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Protective Effects of Phosphate and ATP Pretreatment on Pb-Inhibiting Photosystem Ⅱ Activity

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鉛(Pb)에 依한 光系 Ⅱ 活性抑制에 미치는 燐酸 및 ATP 前處理의 保護效果

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ABSTRACT

The activity of photosystem Ⅱ in isolated chloroplast from the leaves of *Sedum sarmentosum* was measured. The photoreduction rate of DCPIP by photosystem Ⅱ showed the circadian rhythm with a peak at near midday sample for a continuing fine day and at near afternoon between midday and sunset sample for a continuing cloudy day in summer. The optimum light intensity of photoreduction by photosystem Ⅱ in the chloroplast preparation was about $5\sim9\times10^4$ lux. The saturated light intensity was over 9×10^4 lux. Photosystem Ⅱ activity was inhibited by even the lowest concentration of lead. When Pi and ATP of the same concentration as Pb were added to the reaction mixture containing Tris buffer lacking of Pi prior to Pb incubation, photosystem Ⅱ activity was protected from Pb-inhibiting effect by the the pretreament of Pi and ATP. It was assumed that Pb inhibition was probably due to one, P-depriving by the precipitates of $Pb_3(PO_4)_2$ in the reaction mixture and the other, partially Pb-combing with Pi groups of the active site of photosystem Ⅱ.

INTRODUCTION

Lead has been known to mankind since 2500 B.C. Lead toxicity, saturnism was recorded by ancient Greek and Arab physicians; great painters suffered from lead toxicity and thus, lead poisoning is not a by-product of modern technology. Although lead is present at 15 ppm in the earth's crust, it is not uniformly distributed. Seawater contains 5ppb of lead and all the living organisms have it (Venugopal and Luckey, 1978).

We examined and found the precipitation of lead ion and the plant growth (Sung, 1976a), lead contamination of roadside trees in Jinju city (Sung, 1976b), the inhibited effects of Cd, Hg and Pb on the respiration of germinating seeds (Sung, 1979), the inhibited effects of phosphate ion on absorption and toxicity of lead in plants (Sung and Jeong, 1977), the decrease of ATP content by some heavy metals (Al, Cd, Hg and Pb) in plant leaves (Sung and Yang, 1979), similarity of Pb-surplus and P-deficiency on ATP content in plant leaves (Sung and Kwon, 1980), and toxicity of variorus heavy metals on ATP and respiration in germinating seeds (Sung *et al.*, 1981). It was reported that by the interaction between Pb^{2+} and phosphate groups microcrystalline precipitates of $Pb_3(PO_4)_2$ were formed at the internal or on the external surface of the cell (Malone *et al.*, 1974; Schulze and Brand, 1978). These mean for lead to deprive the plants or culture medium of phosphate. It is know that lead toxicity induces partially the deficiency of phosphate, and then phosphorylation related to phospate is damaged (Venugopal and Luckey, 1978).

Heavy metal ion such as lead, osmium and tungsten were deposited at specific sites on the thylakoid membranes of chloroplasts (Sabnis *et al.*, 1969). Photosynthesis and respiration of plant are inhibited by lead (Huang *et al.*, 1974). In addition to lead is known as an inhibitor of PS Ⅱ (photosystem Ⅱ) in isolated chloroplasts (Huang *et al.*, 1974; Miles *et al.*, 1972) and of electron transport, especially in the absence of phosphate (Koeppe and Miller, 1970). Phosphate plays a key role in ATP involved metabolism and is known as a component of sugar phosphate, nucleotide, coenzymes and phospholipids (Lin and Hanson, 1974; Noggle and Fritz, 1976). Lead is able to bind with a membrane protein and other anions (Venugopal and Luckey, 1978; Sung and Jeong, 1977). It was assumed that the supply of exogenous Pi and ATP could be protected from Pb-inhibiting photoreduction in a view point of the above many reports and the similarity of both Pb^{2+} and Cu^{2+} cations. These reports have not found any protective effects of lead upon the light reactions of photosynthesis, optimum light intensity of *Sedum sarmentosum*, and diurnal fluctuation of photoreduction.

The purpose of present study is to investigate the protective effects of Pb-inhibiting PS Ⅱ activity. On the other hand we had determined the optimum light intensity and the diurnal fluctuation of photoreduction in the isolated chloroplast of *Sedum sarmentosum*.

MATERIALS AND METHODS

Plant Material. The fresh stems attached leaves were collected from the plant (*Sedum sarmentosum* Bunge) growing normally on the hill of field between Jinju nd Munsan in Korea during summer, 1980. The stems were placed in a vinyl bag and immediately transferred to the laboratory. The fresh leaves which were detached from the stem were washed three times with distilled water. Surface water of the leaves were removed with

three layers of filter paper and then used for the isolation of chloroplasts.

Isolation of Chloroplasts. The procedure of chloroplasts isolation was modified by the technique of Samuelsson and Öquist (1980). Ten grams of fresh leaves were detached from the plants and washed three times with distilled water. In the next the leaves were prepared with the plant material mentioned above. The homogenation was carried out with 50 ml of cold grinding medium consisted of 0.5 M sucrose 0.01 M NaCl, 0.05 M Tris buffer adjusted to the final pH of 7.5 in a cold blender at full speed for 15 seconds. The homogenate was filtered through two layers of gauze into the chilled centrifuge tube, set in a refrigerated centrifuge, and then accelerated to 1,000 *g* for one minute. The pellet was removed and the supernatant was centrifuged again at 2,000 *g* for 10 minutes and the settled chloroplast were suspended in the cold grinding medium. The supernatant was removed again and the pellet was diluted with the cold solution of Tris buffer. The diluted pellet was centrifuged at 2,000 *g* for 10 minutes with the same procedure mentioned above. Finally the pellet was diluted with Tris buffer to contain 1~2 mg of chlorophyll per ml according to the method of Arnon (1949). The chloroplast preparation was stored at 1±0.5°C and the rate of DCPIP photoreduction as the function of PS Ⅱ activity was measured immediately after the preparation.

Photosystem Ⅱ Activity. The chloroplast stored was used for the measurement of DCPIP photoreduction. The experimental group was divided into 3 groups. In the first group the treatment of Pi, ATP and Pb was divided into 5 subgroups with 2.49 ml of the chloroplast preparation in each cuvette, and 0.5 ml of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} M solution of Pi, ATP, and Pb was added respectively to the cuvette containing the chloroplast preparation. The second group was divided again into 2 subgroups, and in the Pb pre-treatment subgroup, 0.5 ml of 10^{-4} M Pb solution was added to each cuvette containing the chloroplast preparatoin, thereafter 0.5 ml of 10^{-4} M Pb solution was added to it. In the Pb post-treatment subgroup, firstly Pi and ATP mentioned above were added to the cuvette, secondly Pb was added to it. In the third group, the control group was made with adding 0.5 ml of distilled water ot the cuvette containing 2.49 ml of the chlorplast preparation. All the cuvette treated with the mentioned above were incubated at 25°C in the dark for 10 minutes. After incubation, 0.01 ml of 0.3 μmoles DCPIP solution finally was added to every cuvette and content of chlorophyll in the chloroplast preparaton was keptcontinously on 20 μg per ml of it. These procdures were carried out with a cuvette set tightly in 5 ml chamber. Light intensity on the surface of cuvette was 50,000 lux. In order to eliminate heat and infrared light from the lamp, the light was filtered through a screen of water layer saturated with $CuSO_4$ and the cuvettes were blown with electric fan continously. The mixture solution was kept at 25°C. During illumination the PS Ⅱ activity was measured by the rate of DCPIP photoreduction for 4 minutes at one minute interval with Shimadzu UV-190 Double-Beam Spectrophotometer at 610 nm. A diurnal fluctuation of photoreduction rate of DCPIP was determined in the chloroplast preparation

from the sample collected at three hour interval in the field under a continuing fine or a continuing cloudy day conditions during day time in summer in 1980. We had much rainy and cloudy day conditions during the experiment period of diurnal fluctuation for photoreduction. Light intensity was mesured by both horticultural lux meter, Demetra Toyo Japan, Dakemura Electric Works Ltd., and electric lux meter, Sibata Kayaku Kakai Kogyo K.K.

Identificationof Precipitates. A precipitate was observed in the reaction mixture solution during the experiment with increasing lead concentrations. The precipitate in the mixture solution was filtered through two layers of gauze and centrifuged at 100 *g* for 5 minutes prior to the removing of supernatant. The sediment in the tube was diluted with distilled water. Such sediment was centrifuged twice at 1,000 *g*, and separated by paper chromatography. The identification was determined by spectrophotometer.

Ion Exchange. Na ion from Na_3PO_4 was eliminated with Amberlite IR-120 B resin and NO_3 ion from Pb $(NO_3)_2$ eliminared with Amberlite IRA-410 resin. Activation of each resin was carried out according to NaOH and HCl solution. The solution of Pi and Pb by ion exchange resin were used to estimate an effect of Pi and Pb on DCPIP photoreduction in the chloroplast preparation. Pi and Pb were used as the forms of PO_4 and Pb^{2+}, respectively.

RESULTS

Optimum Light Intensity. Because the optimum light intensity for photosynthesis is different with the kinds of plants, the optimum light for photoreduction in chlorplast of *Sedum sarmentosum* must be determined prior to the experiments. The reaction mixture containing the chloroplast preparation was illuminated with the different light levels, such as 0, 1, 5, 9 and 13×10^4 lux. The photoreduction was increased with increasing light intensity from 0 to 9×10^4 lux, but decreased with increasing the light intensity over 9×10^4 lux. The optimum light intensity in the chloroplast preparation for photoreduction was $5\sim9\times10^4$ lux. The saturated light intensity for photoreduction in the material plant was over 9×10^4 lux (data not shown).

Diurnal Fluctuation of Photoreduction. DCPIP photoreduction in chloroplasts was meausred at 3 hour intervals in the progress of time under a continuing fine day and a continuing cloudy daylight conditions (Fig.1). Photoreduction rate was continously increased from morning to near midday under a fine day, at late afternoon under a cloudy day. The photoreduction damped down at the time of maximum light intensity (140,000 lux) for a fine day, however peaked at the time of moderate light intensity (90,000 lux) for a cloudy day. Diurnal fluctuation of photoreduction was different with the condition of daylight intensity. At late afternoon, for a cloudy day it was decreased again. These fluctuations of photoreduction curves (Fig.1) were repeated everyday as

far as the sampling condition is under the one of fine and cloudy day (data not shown). Although we investigated on the cause of photoreduction rhythmicity, we did not found any other reasons for the tendency of photoreduction rhythmicity in the day time.

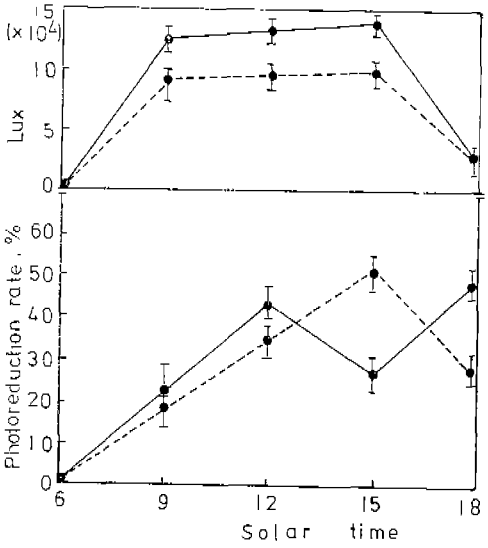

Fig. 1. Diurnal changes of the light intensity (upper) and photoreduction activity of DCPIP in chloroplast of *Sedum sarmentosum* (low) on a continuing fine day (solid line) or a continuing cloudy day (dotted line).

Effects of Pi, ATP and Pb on PS II. Effects of Pi concentrations on DCPIP photoreduction in chloroplasts were measured for 4 minutes at one minute interval (Fig.2-Ⅰ). The photoreduction rate was slightly stimulated by low concentrations of Pi and ATP in chloroplasts, and inhibited by high concentrations of it. The optimum concenrtation of exogenous Pi preparation may be required for photoreduction by chlorplasts. The optimum concentration was below 0.4 M Pi and the high concentration over 0.3 M Pi inhibited photoreduction. We used Tris buffer instead of phosphate, therefore it was thought that the photoreduction of the reaction mixture might require the exogenous Pi. Photosystem Ⅱ itself did not need phosphate, but phosphate seemed to affect PS Ⅱ activity indirectly

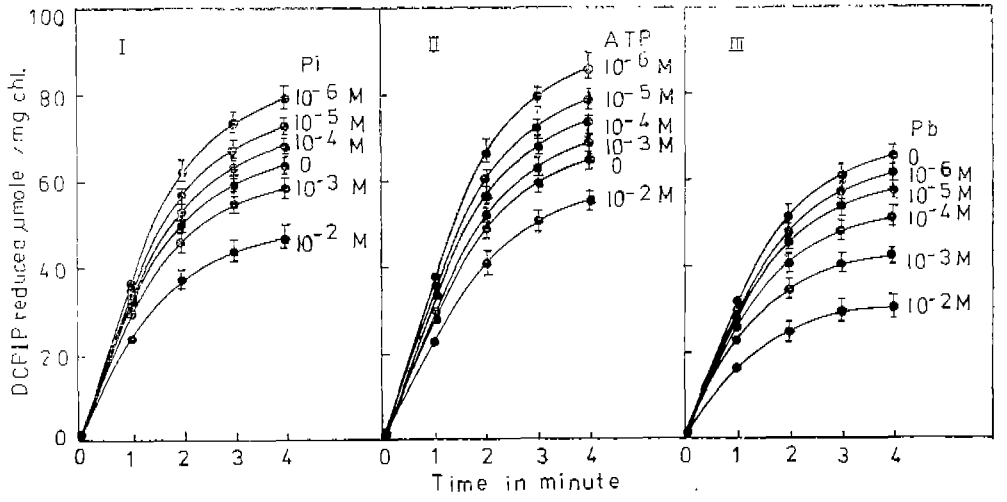

Fig. 2. Effects of Pi concentrations (Ⅰ), ATP concentrations(Ⅱ) and Pb concentrations (Ⅲ) on DCPIP photoreduction in chloroplast.

Effects of ATP concentration on DCPIP photoreduction in chloroplasts were estimated (Fig. 2-Ⅱ). The photoreduction was stimulated by exogenous ATP of the low concentrations, but inhibited by the high concentration of ATP. It was probably due to the necessity of ATP for the indirect stimulation of photoreduction. The stimulation of PS Ⅱ activity at low concentration of ATP was similar to that of Pi (Fig. 2-Ⅰ, 2-Ⅱ). It was supposed that the exogenous ATP energy might be required for the active transport metabolism of thylakoid membrane occuring photoreduction. Fig. 2-Ⅲ shows that the activity of PS Ⅱ (H_2O→DCPIP) decreased after the incubation of reaction mixture with Pb in the darkness for 10 minutes. The extent of inhibiton was increased with increasing Pb concentrations. The concentration of 10^{-3}M Pb inhibited the photoreduction by about 50% after four minute exposure to the light. The increase of inhibition in the progress of time of exposure showed that Pb inhibition was slightly stimulated. Comparative effects of Pi, ATP and Pb concentrations on DCPIP photoreduction in chloroplasts were demonstrated (Fig. 3). At the low concentrations of both Pi and ATP the exogenous ATP stimulated photoreduction more than Pi. Lead always inhibited photoreduction in chloroplasts.

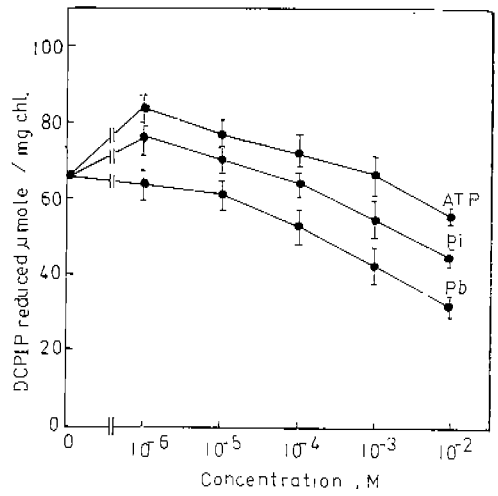

Fig. 3. Comparative effects of Pi, ATP and Pb concentrations on DCPIP photoreduction in chloroplast.

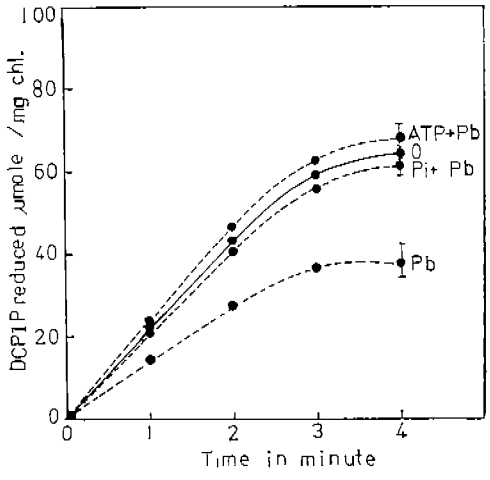

Fig. 4. Protective effects of the pretreatment of Pi and ATP on Pb-inhibiting photoreduction in chloroplast.

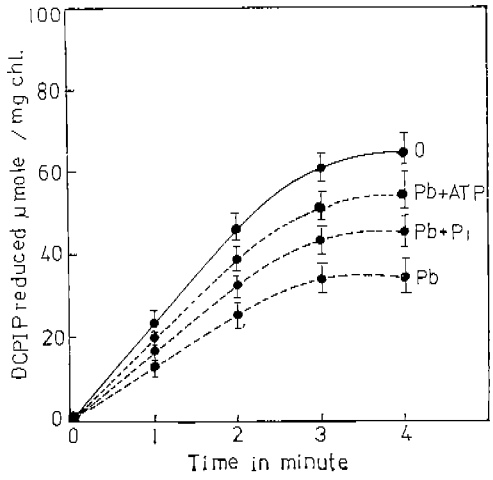

Fig. 5. Effects of Pi and ATP post-treatment on DCPIP photoreduction in chloroplast.

Protective Effects. The protective of Pb-inhibiting photoreduction in chloroplasts by Pi and ATP pretreatments of 0.4 M prior to Pb incubation was demonstrated (Fig. 4). Each 0.4 M treatment of Pi or ATP at the same concentration as Pb protected Pb-inhibiting photoreduction due to Pb post-treatment (Fig. 4), but post-treatment of Pi and ATP did not protect Pb-inhibiting photoreduction due to pretreatment of Pb (Fig. 5). It was thought that non-protection of Pb inhibition was probably due to pre-binding of Pb to thylakoid membrane of PO_4 site.

Precipitates. The precipitate was observed with naked eye in the reaction mixture

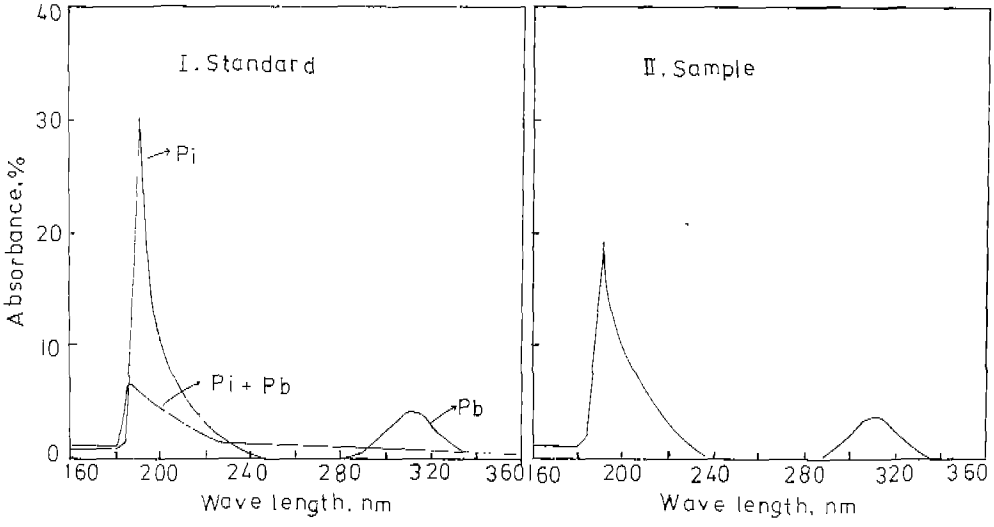

Fig. 6. Absorption spectra of Pi, Pb and its mixture of standard (Ⅰ), and sample (Ⅱ) dissolved with corresponding solvent. Ⅰ, Standard; because of precipitates formed between Pi and Pb, the mixture curve disappeared in the course of time. Ⅱ, Sample; precipitates were dissolved completely with each ion state in corresponding solvent, the mixture curve like standard could not be shown.

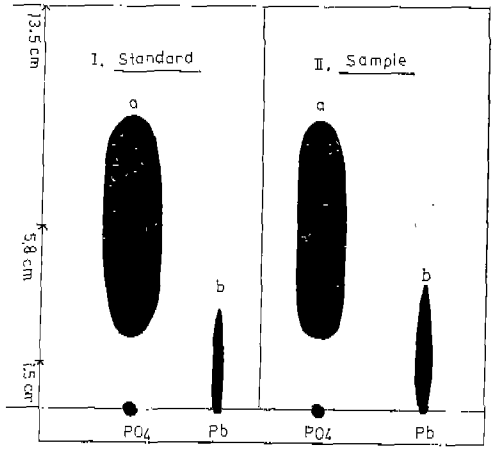

Fig. 7. Paper chromatography of precipitates from chloroplast mixture treated with Pi and Pb. Rf value of precipitates (Pi=0.45, Pb=0.10) was corresponded with Rf value of standard Pi and Pb.

of chloroplast by the artificial supply of both Pb and Pi. We separated the precipitates from the reaction mixture by centrifugation, and identified by UV-absorption and chromatography. The precipitates were formed by Pb supply to the reaction mixture. The precipitates were dissolved in either Pb or Pi solvents. We obtained the standard peak at 315 nm and 195 nm for Pb and Pi ($-PO_4$) in Fig. 6-Ⅰ, and also obtained the peak at the same wave length from the sample of precipitates, respectively (Fig. 6-Ⅱ). Lead and Pi were separated and identified from the precipitates by paper chromatography(Fig. 7). Therefore we found that the precipitates were composed of Pb and PO_4.

DISCUSSION

Recommendable lux value for cultures was written on the manual of Nieukoop lux meter model LU-345 made in Holland; 12,000~15,000 lux, anthurium, marantha and saintpaulia; 15,000~20,000 lux, aglaoanema and philodendron; 20,000 lux, felci and peperonia; 30,000lux, araucaria and cissus; 30,000~40,000 lux, sarsevieria and palme; 50,000 lux, ardisia, begonia, calceolaria, cyclamen, gardenia, kalanchoe, and pandanus; 80,000~100,000 lux, acalypha, croton, ficus and pelargonium. Recommendable lux range for cultures of the above light intensity was summarized as 12×10^3~10^5 lux.

The light intensity used for photosynthetic experiments was different with the workers and plant kinds; 25,000~35,000 lux, at lettuce leaf level for electron transport system (Shimazaki and Sugahara, 1980); 1,300~21,000lux, for circadian clock of *Gonyaulax polyedra* in culture medium (Sweeney, 1976); 28,000 lux for photosynthetic fixation of $C^{14}O_2$ in *Euglena* (Laval-Martin *et al.*, 1979); 30,000 lux for Hill reaction in spinach chloroplast (Ikehara and Sugahara, 1969); 50,000 lux for photophosphorylation in spinach chloroplasts (Shinohara and Sakurai, 1980); 3,000 lux for culture of duckweed at plant level (Tsudzuki and Kondo, 1979); 30,000 lux for photochemical activity of tobacco plants (Haraguchi and Shimizu, 1970); 2 Klux for *Chlorella* photosynthesis (Matsuka and Hase, 1966); 750 W/15 cm for spinach chloroplast (Miles *et al.*, 1972); 1,000 W/m for photosynthetic electoron transport in spinach chloroplast (Samuelsson and Öquist, 1980); 300 W tungsten lamp for photosynthesis in spinach chloroplast (Allen and Whatley, 1978); 1,500 W/12 cm for C_3 and C_4 photosynthetic types (Bolton and Brown, 1980); 2,800 ft-c (1,200 μ einsteins) for *Elodea* photosynthesis (DeGroote and Kennedy, 1977); 500 K ergs/sec·cm^2 for electron transport activity in maize chloroplast (Walker and Izawa, 1979); 5 nE/cm^2·sec, a quantum flux density at the surface of the leaf segments in wheat from 150 W incandescent bulb (Edwards *et al.*, 1978). Because it was found that the optimum light intensity was about 5×10^4 lux in the leaves of our material plant, all measurement of photoreduction was carried out under 5×10^4 lux, 25°C in laboratory. The recommendable lux range (5~9×10^4 lux) of our material plant for photoreduction was higher than that of any other plants. The reason was thought that

this material plant was a kind of CAM (crassulacean acid metabolism) plant. The light intensity was expressed as many different ways by workers; lux, K lux, W/cm, W/m, ft-c, erg/sec·cm^2, K erg/sec·cm^2, nE/cm^2·sec and etc.

We found the fluctuation of photoreduction in chloroplasts for a daylight. Inanaga *et al.* (1980) have reported that photosynthetic rate in the day time was affected by the air temperature of the preceding nights. Night temperature below a certain limit brought about depression of photosynthesis during the succeeding day time. The lower the night temperature, the larger the extent of depression and the logner time required to recover from the depression. However we could not found the correlation between photoreduction rhythms and the air temperature. In *Wolffia microscopica*, the activity of nitrate reductase showed strong diurnal rhythmicity with a peak at the forenoon. The rhythmicity persists under the extended dark conditions as well as under continuous light. The phase of rhythm shifts under inverted light-dark cycles (Bakshi *et al.*, 1978, 1979). Phosphoenol pyruvate carboxylase from the crassulacean plant was able to control a circadian rhythm of CO_2 fixation (Jones *et al.*, 1978). The circadian rhythm in bioluminescence in *Gonyaulax*, a kind of algae has been found by Sweeney (1979) and Kiessig *et al.* (1979). Circadian rhythms in photosynthesis have been observed in a number of higher plants as well as in algae (Hillman, 1976). Several studies have been reported as a rhythm of photosynthetic capacity during the cell cycle of the algae flagellate *Euglena* (Cook, 1966; Walther and Edmunds, 1973). During synchronous growth, photosynthetic CO_2 fixation was rhythmic with a period at 24 or about 30 hours corresponding to the period of cell division rhythm in the population. Futhermore, the rhythm in CO_2 fixation per cell found in nondividing cultures maintained for several weeks with a free running, circadian period of approximately 30 hours (Laval-Martin *et al.*, 1979). An endogenous, circadian rhythm in cellular chlorophyll was found to exist, independently on cell division, under both light regiments and in each individual experiment. It was thought that the photoreduction or photosynthesis rhythmicity was shown as a photosynthetic metabolism cycle under the control of chloroplast DNA or nuclear DNA, a kind of heredity.

Fig. 2 showed the requirement of the exogenous phosphate for photoreduction in isolated chloroplasts. Generally it has been known that the extracted chloroplasts has been suspended or resuspended in phosphate buffer for the adjustment of pH in photosynthetic studies (Takamiya *et al.*, 1980; Brown and Thorpe, 1980; Komor *et al.*, 1978; Anderson and House, 1979). However presence of phosphate strongly affected the pattern of ATP pool fluctuation in dark-light and light-dark transitions. Net O_2 evolution in the light was decreased by phosphate addition. Providing phosphate at steady state conditions in the light in a N_2 atmosphere produced a rapid drop in ATP content, followed by a recovery. In cells treated with DCMU, addition of phosphate accompanied by a rapid and permanent increase in ATP pool (Larsson and Tillberg, 1979). Protoplast

extracts evolved approximately 6 micromoles of O_2 per milligram of chlorophyll before photosynthesis become largely dependent on the exogenous Pi while photosynthesis by chloroplasts had a much stronger dependence on exogenous Pi from the outset. Photosynthesis by chloroplasts from 6-day-old wheat plants under optimum levels of Pi was similar to that with the addition of 5 millimolar inorganic pyrophosphate (PPi) plus 0.2 millimolar ADP. Either PPi or ADP separately inhibited photosynthesis. Chloroplasts from 9-day-old wheat leaves were slightly less sensitive to inhibition by PPi and showed little or no inhibition by ADP (Edwards *et al.*, 1978). At constant levels of orthophosphate (4 mM) and 3-phosphoglycerate (4 mM), a change in ATP concentration from 0.2 to 1 millimolar causes an immediate 4-to 5-fold increase in the rate of ADP-glucose formation (Kaiser and Bassham, 1979). With chloroplasts from young pea leaves, PPi +ADP (or ATP) tends to give higher rate of photosynthesis than with the optimum level of Pi alone (Robinson and Wiskich, 1977; Stankovic and Walker, 1977). In wheat chloroplasts, at least under experimental conditions, both the level of Pi and rate of starch synthesis in the chloroplast in the absence of exogenous Pi must be below (Edwards *et al.*, 1978). PPi has been found to protect against Pi inhibition with spinach chloroplasts (Lilley *et al.*, 1973). Starch synthesis would release Pi and allow photosynthesis to continue but recycling of this nature may well be too slow to permit maximal rate of photosynthesis. Pi uptake and triose-P release from chloroplast, catalyzed by the phosphate transporter, is thought to be required for sucrose synthesis (Walker and Herold, 1977). With chloroplast of spinach and peas, photosynthesis typically starts off at a relatively high rate and proceeds for several minute before exogenous Pi is required (Cockburn *et al.*, 1967a, 1967b, 1968). β-Naphthyl di-, tri- or tetra-phosphate inhibits photophosphorylation of spinach chloroplasts comparatively with ADP, whereas β-naphthyl monophosphate inhibits it comparatively with Pi. The results suggest that the effect of the monophosphate is principally on the Pi sites and that of the di-, tri or tetra-phosphate is on the adenine nucleotide sites on the active site of chloroplast coupling factor 1 (Shinohara and Sakurai, 1980). Lead always inhibited photoreduction in chloroplasts. This was in accordance with the finding of Samuelsson and Öquist(1980) and Cedeno-Maldonado and Swader(1972). PS Ⅱ activity was inhibited even if the concentration of lead is the lowest. Therefore lead act as an inhibitor of photoreduction, PS Ⅱ activity, and the results were in accordance with the report (Miles *et al.*, 1972).

In the present study, protective effect of Pb-inhibiting photoreduction by supply of phosphate was probably due to supplement of free phosphate against depriving of phosphate by lead. Photosystem Ⅰ activity, as measured by electron transport from DCPIP to methyl viologen, was not reduced by lead treatment (Miles *et al.*, 1972). However photosystem Ⅱ was inhibited by lead salts when electron flow was measured from water to methyl viologen and Hill reaction or by chlorophyll fluorescence (Miles *et al.*, 1972). These indicated that the primary site of inhibition was the oxidizing side of PS Ⅱ by the

fluorescence induction curves. That this site was between the primary electron donor of PS Ⅱ and the site of water oxidation could be demonstrated by hydroxylamine restoration of normal fluorescence following lead inhibition (Miles *et al.*, 1972). By them Mg^{2+}, Ca^{2+}, Sr^{2+}, Mn^{2+}, Al^{3+}, Zn^{2+} and Cd^{2+} all either increased or had no effect on fluorescence yield of spinach chloroplasts at room temperature. They tested some of this divalent ions and found, while using their techniques and tomato chloroplasts, that there was an increase in fluorescence compared to a decrease in fluorescence following lead treatment. Lead salts exhibited a marked inhibition of FMN (flavin mononucleotide), methyl viologen, or PMS (phenazine methosulfate)-mediated light driven proton translocation. Sabnis *et al.* (1969) found that a nonenzymatic discrete deposition of lead, osmium and tungsten at specific sites on the thylakoid membrane of chloroplasts was formed. Mn is effective in reducing the sensitivity to Cu poisoning in chloroplast mediated photoreduction and it is generally assumed that the most sensitive site of Cu inhibition is on the oxidizing side of PS Ⅱ (Cenedo-Maldonado and Swader, 1972; Habermann, 1969).

In the present study phosphate is effective in reducing the sensitivity to lead poisoning in chloroplast mediated photoreduction, and it is assumed that the most sensitive site of lead inhibition is on the oxidizing side of PS Ⅱ. On the other hand, lead might be bound to PO_4 of phospholipid in a thylakoid membrane which is not involved in electron transport itself, but it causes the structural changes inside the membrane upon Pb binding and an induction of P-deficiency in the reaction mixture by $Pb_3(PO_4)_2$ compounds, with the subsequent inhibition of electron transport as a result. The addition of phosphate normally reactivated PS Ⅱ activity in Pb inhibiting chloroplasts in absence of phosphate (Fig. 2-1). This tendency was in accordance with Habermann's report(1969) which Cu inhibition of Hill reaction was partially reversed when Mn was added. We used Pb and Pi or ATP instead of Cu and Mn. Our results could be explained by Pb forming precipitates, and so the Pb toxic effects could be decreased or reversed by phosphate. In the case of supply of Pb prior to incubation of Pi and ATP in the reaction mixture Pb-inhibiting PS Ⅱ was not recovered, but the addition of Pi and ATP prior to incubation of Pb could protect PS Ⅱ activity from Pb inhibition. It was thought that Pb binds more firmly to phosphate groups than any other sites when more phosphate is present. If the optimum level of phosphate is present in the chloroplast more than Pb concentration, Pb inhibition of PS Ⅱ activity could be reversed or protected by phosphate (data not shown). Especially the inhibition site of photoreduction was supported by the deposition of lead at specific sites on the thylakoid membranes of chloroplasts as the report (Sabnis *et al.*, 1969).

The results suggest that the effect of Pb^{2+} on photoreduction of PS Ⅱ is an indirect one, whereby $Pb_3(PO_4)_2$ precipitation induces a free or structural phosphate deficiency. In physiological media Pb^{2+} concentration may reach about 10^{-4} M; interaction between Pb and cellular phosphate group is obvious, since the low solubility product of $Pb_3(PO_4)$

about 10^{-53} (Stecher, 1976; Dean, 1979). It was reported that lead could form stable complexes with free thiol-, carboxylate- and phosphate-carrying ligands of biopolymers and membranes; imidazole and amino groups are of very little importance for Pb^{2+} complex formation; lead nucleoside, especially the cytidine complex is very stable; lead readily binds to SH-, PO_4- and COOH-containing ligands of the erythrocyte membrane to increase the mechanical, but decrease the osmotic fragility of erythrocytes; lead alters membrane permeability, and binds to active sites involved in K permeability, and inhibits active transport by blocking K-Na transport ATPase in animal (Venugopal and Luckey, 1978). Schulze and Brand (1978) have reported that in the presence of equivalent Pb^{2-} and phosphate, considerable Pb^{2+} remained in solution. The concentration of phosphate was decreased to an undetectable level when the amount of Pb^{2} is not toxic to *Chlamydomonas*, but kills cells by depriving them of phosphate. Koeppe and Miller (1970) examined the effect of lead salts on corn mitochondria and found an inhibition of electron transport, especially in the absence of phosphate. It was reported that the deposits were formed in the vesicle and on the surface of cell membrane in plants (Malone *et al.*, 1974). The addition of Pb salts to phosphate-containing unicellular culture media caused precipitation of $Pb_3(PO_4)_2$. Cells did not survive when the amount of Pb in the culture exceeded the equivalents of phosphate (Schulze and Brand, 1978). In our experiment it was assumed that the precipitates formed in the reaction mixture was $Pb_3(PO_4)_2$.

According to precipitates formed between Pi and Pb in standard curve in Fig. 6, the mixture curve was disappeared with the progress of time. In sample curve because the precipitate was dissolved completely with each ion state, the mixture curve like standard could not appear. Two peaks of sample dissolved precipitates were corresponded with the standard peak of Pi and Pb. By the paper chromatography of precipitates the precipitate Rf value was corresponded with the standard Rf value of Pi and Pb (Fig. 7). It was identified that the precipitates was composed of $Pb_3(PO_4)_2$ by absorption spectrum and paper chromatography.

Because lead pre-treatment could be able to bind with free phosphate, it was assumed that insoluble phosphate could be induced the blocking of Pi and ATP metabolism for photoreduction. This possibility was thought in the review of significant inhibition of Pi absorption by lead in the previous report (Sung, 1976) and Ksp (constant of solubility products) of lead salts; 1.5×10^{-53} $Pb_3(PO_4)_2$, 1.4×10^{-28} PbS, 4.2×10^{-15} $Pb(OH)_2$ and 1.5×10^{-13} $PbCO_3$ (Stecher, 1976; Dean, 1979). Therefore the results were thought that lead pre treatment could not protect from photoreduction, while pre-treatment of phosphate and ATP could be in compensation for itself Pi and ATP deficiency or blocking of metabolism for photoreduction by lead post-treatment. In the present study it was thought that lead could be bound to the site of PO_4 in chloroplast under the *vitro* condition. It was assumed that the site of PO_4 in thylakoid membrane could be blocked

by lead.

摘 要

本 硏究는 아직 밝혀진 바 없는 돌나물(*Sedum sarmentosum* Bunge) 葉綠體의 光還元에 미치는 照度의 影響, 光還元週期의 有無 및 抽出葉綠體에서 光系 Ⅱ 活性에 미치는 鉛(Pb)의 抑制 影響에 對한 Pi 및 ATP 添加의 保護效果를 究明코저 함에 그 目的이 있다. 實驗은 野外에서 夏節期에 群落을 이루어 自生하고 있는 돌나물의 잎을 採取하여 葉綠體를 抽出하고, 抽出液에 Pi 및 ATP의 前處理와 後處理로서 Pb에 依한 光系活性抑制의 保護效果를 光還元法으로 測定하였다. 抽出葉綠體의 光系Ⅱ 活性은 아침부터 經時的으로 增加하여 快晴한 날은 正午頃에, 흐린날은 正午와 해질 무렵 사이에 peak를 이루었다. 이러한 趨勢는 每日 circadian rhythm으로 나타난다는 것을 發見할 수 있었다. 이 rhythm은 그날의 照度와 相關關係가 없었다. 이 植物의 光系 Ⅱ 活性의 最適照度는 5~9×10^4 lux이며 9×10^4 lux 以上은 飽和照度임을 알 수 있었다. Pb는 光系 Ⅱ의 光化學的 活成에 抑制作用을 나타내었고, 이는 Pi 또는 ATP 前處理로서 保護될 수 있었다. 그러나 Pb를 前處理한 後의 Pi 또는 ATP 添加는 保護效果를 나타내지 않았다. 이는 Pb가 光系 Ⅱ의 活性部位에 不溶態로 結合하여 光系 Ⅱ의 活性을 抑制함을 意味하며 thylakoid 膜의 光系Ⅱ活性部位를 構成하는 Pi 作用基가 部分的으로 Pb와 結合하므로서 光系 Ⅱ의 活性部位가 不活性化됨을 推定할 수 있다.

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