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EFFECTS OF LEAD CHLORIDE ON CHLOROPLAST REACTIONS

KEY WORDS: Lead, Chlorophyll, Chloroplasts, Hill reaction, Fluorescence, Maize, Light reactions, Conformational changes.

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ABSTRACT

In maize chloroplasts, lead chloride causes inhibition or stimulation of photosystem II activity depending on the pH of the reaction media. At pH 7.8, an inhibition of 16 to 39% with 0.75mM to 9mM $PbCl_2$ is observed. At pH 6.5, however, a stimulatory effect of 20% is observed with 9mM $PbCl_2$; this effect is not due to the uncoupling of photophosphorylation, or to the ability of $PbCl_2$ to donate electrons to photosystem II. On the basis of other data, reported here, a common site of inhibition between water and Z (the primary electron donor of photosystem II) for lead treatment and Tris-washing (0.8M, pH 8.0) of chloroplasts is suggested.

Furthermore, lead salts (0.9mM) at both pH 6.5 and 7.8 induced about 20% inhibition of the variable chlorophyll *a* fluorescence even in the presence of 10 μM 3-(3,4 dichlorophenyl)1,1 dimethylurea. Room temperature and 77°K chlorophyll *a* emission spectra show an increase in the ratio of pigment system I to II fluorescence upon the addition of lead salts at both pHs--an opposite effect to that induced by salts of other divalent ions (e.g. Mg^{2+}). Lead salts (0.9mM to 9mM) cause 10 to

20% increase in the 540 nm absorbance change, reflecting a decrease in spaces between thylakoids, over that of untreated chloroplasts. Lead-induced conformational changes in thylakoid membranes of chloroplasts, leading to changes in energy distribution between pigment systems I and II, is suggested to explain the above results.

INTRODUCTION

Lead concentration has been increasing in the atmosphere and soil mainly due to its liberation from exhausts of motor vehicles and its use in industry[1]. Lead accumulation in soils and plants near highways has been documented[2-4]. Plants have been shown to take up and transport lead in their tissues[5-7]. Thus, lead may be expected to affect different physiological and enzymatic processes of plants. Inhibitory effect of lead on enzymes in animals and humans has been reported[8,9]. However, there are very few reports on the effect of lead on plants inspite of the importance of photosynthesis for life (see review in ref. 10). Miles et al.[11] showed that 2.4mM lead chloride caused a very high (90%) inhibition of chloroplast reactions in spinach and tomato; this inhibitory action of lead salts was specific to pigment system II (PSII)[12] activity. In this paper it is shown that this inhibitory effect on PSII activity of maize chloroplasts depends on pH; at pH 7.8 to 8.0, a maximum inhibition of 40% and at pH 6.5 to 6.7, a stimulation of 20% were observed. Comparison of data on the effect of lead salts and of tris-washing (0.8M, pH 8.0) on chloroplasts suggests that both treatments have a common site of action between water and Z (the primary electron donor of PSII). The conclusion of Miles et al.[11] that a site of action for lead salts is on the "oxidizing" side of PSII is, therefore, confirmed.

In this paper, we also present new data on the effect of lead treatment of chloroplasts on chlorophyll a (Chl a) fluorescence emission spectra at 77 and 298°K and on the 540nm absorbance change. Results

from these experiments, combined with those on the time course of Chl a fluorescence in the presence of 3-(3,4 dichlorophenyl) 1,1 dimethylurea (DCMU), suggest that lead chloride controls the transfer of excitation energy from PSII to PSI in a fashion opposite to that induced by other divalent cations like Mg^{2+}, Ca^{2+}, etc.[13-16], but similar to the effect of low (2mM) concentrations of monovalent cations[17].

MATERIALS AND METHODS

Mesophyll (granal) chloroplasts were isolated from 3-4 week-old maize plants. Leaves were homogenized in an Omni mixer with a medium containing 0.05M Tricine-NaOH buffer (pH 7.8), 0.33M sorbitol, 0.6% Carbowax, 0.01M NaCl, 0.001M $MgCl_2$, and 0.1% bovine serum albumin (BSA). The homogenate, filtered through eight layers of cheesecloth, was centrifuged at 1000 X g for 7 minutes. The sedimented chloroplasts were washed once with the homogenizing medium and centrifuged again at 1000 X g for 7 minutes. Finally, chloroplasts were suspended in a medium containing 0.05M Tricine-NaOH buffer (pH 7.8), 0.33M sorbitol, 0.01M NaCl, and 0.001M $MgCl_2$. (This method yielded partially "coupled" chloroplast preparations.) Chlorophyll concentration was determined according to Bruinsma[18].

Tris-washed chloroplasts were prepared according to the method of Yamashita and Butler[19]. Following the Tris-washing (0.8M, pH 8.0), chloroplasts were washed twice with a medium containing 0.05M Tricine-NaOH buffer (pH 8.0), 0.33M sorbitol, 0.1 N NaCl and 0.001M $MgCl_2$. These washings were necessary to remove traces of Tris from the sample since lead salts were found to complex with Tris (from Calbiochem, Harleco, and Sigma Chemical Co.) and form a precipitate.

Hill activity was determined by measuring the reduction of 2,6 dichlorophenol indophenol (DCPIP) spectrophotometrically as described by

Stemler and Govindjee[20], or by measuring oxygen evolution with a Clark electrode connected to a Yellow Springs oxygen monitor (Model 53).

Chlorophyll a fluorescence was measured with a spectrofluorometer described elsewhere[21]. The time course of Chl a fluorescence was measured as described by Munday and Govindjee[22], and emission spectra as by Cho et al.[23] The emission spectra were corrected for the spectral variation of the monochromator and the photomultiplier (EMI 9558B). Other details are given in the legends of figures 1-3.

The 540nm absorbance change was measured with the difference (absorption) spectrophotometer of Sybesma and Fowler[24]. The measuring monochromator was set at 540 nm (half band width, 6.6nm) and a Corning C.S. 4-91 filter was placed before the photomultiplier to eliminate the red actinic beam (650nm interference filter; half band width, 22nm; intensity 1.4×10^4 ergs·cm^{-2} sec^{-1}). Light intensity was measured with a Yellow Springs radiometer (model 63) or with a Bi eppley thermopile (#6161).

When $PbCl_2$ was added to samples, the pH was adjusted with NaOH before the addition of chloroplasts; thereafter samples were incubated for ten minutes before taking measurements.

RESULTS AND DISCUSSION

1. EFFECT OF LEAD ON ELECTRON TRANSPORT IN ISOLATED MAIZE CHLOROPLASTS

At pH 6.5, no significant effect of 0.75mM to 6.0mM $PbCl_2$ was found on the saturating rate of DCPIP photoreduction (Table 1). However, 9mM $PbCl_2$ caused a 20% stimulation. On the other hand, at pH 7.8, 0.75mM to 9mM lead chloride caused a decrease in the rate of DCPIP photoreduction: 16% at 0.75mM, 22% at 6mM, and 39% at 9mM $PbCl_2$. Separate controls containing the same concentrations of NaCl as those of lead chloride were assayed similarly to rule out the role of chloride in in-

TABLE 1

Effect of Various Concentration of $PbCl_2$ on Pigment System II Activity in Strong Light* at pH 6.5 and 7.8

Treatment	µmoles DCPIP reduced/mg Chl/hr (average of nine experiments)	
	pH 6.5†	pH 7.8†
**None	50.6	31.0
+0.5mM $PbCl_2$	50.6	31.0
+0.75mM $PbCl_2$	50.5 ± 2.0	26.0 ± 1.5
+1mM $PbCl_2$	51.5 ± 2.0	25.0 ± 2.0
+6mM $PbCl_2$	55.9 ± 4.5	24.0 ± 1.3
+9mM $PbCl_2$	60.2 ± 1.7	19.0 ± 1.5

*Light intensity, 2 x 10^6 ergs cm^{-2} sec^{-1}; **One ml samples containing 5-8µgm Chl were suspended in a reaction mixture containing Tracine-NaOH buffer (0.05M, pH 6.5 or 7.8), 0.01M NaCl, and DCPIP, 50µM; †after addition of $PbCl_2$, pH was adjusted with NaOH to 6.5 or 7.8.

ducing these effects. No effect of NaCl on DCPIP photoreduction was observed. Our results at pH 7.8 confirm the finding of Miles et al.[11] on the effect of lead salts on PSII activity. However there are differences in the degree of inhibition between our results on maize and that of Miles et al. on spinach and tomato, the inhibition being lower in maize. To check if the stimulatory effect of $PbCl_2$, at pH 6.5, was due to the uncoupling of phosphorylation, we repeated the above measurements at both pHs in the presence of 50mM methylamine hydrochloride (an uncoupler). Similar results to that reported in table 1 were obtained indicating that the stimulatory effect may not be due to uncoupling.

The stimulatory effect of $PbCl_2$ (at pH 6.5) on the saturating rates of PSII activity could be caused by the ability of lead salts to donate

electrons to system II. To test this hypothesis, chloroplasts which have lost their ability to evolve oxygen by tris-washing (0.8M, pH 8.0[19]) were used. This treatment blocks electron flow from water to oxidized Z (Z being the primary electron donor of PSII); perhaps the rate-limiting step is eliminated. If lead salt is added to these chloroplasts and resumption of PS II activity is accomplished (at pH 6.5), one may conclude that lead is capable of donating electrons to oxidized Z (made in light reaction of system II) (cf. ref 25). Tris inhibition has been shown[26] to be pH dependent. This was confirmed (Table 2). When the reaction was run at pH 6.5, the activity of Tris-washed chloroplasts was inhibited by only

TABLE 2

Effect of $PbCl_2$ on DCPIP Reduction in Tris-Washed Chloroplasts at pH 6.5 and 7.8.

Sample	Additions	Rates (μmoles DCPIP reduced/mg Chl/hr) (average of five experiments)	
		pH 6.5	pH 7.8
Chloroplasts	none	30.0	27.0
"	+9mM $PbCl_2$	36 ± 1.0	16.0 ± 1.0
"	+9mM $PbCl_2$ + 0.5mM DPC	--	32.0 ± 1.3
Tris-washed chloroplasts	none	27 ± 0.5	3.5 ± 0.5
"	+0.5mM DPC	68 ± 5.0	22.4 ± 5.0
"	+1mM $PbCl_2$	25 ± 0.8	3.8 ± 0.2
"	+6mM $PbCl_2$	28 ± 2.0	3.1 ± 0.3
"	+9mM $PbCl_2$	27 ± 1.5	3.2 ± 0.4

One ml samples, containing 5-8μgm Chl, were suspended in Tricine-NaOH buffer (0.05M, pH 6.5 or 7.8); NaCl, 0.01M; DCPIP, 50μM.

10% compared to 87% inhibition at pH 7.8. When 50 mM diphenylcarbazide (DPC)--which donates electrons to PS II[27]--was added to Tris-washed chloroplasts at pH 7.8, 88% of the control activity was restored. At pH 6.5, similar addition of DPC leads to a doubling of the DCPIP photo-reduction (stimulation). This is due to the fact that the optimum pH for DPC to donate electrons to PS II is around pH 6.7[27]. When 1, 6 or 9 mM $PbCl_2$ was added to Tris-washed chloroplasts no significant change in activity was observed, thus ruling out the possibility of lead salt acting as an electron donor to PS II. Furthermore, since lead chloride did not cause further decrease in activity of Tris-washed chloroplasts, the site which is inhibited by lead treatment at pH 7.8 must not be beyond that of Tris inhibition. Diphenylcarbazide was shown to relieve inhibition of PS II by lead treatment in chloroplasts. This confirms the suggestion[11] that $PbCl_2$ inhibits PS II between water and Z (the primary electron donor of PS II) as DPC is known to donate electrons directly to Z^+.

To check whether $PbCl_2$ can donate electrons before the site of inhibition by Tris washing, we measured both oxygen evolution and DCPIP photo-reduction in chloroplasts treated with 9 or 12 mM $PbCl_2$ at pH 6.5. If lead chloride donates electrons, we expect a decline in oxygen evolution rate at these concentrations where DCPIP photoreduction was stimulated. Our results did not show any decrease or significant stimulation of oxygen evolution.

2. EFFECT OF $PbCl_2$ ON CHLOROPHYLL A FLUORESCENCE: TRANSIENTS AND EMISSION SPECTRA AT 298°K AND 77°K

Upon illumination with bright light, dark adapted chloroplasts show a rise in chlorophyll a fluorescence yield from an initial level (Fo; reflecting emission from "bulk" pigments) to a maximum level ($F\infty$)[28].

This fluorescence rise reflects the photoreduction of Q (the primary stable electron acceptor of PS II, and the quencher of chlorophyll fluorescence). An accumulation of reduced Q results in closure of the traps and causes high fluorescence yield[29] while an accumulation of oxidized Q, which results either from accelreated rate of electron transport or inhibition of PS II on the water side of Z causes low fluorescence yield. From studies on isolated chloroplasts[13-17] and on algae[30-34] it is clear that not all fluorescence changes are due to changes in the redox state of Q since some changes occur even in the presence of DCMU that blocks electron flow from Q to the next electron carrier in photosynthesis. It has been suggested that these fluorescence changes are controlled by structural alteration of the pigment bed. Thus, studies of fluorescence changes induced by lead in the presence of DCMU were used here as an indicator of conformational changes in chloroplasts; the same effects could be present even in the absence of DCMU.

The presence of 0.9mM to 9mM $PbCl_2$ at both pH 6.5 and 7.8 did not effect the F_o level (data not shown). At pH 6.5 (fig. 1, top) and pH 7.8 (fig. 1, bottom), in the absence of $PbCl_2$ or DCMU, chlorophyll *a* fluorescence rises biphasically from F_o to F_∞ level. However, in the presence of 0.9 mM $PbCl_2$ the level F_∞ is decreased; the variable fluorescence (F_∞ - F_o) is decreased by 20 and 17% compared to that in untreated samples at pH 6.5 and pH 7.8 respectively. The decrease in fluorescence level caused by $PbCl_2$ in the presence of DCMU must not be due to decrease in the rate of electron transfer because DCMU has already prevented the reoxidation of QH to Q[29]. However, this decrease could be due to structural changes in thylakoids such that more excitation energy is spilled over from strongly fluorescent PS II to weakly fluorescent PS I.

To further confirm such an effect, Chl *a* emission spectra at 77°K and 298°K were measured. At 77°K, emission spectra of normal chloroplasts

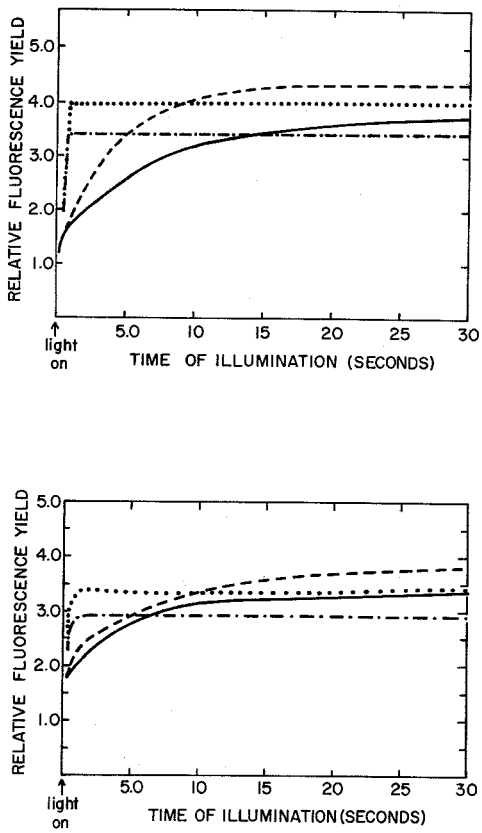

FIG. 1

Effect of $PbCl_2$ and DCMU added separately or together on the time course of Chl a fluorescence in maize chloroplast suspensions at pH 6.5 (top) and at pH 7.8 (bottom). Fluorescence was measured at 685nm (half bandwidth, 6.6nm); - - -, normal chloroplasts without any addition; ———, 0.9mM PbCl; . . . ., 10μM DCMU; -.-., 10μM DCMU and 0.9mM $PbCl_2$; F_o level (between 1.0 and 1.5) not recorded in these plots. Excitation, broad band blue light (C.S. 4-72 and C.S. 3-73) intensity, $6x10^4$ ergs·cm^{-2} sec^{-1}; three ml of samples, containing 15μg Chl ml^{-1}, were suspended in 0.05M Tricine-NaOH buffer.

show a three banded structure with peaks at 735nm (F735), 685nm (F685) and 696nm (F696). F735 is attributed to emission mostly from pigments of system I, while F685 and F696 are mostly emitted from PS II. (For further discussion, see Govindjee et al.[34]). Thus a comparison of the ratios

of F735/F685 and F735/F696 at 77°K of lead-treated and untreated samples could reflect the distribution of absorbed quanta between the two photosystems. At pH 6.5 (fig. 2, top) and in the presence of 0.9mM $PbCl_2$, the ratio F735/F685 is about 2.3 compared to 1.7 in untreated chloroplasts. The ratio F735/F696 is about 3.0 in lead-treated as compared to 2.0 in normal chloroplasts. Thus it is clear that in presence of 0.9mM $PbCl_2$ there is a relative decrease in Chl a fluorescence at 685nm (26% of control) and at 696nm (50% of control) compared to that emitted at 735nm. At pH 7.8 (fig. 2, bottom) 0.9mM $PbCl_2$ caused similar effects although to a lesser extent than at pH 6.5: the ratio F735/F696 was 2.2 compared to 2.0 in untreated chloroplasts. (Note, however, that F750 on spectra normalized at 685nm, increased by 50% upon lead addition.) The ratio F735/F696 was 3.2 in lead-treated and about 2.2 in normal chloroplast suspensions. The emission spectra at both pHs clearly show lead-induced increases in ratio of fluorescence intensity from long wavelength forms of Chl a associated with PS I, to that from short wavelength forms of Chl a, suggesting a spillover of excitation energy from PS II to PS I. This is, perhaps, caused by conformational changes induced by lead salts. Other divalent cations (Mn^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+} and Ni^{2+}) have been shown[35] to induce contractions in chloroplasts and subchloroplast particles similar to those obtained by lowering the pH.

No effect of 0.9mM to 9mM $PbCl_2$ was observed on the absorption spectra of chloroplasts. Thus, lead-induced changes in emission spectra of Chl a fluorescence could not be attributed to lead chloride causing changes in pigment concentration. These fluorescence changes appear to be due to lead-induced conformation changes which affect distribution of absorbed quanta between pigment system I and II.

The above mentioned effect of lead chloride on Chl a fluorescence was also confirmed by measurements of emission spectra at room temperature.

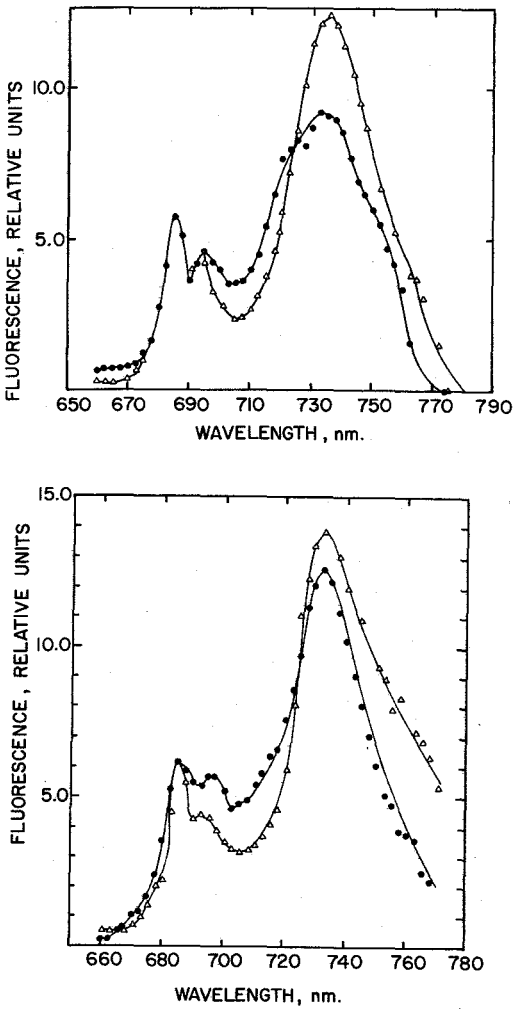

FIG. 2

Effect of P_bCl_2 on the emission spectra of Chl a fluorescence of maize chloroplasts at 77°K at pH 6.5 (top) and pH 7.8 (bottom). Emission spectra were measured with an excitation at 436nm (half bandwidth, 9.9nm); a Corning red (C.S. 2-73) filter was placed before the measuring monochromator to eliminate stray exciting light; -●-, normal maize chloroplasts without any addition; -Δ-, with 0.9mM $PbCl_2$; one ml sample, containing 3μg Chl, was suspended in 0.05M Tricine-NaOH buffer. Curves were arbitrarily adjusted to read the same fluorescence intensity at 685nm. Samples were in complete darkness while being quickly cooled at 77°K.

Normal chloroplasts at room temperature show a main fluorescence band with a peak at 683nm and a broad shoulder at longer wavelengths. Fluorescence emitted at wavelengths between 710nm (F710) and 730nm (F730) has been attributed to emission by pigments mainly of PS I while F685 is emitted mainly from PS II[36]. At pH 6.5 (fig. 3, top) and at pH 7.8 (fig. 3, bottom), 0.9mM $PbCl_2$ caused 35% to 63% increase in fluorescence levels at 720nm relative to those at 683nm. Qualitatively, these results agree with those obtained at 77°K which suggest that lead chloride causes an increase in fluorescence intensity emitted from PS I compared to that emitted from PS II.

3. EFFECT OF $PbCl_2$ ON 540nm ABSORPTION CHANGE

To test whether a structural change is induced by treatment of chloroplasts with $PbCl_2$, we measured 540nm absorbance change. Our results at pH 7.8 showed that 0.9mM to 9.0mM lead chloride or nitrate caused 10% to 20% increase in absorbance over that of control. Light or dark incubation of chloroplasts with lead did not affect the results.

In general it has been shown that absorbancy change at 540 nm is inversely proportional to particle volume. Thus an increase in absorbance indicates that lead caused the shrinkage of chloroplasts. Light induced transmission changes have been correlated more closely with changes of spaces between thylakoids than with changes in their thickness[37]. Thus, lead-induced shrinkage of chloroplasts could indicate tighter packing of thylakoid membranes. Inspite of lack of data on 90° scattering changes that reflect more closely changes in thickness of thylakoids (i.e., distance between PSI and PSII), we could postulate that tighter packing of thylakoids in presence of lead salts might also lead to closer distance between PSI and PSII. This would cause an increase in spill over of energy from pigment system II to I.

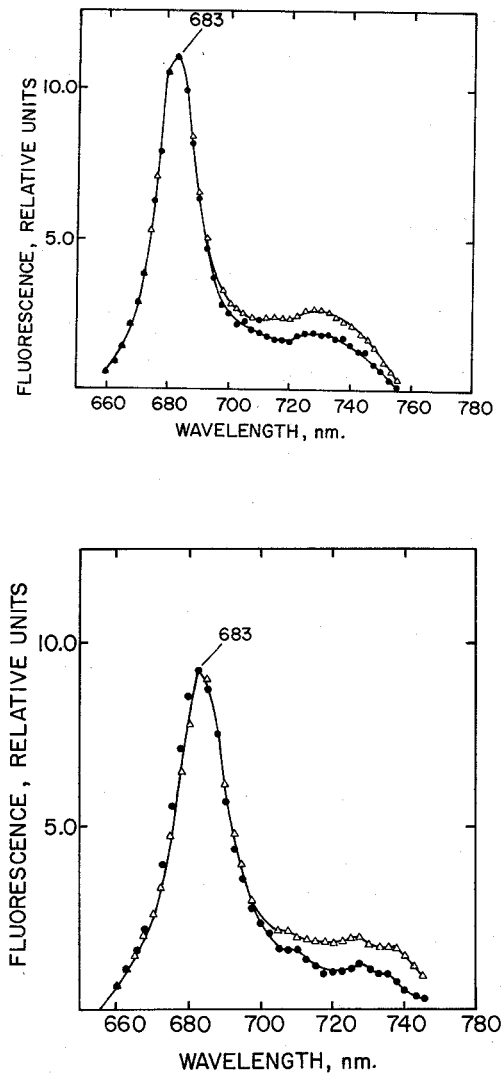

FIG. 3

Effect of $PbCl_2$ on the emission spectra of Chl a fluorescence of maize chloroplasts at room temperature (298°K) at pH 6.5 (top) and pH 7.8 (bottom). Emission spectra were measured with an excitation at 436nm (half bandwidth, 13.2nm); -●-, normal maize chloroplasts without any addition, -Δ-, with 0.9mM $PbCl_2$. Three ml of samples, containing 9µg Chl ml^{-1}, were suspended in 0.05M Tricine-NaOH buffer. Curves were arbitrarily adjusted to read the same fluorescence intensity at 683nm.

CONCLUDING REMARKS

Results of our study on the effects of lead chloride on isolated maize chloroplasts allow us to make several suggestions regarding the mode of action of lead chloride in chloroplasts:

First, lead chloride causes a 20% decrease in variable Chl a fluorescence yield in both the presence and absence of DCMU (fig. 1). This, together with an increase in ratios of fluorescence emitted by pigment system I to that emitted by pigment system II at 77°K (fig. 2) and 298°K (fig. 3) and 10% to 20% increase in absorbance at 540nm, is taken to indicate that lead chloride causes conformational changes of thylakoid membranes of chloroplasts leading to a greater spill over of excitation energy from PS II to PS I; this is in contrast to the decreased spill over upon treatment with magnesium chloride and other divalent salts[11-14]. We do not yet know why Pb^{2+} and Mg^{2+} (both of which are divalent ions) cause opposite effects. The answer may lie in the size of these ions, and the site of their binding.

Second, the inhibitory effect of lead chloride on PS II reactions appears to be similar to inhibition of oxygen evolution in isolated chloroplasts by Tris-washing (0.8M pH 8.0; ref. 19). These two treatments have the same pH dependence[26] to be effective as inhibitors of PS II activity in chloroplasts (tables 1 and 2). At alkaline pH (7.8 to 8.0), both treatments were effective in inhibiting oxygen evolution while no such effects were observed at acidic pH (table 2). Furthermore, addition of an electron donor such as diphenylcarbazide (which is known to donate electrons to PS II) to chloroplasts treated with either lead chloride or 0.8M Tris (table 2), caused a restoration of photosynthetic electron transport activity. Also addition of lead chloride to Tris-washed chloroplasts did not cause further decrease in PS II activity implying that lead does not inhibit beyond the site of Tris inhibition. Thus one

can suggest that both lead chloride and Tris-washing of chloroplasts are probably affecting a site between water and Z (the primary electron donor of PS II).

Third, the concentrations at which lead chloride inhibits PS II activity in isolated chloroplasts are quite variable from one group of plants (C_4 plants, such as maize, this paper) to another (C_3 plants, such as spinach and tomato[11]). In maize a maximum of 40% inhibition of PS II activity is observed (pH 7.8; 9mM $PbCl_2$, this paper) in comparison to 95% inhibition at 2.4mM $PbCl_2$ in spinach and tomato chloroplasts[11].

Fourth, the slight stimulatory effect of 9mM lead chloride at pH 6.5 is not clearly understood at this point since our results showed that neither uncoupling of photophosphorylation nor ability of lead chloride to donate electrons could explain it. Heavy metals such as lead, cadmium, and mercury can replace native metals on active sites of several enzymes and cause activation rather than inhibition of enzymatic activity[10]. However, it remains to be tested whether the stimulation of PSII activity at pH 6.5 to 6.7 is caused by such action of lead ion on photosynthetic enzymes involved in oxygen evolution.

Finally, we note that Daniel Wong (in our laboratory) has observed inhibitory effects of lead salts on system I reactions (oxidation of reaction center P700). All data taken together suggest a multiple and complex effects of lead ions on chloroplast reactions.

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REFERENCES

1. T. J. Chow and J. L. Earl, Science 169, 577 (1970).

2. H. L. Motto, K. H. Daines, D. M. Chilko and C. K. Motto, Environ. Sci. Technol. 4, 231 (1970).

3. M. Singer and L. Hanson, Soil Sci. Amer. Proc. 33, 152 (1969).

4. J. M. Hopkins, R. H. Wilson and B. N. Smith, Naturwissenschaften 59, 421 (1972).

5. H. Cannon and J. Bowles, Science 137, 765 (1962).

6. J. V. Lagerwerff, Soil Sci. 111, 129 (1971).

7. E. A. Schuck and J. K. Locke, Environ. Sci. 4, 324 (1970).

8. J. J. Chilsom Jr., Sci. Amer. 224, 15 (1971).

9. R. M. Sauer, B. C. Zook and F. M. Garner, Science 169, 1091 (1970).

10. B. L. Vallee and D. D. Ulmer, Ann. Rev. Biochem. 41, 91 (1972).

11. C. D. Miles, J. R. Brandle, D. J. Daniel, O. Chu-Der, P. D. Shnare and D. J. Uhlik, Plant Physiol. 49, 820 (1972).

12. Abbreviations: Chl, chlorophyll; DCPIP, 2,6-Dichlorophenol indophenol; DCMU, 3-(3,4 dichlorophenyl)-1,1 dimethylurea; Q, Quencher of chlorophyll fluorescence, also primary electron acceptor for system II; DPC, diphenylcarbazide; PSII, photosystem II; $PbCl_2$, lead chloride.

13. P. H. Homann, Plant Physiol. 44, 932 (1969).

14. N. Murata, Biochim. Biophys. Acta 189, 171 (1969).

15. N. Murata, H. Tashiro and A. Takamiya, Biochim. Biophys. Acta 197, 250 (1970).

16. P. Mohanty, B. Z. Braun and Govindjee, Biochim. Biophys. Acta 292, 459 (1973).

17. E. Gross, Biophys. Soc. Abst. 13, 64a (1973).

18. J. Bruinsma, Photochem. Photobiol. 2, 241 (1963).

19. T. Yamashita and W. L. Butler, Plant Physiol. 43, 1978 (1968).

20. A. Stemler and Govindjee, Plant Physiol. 52, 119 (1973).

21. C. Shimony, J. Spencer and Govindjee, Photosynthetica 1, 113 (1967).

22. J. C. Munday Jr. and Govindjee, Biophys. J. 9, 1 (1969).

23. F. Cho, J. Spencer and Govindjee, Biochim. Biophys. Acta 126, 174 (1966).

24. C. Sybesma and C. F. Fowler, Proc. Natl. Acad. Sci. U.S. 61, 1343 (1968).

25. G. Ben-Hayyim and M. Avron, Biochim. Biophys. Acta 205, 86 (1970).

26. N. Ikehara and E. G. Uribe, FEBS (Fed. Eur. Biochim. Soc.) Letters 9, 321 (1970).

27. L. P. Vernon and E. R. Shaw, Plant Physiol. 44, 1645 (1969).

28. S. Malkin and B. Kok, Biochim. Biophys. Acta 126, 413 (1966).

29. L.N.M. Duysens and H. E. Sweers, In Studies on Microalgae and Photosynthetic Bacteria p. 353 (1963), University of Tokyo Press, Tokyo.

30. T. T. Bannister and G. Rice, Biochim. Biophys. Acta 162, 555 (1968).

31. C. Bonaventura and J. Myers, Biochim. Biophys. Acta 189, 366 (1969).

32. G. Papageorgiou and Govindjee, Biophys. J. 8, 1299 (1968).

33. P. Mohanty and Govindjee, Biochim. Biophys. Acta 305, 95 (1973).

34. Govindjee, G. Papageorgiou and E. Rabinowitch, In Practical Fluorescence Theory, Methods and Techniques. p. 543 (1973), Marcel Dekker, Inc. N. Y.

35. R. A. Dilley and A. Rothstein, Biochim. Biophys. Acta 135, 427 (1967).

36. Govindjee and L. Yang, J. Gen. Physiol. 49, 763 (1966).

37. S. Murakami and L. Packer, J. Cell Biol. 47, 332 (1970).

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