Influence of a novel organometallic Cu(II) complex on the photochemical activity of Photosystem II in spinach

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Photosynthesis is an attractive target for inhibitory compounds, both for the development of new herbicides and for advancing the understanding of photosynthetic processes. The synthetic $[CuL_2]Br_2$ complex was studied for its inhibitory effect on the photosynthetic activity of photosystem II. It was demonstrated that a complex containing a benzothiazole group is an effective suppressor of photosynthetic activity.

Keywords*: Organometallic complexes, DCMU, OJIP, inhibitors*

INTRODUCTION

Herbicides remain the most effective method for controlling weeds. However, the repeated use of the same compounds leads to soil and water pollution, with chemical substances posing a risk of environmental damage (Vítek et al., 2017). Therefore, to mitigate environmental harm, special attention should be given to the development of new, effective, and selective compounds that act through different mechanisms. Photosynthesis is a complex process in which solar energy is converted into the energy of chemical bonds. As a vital process for all photosynthetic organisms, photosynthesis remains an attractive target for the application of inhibitory compounds (Zharmukhamedov et al., 2022). Currently, numerous chemical compounds are capable of inhibiting essential reactions in photosynthesis (Schütte et al., 2017).

However, compounds that affect only one of the metabolic pathways in plants are not very effective due to the evolving resistance of plants to their action. Therefore, the development of a universal inhibitor capable of suppressing a wide range of vital reactions represents a promising approach to addressing the resistance problem (Vass, 2012). In addition, chemical compounds can serve as tools for studying the mechanisms of photosynthetic reactions. Numerous exogenous artificial electron donors and acceptors, as well as inhibitors, are widely used to separate the electron transport chain into distinct regions, allowing for their study without disrupting the thylakoid membrane with detergents.

Copper plays an important role in a variety of metabolic processes in plants, cyanobacteria, and algae (Yruela, 2005). Cu^{2+} ions are essential for plant growth; however, high concentrations of Cu(II) exhibit the highest toxicity among heavy metal cations. It has been demonstrated that components of photosystem II (PSII) are more sensitive to the inhibitory effects of Cu than those of photosystem I (PSI) (Murakami et al., 2014). It is hypothesized that both the donor and acceptor sides of PSII are affected by Cu. Evidence suggests that Cu inhibits the activity of the PSII reaction center, enhances chlorophyll (Chl) degradation, and inhibits the activity of the water-oxidizing complex (WOC). Furthermore, Cu can disrupt PSII photochemical activity and alter the structure of thylakoid membranes, affecting the overall activity of the photosystems (Deng et al., 2014).

In this study, the $[CuL_2]Br_2$ complex (where L =bis{4H-1,3,5-triazino[2,1-b]benzothiazole-2-amine, 4-(2-imidazole)}copper(II) bromide) was investigated for its inhibitory effect on the photochemical activity of spinach. Figure 1 shows the structure of the ligand (A) and the Cu(II)-complex (B).

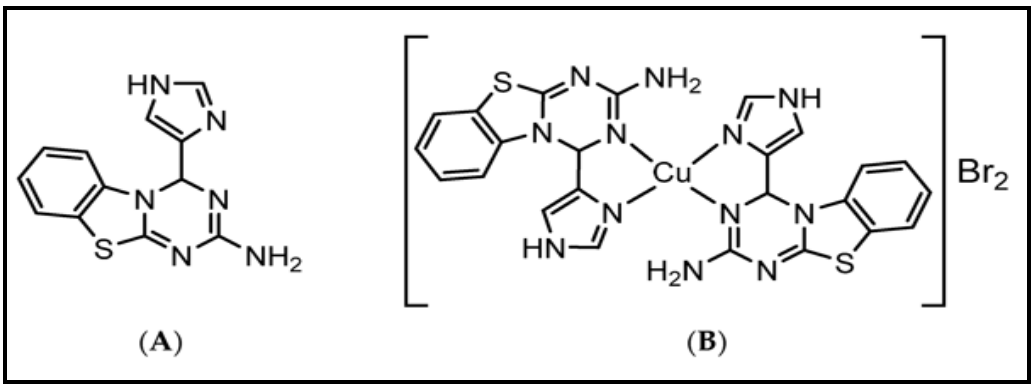

Fig. 1. Ligand (L), 4H-1,3,5-triazino [2,1-b]benzothiazole-2-amine,4-(2-imidazole) (**A**), structure of [Cu(II)L2]Br2 complex (**B**) (Zharmukhamedov et al., 2022).

MATERIALS AND METHODS

Isolation of PSII preparations. PSII-enriched active thylakoid membrane fragments were extracted from leaves as described previously (Chiller & Dau, 2000). The PSII-containing membranes were suspended in medium (A) (50 mM MES-NaOH, pH 6.5, 300 mM sucrose, and 15 mM NaCl) and stored at −80°C. The total chlorophyll concentration in the PSII-containing membranes was measured using 96% (v/v) ethanol (Arnon, 1949).

Spectrophotometric measurements. The absorption spectra of the $[CuL_2]Br_2$ complex were measured using a standard quartz cell (Hellma, Müllheim, Germany) on a two-beam Shimadzu spectrophotometer (Shimadzu UV-1800, Shimadzu Europa GmbH, Duisburg, Germany) in the wavelength range of 200–700 nm at room temperature. The concentration of the $[CuL_2]Br_2$ complex was 0.1 mM. Stock solutions of the $[CuL_2]Br_2$ complex and DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) were prepared by dissolving in DMSO.

Fast induction kinetics of chlorophyll fluorescence. The fast induction kinetics of chlorophyll fluorescence was measured using a MULTI-COLOR-PAM fluorimeter (Heinz Walz GmbH, Pfullingen, Germany). All measurements were conducted at 20°C in a quartz cuvette (1 cm path length) at room temperature, following a dark adaptation period of at least 15 minutes. For the measurements, the chlorophyll concentration was 4 μg mL^{-1}.

RESULTS AND DİSCUSSİON

Original OJIP kinetics. The analysis of the OJIP test shown in Figure 2 reveals that all the investigated agents and their combinations lead to a significant decrease in chlorophyll (Chl) fluorescence intensity, particularly at the F_M level. This results in a decrease in F_V (variable fluorescence). The F_V/F_M ratio is a widely used parameter that characterizes the quantum efficiency of the primary photochemical reaction in PSII (Kalaji et al., 2017, Pospíšil & Dau, 2002). Additionally, small increases in the F_0 level were observed with 3.6 μM $[CuL_2]Br_2$ (kinetics 2), 14.5 μM $[CuL_2]Br_2$ (kinetics 3), and 4 μM DCMU (kinetics 4). An increase in the F_0 level in the presence of DCMU has been previously observed in thylakoids and PSII-containing membranes (Pospíšil & Dau, 2000). In the presence of DCMU with both concentrations of $[CuL_2]Br_2$ (kinetics 5 and 6), the increase in F_0 is smaller than the increase observed with DCMU alone (kinetics 4).

Original OJIP Kinetics Normalized Relative to F_0 ($F_{0.02ms}$). To simplify the analysis and more clearly represent the potential changes caused by the agents added to the control (without other additives), normalization is performed relative to the initial fluorescence level, F_0, using the value of F_0 measured at 20 μs (Kalaji et al., 2017).

The original OJIP kinetics, normalized to F_0, are shown in Figure 3 as $F_t - F_0$ versus time, where F_0 represents the fluorescence at 0.02 ms and Ft represents the fluorescence at time t.

In the presence of both concentrations of $[CuL_2]Br_2$, a decrease in chlorophyll fluorescence intensity (F) is observed throughout the entire OJP kinetics. This decrease particularly affects the F_J level (2–3 ms), with a more noticeable effect observed at 14.5 μM $[CuL_2]Br_2$. The reduction in chlorophyll fluorescence is especially significant at the F_M level, where the kinetics for 3.6 μM and 14.5 μM $[CuL_2]Br_2$ (kinetics 2 and 3) show a decrease compared with the control. The decrease in F_M is particularly pronounced at 14.5 μM $[CuL_2]Br_2$ (kinetic 3). We will refer to these decreases in F (including F_J and F_M) as the "effect of $[CuL_2]Br_2$."

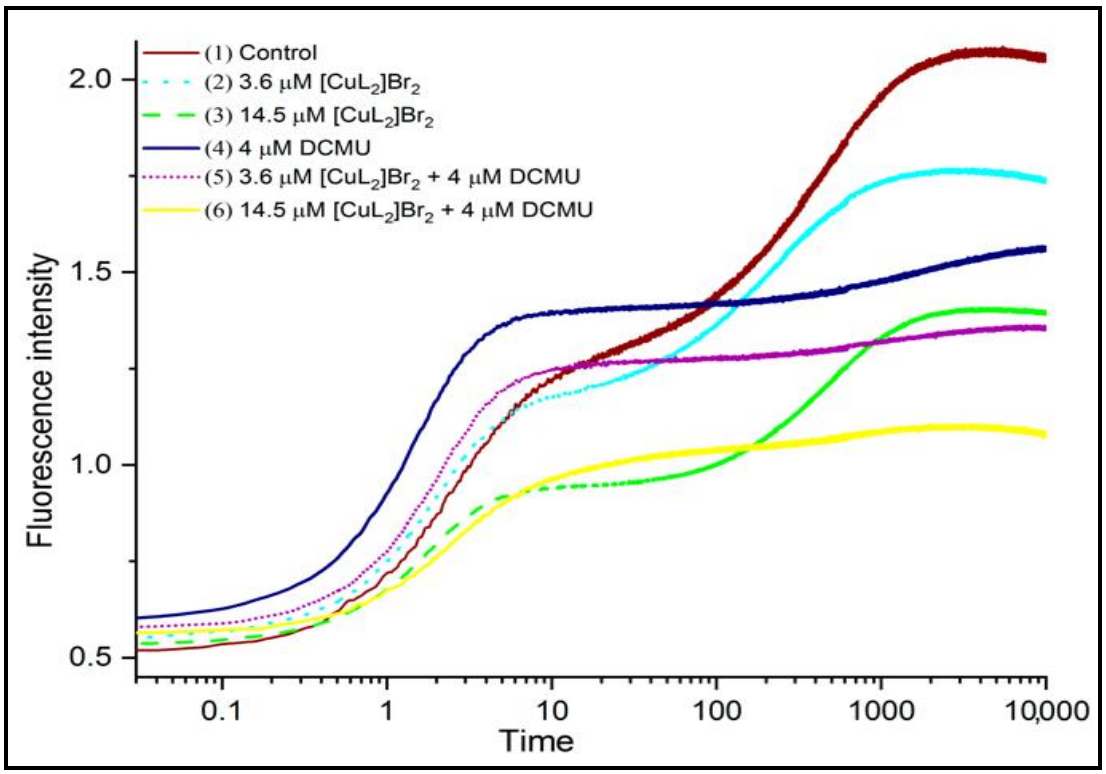

Fig. 2. Original OJIP kinetics, kinetic 1- control sample with PSII membranes in the absence any additions, kinetic 2 - 3.6 μM $[CuL_2]Br_2$; kinetic 3 - 14.5 μM $[CuL_2]Br_2$; kinetic 4 - 4 μM DCMU; kinetic 5 - 3.6 μM $[CuL_2]Br_2$ + 4 μM DCMU; kinetic 6 - 14.5 μM $[CuL_2]Br_2$ + 4 μM DCMU.

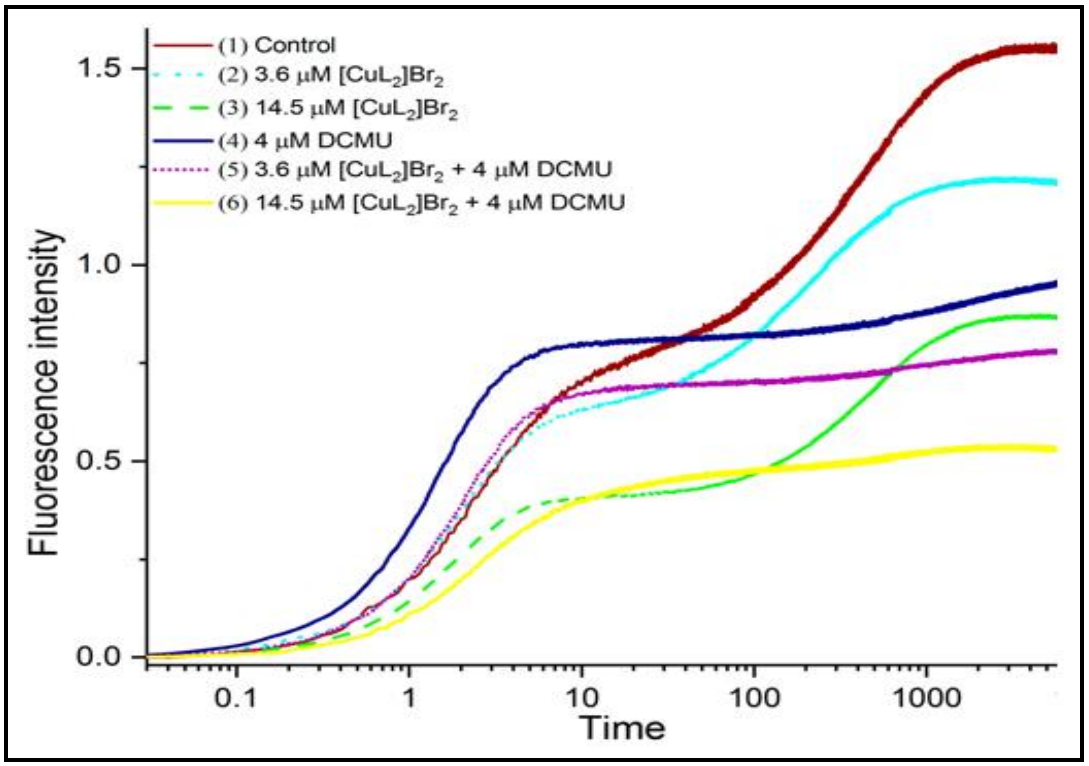

Fig. 3. OJIP kinetics normalized relative to $F_{0.02ms}$, kinetic 1- control sample with PSII membranes in the absence any additions, kinetic 2 - 3.6 μM $[CuL_2]Br_2$; kinetic 3 - 14.5 μM $[CuL_2]Br_2$; kinetic 4 - 4 μM DCMU ; kinetic 5 - 3.6 μM $[CuL_2]Br_2$ + 4 μM DCMU; kinetic 6 - 14.5 μM $[CuL_2]Br_2$ + 4 μM DCMU.

We have determined the percentage change in F_M from the control based on the results obtained in Figure 2. The decrease in F_M for each condition, compared to the control, is as follows:

- Kinetic 2 (3.6 μM $[CuL_2]Br_2$: 22%
- Kinetic 3 (14.5 μM $[CuL_2]Br_2$: 45%
- Kinetic 4 (4 μM DCMU): 38%
- Kinetic 5 (3.6 μM $[CuL_2]Br_2$ + 4 μM DCMU): 50%
- Kinetic 6 (14.5 μM $[CuL_2]Br_2$ + 4 μM DCMU): 66%

Thus, these experimental data indicate that 22% and 45% of the total PSII-containing membranes (kinetic 1 and kinetic 2) are no longer able to carry out the photochemical reduction of the corresponding components on the acceptor side of PSII. This is due to the specific suppressive effect of $[CuL_2]Br_2$ on the components responsible for either charge separation or electron transfer from the donor side to the components of PSII.

In addition, F_M is suppressed in the presence of 4 μM DCMU and, particularly, in the presence of its combinations with both concentrations of $[CuL_2]Br_2$. Moreover, when 14.5 μM $[CuL_2]Br_2$ is combined with 4 μM DCMU, an almost synchronous decrease in chlorophyll fluorescence intensity (F) is observed throughout the entire OJIP kinetics. In the presence of DCMU (without $[CuL_2]Br_2$), we observed an increase in the F_J peak to the so-called F_M peak (DCMU effect). In the presence of DCMU, the entire amount of Q_A in the sample is reduced, resulting in an increase in the J peak to its maximum possible level. At the same time, the F_M value decreases to 62% of the control F_M value. The changes in OJIP kinetics observed in the presence of both DCMU and $[CuL_2]Br_2$ are similar to those recorded in the presence of only DCMU (the "DCMU effect"), but the percentage decrease is even more significant over the entire OJIP kinetics (the "$[CuL_2]Br_2$ effect"). This decrease is especially pronounced in kinetic 6 (14.5 μM $[CuL_2]Br_2$ + 4 μM DCMU).

DISCUSSION

Main inhibitory effect of $[CuL_2]Br_2$ on OJIP transient. The most pronounced and, therefore, undoubtedly the primary effect of the $[CuL_2]Br_2$ complex on the photochemical activity of PSII-containing membranes is the synchronous decrease in fluorescence intensity along the entire JIP kinetics (Figures 2 and 3, kinetics 2, 3). At a concentration of 3.6 μM $[CuL_2]Br_2$, 22% of PSII-containing membranes are completely excluded from the kinetics, but at 14.5 μM $[CuL_2]Br_2$, 45% of PSII-containing membranes are already fully inhibited (in the absence of DCMU). Importantly, $[CuL_2]Br_2$ demonstrates this effect on PSII even in the presence of DCMU, with an efficiency comparable to that observed in its absence. Based on these data, we can conclude that the primary effect of $[CuL_2]Br_2$ on PSII is independent of DCMU. The reduction of F_M observed at both concentrations of $[CuL_2]Br_2$, in the presence and absence of DCMU, suggests that PSII inhibition by $[CuL_2]Br_2$ may occur via the same mechanism of action in both cases. The fact that $[CuL_2]Br_2$ inhibits F_M regardless of the presence of DCMU can be explained by the hypothesis that the site of action of $[CuL_2]Br_2$ on PSII precedes the site of action of DCMU. The simultaneous decrease in the Fm intensity of chlorophyll in the OJIP kinetics, which increases with the concentration of $[CuL_2]Br_2$, may be the result of the destruction of the donor side or the reaction center (RC) of PSII. Similar results in the OJIP test were observed when the donor side of PSII became inactive (Zharmukhamedov et al., 2022).

CONCLUSIONS

According to the results of this study, $[CuL_2]Br_2$ exerts various interesting effects on different regions of PSII. The results demonstrate that the primary effect of $[CuL_2]Br_2$ on PSII is likely associated with the inhibition of the activity of the PSII reaction center. $[CuL_2]Br_2$ effectively reduces the F_M value both in the absence and presence of DCMU. The obtained data may be useful for developing effective herbicides for agricultural applications.

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İspanaqda fotosistem II-nin fotokimyəvi aktivliyinə yeni Cu(II) orqanometalik kompleksinin təsiri

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Fotosintez günəşdən gələn enerji hesabına bitkilərin böyüməsini və inkişafını təmin edir və bitki hüceyrələrinin malik olduğu unikal xüsusiyyətidir. Fotosintetik aparatın (FA) əsas komplekslərindən biri olan fotosistem II (FSII) suyu elektronlara, protonlara və oksigenə parçalayır. $Cu(II)^+$ aqua ionlarının kompleksləri və liqandları FSII-nin fotosintetik fəaliyyətinə tormozlayıcı təsir göstərmək üçün tədqiq edilmişdir. Müxtəlif FSII komponentlərində Cu^{2+} aqua ionlarının müxtəlif təsir saytları və effektləri təyin edilmişdir.

Açar sözlər: *Üzvü-metal komplekslər, DCMU, OJIP, inhibitorlar*

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