Inhibition of oxygen evolution in Photosystem II by Cu(II) ions is associated with oxidation of cytochrome b_{559}

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We have found that elevated copper concentrations, apart from the inhibition of oxygen evolution, changed the initial states distribution of the oxygen-evolving complex. Already at low concentrations, copper ions oxidized the low-potential form of cytochrome b_{559} and also its high-potential form at higher concentrations at which fluorescence quenching was observed. We suggest that the primary target sites in Photosystem II for

copper is tyrosine_z, both cytochrome b_{559} forms and chlorophyll_z, and that these sites are the source of the copper-induced fluorescence quenching and oxygen evolution inhibition in Photosystem II.

Key words: copper, cytochrome b_{559} , fluorescence quenching, oxygen evolution, Photosystem II.

INTRODUCTION

Copper shows the highest toxic effects among heavy metals in plants at elevated concentrations [1–4]. Therefore investigations on the mechanism of copper action on the photosynthetic apparatus are of special interest. In the photosynthetic electron-transport chain, Photosystem II (PSII) is the most sensitive site to Cu^{2+} ions [1–4]. The most apparent effect of the toxic action of copper on PSII is inhibition of oxygen evolution accompanied by quenching of variable fluorescence [5–7]. The different sites of copper action in PSII suggested in the literature certainly depend on the relative copper to reaction centre proportions. It was found that Cu^{2+} ions inhibit both the donor and the acceptor sides of PSII, but the most sensitive site of Cu inhibition was located on the oxidizing side of PSII [8–10], where a reversible inhibition of Tyr_z oxidation by Cu^{2+} has been observed [11,12]. At higher copper concentrations, primary quinone acceptor Q_A [12], pheophytin- Q_A -Fe region [13], non-haem iron [14,15] and secondary quinone acceptor Q_B [16] were identified as the target sites of Cu^{2+} inhibitory action on the acceptor side of PSII. On the other hand, very low, equimolar Cu^{2+} /PSII proportions were recently found to stimulate oxygen evolution [17]. Within PSII, apart from the oxygen-evolving complex, cytochrome b_{559} (cyt b_{559}) in its high-potential (HP) form is the most sensitive component. Despite numerous investigations on the effect of copper on PSII components, only a few were devoted to cyt b_{559} . Yruela et al. [18] found that copper decreased the level of the photoreduced cyt b_{559} and slowed down its rate of photoreduction, whereas Jegerschöld et al. [12] observed conversion of the HP form of cyt b_{559} to the low potential (LP) form at high copper concentration.

In the present study, we investigated the mechanism of Cu^{2+} action on PSII components using fast oxygen electrode and by measuring fluorescence induction kinetics, as well as the effect of copper on the redox state of HP and LP forms of cyt b_{559} . Based

on the obtained results, we tried to correlate the observed effects with the mechanism of fluorescence quenching by copper ions in PSII.

MATERIALS AND METHODS

PSII particles (BBY) were isolated from tobacco (*Nicotiana tabacum*, cv. John Williams Broadleaf or from the Su/su var. Aurea mutant) as described by Berthold et al. [19] with the modifications of Barón et al. [20]. Tobacco thylakoids were isolated according to the method described in [21]. The amperometric measurements of oxygen evolution were performed on PSII particles at a chlorophyll (Chl) concentration of 42 $\mu\text{g}/\text{ml}$ and thylakoids at Chl concentration of 84 $\mu\text{g}/\text{ml}$ in 50 mM Hepes buffer (pH 7.0), containing 10 mM KCl, 5 mM MgCl_2 and 2.5 mM CaCl_2 using a three-electrode system [22]. Saturating light flashes of 5 μs duration at half intensity were provided by a xenon lamp (Stroboscope 1539A; General Radio, West Concord, MA, U.S.A.). The samples were illuminated by 15 flashes, spaced 300 ms apart. The measurements of fluorescence induction kinetics were performed on a home-built fluorimeter using excitation with blue light (BG12 filters) and detection at 685 nm by a monochromator. Fluorescence was measured at a Chl concentration of 50 $\mu\text{g}/\text{ml}$ in the same medium as that for the oxygen evolution measurements. Dark incubation time was 10 min. Cyt b_{559} redox changes in the isolated PSII particles were measured with the SLM Aminco DW2000 spectrophotometer in the dual-wavelength mode at 559–570 nm and Chl concentration of 50 $\mu\text{g}/\text{ml}$ under continuous stirring [23]. In freshly isolated PSII preparations, cyt b_{559} is found mainly in the reduced HP form and partially in the oxidized LP form [24,25]. Therefore the presence of the LP form does not interfere with the measurements of the oxidation of the cyt b_{559} HP form by Cu^{2+} ions. To follow the oxidation of only the cyt b_{559} LP form, 0.2 % Triton X-100

Abbreviations used: Chl, chlorophyll; cyt b_{559} , cytochrome b_{559} ; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; HP, high potential; LP, low potential; PSII, Photosystem II.

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was added to PSII particles. This treatment converts the HP form into the LP form [23–25]. Measurements of oxidation of the LP form of cyt *b*₅₅₉ were performed in the presence of 0.2 % Triton X-100 under anaerobic conditions to avoid autoxidation of the LP form using an oxygen trap composed of glucose oxidase (50 units/ml = 1.5 mg/ml), catalase (500 units/ml = 23.8 µg/ml) and 1 mM glucose. The LP form of cyt *b*₅₅₉ was prereduced by illumination of the sample with white light for 30 s in the presence of 10 µM FMN [26].

RESULTS

Measurements of oxygen evolution under short saturating flashes show that with increasing CuCl₂ concentrations, the oscillation pattern changes and oxygen evolution is inhibited at the highest concentrations used (Figure 1). The maximum of oxygen evolution is progressively shifted from the third flash to the fourth or even to the fifth flash and the oscillations are damped. The obtained results were analysed using the 5S-state model developed by Burda and Schmid [27]. It is well known that the

Figure 1 Effect of CuCl₂ on flash-induced oxygen evolution in PSII particles isolated from tobacco

Chl concentration, 42 µg/ml; 50 mM Hepes (pH 7.0) containing 10 mM KCl, 5 mM MgCl₂ and 2.5 mM CaCl₂. Dark incubation time was 10 min. All amplitudes were normalized to the amplitude of the third flash in the control sample. a.u., arbitrary units.

oxygen-evolving complex accumulates successively four positive charges in the Mn-active site before oxygen is evolved. The redox states of the Mn complex are assigned by S_{*i*}, where the subscript denotes the accumulated charge (*i* changes from 0 to 4). The probability of the non-successful transition S_{*i*} → S_{*i*+1} is given by α_{*i*} (the miss parameter). The probability of oxygen yield, accompanied by a fast S₃ → (S₄) → S₀ transition is described by the parameter *d* [27]. The above parameters, fitted to the observed patterns of oxygen evolution, are given in Table 1. It can be found that copper ≥ 5 µM in CuCl₂ caused an increase of the S₀-state population at the expense of the S₁-state population and lowering of the total miss parameter α_{*i*}. Generally, the non-successful transitions between S₀ → S₁ and S₂ → S₃ states give the main contribution to the α_{*i*} parameter. In our case, the decrease in α_{*i*} originates from more efficient transitions between S₀ → S₁ states. The increase in the α_{*i*} at Cu²⁺ concentrations of 20 µM results from decreasing the efficiency of the transitions between S_{*i*} → S_{*i*+1} states that is accompanied by the decrease in the oxygen evolution yield. Copper concentrations over the range 1–10 µM do not change significantly the total amount of oxygen evolved. Copper ions at 1 and 5 µM cause a small decrease in the parameter *d*, which suggests a less efficient fast S₃ → S₀ transition. On the other hand, at 20 µM Cu²⁺ the fast mode of oxygen evolution is decreased 5-fold when compared with 10 µM Cu²⁺.

To compare the accessibility of copper target sites in PSII particles and intact thylakoids, we analysed the effect of increasing copper concentrations on oxygen evolution pattern in tobacco thylakoids. Since thylakoids show higher Chl to PSII ratio (400–700) [28–30] than PSII particles (250–300) [30], to keep the copper to PSII ratio similar for both PSII particles and thylakoids, we used higher Chl concentration of thylakoids than for PSII particles (Table 2). It is evident that, as for PSII particles, copper ions in thylakoids at a concentration of 20 µM cause a decrease in total oxygen evolution by approx. 50 %. At ≥ 20 µM Cu²⁺, an increase in the α_{*i*} and a decrease in the probability of fast O₂ evolution are observed for both preparations. In contrast with PSII particles, for thylakoids, eight times higher concentrations of Cu²⁺ were required to increase the S₀ state population at the expense of the S₁ state (see Tables 1 and 2). These results suggest that there are at least two Cu²⁺ sites in PSII responsible for the inhibition of oxygen evolution. One binding site has the same accessibility for copper ions both in PSII particles and thylakoids, whereas the other one is more easily accessible in the isolated PSII particles.

The difference in the S₀ to S₁ ratio between untreated PSII particles and untreated thylakoids (see Tables 1 and 2) suggests that the dark stable Tyr_D radical, which is known to take part in the dark oxidation of the S₀ to S₁ state [31], is not so active in this

Table 1 Parameters of the 5S-state model fitted to the experimental data of CuCl₂-treated PSII particles isolated from tobacco and total oxygen evolution of these samples

S_{*i*} (*i* = 0, 1, 2); α_{*i*}, total miss parameter (the sum of probabilities of unsuccessful transitions between S_{*i*} → S_{*i*+1}); *d*, probability of the fast S₃ → (S₄) → S₀ transition associated with oxygen evolution; and total oxygen evolution is the sum of oxygen-evolution signals at all 15 flashes and normalized to the control samples. Chl concentration, 42 µg/ml; 50 mM Hepes (pH 7.0) containing 10 mM KCl, 5 mM MgCl₂ and 2.5 mM CaCl₂. Dark incubation time, 10 min. The reported values are the means calculated from 20 experiments. The given errors are the maximal errors obtained from the fitting procedures for 20 replicates [17]. a.u., arbitrary units.

CuCl ₂ (µM)	S ₀ (%)	S ₁ (%)	S ₂ (%)	α _{<i>i</i>}	<i>d</i>	Total O ₂ evolution (a.u.)
0.0	19.1 ± 0.5	74.5 ± 0.9	6.9 ± 0.5	1.46 ± 0.01	0.38 ± 0.01	1.00 ± 0.03
1.0	15.2 ± 0.5	79.9 ± 0.9	5.6 ± 0.5	1.58 ± 0.01	0.30 ± 0.01	0.93 ± 0.03
5.0	32.7 ± 0.6	60.9 ± 0.9	7.1 ± 0.6	1.03 ± 0.01	0.30 ± 0.01	1.00 ± 0.03
10.0	33.3 ± 0.6	63.4 ± 1.0	2.3 ± 0.5	1.06 ± 0.01	0.35 ± 0.01	0.97 ± 0.03
20.0	55 ± 0.7	36 ± 1.1	6.2 ± 0.5	1.80 ± 0.01	0.07 ± 0.01	0.54 ± 0.03
100.0	—	—	—	—	—	~ 0

Table 2 Parameters of the 5S-state model fitted to the experimental data of CuCl_2 -treated tobacco thylakoids and total oxygen evolution of these samples

Chl concentration, $84 \mu\text{g/ml}$; 50 mM Hepes (pH 7.0) containing 10 mM KCl, 5 mM MgCl_2 and 2.5 mM CaCl_2 . Dark incubation time, 10 min. Other conditions are as in Table 1. The reported values are the means calculated from ten experiments. The given errors are the maximal errors obtained from the fitting procedures for ten replicates.

CuCl_2 (μM)	S_0 (%)	S_1 (%)	S_2 (%)	α_1	d	Total O_2 evolution (a.u.)
0.0	5.8 ± 0.5	86.7 ± 1.1	6.6 ± 0.5	0.60 ± 0.01	0.81 ± 0.01	1.00 ± 0.03
10.0	7.0 ± 0.6	76.2 ± 1.2	14.4 ± 0.6	0.60 ± 0.01	0.81 ± 0.01	0.98 ± 0.03
20.0	3.0 ± 0.6	68.6 ± 1.1	29.0 ± 0.7	0.80 ± 0.01	0.71 ± 0.01	0.45 ± 0.03
40.0	23.7 ± 0.8	70.7 ± 1.3	6.7 ± 0.5	0.97 ± 0.01	0.50 ± 0.01	0.32 ± 0.03
80.0	26.3 ± 0.9	56.6 ± 1.0	17.5 ± 0.5	2.17 ± 0.01	0.24 ± 0.01	0.19 ± 0.03

Figure 2 Effect of CuCl_2 on fluorescence induction in PSII particles

Chl concentration, $50 \mu\text{g/ml}$; 50 mM Hepes (pH 7.0) containing 10 mM KCl, 5 mM MgCl_2 and 2.5 mM CaCl_2 . a.u., arbitrary units.

respect in PSII particles as in thylakoids. This is probably due to structural changes of the oxygen-evolving complex or the partial reduction of Tyr_D in the isolated PSII particles.

The inhibition of oxygen evolution by Cu^{2+} ions is accompanied by quenching of Chl fluorescence from PSII (Figure 2). This quenching effect could be explained by donor-side inhibition due to the known Cu^{2+} action on Tyr_Z [11,12]. The reason for fluorescence quenching under donor-side inhibition is attributed to the formation of the Chl_Z^+ radical, which is a strong fluorescence quencher in PSII [32]. However, formation of this radical is dependent on the redox state of cyt b_{559} [33]. The effect of Cu^{2+} on the redox state of cyt b_{559} clearly shows that Cu^{2+} caused oxidation of both the LP and the HP forms of cyt b_{559} in the dark (Figure 3). Oxidation of the cyt b_{559} LP form occurred at considerably lower Cu^{2+} concentrations than the oxidation of the HP form. Within the concentration range used, Cu^{2+} ions did not change the level of the HP form, i.e. Cu^{2+} did not cause any conversion of the HP to LP form of cyt b_{559} (results not shown). Comparison of Cu^{2+} concentrations that inhibited oxygen evolution (Figure 1) and cause fluorescence quenching (Figure 2) with those that oxidized the HP form of cyt b_{559} (Figure 3), shows that all these effects take place at similar Cu^{2+} concentrations.

Figure 3 Dark oxidation of cyt b_{559} HP and LP forms by CuCl_2 in PSII particles measured at 559–570 nm

The redox changes of the LP form were performed under anaerobic conditions in the presence of 0.2% Triton X-100. Before CuCl_2 addition, the LP form was reduced by illumination of the sample for 30 s in the presence of $10 \mu\text{M}$ FMN. Chl concentration, $50 \mu\text{g/ml}$; 50 mM Hepes (pH 7.0) containing 10 mM KCl, 5 mM MgCl_2 and 2.5 mM CaCl_2 .

This indicates that these effects could have a similar molecular origin. When comparing the effect of Cu^{2+} ions on fluorescence quenching and cyt b_{559} oxidation, it should be taken into account that the dark incubation time before fluorescence measurements was 10 min and that the apparent fluorescence quenching (F_v/F_m , where F_v is the variable fluorescence and F_m the maximal fluorescence) by Cu^{2+} ions depends also on the light intensity used.

We did not observe any release of the extrinsic PSII proteins (17, 23 and 33 kDa) at the CuCl_2 concentrations applied (results not shown).

DISCUSSION

The inhibitory effect of Cu^{2+} on the oxygen evolution and fluorescence quenching at the higher Cu^{2+} concentrations may be attributed to donor-side inhibition, quite probably by blocking the Tyr_z function. In consequence, the electron donation from the HP or LP forms of the cyt b_{559} via the redox-active Chl_z molecule is activated. If both cyt b_{559} forms are oxidized, Chl_z molecule forms a stable cation radical, which is a strong fluorescence quencher of PSII [32]. Under non-inhibitory conditions, the LP remains mainly oxidized, whereas the HP form is reduced. Under such conditions, the excitation energy from light-harvesting complex is directed via the Chl_z molecule to the reaction centre. When the Chl_z^+ radical is formed, the excitation energy is dissipated by this radical preventing PSII against overexcitation [33]. Our finding that Cu^{2+} oxidized both cyt b_{559} forms suggests that this process might stimulate the formation of Chl_z radical and fluorescence quenching in PSII. The non-specific antenna quenching of Chl fluorescence by Cu^{2+} , which is a paramagnetic ion, is not significant in our case, since quenching of the initial fluorescence (F_0) by Cu^{2+} observed in many studies was not found or was weak [5–7,34]. Similar effects as for Cu^{2+} ions on oxygen evolution and fluorescence quenching are caused by carbonyl cyanide *p*-trifluoromethoxyphenyl-hydrazone (FCCP), which induces cyt b_{559} HP oxidation and catalyses electron transfer from Chl_z to Tyr_z [35]. This electron transfer inhibits electron donation from the oxygen-evolving complex and facilitates Chl_z^+ formation. The oxidation mechanism of the HP form of cyt b_{559} by Cu^{2+} is probably similar to that caused by FCCP, i.e. by deprotonation of the HP form. FCCP is a well-known protonophore but it is not capable of oxidizing directly the HP form. It was suggested that FCCP deprotonates the surrounding groups of the haem of the cytochrome, and the cytochrome, in an unstable deprotonated form, undergoes autooxidation [24,36]. Substitution of protons in the amino acid groups that can be protonated or deprotonated has already been suggested as the mechanism of inhibitory action of Cu^{2+} [37]. It was recently found that the histidine co-ordinating haem of the cyt b_{559} HP form is protonated and any treatment that releases this proton induces conversion of the cytochrome to the LP form via the intermediate-potential form [38]. Copper ions are known for their affinity to histidine residues; therefore, it seems reasonable to assume that Cu^{2+} ions substitute for the proton bound to the histidine residue co-ordinating haem of cyt b_{559} HP form and induce its oxidation to the LP form. It should also be mentioned that direct oxidation of the cyt b_{559} HP form by Cu^{2+} ions is thermodynamically unfavourable because of the midpoint redox potential of Cu^{2+} ($E_{m7} = +0.167$ V), which is lower than that of the cyt b_{559} HP form ($E_{m7} = +0.33$ – 0.40 V).

The observed reversibility of oxygen evolution inhibition and fluorescence quenching [5], together with the reversibility of Tyr_z

Scheme 1 Model of electron transfer pathways within PSII showing the possible interaction sites of copper responsible for the inhibition of oxygen evolution and fluorescence quenching

Electron-transfer routes ($\rightarrow$), excitation energy transfer ($\dashrightarrow$), interconversion between cyt b_{559} HP and LP forms ($\leftrightarrow$) and Cu^{2+} -interaction sites ($\dashrightarrow$).

inhibition by Cu^{2+} [12], could suggest that Cu – Tyr_z interaction is the only reason for both the observed effects. However, Cu -stimulated fluorescence quenching is also observed in Tris -treated PSII particles, where MnCl_2 was used as an electron donor [7]. At the applied concentrations of MnCl_2 (2 mM) in those experiments, this electron donor is supposed to donate electrons directly to P680 (low-affinity site) at 60 % and via Tyr_z (high-affinity site) at 40 % of the total electron donation rate [39]. This indicates that fluorescence quenching by Cu^{2+} occurred effectively, although the electron flow through Tyr_z was mostly omitted. The oxidation of both cyt b_{559} forms cannot be the only reason for fluorescence quenching as well, since ferricyanide, which oxidized both cyt b_{559} forms more efficiently than Cu^{2+} (results not shown), is not an efficient fluorescence quencher of PSII, at least at room temperature (22 °C) [40]. Another interaction site of Cu^{2+} within PSII, responsible for fluorescence quenching, is probably the Chl_z molecule which could form a Chl_z – Cu charge-transfer complex or could be directly oxidized by Cu^{2+} , giving the Chl_z^+ cation radical which quenches fluorescence. Such a Chl_z – Cu complex or a Cu -induced Chl_z^+ radical, stable at room temperature, was already observed in ESR studies [12]. Thus the inhibition of electron donation from the oxygen-evolving complex at the Tyr_z site, cyt b_{559} oxidation and the enhanced Chl_z oxidation are responsible for the observed Cu -induced fluorescence quenching and the inhibition of oxygen evolution. The possible interaction sites of Cu^{2+} with the components of PSII, discussed above, are shown in Scheme 1.

In summary, we have shown that apart from the inhibition of total oxygen evolution, the increased copper concentrations changed the distribution of initial S-states of the oxygen-evolving complex and decreased the α_i parameter. Copper ions oxidized directly the cyt b_{559} LP form at low concentrations (1–10 μM) and the HP form at higher concentrations (10–100 μM), probably by deprotonation of this labile cyt b_{559} form. Oxygen-evolution inhibition and the oxidation of cyt b_{559} by copper ions were found to correlate with the fluorescence quenching of PSII. We

also suggest the enhanced formation of the Chl_z⁺ radical due to its direct interaction with Cu²⁺ ions. This could be responsible for fluorescence quenching and would result in the stimulation of the cyclic electron transport around PSII and dissipation of the excess of absorbed light energy.

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REFERENCES

- 1 Droppa, M. and Horváth, G. (1990) The role of copper in photosynthesis. *Plant Sci.* **9**, 111–123
- 2 Barón, M., Arellano, J. B. and Gorgé, J. L. (1995) Copper and Photosystem II: a controversial relationship. *Physiol. Plantarum* **94**, 174–180
- 3 Prasad, M. N. V. and Strzalka, K. (1999) Impact of heavy metals on photosynthesis. In *Heavy Metal Stress in Plants* (Prasad, M. N. V. and Hagemeyer, J., eds.), pp. 117–138, Springer Verlag, Berlin
- 4 Maksymiec, W. (1997) Effect of copper on cellular processes in higher plants. *Photosynthetica* **34**, 321–342
- 5 Hsu, B.-D. and Lee, J.-Y. (1988) Toxic effects of copper on Photosystem II of spinach chloroplasts. *Plant Physiol.* **87**, 116–119
- 6 Samson, G., Morissette, J.-C. and Popovic, R. (1988) Copper quenching of the variable fluorescence in *Dunaliella tertiolecta*. New evidence for a copper inhibition effect on PS II photochemistry. *Photochem. Photobiol.* **48**, 329–332
- 7 Arellano, J. B., Lazaro, J. J., Lopez-Gorge, J. and Baron, M. (1995) The donor side of Photosystem II as the copper-inhibitory binding site. *Photosynth. Res.* **45**, 127–134
- 8 Haberman, H. M. (1969) Reversal of copper inhibition in chloroplasts reactions by manganese. *Plant Physiol.* **44**, 331–336
- 9 Cedeno-Maldonado, A. and Swader, J. A. (1972) The cupric ions as an inhibitor of photosynthetic electron transport in isolated chloroplasts. *Plant Physiol.* **50**, 698–701
- 10 Vierke, G. and Stuckmeier, P. (1977) Binding of copper(II) to proteins of the photosynthetic membrane and its correlation with inhibition of electron transport in class II chloroplasts of spinach. *Z. Naturforsch.* **32**, 605–610
- 11 Schröder, W. P., Arellano, J. B., Bittner, T., Baron, M., Eckert, H. J. and Renger, G. (1994) Flash induced absorption spectroscopy studies of copper interaction with Photosystem II in higher plants. *J. Biol. Chem.* **269**, 32865–32870
- 12 Jegerschöld, C., Arellano, J. B., Schröder, W. P., van Kan, P. J., Barón, M. and Styring, S. (1995) Copper(II) inhibition of electron transfer through Photosystem II studied by EPR spectroscopy. *Biochemistry* **34**, 12747–12754
- 13 Yruela, I., Gatzen, G., Picorel, R. and Holzwarth, A. R. (1996) Cu(II)-inhibitory effect on Photosystem II from higher plants. A picosecond time-resolved fluorescence study. *Biochemistry* **35**, 9469–9474
- 14 Singh, D. P. and Singh, S. P. (1987) Action of heavy metal on Hill activity and O₂ evolution. *Plant Physiol.* **83**, 12–14
- 15 Jegerschöld, C., McMillan, F., Lubitz, W. and Rutherford, A. W. (1999) Effect of copper and zinc ions on Photosystem II. Studies by EPR spectroscopy. *Biochemistry* **38**, 12439–12445
- 16 Mohanty, N., Vass, I. and Demeter, S. (1989) Copper toxicity affects Photosystem II electron transport at the secondary quinone acceptor (Q_B). *Plant Physiol.* **90**, 175–179
- 17 Burda, K., Kruk, J., Strzalka, K. and Schmid, G. H. (2002) Stimulation of oxygen evolution in photosystem II by copper(II) ions. *Z. Naturforsch.* **57c**, 853–857
- 18 Yruela, I., Pueyo, J. J., Alonso, P. J. and Picorel, R. (1996) Photoinhibition of Photosystem II from higher plants – effect of copper inhibition. *J. Biol. Chem.* **271**, 27408–27415
- 19 Berthold, D. A., Babcock, G. T. and Yocum, C. F. (1981) A highly resolved, oxygen evolving Photosystem II preparation from spinach thylakoid membranes. *FEBS Lett.* **134**, 231–234
- 20 Barón, M., Arellano, J. B., Schröder, W. P., Lachica, M. and Chueca, A. (1993) Copper binding sites associated with Photosystem II preparations. *Photosynthetica* **28**, 195–204
- 21 Robinson, H. H. and Yocum, C. F. (1980) Cyclic photophosphorylation reactions catalyzed by ferredoxin, methyl viologen and anthraquinone sulfonate. *Biochim. Biophys. Acta* **590**, 97–106
- 22 Schmid, G. H. and Thibault, P. (1979) Evidence for a rapid oxygen uptake in tobacco chloroplasts. *Z. Naturforsch.* **34**, 414–418
- 23 Kruk, J. and Strzalka, K. (2001) Redox changes of cytochrome *b*-559 in the presence of plastoquinones. *J. Biol. Chem.* **276**, 86–91
- 24 Ortega, J. M., Hervas, M. and Losada, M. (1988) Redox and acid–base characterization of cytochrome *b*-559 in photosystem II particles. *Eur. J. Biochem.* **171**, 449–455
- 25 Whitmarsh, J. and Pakrasi, H. B. (1996) Form and function of cytochrome *b*-559. In *Oxygenic Photosynthesis: The Light Reactions* (Ort, D. R. and Yocum, C. F., eds.), pp. 249–264, Kluwer Academic Publishers, Dordrecht
- 26 Klein, J., De Las Rivas, J. and Barber, J. (1995) Indirect reduction of cytochrome *b*-559 in isolated reaction centers of PSII by exogenous flavins. *Bioelectrochem. Bioenerg.* **38**, 9–14
- 27 Burda, K. and Schmid, G. H. (1996) A new method of determining a number of S-states in the Kok model. *Z. Naturforsch.* **51**, 329–341
- 28 Melis, A. (1996) Excitation energy transfer: functional and dynamic aspects of Lhc (cab) proteins. In *Oxygenic Photosynthesis: The Light Reactions* (Ort, D. R. and Yocum, C. F., eds.), pp. 523–538, Kluwer Academic Publishers, Dordrecht
- 29 Kruk, J. and Strzalka, K. (1999) Dark reoxidation of the PQ-pool proceeds via the low-potential form of cytochrome *b*-559 in spinach thylakoids. *Photosynth. Res.* **62**, 273–279
- 30 Haumann, M. and Junge, W. (1996) Protons and charge indicators in oxygen evolution. In *Oxygenic Photosynthesis: The Light Reactions* (Ort, D. R. and Yocum, C. F., eds.), pp. 165–192, Kluwer Academic Publishers, Dordrecht
- 31 Vass, I. and Styring, S. (1991) pH-dependent charge equilibria between tyrosine-D and the S states in Photosystem II. Estimation of relative midpoint redox potentials. *Biochemistry* **30**, 830–839
- 32 Schweitzer, R. H. and Brudvig, G. W. (1997) Fluorescence quenching by chlorophyll cations in Photosystem II. *Biochemistry* **36**, 11351–11359
- 33 Stewart, D. H. and Brudvig, G. W. (1998) Cytochrome *b*₅₅₉ of Photosystem II. *Biochim. Biophys. Acta* **1367**, 63–87
- 34 Boucher, N. and Carpentier, R. (1999) Hg²⁺, Cu²⁺ and Pb²⁺-induced changes in Photosystem II photochemical yield and energy storage in isolated thylakoid membranes: a study using simultaneous fluorescence and photoacoustic measurements. *Photosynth. Res.* **59**, 167–174
- 35 Heimann, S., Klughammer, C. and Schreiber, U. (1998) Evidence for one cytochrome *b*-559 per PSII in spinach chloroplasts. In *Photosynthesis: Mechanisms and Effects*, vol. II (Garab, G., ed.), pp. 1089–1092, Kluwer Academic Publishers, Dordrecht
- 36 Arnon, D. I. and Tang, G. M.-S. (1988) Divergent pathways of photosynthetic electron transfer: the autonomous oxygenic and anoxygenic photosystems. *Proc. Natl. Acad. Sci. U.S.A.* **85**, 9524–9528
- 37 Yruela, I., Montoya, G. and Picorel, R. (1992) The inhibitory mechanism of Cu(II) on the Photosystem II electron transport from higher plants. *Photosynth. Res.* **33**, 227–233
- 38 Roncel, M., Ortega, J. M. and Losada, M. (2001) Factors determining the special redox properties of photosynthetic cytochrome *b*559. *Eur. J. Biochem.* **268**, 4961–4968
- 39 Kullander, C., Fredriksson, P.-O., Sayre, R. T., Minagawa, J., Crofts, A. R. and Styring, S. (1995) Electron donation from exogenous donors to Photosystem II studied in *Chlamydomonas reinhardtii* mutants. In *Photosynthesis: From Light to Biosphere*, vol. II (Mathis, P., ed.), pp. 321–324, Kluwer Academic Publishers, Dordrecht
- 40 Okayama, S. and Butler, W. L. (1972) The influence of cytochrome *b*559 on the fluorescence yield of chloroplasts at low temperature. *Biochim. Biophys. Acta* **267**, 523–529

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