles with Cu (II) in light we have demonstrated that light is essential for the damage due to Cu (II) and that this furthermore is irreversible.

*Abbreviations:* DCMU-3-(3,4-dichlorophenyl)-l,1-dimethyl urea; DCIP-2,6-dichlorophenolindophenol; DPC-1,5-diphenilcarbazide; $F_o$-initial non-variable fluorescence; $F_I$-intermediate fluorescence yield; $F_m$-maximum fluorescence yield: $F_v$-variable fluorescence yield; Mes-2,-(N-morpholino)ethanosulfonic acid; OEC-oxygen-evolving complex; P680-Primary electron donor chlorophyll; Pheo-pheophytin; PPBQ-phenyl-p-benzoquinone; PS II- Photosystem II; SiMo-Silicomolybdate; $Q_B$-secondary quinone acceptor; QA-primary quinone acceptor; Tris-N-tris(hydroxymethyl)amino ethane; $Tyr_z$-electron carrier functioning between P680 and the Mn cluster

## Introduction

The Cu (II)-inhibitory site in PS II remains a controversial point regarding metal toxicity in photosynthetic electron transport (see reviews Droppa and Horvath 1990; Barón et al. 1995). Its elucidation has become important not only in relation to the ecological problem of heavy metal contamination, but also from a theoretical point of view, to clarify the role of the different components of the electron transport chain. Some authors locate the target of Cu (II)-inhibition in PS II on the oxidizing side. Samson et al. (1988) suggest an interaction of Cu (II) at or beyond the PS II primary electron carrier $Tyr_Z$. Shioi et al. (1978a,b) indicate that Cu (II) inactivates the PS II donor side after the DPC electron donating site. Vierke and Struckmeier (1977, 1978) point out that Cu (II) could inhibit a component very close to the oxygen evolving complex through a Cu (II)-binding to a membrane protein residue. Haberman (1969), Cedeño-Maldonado and Swader (1972) and Samuelson and Öquist (1980) also indicate that the most sensitive site of PS II for Cu (II)-inhibition is the oxidizing side. On the contrary, other studies conclude that Cu (II) influences the PS II electron transport on the acceptor side. Yruela et al. (1991, 1993) propose a target at the Pheo-QA-$Fe^{+2}$. Mohanty et al. (1989) suggest that Cu (II) binds to the reaction center and induces a structural alteration of the $Q_B$ binding protein with a loss in the $Q_B$ function. The PS II reaction center was also considered the Cu (II)-inhibitory binding site by Renganathan and Bose (1989) and Hsu and Lee (1988). Even the cytochromes and the non-heme $Fe^{+2}$ were proposed by Singh and Singh (1987) as tentative sites of Cu (II) action.

Despite all these studies the mechanism of Cu (II)-inhibition remains unclear. This may be due to the fact that our knowledge of PS II structure is still incomplete. Moreover, the uncertainty concerning the action sites of some electron donors and acceptors used to test the Cu (II)-inhibitory patterns, does not make the task any easier. In addition, some of those reagents and buffers used could interact chemically with Cu (II), thus producing unreliable results.

We have made an attempt to locate the site of Cu (II) inhibition by means of different techniques: flash-induced absorption spectroscopy (Schröder et al. 1994), EPR spectroscopy (Jegerschöld et al. 1995) and the polarographic and fluorescence experiments using for the present paper. Based on these works, we propose that Cu (II) creates a lesion on the PS II donor side, near the reaction center.

## Material and methods

*Preparation of PS II particles*

Spinach plants (*Spinacia oleracea* L.) were purchased in a local market. PS II particles capable of O2 evolution were prepared as in Berthold et al. (1981), with modifications by Barón et al. (1993) and Arellano et al. (1994), to eliminate nuclear contaminations. The PS II preparation was stored at -80 °C in 5 mM MgC12, 15 mM NaC1, 400 mM sucrose, 50 mM MES-NaOH buffer (pH 6.5).
Tris-treated PS II particles were obtained according to Ljungberg et al. (1986). After treatment, the particles were washed with 10 mM NaC1 and 20 mM MES-NaOH (pH 6.5) and suspended in the same buffer.

*Oxygen evolution measurements*
O2 evolution was measured with a Clark-type electrode fitted with a circulating water jacket at 20°C, under continuous illumination with saturating actinic red light. The standard assay medium was 10 mM NaC1, 20 mM MES-NaOH (pH 6.5 buffer), containing 0.4 mM PPBQ or 0.1 mM SiMo as electron acceptors and PS II particles with a chlorophyll concentration corresponding to a 10 $\mu$g Chl/ml for PPBQ and 20 $\mu$g/ml for SiMo assays. The assay medium was kept for 1 min in dark, except for SiMo which was added immediately before the light was turned on.

*Modulated fluorescence measurements*
Chlorophyll fluorescence induction curves of Tris washed-PS II particles were measured with a PAM fluorometer (Walz, Effeltrich, Germany), as previously described (Schreiber et al. 1986). The initial fluorescence ($F_o$) was determined using the PAM 101 unit with a light emitting diode applied at a frequency of 1.6 KHz. The integrated intensity of the modulated measuring beam was 10 mW/m², which was low enough to prevent appreciable induction transients. The maximal fluorescence ($F_m$) was determined using the PAM 103 unit. The modulated measuring beam had a frequency of 100 KHz and the saturating actinic blue light (Schott BG 37 filter) was emitted by a Schott cold light source (KL 1500/KL 1500-T). The fluorometer detector was protected by a far red cutoff filter (Schott RG-9). The kinetics were recorded with a computer program DA- 100, version 1.06a., supplied by Walz.
The standard assay medium was 10 mM NaC1, 20 mM MES-NaOH (pH 6.5) buffer containing 2 mM MnC12 as electron donor. Tris-washed PS II particles corresponding to a chlorophyll concentration of 10 µg/ml were also added.

*Copper, pH and DCMU effects*
The influence of pH on O2 evolution and fluorescence emission was tested using 10 mM NaC1, 20 mM MES-NaOH buffers in 5-7 pH range. Cu (II)-inhibitory concentrations from

5 μM to 100 μM were assayed for the different pHs. DCMU concentrations 1-100 μM were used to block the QB site.

The reversal of Cu (II)-induced inhibition was tested after incubation in dark of PS II particles with 30 μM Cu (II) during 90 s and a posterior pre-illumination of these samples with saturating actinic red light for 1-30 s. Then, EDTA 5 mM was added and, after 1 min incubation in dark, $O_2$ evolution was measured.

**Results**

The $O_2$ evolution of Cu (II)-treated PS II particles was determined using the $H_2O \rightarrow$ PPBQ and $H_2O \rightarrow$ SiMo Hill reactions. The rates for control preparations were, respectively, 540 and 95 μM $O_2$ mg $Ch1^{-1}$ $h^{-1}$. It is well known that substituted quinones are better electron acceptors than SiMo. The dependence of the oxygen evolution rate on SiMo concentration showed that 0.1 mM was the optimal concentration for the acceptor since the lower ones are not saturating and higher concentrations possess an inhibitory effect (preliminary assays not shown). The effect of toxic Cu (II) is shown in Fig. 1, where a decrease in PS II activity corresponds to an increase in Cu (II) concentrations. The I50 values for inhibition were 9 μM Cu (II) for $H_2O \rightarrow$ PPBQ and 20 μM for $H_2O \rightarrow$ SiMo. These differences will be discussed later.

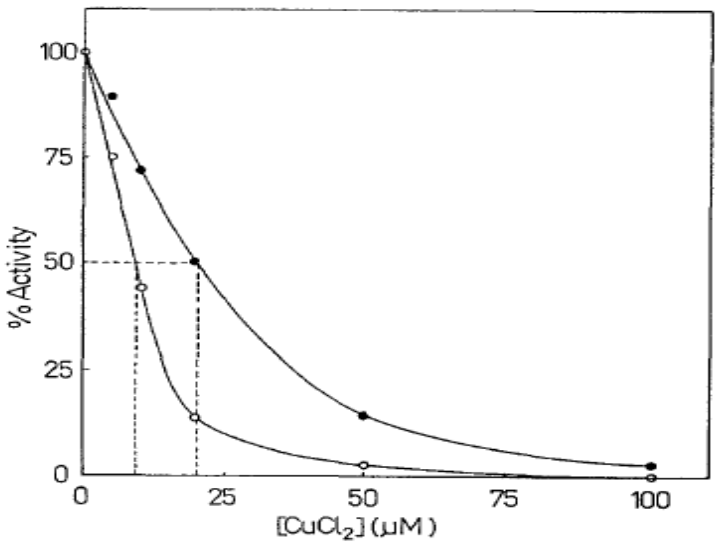


Fig. 1. Cu (lI)-inhibition of oxygen evolution of PS II particles in the $H_2O \rightarrow$ PPBQ (○) and $H_2O \rightarrow$ SiMo (●) Hill reactions.

In comparison with PPBQ, which is a typical electron acceptor on the $Q_B$ site, SiMo seems to accept electrons both at the $Q_B$ niche and at a previous site in the Pheo-$Q_A$-$Fe^{+2}$ non-

heme domain (Böger 1982; Graan 1986). Our PS II preparations exhibit about 50% of the SiMo-mediated PS II electron transport activity insensitive to DCMU, even when 100 μM DCMU was used (data not shown). When the toxic effect of Cu (II) on the $H_2O \rightarrow$ SiMo Hill reaction was studied in the presence of 20 μM DCMU that is enough to block the $Q_B$ niches, the inhibition curve was identical to the SiMo one shown in Fig. 1, and the activity decreases until a total inhibition at 100 μM Cu (II). That suggests that the Cu-induced inhibition may take place before a SiMo-binding place which is DCMU-insensitive

The pH value of the assay medium was critical for the study of the inhibitory effect of Cu (II) on the Hill reactions. The pH dependence pattern of $O_2$- evolving capacity in PS II particles, with PPBQ as electron acceptor (Fig. 2), shows an optimal value at pH 6-7, whereas higher or lower pHs inhibit the activity. Thus, the toxic effect of copper was studied at 9 μM Cu (II) ($I_{50}$ at pH 6.5). Figure 2 also displays the influence of pH on the Cu (II) inhibitory effect on $O_2$ evolution. For these assays we considered a 100% value the corresponding activity in the control experiments at the same pH value. The toxic effect decreased with the pH and almost completely disappeared at pH 5.0. In this figure, alkaline pH is not shown as this pH range interferes with the Cu (II) solubility and is not relevant for our studies. Schlodder and Meyer (1987) have described a negative effect of alkaline pHs on the OEC, due to a disorganization of the Mn cluster.

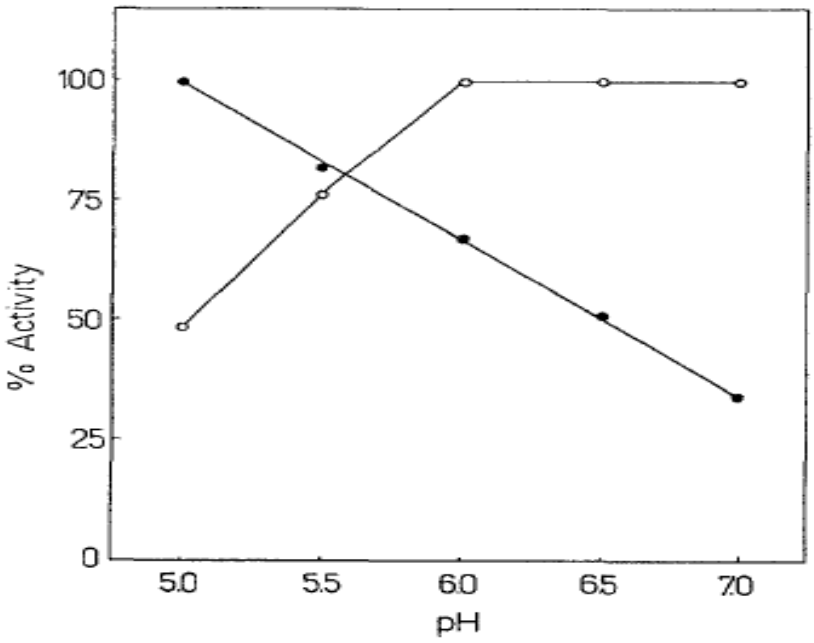


Fig. 2. Effect oh pH on oxygen evolution of PS II particles in the H2O → PPBQ reaction with (○) or without (●) Cu (II). The Cu (II) concentration is 9 raM, the 150 at pH 6.5.

The effect of Cu (II) on the fluorescence induction of Tris-washed PS II particles is shown in Fig. 3. Tris washing of PS II particles resulted in a simultaneous loss of oxygen evolution and variable fluorescence ($F_v$). The remaining fluorescence could be attributed to the 'inactive' $Q_B$-non reducing centers (Hsu and Lee 1991; Cao and Govindjee 1990). Using Lavergne's nomenclature (Lavergne 1982), PS II-$Q_B$ non reducing centers are photochemically competent, but unable to transfer electrons efficiently from $Q_A$ to the secondary electron acceptor, $Q_B$. This feature distinguishes them from the plastoquinone reducing centers, $Q_B$ reducing centers. $F_v$ was recovered by using 2 mM $MnCl_2$ as electron donor, with a ratio $F_m/F_o$ of about 4. The initial fluorescence ($F_o$) and $F_v$ are lowered by copper, although the rise kinetics were less sensitive. The 150 value for Fv inhibition was 30 μM Cu (II), higher than that observed for the Hill reactions $H_2O$ SiMo and $H_2O \rightarrow$ PPBQ. The $F_o$ decrease could be a consequence of a non-specific quenching due to Cu which is a paramagnetic ion. There is a Cu (II)-insensitive component in $F_v$, which was not inhibited even at 100 μM, a concentration that totally inhibited the oxygen-evolving capacity of PS II particles. This could be explained by PS II reaction centers which are not inhibited by Cu (II), presumably photochemically competent but ineffective in net electron flow and water oxidation. In this case, there is a similarity between the effect of Cu and Tris on PS II.

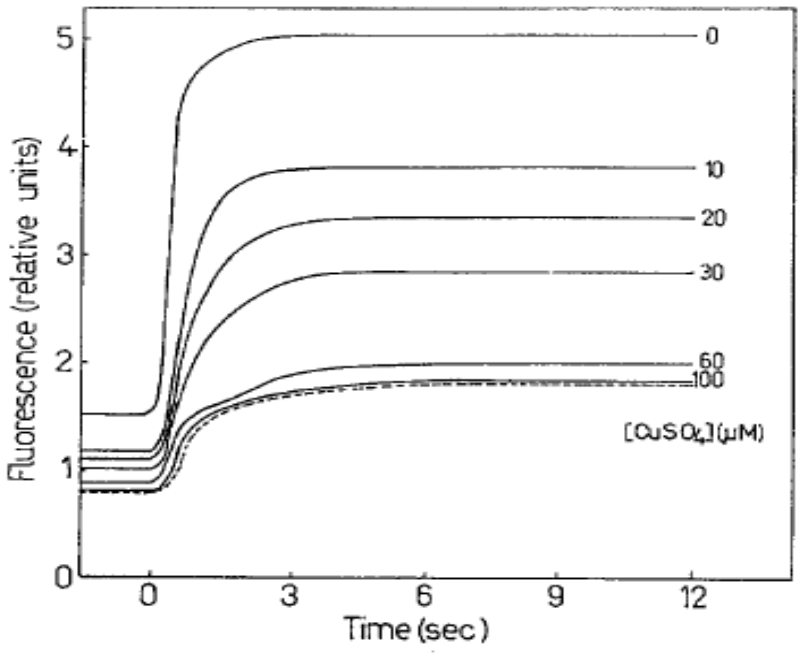


Fig. 3. Effect of Cu (II) on the fluorescence induction kinetic of Tris-washed PS II particles in the presence of 2 mM $MnCl_2$ as electron donor. The broken line represents the fluorescence induction in the absence of $MnC1_2$ and copper.

We have also tested pH influence on the fluorescence induction kinetic of Tris-washed PS II particles, using 2 mM $MnCl_2$ as electron donor (Fig. 4), as well as the effect of toxic Cu (II) at different pH levels on $F_v$ inhibition in the same preparations (Fig. 5). In the first case, $F_o$ was unaffected in the pH range 5-7, changes in $F_v$ were slight and the kinetics rise was slowed down by the pH decrease. In the second set of experiments, Tris-washed PS II particles treated with 30 μM Cu (II) ($I_{50}$ value for $F_v$ inhibition at pH 6.5), were more sensitive with pH rise. A detailed analysis of the fluorescence induction kinetics of Fig. 5 also shows that the fluorescence rise from $F_o$ to $F_I$ (fluorescence intermediate yield), is virtually unaffected by Cu at different pH values. This fluorescence component is attributed to the $Q_A$ reduction in the PS II $Q_B$-non reducing centers and we have already mentioned that it is not suppressed by Tris washing (Hsu and Lee 1991; Govindjee 1990) neither by other treatments which also result in a irreversible inhibition of oxygen evolution (Melis 1985; review Melis et al. 1991). However, the increase from F~ to Fro, probably due to the activity of the $Q_B$-reducing centers, appears more sensitive to Cu (II) at high pHs. This strengthens the former arguments that some reaction centers are less sensitive to Cu (II) toxicity.

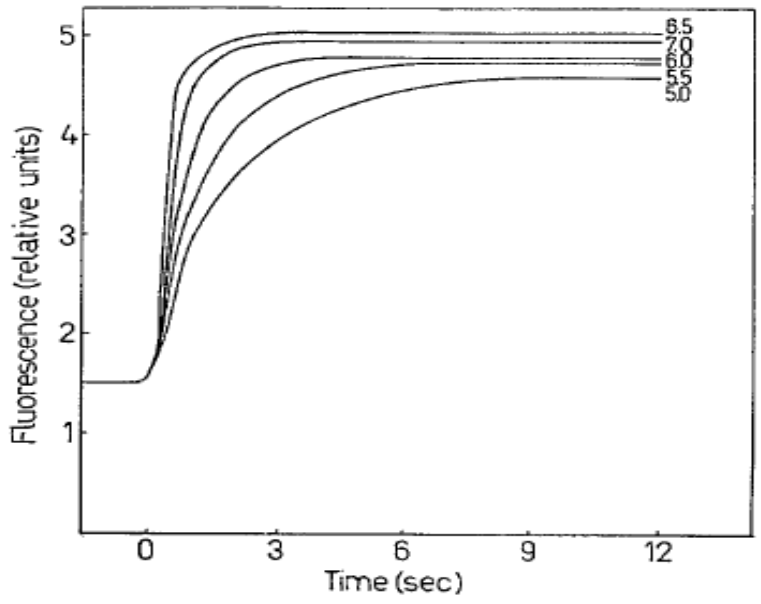


Fig. 4. Influence of pH on the fluorescence induction kinetic of Tris-washed PS II particles with 2 mM $MnC1_2$ as electron donor.

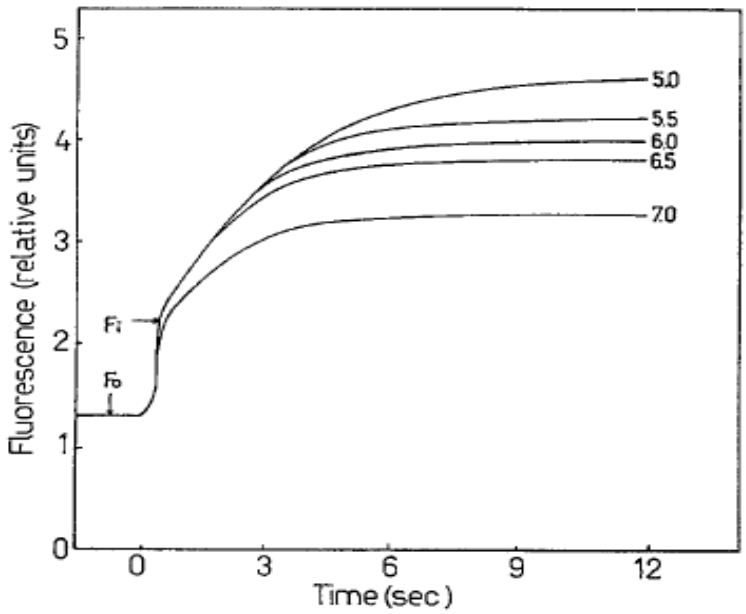


Fig. 5 Influence of pH on the fluorescence induction kinetic of Tris-washed PS II particles with 2 mM $MnCl_2$ as electron donor, in the presence of 30 mm Cu (II) $I_{5o}$ for $F_v$ inhibition at pH 6.5).

The effect of pH on PS II inhibition, both of $O_2$ evolution and fluorescence, indicates that Cu (II) and $H^+$ might compete for the same binding site. The fact that PS II preparations, with an inactivated OEC by Tris wash, are also sensitive to toxic Cu (II), challenges that the donor-side effect described in this work originates from a limited donor capacity.

Another polemic point proved to be the reversibility and light-dependence of Cu (II) toxicity. We have incubated PS II particles with 30 μM Cu (II) in the dark, which were then pre-illuminated for different periods of time, before a treatment with 5 mM EDTA, a Cu (II)-chelating agent. The reversal by EDTA of Cu (II)-inhibition (Fig. 6) was determined by measuring the $O_2$ evolving capacity ($H_2O \rightarrow$ PPBQ). The control corresponds to a PS II preparation without Cu (II) addition, whereas the lowest trace refers to PS II particles treated by 30 μM Cu (II) but without EDTA treatment. The reversibility of the inhibition by EDTA was shown to be dependent on the incubation time with Cu (II) in light. After exposing the Cu (II)-treated PS II particles to preillumination periods longer than 30 s, the damage was totally irreversible, even after EDTA incubation longer than 1 rain (data not shown).

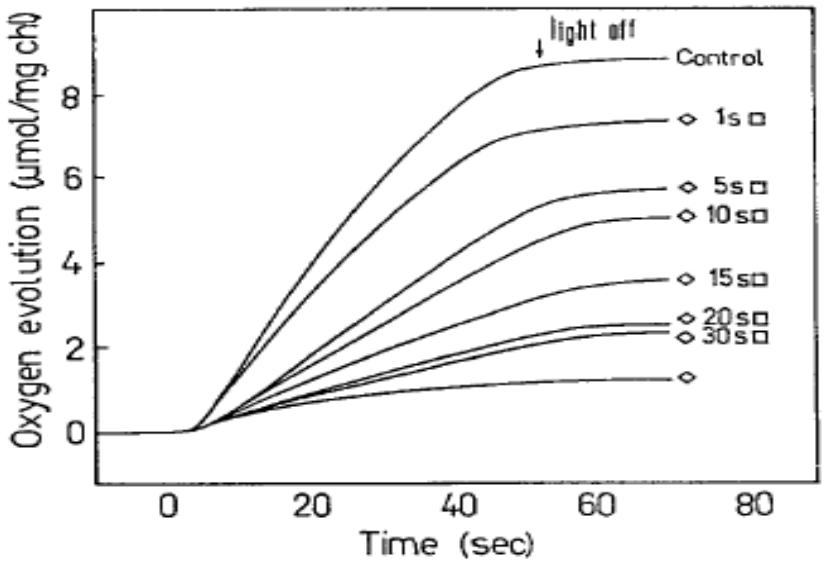


Fig. 6. Analysis of the oxygen evolution ($H_2O \rightarrow$ PPBQ) of PS II particles incubated in dark with 30 mM Cu (II) during 90 s (◊), preilluminated at different period of times (1-30 s), and finally treated with 5 mM EDTA (□). The control corresponds to a PS II preparation without Cu (II) and the lowest trace to a Cu (II)-treated one without EDTA treatment.

**Discussion**

Our results regarding $O_2$-evolving capacity ($H_2O \rightarrow$SiMo and $H_2O \rightarrow$ PPBQ) of PS II particles and fluorescence induction curves of Tris-washed ones, in the presence of toxic Cu (II) concentrations, exclude the $Q_B$ site and the water splitting system as the main Cu (II)-inhibitory targets in PS II. A second conclusion is the dependency of the toxic effect on the pH, which would suggest that Cu (II) can interact with amino acid residues which can be protonated and deprotonated. In addition, our results clearly show that Cu (II) damage on the electron transport chain is lightdependent, becoming totally irreversible after 30 s illumination.

It is also worth noting that Cu (II) inhibits the DCMU-insensitive $H_2O \rightarrow$ SiMo activity. These results are similar to those obtained by Renganathan and Bose (1989). SiMo is an artificial electron acceptor with a very controversial binding site in PS II. However, this acceptor has been long considered the only specific one for testing the PS II donor side in a Hill reaction (Barber et al. 1987; Takahashi et al. 1987; Yruela et al. 1991).

In conjunction with data provided by chlorophyll fluorescence we can obtain more reliable conclusions. The decrease of Fv obtained in the Tris-washed preparations treated with Cu

(II), is usually interpreted in terms of inhibition in the PS II donor side (Schreiber and Neubauer 1987; Hsu and Lee 1988, Renganathan and Bose 1989; Krieger et al. 1992; Renger et al. 1993). This may be consistent with the inhibition of the DCMU-insensitive $H_2O \rightarrow$ SiMo activity.

Since Cu (II) inhibits the activity of a PS II with an oxygen-evolving complex previously inactivated by Tris washing or heat treatment (present paper; Hsu and Lee 1988; Mohanty et al. 1989; Renganathan and Bose 1989), the PS II Cu (II)-inhibitory site, must be located on the donor side, close to the reaction center.

The clear influence of pH on Cu (II) inhibition provides some indications as to the nature of the Cu (II)-binding site. Steemann-Nielsen et al. (1969) and Yruela et al. (1992) also found that PS II is more sensitive to Cu (II) inhibition with pH rise, suggesting that Cu (II) and $H^+$ compete for the same binding site. One reliable explanation might be that of a metal interaction with some amino acids which are susceptible to being protonated and deprotonated. Yruela et al. (1992, 1993) located these in the Pheo-$Q_A$-$Fe^{2+}$ domain. We have demonstrated that the optimal pH range for the oxygen evolution of PS II particles is 6-7, whereas lower and higher pHs have a negative effect. Schlodder and Meyer (1987) considered that the inhibition at acid pHs is reversible and due to the protonation of a group on the donor side with a pka about 4.5. Therefore, in the experiments with Cu (II)-treated preparations, a recovery of the activity at increasing pHs would be expected. However, inhibition increases even at the optimal pHs, thus supporting the idea of $H^+$ and Cu (II) competing for the same ligand on the donor side. We have also shown in control preparations that $F_v$ did not change in the pH range of 5-7, but the rise kinetics slowed down at decreasing pHs values. This might be related to a slower kinetic donation of $Tyr_z$ to P680 (Conjeaud and Mathis 1980). In Cu (II)-treated preparations, an increase was not observed in the rise kinetics of $F_v$ parallel to the pH increase. This could indicate that the electron donation from $Tyr_z$ to P680 is still blocked by the action of Cu (II). Our recent experiments by flash-induced absorption spectroscopy in PS II particles, core complexes, as well as, in Tris washed preparations, suggests an inhibition by Cu (II) of the electron transfer between $Tyr_z$ and P680 with no changes in the charge separation between P680 and $Q_A$ (Schröder et al. 1994). The effect of Cu remained by the addition of the exogenous PS II donor, $NH_2OH$. The fact that Tris-washed PS II revealed in all our experiments a similar behaviour to the oxygen-evolving one in relation to Cu (II), shows that the effect by toxic Cu (II) described in this work is not related to a limited donor capacity from the OEC.

To interpret the donor-side dependent quenching of the fluorescence induced by Cu (II), different mechanisms could be discussed: Accumulation of P680 radical cation and

dissipative photochemical quenching via electron flow around PS II, involving alternate donors to P680.

In the first case, it was reported (Butler 1972) that $P680^+$ is a strong fluorescence quencher. Treatments which primarily affect components at the PS II donor side (Tris washing, heat) are shown to suppress Fm and to slow down the reduction rate of $P680^+$ by the donor side (Schreiber and Neubauer 1987). We have already shown that Cu (II) and Tris treatments have similar effects. Both slow down the $P680^+$ reduction with a shift of the ns kinetics to the μs-time domain. However, whereas Cu blocks the electron transfer from $Tyr_z$ to P680, Tris only retards it (Schröder et al. 1994). In these conditions the development of P680 quenching could be possible.

The second mechanism is developed when normal electron donation to $P680^+$ is inhibited and the reaction center could also be reduced by alternate donors. This would also be plausible for Cu (II), because we have recently seen by EPR spectroscopy that Cu (II) treatment of PS II preparations induced the appearance of a new, as yet unidentified donor which could reduce $P680^+$ (Jegerschöld et al. 1995).

Our results regarding a Cu (II)-insensitive component of $F_v$ agree with those of Renganathan and Bose (1989), who propose that Cu (II) does not inhibit a fraction of PS II centers, which contribute to $F_v$ but do not participate in $Q_B$ reduction. The relative insensitivity of the rise kinetics of $F_v$ to Cu (II), mainly from $F_o$ to $F_I$, corresponding to the activity of $Q_B$-non reducing centers, in our pH dependence experiments, also support this theory. Samson et al. (1988) likewise suggested that PS II reaction centers are heterogeneous in their susceptibility to the inhibitory effect of Cu (II), since they found that the quantum yield of PS II photochemistry was lowered and the rate of $Q_A$ photoreduction remained unaffected by Cu (II). Some reports have shown the different sensitivity to some stress factors (i.e. photoinhibition) between the two PS II subpopulations (Mäenpää et al. 1987; Briantais et al. 1988; Lapointe et al. 1993). They support that the Photosystem II heterogeneity plays a role in the recovery of PS II after stress-induced damages and the $Q_B$-non reducing centers are involved in different mechanisms of limiting damage (cyclic electron flow, D1 repair cycle, etc.) (Falkowski et al. 1986; Neale and Melis 1990; Melis 1991).

In our case, preliminary results concerning the activity of $Q_B$-reducing and non-reducing centers under Cu (II) toxicity conditions, show that the relative amount of PS II non-reducing centers increases with the Cu concentration.

The results presented in this paper and in two recent works show a Cu (II)-inhibitory site on the PS II donor side very close to the reaction center. However, this does not exclude the possibility of Cu action at other sites in the electron transport chain. Some secondary

effects of copper upon incubation at high concentration either on the PS II acceptor side or on the OEC can not be discounted.

In the first case, the possibility that Cu (II) influences the $Q_B$ function by affecting components beyond $Q_B$ can be ruled out, because we have recently demonstrated by different biophysical methods, that the charge separation between $P680^+$ and $Q_A$ is not affected by Cu (II). The use of DCBQ as electron acceptor in some assays does not change this result. A secondary modification at the $Q_B$ site, reflected in experiments by Renger et al. (1993) by changes of the affinity to atrazine binding-site without affecting the number of binding-sites is, taking into account our results, a reasonable possibility. In agreement with Mohanty et al. (1989), we could suggest that the Cu (II)-binding in the neighbouring of the reaction center, for example near $Tyr_z$, could induce indirect alterations of the $Q_B$ site. The fact that the $I_{50}$ value for the inhibition by Cu of the reaction $H_2O \rightarrow$ SiMo is higher than the $I_{50}$ of the reaction $H_2O \rightarrow$ PPBQ could be explained by the existence of this secondary action site. Renganatan and Bose (1989) using SiMo and DCIP as electron acceptors justified the differences between the $I_{50}$ values for Cu inhibition by the existence of PS II centers insensitive to the Cu action.

Regarding OEC disturbances induced by Cu (II), we have seen in our EPR experiments (Jegerschöld et al. 1995), a disorganization of this complex at higher Cu concentrations than those used in the present work.

However, the toxic effect by Cu on the PS II electron transport that we have described in different reports could not be explained by the $Q_B$-site or the OEC as main targets.

The irreversibility and light-dependence of the Cu (II) action on PS II agree with the works by Steemann-Nielsen (1969), Cedeño-Maldonado and Swader (1972), and those of Samuelson and Öquist (1980). On the contrary, Haberman (1969), Gross et al. (1970) and Gupta (1986) showed a reversibility by addition of $Mn^{+2}$, whereas Hsu and Lee (1988) demonstrated a recovery of activity after centrifugation to eliminate the excess Cu (II). The explanation was given by Renganathan and Bose (1989, 1990), who found that different buffers commonly used to test Cu (II) toxicity in chloroplasts and PS II particles bind Cu (II) in a high proportion and prevent it reaching its target. Under such conditions it is relatively easy to reverse the action of toxic Cu (II). In our assays we have avoided buffers and electron acceptors that react with Cu (II). The increase in the damage with the time of light exposure that we have found and the consequent unrecovery of the PS II activity by EDTA, 133 showed that Cu (II) inhibition was light-stimulated. We suggest that the donor-side inhibition by Cu generates some kind of photoinhibitory damage on PS II that is enhanced by light. We have already shown that some common responses of PS II to Cu

toxicity and photoinhibition (the insensitivity of some PS II centers, the appearance of new electron donors to the reaction center, etc) could exist.

In conclusion, our results suggest that Cu (II) blocks the electron transport on the donor side of PS II, which results in irreversible damage that is enhanced by light. Our studies reconcile the donor-side and reaction center theories of Cu (II)-target on PS II. However, we believe that Cu could interfere with several sites on PS II. In view of the findings reported here, a re-examination of the earlier data and conclusions is appropriate.

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