ENVIRONMENTAL LETTERS, 6(1), 1-12 (1974)

EFFECTS OF CADMIUM NITRATE ON SPECTRAL CHARACTERISTICS AND LIGHT REACTIONS OF CHLOROPLASTS

KEY WORDS: Cadmium, Chlorophyll, Chloroplasts, Absorption, Fluorescence, Maize, Light reactions

Maarib Bakri Bazzaz and Govindjee
Institute for Environmental Studies and
Department of Botany, University of Illinois, Urbana, Illinois 61801

ABSTRACT

In mesophyll chloroplasts from maize, photosystem (PS) II activities (2,6 dichlorophenol indophenol photoreduction and the variable fluorescence yield of chlorophyll (Chl) a) are inhibited strongly by 0.5 mM cadmium, supplied as $Cd(NO_3)_2$. However, diphenylcarbazide restores these activities suggesting that cadmium inhibits PSII activity at the oxygen evolving site between water and Y (the first secondary electron donor of PSII). PSI activity (light induced P700 oxidation) is insensitive to cadmium. Furthermore, absorption and fluorescence studies showed that cadmium causes a 20% to 30% decrease in the total chlorophylls, 15% to 25% decrease in the ratio of Chl a/Chl b and a 20% to 25% decrease in the ratio of the short to the long wavelength forms of Chl a.

INTRODUCTION

The release of Cd[1] from smelters, combustion of fuels, degradation of tires, phosphate fertilizers and pesticides makes it a major environmental contaminant. Human uptake, absorption, retention and subsequent toxicity by Cd from air, water and food are known[2]. The Cd aerosal settles down with dust and precipitation (see Kitamura, cited in ref. 2),

contaminating soils and plants; it accumulates in plants and soils near highways[3] and smelters[4] and is taken up by plants grown in Cd rich soils[5,6,7]. Cadmium has been shown to inhibit growth in several species of higher plants[8] and Chlorella[9]. Plants grown in hypotonic media accumulate Cd in their roots and leaves[10]; detached plant leaves immersed in 2 ppm Cd salts show a reduced rate of gas exchange in light as measured with an infra red gas analyzer (F. Bazzaz, 1973, submitted for publication), probably attributable to increased stomatal resistance. In order to clearly answer the question whether photosynthesis per se is inhibited by Cd, and if so, what are the site(s) of its action, we studied the effect of Cd $(NO_3)_2$ on photosynthetic reactions in isolated chloroplasts from maize.

Our results show that indeed Cd^{2+} is a potent inhibitor of photosynthesis in chloroplasts; 0.5mM $Cd(NO_3)_2$ caused a complete inhibition of pigment system (PS)II reactions, in addition to changes in concentration and composition of pigments.

MATERIALS AND METHODS

Mesophyll chloroplasts were isolated from maize leaves as described previously[11]. However, 0.05M HEPES buffer (pH, 7.6) was substituted for Tris-HCl buffer in the isolation medium, and 0.1% BSA was added to the homogenizing but not to the suspension medium. The concentration of chlorophylls was determined according to Arnon[12].

The reduction of DCPIP was measured spectrophotometrically as described by Stemler and Govindjee[13]. The effect of KNO_3 or $Ca(NO_3)_2$ on DCPIP photoreduction was tested to check whether the effect of cadmium was specific or simply due to osmotic effect of salts on chloroplasts. Other details are given in the legend of figure 1.

Absorption spectra were measured with a Bausch and Lomb spectronic 505 spectrophotometer equipped with an integrating sphere. Chlorophyll

emission spectra were measured with a spectrofluorometer described in ref. 14, the time course of Chl a fluorescence as in ref. 15, and the excitation spectra of Chl a fluorescence as in ref. 16. The excitation spectra were corrected for the spectral variation of the monochromator; other details are given in the legend of figure 4.

Light induced absorbance change for the reaction center of pigment system I (P700) was measured by a split-beam difference spectrophotometer[17]. The 703nm measuring beam, obtained through a monochromator, had a band width of 9.0nm. The photomultiplier (Amperex 56 CVP) was protected by a 703nm interference filter (band width, 12.5nm). Samples were illuminated with 729nm (band width, 9.0nm) light (incident intensity, 2×10^4 ergs cm^{-2} sec^{-1}).

RESULTS AND DISCUSSION

I. INHIBITION OF SYSTEM II BEFORE DIPHENYLCARBAZIDE DONATION SITE

A. Dichlorophenol Indophenol Photoreduction

Figure 1A shows the effects of different concentrations of $Cd(NO_3)_2$ KNO_3 and $Ca(NO_3)_2$ on DCPIP photoreduction in maize chloroplasts. 0.5mM $Cd(NO_3)_2$ inhibited this activity by 85%, whereas the other two salts, at the same concentration, decreased the same activity by only 10%. A short incubation period of chloroplasts with $Cd(NO_3)_2$ was necessary to obtain maximum inhibition (Fig. 1B); at 0.25mM $Cd(NO_3)_2$, 50% inhibition was observed after one minute, and a 70% (maximum) inhibition was attained after 15 minutes of incubation.

The rate of DCPIP photoreduction in untreated chloroplasts was 55 µmoles mg^{-1} Chl hr^{-1}. When chloroplasts were incubated for 10 minutes with 0.6mM $Cd(NO_3)_2$, this rate was reduced to 7µmoles mg^{-1} Chl hr^{-1}. However, when both $Cd(NO_3)_2$ and 0.15mM DPC were added together, the rate was 50µmoles mg^{-1} Chl hr^{-1} showing the absence of inhibition by Cd-

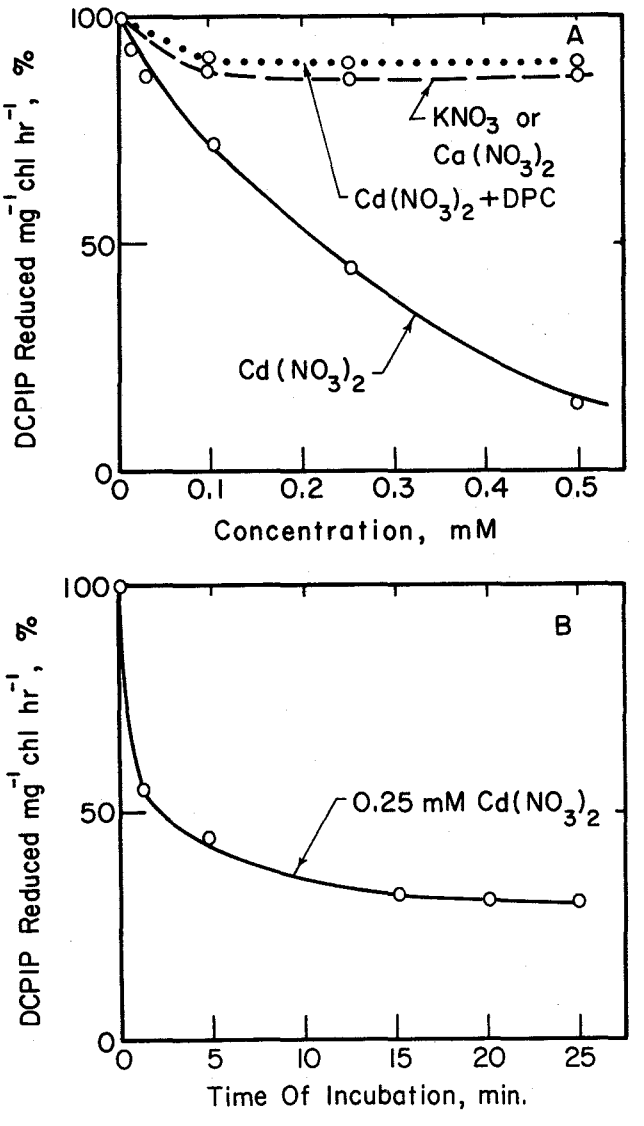

FIG. 1

(A): Effects of $Cd(NO_3)_2$ (———), KNO_3 or $Ca(NO_3)_2$ (----) and $Cd(NO_3)_2$ + DPC (.....) on saturated rates of DCPIP photoreduction in maize chloroplasts. Reaction mixture contained: 0.05M HEPES buffer (pH 7.6), $5x10^{-5}$M DCPIP, 0.01M NaCl and 2.5 µg Chl. Chloroplasts were incubated for ten minutes in dark before measurements; other details in the text. 100% of control corresponds to 54 µmoles DCPIP reduced mg^{-1} Chl hr^{-1}. Each point represents the average of five measurements.
(B): Effects of 0.25mM $Cd(NO_3)_2$ on the rates of DCPIP photoreduction in chloroplasts at different incubation periods.

$(NO_3)_2$ for electron flow from DPC to DCPIP. 10μM 3-(3,4 dichlorophenyl)-1,1 dimethyl urea (DCMU) caused complete inhibition of this reaction. The above results were confirmed in five experiments. We conclude that the inhibition of PSII activity by $Cd(NO_3)_2$ treatment is due to the inhibition of a reaction before the site of electron donation by DPC. [The latter is known to feed electrons to chloroplasts whose oxygen evolving ability is impaired by Tris washing (0.8M, pH 8.0)[18], the donation site being close to the first secondary electron donor (Y) of PSII, the primary electron donor being defined as the reaction center chlorophyll of PSII, see Butler,[19] .]

B. Chlorophyll a Fluorescence Induction

When dark adapted chloroplasts are illuminated with strong blue light, the chlorophyll a fluorescence level rises instantly to F_0 (which reflects fluorescence intensity emitted from bulk pigments of PSII and PSI); thereafter it rises biphasically to a final level F_∞[20]. The fluorescence rise from F_0 to F_∞ reflects the reduction of the "primary" electron acceptor of PSII (Q) to QH under conditions when the primary electron donor (Chl a_{II}, P680) is restored to its reduced state[19]. If cadmium causes inhibition of PSII at the water oxidation level, the reduction of Q to QH would be impaired due to lack of electron (hydrogen) supply, and a reduced level of variable to constant fluorescence would be expected. Indeed, this was observed (Fig. 2); the variable fluorescence $(F_\infty - F_0)$was inhibited almost completely with 0.5mM $Cd(NO_3)_2$. However, when DPC was added together with $Cd(NO_3)_2$, the kinetics of the fluorescence rise and the intensity at F_∞ were restored almost completely (Fig. 2), confirming that the site of inhibition by Cd was before the DPC donating site. The above results were confirmed in five experiments.

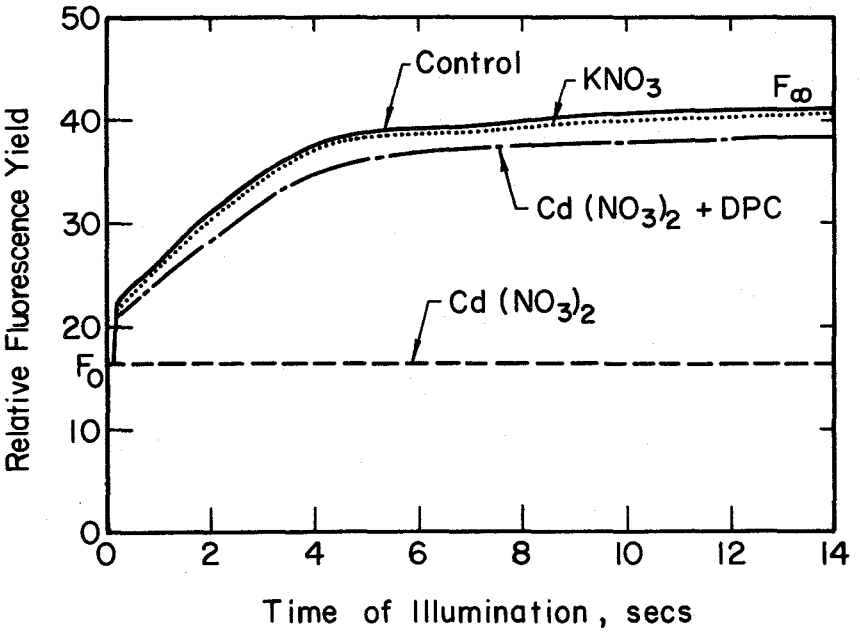

FIG. 2

Effects of 0.5mM $Cd(NO_3)_2$ (- - -), 0.5mM KNO_3 (· · ·), and 0.5mM $Cd(NO_3)_2$ plus 0.5mM DPC (——) on the time course of Chl a fluorescence yield at 685 nm. Chloroplasts containing 15 μg Chl ml^{-1} were suspended in 0.05M HEPES buffer (pH, 7.6).

II. ABSENCE OF INHIBITION ON SYSTEM I: P700

In untreated chloroplasts, PSI (729nm) light caused an absorbance decrease of 7.05×10^{-4} at 703nm (P700), while in the presence of 0.5mM and 2.5mM $Cd(NO_3)_2$, it was 6.2×10^{-4} and 7.8×10^{-4} respectively. The slight differences between the control and Cd treated chloroplasts are not significant. It is clear from these data that PSI activity is not sensitive to the concentrations of cadmium that inhibit water oxidation almost completely. This result was confirmed in three experiments.

III. EFFECTS ON PIGMENTS

A. Absorption Spectra and Extraction of Pigments

Figure 3 shows the absorption spectra (630-750nm region) of control (dashes and dots) and Cd treated (1mM, 20 mins incubation; dashes only) chloroplasts containing about 20μg Chl ml^{-1} suspension; the solid curve represents an absorption spectrum of the control but diluted to match the absorption at 660 nm with that of the Cd treated sample. The sample to which $Cd(NO_3)_2$ was added reflected about 30% decrease in absorbance. Furthermore, the ratio of A678 (absorption at 678nm, mainly due to Chl a) to A650 (mainly due to Chl b) is slightly higher (1.9) in the control than in the Cd treated sample (1.7). This difference (12%) was confirmed in all of the measurements on 20 different samples. It is clear that cadmium causes a decrease in the ratio of Chl a/Chl b, as shown by the difference absorption spectrum of Cd treated (dashed curve) minus control (solid line) chloroplast suspensions (see insert, Fig. 3). This spectrum shows two small positive absorption bands at 695nm (Chl a 695) and 645nm (Chl a, Chl b?), and a large negative asymmetric broad band (half band width, 20nm) at 677.5nm (Chl a 670 and Chl a 680). These data further suggest that there has been a change in the ratio of short to long wavelength forms of Chl a.

The effect of different concentrations of Cd on A678/A650 showed a maximum decrease of 25% at 0.5mM $Cd(NO_3)_2$ which corresponds to 8μM Chl/50μM Cd. (The K_m of this effect was 8μM Chl/10μM Chl.) Furthermore, measurements on the acetone extracts of untreated and Cd treated chloroplast suspensions showed that A678/A650 of 1.9 (control) reflected Chl a/Chl b ratio of 4.2 (extract) while A678/A650 of 1.7 (Cd treated) corresponded to Chl a/Chl b ratio of 1.6. A decrease of 42% in Chl a/

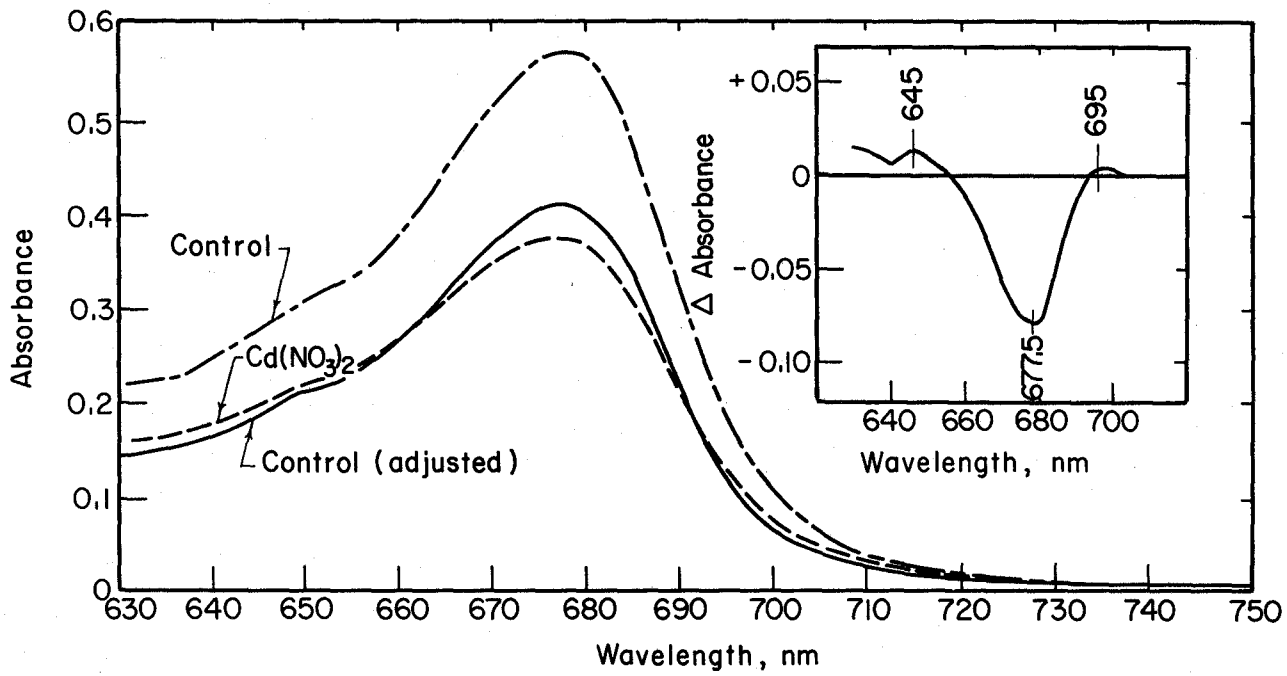

FIG. 3.

Absorption spectra of suspensions of control and $Cd(NO_3)_2$ treated maize chloroplasts. (— · —), 20μg Chl ml^{-1} (control); (——), 13μg Chl ml^{-1} (control); (---), 20μg Chl ml^{-1} with 1mM $Cd(NO_3)_2$. Insert: difference absorption spectrum of Cd treated (---) minus control (——) samples.

Chl b ratio was thus obtained upon Cd treatment. Although these results show an actual decrease in ratio of Chl a/Chl b when $Cd(NO_3)_2$ was added to chloroplasts, the absolute change may have been exaggerated if Cd complexed with some form of Chl a and, thus, did not permit its full extraction with 80% acetone.

B. Excitation Spectra of Chlorophyll a Fluorescence

Figure 4A shows the room temperature action (excitation) spectra for Chl a fluorescence at 740nm (F740) for control and for Cd treated chloroplasts. The ratio of the intensity of fluorescence excited by Chl a (678nm) to that by Chl b (650nm) is lower in Cd treated sample in agreement with the results on A678/A650 noted above. The intensities of fluorescence excited by wavelengths higher than 687nm are higher in Cd treated samples, also in agreement with the results from the absorption spectra. The difference spectrum of the two excitation spectra shows two positive bands at 700nm (Chl a 695) and 640nm[21] (Chl a, Chl b?) and a negative band around 680 nm (Chl a 670 and Chl a 680) (see Fig. 4B). This result is similar to that of the difference absorption spectrum (insert, Fig. 3) suggesting that cadmium does not elicit significant changes in the efficiency of excitation energy transfer. This is confirmed by the parallel decrease in Chl a absorption and fluorescence excited by 678nm compared to the same for Chl b (650nm). Experiments at 77°K showed similar results (not shown).

ACKNOWLEDGMENT

This work was supported by National Science Foundation RANN Grant GI 31605. We thank Renee Basta for technical assistance.

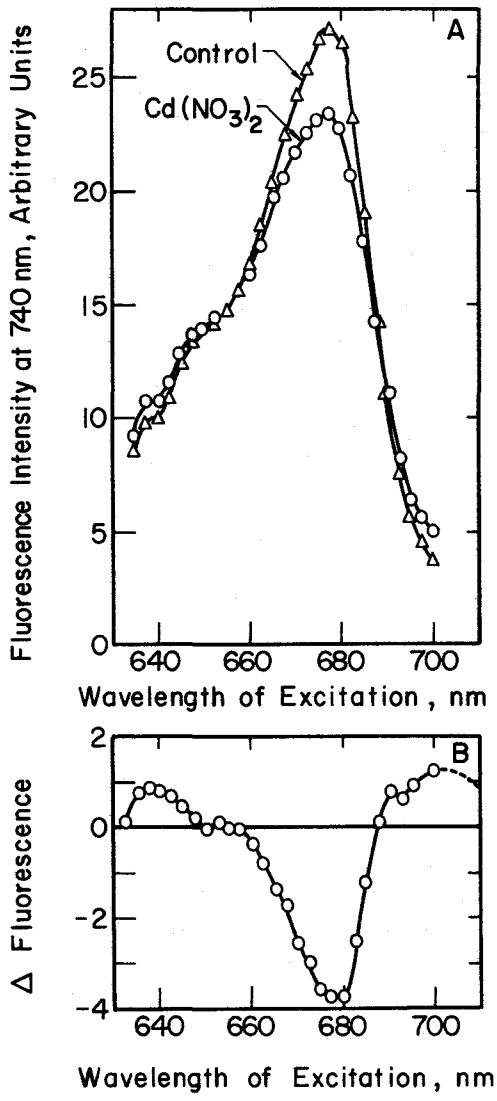

FIG. 4

(A): Room temperature fluorescence excitation spectra of F740 of untreated (-Δ-) and treated (0.5mM $Cd(NO_3)_2$, -o-) chloroplast suspensions containing 26μg Chl ml^{-1} suspended in 0.05 HEPES buffer (pH, 7.6). The exciting slit had a half band width of 3.3 nm and the measuring slit of 16.5nm.
(B): Difference fluorescence excitation spectrum of untreated (-Δ-) minus Cd treated (0.5mM, -o-) chloroplast suspensions.

REFERENCES

1. Abbreviations: BSA, bovine serum albumin; Cd, cadmium; Chl, chlorophyll; DCPIP, 2,6-dichlorophenol indophenol; DCMU, 3-(3,4 dichlorophenyl)-1,1 dimethyl urea; DPC, diphenylcarbazide; HEPES, N-2-hydroxyethylpiperazine-N'-propanesulfonic acid; P700, reaction center of photosystem I.

2. L.Friberg, M. Piscator and G. Nordberg, In Cadmium In The Environment 166P (1971), Chemical Rubber Co. Press, Cleveland, Ohio.

3. J.V. Lagerwerff and A. A. W. Specht, Environ. Sci. Technol. 4, 583 (1970).

4. M. J. Buchauer, Environ. Sci. Technol. 7, 131 (1973).

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

6. H. A. Schroeder and J. J. Balassa, Soil Science 140, 819 (1963).

7. N. Yamagata and I. Shigematsu, Bull. Inst. Pub. Health 19, 1 (1970).

8. A. L. Page, F. T. Bingham and C. Nelson, J. Environ. Quality 1, 288 (1972).

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

10. F. Haghiri, J. Environ. Quality 2, 93 (1973).

11. M. B. Bazzaz and Govindjee, Plant Physiol. 52, 257 (1973).

12. D. I. Arnon, Plant Physiol. 24, 1 (1949).

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

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

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

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

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

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

19. W. L. Butler, Proc. Natl. Acad. Sci. U.S. 69, 3420 (1972).

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

21. The positive band around 640 nm both in the difference excitation and absorption spectra of Cd treated minus control samples is perhaps due to the second band of the long wavelength (or aggregated form of) Chl a (see Brody and Brody, 22).

22. S. S. Brody and M. Brody, Nat. Acad. Sci. Nat. Res. Council Publ. 1145, 455 (1963).

Received October 18, 1973
Accepted October 29, 1973