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Photosynthesis of *Chlorella vulgaris* as affected by diaqua(4-chloro-2-methylphenoxyacetato)copper(II) complex

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Abstract

Inhibitory effect of diaqua(4-chloro-2-methylphenoxyacetato)copper(II) complex (MCPACu) on oxygen evolution rate in algae *Chlorella vulgaris* was found. Electron paramagnetic resonance and fluorescence spectroscopy showed that the sites of action of MCPACu are Z^+ and Y^+ intermediates on the donor side of photosystem 2 as well as the manganese cluster in the oxygen evolving complex.

Additional key words: algae, copper toxicity, electron paramagnetic resonance, oxygen evolution rate, photosystem 2.

Introduction

Due to industrial pollution and agricultural practices phytotoxic amounts of heavy metals can contamine the soil as well as aquatic ecosystems. The most general symptoms of their phytotoxicity are lower yield and chlorosis (Foy *et al.* 1978).

Copper(II) ions are known to inhibit photosynthetic processes in plants (Mohanty *et al.* 1989, Yruela *et al.* 1991, 1992, 1993, Maksymiec *et al.* 1994) and in green algae (Samson *et al.* 1988). Some authors located the Cu^{2+} binding site at various parts of the acceptor side of PS 2 (Mohanty *et al.* 1989, Yruela *et al.* 1991, 1993, Maksymiec *et al.* 1994), however Samson *et al.* (1988) suggested that Cu^{2+} impairs the PS 2 photochemistry by affecting the water-oxidizing site of the donor side of PS 2 and Hsu and Lee (1988) situated Cu^{2+} site of action into the core of PS 2. Shioi *et al.* (1978a,b) found two sites of Cu^{2+} action, namely the donor side of PS 2 and ferredoxin which is situated in the acceptor side of PS 1.

Diaqua(4-chloro-2-methylphenoxyacetato)copper(II) complexes with selected

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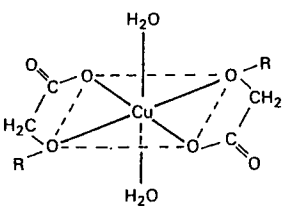
Abbreviations: Chl - chlorophyll; DMSO - dimethyl sulfoxide; EPR - electron paramagnetic resonance; IC_{50} - concentration of MCPACu causing 50 % OER inhibition with respect to the control; MCPACu - diaqua(4-chloro-2-methylphenoxyacetato)copper(II) complex [$(Cu(Cl4Me2PhOAc)_2 (H_2O)_2]$; OER - oxygen evolution rate; PS - photosystem.

O- and N-donor ligands show important herbicidal activity which is characteristic for the organic aryloxyacetate skeleton and also relatively low fungicidal activity against phytopatogene fungi (Blahová *et al.* 1982, 1992). It was found that the aqua(aryloxyacetato)copper(II) complexes inhibit oxygen evolution rate in spinach chloroplasts and the site of action of these inhibitors of photosynthesis are Z^+ and Y^+ intermediates on the donor side of PS 2 as well as the manganese cluster in the oxygen evolving complex (Kráľová *et al.* 1994). The aim of this work is investigating the effect of diaqua(4-chloro-2-methylphenoxyacetato)copper(II) complex (MCPACu) on photosynthetic processes in *Chlorella vulgaris*.

Materials and methods

The studied compound MCPACu was prepared according to Krätsmár-Šmogrovič and Jokl (1965).



R = 4-chloro-2-methylphenyl

Chlorella vulgaris was stationarily cultivated (3 weeks, 16 h photoperiod) in cultivation medium described by Sidóová *et al.* (1992).

The oxygen evolution rate (OER) in algal suspensions (*Chlorella vulgaris*) was measured at 24 °C using a Clark type electrode (*SOPS 31 atp, Chemoprojekt*, Prague, Czech Republic) in a chamber constructed according to Bartoš *et al.* (1975). Prior to the OER measurements the suspensions were accomodated in the dark (4 h). The samples were then illuminated with a 250 W halogen lamp through a water filter (irradiance 450 μmol(PAR) m^{-2} s^{-1}).

The EPR spectra were registered by an *ESR 230* apparatus (*ZWG AdW*, Berlin, Germany) which operates in the X band at 24 °C. The concentration of algal suspension was about 2.1 mg(Chl) cm^{-3} for the algal system, the used microwave power 5 mW, the modulation amplitude 5×10^{-4} T. The samples were illuminated directly in the resonator cavity with a 250 W halogen lamp and they were protected against warming by a water filter.

The fluorescence emission spectra were recorded by fluorescence spectrophotometer *F-2000* (*Hitachi*, Tokyo, Japan) at room temperature. The samples of *Chlorella vulgaris* (10 mg(Chl) dm^{-3}) were excited at 436 nm, *i.e.* at the wavelength causing mainly excitation of Chl *a*, using a 10 nm slit. The samples were kept in the dark for 10 min prior to the measurements.

Because of low water solubility of MCPACu, it was dissolved in DMSO. The applied concentration of DMSO (10 %, v/v) did not affect the photochemical activity of algae.

Results and discussion

MCPACu inhibited OER in *Chlorella vulgaris* (Fig. 1). The corresponding IC_{50} value of MCPACu for algal suspension containing 15 mg(Chl) dm^{-3} is 1.46 mmol dm^{-3}. As manifested in Fig. 2 MCPACu decreases the fluorescence intensity at 684 nm belonging to the pigment protein complex of PS 2 (Kráľová *et al.* 1994). Thus, it can be assumed that MCPACu interacts with PS 2 causing fluorescence quenching of Chl *a* in the pigment protein complex of PS 2. At constant concentration of MCPACu Chl *a* fluorescence intensity shows a time-dependent decrease (Fig. 2).

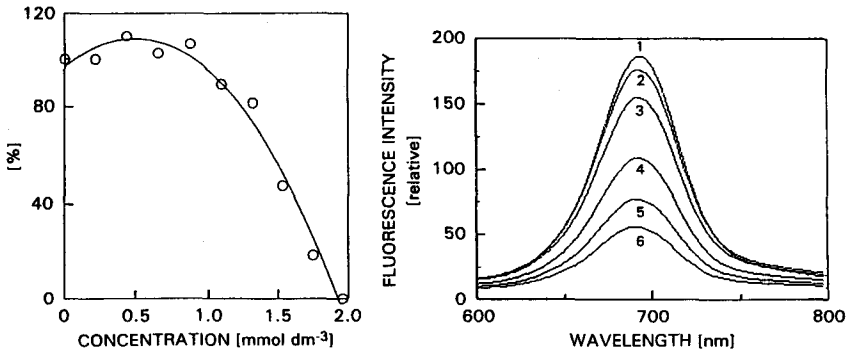


Fig. 1. Dependence of oxygen evolution rate (OER) upon MCPACu concentration in *Chlorella vulgaris* suspension [15 mg(Chl) dm^{-3}]. The composition of the cultivation medium applied in the suspension is described in Sidóová *et al.* (1992). 100 % activity is 0.15 mmol(O_2) g^{-1}(Chl) s^{-1}.

Fig. 2. Fluorescence spectra of *Chlorella vulgaris*: untreated (*curve 1*) and treated with 0.48 mmol dm^{-3} MCPACu (*curves 2 - 6*: 10, 20, 30, 40, 50 min after addition of MCPACu)

The damage of PS 2 by MCPACu was detected also by EPR spectroscopy. The investigation of EPR signals I and II belonging to photosystems PS 1 and PS 2 showed that due to the effect of MCPACu the signal II belonging to Z^+/Y^+ intermediates situated on the donor side of PS 2 had completely disappeared (Fig. 3B versus 3A, full lines). On the other hand, the intensity of signal I belonging to PS 1 remained low also after illumination (Fig. 3B, dashed line). This can be explained by the interaction of MCPACu with ferredoxin (Shioi *et al.* 1978b) causing interruption of the electron flow from PS 1 to the final acceptor NADPH.

Contrary to these findings, in spinach chloroplasts the intensity of signal I in the light obtained in the presence of MCPACu was pronouncedly higher than that of untreated chloroplasts (Kráľová *et al.* 1994) due to the interruption of the electron transport between the photosynthetic centres. This indicates that the electron flow in PS 1 is not damaged. This different effect of MCPACu on signal I in the algal and chloroplast suspensions can be caused by some differences between two applied model photosynthesizing organisms (intact *Chlorella vulgaris* cells and spinach chloroplasts broken up to 90 %).

From the viewpoint of MCPACu action on photosynthetic apparatus it can be

assumed that Cu^{2+} ions of this compound have a certain possibility to exchange its ligands with subsequent formation of coordination compounds with the aminoacids taking place in PS 1 and PS 2 proteins. This assumption is supported also by the finding that the EPR signals belonging to Z^+/Y^+ intermediates disappeared due to interactions of MCPACu with PS 2. These intermediates are tyrosine cation radicals taking place in PS 2 proteins D_1 and D_2, respectively (Barry and Babcock 1988). In the presence of MCPACu the photosynthetic apparatus can be affected also by replacing of bivalent metal ions situated in PS 1 and PS 2. This phenomenon was confirmed in our previous paper (Kráľová *et al.* 1994) where Cu^{2+} released Mn^{2+} from the manganese cluster on the donor side of PS 2 into interior of spinach thylakoids. The released Mn^{2+} were confirmed by EPR spectroscopy. Similar effect of MCPACu, however with lower intensity, was observed also with the studied algal photosynthetic system (not documented).

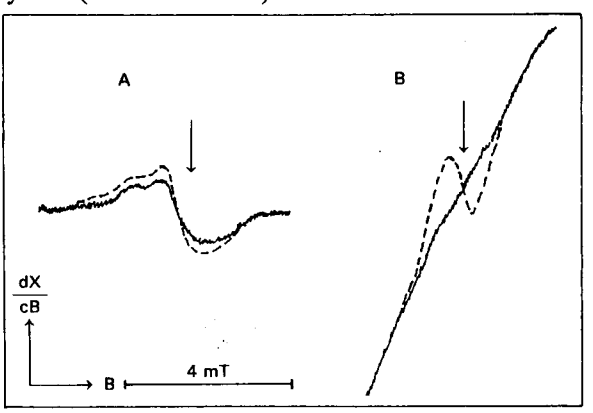


Fig. 3. EPR spectra of untreated algal suspension (*A*) and in the presence of 0.05 mol dm^{-3} of MCPACu (*B*). The full lines correspond to the suspensions kept in the dark, the dashed lines to the illuminated ones. The arrows denote g = 2.0026.

It can be concluded that the site of action of MCPACu is the donor side of PS 2 what is in good accordance with the findings of Samson *et al.* (1988), Hsu and Lee (1988) and Shioi *et al.* (1978a, 1978b). This was confirmed by disappearance of the EPR signal II, by the presence of the EPR signal belonging to the free Mn^{2+} and by the decrease of chlorophyll fluorescence intensity. The site of action situated on the acceptor side of PS 2 reported by Yurela *et al.* (1991, 1992, 1993) and Mohanty *et al.* (1989) was not confirmed. The proposed site of action situated in the donor side of PS 2 was supported also by the fact that in the presence of MCPACu the photoreduction of 2,6-dichlorophenol-indophenol in chloroplasts was restored by diphenylcarbazide (Kráľová *et al.* 1994). This could not take place if the side of action of Cu^{2+} would be situated in the acceptor side of PS 2, between pheophytin and the secondary quinone acceptor Q_B. According to Shioi *et al.* (1978b), a further site of action of the studied compound, namely ferredoxin in the acceptor side of PS 1, has been confirmed (the increase of the intensity of the EPR signal I in the light was not observed). However, the explanation of the different effects of MCPACu on PS 1 in algae and spinach chloroplasts requieres a further detail study.

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