

Polyamine Alleviates Inhibition of O₂ Evolution by Cd for Spinich

Tae Wan Kim*, Ju-Young Lee*, Yong-Se Lee**, Seon-Woong Hwang* and Young-Sang Yoon***

ABSTRACT

Polyamine concentrations were clearly enhanced in leaves and chloroplasts by Cd treatment, but not in thylakoid and PSII. It appeared that newly synthesized polyamines by Cd are distributed in stromal space. The accumulated polyamines in stromal space could not be adjacent to thylakoid membranes, suggesting that they are already saturated. The levels of putrescine and spermine were about 36 and 20 fold lower in chloroplast than in whole leaf cells respectively, whereas agmatine level was only 3.7 fold lower. The inhibitory effect of Cd on O₂ evolving process was obviously alleviated by 0.2mM spermine supplement. Polyamines stimulated O₂ evolution within the range of 0.5mM in spinach thylakoids. It was also found that stimulating effect of polyamines is about 2 fold higher in dicothyledonous spinach than in monocotyledonous wheat at same concentrations. Furthermore, the enhanced activity of O₂ evolution was lowered rather by agmatine treatment than by putrescine treatment in wheat, suggesting a difference between monocot and dicot.

Introduction

Cadmium(Cd) is one of the important heavy metal pollutants and its toxic effects on plants are well documented. Earlier investigations have demonstrated a remarkable reduction in the overall rate of photosynthesis by Cd(Bhardwaj et al. 1989; Chugh and Sawhney 1999; Sheoran et al. 1990). Several studies illuminated that inhibition of photosynthesis cannot entirely be attributed to the direct interference of the heavy metal with photoreactions since CO₂ fixation through ribulose biphosphate carboxylase(RuBisCO) is also inhibited by Cd(Weigl H. J. 1985). The diamine putrescine(Put) and cadaverine(Cad) and the polyamine agmatine(Agm), spermidine(Spd) and spermine(Spm) are widely distributed

in bacteria, animals and plants. Under a wide range of stress conditions such as K deficiency, low pH, chilling injury and ozone treatment, polyamine is actively synthesized. The induction of Put has been thought to be a protective response against stress. The binding of polyamines to macromolecules such as nucleic acids(Fink and Pettijohn 1975), phospholipids(Chapel et al. 1984) and proteins(Metha et al. 1991) may indicate their role in response to stress. Their binding to photosystem II(PS II) of spinach and chlorophyll(chl) a/b protein was investigated by Del Duka et al. (1994). It has also been suggested that Cd interacts with the protein subunits of light harvesting complex-II(Van Assche and Clijsters 1990). Kotzabasis and co-workers reported that Put, Spd, and Spm are present in intact chloroplasts and thylakoid membranes(1993).

* Department of Plant Nutrition, National Institute of Agricultural Science and Technology, Suwon, Korea

** Department of Agronomy, College of Agriculture, Taegu University, Kyongsan, Korea

*** Dept. of Plant Resources, Kongju National Univ. Kongju, Korea

Phytotoxicity of Cd involves lipid peroxidation in chlorophyll degradation process. Kim and Heinrich(1995) have found that Spm has a protective effect on chlorophyll degradation within intact chloroplast. However, the role of polyamines in O₂-evolution during the photosynthesis process has not been clearly determined. In view of the above, we therefore tried to clarify the role of polyamines in O₂-evolution under Cd-stress.

Materials and Methods

Wheat(*T. aestivum* cv. Geurumil) seeds were germinated in a petri dish for 5 days. The seedlings were transplanted and cultured in a chamber(Conviron) under a growth condition of 25/15C and 12/12h (day/night) until chloroplasts and their components were isolated. Seedlings were grown in strength of Hoagland's solution with or without 100 M Cd. Spinach plants were cultured under same condition but 20/10C. Chloroplasts, thylakoids and photosystem II were isolated from young leaves of 2-week-old wheat seedling and 4-week-old spinach by the methods of Kim and Heinrich 1995; Kotzabasis et al. 1993; Ono et al. 1986. O₂ evolution was measured with a Clark-type oxygen electrode(YSI Co. Model 4004) at 25C. The reaction mixture contained 25 mM HEPES(pH 7.5), 100 mM sucrose, 5 mM MgCl₂, 1 mM NH₄Cl, 3 mM potassium ferricyanide and thylakoid fractions(0.15-0.20 mg chlorophyll) in a volume of 3.3 ml. Actinic light was obtained from a 300 W tungsten lamp after passing it through a 1% CuSO₄ solution(10 cm thick). As the O₂ evolution increased steeply with increasing irradiance, all O₂ evolution experiments were carried out under a constant value at about 10 W m⁻². Polyamines and Cd were added in indicated experiments. Each experiment was conducted 40 min after preincubation with or without Cd in darkness. Isolations of subfractions were carried out 6 h after illumination for polyamine analysis but 24 h after dark treatment for O₂ evolution experiments. Free and covalently-bound polyamines were extracted after

hydrolysis of the various preparations as described by Flores and Galston(1982). The benzoylated polyamines were detected by HPLC using a narrow bore reverse phase C18 column and a two-solvent system containing acetonitrile and deionized sterile water. Polyamines were eluted using a changeable acetonitrile gradient(40 to 65%, v/v).

Results and Discussions

Polyamine concentrations are clearly enhanced in leaves and chloroplasts by Cd treatment, but not in thylakoid and PS II(Table 1). It may mean that newly synthesized polyamines by Cd are distributed in stromal space. Furthermore, the polyamines do not actively bind to thylakoid membranes in spite of their increased concentration in chloroplast. Thus, we postulate that on the basis of chlorophyll content, maximal binding capacity of polyamines to thylakoid membranes may be less than 5 nmole in monocot wheat. Namely, the accumulated polyamines in stromal space could not be adjacent to thylakoid membranes, suggesting that they are already saturated. The increase in endogenous polyamine levels has been considered as a protective response against Cd in potato leaves(Stroinski and Szczotka 1989). Our results showed that the increase of endogenous polyamines occurs not only in whole leaf cells but also in chloroplasts. The levels of Put and Spm are about 36 and 20 fold lower in chloroplasts than in whole leaf cells respectively, whereas Agm level is 3.7 fold lower(Table1). Put was the dominant polyamine in the PS II from wheat as earlier observation by Kotzabasis et al. (1993). Cd treatment exhibited an effect on the increase in the concentration of Agm, Spd and Put within chloroplast. Our result also shows that Cd does not bind to PS II. Drmemann et al.(1996) demonstrated that the bound polyamines in plastid isolated membranes from unicellular green alga *Scenedemus obliquus* during various developmental stages of chloroplasts were considerably redistributed after the transition from dark to light. In this

study, the isolation of chloroplasts was identically conducted 6 h after the start of irradiance. Thus the difference in polyamine levels may be considered as an effect of Cd. To clarify the physiological role of specific enhancement in Put, Spm and Agm after Cd treatment, we have measured the O₂ evolution activity using intact thylakoid membranes. The inhibitory effect of Cd on O₂ evolving process was obviously alleviated by 0.2 mM Spm supplement(Fig. 1). Polyamines stimulated O₂ evolution within the range of 0.5 mM in spinach thylakoids. In this range the activation curves of Spm and Agm appeared to be exponential. It was also found that the stimulating effect of polyamines is about 2 fold higher in dicotyledonous spinach than in monocotyledonous wheat at same concentrations.

Table 1. Concentrations of the polyamines in wheat leaves, chloroplasts, thylakoids and PS II. Concentrations of the polyamines in leaf and chloroplast are represented as the sum of free and bound form. The others means the concentration of bound form

Polyamines		Cd treatment (μ M)		B/A ratios
		0(A)	100(B)	
Leaf (nmole g ⁻¹ fresh weight)	Put	882	1,437	1.63
	Cad	63	210	3.33
	Spd	273	354	1.30
	Spm	696	932	1.34
	Agm	380	420	1.11
	Total	2,294	3,353	1.46
Chloroplast (nmole mg ⁻¹ chl.)	Put	15.4	39.8	2.58
	Cad	5.8	7.8	1.34
	Spd	13.3	41.9	3.15
	Spm	29.4	46.3	1.57
	Agm	15.1	110.3	7.30
	Total	79	246.1	3.1
Thylakoid (nmole mg ⁻¹ chl.)	Put	2.30	2.54	1.10
	Cad	0.08	0.06	0.75
	Spd	0.31	0.38	1.23
	Spm	0.24	0.32	1.33
	Agm	0.35	0.40	1.14
	Total	3.28	3.70	1.12
PS II (nmole mg ⁻¹ chl.)	Put	1.14	1.26	1.63
	Cad	trace	trace	3.33
	Spd	0.24	0.22	1.30
	Spm	0.13	0.16	1.34
	Agm	0.08	0.09	1.11
	Total	1.59	1.73	1.46

Furthermore, the enhanced activity of O₂ evolution was lowered rather by Agm treatment than by Put treatment in wheat(Fig. 2), suggesting a difference between monocot and dicot.

Askar and Treptow showed that diamine-amino-transferase can especially transfer an amino group of Put to -oxoglutaric acid, resulting in the formation of glutamic acid, which in turn, is the precursor molecule for the C₅-pathway and thus for chlorophyll biosynthesis. Earlier observations showed that exogenous polyamine addition in senescent leaf retained the chlorophyll loss in various higher plants(Popovic 1979; Kim and Heinrich 1995). Their experiments were conducted in darkness. In this case the senescence process is far away compared to normal chloroplast developmental processes and chlorophyll biosynthesis of which most regulatory enzymes are subjected to light regime. It becomes clear that the role of polyamines ought to be important in the protective mechanism of photosensory apparatus under dark and light. Such a study will be the subject of future work. Heavy metals such as Cd preferably bind to PS II rather than to PS I(Van Assche and Clijsters 1990). Stiborova(1988) indicated

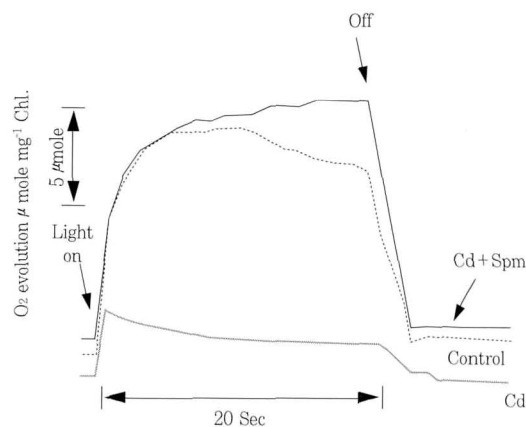


Fig. 1. Time courses of O₂ evolution. Spinach thylakoid suspensions were preincubated with or without 100 μ M Cd or 0.2 mM Spm in the dark for 40 min and then illuminated with actinic light(10 W m⁻²) at 25°C, Chl concentration in these reaction mixtures were 200 μ g.

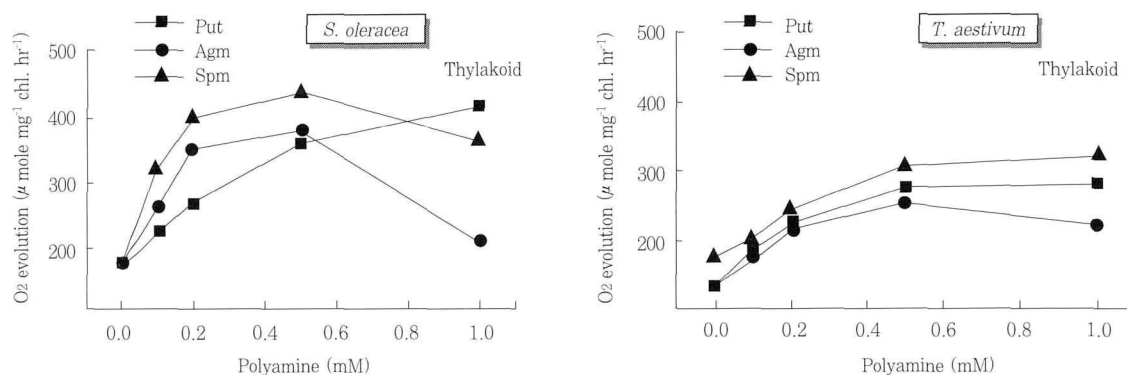


Fig. 2. Changes in O₂ evolution by exogenous polyamines in spinach (*S. oleracea*) and wheat (*T. aestivum*) thylakoid membranes. Thylakoid suspension are pre-incubated with or without 0.1mM to 1.0mM polyamines in the dark for 40 min and then illuminated with actinic light (10 W m⁻²) through 1% CuSO₄ at 25°C. Chlorophyll concentration in these reaction mixtures (3.3ml) were 150 to 200 μg. The values (μmole mg⁻¹ chl h⁻¹) are presented as the mean of triplicated experiments.

that Cd could bind to numerous stromal proteins resulting in the loss of their functions. Nevertheless, our in vitro data give evidence for an enhancing role of polyamines in the O₂ evolution under light condition. Recently, Chugh and Sawhney (1999) demonstrated that the photosynthetic activity per plant by Cd was depressed to a much greater extent than chlorophyll content was. Such result was investigated from leaf disc test as earlier observations (Kim and Heinrich 1995; Popovic et al. 1979). Metabolic processes are interdependent in vivo and thus it is difficult to extrapolate with certainty present in vitro data in intact chloroplast and thylakoid. In this context, we would conclude that on the one hand, the polyamine increase within chloroplast by Cd may enrich the substrates for a newly synthesized chlorophyll resulting in retention of chlorophyll level, and on the other hand, their polycationic characteristics function as a protector on membrane against Cd. Namely, Cd might be repulsed from thylakoid. Thus, O₂ evolution might regain its activity by exogenous polyamines in our in vitro experiment. The polyamine concentration of mM is physiologically excess level within chloroplast. In further works it is needed to investigate O₂ evolution during the polyamine degradation and transamination.

References

- Askar, A. and H. Treptow. 1986. Biologend Amine in Lebensmitteln. Verlag Eugen Ulmer, stuttgart. ISBN 3-8001-2132-8
- Bhardwaj R., 1989. Plant Physiol. Biochem. 16:40-48
- Chapel, M., J. Teissie and G. Alibert. 1984. Electrofusio of spermine treated plant protoplasts. FEBS Lett. 173:331-336
- Del. Duka, S., V. Tidu, R. Bassi, C. Esposito and D. srafini-Fracassini. 1994. Identification of chlorophyll a/b proteins as substrates of transglutaminase activity in isolated chloroplasts of Helianthus tuberosus L. Planta 193:283-289
- Domemann, D., E. Navakoudis and K. Kotzabasis. 1996. Changes in the polyamine content of plastidial membranes in light-and dark-grown wildtype and pigment mutants of the unicellular green alga Scenedesmus obliquus and their possible role in chloroplast photodevelopment. J. Photochem. Photobiol. 36:293-299
- Fink, L. and D. E. Pettijohn. 1975. Polyamine stabilize DNA folds, Nature 253:62-63
- Flores, H. E. and A.W. Galston. 1982. Analysis of polyamines in higher plants by high performance liquid chromatography. Plant Physiol. 69:701-706
- Kin, T.W. and G. Heinrich. 1995. Effects of Sr 2t, Ca 2t, and spermine on thylakoid protein and chlorophyll degradation during dark incubation senescence of sugar beet leaf discs. Photosynthetica 31:315-319
- Kotzabasis, K., C. Fontinou and D. Ghanotakis. 1993. Polyamines in the photosynthetic apparatus, Photosystem II highly resolved subcomplexes are enriched in spermine. Photosynthesis Res. 38:83-88

- Kotzabasis, K., M. D. Christakis-Hampass and K. A. Roubelakis-Angelakis. 1993. A narrow-bore HPLC method for the identification and quantification of free, conjugated and bound polyamines. *Anal. Biochem.* 214:484-489
- Metha, A. M., R. A. Saffner, G. W. Schaeffer and A. K. Matoo. 1991. Translational modification of an 18 kDa polypeptide by spermine in rice cell suspension cultures. *Plant Physiol.* 95:1294-1297
- One, T. A. 1986. *Plant Physiol.* 80:85-90
- Popovic, R. B., D. J. Kyle, A. S. Cohen and S. Zalik. 1979. Stabilization of thylakoid membranes by spermine during stress-induced senescence of barley leaf discs. *Plant Physiol.* 64:721-726
- Sheoran I. S. 1990. *Plant Soil.* 129: 243-249
- Stiborova, M. 1998. Cd²⁺ ions affect the quaternary structure of ribulose-1, 5-bisphosphate carboxylase from barley leaves. *Biochem. Physiol. Pflanzen.* 183:371-378
- Stroinski, A. and Z. Szczotka. 1989. Effect of cadmium and *Phytophthora infestans* on polyamine levels in potato leaves. *Physiol. Plant.* 77:244-246
- Van Assche, F. and H. Clijsters. 1990. Effects of metals on enzyme activity in plants. *plant cell Environ.* 13:195-206
- Weigl, H. J. 1985. *J. Plant Physiol.* 119:179-189

Polyamine Alleviates Inhibition of O₂ Evolution by Cd for Spinich

Tae Wan Kim*, Ju-Young Lee*, Yong-Se Lee**, Seon-Woong Hwang* and Young-Sang Yoon***

시금치에서 Polyamine에 의한 카드뮴의 산소방출억제 경감효과

김태완* · 이주영* · 이용세** · 황선웅* · 윤영상**

카드뮴은 잎과 엽록체내 폴리아민함량을 증가시키지만 틸라코이드와 광포집체II내에서는 증가효과가 없었다. 카드뮴에 의해 새로 생합성된 폴리아민은 스트로마 공간상에 분포하는 것으로 보였다. 폴리아민은 이미 포화되더라도 틸라코이드막에 결합하지는 않는 것으로 밝혀졌으며 엽록체내 putrescine과 spermine은 잎세포 내에서 보다 각각 36배와 20배 적었던 반면 agmatine은 3.7배 적었다.

카드뮴의 산소방출 억제효과는 0.2mM spermine 첨

가로 현저히 완화되었다. 폴리아민은 또한 시금치 틸라코이드에서 0.5mM 농도이하에서 산소방출을 촉진하였다. 이러한 촉진효과는 동일농도에서 단자엽보다는 쌍자엽에서 2배정도 높았다. 더욱이 쌍자엽 밑에서 상승효과는 putrescine보다는 agmatine처리에서 오히려 낮았다. 이러한 결과로부터 단자엽과 쌍자엽 식물간에 폴리아민의 산소방출 과정에서 다른 효능을 보임을 알 수 있었다.

Key words : Polyamine, O₂ evolution, Cd.

* 농업과학기술원 식물영양과(Department of Plant Nutrition, National Institute of Agricultural Science and Technology, Suwon, Korea)

** 대구대학교 농학과(Department of Agronomy, College of Agriculture, Taegu University, Kyungbuk, Kyongsan, Korea)

*** 공주대학교 식물자원과(Dept. of Plant resources, Kongju National Univ. Kongju, Korea)