S10-008

Two different ways of stimulating photosynthetic oxygen evolution by copper(II) ions and trivalent lanthanides.

K Burda[1], J Kruk[2], K Strzałka[2] and GH Schmid[3]

[1]*The Henryk Niewodniczański Institute of Nuclear Physics, ul. Radzikowskiego 152, 31-342 Kraków, Poland; fax: (+48 12) 6371881, e-mail: burda@alf.ifj.edu.pl*
[2]*Department of Plant Physiology and Biochemistry, The Jan Zurzycki Institute of Molecular Biology, Jagiellonian University, Al.Mickiewicza 3, 31-120 Kraków, Poland*
[3]*Fakultät für Biologie, Lehrstuhl Zellphysiologie, Universität Bielefeld, D-33615 Bielefeld, Germany*

Keywords: photosystem II, oxygen evolution, lanthanides, copper ions.

Abstract
We have studied the influence of lanthanides, which are suitable probes for Ca^{+2} binding sites as well as the influence of copper ions on the water oxidation process in photosystem II - preparations from tobacco.
The inhibitory action of copper and lanthanides on photosystem II is well known but we have observed that they may stimulate oxygen evolution if they are applied in equimolar proportions to photosystem II reaction centers. The molecular mechanism of copper action is however different than that of lanthanides. We have found that there are two Eu(Dy) – binding sites one with high affinity in the core of PS II and the other one with low affinity in the 33 kDa extrinsic protein or the contact surface of the 23 and 33 kDa peptides. The binding of lanthanides is reversible under the action of calcium ions. Copper ions stimulate O_2 evolution even in the presence of Ca^{2+} excess. Taking into account this observation and the analysis of copper content in PS II preparations from the wild type tobacco and a tobacco mutant deficient in light-harvesting complex as well as the inhibitory effect of azide on oxygen evolution in thylakoids we suggest that Cu^{2+} is a native component of photosystem II involved in the water splitting process.

Introduction

Photosystem II (PS II) contains the oxygen-evolving complex (OEC) which catalyzes the light driven reaction of four electron extraction from water molecules. It is situated on the luminal side of the thylakoid membrane. Intrinsic proteins D1, D2 and cytochrome b_{559} constitute the core of PS II. Among the extrinsic peptides the 33 kDa, 24 kDa and 17 kDa peptides have been recognized to be involved in the water splitting process (for review see: Renger, 2001).
It is established that manganese, chloride and calcium are necessary for the proper functioning of the oxygen-evolving complex (Debus, 1992). However, only the role of the Mn cluster is understood. The knowledge on Cl^- and Ca^{2+} action is still poor. It has been suggested that calcium takes part in the incorporation and function of the manganese complex (Ono and Inoue, 1983, 1984). Lanthanides, being suitable probes for Ca^{2+}- binding sites in photosystem II, have been found to produce a destruction of the water splitting enzyme activity (Ghanotakis et al., 1985; Bakou and Ghanotakis, 1993). Among other heavy metals, copper has been shown to inhibit oxygen evolution (Cedeño-Moldonado and Swader, 1972; Droppa and Horváth, 1990; Barón et al., 1995).

We have observed that Eu^{3+} (Dy^{3+}) in an equimolar amount to the PS II reaction center (RC) or Cu^{2+} in an equimolar concentration to PS II RC stimulate oxygen evolution. Eu^{3+} and Dy^{3+} most probably stabilize the 33 kDa protein structure whereas there are indications that Cu^{2+} might be a native component of photosystem II.

Materials and methods

Photosystem II particles were isolated from tobacco (*Nicotiana tabacum* var. John Williams Broadleaf or from the Su/su var. Aurea mutant) according to the method of Berthold et al. (1981) with the modification of Barón et al. (1993). The amperometric oxygen evolution measurements were performed with PS II particles at a chlorophyll (Chl) concentration of 42 μg/ml in 50 mM Hepes buffer, pH 7.0 containing 10 mM KCl, 5 mM $MgCl_2$ and 2.5 mM $CaCl_2$ using the three electrode system of Schmid and Thibault (1979). In the case of measurements with various $EuCl_3$ and $DyCl_3$ concentrations $CaCl_2$ was omitted in the buffer. Samples were incubated in the presence of Eu^{3+}, Dy^{3+} and Cu^{2+} for 5 min on ice and then 5 min at room temperature in darkness before putting them on the electrode. The 17 kDa and 23 kDa peptides were removed by NaCl washing and the 33 kDa subunit was removed by $MgCl_2$ washing.

Saturating light flashes of 5 μs duration at half intensity were provided by a xenon lamp (Stroboscope 1539A from General Radio). Samples were illuminated by 15 flashes spaced 300 ms apart. The measurements of fluorescence induction kinetics were performed with a home-built fluorometer using excitation with blue light (BG12 filters) and detection at 685 nm through a monochromator. Fluorescence was measured at Chl conc. of 50 μg/ml in the same medium as that for the oxygen evolution measurements.

The 33 kDa protein was prepared as described by Jansson (1984). The content of Cu, Mn, Dy and Eu ions in PS II was determined by AAS (Atomic Absorption Spectroscopy) or ICP (Induced Coupled Plasma).

Results and Discussion

In Fig.1 we present the oscillatory pattern of oxygen evolution by PS II membranes incubated with different europium chloride concentrations. For 0.2 μM $EuCl_3$ (1 Eu^{3+}/PSII RC) we have obtained a stimulation of O_2 yield whereas a two times higher concentration led to inhibition of oxygen evolution. We have also observed a stimulatory effect for Dy^{3+} at the same concentrations as for Eu^{3+}.

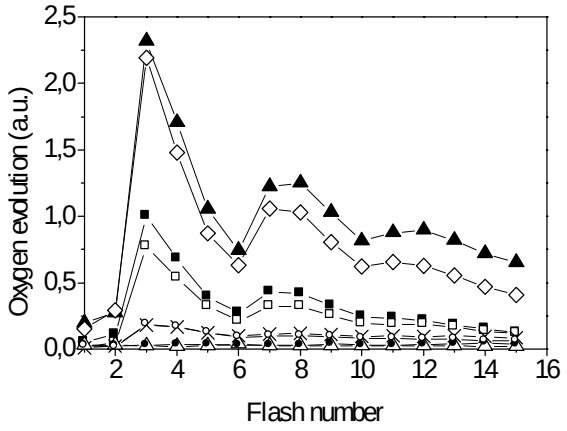

Fig. 1

—■— PS II control (PS_c)

—□— PS II depleted of the 23 kDa and 17 kDa peptides ($PS_{23,17}$)

—×— PS II depleted of the 33 kDa, 23 kDa and 17 kDa peptides ($PS_{33,23,17}$)

—◇— PS_c + 0.8μM $CaCl_2$

—▲— PS_c + 0.2μM $EuCl_3$

—△— PS_c + 0.4μM $EuCl_3$

—•— $PS_{23,17}$ + 0.2μM $EuCl_3$

—○— $PS_{23,17}$ + 0.2μM $EuCl_3$ + 4.8μM $CaCl_2$

More detailed studies of lanthanides on the process of water splitting are given in (Burda et al., 1996). We suggested two Eu (Dy) – binding regions: one with a low affinity for lanthanides (calcium) on the 33 kDa protein or on the contact surface of the 33 kDa and 23 kDa peptides and a second one with high affinity located close to the Mn complex and Tyr_Z. Substitution of Ca^{2+} by Eu^{3+} (Dy^{3+}) at the low affinity binding site causes a stimulation of oxygen evolution whereas binding at the high affinity binding site results in total inhibition of O_2 yield. Higher concentrations of lanthanides lead to extraction of the extrinsic proteins.
Results of our investigations on the number of lanthanides specifically bound to PS II particles and to the 33 kDa protein using the ICP method are is given in Table 1.

Table.1.

Samples treated with $EuCl_3$ and $DyCl_3$.	Content of Eu^{3+} (Dy^{3+}) / PS II RC (33 kDa protein)	
	dialyzed	washed with EDTA
PS II particles depleted of extrinsic peptides	51.2±1.7 (40.2±1.8)[a]	0.81±0.04 (0.82±0.1)[a]
33 kDa protein	1.08±0.07 (1.19±0.04)[a]	≤ 0.10

[a]Values in brackets are given for Dy^{3+}.

In the case of PSII particles depleted of the extrinsic peptides a high affinity binding site only for one lanthanide ion has been detected, which could not be removed under EDTA treatment. On the other side we have only found one loosely bound Eu^{3+} (Dy^{3+}) with the protein 33kDa. The data confirms our earlier hypothesis on the existence of two distinct binding sites of lanthanides (calcium) on the donor side of PS II and the possible mechanism of lanthanide action within photosystem II.
Measurements of oxygen evolution under short saturating flashes in PS II membranes treated with $CuCl_2$ and $CuSO_4$ are shown in Fig. 2.

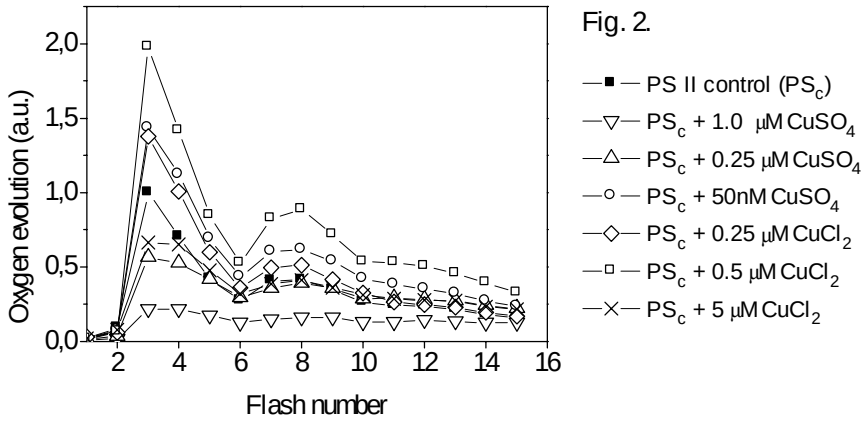

Fig. 2.

Copper ions at concentrations corresponding to 1.5 – 3 Cu^{2+}/PS II RC stimulate oxygen evolution almost two-fold. With increasing concentrations of copper ions the O_2 yield decreases. The Cu^{2+} concentrations which stimulate the process of water splitting had no effect on the fluorescence kinetic and they did not change the dark distribution of the redox states of the Mn complex (S_i) nor the transition probabilities between the $S_i \rightarrow S_{i+1}$ calculated from the 5S-state model (Burda and Schmid, 1996). Higher Cu^{2+} concentrations quenched the variable fluorescence and changed the S-states dark distribution and transitions between them. We have not observed any release of extrinsic proteins in PS II membranes treated with copper ions.

In order to answer the question whether the stimulation of oxygen evolution at low Cu^{2+} concentrations results from natively PS II bound Cu^{2+} , we determined the native Cu and Mn content in PS II particles isolated from wild type tobacco and from the LHC - deficient tobacco mutant. Independently on the detergent treatment and the studied species we obtained 1-2 Cu atoms per 4 Mn atoms. Additionally, the nearly two-fold inhibition of oxygen evolution by azide in fresh thylakoids gives the evidence for a specific Cu-binding site within PS II or another metal binding site, which can be easily substituted by Cu^{2+}. The most potential candidates are manganese and calcium cations. However, manganese substitution by Cu^{2+} is improbable at such low copper concentrations and could result in the inhibition of oxygen evolution. Calcium substitution by Cu^{2+} at a non-specific binding site can also be excluded because Cu^{2+} stimulates the process in the presence of excess of calcium ions (0.5 μM $CuCl_2$ versus 2.5 mM $CaCl_2$). The stimulatory effect of O_2 evolution by Eu^{3+} and Dy^{3+} has been observed but in the absence of external Ca^{2+} cations.

The involvement of Cu^{2+} in photosynthetic oxygen evolution has been already suggested earlier (Lightbody and Krogmann, 1967; Barr and Crane, 1976). Evidence for Cu^{2+} involvement came from observations of the inhibitory action of chelators and the analysis of copper content in PS II preparations. The experiments presented here clearly demonstrate stimulation of oxygen evolution by copper II ions. The exact mechanism of Cu^{2+} action, however, has to be elucidated.

Acknowledgement: This work was supported by grant No. 6 P04A 03817 from the Committee for Scientific Research of Poland (KBN).

References

Bakou A, Ghanotakis DF (1993) *Biochimica et Biophysica Acta* **1141**, 303-308.
Barón M, Arellano JB, Schröder WP, Lachica M, Chueca A (1993) Photosynthetica **28**, 195-204.
Barón M, Arellano JB, Gorgé JL (1995) *Physiol. Plantarum* **94**, 174-180.
Barr R, Crane FL (1976) *Plant Physiology* **57**, 450-453.
Berthold DA, Babcock GT, Yocum CF (1981) *FEBS Letters* **134**, 231-234.
Burda K, Strzalka K, Schmid GH (1996) *Zeitschrift für Naturforschung* **50c**, 220-230.
Burda K, Schmid GH (1996) *Zeitschrift für Naturforschung* **51c**, 329-341.
Cedeño-Moldonado A, Swader JA (1972) *Plant Physiology* **50**, 698-701.
Debus RJ (1992) *Biochimica et Biophysica Acta* **1102**, 269-352.
Droppa M, Horváth G (1990) *Critical Review Plant Science* **9**, 111-123.
Ghanotakis DF, Babcock GT, Yocum CE (1985) *Biochimica et Biophysica Acta* **809**, 173-180
Jansson Ch in *Advances in Photosynthesis Research*, Sybesma C (ed.) Dr W Junk Publishers, The Netherlands, 1984, pp. I3.375-I3.378.
Lightbody JL, Krogmann PW (1967) *Biochimica et Biophysica Acta* 131, 508-515.
Renger G (2001) *Biochimica et Biophysica Acta* **1503**, 210-228.
Schmid GH, Thibault P (1979) *Zeitschrift für Naturforschung* **34c**, 414-418.