

Cd²⁺ effect on photosynthetic apparatus in *Synechococcus elongatus* and spinach (*Spinacia oleracea* L.)

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Abstract

Thylakoid membranes (TM) of the cyanobacterium *Synechococcus elongatus* were exposed for 30 min to the influence of 0, 10, 100, and 1 000 mM CdCl₂ (= Cd₀, Cd₁₀, Cd₁₀₀, and Cd₁₀₀₀). Cd₁₀ and Cd₁₀₀ caused some increase in activity of photosystem 2, PS2 (H₂O → DCPIP), while distinct inhibition was observed with Cd₁₀₀₀. We also observed a similar effect when measuring oxygen evolution (H₂O → PBQ + FeCy). Chloroplasts of spinach (*Spinacia oleracea* L.) were incubated for 30 min with 0, 15, 30, and 60 mM CdCl₂ (= Cd₀, Cd₁₅, Cd₃₀, and Cd₆₀). All concentrations studied inhibited the PS2 activity, the effect being stronger with increasing concentration of Cd²⁺. The photosynthetic oxygen evolution activity was also influenced most distinctly by the highest concentration employed, *i.e.* Cd₆₀. Electrophoretic analysis of the protein composition of cyanobacterium TM showed chief changes in the molecular mass regions of M_r 29 000 and 116 000, while with spinach chloroplasts the most distinct differences were observed in the regions of M_r 15 000 and 50 000. Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) activity in cyanobacterial spheroplasts still remained on the 40 % level in the case of Cd₁₀₀₀, but it decreased down to approx. 2.5 % in the Cd₆₀ sample of spinach chloroplasts.

Additional key words: cyanobacteria; heavy metals; photochemical activity; photosystem 2; proteins; ribulose-1,5-bisphosphate carboxylase/oxygenase.

Introduction

Contemporary fast development of industry is accompanied by rising amounts of toxic heavy metals, including cadmium, in the environment. The toxic effects of cadmium on the human and animal organism have been known for a long time. However, negative effects are also found in autotrophic organisms, which moreover can serve as entrance pathways into other organisms in the food chain.

Cadmium ions (Cd²⁺) are taken up by plants through the root system, which thus is probably the first site of the negative influence of these ions (Weigel and Jäger 1980, Krupa and Baszyński 1995). Cd²⁺ also influence the electron transport chain of photosynthesis, particularly in the vicinity of the multiprotein complex of photosystem 2

(PS2): is not yet clear if either on the donor or the acceptor side (Clijsters and Van Assche 1985, Baszyński 1986a,b). The oxygen evolving complex (OEC) is likewise very sensitive to the effect of Cd²⁺. Changes take place in the polypeptide composition of the OEC or toxic metal ions interact with those ions which are essential for the chief function of OEC, *i.e.* Mn²⁺, Ca²⁺, and Cl⁻ (Bernier *et al.* 1993, Ouzounidou *et al.* 1993, Skorzyńska and Baszyński 1993, Maksymiec *et al.* 1994, Krupa and Baszyński 1995). Using electron emission spectroscopy, Šeršeň and Král'ová (2001) observed a direct influence of Cd²⁺ on the Mn ion cluster situated in OEC.

Photosystem 1 (PS1) is often described as relatively insensitive to the effect of Cd²⁺, but some negative

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Abbreviations: BSA – bovine serum albumin; Chl – chlorophyll; DCPIP – 2,6-dichlorophenolindophenol; DPC – 1,5-diphenylcarbazide; DTT – 1,4-dithio-D,L-threitol; EDTA – ethylenediaminetetraacetic acid; FeCy – ferricyanide; HEPES – N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]; OEC – oxygen evolving complex; PBQ – phenyl-p-benzoquinone; PEPC – phosphoenolpyruvate carboxylase; PS – photosystem; RuBP – ribulose-1,5-bisphosphate; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase; SDS – sodium dodecylsulfate; SDS-ELFO – sodium dodecylsulfate electrophoresis; TM – thylakoid membranes; Tris-HCl – tris(hydroxymethyl)-aminomethane.

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manifestations can be found in this case too. Plants exposed to the influence of Cd^{2+} exhibit a deficit of iron, due to which electron flow around PS1 is inhibited, as Siedlecka and Baszyński (1993) confirmed by *in vivo* experiments on maize plants. Šeršeň and Král'ová (2001) also found that 1 mM CdCl_2 is sufficient to totally inhibit photooxidation of reduced 2,6-dichlorophenolindophenol ($\text{DCPIP}_{\text{red}}$).

Negative effects of Cd^{2+} can also be observed in the so-called carboxylation phase of photosynthesis. In general, the toxicity of Cd^{2+} here leads to inactivation of enzymes by means of reactions with their thiol groups (Fuhrer 1982). The main target of the influence of toxic metals are two key enzymes of the photosynthetic fixation of carbon dioxide, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) and phosphoenolpyruvate carboxylase (PEPC) in *in vivo* as well as *in vitro* systems. Cd^{2+} ions lower the activity of RuBPCO and damage its structure by substituting for Mg^{2+} ions, which are important cofactors of carboxylation reactions and, last but not least, Cd^{2+} can shift RuBPCO activity towards oxygenation reactions (Siedlecka *et al.* 1998). High Cd^{2+} concentrations also lead to irreversible dissociation of the large and small RuBPCO subunits, the consequence being total inhibition of the enzyme (Stiborová 1988, Malik *et al.* 1992).

The influence of Cd^{2+} on cyanobacteria and algae has also been studied. Not only does Cd^{2+} ions deteriorate cell division (Laube *et al.* 1980), but also influences their ultrastructure (Fernandez-Piñas *et al.* 1995). Bascik-Remisiewicz and Tukaj (2002) studied the toxicity of Cd^{2+} in dependence on medium composition, pH, and CO_2 content. They found that a relation between Cd^{2+}

toxicity and pH can only be observed in algae cultivated at lower CO_2 concentrations. Toxicity of Cd^{2+} decreased with rising pH of the culture medium.

Since plants lack the mobility of the majority of animals, plants possess a potent physiological capacity to adapt to unfavourable environmental conditions. Plants have developed several protective mechanisms against heavy metal action (Tomsett and Thurman 1988, Florijn *et al.* 1993). The first is immobilisation of Cd^{2+} in the cell wall by the extracellular saccharides. This mechanism functions, first of all, in root systems (Nishizono *et al.* 1989, Verkleij and Schat 1990, Wagner 1993, Bekasova *et al.* 2002). The other mechanism is formation of phytochelatins, specific binding polypeptides (class III metallothioneins), which due to the presence of $-\text{SH}$ groups in their structure are able to chelate Cd^{2+} ions and hinder their circulation in the cytosol (Grill *et al.* 1985). Cyanobacteria also make use of this detoxification process, but the polypeptides produced are classified as class II metallothioneins. Other protective mechanisms include compartmentation and production of the so-called stress proteins.

Tůmová and Sofrová (2002) studied the response of intact cyanobacterial cells and of their photosynthetic apparatus to Cd^{2+} present in the cultivation medium up to 24 h. The aim of the present work was to compare the influence of Cd^{2+} on the photosynthetic apparatus of different photoautotrophic organisms: the cyanobacterium *Synechococcus elongatus* as a representative of prokaryots and spinach (*Spinacia oleracea L.*) as a representative of higher plants, moreover, using not intact cells but isolated chloroplasts and thylakoid membranes (TM).

Materials and methods

Material: Cultivation of the cyanobacterium *Synechococcus elongatus*, isolation of spheroplasts and TM were carried out as described by Tůmová and Sofrová (2002). Chlorophyll (Chl) *a* was analysed using the Ogawa and Vernon method (1971).

Isolation of spinach chloroplasts: Approximately 70 g washed leaves of market spinach were homogenised in type *ETA 012* (Czech Republic) household mixer for 15 s in 350 cm³ of MHM medium (5 mM MgCl_2 , 40 mM HEPES, and 500 mM D-mannitol; pH made up to 6.5 value using 1 M NaOH). The homogenate was filtered through 8 gauze layers. The filtrate thus obtained was centrifuged at 2 750×g, 1 min, 4 °C using a type *MPW-360* centrifuge (*Mechanika Precyzyjna*, Warszawa, Poland) and washed with 25 cm³ of MHM medium. The suspension was centrifuged at 2 750×g for 5 min at 4 °C using the same centrifuge. The sediment was resuspended in 1–2 cm³ of MHM medium and stored at –20 °C. The Chl (*a+b*) content was measured spectrophotometrically by the method of Arnon (1949).

Incubation with Cd^{2+} : Volumes of 2 M CdCl_2 in MHM were added to samples of cyanobacterial TM and spinach chloroplasts so as to achieve a desired final Cd concentration in the total volume of 1 cm³ suspension containing approx. 1 mg Chl and 0, 10, 100, and 1 000 mM CdCl_2 (Cd_0 , Cd_{10} , Cd_{100} , and Cd_{1000}) in the case of the cyanobacterium, and 0, 15, 30, and 60 mM CdCl_2 (Cd_0 , Cd_{15} , Cd_{30} , and Cd_{60}) in the case of spinach chloroplasts. The samples thus prepared were incubated with Cd^{2+} in the dark for 30 min at 4 °C and then washed with MHM.

Photochemical activities: Photochemical activities of PS2 in suspensions of cyanobacterial TM and spinach chloroplasts were measured as the photoreduction of 2,6-dichlorophenolindophenol (DCPIP, stock solution 2 mg DCPIP per 1 cm³ H₂O) in the presence or in the absence of 1,5-diphenylcarbazide (DPC, stock solution 12 mg DPC per 1 cm³ 100 % methanol) at 600 nm using a *Spekol 11* spectrophotometer (*Carl Zeiss*, Jena, Germany) in RMh buffer (10 mM KCl and 40 mM HEPES, pH made up to 6.5 value using 1 M NaOH). The reaction

mixture contained 20 µg Chl, 30 µM DCPIP, and where appropriate 0.3 mM DPC, in a total volume of 2 cm³ of RMH buffer.

The rate of oxygen evolution was measured using a Clark oxygen electrode (Hansatech, UK). The reaction cell was irradiated with red radiation from three lamps at 720 µmol m⁻² s⁻¹ and tempered to 30 °C. The reaction mixture contained sample volumes corresponding to 10 µg Chl, 1 mM potassium ferricyanide (FeCy), and 0.3 mM phenyl-p-benzoquinone (PBQ) in a total volume of 1 cm³ of the RMH medium.

SDS electrophoresis: A vertical 14 % (m/v) polyacrylamide gel was used in electrode buffer solution, containing 17 mM Tris-HCl, 1.3 % glycine, and 0.1 % SDS, pH 8.3. The same solution was used as upper and lower electrode buffer solution. Samples for electrophoresis were first resuspended in sample buffer solution containing 0.763 % Tris-HCl, pH 6.8, 2 % SDS, 10 % (m/v) glycerol, 5 % (v/v) mercaptoethanol, and a trace amount (0.003 %) of bromophenol blue in the ratio Chl : sample buffer 1 : 4 [µg : mm³]. The samples were then incubated for 3 min at 100 °C. Sample amounts corresponding to 3.75 µg Chl were applied to the gel. SDS-ELFO was carried out at 10 mA and 15±2 °C for 4 h. Proteins were detected using Coomassie Brilliant Blue R-250 (0.25 % solution in a mixture of ethanol : glacial acetic acid : water in a volume ratio of 25 : 10 : 65 containing 1 % trichloroacetic acid). A mixed protein standard (Sigma Co., St. Louis, MI, USA) containing α-lactalbumin (14 200), trypsin inhibitor (20 100), carbonic anhydrase (29 000), ovalbumin (45 000), bovine serum albumin (66 000), phosphorylase b (97 400), β-galactosidase (116 000), and myosin (205 000) was used.

Results

Photochemical activities: In the range of 0 to 30 min, the duration of incubation did not significantly influence the evolution of Cd²⁺ inhibition (Table 1). Consequently, penetration of Cd²⁺ ions into chloroplasts was fast. The control value decreased with time, *i.e.* incubation proper in the dark at 4 °C partly decreased the PS2 photochemical activity. Notwithstanding, the rate of inhibition was always near 50 %.

1 000 mM CdCl₂ exhibited the greatest inhibitory effect on the PS2 photochemical activity of *S. elongatus* TM (Table 2). We recorded a decrease of approx. 75 % of the activity measured as DCPIP photoreduction and about 95 % of the oxygen evolution activity. A slight rise in DCPIP photoreduction and oxygen evolution was recorded with Cd₁₀ and Cd₁₀₀. The activity of samples measured in the presence of the artificial electron donor DPC *versus* the H₂O → DCPIP activity increased significantly in the Cd₁₀₀₀ sample. A distinct decrease of DCPIP photoreduction in the suspension of spinach chloroplasts was recorded in the samples Cd₃₀ and Cd₆₀, while oxygen

MALDI-TOF mass spectroscopy: First, SDS-ELFO of samples of chloroplasts Cd₀ to Cd₆₀ incubated was performed (see SDS-ELFO) and then the destained gel was transferred into 1 % (v/v) acetic acid. Mass spectra were measured with a *Bruker BIFLEX* mass spectrometer, with time-of-flight detection (*Bruker-Franzen*, Bremen, Germany) as described by Bezouška *et al.* (1999).

RuBPCO carboxylation activity was determined by the following method (modified after Slack and Hatch 1967). Into a total volume of 0.5 cm³ the following were added in succession: 100 mm³ suspension of cyanobacterial spheroplasts or spinach chloroplasts, corresponding to about 100 µg Chl, 250 mm³ stock buffer solution (200 mM Tris-HCl pH 8.1, 2 mM EDTA, 60 mM MgCl₂, and 10 mM DTT), 70 mm³ distilled water, and 25 mm³ 100 mM NaHCO₃ solution. The samples were incubated at room temperature for 10–15 min, and then, 5 mm³ NaH¹⁴CO₃ (*Amersham Bioscience*, UK) of 3.7 MBq per mmol specific activity and 50 mm³ of 10 mM RuBP solution were added. The samples were incubated at room temperature for 5 min. The reaction was stopped by adding 0.5 cm³ of 50 % (v/v) HCl and the microtubes with the samples were left to stand open overnight in the fume cupboard for the non-reacted CO₂ to volatilize. After adding 10 cm³ *Eco-Plus* scintillation solution (*Roth*, Germany) the radioactivity of the samples was measured with the *Beckman LS 6000 SE* (USA) liquid scintillation counter. The activity of RuBPCO was calculated from amount of non-reacted substrate, obtained on the basis of sample radioactivity measurements. The protein content was measured according to Lowry (1951).

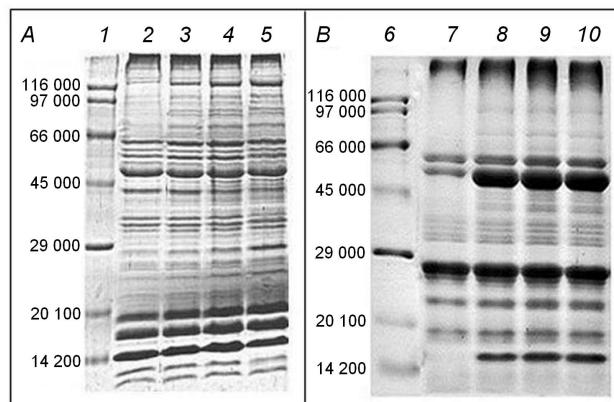


Fig. 1. Electrophoretic separation of proteins of cyanobacterial thylakoid membranes (TM) (A) and spinach chloroplasts (B) on 14 % polyacrylamide gel in SDS medium. Lanes: 1 – mixed standard, 2 – Cd₀, 3 – Cd₁₀, 4 – Cd₁₀₀, 5 – Cd₁₀₀₀, 6 – mixed standard, 7 – Cd₀, 8 – Cd₁₅, 9 – Cd₃₀, 10 – Cd₆₀.

Table 1. Influence of the duration of incubation on DCPIP photoreduction ($\text{H}_2\text{O} \rightarrow \text{DCPIP}$) [$\text{mmol}(\text{DCPIP red.}) \text{ kg}^{-1}(\text{Chl}) \text{ s}^{-1}$] in spinach chloroplasts exposed to 60 mM CdCl_2 for 10, 20, 30, and 40 min. Arithmetic mean \pm standard deviation ($n = 3$).

Time [min]	CdCl_2 [mM]	PS2 activity $\text{H}_2\text{O} \rightarrow \text{DCPIP}$	[%]
0	0	40.0 \pm 0.6	100.0 \pm 1.5
	60	20.3 \pm 2.4	50.8 \pm 12.0
10	0	35.6 \pm 1.9	100.0 \pm 5.2
	60	18.1 \pm 0.4	50.8 \pm 2.3
20	0	38.1 \pm 1.8	100.0 \pm 4.8
	60	17.5 \pm 0.8	46.0 \pm 4.6
30	0	33.6 \pm 0.3	100.0 \pm 0.8
	60	18.8 \pm 0.8	56.0 \pm 4.1

Table 2. Photoreduction of DCPIP ($\text{H}_2\text{O} \rightarrow \text{DCPIP}$, DPC \rightarrow DCPIP) [$\text{mmol}(\text{DCPIPred}) \text{ kg}^{-1}(\text{Chl}) \text{ s}^{-1}$] and oxygen evolution activity [$\text{mmol}(\text{O}_2) \text{ kg}^{-1}(\text{Chl}) \text{ s}^{-1}$] of cyanobacterial TM and spinach chloroplast suspensions incubated with different CdCl_2 concentrations for 30 min. The column of activity differences expresses the activity difference measured in the presence and without addition of DPC as artificial electron donor. Arithmetic mean \pm standard deviation ($n = 3$).

Organism	Sample	PS2 activity		DPC \rightarrow DCPIP (B) [%]	B/A [%]	Oxygen production	
		$\text{H}_2\text{O} \rightarrow \text{DCPIP}$ (A) [%]				$\text{H}_2\text{O} \rightarrow \text{FeCy} + \text{PBQ}$	[%]
<i>Synechococcus</i>	Cd_0	21.4 \pm 0.9	100.0 \pm 4.2	24.4 \pm 1.7	100.0 \pm 7.0	114.0	82.0 \pm 2.9
	Cd_{10}	24.2 \pm 0.5	113.1 \pm 2.1	27.5 \pm 2.3	112.7 \pm 8.4	113.6	89.3 \pm 3.7
	Cd_{100}	23.1 \pm 1.5	108.0 \pm 6.5	24.5 \pm 0.6	100.4 \pm 2.4	106.1	89.1 \pm 3.3
	Cd_{1000}	5.3 \pm 0.8	24.8 \pm 15.1	9.8 \pm 0.5	40.2 \pm 5.1	184.9	4.0 \pm 0.7
<i>Spinacia</i>	Cd_0	46.4 \pm 2.8	100.0 \pm 6.0	48.7 \pm 0.2	100.0 \pm 0.4	105.0	20.7 \pm 1.1
	Cd_{15}	39.6 \pm 0.4	85.3 \pm 1.0	42.8 \pm 1.1	87.9 \pm 2.6	108.1	21.0 \pm 0.3
	Cd_{30}	28.9 \pm 0.9	62.3 \pm 3.1	30.3 \pm 0.4	62.2 \pm 1.3	104.8	20.4 \pm 1.4
	Cd_{60}	26.0 \pm 0.6	56.0 \pm 2.3	29.0 \pm 1.0	59.5 \pm 3.4	111.5	12.3 \pm 1.0

these proteins and comparison with spectra contained in the data base *ProFound* provided their precise molecular masses. The size of regions of M_r 15 000 corresponds to the small subunit of the enzyme RuBPCO, the M_r of which is 14 610. A mixture of two proteins was ascribed to the M_r region around 50 000, *i.e.* the large RuBPCO subunit of M_r 50 410 and the β -subunit of ATP-synthase of M_r 50 290.

While the amount of some proteins in suspensions of cyanobacterial TM as well as in spinach chloroplasts determined by SDS-ELFO increased with the rising Cd^{2+} content, specific activity of RuBPCO measured by means of radioactively labelled $\text{NaH}^{14}\text{CO}_3$ showed, in contrary, a rapid decrease in both cases (Table 3).

Carboxylation activity of RuBPCO: While the specific carboxylation activity of spinach RuBPCO decreased at

evolution activity was not influenced significantly even in the Cd_{30} sample. The strongest inhibition was found in Cd_{60} , where both activities decreased by approx. 43 and 41 %, respectively. In this case, the presence of the non-physiological electron donor DPC had only a slight stimulating effect, most marked in Cd_{60} .

Protein composition of TM: In *S. elongatus*, electrophoresis showed several changes in protein pattern but the most significant changes were found in the relative molecular mass (M_r) regions of approx. 29 000 and 116 000. In these two bands, the intensity of the changes increased with rising Cd concentration in the sample (Fig. 1A). In spinach chloroplasts, we found the most marked changes in the M_r regions around 15 000 and 50 000 (Fig. 1B). Measurement of the mass spectra of

the highest concentration used, *i.e.* Cd_{60} , down to approx. 2.5 %, in *S. elongatus* it still remained on the 40 % level in the Cd_{1000} sample (Table 3).

Table 3. Specific activity of RuBPCO [$\mu\text{mol}(\text{RuBP}) \text{ s}^{-1} \text{ kg}^{-1}(\text{protein})$] determined in suspensions of cyanobacterial spheroplasts and spinach chloroplasts incubated in different CdCl_2 concentrations. Arithmetic mean \pm standard deviation ($n = 3$).

Organism	Sample	RuBPCO	[%]
<i>Synechococcus</i>	Cd_0	83.3 \pm 10.0	100.0 \pm 12.4
	Cd_{100}	31.7 \pm 8.3	37.5 \pm 15.6
	Cd_{1000}	33.3 \pm 6.7	40.5 \pm 17.5
<i>Spinacia</i>	Cd_0	345.0 \pm 26.7	100.0 \pm 7.6
	Cd_{15}	75.0 \pm 3.3	21.7 \pm 3.4
	Cd_{60}	8.3 \pm 1.7	2.5 \pm 12.3

Discussion

In *S. elongatus* the lower CdCl₂ concentrations (Cd₀ to Cd₁₀₀) had rather an activating effect on TM, even though Cd is one of the most toxic metals. We recorded a distinct inhibitive effect only with Cd₁₀₀₀: PS2 photochemical activity decreasing by about 75 %. We observed a similar effect in oxygen evolution measurements: Cd²⁺ ions again caused a slight activity rise in samples Cd₁₀ and Cd₁₀₀, while approx. only 5 % of the original activity was found in the Cd₁₀₀₀ sample. Cyanobacteria accumulate large amounts of toxic metals relatively quickly. For example, the cyanobacterium *Chroococcus paris* can contain up to 53 g(Cd²⁺) kg⁻¹(dry mass), 90 % of the metal content being bound within the first minute (Les and Walker 1984). Although the degree of toxic metal accumulation by cyanobacteria depends on the species, physiological conditions, and metal ion concentration in the medium as well as on the physico-chemical properties of the medium, *i.e.* pH and temperature (Kostyaev *et al.* 1980, Les and Walker 1984, Trevors *et al.* 1986, Koelmans *et al.* 1996), cyanobacteria can be used as a tool for improving the quality of soil and water.

Since it is evident from measured data that Cd²⁺ ions inhibit photochemical activity in the vicinity of PS2, we attempted to find out by measuring these activities in the presence of the non-physiological electron donor DPC whether the disturbance of electron transport takes place at the site of electron transfer from the natural electron donor, *i.e.* water to the PS2 reaction centre or whether the disturbance is localized in a site between the PS2 reaction centre and plastoquinone. We conclude that the site of the effect of Cd²⁺ probably is the oxidative (donor) side of PS2. We derive this conclusion from rising photochemical activity measured in the presence of DPC. This rise was as high as 85 % in the Cd₁₀₀₀ sample. Therefore, DPC in cyanobacteria is able to supply electrons to damage PS2 with relatively high efficiency.

In spinach chloroplasts, we found that DCPIP photoreduction decreased with Cd²⁺ concentration, even though we used CdCl₂ concentrations ten times lower than in

S. elongatus TM. From this point of view, cyanobacterial TM are far more resistant to the influence of Cd²⁺ than spinach chloroplasts.

Studying the influence of CdCl₂ on spinach chloroplasts by electron paramagnetic resonance, Šeršeň and Král'ová (2001) found direct interaction of CdCl₂ with the complex of manganese ions which is situated in OEC, CdCl₂ causing release of manganese ions from this complex. This inactivates electron transfer from water to the PS2 reaction centre. Our measurements did not prove an unambiguous effect of DPC, therefore we cannot corroborate the results reported by Šeršeň and Král'ová.

In cyanobacteria we observed a rise of protein amounts in regions of M_r around 116 000 and 29 000. Although photochemical activities of cyanobacterial TM samples decreased, we found by electrophoresis no proteins the content of which would decrease with the rising Cd²⁺ concentration in the sample.

In experiments with spinach chloroplasts, protein band intensities in regions of M_r 15 000 and 50 000 increased with the rising CdCl₂ concentration. Mass spectroscopy confirmed that the regions involved correspond to the small and large subunits of RuBPCO. Stiborová (1988) also confirmed irreversible dissociation of the subunits of this enzyme under the influence of Cd²⁺ ions. In spinach we found by measuring the carboxylation activity of RuBPCO using the substrate labelled with NaH¹⁴CO₃ that the specific activity of the enzyme in the samples rapidly decreased with the rise of Cd²⁺ ion concentration (down to 2.5 % of the original enzyme activity in the sample Cd₆₀). We also studied carboxylation activity in *S. elongatus*, where however the enzyme retained approx. 40 % of the original activity even in the Cd₁₀₀₀ sample. Hence, in this case the cyanobacterium was more resistant to the influence of Cd²⁺ ions than spinach. Our results also confirmed that cyanobacteria possess a high binding capacity to ions of toxic metals and could be used to remediate environmental contamination.

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