J. Plant Biol. 38(1): 11~18 (1995)

Mercury-Induced Light-Dependent Alterations of Chlorophyll a Fluorescence Kinetics in Barley Leaves

Lee, Choon-Hwan*, Hosik Chang, Suk-Bong Ha and Chin Bum Lee[1]
Department of Molecular Biology, Pusan National University, Pusan 609-735, Korea; and
[1]Department of Biology, Dongeui University, Pusan 614-714, Korea

Mercury-induced changes in Chl a fluorescence induction kinetics of scratched barley leaf segments were dependent on the presence of light. By the treatment of 50 μM $HgCl_2$ under light condition, Fm and Fp were decreased. However, they were not significantly reduced under dark condition even after 2 h of mercury treatment. Under dark condition the decrease in variable fluorescence (Fv) after P transient was blocked within 20 min of the treatment. The analysis of fast fluorescence rise curve suggests that the inhibitory site of mercury under both light and dark conditions is not at Q_B binding site and the inhibition does not involve the increase in inactive PSII centers. Under light condition the decrease in Fp was partially recovered by addition of 50 μM NH_2OH. These results suggest that a major inhibitory site of mercury under dark condition is at the reducing side of PSII and the site under light condition is at the oxidizing side of PSII possibly in addition to the one under dark condition. Under both light and dark conditions, energy-dependent quenching (qE) was almost completely repressed within 20 min of mercury treatment and noticible change in Fo was not observed. The qE repression is probably due to the blockage of transthylakoid ΔpH formation.

Keywords: barley leaf, Chl fluorescence, mercury toxicity, light

Mercury has inhibitory effects on photosynthetic electron transport in higher plants (Honeycutt and Krogmann, 1972; Kimimura and Katoh, 1972; Tripathy and Mohanty, 1980), algae and cyanobacteria (De Filippis *et al.*, 1981; Murthy *et al.*, 1990; Samson and Popovic, 1990; Kim *et al.*, 1994). Mercury inhibits both PSII and PSI electron transport activities. The major site of mercury inhibition on PSII has been proposed to be associated with water-splitting system (Samson snd Popovic, 1990; Chun *et al.*, 1994). Sakurai *et al.* (1991) reported mercury inhibition of Fe-S center B of PSI.

Chl a fluorescence emitted from isolated thylakoids and intact leaves reflects the primary processes of photosynthesis, intrathylakoidal proton transfer, photophosphorylation and CO_2 assimilation (Krause and Weis, 1988).

In isolated barley chloroplasts, the activity of PSII was more severely damaged than that of PSI and one of the primary inhibition sites was on the oxidizing side of PSII (Moon *et al.*, 1992; Chun *et al.*, 1994). Significant reduction in transthylakoidal proton gradient formation was also observed. However, any comparable damages in photosynthetic apparatus was not observed in intact barley seedlings when mercury was added in their growth medium (Lee *et al.*, 1992).

In this report, we investigated the primary inhibition sites of mercury on the photosynthetic apparatus of detached barley leaves. Interestingly the mercury inhibition under light condition was different from that under dark condition.

MATERIALS AND METHODS

Plant materials and chemical treatments

Barley (*Hordeum vulgare* L. cv. Albori) plants were germinated and grown at 25℃ under continuous

*Corresponding author: Fax +82-51-513-9258
© 1995 by Botanical Society of Korea, Seoul

white light from fluoresent tubes giving PAR of 65-80 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Two centimeter segments, 2 cm below the tip of the primary leaves, were taken from 8-9 day old plants, and scratched by shaking in a flask filled with fine powders of quartz sand. The scratched leaves were left in a petridish filled with distilled water for 30 min and treated with chemicals. Normally shaking the flask by waving a hand up and down about fifty times did not cause any detectable changes in their fluorescence emission and was enough to remove diffusion barriers on the leaf surfaces.

The light intensity used for the treatment of mercury under light condition was the same as that used in the growth chamber. Otherwise it is mentioned in the text. For the treatment under dark condition, petridishes containing leaf segments were shaded by wrapping with two sheets of aluminum foil. Before measurement of fluorescence emission, leaf segments were dark-adapted for 10 min after moisture on the leaf surfaces was blotted out. Leaf segments were treated with 5 mM hydroxylamine (NH_2OH) or 10 mM dithiothreitol (DTT) during the dark adaption period. For DTT treatment, the leaf segments were dark-adapted for 20 min.

Measurement of fluorescence induction kinetics

Modulated Chl a fluorescence emission from the two segments of leaves was measured by using a PAM Chl fluorimeter (Walz, Germany). Actinic light was provided by light emitting diodes (H2000, Stanley, Japan), and the intensity was adjusted to 160 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The intensity of the modulating beam was 0.05 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Saturation light (1900 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was provided by a halogen lamp (KL 1500, Schott, Germany). The data were collected and handled with computer programs written in Basic and Assembly languages in an IBM compatible personal computer equipped with a data acquisition board (DAS16G, Metrabyte, USA).

Fluorescence rise kinetics and derivative analysis

For the analysis of fluorescence rise kinetics, 15,000 data were collected for 3 s and they were averaged to give 3000 points. After turning off the actinic light source, a saturation pulse was given for 0.8 s to measure maximal fluorescence level (Fm). The initial fluorescence (Fo) was measured with the modulating beam alone. For the derivative analysis, the data was averaged further to give 150 points and the rate of fluorescence change (dFv/dt) was calculated.

Fluorescence quenching analysis

Quenching coefficients were calculated according to Oxborough and Horton (1988). For the quenching analysis, at least three consecutive saturation pulses were given to measure photochemical quenching coefficient (qQ), and the duration of each pulse was 0.8 s. To measure nonphotochemical quenching coefficient (qN) and energy-dependent quenching coefficient (qE), saturation pulses were given for 0.8 s in 20 s intervals after the actinic light source was turned off.

RESULTS AND DISCUSSION

Fluorescence induction parameters and mercury effect

In isolated barley chloroplasts, both whole chain electron transport and PSII-supported O_2 evolution were inhibited about 50% of the control by the treatment of 50 μM of $HgCl_2$ (Moon *et al.*, 1992; Chun *et al.*, 1994). This inhibitory effect of mercury in isolated chloroplasts was almost instantaneous. However, in scratched leaves the inhibitory effect depended on both the concentration of mercury (data not shown) and the duration of the treatment as shown in Figs. 1 and 2. Fm was decreased 40% of the control after 40 min and 70% after 120 min of mercury treatment, but no noticeable increase in Fo was observed within 45 min of the treatment, although significant increase in Fo was observed in isolated chloroplasts (Chun *et al.*, 1992). This indicates that the antenna pigment bed system is not altered significantly in intact leaves.

Fluorescence rise curve and derivative analysis

Time-dependent changes in fluorescence rise curves from the mercury treated leaf segments are

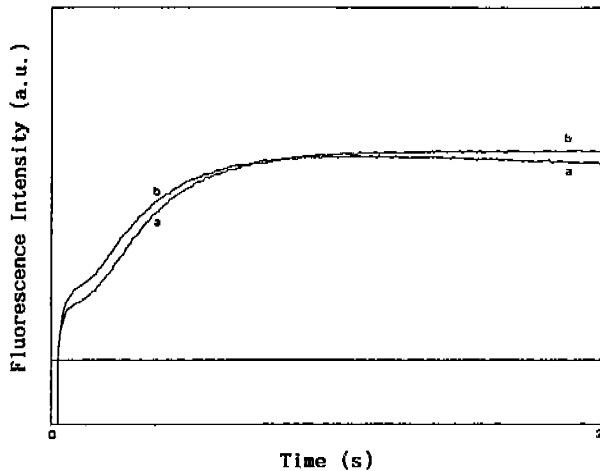

Fig. 6. The inhibitory effect of $HgCl_2$ on fluorescence rise kinetics of barley leaf segments under dark condition. (a) control, (b) 50 μM $HgCl_2$ treated for 50 min.

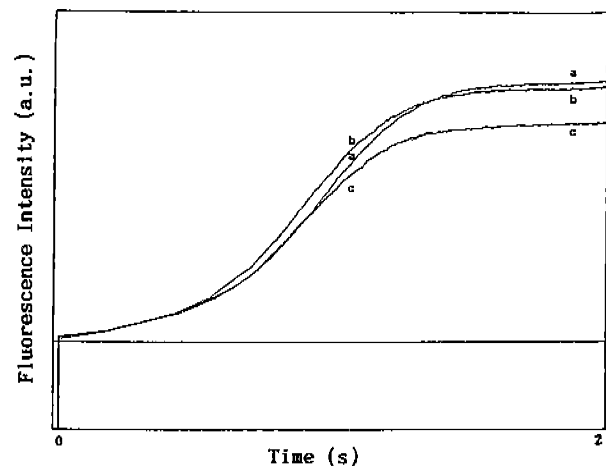

Fig. 7. The inhibitory effect of $HgCl_2$ on fluorescence rise kinetics of DCMU-poisened barley leaf segments under dark condition. (a) control, (b) 50 μM $HgCl_2$ treated for 60 min, (c) 50 μM $HgCl_2$ treated for 90 min. Samples were treated with 50 μM DCMU during the 10 min dark incubation period. Horizontal axis is in logarithmic scale.

not reach Fm and there was no significant changes in the fluorescence rise curve around the D transient (Fig. 6). This result suggests that the primary inhibitory site of mercury on the donor side of PSII under dark condition is not at the Q_B binding site. As shown in Fig. 5, Fm was decreased less than 10% by the treatment of mercury under dark condition and no significant changes in Fo was observed.

In the semilogarithmic plot of fast induction curves of DCMU-poisoned leaf segments, there was no noticeable changes in O-I slope, but I-P slope was slightly decreased as the treatment time proceeds (Fig. 7). This indicates that mercury has little inhibitory effect on electron donor side of PSII within 60 min after the treatment under dark condition.

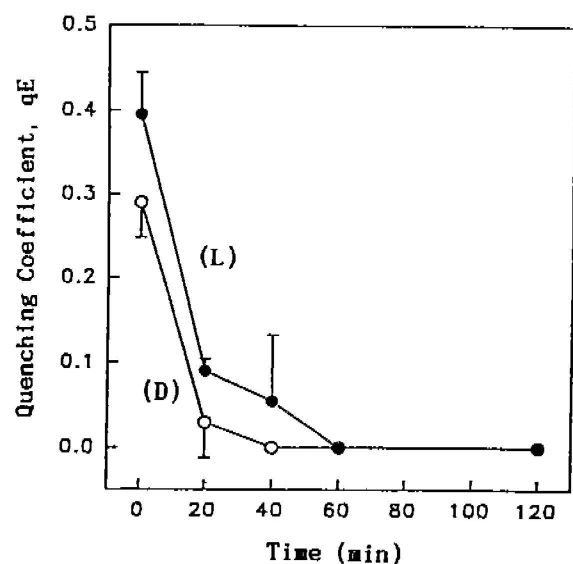

Fig. 8. Time-dependent changes in energy-dependent quenching coefficient (qE) of barley leaf segments treated with 50 μM $HgCl_2$. (L) under light, (D) under dark condition.

Fluorescence quenching analysis

Both qQ and qN were decreased by the treatment of mercury under both light condition (Fig. 1) and dark condition (Fig. 5). Decrease in qN was mainly due to the inhibition of qE formation by mercury (Figs. 1, 5, 8). qE was almost completely repressed within 20 min by the mecury treatment under both light and dark conditions. qE is known to arise when pH gradient is present across the thylakoid membrane (Horton and Hague, 1988). The qE formation is also dependent on the conversion of violaxanthin to zeaxanthin under light (Demming-Adams *et al.*, 1990). They reported that an inhibitor of violaxanthin deepoxidase, dithiothreitol (DTT), inhibited a portion of qE formation by raising variable fluorescence (Fv) in spinach leaves and chloroplasts. In DTT-treated barley leaf segments, both increase in Fv and rapid qE relaxation were observed (Fig. 9). However, the treatment of mercury under both light and dark conditions showed no indication

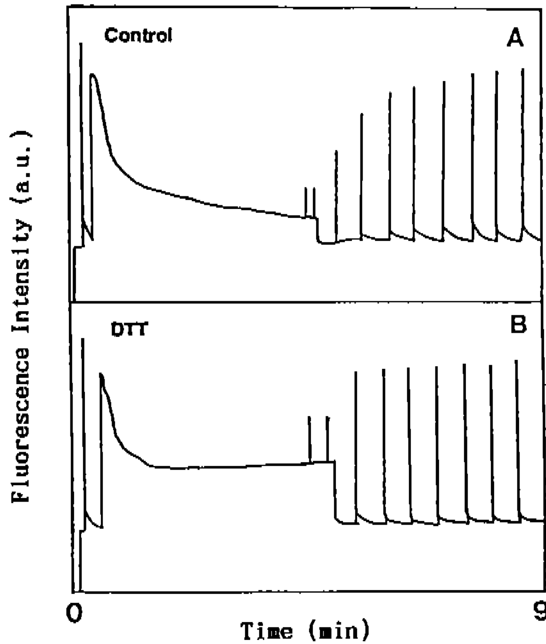

Fig. 9. Effect of dithiothreitol on Chl fluorescence induction transients of barley leaf segments. (A) control, (B) 10 mM dithiothreitol (DTT) treated for 20 min under dark.

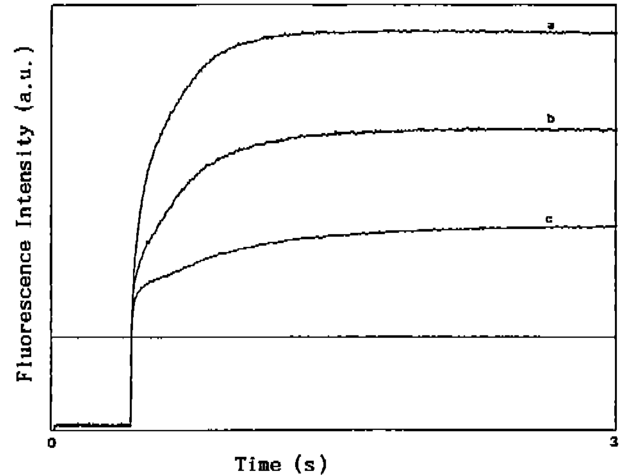

Fig. 10. Effect of light intensity on fluorescence rise kinetics of barley leaf segments treated with 50 μM $HgCl_2$. (a) dark, (b) light, 3 $\mu mol \cdot m^{-2} \cdot s^{-1}$, (c) light, 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

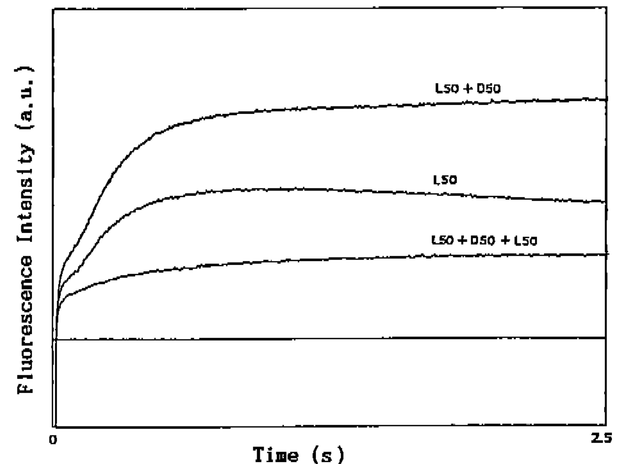

Fig. 11. Light-dependent effect of 50 μM $HgCl_2$ on fluorescence rise kinetics of barley leaf segments. (L50) treated under light for 50 min, (D50) treated under dark for 50 min.

of fast qE relaxation (Figs. 1 and 5). Therefore the possiblity of mercury inhibition of zeaxanthin formation without affecting transthylakoid ΔpH formation can be rejected. Similar to the report of Chun *et al.* (1994) using isolated barley chloroplasts, this result suggests that mercury reduces qE formed by the transthylakoid ΔpH. However we do not know mercury inhibits zeaxanthin formation as well, because thylakoid membrane must be in the energized state in order for zeaxanthin to be active in thermal energy dissipation (Demming-Adams and Adams III, 1992).

Light and mercury effects

The light-dependent effects of mercury are probably due to photoinhibition. To see the effect of light intensity, the light intensity was lowered from 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 3 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Comparing with dark control, a noticeable decrease in Fp was still observed from the leaf segments treated with mercury under very-weak light condition (Fig. 10). Interestingly the decrease in fluorescence yield under light condition could be recovered under dark condition (Fig. 11).

A possible light-specific inhibitory site of mercury, which is repairable and protected from mercury under dark condition, is the oxygen-evolving complex. As mentioned earlier, Q_B-binding protein is probably not a candidate although it is known to be primarily damaged during photoinhibition and repaired very rapidly.

ACKNOWLEDGEMENTS

This work was supported by a research grant from Ko-

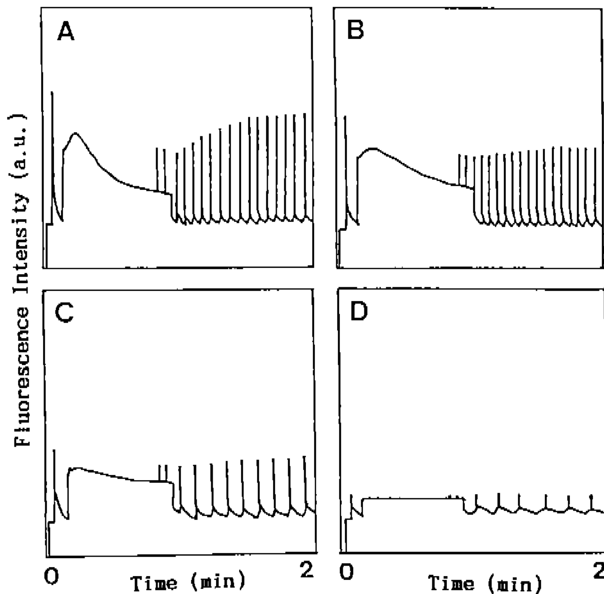

Fig. 1. Time-dependent changes in Chl a fluorescence induction transients of barley leaf segments treated with 50 μM $HgCl_2$ under light condition. (A) 0 min, (B) 20 min, (C) 40 min, (D) 120 min after the treatment.

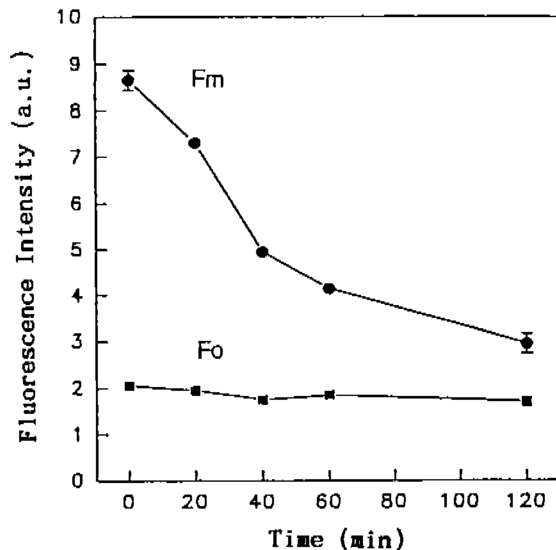

Fig. 2. Time-dependent changes in Fm and Fo in barley leaf segments treated with 50 μM $HgCl_2$ under light condition.

shown in Fig. 3. This alteration of the curves could be quantitatively described by analyzing their first derivative (dFv/dt) plots as shown in Fig. 3(A-b). Parameters obtained from the analysis of the curves are listed in Table 1.

Fluorescence magnitude at the P transient (Fp)

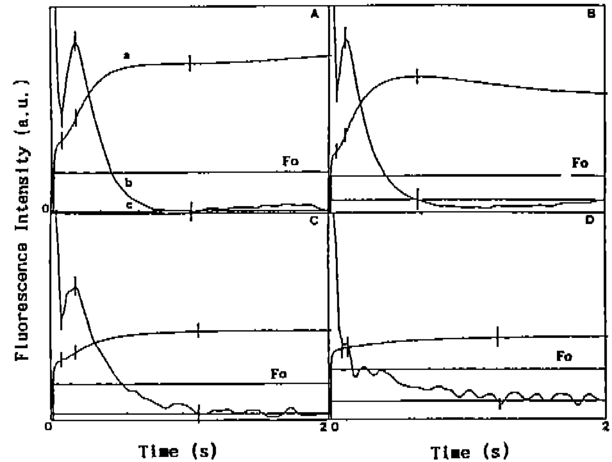

Fig. 3. Time-dependent changes in fluorescence rise curves from barley leaf segments treated with 50 μM $HgCl_2$ under light condition. (A) 0 min, (B) 15 min, (C) 45 min, (D) 120 min after the treatment. (a) original curve, (b) smoothed first-derivative curve, (c) horizontal line where the first derivative is zero (vertical axis scale is normalized).

was quenched by mercury, but the value at the D transient (Fd) did not show significant changes. Because the increase in Fd-Fo could be ascribed to the increase in the portion of inactive center according to Cao and Govindjee (1990), the inhibitory effect of mercury is not primarily related to the increase in the portion of inactive center and/or the inactive center may be less sensitive to mercury inhibition.

The slope between D and P transients or Fr (maximum rate of fluorescence increase in D-P curve) could be accurately measured at a maximum point of the first derivative plot. Fr was increased slightly after 15 min of the treatment and decreased thereafter. The initial increase in Fr is possibly due to partial blockage of electron transport chain at the reducing side of PSII. Murthy *et al.* (1990) reported that mercury treatment at low concentration enhanced fluorescence in a similar manner to diuron in cyanobacteria. The extent of dip in the fluorescence rise curve could be quantitatively described by using a new parameter, ΔS(D-Fr) (absolute difference between the first minimum value and the first maximum after the first minimum value in the dFv/dt curve). This ΔS(D-Fr) reflects the equilibration time between first electron acceptor of PSII, Q_A and Q_B. Significant changes in this value could be expected if mercury affected at or near Q_B binding site. How-

Table 1. Changes in parameters from the analysis of fast fluorescence induction kinetics in bareley leaf segments treated with 50 μM $HgCl_2$

Duration of treatment (min)	Fp (V)	Fp/Fm	Fd (V)	Fr (V/S)	ΔS(D-Fr) (V/S)
0	1.01(0.05)	0.74(0.33)	0.49(0.02)	1.99(0.14)	0.99(0.05)
15	0.92(0.06)	0.79(0.03)	0.42(0.04)	2.37(0.17)	1.10(0.04)
30	0.73(0.03)	0.77(0.04)	0.35(0.03)	1.60(0.05)	0.94(0.03)
60	0.79(0.07)	0.85(0.05)	0.40(0.01)	1.49(0.08)	0.63(0.08)
80	0.67(0.03)	0.90(0.04)	0.49(0.01)	0.77(0.03)	0.16(0.03)
120	0.56(0.02)	0.98(0.01)	0.48(0.02)	0.22(0.03)	n.d.[a]

[a]n.d.=not correctly determined. *Values inside parenthesis are SD from 3 measurements.

ever after 15 min of the treatment, there was no significant changes in the fluorescence rise curve around the D transient (Fig. 3(B)) and ΔS(D-Fr) did not change significantly (Table 1). This result suggests that the primary inhibitory site of mercury on the donor side of PSII within 120 min is not at the Q_B binding site. Similar results were observed in *Ulva pertusa* (Kim *et al.*, 1994). Sakurai *et al.* (1991) reported that mercury selectively inactivates Fe-S center B of PSI in spinach thylakoids and Chun *et al.* (1994) reported a slight decrease in PSI electron-transport activities in mercury-treated barley chloroplasts.

Recovery of mercury effect by hydroxylamine

Table 1 shows Fp quenching and the decrease in Fr after 30 min of the mercury treatment. When 5 mM hydroxylamine was added as an electron donor instead of water, the decrase in fluorescence yield caused by mercury could be partially recovered (Fig. 4). This result indicates that one of the inhibitory sites of mercury in PS II is located at or near the oxygen-evolving complex. Similar results have been reported in spinach chloroplasts (Honeycutt and Krogmann, 1972), in *Dunaliella tertiolecta* (Samson and Popovic, 1990), and in isolated barley chloroplasts (Chun *et al.*, 1994).

Mercury effects under dark condition

Under dark condition the inhibitory effects of mercury was somewhat different from those under light

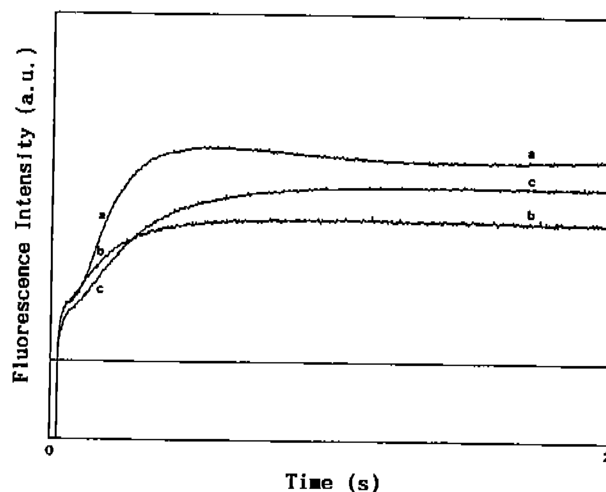

Fig. 4. The effect of hydroxylamine and $HgCl_2$ on fluorescence rise kinetics of barley leaf segments under light condition. (a) control, (b) 50 μM $HgCl_2$ treated for 60 min, (c) 50 μM $HgCl_2$ treated for 60 min and 5 mM NH_2OH was treated during the 10 min dark incubation period.

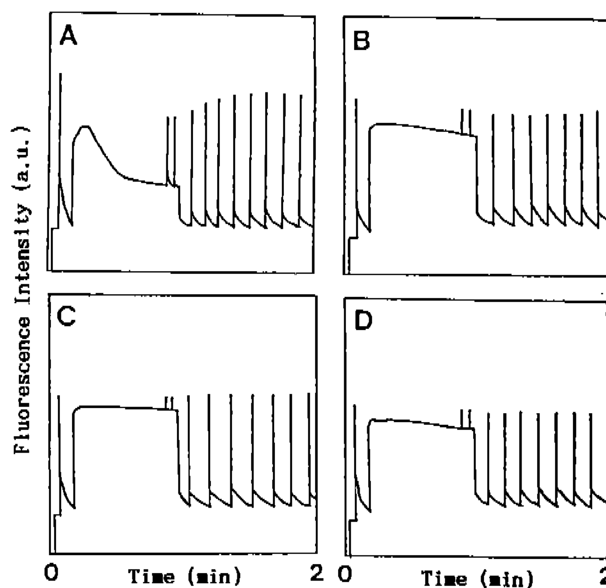

Fig. 5. Time-dependent changes in chlorophyll fluorescence induction transients of barley leaf segments treated with 50 μM $HgCl_2$ under dark condition. (A) 0 min, (B) 20 min, (C) 40 min, (D) 120 min after the treatment.

condition (Fig. 5). By the treatment of mercury for more than 20 min, the decrease in fluorescence intensity (P-S curve) was almost completely inhibited, which was also observed in the fast fluorescence rise curve (Fig. 6). Although these results are similar to the effect of diuron, the fluorescence intensity did

rea Science and Engineering Foundation (913-0401-016-2) and in part by a research grant from Korea Science and Engineering Foundation (91-0500-16). We thank Yeongnam Crop Experiment Station, Rural Development Administration for generous supply of barley seeds.

LITERATURE CITED

Cao, J. and Govindjee. 1990. Chlorophyll a fluorescence transient as an indicator of active and inactive photosystem II in thylakoid membranes. *Biochim. Biophys. Acta* **1015**: 180-188.

Chun, H.S., C.-H. Lee and C.B. Lee. 1994. Mercury-induced alterations of chlorophyll a fluorescence kinetics in isolated barley (*Hordeum vulgare* L. cv. ALBORI) chloroplasts. *J. Photosci.* **1**: 47-52.

De Filippis, L.F., R. Hampp and H. Ziegler. 1981. The effects of sublethal concentrations of zinc, cadmium and mercury on *Euglena*. II. Respiration, photosynthesis and photochemical activities. *Arch. Microbiol.* **128**: 407-411.

Demming-Adams, B. and W.W. Adams III. 1992. Photoprotection and other responses of plants to high light stress. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **43**: 599-626.

Demming-Adams, B., W.W. Adams III, U. Heber, S. Neimanis, K. Winter, A. Kruger, F. Czygan, W. Bilger and O. Björkman. 1990. Inhibition of zeaxanthin formation and of rapid changes in radiationless energy dissipation by dithiothreitol in spinach leaves and chloroplasts. *Plant Physiol.* **92**: 293-301.

Honeycutt, R.C. and D.W. Krogmann. 1972. Inhibition of chloroplast reactions with phenylmercuric acetate. *Plant Physiol.* **49**: 376-380.

Horton, P. and A. Hague. 1988. Studies on the induction of chlorophyll fluorescence in isolated barley protoplast. IV. Resolution of non-photochemical quenching. *Biochim. Biophys. Acta* **932**: 107-115.

Kim, M.G., S.K. Kang, C.-H. Lee, C.B. Lee and I.K. Chung. 1994. Characteristics of fluorescence induction kinetics from *Ulva pertusa* Kjellman and effects of mercury. *Korean J. Phycol.* **9**: 237-246.

Kimimura, M. and S. Katoh. 1972. Studies on electron transport associated with photosystem I. I. Functional site of plastocyanin; Inhibitory effects of $HgCl_2$ on electron transport and plastocyanin in chloroplasts. *Biochim. Biophys. Acta* **283**: 279-292.

Krause, G.H. and E. Weis. 1988. The photosynthetic apparatus and chlorophyll fluorescence. An introduction. *In* Application of chlorophyll fluorescence. H.K. Lichtenthaler (ed.). Kluwer Acad. Pub., Dordrecht, pp. 3-11.

Lee, C.-H., H. Chang, I.K. Chung, H.S. Chun, C.B. Lee, B.Y. Moon and J.A. Lee. 1992. Toxic effects and distribution of mercury in barley seedlings. *Korean Environ. Sci. Soc.* **1**: 13-21.

Moon, B.Y., H.S. Chun, C.-H. Lee and C.B. Lee. 1992. Mercury-specific effects on photosynthetic apparatus of barley chloroplasts compared with copper and zinc ions. *Korean Environ. Sci. Soc.* **1**: 1-11.

Murthy, S.D.S., N.G. Bukhov and P. Mohanty. 1990. Mercury-induced alterations of chlorophyll a fluorescence kinetics in cyanobacteria: Multiple effects of mercury on electron transport. *J. Photochem. Photobiol.* **6**: 373-380.

Oxborough, K. and P. Horton. 1988. A study of the regulation and function of energy-dependent quenching in pea chloroplasts. *Biochim. Biophys. Acta* **934**: 135-143.

Sakurai, H., K. Inoue, T. Fujii and P. Mathis. 1991. Effects of selective destruction of iron-sulfur center B on electron transfer and charge recombination in photosystem I. *Photosynth. Res.* **27**: 65-71.

Samson, G. and R. Popovic. 1990. Inhibitory effects of mercury on photosystem II photochemistry in *Dunaliella tertiolecta* under *in vivo* conditions. *J. Photochem. Photobiol. B: Biology.* **5**: 303-310.

Tripathy, B.C. and P. Mohanty. 1980. Zinc-inhibited electron transport of photosynthesis in isolated barley chloroplasts. *Plant Physiol.* **66**: 1174-1178.

(Received March 5, 1995)

水銀 處理에 의한 보리 잎 葉綠素 螢光의 光依存的 變化

李 春 煥* · 張 皓 植 · 河 奭 鳳 · 李 鎭 範[1]
釜山大學校 自然科學大學 分子生物學科, [1]東義大學校 自然科學大學 生物學科

적 요

보리 잎 절편에서 엽록소 a 형광유도 과정에 미치는 수은의 억제효과와 광의 존재 유무에 따른 작용의 차이를 조사하였다. 광조건하에서 50 μM $HgCl_2$의 처리로 Fm와 Fp가 감소하였으나 Fo는 크게 변하지 않았다. 그러나 암소에서 Fm와 Fp는 수은처리 2시간 후에도 크게 감소하지 않았으며, 수은 처리 후 20분부터 P(peak) 이후에 나타나는 Fv의 감소가 거의 완전히 억제되었다. 암소 및 광조건하에서 수은처리에 따른 빠른 형광증가곡선의 변이를 분석한 결과는 수은처리로 불활성 광계 II가 크게 증가하지 않으며, 수은의 작용부위가 Q_B 결합부위가 아님을 시사하였다. 광조건하에서 감소한 Fp는 50 μM NH_2OH의 처리로 일부 복구되었다. 이상의 결과로 보아 암소에서 수은의 주 작용부위는 광계 II 이후의 전자전달계에 있는 것으로 사료되며, 광조건하에서 수은의 주 작용부위는 암소에서의 작용부위와 아울러 광계 II 이전의 산소발생기구 부근에 있는 것으로 사료된다. 또한 수은은 광의 존재유무에 관계없이 처리 후 20분 이내에 에너지의존 형광소멸계수(qE)를 거의 완전히 억제시켰으며, qE의 감소는 틸라코이드막 내외의 수소이온농도 구배형성의 억제에 의한 것으로 사료된다.

주요어: 보리 잎, 엽록소 형광, 수은 독성, 광

*교신저자: Fax (051) 513-9258