Copper effect on the protein composition of photosystem II

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Abbreviations – Chl, chlorophyll; CP43 and CP47, antenna complexes of photosystem II: DCBQ, 2,6-dichlorobenzoquinone; EPR, electron paramagnetic resonance; Fm, maximum Chl *a* fluorescence; Fo, mínimum Chl *a* fluorescence; Fv, variable Chl a fluorescence; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulphonie acid; MES, 2-(N-morpholino)ethanesulphonic acid; $P_{680,}$ primary electron donor of photosystem II reaction centre; PFD, photon flux density; Pheo, pheophytin; PS, photosystem; Q_A and $Q_{B,}$ primary and secondary plastoquinone electron acceptors of photosystem II; RC, reaction centre; SDS, sodium dodecyl sulphate; Tyr_z tyrosine 161 of the D1 polypeptide of photosystem II.

Abstract

We provide data from *in vitro* experiment on the polypeptide composition, photosynthetic electron transport and oxygen evolution activity of intact photosystem II (PSII) preparations under Cu(II) toxicity conditions. Low Cu(II) concentrations (Cu(II) per PSII reaction centre unit < 230) that caused around 50% inhibition of variable chlorophyll *a* fluorescence and oxygen evolution activity did not affect the polypeptide composition of PSII. However, the extrinsic proteins of 33, 24 and 17 kDa of the oxygen-evolving complex of PSII were removed when samples were treated with 300 μM $CuCl_2$ (Cu(II) per PSII reaction centre unit = 1400). The LHCII antenna complex and D1 protein of the reaction centre of PSII were not affected at these Cu(II) concentrations. The results indicated that the initial inhibition of the PSII electron transport and oxygen-evolving activity induced by the presence of toxic Cu(II) concentrations occurred before the damage of the oxygen-evolving complex. Indeed, more than 50% inhibition could be achieved in conditions where its protein composition and integrity was apparently preserved.

Introduction

A significant part of the environmental pollution caused by modern human activities arises from the release of heavy metals into the environment. Some heavy metals such as Cu are essential as micronutrients, however, they are toxic at high concentrations for photosynthetic organisms (Clijsters and van Asche 1985, Maksymiec 1997). The copper-inhibitory effect on photosynthetic electron transport has been previously reported in planta, green alga and cyanobacteria. Extensive *in vitro* studies have shown that photosystem II (PSII) is more susceptible to copper toxicity (for review sea Droppa and Horváth 1990, Barón et al. 1995a) than photosystem I (PSI) (Ouzounidou et al. 1997). However, the precise location of the Cu(II)-binding site on PSII and the underlying mechanisms of copper inhibition are still the subject of debate. Both the acceptor and donor side have been proposed as copper-inhibitory sites. On the PSII reducing side, the Q_B binding site (Mohanty et al. 1989) and the Pheo-Fe-Q_A, domain (Yruela et al. 1991, 1992, 1993, 1996a) have been reported as the most sensitive sites for Cu(II) toxicity. In addition, the copper ion was shown to impair the function of the oxidising side (Cedeño-Maldonado and Swader 1972, Vierke and Struckmeier, 1977, Shioi et al. 1978a,b, Bohner et al. 1980, Samuelsson and Öquist 1980). Some authors (Schröder et al. 1994, Arellano et al. 1995) have suggested that the electron flow from tyrosine (Tyr_Z) to $P680^+$, is blocked at toxic Cu(II) concentrations. Králová et al. (1994) and Sersen et al. (1997) have proposed that Cu(II) interacts not only with Tyr_z, but also with Tyr_D on D2 protein. A possible direct interaction between copper and calcium at the oxidising side of PSII was also shown both *in vitro* (Sabat, 1996) and *in vivo*

(Maksymiec and Baszynski, 1999). Additional effects of Cu(II) toxicity on both the donor side, affecting the Mn-cluster and the extrinsic proteins of the oxygen-evolving complex, and the acceptor side, interacting with the non-heme Fe^{2+} have been reported (Renger et al. 1993, Jegerschóld et al. 1995, 1999, Sersen et al. 1997). The interaction of Cu(II) toxicity with photoinhibitory and recovery proceses on PSII has been also investigated (Yruela et al. 1996b, Pätsikkä et al. 1998) demonstrating how Cu(II) enhances the adverse effects of light.

At present, most of the evidente for Cu(II) inhibition of the photosynthetic activity come from experiments *in vitro*. An extensive analysis of the literature reveals that the experimental conditions used in different publications differ significantly, particularly the copper concentration. The ratio between Cu(II) and the PSII reaction centre (RC) unit (Cu(II)/PSIIRC) was very variable depending on the techniques used in the measurements (i.e. oxygen evolution activity, fluorescence, flash-induced absorption spectroscopy, thermoluminescence, electron paramagnetic resonante (EPR), Fourier transform infrared (FTIR) spectroscopy, photoacoustic. This fact makes it difficult to compare and interpret the published results and makes it necessary to distinguish Cu(II) effects *in vitro* on PSII at low and high Cu(II) concentrations. It is also needs to be established which physiological effects of copper on photosynthesis *in vivo* result from inhibition of the electron transport on PSII.

It was shown *in vivo* that the decrease of the photochemical activity caused by Cu(II) was accompanied by an alteration of the structure and composition of the thylakoid membranes, which can influence the conformation and function of the photosystems (Baszynski et al. 1988, Ouzounidou et al. 1992, Lidon et al.

1993). Baszynski and Krupa (1995) have proposed that those proceses induced by different heavy metals could involve either the destruction of the oxygen-evolving complex polypeptide composition or the interaction with ions necessary for proper functioning of the complexes such as Mn^{2+}, Ca^{2+} and Cl^{-}.

Considering that loss of oxygen-evolving complex extrinsic proteins induced by toxic Cu(II) concentrations is a mechanism that can operate *in vivo* and *in vitro*, we have addressed the question of how different Cu(II)/PSIIRC ratios affected both the PSII activity and the integrity of the oxygen-evolving complex, correlating the inactivation of the photosynthetic activity of PSII with changes in the polypeptide composition of PSII in the presence of Cu(II) from *in vitro* experiments.

Materials and methods

Preparation of thylakoids and oxygenic PSII membranes

Thylakoids and oxygenic PSII membranes were prepared from market spinach. Thylakoids were isolated as described by Nelson et al. (1970) with some modifications (Yruela et al. 1991). Tricine was used instead of N-2-hydroxyethylpiperazine-N'-2-ethanesulphonie acid (HEPES)-NaOH to avoid chemical interference with Cu(II) (Renganathan and Bose, 1990). The thylakoid fraction was resuspended in 10 mM NaCl and 10 mM HEPES-NaOH (pH 7.5) at a chlorophyll (Chl) concentration of 1.5 mg ml^{-1}. Oxygenic PSII membranes were isolated by the method of Berthold et al. (1981) with the

modification of Yruela et al. (1991). Samples were resuspended in 0.4 M sucrose. 15 mM NaCl. 5 mM $MgCl_2$, and 50 mM 2-(N-morpholino)ethancsulphonie acid (MES)-NaOH (pH 6.0). All purification steps performed at 4°C under dim light. Chl *a* concentration was determinad as described by Arnon (1949). Samples were frozen in liquid N_2 and stored at - 80°C until use. Thylakoids and PSII membranes preparations exhibited oxygen evolution rates of 240 and 560 µmol 0_2 mg^{-1}Chl h^{-1}, respectively, in the presence of 0.5 mM 2.6-dichlorobenzoquinone (DCBQ) as artificial electron acceptor.

Inhibition with Cu(II)

Thylakoids were resuspended at a final Chl concentration of 10-15 µg ml^{-1} in 10 mM NaCl and 25 mM HEPES-NaOH (pH 7.5). PSII membranes were resuspended at a final Chl concentration of 10 µg ml^{-1} in 10 mM NaCl and 25 mM MES-NaOH (pH 6.5). Both types of samples were dark-adapted for 30 min at 4°C and incubated with 0-40 µM $CuCl_2$, for 20 min at 4°C with stirring before both fluorescence and oxygen evolution activity measurements.

Oxygen evolution activity

Oxygen evolution activity was measured with an oxygen electrode (Hansatech, Norfolk, UK) fitted with a water jacket thermostated at 25°C. The standard assay medium consisted of 300 mM sucrose, 10 mM NaCl and 25 mM MES-NaOH (pH 6.5). DCBQ at a saturating concentration of 0.5 mM was used as an artificial electron acceptor. Actinic light from two projector lamps

placed on both sides of the electrode cuvette was filtered through 9.5 cm of water. The photon flux density (PFD) on the surface of each side of the sample cuvette was 2200, 950 and 450 µmol m^{-2} s^{-1}. DCBQ was disolved in ethanol and added just before measuring oxygen evolution activity. The ethanol concentration in the sample was less than 0.1%.

Chl *a* fluorescence induction

Chl *a* fluorescence induction curves were recorded with a Hansatech photodiode detector connected to a digital storage oscilloscope. The sample was illuminated with a blue green actinic light from a 150 W tungsten lamp powered with a stabilised power supply and passing through 1 KG1 and 3 KG3 Schott infrared filters plus a 620 nm low-pass filter. Light was passed through a Copal photographic shutter (2 ms opening time) and a Schölly fibre optic guide. The detector was protected by a 3 mm Schott RG 665 filter plus a 680 nm (10 nm bandpass) interference filter. The sample was kept stirred in an oxygen electrode cuvette and the PFD was 1000 µmol m^{-2} s^{-1} on the surface of the cuvette. This PFD was high enough to reduce the PSII acceptor side in cells and thylakoids. PSII fluorescence was monitored for 1.5 s. All measurements were done at 24°C.

SDS-PAGE of PSII proteins and immunological techniques

Samples at 150 µg Chl ml^{-1} were treated with 5, 50 and 300 µM $CuCl_2$ for 20 min

in the dark at 4°C, centrifuged at 45000 x *g* for 60 min and both the sediment and the supernatant were taken. SDS-PAGE of the different SDS-PAGE fractions was carried out basically as in Laemmli (1970). Equal amounts of PSII membranes and supernatant liquid were loaded onto the gel. We ran 12-22% acrylamide linear gradient gel containing 4 M urea in the resolving gel. Samples were denatured at room temperature in sample buffer containing 50 mM Tris-OH (pH 7.2), 2% sodium dodecyl sulphate (SDS), 2 M urea and 40 mM dithiothreitol. Gels were stained with Coomassie brilliant blue R-250. Protein concentration was determined by the method of Markwell et al. (1981). For immunoblotting, the same fractions obtained after Cu(II) treatment were analysed under denaturing conditions in continuous 15% acrylamide gels in the presence of 4 M urea. Gels were transferred onto nitro-cellulose membranes with a BioRad transfer system and probed with antiserum against the LHCII antenna complex, the D1 and the 33 kDa extrinsic protein of PSII raised in rabbit against the same proteins from spinach. Bands were revealed by the peroxidase method.

Results

The effect of Cu(II) concentration on the oxygen evolution activity of PSII membrane and thylakoid preparations was measured (Fig. 1). To calculate the Cu(II) per PSII reaction centre unit (Cu(II)/PSIIRC) ratio in PSII membranes we considered 250 Chl/RC (Berthold et al. 1981). The loss of activity by Cu(II) action was faster in PSII membranes compared to thylakoids. The finding was

independent of the light intensity used during the measurements. A 50% inactivation occurred with ~7 μM (Cu(II)/PSIIRC = 230) and ~27 μM $CuCl_2$, in PSII membranes and thylakoids, respectively. Such differences could be due to the fact that the Cu(II) binding site is more accessible in PSII-enriched membranes than in thylakoids due to their different morphology and/or composition. The fact that similar inactivation was measured using high and low light intensities for each system supports this suggestion. According to that, we have also observed that the dependence of the incubation time on the Cu(II) inactivation differed between both type of samples (Fig. 2). The Cu(II) effect on chlorophyl a fluorescence parameters was also measured. Fig. 3A displays the Fo and Fv/Fm values of the thylakoid preparations incubated with variable amounts of $CuCl_2$. Thylakoids with no addition of Cu(II) had a Fv/Fm value of 0.79 which is indicative of an undamaged photosynthetic apparatus (Krause and Weis 1991). The variable fluorescence (Fv) and Fv/Fm (Fig. 3A, inset) was markedly reduced with increasing Cu(II) concentrations. However, the initial fluorescence (Fo) remained unchanged (data not shown). These data are in accordance with those reported by other authors (Shioi et al. 1978b, Bohner et al. 1980. Hsu and Lee 1988, Samson et al. 1988, Mohanty et al. 1989). A 50% inhibition of Fv occurred in the presence of ~20 μM $CuCl_2$, whereas only~7 30% of PSII are able to emit variable fluorescence after treatment with 40 μM $CuCl_2$. The results were consistent with those showed in Fig. 1. Addition of Cu(II) to a suspension of thylakoids pre-incubated with DCMU also decreased the Fv and Fv/Fm values (Fig. 3B) in agreement with oxygen evolution measurements reported in previous works (Yruela et al. 1991, 1992). The slightly less inhibition in the presence of DCMU could be explained by Cu(II) having a

high affinity for ligands with amine groups (Renganathan and Bose 1990) such as DCMU, thus decreasing the active Cu(II)-inhibitory concentration in the sample.

Fig. 4 shows the Cu(II) effect on the polypeptide composition of oxygen-evolving PSII preparations. Samples were incubated in the dark with 5, 50 and 300 μM $CuCl_2$ corresponding to a Cu(II)/PSIIRC ratio of 23, 230 and 1400, respectively. After Cu(II) treatment the PSII suspensions were centrifuged to separate the released proteins, and the pellet and supernatant fractions were analysed by SDS-PAGE. The results are representative of 3 independent experiments. Fig. 4A shows that 50 μM $CuCl_2$ (Cu(II)/ PSIIRC = 230) does not remove any specific polypeptides of PSII, since the supernatant fractions obtained after Cu(II) incubation did not contain any free proteins (Fig. 4A, lanes 3 and 5). However, the highest concentration used, 300 μM $CuCl_2$ (Cu(II)/PSIIRC = 1400), caused the release of the 33, 24 and 17 kDa polypeptides (Fig. 4A, lane 7), that could correspond to the extrinsic proteins that stabilise the oxygen-evolving complex and regulare its activity (Enami et al. 1989, 1991, Hankamer and Barber 1997). In these conditions, the loss of a proteins with apparent weight approximately of 43 and 47 kDa were also observed (Fig. 4A, lane 6). The loss of these proteins differed from each other. The fact that the 43 kDa protein lost due to Cu(II) action did not appear in the soluble phase (Fig. 4A, lane 7) could indicate that it is degraded during the treatment. In contrast, the 47 kDa was lost by release to the soluble phase. Interaction between the 47 and 33 kDa proteins of the oxygen-evolving complex has been shown (Bricker et al. 1988, Hankamer and Barber 1997). Barón et al. (1995b) reported that Cu(II) stress *in vivo* had an effect on proteins associated with the oxygen-evolving

complex. The immunoblot assays confirmed that the 33 kDa protein released after incubation of the PSII preparations with 300 μM $CuCl_2$ (Fig. 4A and B, lane 7) corresponds to the extrinsic protein of the oxidising side of PSII, and show that this protein is only affected when PSII membranes are incubated with high Cu(II) concentrations. The effect of Cu(II) on the LHCII antenna complex and D1 protein of PSII was also analysed, being both unaffected even in the presence of the highest Cu(II) (Fig. 4B). These data indicate a specific effect of high Cu(II) concentration on the oxygen-evolving complex proteins. The oxygen evolution activity of the samples analysed by SDS-PAGE was also measured (Table 1). The data indicated that a initial inhibition of photosynthesis as oxygen evolution activity occurs before any damage in the protein composition of the oxygen-evolving complex of PSII and at least 50% activity can be inhibited without affecting its integrity.

Discussion

The copper effect on the electron transport activity of PSII *in vitro* has been reported (sea reviews Droppa and Horváth 1990. Barón et al. 1995a). In those works, thylakoids, oxygenic and non-oxygenic PSII-enriched membranes were used. Based on those investigations both the acceptor (Yruela et al. 1991, 1993, 1996a) and the donor sides (Schröder et al. 1994, Arellano et al. 1995, Horváth et al. 1998) of PSII were proposed as the primary target of the Cu(II) inhibition.

The concentrations of Cu(II) and Chl in the inhibitory treatments reported in the literature differ depending on the techniques used for the

measurements. concentrations of Cu(II) and Chl between 5 and 100 μM and 6 and 115 μM, respectively, have been reported in oxygen evolution (Yruela et al. 1991. 1993). fluorescence (Arellano et al. 1995, Yruela et al. 1996a), flash-induced absorption spectroscopy (Schröder et al. 1994), thermoluminiscence (Horváth et al. 1998), FTIR spectroscopy (Szalontai et al. 1999) and photoacoustic (Boucher and Carpentier 1999) measurements. Those concentrations provided a variable Cu(II)/PSIIRC ratios from 210 to 700, being difficult to compare the results from different experiments. Moreover, in EPR measurements (Jegerschöld et al. 1995, 1999) the amounts of Cu(II) and Chl were even higher, giving a Cu(II)/PSIIRC ratio from 1000- to 1400-fold.

Leas effort has been done to study the copper effect on the polypeptide composition of PSII. However, it was reported that Cu(II) stress *in vivo* (Barón et al. 1995b, Baszynski and Krupa 1995) had an effect on proteins associated with the oxygen-evolving complex, disturbing the proper functioning of the Mn cluster, and the associated Ca^{2+} and Cl^{-} ions. In addition, the *in vitro* dissociation of the oxygen-evolving complex proteins and displacement of the native cofactors (Ca^{2+} and Cl^{-} and Mn) by heavy metals such as Pb(II) and Zn(II) (Rashid et al. 1994) were described. Studies with divalent ions as Cu(II), Hg(II) and Pb(II) also showed a donor side inhibition and possible recurrence of cycle electron transport around PSII under toxic conditions (Boucher and Carpentier, 1999).

In the present work, we have analysed the changes in isolated PSII membrane polypeptide composition in the presence of variable Cu(II) concentrations. The data indicated that low Cu(II) concentrations

(Cu(II)/PSIIRC < 230) that caused — 50% inhibition of the oxygen evolution activity did not affect the polypeptide composition of PSII. However, the 33, 24 and 17 kDa extrinsic proteins of PSII can be removed by the effect of higher Cu(II) concentrations. It is well known that treatments with high salí concentrations (i.e.. NaCI) affect the integrity of PSII, particularly by the loss of the 3 extrinsic proteins of the oxygen-evolving complex (Kuwabara and Murata 1983). We have obtained a similar effect at the highest Cu(II) concentrations, with Cu(II)/PSIIRC ratios higher than 230. This degree of toxicity was used in EPR experiments and was demonstrated that Cu(II) provoked a loss of Mn and alteration of Cyt b_{559} potential and probable displacement of Ca^{2+} and Cl^{-} (Jegerschöld et al. 1995). On the contrary, much lower Cu(II) concentration did not modify the ratio of Cyt_{red}/ Cyt_{ox} in PSII membranes (Yruela et al. 1996b). On the other hand, the highest Cu(II) concentration caused the loss of 43 and 47 kDa proteins. The CP43 antenna protein can be removed from the PSII core complex by treatment with either chaotropic agents such as potassium thiocyanate (Yamaguchi et al. 1988), or lithium perclorate (Ghanotakis et al. 1989) or by additional detergent treatments (Akabori et al. 1988). However, those treatments remove CP43 protein from PSII preparations without the removal of CP47 protein. The fact that high Cu(II) concentrations cause the loss of both antenna complexes indicate that its effect is stronger. It is known that histidyl residues are axial ligands for Chl molecules stabilizing pigment-protein complexes. Bricker (1990) has reported that histidyl residues are located near the predicted membrane surface of such complexes. Since Cu(II) has a high affinity for amine, triazole or imidazole nitrogen atoms and it is used in immobilised

metal affinity chromatography (IMAC) for photosynthetic pigment-protein complexes purification (Sulkowski 1985, Vacha et al. 1995) we suggest that Cu(II) could interact with those amino acids destabilizing the 47 and 43 kDa proteins.

The results reported here should be considered in the interpretation of published data and the design of new experiments on the location of the primary Cu(II)-inhibitory site, distinguishing between Cu(II) effects *in vitro* on PSII at low and high Cu(II) concentrations.

Considering the data reported in this work, the presente of high Cu(II) concentrations can significantly modify the oxygen-evolving complex of PSII, dissociating the Mn cluster and associated cofactors. In these conditions, the donor side of PSII would be more exposed to Cu(II) action and the inactivation of electron transport at this region could occur. To gain further insight, it is necessary to know the changes in oxygen-evolving complex protein composition under Cu(II) toxicity *in vivo*, because such changes could be related to the physiological effects of this heavy metal on photosynthesis *in vivo.*

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Table 1. Effect of Cu(II) treatment on oxygen evolution activity and protein composition of oxygenic PSII-enriched membranes. The oxygen evolution activity was measured in PSII membranes particles (10 µg Chl ml^{-1}) in the presence of 0.5 mM DCBQ as artificial electron acceptor. Samples were dark-adaptad for 30 min at 4°C and subsequently incubated with $CuCl_2$, for 20 min at 4°C before the measurements. The PFD on the surface of each side of the sample cuvette was 2 200 µmol m^{-2} s^{-1}. Protein composition was determined by SDS-PAGE (12-22%) acrylamide linear gradient). PSII membrane particles were incubated with $CuCl_2$ for 20 min in the dark at 4°C. Gels were stained by Coomassie brilliant blue R-250. Values represent means of 3 independent experiments.

Samples	Cu(II)/PSIIRC	Oxygen evolution (µmol O_2 mg^{-1} Chl h^{-1})	Protein released
Non-treated	-	560 ± 15 (100%)	
Cu(II)-treated			
5 µM $CuCl_2$	23	454 ± 7 (81%)	
50 µM $CuCl_2$	230	268 ± 5 (48%)	
300 µM $CuCl_2$	1 400	78 ± 11 (14%)	33, 24, 17 kDa

Figure Legends

Fig. 1. Effect of increasing amounts of Cu(II) on oxygen evolution of PSII membranes (10 μg ml^{-1}) (●, ▲, ■) and thylakoids (15μg ml-') (O, Δ, ❑). The activity was measured in the presence of 0.5 mM DCBQ as artificial electron acceptor. Samples were darkadapted for 30 min at 4°C and subsequently incubated with $CuCl_2$ for 20 min at 4°C before the measurements. The PFD on the surface of each side of the sample cuvette during the oxygen evolution activity measurements was 2200 **(●,** O), 950 (▲, Δ) and 450 (■,□)μmol m^{-2} s-'. Values represent means +SE (n=4).

Fig. 2. Dependence on incubation time of the oxygen evolution activity of thylakoids (0) and PSII membranes (0) treated with 15 and 5.5 μM $CuCl_2$, respectively. The activities were measured in the presence of 0.5 mM DCBQ as an artificial electron acceptor. The PFD on the surface of each side of the sample cuvette during the oxygen evolution activity measurements was 2200 μmol m^{-2} s^{-1}. Chl concentrations were 15 and 10 μg ml^{-1} for thylakoids and PSII membranes, respectively. Values represent means ± SE (n = 4).

Fig. 3. Relative values of Fv and Fv/Fm chlorophyll *a* fluorescence parameters in thylakoids treated with different $CuCl_2$ concentrations in the absence (A) and presence (B) of 0.5 μM DCMU. Samples at 10 μg ml^{-1} Chl were dark-adapted for 30 min at 4°C and subsequently incubated with $CuCl_2$ for 20 min at 4°C before the measurements in the absence of DCMU. The DCMU was added immediately before

the measurements. The PFD during the fluorescence measurements was 1000 μmol m^{-2} s^{-1}. Values represent means ± SE (n = 4).

Fig. 4. (A) SDS-PAGE and (B) immunoblots with antiserum anti-LHCII antenna complex, anti-33 kDa extrinsic protein and anti-D1 reaction centre subunit of PSII membrane preparations incubated with $CuCl_2$ in the dark for 20 min at 4°C (lanes 1, 2, 4, 6) and supernatant fraction obtained by centrifugation of PSII membranes after Cu(II) treatment (lanes 3, 5, 7). Lane 1: control; lanes 2 and 3: 5 μM (Cu(II) /PSIIRC=23); lanes 4 and 5: 50 μM (Cu(II) / PSIIRC lanes 6 and 7: 300 μM Cu(II) /PSIIRC = 1400). Values are the average of 3 independent experiments.

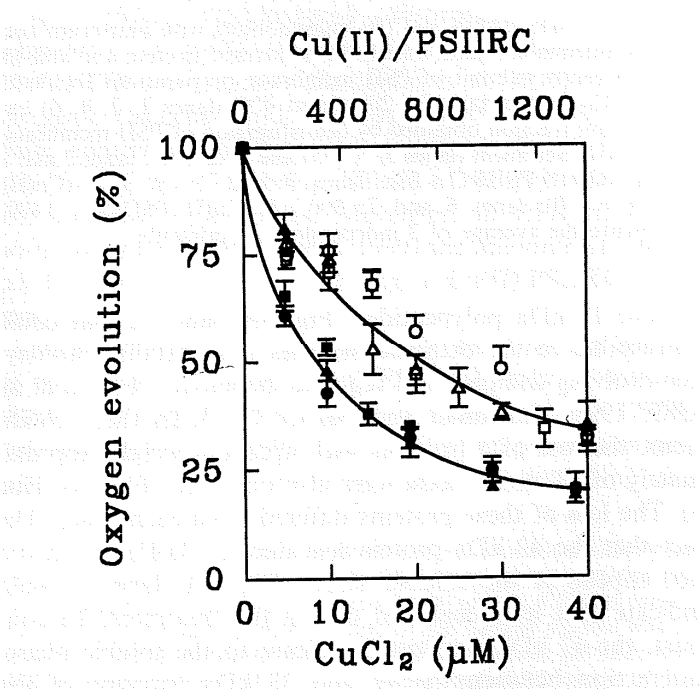
Cu(II)/PSIIRC
0
400
800
1200
100
75
50
25
0
Oxygen evolution (%)
0
10
20
30
40
CuCl2 (μM)

Fig. 1

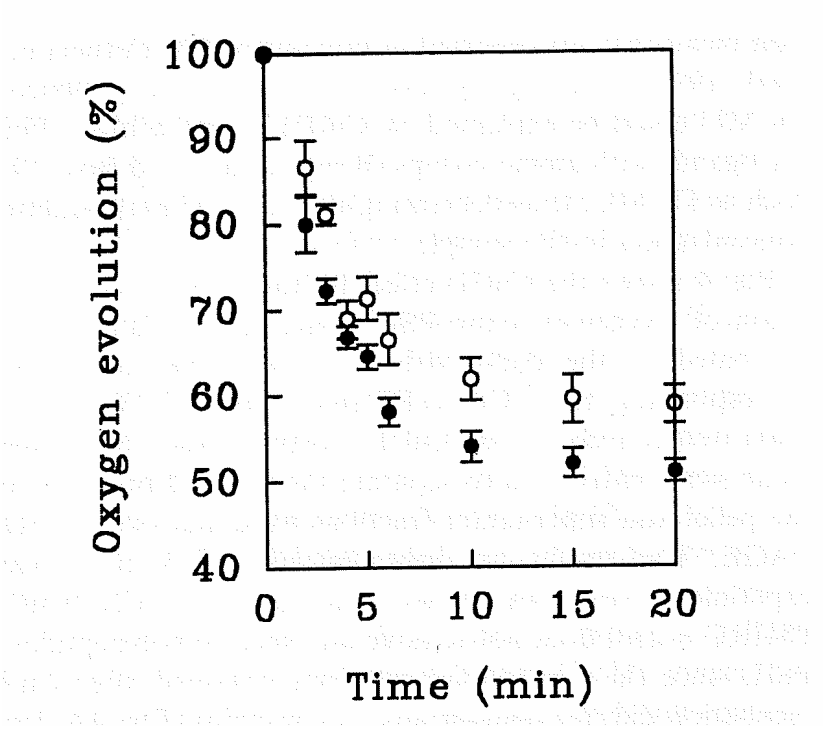

Fig. 2

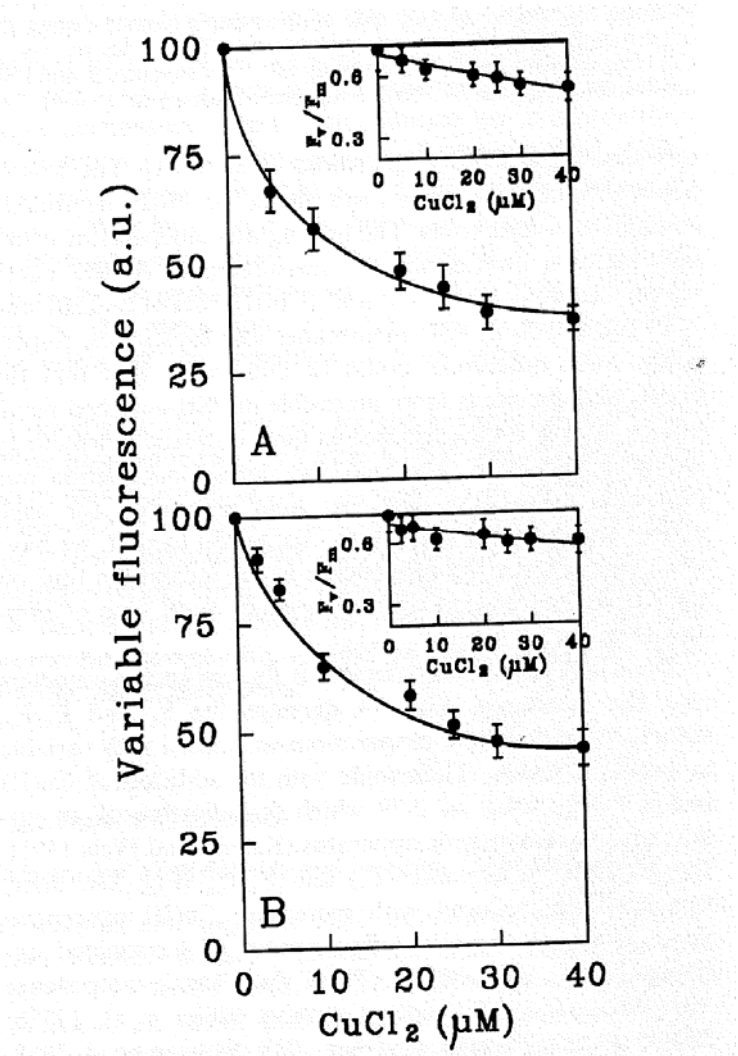
Variable fluorescence (a.u.)
100
75
50
25
0
A
B
F_v/F_m
0.6
0.3
0 10 20 30 40
$CuCl_2$ (μM)
0 10 20 30 40
$CuCl_2$ (μM)

Fig. 3

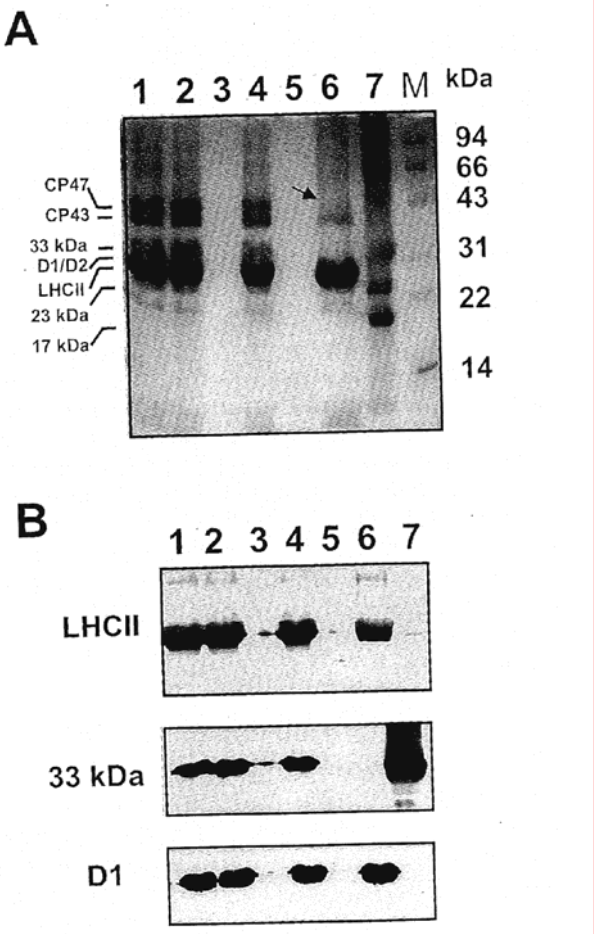
A
1 2 3 4 5 6 7 M kDa
94
66
43
31
22
14
CP47
CP43
33 kDa
D1/D2
LHCII
23 kDa
17 kDa
B
1 2 3 4 5 6 7
LHCII
33 kDa
D1

Fig. 4