



ACTION OF SELECTED HEAVY METALS ON PHOTOSYNTHETIC ELECTRON TRANSPORT ACTIVITIES OF MAIZE THYLAKOID MEMBRANES.

Srinivasulu.P. and Murthy, S.D.S.

Department of Biochemistry, Sri Venkateswara University, Tirupati-517502, Andhra Pradesh, India.

Email: sdsurthy@rediffmail.com

ABSTRACT: In this investigation an attempt has been made to study the effect of mercury (5-20 μM), or nickel (20-100 μM) as partial electron transport activities using maize thylakoid as experimental system. Between two photosystems, photosystem II catalyzed electron transport is more sensitive to heavy metal ions when compare to that of photosystem I. Between Hg or Ni, mercury seems potent inhibitor of Hill reaction and induces around 50% loss at 15 μM and 50 μM respectively. Photosystem I catalyzed electron transport is more sensitive to the action of mercury than that of Ni. Thus two selected heavy metal ions show the action differently on electron transport in maize thylakoid membranes.

Key words: Electron transport, Maize plant, Mercury, Nickel, Photosynthesis.

INTRODUCTION

Heavy metals are phytotoxic, leads to environmental pollution and as plant can easily assimilate the heavy metals like Zn, Cd, Ag, Cr, Ni [1,2,3]. In addition to this, chlorophyll biosynthesis is inhibited by these metals in higher plants eg: Pb or Hg [4, 5] Cr [6] Ag [7]. These heavy metals are known to interfere with a variety of photochemical functions at multiple sites [8,9]. The photosystem (PS) II supported electron transport activity is more susceptible to heavy metals like Hg²⁺ [10, 11] Cr⁶⁺ [12]. PS I catalysed electron transport is less sensitive compare to that of PS II. To know the mechanism of electron transport in thylakoids, Tripathy and Mohanty (1981) stabilised the chloroplast membrane with glutaraldehyde (GA) [13]. Under Zn and Pb stress they have seen the damage even after fixation with GA. It indicates that the inhibition in electron transport is not due to alteration in the thylakoids but it could be due to loss of electron transport activity. Therefore in this investigation an attempt has been made to characterize the effect of selected metal ions (Hg/Ni) effect on photosynthetic electron transport by isolating the thylakoid membranes from maize leaves and then exposing them to selected heavy metals. The effect of selected heavy metals was studied using polarography in terms of partial electron transport measurements to identify the target site in photosynthetic apparatus in maize thylakoids.

MATERIALS AND METHODS

Maize seedlings were raised on petri plates under continuous white light (16 Wm⁻²) at 25°C. Half strength Hoagland solution was supplied at 4-day intervals to the seedlings. Thylakoids have been isolated from 8-day old seedlings and they were exposed to different concentrations of Hg (5-20 μM) or Ni (20-80 μM) for 5min in dark. The chloroplast isolation and the polarographic measurement of the partial photochemical activities were done as described earlier [14] with slight modifications. The assay mixture for the measurement of whole chain electron transport activity contained reaction buffer, 0.5 mM MV and 1 mM Na-azide. The assay mixture for the measurement of PS II mediated oxygen evolution activity contained 0.5 mM PBQ in three ml of the 25mM reaction buffer. For measurement of PS I activity, the reaction mixture contained 0.1 mM DCPIP, 5mM ascorbate, 1 mM azide, 0.010 mM DCMU and 0.5 mM MV. In all these assays chloroplasts equivalent to 30 μg was used. The assays were conducted at 25°C under saturating light intensity (400Wm⁻²).

RESULTS AD DISCUSSION

In this present study, effect of selected heavy metals (Hg/Ni) on photosynthetic electron transport has been analyzed by using oxygen electrode. After isolation of thylakoid from maize they were incubated for 5min under constant stirring with either Hg (5-20 μM) or Ni(20-80 μM) before they measuring the partial electron transport activities. Control thylakoid membrane exhibited the whole chain electron transport activity (H₂O→MV) level to 176 $\mu\text{moles O}_2 \text{ mg Chl}^{-1} \text{ h}^{-1}$ (Table 1).

Mercury treatment caused concentrations dependent inhibits and at 10 μ M almost 42% loss was noticed in whole chain electron transport activity. Further rise of concentrations of mercury to 20 μ M brought 82% inhibition. But with Ni treatment, 56% loss in WCE was observed with 60 μ M of concentrates of Ni. Thus there is a difference in the sensitivity of WCE and mercury seems to be potent inhibitor. To verify above proposition individual electron transport assays mediated by either PS II or PS I has been made. Control activity mediated by PS II in untreated samples showed 226 μ moles of O₂ evolved (Table 3 and 4). The treatment mercury caused 58% loss in Hill activity with 15 μ M concentrations. The possible reasons for the loss of PS II activity could be either alterations at OEC or reducing side of PS II [10]. But when the treatment was given with Ni (20- 80 μ M), 45% loss was observed with 40 μ M concentration of Ni. The reason for the loss in PS II activity cause alterations at WOC of PS II in maize thylakoids as each suggests by Tripathy and Mohanty [13]. To determine the variation in susceptibility of PS II and PS I, the electron transport mediated by PS I has been measured using reduced DCPIP as donor and MV as an acceptor (Table 5 and 6). Control thylakoids membranes exhibited the PS I catalyzed electron transport activity equal to that of 354 μ moles of O₂ consumed. The treatment of thylakoids with mercury induced 52% loss in PS I catalyzed electron transport of maize system. The possible reasons for the loss of PS I activity could be replacement of Cu by Hg in Pcy as indicated by Katoh and Takamiya [15]. But surprisingly with Ni treatment only marginal inhibition was noticed even at 60 μ M of Ni concentrations in PS I catalysed electron transport. Thus both mercury and nickel are showing differential effects on both PS II /PS I electron transport activity based on the nature and concentration of heavy metal used during the treatment.

Table 1: Effect of mercury on whole chain electron transport of thylakoid membranes isolated from maize leaves.

Concentration μ M	Whole chain electron transport activity (H ₂ O \rightarrow MV) μ moles of O ₂ consumed mg Chl ⁻¹ h ⁻¹	Percent loss
Control	176 \pm 14	0
5	133 \pm 11	24
10	102 \pm 9	42
15	58 \pm 4	67
20	32 \pm 2	82

Table 2: Effect of Nickel on whole chain electron transport of thylakoid membranes isolated from maize leaves.

Concentration μ M	Whole chain electron transport activity (H ₂ O \rightarrow MV) μ moles of O ₂ consumed mg Chl ⁻¹ h ⁻¹	Percent loss
Control	178 \pm 16	0
20	146 \pm 11	18
40	117 \pm 10	34
60	78 \pm 6	56
80	59 \pm 4	77

Table 3: Effect of mercury on photosystem II catalyzed electron transport of thylakoid membranes isolated from maize leaves.

Concentration μ M	PS II catalyzed electron transport activity (H ₂ O \rightarrow PBQ) μ moles of O ₂ evolved mg Chl ⁻¹ h ⁻¹	Percent loss
Control	223	0
5	174	22
10	136	39
15	94	58
20	65	71

Table 4: Effect of Nickel on photosystem II catalyzed electron transport of thylakoid membranes isolated from maize leaves.

Concentration μM	PS II catalyzed electron transport activity ($\text{H}_2\text{O} \rightarrow \text{PBQ}$) μ moles of O_2 evolved $\text{mg Chl}^{-1} \text{h}^{-1}$	Percent loss
Control	226	0
20	176	22
40	124	45
60	84	63
80	50	78

Table 5: Effect of mercury on photosystem I catalyzed electron transport in thylakoid membranes isolated from maize leaves.

Concentration μM	PS I catalyzed electron transport activity ($\text{DCPIP}_{\text{H}_2} \rightarrow \text{MV}$) μ moles of O_2 consumed $\text{mg Chl}^{-1} \text{h}^{-1}$	Percent loss
Control	354	0
5	311	12
10	276	22
15	223	37
20	153	52

Table 6: Effect of Nickel on photosystem I catalyzed electron transport in thylakoid membranes isolated from maize leaves.

Concentration μM	PS I catalyzed electron transport activity ($\text{DCPIP}_{\text{H}_2} \rightarrow \text{MV}$) μ moles of O_2 consumed $\text{mg Chl}^{-1} \text{h}^{-1}$	Percent loss
Control	352	0
20	327	7
40	310	12
60	289	18
80	281	20

ACKNOWLEDGEMENTS: SDS is thankful to UGC for providing one time grant (2013-14).

REFERENCE:

- [1] De Filippis, L.F. and Pallaghy, C.K. 1994. Heavy metals : sources and biological effects. In: algae and water pollution. (Rai, L.C., et al., ed.) pp.31-37, E.Schweizerbartsche Verlagsbuch-Handlung, Stuttgart.
- [2] Heng, L.Y., Jusoh, K., Ling, C.H.M. and Idris, M. 2004. Toxicity of single and combinations of lead and cadmium to the cyanobacteria *Anabaena flos-aquae*. Bull. Environ. Contam. Toxicol. 72: 373-379.
- [3] Lamaia, C., Kruatrachuea, M., Pohethitiyooka, P., Upathamb, E.S. and Soonthornsarathoola, V. 2005. Toxicity and accumulation of lead and cadmium in the filamentous green algae *Cladophora fracta*: A laboratory study. Sci.Asia. 31:121-127.
- [4] Prasad, D. D. K., and Prasad, A. R. K. 1987a. Effect of lead and mercury on chlorophyll synthesis in mung bean seedlings. Phytochemistry. 26:881 -883.
- [5] Prasad, D. D. K., and Prasad, A. R. K. 1987 b. Altered δ -aminolevulinic Acid Metabolism by Lead and Mercury in Germinating Seedlings of Bajra (*Pennisetum typhoideum*). J. Plant. Physiol, 127: 241-249.
- [6] Panda, S.K., Chaudhury, I. and Khan, M.H. 2003. Heavy metals induce lipid peroxidation and effects antioxidants in wheat leaves. Biol.Plant. 46 :289-294
- [7] Rai, L.C. 1989. Silver toxicity in a nitrogen -fixing cyanobacterium. Interaction with chromium, nickel and lead. Biometals. 2: 122- 128.

- [8] Murthy, S. D. S. and Mohanty, P. 1991. Mercury induced alteration of energy transfer in phycobilisome by selectively affecting the pigment protein, phycocyanin in the cyanobacterium, *Spirulina platensis*. *Plant.Cell. Physiol.*, 32: 231-237.
- [9] Dixit,V, Pandey,V. and Shyam, R. 2002. Chromium ions inactivate electron transport and enhance superoxide generation *in vivo* in pea (*Pisum sativum L.cv: Azad*) root mitochondria. *Plant Cell Env.* 25 : 687-693.
- [10] Honeycutt, R. C, and Krogmann, D. W. (1972). Inhibition of chloroplast reactions with phenylmercuric acetate. *Plant. Physiol.*, 49:376-380.
- [11] Murthy, S.D.S. 1991. Studies on bioenergetic processes of cyanobacteria: Analysis of the effect of selected heavy metal ions on energy linked process. Ph.D thesis, Jawaharlal Nehru University, New Delhi.
- [12] Nash, S.M.B, Quayle,P.A., Schreiber, U. and Muller,J.F. 2005. The selection of model microalgal species as biometrial for a novel aquatic phytotoxicity assay. *Aquat. Toxicol.* 72: 315-326.
- [13] Tripathy, B. C., and Mohanty, P. 1981. Stabilization by glutaraldehyde fixation of chloroplast membrane structure and function against heavy metal ion induced damage. *Plant. Sci .Lett.*, 22: 253-261.
- [14] Sabat, S. C, Mohanty, N, & Mohanty, P. 1986. Heat-induced alteration in electron donation site(s) of ascorbate and ascorbate-reduced catechol in the electron transport chain of *Amaranthus* chloroplasts. *Indian J. Biochem. Biophys*, 23(5): 266-269.
- [15] Katoh,S. and Takamiya,A. 1964. Nature of copper-protein binding in spinach plastocyanin. *J.Biochem.* 55: 378-387.

International Journal of Plant, Animal and Environmental Sciences

