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Zinc Induced Alterations in the Photosystem II Mediated Photochemistry of Cyanobacterium S*pirulina Platensis*

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ABSTRACT

In this investigation an attempt has been made to identify and correlate the polarographic measurements with the observations of fluorescence kinetics to identify the target site present in photosystem (PSII) II catalyzed electron transport in the cyanobacterium *Spirulina platensis*. Oxygen exchange measurements using Clark type oxygen electrode have showed 35% loss in whole chain activity and 45% loss in PSII activity at 120 µM concentration of zinc sulphate. Further to identify the specific targets sites within the PSII photochemistry, the reaction mixture component water was replaced with hydroxylamine and the results indicated that there could be damage at water oxidation complex. PAM Chl *a* fluorescence kinetic measurements revealed the decrease in variable fluorescence and increase in Initial fluorescence indicating that light harvesting complex of PSII could be the target for zinc stress at 120 µM. Thus loss of manganese in water oxidation complex and alterations in light harvesting complex of PSII are responsible for the altered PSII photochemistry in the above cyanobacterium under zinc stress.

Keywords: Chl *a* fluorescence, Electron transport, Light harvesting complex (LHC), *Spirulina platensis,* Zinc stress (Zn).

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INTRODUCTION

Heavy metal exposure induces alterations in the basic metabolic events in plants and photosynthetic organisms. Photosynthesis is one of the major physiological processes known to be affected severely by heavy metal stress like Zinc (Zn), Cadmium (Cd), Lead (Pb) and Chromium (Cr) [1]. Several studies have been done to understand the effect of heavy metals on alterations in morphological and physiological changes in plants. The effect of metal ions on higher plants includes disruption of many physiological functions by binding to protein sulfhydryl groups and substituting the essential ions. Among the various micronutrient elements Zn stands as an essential micronutrient influencing vast number of physiological mechanisms [1, 3-7]. Zn is a common deficient as well as phytotoxic element in agricultural soils [8]. It is the second most abundant transition metal after iron (Fe) in the soil. First group microelements includes, Zn, manganese (Mn) and magnesium (Mg) which are essential and responsible to carry out numerous physiological processes, but at high concentrations they become strongly toxic and decline plant growth. The second group includes Cd, Pb and mercury (Hg), which disrupts physiological processes even at low concentrations [9]. Among the first group elements Zn acts as an essential trace element for plant cell physiological processes, and also in many living systems.

Zn plays structural and ⁄or catalytic roles in many metabolic processes, and is the only metal present in all enzyme classes [10, 11]. Because of phytotoxic ability reduced yields was observed [7, 12] chiefly due to fall in Net photosynthetic efficiency, due to alterations in the photochemical reactions (2). Photochemical reactions such as biosynthesis of chlorophylls (Chl) [13] and cell membrane integrity (14) are largely influenced processes due to Zn phytotoxicity.

At molecular level critical damage in the Chl structure, due to Mg ion replacement with heavy metals, such as Zn (15) Hg^{+2}, $Cu^{+2}$and Pb^{+2} [16]were reported largely. Decline in relative fluorescence yield of Fv/Fm in *Chlorella pyrenoidosa* with increased Zn ion concentrations was identified by Plekhanov and Chemeris [17]. In conclusion several investigators manifested not only inhibition of electron transport of photosystems in photosynthetic mechanism, but also reduced photosynthetic membrane energization in thylakoids.

By considering all the above mentioned literature an attempt has been made to identify the targets for Zn action in photosynthetic electron transport by correlating results with spectral, polarographic and Chl *a* fluorescence Kinetic measurements by using *Spirulina platensis* as experimental material by treating the cells for 24 hrs with zinc sulphate ($ZnSO_4$) of different concentrations.

MATERIALS AND METHODS

Spirulina platensis was grown axenically in the Zarrouk's medium (18) at 25± 2 ^{o}C under continuous white light of 10 W m^{-2}. The cells were suspended in 25 mM HEPES- NaOH buffer (pH 7.5) at a Chl concentration of 200µg mL^{-1}. Samples were exposed to different concentrations of $ZnSO_4$ (30-120 µM) for 24h under continues illumination. After treatment the

cells were collected by centrifuging at 6000 xg for 10 min. Then the pellets were suspended in the above reaction buffer. The assay mixture for whole chain electron transfer measurement contained 25 mM HEPES-NaOH buffer, (pH 7.5), 0.5 mM Methylviologen (MV) and 1 mM Na-azide [18].Whenever there is a requirement of water, a physiological donor is replaced by hydroxyl amine (0.1mM) to identify the damage at water oxidation complex (WOC). The reaction mixture for PSII catalyzed electron transfer activity measurement contained para-benzoquinone (pBQ) 0.5 mM pBQ [19]. By using neutral density filters light intensity was varied from 24-416 Wm^{-2} to identify the target site of Zn in PSII [20]. The chlorophyll content from cells was estimated using methanol by following the method of Mackinney [21]. Chlorophyll *a* fluorescence kinetics was measured by using PAM kinetic fluorimeter by following the method of Murthy *et al.,* [22].

RESULTS

Robinson *et al.,* [23] showed that MV is having access to the thylakoid membranes in intact cells of *Spirulina*. Whole chain electron transport activity has been measured in intact cells using MV as terminal acceptor ($H_2O \rightarrow MV$). Control cells with out heavy metals exhibited a high rate of oxygen consumption (260 µmol oxygen was consumed mg $Chl^{-1}h^{-1}$). Zn is able to inhibit whole chain electron transport activity by 35% at high concentration of 120µM (Table-1).The partial inhibition of whole chain electron transport activity in case of Zn is due to its inhibitory effect on PSII reaction centre as reported earlier by Tripathy and Mohanty [24]. To find out whether the specific target site of inhibition is at WOC or not, hydroxyl amine has been supplied as a donor in reaction buffer for PSII instead of water. This measurement clearly indicated that there is no loss in whole chain electron transport activity when hydroxyl amine acted as donor instead of water (Fig-1). To further confirm the effect of Zn on PSII, an attempt has been made to study the Zn effect on PSII catalyzed pBQ Hill reaction (Table-2). pBQ is lipophilic in nature and easily enters into the intact cells of *Spirulina*. pBQ accepts electrons from PQ pool as reported by Warburg and Luthgens [25] and Trebst, [26]. Hill reaction studies with pBQ in control cells exhibited a high rate of oxygen evolution of 392 µ mol oxygen was evolved mg $Chl^{-1}h^{-1}$ (Table-2). The treatment of Zn at high concentrations of 120 µM brought 32% inhibition in the Hill activity of intact cells. The possible reason for the loss of PSII activity could be due to alterations in the WOC, which is also evidenced from the measurements of hydroxylamine mediated whole chain electron transport measurements (Table-1& Fig-1). Similar observations have been made in the above cyanobacterium under mercury stress by Murthy [19]. To examine whether the inhibition induced by Zn in Hill activity is linked to alterations in the energy transfer or not an attempt has been made to study the inhibition caused by Zn (120 µM) at different intensities of light 24-416 Wm^{-2} (Table-3).

To identify the changes in light harvesting (LHC) complex of PS II under Zn stress, PAM kinetic fluorimeter has been employed to measure the Chl*a* fluorescence in intact cells (Table-4) upon excitation with weak light the fluorescence has reached to a distance called F_o in control sample. The Zn treatment caused an enhancement in F_o values from 5.1 to 6.2 Cm. This increase in F_o is due to alterations in the LHC of PSII. Further excitation with strong actinic red light caused further increase of fluorescence to F_m (maximum fluorescence). The difference between F_m and F_o is known as F_v (variable fluorescence). In control sample this value is equal

to 4.4 Cm. Zn toxicity caused decrease in the F_v value to 2.1 Cm. This loss is due to inhibition of PSII photochemistry by Zn in treated samples. Murthy *et al*., [22] showed that mercury causes inhibition in photosynthetic electron transport of *Spirulina* cells at multiple sites which is evident from their Chl *a* fluorescence kinetic measurements. Plekhanov and Chemeris [17] has reported similar type of alterations in fluorescence kinetic measurements in *Chlorella pyrenoidosa* an photosynthetic green algae. Thus there is a correlation between PAM Chl *a* fluorescence kinetics and polorographic measurements with intact cells under the influence of Zn. In summary Zn at higher concentrations 120 µM causes alterations in LHC of PSII and induces inhibition in photosynthetic electron transport activity of PSII by acting at a site in WOC most probably by replacing the Mn ions in WOC in the cyanobacterium *Spirulina platensis*. Allen *et al*., [27] and Hsieh *et al*., [28] reported similar type of manganese replacement in PS II and its photochemical loss in *Chlamydomonas reinhardtii*.

Table 1: Effect of zinc on whole chain electron transport activity of *Spirulina platensis* intact cells.

Zinc concentration (µM)	Whole chain electron transport activity ($H_2O \rightarrow MV$) µmoles of O_2 consumed mg Chl^{-1} h^{-1}	Percent loss
Control	260±24	0
30	248±21	5
60	210±18	20
90	186±16	28
120	170±14	35

Table 2: Effect of zinc on PS-II catalyzed electron transport activity of *Spirulina platensis* intact cells.

Zinc concentration (µM)	PS-II electron transport activity ($H_2O \rightarrow PBQ$) µmoles of O_2 evolved mgChl^{-1} h^{-1}	Percent loss
Control	392±37	0
30	360±62	8
60	344±56	22
90	266±72	32
120	215±64	45

Table 3: Variation of inhibitory pattern of PS-II activity catalyzed electron transport activity in control and treated samples at different illumination conditions in *Spirulina platensis*.

Light intensity Wm^{-2}	PS-II catalyzed electron transport activity ($H_2O \rightarrow PBQ$) µmoles of O_2 evolved mgChl^{-1} h^{-1}		Percent loss
	Control	Zinc-Treatment (120 µM)	
10	36±3	25±2	29
100	99±8	67±6	32
200	198±18	124±10	37
400	390±37	214±19	45

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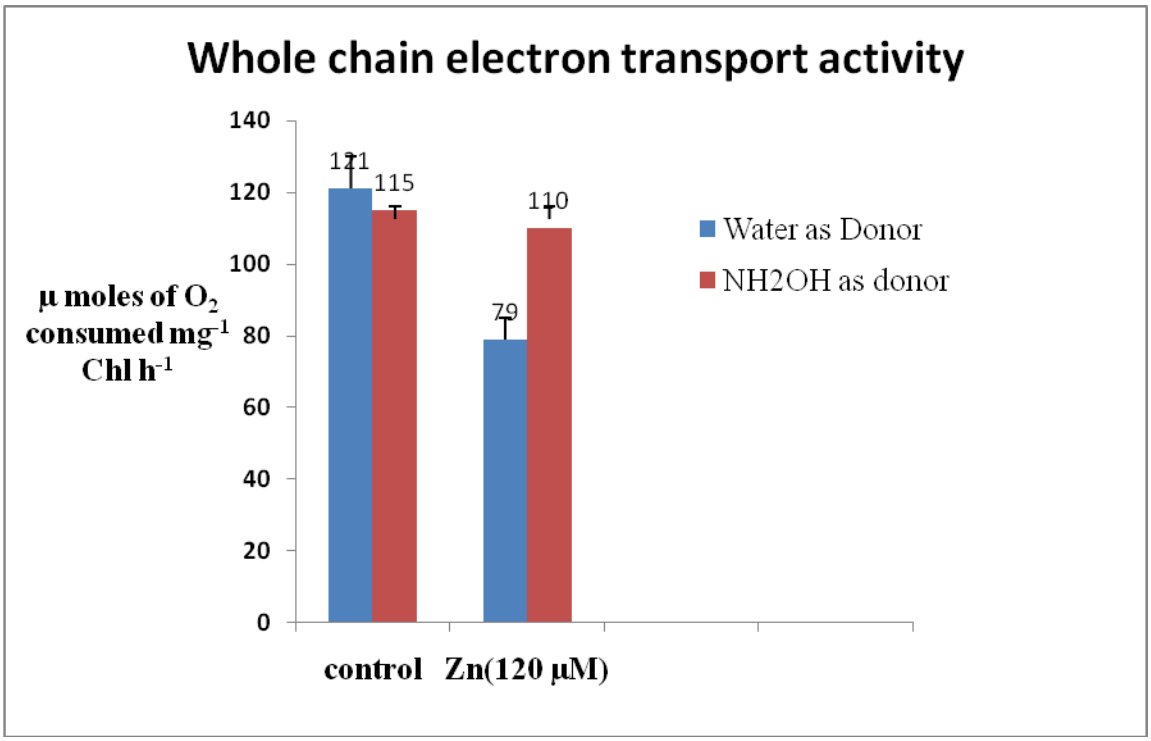
Table 4: Effect of zinc on Chl *a* fluorescence kinetics of *Spirulina platensis* intact cells. The samples were excited with very low light intensity to measure the initial fluorescence (Fo) and with strong red light to measure variable fluorescence (Fv).

Zinc concentration (μM)	Fluorescence parameters (in terms of distance, Cm)		
	Fo	Fv	Fm
Control	5.1	4.4	9.5
30	5.4	4.2	9.6
60	5.6	3.4	9.0
90	5.8	2.8	8.6
120	6.2	2.1	8.3

Figure: 1 Effect of zinc on whole chain electron transport activity of *Spirulina platensis* mediated by different donors.



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