Effects of Cu(II) Complexes on Photosynthesis in Spinach Chloroplasts. Aqua(aryloxyacetato)copper(II) Complexes

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Abstract. The inhibitory effect of 14 aqua(aryloxyacetato) copper(II) complexes on oxygen evolution rate in spinach chloroplasts has been investigated. The inhibitory effect of these effectors on photosynthesis was confirmed by Hill reaction as well as by EPR and fluorescence spectroscopies. The results of the EPR study showed that the sites of action of the studied effectors are Z^+ and Y^+ intermediates at the donor side of the photosystem (PS) 2. The EPR study also showed that another site of action is the oxygen evolving complex, namely its manganese cluster. The above suggestions were supported by the results of the fluorescence study as well. Based on the restoring of the photosynthetic electron transport to 2,6-dichlorophenol-indophenol in chloroplasts inhibited by the studied Cu(II) complexes using sym-diphenylcarbazide it can be assumed that the own core of PS2 (P680) and a part of the electron transport chain – at least up to plastoquinone – remain intact.

Key words: Copper(II) complexes — Photosynthesis inhibition — Spinach chloroplasts — EPR spectroscopy — Fluorescence spectroscopy

Introduction

Considering the increasing problem of environmental pollution by heavy metals, the study of their effects upon ecosystems is of great importance. Cations of the heavy metal copper (Cu(II)) are well known to inhibit the photosystem 2 (PS 2) electron transport in higher plants (Hsu and Lee 1988; Mohanty et al. 1989) and in green algae (Samson et al. 1988). However, the Cu(II)-binding site and the underlying inhibitory mechanisms are still controversial. Mohanty et al. (1989), Hsu and Lee (1988), Singh and Singh (1987), Yruela et al. (1991, 1993), and Maksymiec et al. (1994) located the Cu(II) binding site on various sites of the acceptor side of PS 2. Samson et al. (1988) suggested that Cu(II) impairs the PS 2 photochemistry by

affecting the water-oxidizing site on the donor side of PS 2.

Copper salts of phenoxyacetic acid and of its substituted derivatives are interesting not only from the viewpoint of their coordinative chemical properties (Prout et al. 1968; Goebel and Doedens 1972; Smith and Reilly 1981) but also because of a variety of their biological activities. The 4-chloro- and 4-chloro-2-methyl-phenoxyacetato copper(II) complexes with some O- and N-donor ligands have been shown to have important herbicidal activity which is typical of the organic aryloxyacetate skeleton (Blahová et al. 1982, 1992). These compounds exhibit relatively low fungicidal activity against phytopatogenic fungi (Blahová et al. 1982, 1992). Interesting results were obtained by studying antimicrobial activities of some aqua(carboxylato)copper(II) complexes and (3,5-dimethylpyrazole)-(aryloxyacetato)copper(II) complexes (Sokolík et al. 1992). The values of minimum inhibitory concentrations of these effectors vary in the range of 400–800 $\mu g.cm^{-3}$ for *Escherichia coli*, 400–600 $\mu g.cm^{-3}$ for *Candida albicans* and 300 $\mu g.cm^{-3}$ for *Staphylococcus aureus* (Blahová et al. 1993). The anti-inflammatory activity of selected mononuclear aqua(aryloxyacetato)copper(II) complexes was investigated by Sokolík et al. (1993) and Blahová et al. (1994); the biological activities of the most effective compounds were reported to be comparable with that of copper(II)salicylate tetrahydrate.

This work is aimed at investigating the effect of 14 aqua(aryloxyacetato)copper(II) complexes on photosynthetic processes in spinach chloroplasts.

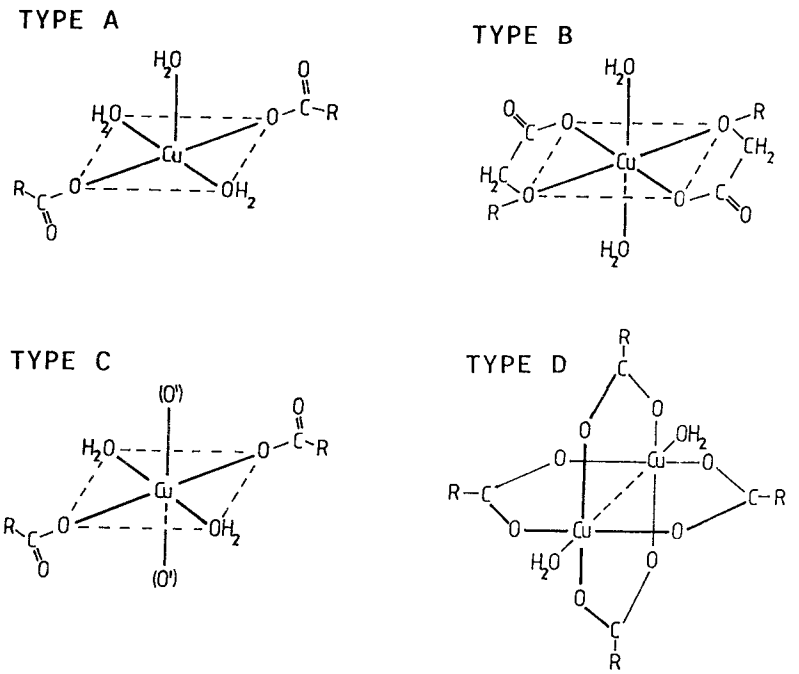
Materials and Methods

The aqua(aryloxyacetato)copper(II) complexes studied were prepared at room temperature, using the method described by Kratsmár-Šmogrovič and Jokl (1965), by the reaction of Cu^{2+} ions with the corresponding aryloxyacetate ions in aqueous solution (pH approximately 4.5). The stoichiometric composition, the elemental analysis (C, H, Cu) and the physico-chemical properties of the studied compounds are described in Blahová et al. (1972, 1980, 1986, 1992), Kratsmár-Šmogrovič et al. (1973), Plesch et al. (1973). The structural types of the individual aqua(aryloxyacetato)copper(II) complexes studied were determined by magnetic measurements and by the parameters of their electron and EPR spectra in the solid state (Scheme 1).

$CuSO_4.5H_20$ (p.a) was obtained from Lachema, Brno.

The effects of the studied compounds on oxygen evolution rate in spinach chloroplasts were investigated spectrophotometrically (Specord UV VIS Zeiss Jena, Germany) in the presence of the electron acceptor 2,6-dichlorophenol-indophenol, according to Kráľová et al. (1992). Because of low water solubility of the studied compounds, they were dissolved in dimethyl sulfoxide (DMSO). The applied concentration of DMSO did not affect the photochemical activity of spinach chloroplasts. The inhibitory activity of the compounds studied has been expressed in terms of IC_{50} values, i.e. by the concentration of the effector (in $mol.dm^{-3}$) causing a 50% decrease of the parameter studied, as compared to control samples.

EPR measurements were carried out with ERS 230 (WG, Akademie der Wissen-



Scheme 1. Structural types of the studied Cu(II) complexes

Type A – $[Cu(R\text{-}COO)_2(H_2O)_3]$
R = phenoxymethyl (I), 4-methylphenoxymethyl (IV), 4-chloro-3-methylphenoxymethyl (X)
Type B – $[Cu(R\text{-}COO)_2(H_2O)_2]$
R = 2-methylphenyl (II), 3-methylphenyl (III), 3-chlorophenyl (VII), 4-chlorophenyl (VIII), 4-chloro-2-methylphenyl (IX), 2-nitrophenyl (XIV)
Type C – $[Cu(R\text{-}COO)_2(H_2O)_2]$. 2 H_2O
R = 2-chlorophenoxymethyl (VI), 2,4-dichlorophenoxymethyl (XI), 1-naphtoxymethyl (XII), 2-naphtoxymethyl (XIII)
Type D – R = 2-chlorophenoxymethyl (V)

schaften, Berlin, Germany) operating in X-band at 5 mW of microwave power and 5.10^{-4} T of the magnetic field modulation. EPR spectra of untreated spinach chloroplasts as well as those in the presence of the compounds tested (0.05 mol.dm^{-3}) were recorded both in the dark and in the light. The chlorophyll (Chl) content in the samples was 4 mg.cm^{-3}. Irradiation was carried out with a 250 W halogen lamp through a water filter.

Fluorescence measurements were performed with an F-2000 spectrophotometer (Hitachi, Japan) at room temperature (approximately 24 °C). Chloroplast suspensions containing 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.

Results

The aqua(aryloxyacetato)copper(II) complexes studied inhibit oxygen evolution rate in spinach chloroplasts. The corresponding IC_{50} values for the tested effectors and the types of their structures are summarized in Table 1. The biological activities of the 14 investigated copper(II) complexes varied within a relatively narrow concentration range (IC_{50} values: 4.58 μmol.dm^{-3} for compound V, and 22.59 μmol.dm^{-3} for compound II) and are comparable with the IC_{50} value determined for $CuSO_4.5H_2O$ (13.21 μmol.dm^{-3}). After storage of the solutions of the studied copper(II) complexes in DMSO for 24 h, the IC_{50} values increased about threefold. Thus, the stability of the complexes in solution is insufficient, and the products formed after degradation are less efficient inhibitors of photosynthesis.

Table 1. IC_{50} values for oxygen evolution rate in spinach chloroplasts in the presence of aqua(aryloxyacetato)copper(II) complexes. Symbols: PhOAc, phenoxyacetate anion; Me, methyl; Cl, chloro; NO_2, nitro; NOAc, naphtoxyacetate anion

N	Compound	$IC_{50}.10^6$ (mol.dm^{-3})	Type of structures
I	$Cu(PhOAc)_2.3H_2O$	8.93	A
II	$Cu(Me2PhOAc)_2.2H_2O$	22.59	B
III	$Cu(Me3PhOAc)_2.2H_2O$	13.16	B
IV	$Cu(Me4PhOAc)_2.3H_2O$	11.20	A
V	$Cu(Cl2PhAc)_2$	4.58	D
VI	$Cu(Cl2PhOAc)_2.4H_2O$	9.22	C
VII	$Cu(Cl3PhOAc)_2.2H_2O$	9.98	B
VIII	$Cu(Cl4PhOAc)_2.2H_2O$	7.87	B
IX	$Cu(Cl4Me2PhOAc)_2.2H_2O$	15.38	B
X	$Cu(Cl4Me3PhOAc)_2.3H_2O$	11.64	A
XI	$Cu(diCl2,4PhOAc)_2.4H_2O$	10.50	C
XII	$Cu(1\text{-}NOAc)_2.4H_2O$	10.79	C
XIII	$Cu(2\text{-}NOAc)_2.4H_2O$	18.71	C
XIV	$Cu(2\text{-}NO_2PhOAc)_2.2H_2O$	7.27	B

The effects of the studied compounds on the set of photosynthetic centres PS 1 and PS 2, showing EPR signals in the region of free radicals (so-called signal I and signal II) were studied using EPR spectroscopy. Fig. 1 illustrates the effect of compound IX on EPR spectra of spinach chloroplasts. From Fig. 1 it is obvious that compound IX decreased the intensities of EPR signal II_{slow} and signal $II_{very\ fast}$, belonging to intermediates Y^+ and Z^+ respectively, which are situated on the donor

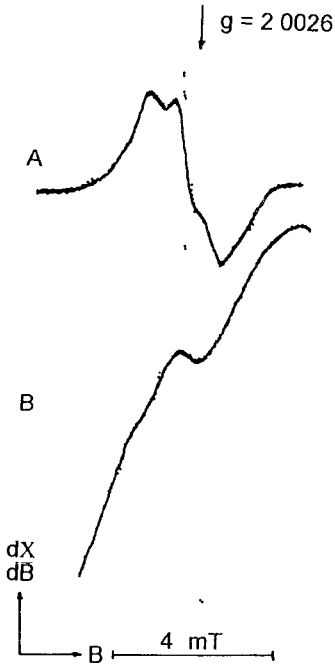


Figure 1. EPR spectra of untreated spinach chloroplasts (*A*) and of chloroplasts treated with 0 05 mol dm^{-3} Cu (Cl4 Me2PhOAc)$_2$ 2H_2O (compound IX) (*B*) The full lines correspond to chloroplasts kept in the dark the dotted lines to the illuminated chloroplasts The dotted spectrum in *B* was recorded at sensitivity two times lower than recordings of the other spectra

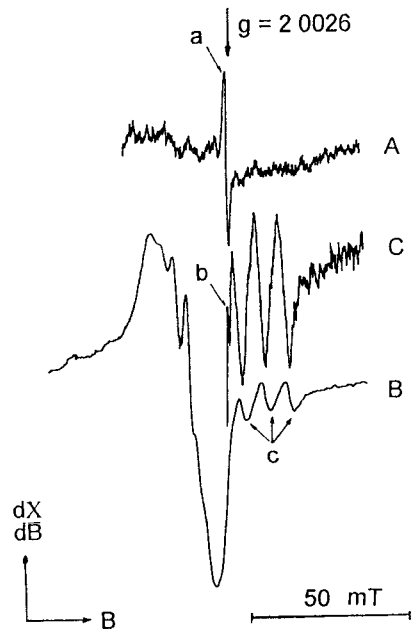


Figure 2. EPR spectra of untreated spinach chloroplasts (*A*) and in the presence of 0 025 mol dm^{-3} Cu(Cl4Me2PhOAc)$_2$ 2H_2O (compound IX) and 10% dimethyl sulfoxide (*B*, *C*) Spectrum *B* was recorded at four times lower sensitivity The arrows marked "*a*" point to signals I and II, that marked "*b*" signal I, and those marked "*c*" the signal of Mn^{2+} ions

side of PS 2 (Fig 1*B*, full line) On the other hand, after illumination of treated chloroplasts, a very pronounced increase of signal I, belonging to the chlorophyll dimer in PS 1, could be observed (Fig 1*B*, dotted line)

The effects of the aqua(aryloxyacetato)copper(II) complexes on the photosynthetic centres of chloroplasts were investigated by studying of Chl_a fluorescence The decrease of the fluorescence maximum at 686 nm, belonging to the pigment protein complexes in PS 2, in the presence of compound IX suggests the inhibitory effect of this effector on spinach chloroplasts (Fig 3)

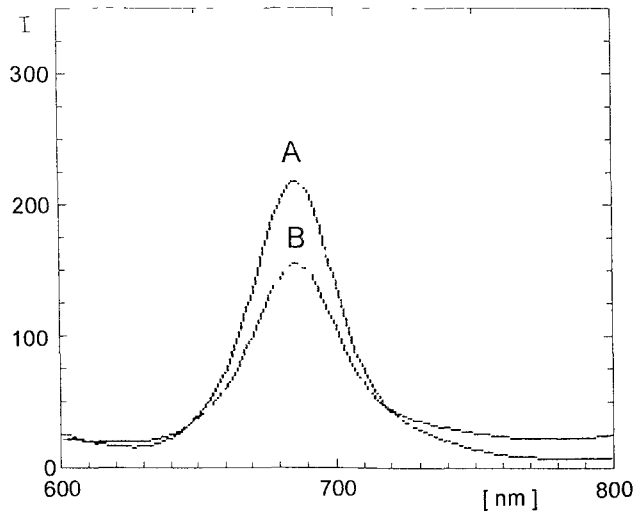


Figure 3. Fluorescence spectra of untreated spinach chloroplasts (*A*) and of chloroplasts treated with saturated aqueous $Cu(Cl4Me2PhOAc)_2 . 2\ H_2O$ (compound IX) solution (*B*).

Discussion

Aqua(aryloxyacetato)copper(II) complexes are composed from two biologically active components, copper(II) and the aryloxyacetic part which is typical of the group of plant growth regulators of the auxine type. Also, it is probable that the resulting biological activity of the studied effectors is affected by both components. The IC_{50} values for the inhibition of oxygen evolution rate in spinach chloroplasts by polysubstituted phenoxyacetic acids 2,4-dichlorophenoxy (2,4-D) and 2-methyl-4-chlorophenoxyacetic acid (MCPA) are one to two orders higher than those obtained for the studied aqua(aryloxyacetato)copper(II) complexes (3.16 and 9.33 $mmol.dm^{-3}$ respectively) (Kráľová et al. 1993). This means that, from the viewpoint of biological effectiveness, the presence of copper(II) in the studied effectors is of great importance. However, the decrease of the inhibitory activity due to the degradation of the complex after its storage in DMSO solution over 24 h indicates that the copper(II) complex is more active than its individual components. Similar to the inhibitory activity of the studied effectors on oxygen evolution rate in spinach chloroplasts (Table 1), also their antimicrobial activities showed only relatively small variations depending on the structure of the aryloxyacetic part of the effector (Blahová et al. 1993).

EPR experiments documented the effects of the aqua(aryloxyacetato) copper(II) complexes on the photosynthetic centres. The decrease of the intensity of signal II_{slow}, belonging to the Y^+ intermediate, namely to the cation radical

tyrosine 160 in the donor part of protein D_2 in PS 2, and the decrease of signal $II_{very\ fast}$, belonging to Z^+ intermediate, namely to the cation radical of tyrosine 161 in the donor part of D_1 protein in PS 2, suggested that the site of action of the studied copper(II) complexes is the donor side of PS 2. This was supported also by the presence of EPR signal, belonging to free Mn^{2+} ions released from the manganese cluster located in the oxygen evolving complex on the donor side of PS 2. The effect of compound IX on manganese cluster of spinach chloroplasts is illustrated by the EPR spectra (Fig. 2). Interactions of compound IX with the manganese cluster resulted in the release of free Mn^{2+} ions into the interior of the thylakoid membranes. The signal of free Mn^{2+} ions was seen in the EPR spectra of chloroplasts treated with compound IX (Fig. 2, lines *B*, *C*). This signal, consisting of six lines, was superposed onto the EPR signal of compound IX (Fig. 2, line *B*). Due to a high intensity of the EPR spectrum of compound IX from the six lines corresponding to Mn^{2+} only three could be resolved.

No effects of the studied copper(II) complexes were observed on PS 1. This is documented by the EPR spectra of chloroplasts treated with effector IX (Fig. 1*B*, dotted line) where a very intensive EPR signal I appeared during illumination. The increase of signal I, belonging to the Chl_a dimer of P 700^+, was caused by the damage to PS 2. Due to this injury PS 2 could not supply the electrons to PS 1 and was unable to reduce P 700^+ to its neutral form, and consequently an intensive increase of signal I can be observed in EPR spectra (Šeršeň et al. 1990). In contrast to the studied aqua(aryloxyacetato)copper(II) complexes affecting only PS 2, the corresponding uncomplexed aryloxyacetic acids (Gribova et al. 1985; Kráľová et al. 1993) impair both photosynthetic centres, PS 1 and PS 2.

The results of fluorescence measurements support the findings obtained with EPR spectroscopy concerning the site of action of the studied Cu(II) complexes. The decreased intensity of the emission band at 686 nm, belonging to the pigment-protein complexes in PS 2 (Atal et al. 1991) (Fig. 3), suggests PS 2 as the site of action of the studied complexes.

Upon addition of sym-diphenylcarbazide (0.5 $mmol.dm^{-3}$) to chloroplasts inhibited by the studied Cu(II) effectors the oxygen evolution rate was restored up to 90%. Since the site of action of this artificial donor of PS 2 is an intermediate in Z/Y on the donor side of PS 2 (Jagerschöld and Styring 1991) it can be assumed that, in the presence of the studied effectors, the own core of PS 2 (P 680) and a part of the elecron transport chain – at least up to plastoquinone – remain intact.

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