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MERCURY INDUCED ALTERATIONS IN PHOTOSYSTEM-II PHOTOCHEMISTRY IN MAIZE PLANTS

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Abstract:

Mercury is a toxic heavy metal and it inhibited PS-II catalysed electron transport activity in a dose dependent manner. 50% loss was noticed with the treatment of 15µM $HgCl_2$. Spectral measurements clearly indicated that there is an enhancement in F_o value due to changes in LHC-II complex of PS-II in maize leaves. There is a positive correlation between the loss of PS-II and Chl a fluorescence which clearly demonstrates that the inhibitory site lies near PS-II reaction centre of maize thylakoids.

Key words: Electron transport; Fluorescence; Mercury;Maize plants; Photosystem II.

INTRODUCTION:

Heavy metals like Zn, Cd, Ni, Cu, Hg, Co and Pb can inhibit photosynthetic electron transport at multiple sites (Van Assche and Cl ijsters, 1980; Cl ijsters and Van Assche, 1986; Mohanty *et al*., 1989). Photosynthetic organisms showed reduction in PS II supported electron transport activities when they were grown *in vivo* in the presence of supra optimal level of heavy metals like Cd (Baszynski *et al.*, 1980), Zn (DeFilippis *et al.*,1981b). Majority of the observations on the effect of heavy metal ions on partial photochemical reactions are made in isolated systems *in vitro*. The PS II supported electron transport activity is more susceptible to heavy metals like Zn, Ni (Tripathy *et al*, 1981), Co (Tripathy *et al*., 1983), Cu, Cd and Zn, Cd (Bazzaz and Govindjee, 1974a) Cu (Cedeno- Maldonado *et al.*, 1972; Shioi *et al.*, 1978a; Shioi *et al.*, 1978b; Samuel son and Oquist, 1980; Renganathan and Bose, 1989; Mohanty *et al.*, 1959al Co, Ni, and Zn (Mohanty *et al.*, I 989b) Pb (Bazzaz and Gov i nd j ee, 1974b) and Hg^{2+}(Honeycutt and Krogmann, 1972; Samson and Popovic, 1990). In addition to this it was also shown that this heavy metal ion induced inhibition is dependent on the illuminating intensity (Zn, Cu: Cedeno- Maldonado *et al.*, 1972). At light limiting conditions, the extent of inhibition was found to be 1ess than that at light saturating conditions. Sites of heavy metal action in PS II catalyzed electron transport. Restoration of Hill activity by semicarbazide in the heavy metal ion treated plant chloroplasts (Zn: Van Assche and Clijsters, 1980; Van Assche and Clijsters, 1986) and by $MnCl_2$ (Cd: Baszynski *et al.*, 1980) indicates that the water splitting site is the site of action of heavy metals. In Cd treated maize chloroplasts restoration of Hill activity by DPC suggests that OEC is the site of action (Cd: Bazzaz and Govindjee, 1974b). In this investigation the effects of mercury on PS-II photochemistry as been compared with spectral alterations in maize plants.

MATERIALS AND METHODS:

Healthy seeds of Maize (*Zea mays. L*) were obtained from Regional Agricultural research station, Tirupati. The seedlings were randomly placed in plastic trays and grown in a growth chamber providing with fluorescence light (Phillips, India) with a light intensity of 30- 35 µ moles m^{-2} s^{-1} at 25 ± 1^0C. Fully expanded 8^{th} day leaf segments (4-5 cm long) were cut from apical region and used for treatment. Isolation of thylakoid membranes was done according to the procedure described by Sabat *et al.* (1986). PS II catalyzed electron transport assay (H_2O → *p*-BQ) activity was measured as O2 evolution in the thylakoid membranes. The 2 ml reaction mixture contains reaction buffers [50 mM HEPES-NaOH (pH 7.5),100 mM sucrose, 2mM $MgCl_2$ and 5 mM KCl], 0.5 mM freshly prepared p-BQ and thylakoid membranes equivalent to 40 µg of Chl. Fluorescence emission and excitation spectras were recorded by using Jasco FP777 Spectrofluorimeter. PAM kinetic fluorimeter is used for measurements.Lipid peroxidation has been measured according to the method of Carmak and Horst (1991).

RESULTS:

PS II Activity has been measured with oxygen electrode using pBQ as electron acceptor of PS II in terms of oxygen evolution. Control thylakoid membrane exhibited the PS II activity equal to 226 µM of O_2 evolved $mg^{-1}Chl^{-1}h^{-1}$. The treatment different concentrations of Hg (5-20 µM) induced dose dependent inhibition in oxygen evolution and 57% loss was observed at 15 µM Hg. For their enhancement in these 15 µM to20 µM caused 71% inhibition in PS II activity (Table 1).

Table. 1: Effect of mercury on PS II electron transport in maize thylakoidmembranes

$HgCl_2$ (µM)	**PS II catalyzed electron transport $H_2O \rightarrow pBQ$ µ molesof O_2 evolved mg Chl^{-1} h^{-1}**	**Percentage loss**
Control	226 ± 23	0
5	179± 15	21
10	133±11	41
15	97 ±7	57
20	65 ±4	71

Chl *a* fluorescence emission at room temperature originates from PS II only. The fluorescence emitted from PS I at room temperature is always weak. Therefore to correlate the PS II photochemistry with fluorescence of Chl *a*, fluorescence measurement has been measured for thylakoid membrane before and after treating with mercury individually.

The control thylakoid membranes have been exited at 440nm height control fluorescence emission spectra exhibited an emission peak at 677nm an emanating from PS II to chlorophyll. The treatment of Hg caused combination depends on the decrease in fluorescence intensity. In control sample Chl *a* fluorescence equal to 73 relative units was noticed. The treatment with Hg caused decrease in the fluorescence intensity to 38% (Table.2**).**

Table.2: Mercury induced alterations in Chl *a* fluorescence emission of maize thylakoid membranes

Concentration (µM)	**Chl *a* fluorescence emission**	**Percentage increase**
Control	65	0
5	72	15
10	83	30
15	80	38
20	76	42

In Chl a kinetics,upon excitation of sample with weak light caused enhancement raise in the fluorescence to a level of 2cm. This position is caused Fo original fluorescence, after excitation of strong light it exhibited for the raise to 6.5cm which is called as Fm, maximum fluorescence. The difference between F_m to F_o is called F_v, variable fluorescence. This Fv is an indirect indicator of PSII Photochemistry. Fo is an indicator of on the states of LHC of PSII, the treatment of sample with Hg (5µM) caused increase in the F_o value from 2 to 2.6 and decrease in the F_v values from 4.3 to 2.1, this decrease in the F_v value is around 50% (Table. 3).

Table.3: Effect of Mercury on Chl *a* fluorescence kinetics in maize thylakoid membranes.

Concentration (µM)	Fluorescence parameter (in terms of distance, cm)		
	Fo	Fv	Fm
Control	2.0	4.3	6.3
5	2.2	4.4	6.6
10	2.3	3.0	5.3
15	2.7	2.2	4.9
20	2.6	2.1	4.7

From the above, it is clear that PSII is main target for Hg.Photosynthetic electron transport takes place in the thylakoid membrane of chloroplast. Sulpholipids, galoctolipids, phospholipids are responsible for packing of thylakoid membranes to disstarch photo functions, where in our study an attempt has made to analyse the alterations in the thylakoid membranes to measure the lipid peroxidation thylakoid membranes are treated with TCA and TBA and formed MDA was measured. In control thylakoid membranes the formed MDA was observed to the 44nm of MDA gm^{-1} fresh weight of the sample. The treatment of Hg caused concentrations dependent enhancement in the lipid peroxidation at 20 µM, 96% increase was noticed (Table. 4).

Table.4: Effect of mercury on lipid peroxidation of thylakoidmembranes of maize primary leaves.

Concentration (µM)	Lipid peroxidation n moles MDA / g.f.w	Percentage enhancement
Control	46	0
5	57	24
10	71	54
15	82	78
20	89	93

DISCUSSION:

Metal ions are essential of plant growth and development in largequantities as well as in smaller quantities, based on the requirements they are classified into two categories namely macro nutrients(larger quantities), smaller quantities (micro nutrients), K, Ca, Mg etc…come under macronutrients, where as Cu, Mn, Fe etc., come under micro nutrients. But some metal ions which are not essential get deposited in both aquatic and terrestrial environment due to human interaction with nature. Indiscriminate disposal of waste form thermal power plants and factories is responsible for the accumulation of heavy metals in the environment. They are Hg, Cd, Pb, Ni etc. These heavy metals interact with the environment and cause decrease in the plant productivity to verify the above preposition in their investigation maize seedlings where taken as experiment material and the effect of selected metals (Hg-Ni) was studied on photosynthetic electron transport and energy transfer process using both polorographic and spectral measurements. Thetreatment of Hg induced 50% loss in the whole chain electron transport activity at 15µM concentration. This inhibition in whole chain electron transport could be due to alterations at 3 levels, that is inhibition at intersystem electron transport carrier as reported earlier for Hg (Katoh and takamiya, 1964; Honey cut and and Krogmonn, 1972) PS II level as reported for Hg (Golbeck *et al.*, 1977). The inhibition in PS II catalyzed transfer activity was 57% with 15 µM of $HgCl_2$.

The observed inhibition in PS II catalyzed electron transport by Hg could be due to the presence of inhibitory site at either oxidizing side as reported by Samson and Popovic1990 or near PS II reaction centre as observed by Honey cut and Krogmann (1972). The observed inhibition in PS I catalyzed electron transport by Hg could be due to the presence of inhibition sites at plastocyaninas reported by Honey Cutt and Krogmann (1972) or at P700 as observed by Golbeck *et al*., (1977). In addition to these concentrations of Hg andNi are able to induced alterations in florescence emission properties. The addition of lower case of Hg (12 µM) caused decrease in the flourescence intensity indicating the inhibition of PS II Photochemistry. The decrease in the fluoroscence intensity is related loss of PS II catalyzed Electron transport activity as a clear reported in case of high temperature treatment of chlorella cells (Papageorgious 1975; Singhal *et al*., 1981). Murthy *et al*., 1990 also reported loss in the chlorophyll flouroscence is related to PS II photochemistry in the cyanobacterium spirulina platensis under Hg stress. To identify the target for the above heavy metals in PS II, Chl *a* fluorescence kinetics has been measured using PAM kinetic fluorimeter. The treatment of Hg induced alterations in Chl *a* fluorescence kinetics in terms of raise in Fo and decrease in Fv. Raise in Fo in the case of

cyanobacteria has been earlier reported by Murthy *et al.*, (1990) under Hg stress. They indicated that Hg is able to induced alerations in the phycobilin protein(LHC) and affect the energy transfer from LHC to PS II reaction centre also indicated that high temperature treatment induces alterations in Chl *a* fluorescence kinetics of emaranthus thylakoid membranes in terms of increased Fo and Fv. Thylakoid membranes are made up of MGDG, DGDG, sulpholipids and phospholipids to maintain the proper interaction of polypeptides and smooth functioning of photosynthetic electron transport. Environmental stress like temperature senescence is known to alter thethylakoid membrane fluidity and affect the photosynthesis. This fluidity of lipids can be altered due to the reaction of superoxy and peroxy radicals with thylakoid lipids. This interaction with membrane lipids and induce lipid peroxidation. To confirm the above preposition lipid peroxidation has been measured before and after treating thylakoid membranes with metal ions. Hg caused 90% enhancement in the lipid peroxidation.

REFERENCES:

1. Baszynski, T., Wajda, L., Krol, M., Walinska, D., Drupa, Z. and Tukendorf, A. (1980). Photosynthetic activities of cadmium treated tomato plants. *Physiol. Plant*. 46:365-370.
2. Bazzaz, M.B. and Govindjee, (1974a). Effects of cadmium nitrate on spectral characteristics and light reactions of chloroplasts. *Environ. Lett***.** 6: 1-12.
3. Bazzaz, M.B. and Govindjee (1974b). Effect of lead chloride on chloroplast reactions. *Environ. Lett*. 6: 175-191.
4. Carmak, I. and Horst, J. H. (1991) Effect of aluminium on lipid peroxidation, SOD, CAT and peroxidase activities in root tips of soybean. *Physiol Plant*.83**:** 463-468.
5. Cedeno-Maldonado, A., Swader. J.A. and Heath, R.L. (1972) The cupric ions as an inhibitor of photosynthetic electron transport in isolated chloroplasts. *Plant Physiol*. 50: 698-701.
6. De Filippis, L.F., Hampp, R. and Ziegler, H. (1981b) The effect of zinc, cadmium and mercury on *Euglena*. Adenylates and energy changes. *Z. Pf1anzenphysio1*.103: 1-7.
7. Golbeck, J.H., Stephen, L. and San-Pietro, A. (1977) Isolation and characterization of a sub chloroplast particle enriched in iron-sulfur protein and P700. *Arch. Biochem. Biophys*. 178: 140-150.
8. Honeycutt, R.C. and Krogmann, D.W. (1972) Inhibition of chloroplast reactions with phenyl mercuric acetate. *Plant Physiol*. 49: 376-380.
9. Katoh, S. and Takamiya, A. (1964) Nature of copper-protein binding in spinach plastocyanin. *J. Biochem*.**55**: 378-387. 107
10. Mohanty, N., Vass, I. and Demeter, S. (1989a) Copper, toxicity affects photosystem II electron transfer at the secondary quinone acceptor, Q_B. *Plant Physiol*. 90:175-179.
11. Mohanty, N., Vass, I. and Demeter, S. (1989b) Impairment of photosystem II activity at the level of secondary quinone electron acceptor in chloroplasts treated with cobalt, nickel and zinc ions. *Physiol. Plant*. 16:386-390.
12. Murthy,S.D.S.,Bukhov,N.G. and Mohanty,P.(1990) Mercury induced alterations in chlorophyll *a* fluorescence in cyanobacteria; multiple effects of mercury on electron transport. *J.Photochem. Photobiol(Biology)*.**6**:373-378.
13. Papageorgiou, G. C. (1975) Chlorophyll fluorescence: An intrinsic probe of photosynthesis. In: Bioenergetics of photosynthesis, (Govindjee, edt.), pp. 320-366. Academic Press, New York.
14. Renganathan, M. and Bose, S. (1989) Inhibition of primary photochemistry of photosystem II by copper in isolated pea chloroplasts. *Biochim. Biophys. Acta*. 974: 247-253.
15. Sabat,S.C., Mohanty,N. and Mohanty,P.(1986)Heat induced alteration in electron donation site(s)of ascorbate and ascorbate reduced catachol in the electron transport chain of *Amaranthus* chloroplasts.*Ind.J.Biochem.Biophys*.23:266-269.
16. Samson, G., Morissette, J. C. and Popovic, R. (1989) Copper quenching of variable fluorescence in *Dunaliella tertiolecta*. New evidence for a copper inhibition effect on PS II photochemistry. *Photochem. Photobiol*. 48: 329-332.
17. Samson, G. and Popovic, R. (1990) Inhibitory effects of mercury on photosystem II photochemistry in *Dunaliella tertiolecta* under in vivo conditions. *J. Photochem. Photobiol. B. (Biol.)*. 5: 303-310.
18. Samuelson, G. and Oquist, G. (1980) Effects of copper chloride on photosynthetic electron transport and chlorophyll-protein complex of Spinach oleracea. *Plant Cell Physiol*. 21: 445-454.
19. Tripathy,B.C.,Bhatia,B. andMohanty,P.(1981)Inactivation of chloroplast photosynthetic electron transport activity by Ni^{2+}.*Biochim. Biophys.Acta*.638:217-224.
20. Tripathy, B.C.,Bhatia,B. andMohanty,P.(1983) Cobalt ions inhibits electron transport activity of photosystem II with out affecting photosystem I. *Biochim.Biophys.Acta*.722:88-93.
21. Shioi, Y., Tarnai, H. and Sasa, T. (1978a) Effects of copper on photosynthetic electron transport in spinach chloroplasts. *Plant Cell Physiol*. 19: 203-209.
22. Shioi, Y., Tarnai, H. and Sasa, T. (1978b) Inhibition of photosystem II in green alga (*Ankistrodesmus falcatus*) by copper. *Physiol. Plant* 44: 434-438.
23. Singhal,G.S.,Mohanty,P. and Govindjee (1981) Effect of preheating intact cells on pigments revealed by absorption and fluorescence.*Z.Planzenphysiol*.**103**:217-228.
24. Van Assche F. and Clijsters, H. (1980) Zinc mediated effects on leaf CO_2 diffusion conductances and net photosynthesis in *Phaseolus vulgaris* L. *Photosynth. Res.* 1: 171-180.
25. Van Assche, F. and Clijsters, H. (1986) Inhibition of photosynthesis in *Phaseolus vulgaris* by treatment with toxic concentration of zinc: effects on electron transport and photophosphorylation. *Plant Physiol*. 66: 717-721.