Advances in Biology & Earth Sciences
Vol.3, No.1, 2018, pp.39-46

NA-ASCORBATE IN PROTECTION OF PS II ACTIVITY, SUPPRESSED BY COPPER IONS

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Abstract. The effect of restoration by Na-ascorbate the functional characteristics of PS II suppressed by Cu^{2+} were investigated. After incubation of wheat seedlings (*Triticum aestivum* L.) in $CuSO_4$ solution on induction curve of msec delayed light fluorescence of chlorophyll a (msec DF of Chl a) the changes of transition on dependence from concentration and time of Cu^{2+} ions action were observed. The toxic action of Cu^{2+} manifest itself on the part of induction curve of fluorescence, that reflect, probably the formation of primary radical pair on donor side of PS II. The most aggressiveness of Cu^{2+} is shown to manifest on the transport electron chain within Q_A-Q_B. Na-ascorbate to restored the effectiveness of electron transport chain of PS II probably by means of hydroxyl radicals quenching, formed under stress.

Keywords: *Na-ascorbate, electron transport, oxidative stress.*

Abbreviations: *PS II-photosystem II, HM-heavy metal, msec DF Chl a-millisecond delayed light fluorescence of Chlorophyll a, RC-reaction centre.*

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Manuscript received*: 31 January 2018*

1. Introduction

The determination of a mechanism plant adaptation to damaging factors of medium, in particular, to toxic action of heavy metals (HM) is known to be the main problem in biology. Cu^{2+} is known to belong to high level of toxicity. Cu^{2+} is transportated from biological membrane as an univalence ion and due it presence in most protein molecules are actively involved to cell metabolism (Changela *et al*., 2003; Moustakas *et al*., 1998).

Interrelation between Cu^{2+} excess and photosynthesis is investigated long time but its toxic action mechanisms *in vivo* remained an object of debate (Shainberg *et al*., 2001). Deficiency in Cu^{2+} greatly affect on energetic metabolism in electron transport chain of photosystem since inhibit of Cu^{2+} - containing transfers of electrons plastocyanin and cytochromeoxidase. The high concentrations of Cu^{2+} are known to inhibit the photosynthetic transport of electrons especially in PS II. Both the deficit of Cu^{2+} and its excess have influence on biosynthesis of pigments and lipid exchange and accordingly on chloroplast ultrastructure. These negative effects was found to be an unfavorable factor for photosynthesis and in particular on activity of PS II (Barón *et al*., 1995; Nesterov & Rosentsvet, 2008; Yruela, 2009; Burzynski & Zurek, 2007; Li *et al*., 2010; Vodka *et al*., 2013).

The high content of Cu^{2+} in plants lead to oxidative stress that is detected by monitoring some of components in activity and content of antioxidative mechanism changes.

Activity of ascorbate-reductase and catalase was redoubled, activity of glutathione-reductase was inhibited by 60% from control. Cu^{2+} is also influenced on primary processes of photosynthesis (Shainberg *et al.,* 2001).

To reveal of site Cu^{2+} action in photosynthetic apparatus the kinetic analysis of fluorescence of Chl a, absorption and evolution of O_2 were used. The primary photochemical reactions of PS II in seedling of maize adaptated to darkness were insignificant affected at presence of Cu^{2+}. These reactions were damaged by toxicity of Cu^{2+} in adaptated to light leaves. The primary target for Cu^{2+} as it was supposed to be RC of PS II and its ability to adaptate to strong light. The denaturation of PS II under Cu^{2+} high concentrations leads to significant loss of mediated by PS II of transport electrons under continuous illumination and inhibition of oxygen evolution (Ouzounidou *et al.,* 1997). Sublethal concentrations of Cu^{2+} can nonspecifically slow down of D_1 protein synthesis of PS II in Chlorella cells thus to promote to inactivation of PS II on the light (Vavilin *et al*., 1995). The significance of fluorescent characteristics investigated on the spinach chloroplasts has been strongly decreased at the presence of Cu ions. The results of investigations to coordinate with predominately inhibition of donor side of PS II (Boucher & Carpentier, 1999; Dovidkov *et al.,* 1999; Aliyeva & Magomedova, 2004). One of mechanism leading to inhibition of photosynthesis by heavy metals in concentration appropriated with environment is the substitution of Mg^{2+} in chlorophyll molecules. The authors make a conclusion that light-harvesting complex of PS II is a main target for heavy metals.

The inhibition of RC of PS II is a result of specific enters of heavy metals into pheophytin (Küpper *et al.,* 2002). The toxicity of heavy metals is to be conditional upon their ability to inactivate enzymes and other macromolecules, connected with SH groups and block of prostatic groups by replace of functionally importants ions of metals (Clemens, 2006; Joshi & Mohanty, 2004). Initiating generation of active oxygen radicals such as $O_2^{\cdot-}$, H_2O_2 и $OH^{\cdot}$ caused damage of macromolecules and cell structures (Wang *et al*., 2009; Ghnaya *et al*., 2009).

It is known, that main target under oxidative stress is known to be photosynthetic membrane of chloroplast thylakoids and very vulnerable are chlorophyll-protein complexes of PS II due their structural peculiarity (Kurbanova *et al*., 2006; Kurbanova *et al*., 2010). To damaging action of active forms of oxygen resist of antioxidant protection system (AO), that eliminate of oxygen active forms and remove of compounds, damaged at spontaneous oxidation by oxygen. The plants have evolved protective enzymatic and nonenzymatic mechanisms to scavenge ROS. Carotenoids, ascorbate, glutathione, α-tocoferol, flavonoids are known as nonfermentative protective systems (Shao *et al*., 2008; Shesnokova *et al*., 2006; Paradiso *et al.,* 2008; Enikeev *et al*., 2013).

The aim of presented work was determination of protective and regulatory role of exogenous Na-ascorbate in protection of electron transport chain of PS II from action of Cu^{2+}.

2. Material and methods

The 7-days wheat seedlings (*Triticum aestivum* L.) grown at factorostate conditions (t 26°C, humidity 80%) were object of investigations.

Experimental conditions. The wheat seedlings were incubated in $CuSO_4$ solution at concentrations 13mg/l and 26mg/l during 1 and 3 twenty-four hours. The excision from leaves these seedlings were diped to water (as a control) and Na-ascorbate solution (50mg/l) for 30 minutes.

Method. The photochemical activity of PS II was investigated by msec delayed fluorescence of Chl a (msec DF of Chl a). The recombinated fluorescence of Chlorophyll as it was shown by many investigators is an informative method for determination of reactions occurring on donor and acceptor sites of electron transport chain of PS II (Burzynski & Zurek, 2007).

The excitions of leaves were located to special holder and were illuminated by constant white light (250W $m^{-2}S^{-1}$). The light was passed from holes on rotating drum of phosphorscope, so that after 0.3 msec excitation was followed by 1.25 registration of delayed light emission. The experiments were performed in three replicates.

3. Results

The recombination fluorescence of chlorophyll (delayed fluorescence at millisecond time range (msec DF Chl a) is known to be informative method for determination of reactions occurring on the donor and acceptor sides of electron transport chain of PS II (Küpper *et al*., 2002; Burkhead *et al.*, 2009). The photochemical processes activity in wheat leaves after treatment with $CuSO_4$ solution were evaluated by method of msec DF of Chl a induction, determinated activity of electron transport chain within of PS II. Change of these parameters demonstrates of stress level in PS II complexes. The activity fall after 1 and 3 twenty-four hours incubation of seedlings at $CuSO_4$ solution in concentration 13 and 26mg/l was observed as on donor side (f.ph) and also on acceptor side (sl.ph) of electron transport chain of PS II. The suppressing effect of Cu^{2+} was noted on sl.ph and was dependent from Cu^{2+} concentration in medium but not depended from time of action. The observed rise of stationary phase (st.ph) on induction curve composed 62% from control at both concentrations and twenty-four hours incubation and was increased nearly on 100% during 3-days incubation in Cu^{2+} solution at concentration 13mg/l. The activity of sl.ph and st.ph sharp rise on induction curve indicate to that of place of action of Cu^{2+} may be the region of electron transport on Q_A-Q_B in cytochrome b_6/f complex. Thus, it may be assumed that acceptor side of electron transport chain is more susceptible to Cu^{2+} action (Table 1). Under washing (as an control) and action of Na-ascorbate the restoration of both phases activity was observed, moreover the st.ph under action of Na-asc unlike to water approximate to control (Table 1).

For information from state of electron transport chain of PS II and equilibrium between donor and acceptor sides at the changes of significance of size relation f.ph. and sl.ph. to stationary phase on induction curve were considered. During one-day infiltration of seedlings in Cu^{2+} solution the value of f.ph/st.ph was decreased on 40% and 45% (accordingly concentration) relatively to control. The value of sl.ph/st.ph was found to decreased on 49% and 65%. As far as the time was increased the action of Cu^{2+} ions on the f.ph/st.ph was less aggressive. The drop of these values make up only 11 and

19% (accordingly concentration) relatively to control. The value of s.ph/st.ph drops on 41 and 48% as compared with control (Fig. 1 and 2).

Table 1. The changing of activities of fast, slow and stationary phase (f.ph., sl.ph., st.ph.) of msec DF of Chl a of PS II under action of $CuSO_4$ (13mg/l, 26mg/l) during: A-1 twenty-four hours; B-3 twenty-four hours and at result restoration under treatment with Na-ascorbate. The results reflects the comparison of measurement, observed in 3 independent experiments. The dimensions are expressed in relative units.

A

	f.ph		sl.ph		st.ph	
Control	22		18,5		0,8	
	13 mg/l	**26 mg/l**	**13 mg/l**	**26 mg/l**	**13 mg/l**	**26 mg/l**
$CuSO_4$	8,9±0,2	9,4±0,3	7±0,6	5,3±0,4	1,3±0,06	1,3±0,06
$CuSO_4$→ water	11±0,6	14±0,6	9,5±0,3	10,4±0,2	1,3±0,1	1,1±0,06
CuSO4→ Na-asc	10,6±0,8	10,9±0,1	7,3±0,2	5,4±0,3	0,7±0,06	0,6±0,06
	B					
$CuSO_4$	17,8±0,1	11,3±0,2	13,5±0,3	8,5±0,3	1,7±0,4	1,2±0,1
$CuSO_4$→ water	21±0,6	17,5±0,3	11,7±0,6	10±0,6	1,3±0,06	1,3±0,06
CuSO4→ Na-asc	18,8±0,4	12,8±0,13	13,1±0,06	9,5±0,3	0,9±0,06	0,8±0,06

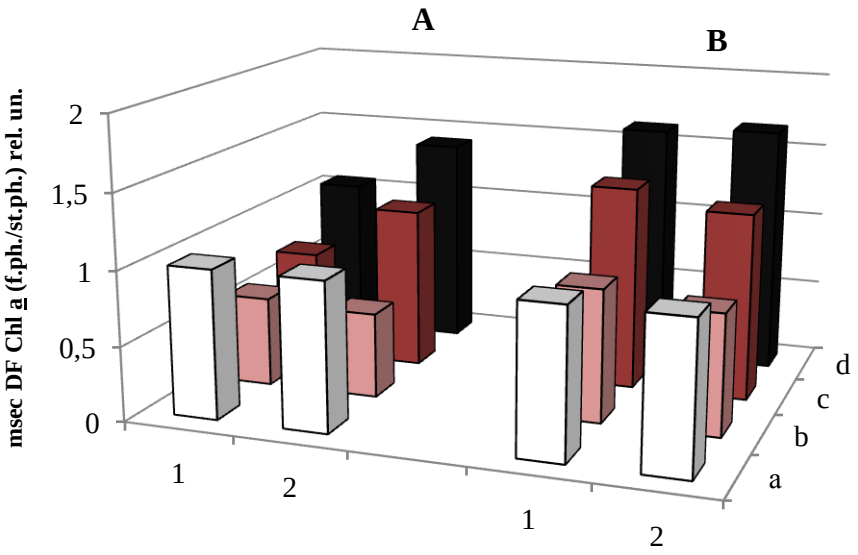

Fig. 1. The columns on figure reflects the relation of fast phase to stationary phase of msec DF Chl a in PS II under action of Cu^{2+} in concentration 1) 13mg/l; 2) 26mg/l during A) 1 twenty four hours; B) 3 twenty four hours; a) control; b) $CuSO_4$; c) washed by water; d) treated with Na-asc. The relation value in control taking up to 1.

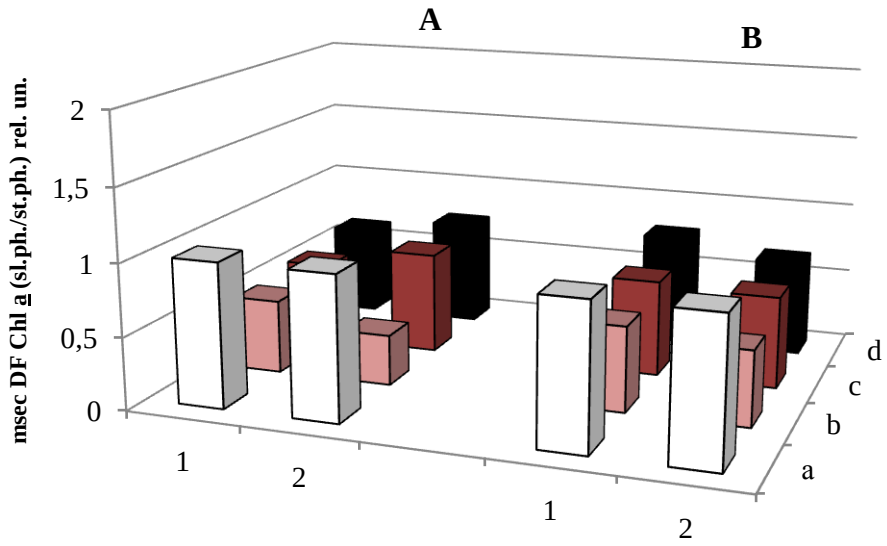

Fig. 2. The columns on figure reflects the relation of slow phase to stationary phase of msec DF Chl a in PS II under action of Cu^{2+} in concentration 1) 13mg/l; 2) 26mg/l during A) 1 twenty four hours; B) 3 twenty four hours; a) control; b) $CuSO_4$; c) washed by water; d) treated with Na-asc. The relation value in control taking up to 1.

The value of f.ph/st.ph was raised on 12% at $CuSO_4$ concentration 13mg/l and at 26mg/l concentration of $CuSO_4$ exceeds the value of given value in control. The value of sl.ph/st.ph increased on 5 and 30% (accordingly concentration) and on 8 and 12% under increasing infiltration time relatively to action of Cu^{2+}. The value of f.ph/sl.ph excess the control value under increasing of infiltration time and is not dependent from concentration of Cu^{2+} (Fig. 1 and 2) after action of Cu^{2+} ions the leaves were treated with Na-asc solution. The value of f.ph/st.ph excess the control independently from concentration of Cu^{2+} solution and time of infiltration. The restoration of sl.ph/st.ph was significantly below.

4. Discussion

The comparative investigations of toxic action of Cu^{2+} ions at dependence from concentration and time of incubation revealed the suppression of primary charge separation with formation of primary radical pair $P680^{+}Phe^{-}$, occurring on donor side of PS II chain. The suppression of this reaction is not depended from concentration. The increasing of infiltration time (3 twenty-four hours) has decreased the toxicity of Cu^{2+} on this processes by 1.5 and 1.4 times (Fig. 2).

The Cu^{2+} action on process linked with electron flow stability to primary quinone acceptor Q_A on acceptor side of PS II chain has another character was dependent from concentration and time of action. The obtained results are confirmed by literature data that Cu^{2+} ions affect both donor and acceptor side of PS II suppressing the electron transport on the level P680 TyrZ on donor side. On the acceptor side Cu^{2+} interact with primary quinone acceptor Q_B replacing an nonheme iron on the part of pheophytin (Phe) – Q_A – Fe and with secondary quinone acceptor Q_B (Schröder *et al.*, 1994; Jegerschöld *et al.*, 1999; Mohanti *et al.*, 1989).

The toxic action of Cu^{2+} leads to disturb the equilibrium between photosystems and displacement of redox state of Q_A promoting the electrons flow to PS I. This results

by rise of st.ph (Table 1) and phases drop to stationary level. The fluorescent characteristics drop appears to be connected with Chl disruption by Cu^{2+} in chlorophyll-protein complexes of PS II (Kurbanova *et al*., 2010). The observed inactivation of PS II chain at presence of Cu^{2+} with the increasing the time of action may be indirectly explained by accumulation of metal by leaves under prolonged action of salt high concentrations appears to be associated with changes of cell envelope permeability or by inclusion of plant cell adaptive possibilities (Shesnokova *et al*., 2006; Burkhead *et al.*, 2009; Küpper *et al.*, 2009). The incorporation of Cu^{2+} in restoration of superoxide O_2 and hydrogen peroxide to hydroxyl radicals (*OH) was supposed that as an all heavy metals excess of Cu^{2+} in plant cell leads to oxidative stress. Due to high reactivity of *OH-radicals there is not a biological mechanism that may protect of important cell components from effect of *OH. The survival of plant cell at that conditions depend from presence of complex protective system, regulating of cell concentration of free ions Cu^{2+}, O_2 and H_2O_2, limitating formation of *OH-radicals (Halliwell & Gutteridge, 1985).

Na-asc as it is generally accepted is a strong inactivator of free radicals (Ganiyeva *et al*., 2015) has a decisive role in protection of chloroplasts of plant cells from oxidative stress, possibly by quenching of O^* and OH^* radicals.

It may be supposed that Na-ascorbate promote to maintenance of oxidation-restoration equilibrement between photosystems that is evidenced by restoration of stationary phase of msec DF of Chl a to norm.

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Published online: 16.04.2018