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# Effects of Inhibitors on Photoswelling in Spinach Chloroplasts.

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## INTRODUCTION

In our previous investigation(*1*), it has been found that chloroplasts suspended in a medium containing NaCl and Tris-HCI swelled spontaneously in the dark, and that this swelling was stimulated by the action of light, and then within about two hours after illumination the swollen chloroplasts began to shrink markedly. The swelling described above is the same phenomenon as light-induced high-amplitude swelling described by PACKER *et al*(*2*) and as photoswelling by NISHIDA(*3*). Hence, this swelling in the light is denoted as photoswelling in this paper. The photoswelling also was observed in the medium lacking photophosphorylation cofactors, and stimulated greatly by adding phenazine methosulfate(PMS) into the medium(*1, 2*) and by tonicity of NaCl(*1*), but not affected by adding $MgCl_2$ and ADP(*2*). Phosphate and phenylmercuric acetate(PMA) suppressed the photoswelling(*1, 4, 5*). On the basis of these data, the photoswelling may be a light-dependent but not an energy-dependent process, and is probably associated with electron flow in chloroplasts upon illumination. However, there are many points which are uncertain in the nature of photoswelling.

In order to elucidate the character of photoswelling, the time course experiments on the influences of cofactors and inhibitors of photosynthesis were initiated in chloroplasts suspended in the most simple medium, containing NaCl and Tris-HCl, in which photoswelling was observed. Effects of these inhibitors on the shrinkage subsequent to photoswelling, the second phenomenon in the time course for the volume changes of the chloroplasts illuminated in the medium, were omitted in this paper for reason of the fact that this shrinkage is associated with breaching of chlorophyll(*1*). From the

results of our experiments the photoswelling was closely associated with the light-induced electron transport, especially electron transport mediated with PMS. The photoswelling in the presence of PMS was enhanced by ammonium chloride, methylamine and quinacrine, but was inhibited by PMA and shrinkage of chloroplasts was observed. Details of our experiments are described below.

## MATERIALS AND METHODS

*Preparation of samples.*

Spinach chloroplasts were prepared in 0.75 M Tris-HCl buffer, *p*H 7.5, containing 0.3 M MaCl (NaCl-Tris solution) as reported earlier(*1*). The isolated chloroplasts were finally resuspended in the same NaCl-Tris solution used in the preparation and ajusted to 0.5-1.0 mg of chlorophyll per ml. The chlorophyll was spectrophotometrically determined by the method of ARNON(*6*). This chloroplast suspension was diluted appropriately with a filtered, dust-free NaCl-Tris solution containing various reagents and then subjected to the volume measurements.

*Determination of chloroplast volume.*

The chloroplast volume was measured electrically with a coulter counter model B. The minute orifice of 50 $\mu$ diameter was chosen in this series of experiments following the recommendation by BRECHER *et al*(*7*). This instrument determines the number and size of particles as a result of the difference in electrical conductivity between the particle and the suspending medium when the particles pass through a minute orifice. The height of the electrical pulses is approximately proportional to the particle volume. The relative frequency distribution curve of particle size can be obtained automatically within 100 sec by recording on the plotter of the instrument.

The mean chloroplast volume was obtained by calculating the arithmetrical mean of the relative frequency distribution curve, as follows. The expression for the mean chloroplast volume (*MCV*) is :

$$MCV(\mu^3)=MW \times \alpha$$

where *MW* is the "mean electric window" and $\alpha$ is the factor which converts window numbers to $\mu^3$ for the particular aperture, aperture current, amplification and components of medium. In this paper, the value of the factor $\alpha$ was determined by calibrating with standard particles of polyvinyltoluene latex (diameter, 3.49 $\mu$, Dow Chem. Co.) and solving for $\alpha$ in above equation. The expression for the mean electric window is :

$$MW=(f \cdot n/f)-0.5$$

where $n$ is the number of the electric window switched automatically in 25 divisions

at regular interval period and $f$ is the number of chloroplasts counted in each window. Since the height of the ordinates on the graphout are proportional to $f$, the reading on the graph can be substituted for actural chloroplast number. All chloroplasts counted in a window have the volume of chloroplasts midway between the upper and lower limits of the window. Therefore, 0.5 is reduced from first terms in order to calibrate as all chloroplasts counted at mid-point in each window. The first 2 or 3 windows (largely background noise) are excluded in case of calculation for the mean window. In some experiments, the change of the number of the chloroplasts having a volume greater than the volume at the distribution maximum in the dark was followed with time after illumination (*ref. 8*). It is the defect that the volume itself of chloroplasts is not determined by this method. But this method can be used to measure the volume changes at intervals of a shorter period as compared with the method calculating the arithmetrical mean chloroplast volume from relative frequency distribution curve as described above. The changes of chloroplast volume in this paper were expressed as percentage from the mean volume or the number having a volume greater than the volume at the distribution maximum at zero illumination time.

The experiments were performed with chloroplast suspension diluted to 0.5-1.0 $\mu$g of chlorophyll per ml of NaCl-Tris solution with or without reagents. The concentration of reagents is indicated under the figures in the Results section. Unless otherwise

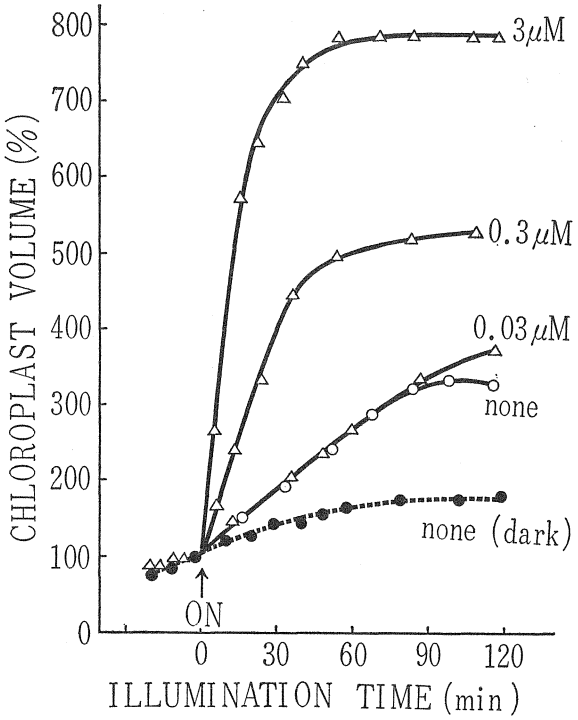


Fig. 1. Effect of PMS and its concentration on photoswelling.

stated, temperature of the chloroplast suspension was kept at 21° and light intensity with incandescent lamp was approximate 30,000 lux on the surface of vessel containing the chloroplast suspension. The measurements were started after 10-20 minutes of incubation in the dark at 21°.

## RESULTS

*Effects of electron transport cofactors.*

Phenazine methosulfate(PMS), one of the cofactors of electron transport in cyclic photophosphorylation, affected noticeably at 20 $\mu$M on the photoswelling in chloroplasts (*1*, *2*). In the presence of PMS at 3 $\mu$M, chloroplasts started to swell noticeably upon illumination, and reached within 60 minutes a steady level about 7.5 folds as great as the initial volume(Fig. 1). The same time course for photoswelling was obtained in the presence of PMS at 15, 20, and 30 $\mu$M, respectively. The maximum value of steady level in photoswelling was 6 to 10 folds at 3 $\mu$M as great as the initial volume; 8.5 folds at 15 $\mu$M, 6 to 10 folds at 20 $\mu$M and 9.5 folds at 30 $\mu$M. There was no correlation between the concentration of PMS and the maximum value of steady level in photoswelling accelerated by PMS at 3 $\mu$M above. The acceleratieve effect of PMS on photoswelling reduced by decreasing its concentration in range from 3 $\mu$M to 0.3 $\mu$M,

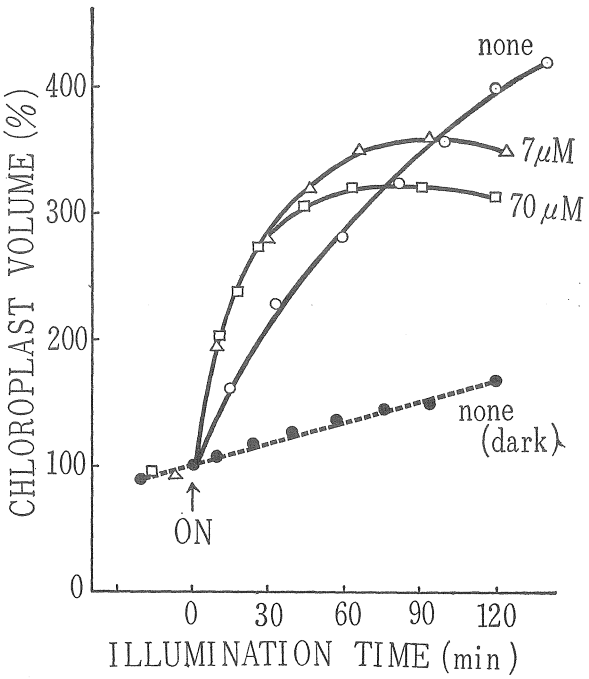


Fig. 2. Effect of FMN and its concentration on photoswelling.

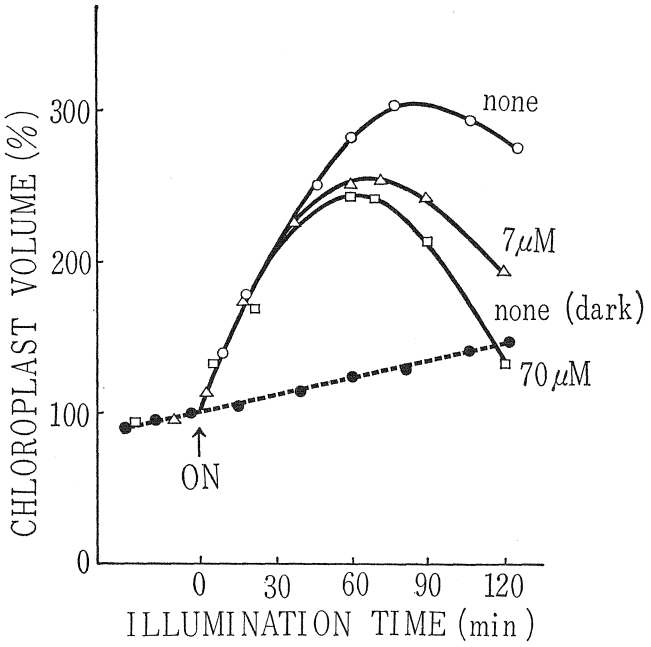


Fig. 3. Effect of vitamin $K_3$ and its concentration on photoswelling.

and disappeared at 0.03 $\mu$M.

In order to examine effect of the other cofactors in cyclic electron transport like PMS, use was made of flavin mononucleotide(FMN) and vitamin $K_3$. In the presence of FMN, the photswelling was slightly accelerated, and its maximum value reached within shorter period after illumination and was lower than that of control (Fig. 2). Vitamin $K_3$ had no effect on the photoswelling, but, as in the presence of FMN, the maximum value of the photoswelling was lower than that of control (Fig. 3). It is supposed that both FMN and vitamin $K_3$ switch over the photoswelling to the subsequent shrinking faster than in control. Indigo carmine, one of very sensitive dyes to oxidizing agents, was tested for its effect on the light-induced volume changes of chloroplasts. The time course for the volume changes in the presence of indigo carmine was essentially identical with the time course in the presence of FMN (*ref.* Fig. 2). The effect of this dye showed no appreciable differences in the rate and extent of photoswelling in the range of concentration from 70 to 700 $\mu$M, but was slightly reduced at 7 $\mu$M in comparison with that at 70 $\mu$M above.

Figure 4 illustrates the time course for light-induced volume changes of chloroplasts suspended in NaCl-Tris solution with ferricyanide, one of the cofactors of electron transport in non-cyclic photophosphorylation. The rate and extent of photoswelling at 3 $\mu$M of ferricyanide was usually similar to, and sometimes higher than, the rate and extent in control. At 30 $\mu$M, the rate of photoswelling was slightly

stimulated, but the extent was similar to, and sometimes lower than, that in control. 2,6-Dichlorophenol indophenol (DPIP) was also used as electron acceptor. The results in the range of concentration at 3.3-165 $\mu$M were essentially identical with the time course in the presence of 30 $\mu$M of ferricynaide (*ref.* Fig. 4).

Accelerating effects of these electron transport cofactors except for vitamin $K_3$ on the photoswelling suggest that this phenomenon depends on light-induced electron flow activities. In particular, PMS stimulated conspicuously on photoswelling at lower concentration than that of other cofactors used. This shows that photoswelling may be closely associated with electron transport mediated with PMS.

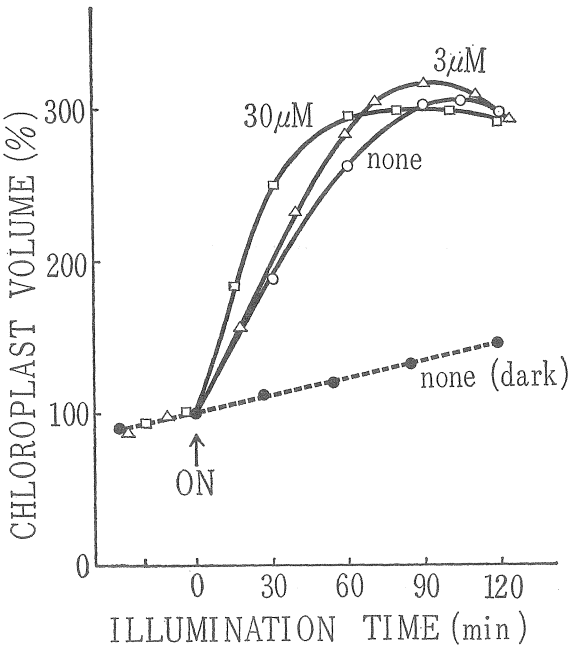


Fig. 4. Effect of ferricyanide and its concentration on photoswelling.

*Effects of inhibitors of electron transport.*

For further investigation on the dependency of the photoswelling upon electron flow activities induced by the action of light, use was made of 3-(3, 4-dichlorophenyl) -1,1-dimethylurea (DCMU) and *o*-phenanthroline as inhibitor of electron transport in photosynthesis. It can be considered that there is no effect of DCMU on the swelling in the dark although the volume in the presence of DCMU was usually similar to, and sometimes slightly higher than, that in control. However, the photoswelling of chloroplasts illuminated in NaCl-Tris solution was suppressed remarkably by DCMU added before illumination. This suppressive effect of DCMU at 10 $\mu$M was reversed virtually by further adding DPIP(33.3 $\mu$M) and ascorbate(666 $\mu$M), which slightly affected on

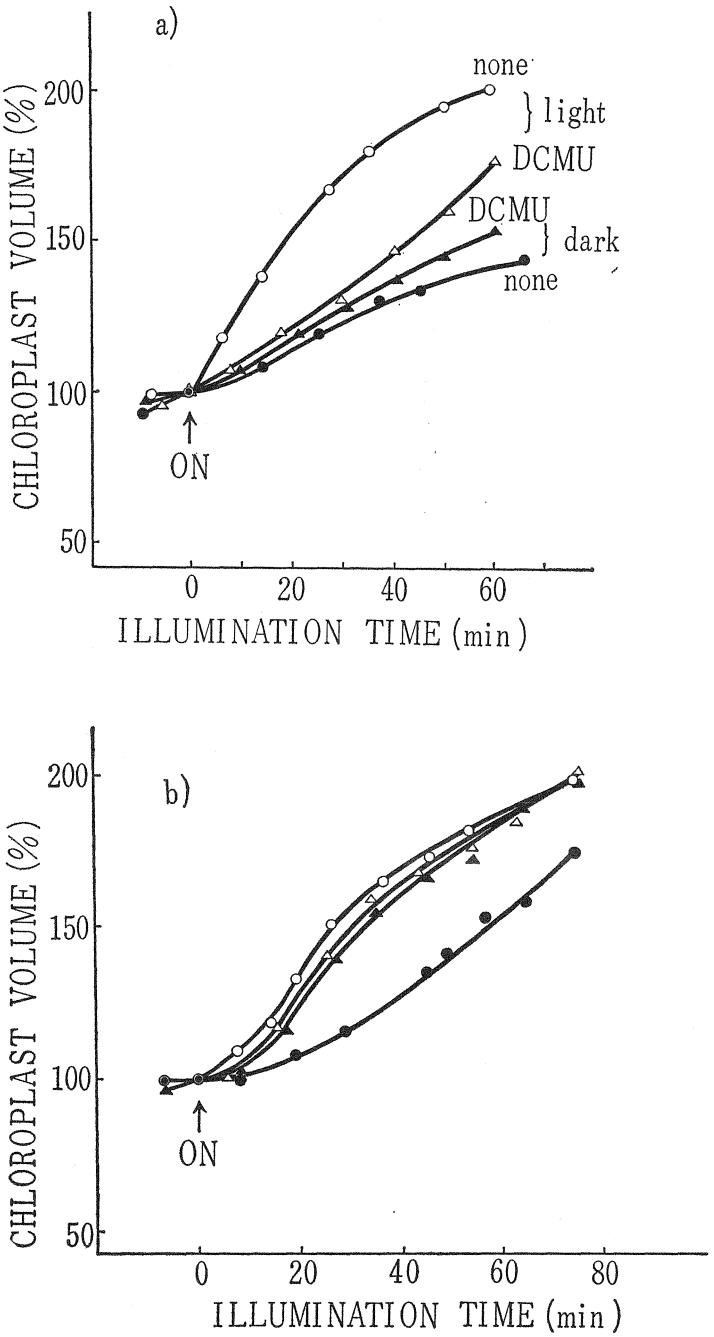


Fig. 5. Effect of DCMU on photoswelling. a) Inhibition by DCMU at 10 $\mu$M. b) Reverse of its inhibition by DPIP and ascorbate. DCMU, 10 $\mu$M; DPIP, 33.3 $\mu$M; ascorbate, 666 $\mu$M, are added. DCMU-DPIP-ascorbate (▲), DPIP-ascorbate (△), DCMU (●), none (○).

the photoswelling. These data are shown in Fig. 5. On the other hand, in the presence of *o*-phenanthroline the photoswelling was affected acceleratively rather than suppressively. When DPIP and ascorbate was added to *o*-phenanthroline, the photoswelling was enhanced (Fig. 6).

These different effects between DCMU and *o*-phenanthroline were investigated on the photoswelling accelerated by PMS. DCMU suppressed the photoswelling accelerated by PMS and approximate 80 per cent of its suppression was reversed by adding further DPIP and ascorbate (Fig. 7). *o*-Phenanthroline also showed similar influence on the photoswelling by PMS. But the reversal extent by further adding DPIP and ascorbate was larger than that in the presence of DCMU, and the volume reversed was equal to, or rather larger than, the volume in the presence of PMS alone (Fig. 8). The photoswelling in isoiated chloroplasts was closely related in the activities of cyclic and non-cyclic electron flow triggered by light. We are greatly interested in different influ ence between DCMU and *o*-phenanthroline on the photoswelling with or without PMS.

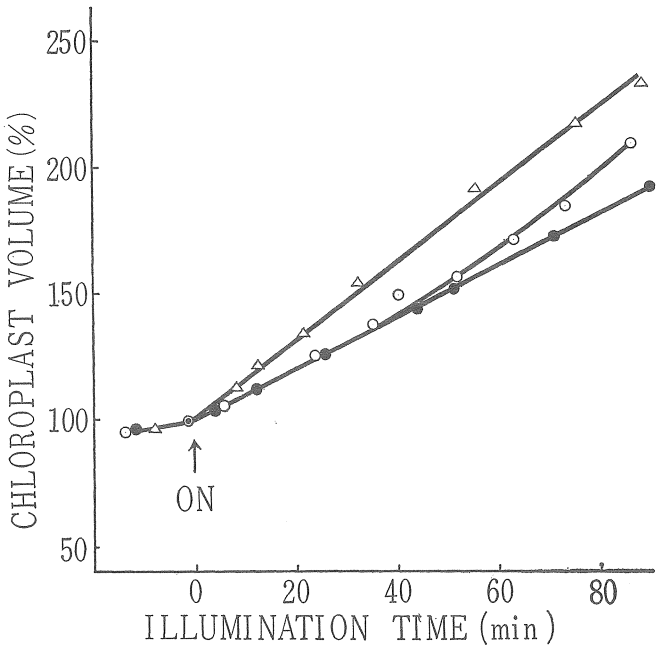


Fig. 6 Enhancement of photowelling by combination of *o*-phenanthroline, DPIP and ascorbate. *o*-phenanthroline, 1 mM; DPIP, 33.3 μM; ascorbate, 666 μM, are added. *o*-phenanthroline (○), none (●). *o*-phenanthroline+DPIP+ ascorbate (△).

*Effects of inhibitors in photophosphorylation.*

The photoshrinkage or light-induced low-amplitude shrinkage, which is an opposite volume change to photoswelling, is also affected by the activities of electron transport, and is suppressed by inhibitors of photophosphorylation (*8-14*). If photcswelling

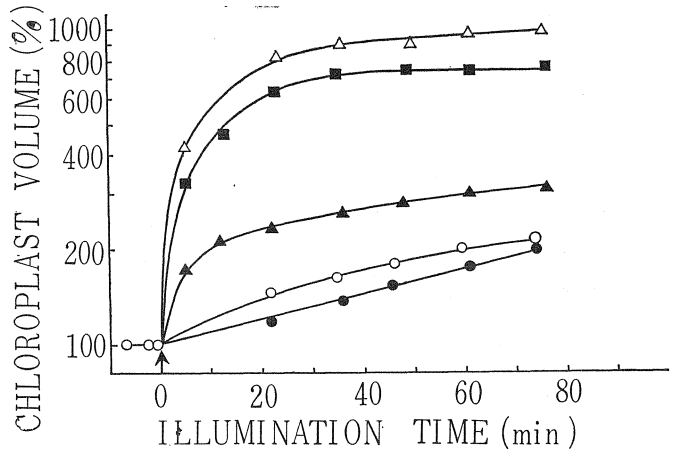


Fig. 7. Inhibition of DCMU on photoswelling by PMS, and reverse of its inhibition by adding DPIP and ascorbate.
Added: PMS, 30 $\mu$M; DCMU, 50 $\mu$M; DPIP, 33.3 $\mu$M; ascorbate, 666 $\mu$M. PMS−DCMU− DPIP−ascorbate (■), PMS−DCMU (▲), PMS (△), DCMU (●), none (○).

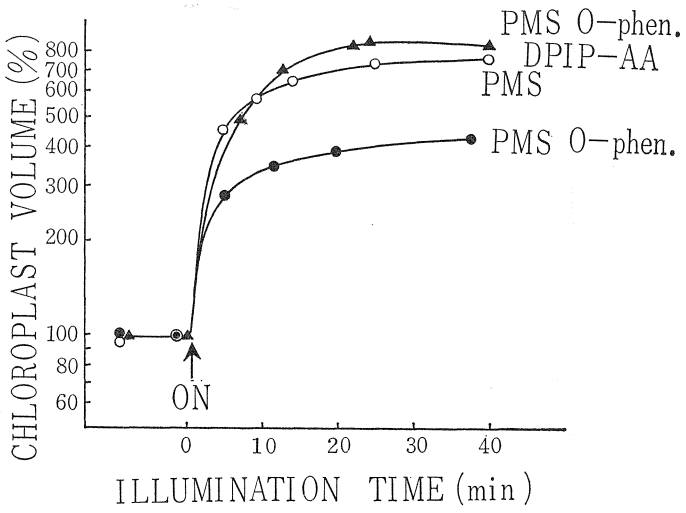


Fig. 8. Inhibition of *o*-phenanthroline on photoswelling by PMS, and reverse of its inhibition by adding DPIP and ascorbate. Added: *o*-phenanthroline, 1 mM; PMS, 30 $\mu$M; DPIP, 33.3 $\mu$M, ascorbate, 666 $\mu$M.

compete with photoshrinkage, the rate and extent of photoswelling may be stimulated by adding uncouplers or inhibitors of photophosphorylation in the chloroplast suspension.

Ammonium chloride was found to have no effect on the light-induced high-amplitude swelling in chloroplasts (*2*). In our previous investigation (*1*), it was shown that ammonium chloride had no effect on the pattern of the time course for the light-induced volume changes of chloroplasts suspended in NaCl-Tris solution. But these data show that ammonium chloride stimulate slightly the rate and extent of the volume changes. Therefore, further detail experiments were performed on this effect. Ammonium chloride stimulated the photoswelling of chloroplasts illuminated in NaCl-Tris solution with increase of its concentration(Fig. 9). Thus enhancement of photoswelling was also obtained by adding methylamine to the suspension. This enhancement effect of ammonium chloride or methylamine obviously strengthened photoswelling when electron flow cofactors were added into chloroplast suspension with these uncouplers. Figure 10 shows the effect of PMS on the photoswelling induced by methylamine, and Fig. 11 shows the effect of DPIP and ascorbate on the photoswelling induced by ammonium chloride.

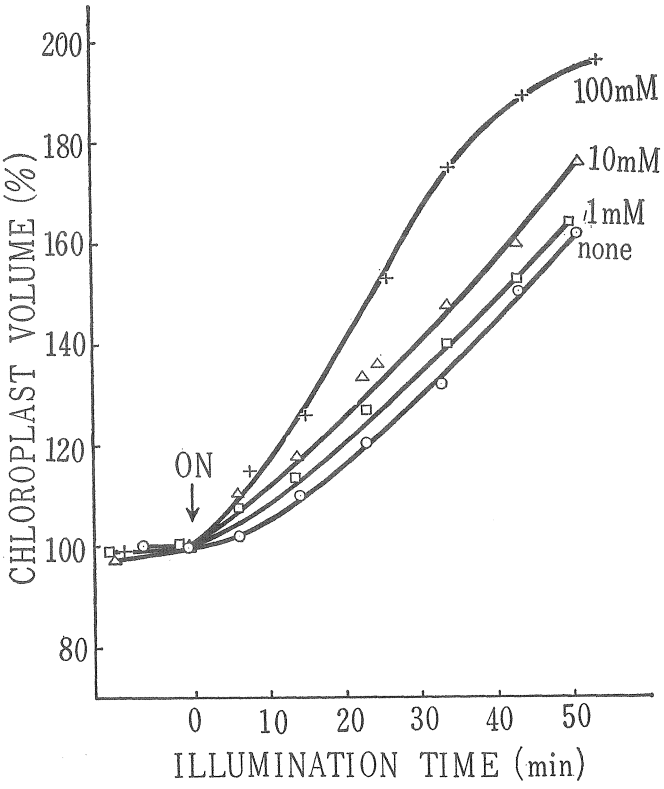


Fig. 9. Effect of ammonium chloride and its concentration on photoswelling.

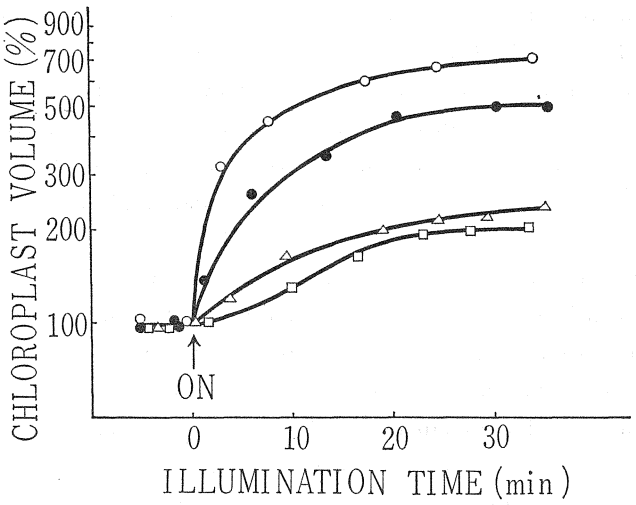


Fig. 10. Enhancement of photoswelling with or without PMS by methylamine. Added: PMS, 30 $\mu$M, methylamine, 100 $\mu$M. PMS+methylamine (○), PMS (●). methylamine (△), none (□).

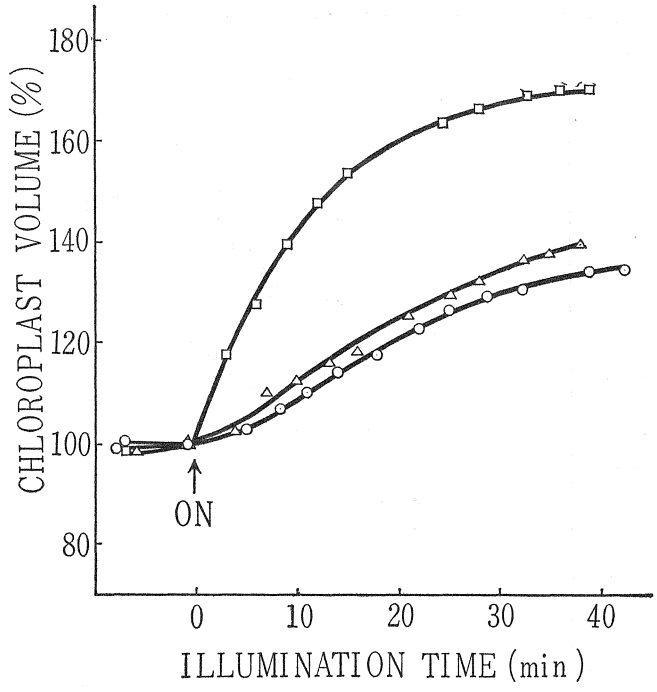


Fig. 11. Effect of ammonium chloride and DPIP and ascorbate on photoswelling. Added: ammonium chloride, 10 mM; DPIP, 33.3 $\mu$M; ascorbate, 666 $\mu$M. $NH_4Cl$–DPIP–ascorbate (□), DPIP–ascorbate (△), none (○).

Dicumarol has been known as an uncoupler of oxidative phosphorylation, which inhibits electron flow at higher concentration as well, and also has been found to be an uncoupler of photophosphorylation(*14*, *16*). This compound, therefore, was tested for its effect on the volume changes of chloroplasts illuminated in NaCl-Tris solution (Fig. 12). No significant difference in the rate and extent of the photoswelling was observed between chloroplasts without dicumarol and with at lower concentration (10$\mu$M). But dicumarol at 50 and 100 $\mu$M increased the rate and extent of the photoswelling. The enhancement effect of dicumarol at 50 $\mu$M above, which agreed with uncoupling concentration (50 $\mu$M) reported by HIND and JAGENDORF(*13*), is considered as resulting from inhibition of endogenous photophosphorylation in chloroplasts.

Quinacrine, one of uncouplers acting at terminal step in ATP formation, was used in experiments on the volume changes of chloroplasts suspended in NaCl-Tris solution. The results are shown in Fig. 13. Incubating with quinacrine in the dark, the swelling of chloroplasts was accelerated by increasing its concentration, and then the swollen chloroplasts by this uncoupler shrunk remarkably upon illumination. The rate of shrinkage under illumination was reduced with increase of quinacrine concentration. The similar effect of this uncoupler has been reported by IZAWA and GOOD(*18*) with electron microscopy on the volume changes of chloroplasts suspended in tricine-NaOH (0.05 M) and sucrose (8 mM). When the higher concentration of quinacrine (1 mM) was added, chloroplasts maintained only the rate of dark swelling accelerated by this

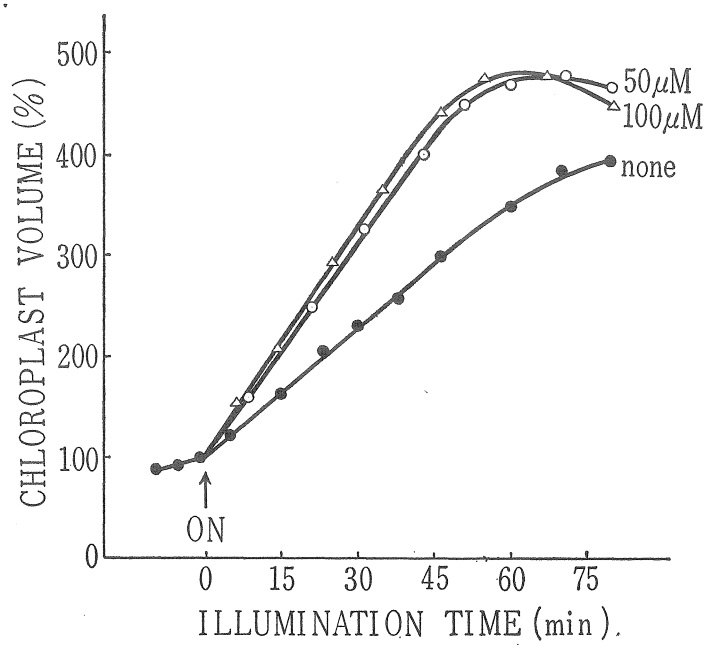


Fig. 12. Effect of dicumarol and its concentration on photoswelling.

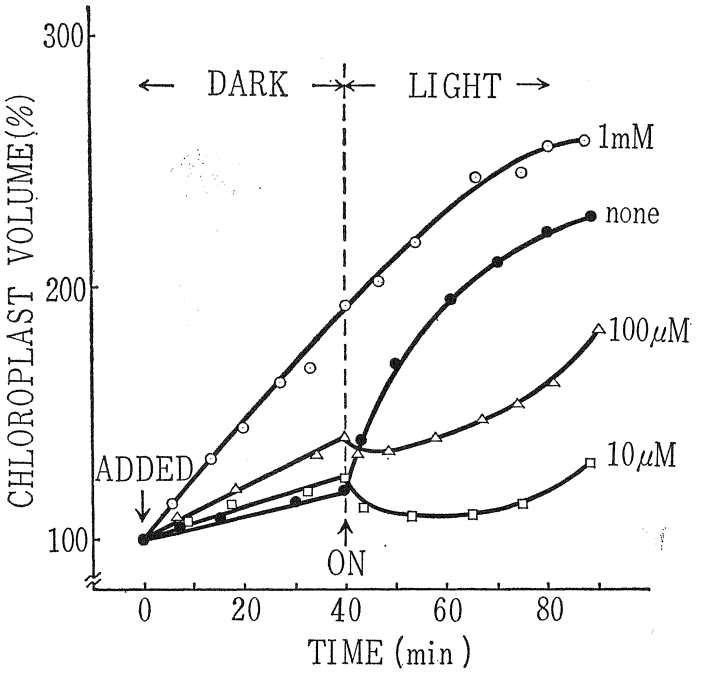


Fig. 13. Effect of quinacrine and its concentration on volume changes in the dark and light.

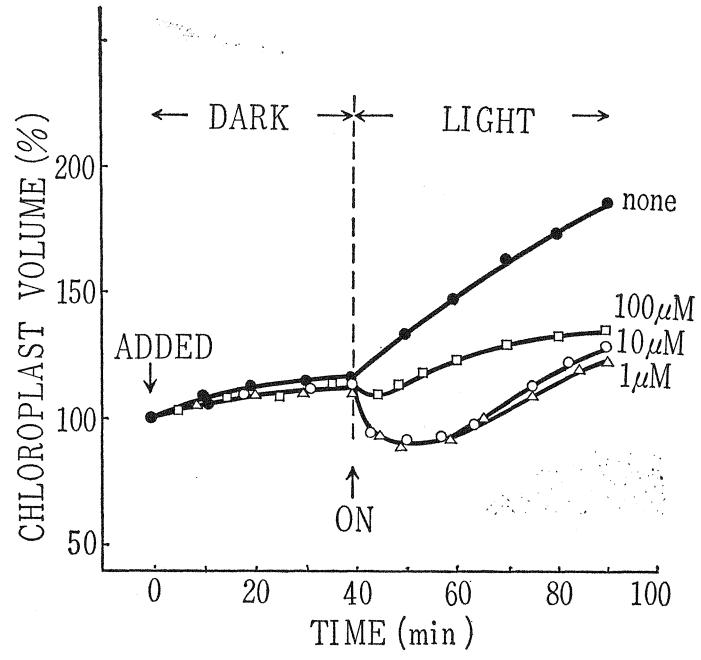


Fig. 14. Effect of phenylmercuric acetate and its concentratration on the volume changes in the dark and light.

uncoupler and the response to the light disappeared(Fig. 13).

It has been proposed by SIEGENTHALER(*14*) that phenylmercuric acetate(PMA) acted like quinacrine at a site beyond the formation of high energy intermediates from investigation of the action of PMA on light-induced shrinkage and on photosynthetic reactions in spinach chloroplasts. For the purpose of comparison with effect of quinacrine, the influence of PMA was examined in the volume changes of chloroplasts suspended in NaCl-Tris solution. The results are shown in Fig. 14. PMA had no effect on the dark swelling, even in higher concentration of this uncoupler. This is distinct from the effect of quinacrine. Like quinacrine, however, PMA suppressed photoswelling under the light condition; that is, when PMA was added, chloroplasts shrunk rapidly upon illumination and the extent of shrinkage was larger rather than that in the presence of quinacrine. The extent of shrinkage by PMA was essentially identical at 1 and 10 $\mu$M, but reduced with increasing its concentration. The shrinkage by PMA was scarcely observed at 100 $\mu$M though photoswelling was still suppressed. The shrinking effect of PMA in the light has already been reported by SIEGENTHALER and PACKER(*5*) using packed volume method.

The suppressive effect of PMA and quinacrine on photoswelling can be considered

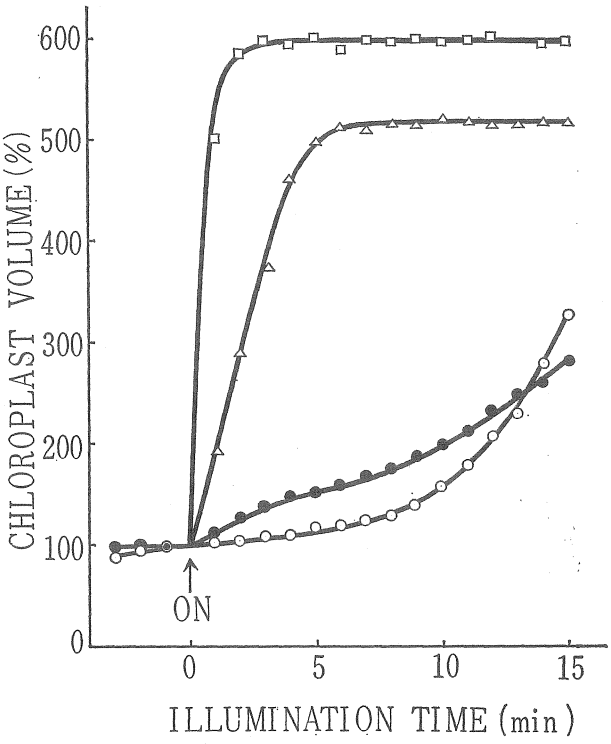


Fig. 15. Effect of quinacrine on the photoswelling with or without PMS. Added: PMS, 30 $\mu$M, quinacrine, 100 $\mu$M. quinacrine −PMS (□), PMS(△), quinacrine (○),none (●).

to be that these uncouplers cause accumulation of high energy intermediates (or states) in chloroplasts and consequently promote shrinkage, since photoshrinkage is related to the formation of high energy intermediates(or states) preceding ATP synthesis (*10*, *14*, *15*). This suggestion has been proposed by SIEGENTHALER (*14*) and DILLEY and VERNON (*10*). Their experiments were performed by using light-scattering method in chloroplasts with PMS(*14*) and trimethyl-1,4-benzoquinone(*10*) as electron transport cofactors but without photophosphorylation cofactors. The experiments, therefore, were carried out with coulter counter in order to confirm the effect of these uncouplers on the volume changes of chloroplasts with PMS as electron flow cofactors. Unexpectedly, as shown in Fig. 15 and 16, the different effect between PMA and quinacrine was found on the volume changes in the presence of PMS. When PMS and PMA were combined, chloroplasts shrunk more greatly than the volume in the presence of PMA alone. While the shrinking effect of quinacrine disappeared by addition of PMS, on the contrary, quinacrine enhanced the rate and extent of the photoswelling which was accelerated by PMS. The results with coulter counter agreed as to the effect of PMA with the observation using light-scattering(*14*), but disagreed as to the effect of quinacrine with observation using light-scattering(*10*, *17*). This discrepancy may be

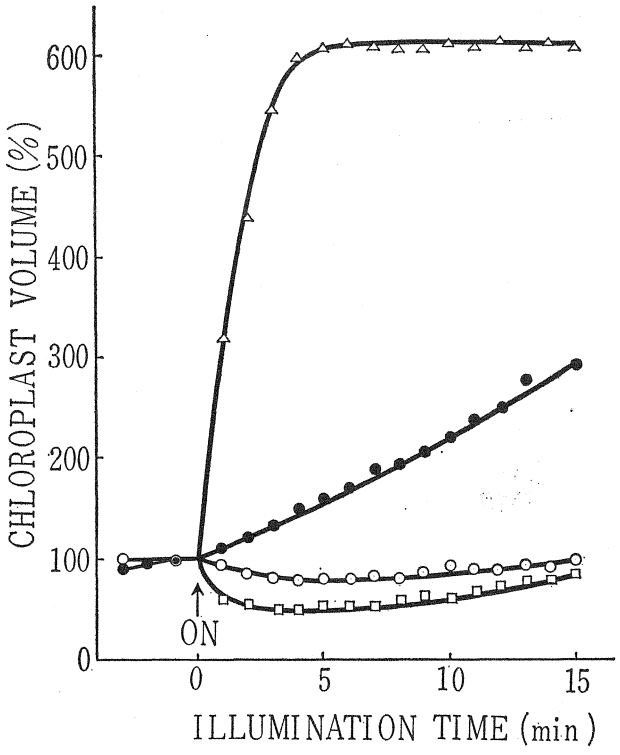


Fig. 16. Effect of phenylmercuric acetate on photoswelling with or without PMS. Added: PMS, 30 $\mu$M. PMA, 50$\mu$M. PMS −PMA (□), PMS (△), PMA (○) none (●).

due to the difference of method measuring the volume or of electron transport cofactor used. The chloroplast volume itself can be directly determined by coulter counter rather than by light-scattering method. Therefore, in view of the fact that the two oppositing volume changes of chloroplasts occured upon illumination when PMS was combined with PMA or quinacrine, it is assumed that PMA and quinacrine operate with a different mechanism in certain situations in which photosynthetic activities are concerned in the volume changes of chloroplasts.

## DISCUSSION

The photoswelling of chloroplasts in the medium without the cofactors of electron transport as shown in this paper has been found in our previous experiments(*1*) and also by PACKER *et al*(*2*), and SIEGENTHALER and PACKER(*5*) under the same conditions. These results suggest that photoswelling may be induced by endogenous photochemical systems in chloroplasts. Furthermore, the facts that photoswelling is affected by exogenous photochemical cofactors or inhibitors, indicate the dependence of photoswelling upon the photochemical activities triggered by the action of light. The importance of electron transport on photoswelling was confirmed by NISHIDA(*3*) and SIEGENTHALER and PACKER(*5*) although no kinetic study was performed on the relationships between photoswelling and electron transport systems in chloroplasts.

In our experiments photoswelling was stimulated by adding the electron flow cofactors, such as PMS, FMN, indigo carmine and ferricyanide; above all, the enhancement by PMS was specifically conspicuous in comparison with the effect of other cofactors. It has been found by NISHIDA *et al*(*1*) and PACKER *et al*(*2*) that PMS is an activator of photoswelling. Their experiments were performed at concentration of 20 $\mu$M and 30 $\mu$M, while our experiments showed that the enhancement by PMS was caused at more dilute concentration of 0.3 $\mu$M (Fig. 1). This result suggests that photoswelling has specific sensibility for PMS, and hence it was used in the experiments performed to compare the photoswelling by various inhibitors of photosynthesis. When DCMU or *o*-phenanthroline was added, the photoswelling by PMS was partially suppressed but still stimulated greater than the photoswelling without PMS. By further addition of DPIP and ascorbate, the suppression of DCMU or *o*-phenanthroline was reversed to the near level of photoswelling with PMS only. This suggests that photoswelling may depend on both cyclic and non-cyclic electron transport.

However, no significant difference was observed between the enhancement effect of FMN and ferricyanide, and vitamin $K_3$ had no effect on the photoswelling (Fig. 2 and 3). The difference of reversal extent by adding DPIP and ascorbate was found on the photoswelling with PMS between in the presence of DCMU and *o*-phenanthroline

(Fig. 7 and 8). It is unreasonable as for influence of PMS on photoswelling to think that PMS serves only as a cofactor in cyclic electron transport in chloroplasts. PMS is a non-physiological cofactor in distinction from FMN and vitamin $K_3$ and hence PMS has a posibility of service as a fast bypass for electron transport around the site which is rate-limiting in the system. The reason why PMS is sensitive to photoswelling and the different effect of DCMU and *o*-phenanthroline on the photoswelling with or without PMS, can not be clear from the results of our experiments but is an interesting problem as to elucidating the mechanism of photoswelling.

When uncouplers of photophosphorylation, such as ammonium chloride, methylamine and dicumarol, were added to chloroplast suspension, photoswelling was enhanced with increase of their concentration, and was more noticeably enhanced in the presence of electron transport cofactors (Fig. 10 and 11). The enhancement effect of ammonium chloride has already been observed by Nobel (*19*) using the packed volume method, and the effect of methylamine has been observed by Izawa and Good (*17*, *18*) with light-scattering and electron microscopy. These uncouplers, in general, inhibit photophosphorylation while stimulating the electron transport in chloroplasts. The enhancement effects of the uncouplers, therefore, were probably caused by acceleration of electron transport, and by uncoupling the phosphorylation in chloroplasts.

If photoswelling compete with photoshrinkage, which is associated closely with the formation of high energy intermediates(or states) preceding ATP synthesis (*see review 20*), photoswelling may be reduced by quinacrine and PMA, which inhibit the terminal step(s) in ATP formation but not inhibit the high energy intermediates(or states) formed in a prior reaction. This suggestion was confirmed from facts that shrinkage of illuminated chloroplasts in the presence of these uncouplers was measured with coulter counter (Fig. 13 and 14), as observed with packed volume(*5*), light-scattering(*17*) and elecron microscopy(*18*). The idea has also been proposed by Dilley and Vernon(*10*) and Siegenthaler(*14*) by measuring with light-scattering method the shrinkage of chloroplasts in the presence of electron transport cofactor and quinacrine or PMA. However, when chloroplast volume was measured with coulter counter, the illuminated chloroplasts in the simultaneous presence of PMS and quinacrine swelled greatly more than the volume in the presence of PMS alone (Fig. 15). The discrepancy may be caused by difference in the method measuring the volume or in the electron transport cofactor used, that is, PMS and trimethyl-1,4-benzoquinone (*10*) and ferricyanide(*17*). While PMA was enhanced shrinkage with or without exogenous electron transport cofactors in different method measuring the volume (Fig. 16, *ref. 5* and *14*). It may be assumed that PMA and quinacrine have dissimilar effects on a mechanism of photoswelling rather than photophosphorylation mediated by PMS.

The chloroplasts isolated by NaCl-Tris solution was the stripped type, which have lost their bounding membranes (outer limiting membranes) and a considerable portion

of their stroma, but which have retained the fretwork of their lamellar systems intact, as reported by LEECH(*22*). MURAKAMI and NOBEL(*23*) have elucidated with electron microscopy that the swelling of spinach chloroplasts suspended in Tris-HCl buffer with NaCl was caused by swelling thylakoids in the lamellar systems, and that this type of swelling was much more pronounced upon illumination. Mechanisms of volume changes in chloroplasts has been considered by which light, the electron- and energy-transfer reactions initiated, and the products formed can control the movement of water and ions(*2*, *5*, *17*, *21*). Therefore, mechanisms of photoswelling can be considered to depend on the results that ions and salts are influxed by coupling with the light-induced electron and energy transfer activities into lamellar systems, and consequently water osmotically is influxed into them.

Several investigations under conditions causing photoswelling have been reported on the light-induced uptake of calcium and strontium(*25*, *26*) and ammonium ions (*27*). There are no investigations proving directly the light-induced uptake of sodium and chloride ions which are component in suspending medium used here.

However, NOBEL and PACKER(*28*) studied on the light-induced uptake of various ions in chloroplasts suspended in 175 mM KCl, 50 mM Tris-HCl(*p*H 7.5), 5 mM $MgCl_2$, 10 $\mu$M PMS, 3 mM ATP and 5 mM reduced lipoic acid. They found that sodium ion uptake depend on adenosine triphosphatase activities triggered by light. Their medium used perhaps appear to cause the swelling of chloroplasts upon illumination. MURAKAMI and NOBEL(*23*) proposed that photoswelling base on a hydrogen-sodium exchange with light-induced electron transport systems from resarch of the role of lipid components in the conformation of the membrane structure of isolated chloroplasts. The light-induced chloride ion uptake would be expected if the essential accompaniment of a proton extruded to one side of a membrane is an anion transported to the same side simultaneously, although PACKER *et al*(*21*) have put a question mark, in their unified mechanism, against the light-induced uptake of chloride ion by the chloroplast membrane. Basically, the swelling occurring in a strong elctrolyte would be due to the uptake of anion, accompanied by the movement of proton, by the appropriate redox system in the chloroplast membrane.

From the results described above, it can be considered that photoswelling in NaCl-Trissolution presented here may depend on light-induced uptake of sodium and chloride ions, which may be affected noticeably by activities of PMS-mediated electron transport in chloroplasts. Moreover, it may be assumed that PMA and quinacrine have dissimilar effects on the light-induced ion uptake depending on the utilization of energy transfer systems.

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## SUMMARY

The effects of cofactors and inhibitors of photosynthesis on the photoswelling in chloroplasts suspended in Tris-HCl buffer containing 0.3 M NaCl was investigated by determining the volume with a coulter counter. Photoswelling was stimulated by adding the electron transport cofactors, such as phenazine methosulfate(PMS), flavin mononucleotide, indigo carmine and ferricyanide; above all, the enhancement by PMS was specifically conspicuous in comparison with the effect of other cofactors. Vitamin $K_3$ had no effect on the photoswelling. The photoswelling in the presence of PMS was partially suppressed when 3-(3,4-dichlorophenyl)-1,1-dimethylurea or *o*-phenanthroline was added, and this suppression was reversed by further addition of 2,6-dichlorophenol indophenol and ascorbate. Ammonium chloride, methylamine and dicumarol enhanced photoswelling with increase of their concentration and enhanced more noticeably in the presence of PMS. Phenylmercuric acetate(PMA) and quinacrine suppressed photoswelling in the absence of electron transport cofactors. However, in the presence of PMS, quinacrine enhanced photoswelling while PMA induced markedly photoshrinkage rather than photoswelling. The relationship between photoswelling and photochmeical reactions in chloroplasts was discussed.

## REFERENCS

(*1*) K. NISHIDA, N. TAMAI and K. RYOYAMA. 1966. Light-induced high-amplitude swelling and shrinking in isolated spinach chloroplasts. *Plant & Cell Physiol.*, **7**, 415-428.

(*2*) L. PACKER, P. A SIEGENTHALER and P. S. NOBEL. 1965. Light-induced volume changes in spinach chloroplasts. *J. Cell Biol.*, **26**, 593-599.

(*3*) K. Nishida. 1968. Effects of inhibitors on the volume changes of isolated chloroplasts upon illumination. In *Comparative Biochemistry and Biophysics of Photosynthesis*. Univ. Tokyo Press, Tokyo. pp. 97-105.

(*4*) L. PACKER and P. A. SIEGENTHALER. 1965. Light-dependent volume changes and reactinos in chloroplasts. II. Action of anions. *Plant Physiol.*, **40**, 1080-1085.

(*5*) P. A. SIEGENTHALER and L. PACKER. 1965. Light-dependent volume changes and reactions in chloroplasts. I. Action of alkenylsuccinic acids and phenylmercuric acetate and possible relation to mechanism of stomatal control. *ibid.*, **40**, 785-791.

(*6*) D. I. ARNON. 1949. Copper enzyme in isolated chloroplasts. Polyphenolase in *Beta vulgaris*. *Plant Physiol.*, **24**, 1-15.

(*7*) G. BRECHER, E. F. JAKOBEK, M. A. SCHEIDERMAN, G. Z. WILLIAMS and P. J. SCHMIDT. 1962. Size distribution of erythrocytes. *Ann. New York Acad. Sci.*, **99**, 242-261.

(*8*) M. ITOH, S. IZAWA and K. SHIBATA. 1963. Shrinkage of whole chloroplasts upon illumination. *Biochim. Biophys. Acta*, **66**, 319-327.

(*9*) L. PACKER. 1963. Structural changes correlated with photochemical phosphorylation in chloroplast membranes. *ibid.*, **75**, 12-22.

(*10*) R. A. DILLEY and L. P. VERNON. 1964. Changes in light-absorption and light-scattering

properties of spinach chloroplasts upon illumination : Relationship to photophosphorylation. *Biochemistry*, **3**, 817-824.

(*11*) L. PACKER and R. H. MARCHANT. 1964. Action of adenosine triphosphate on chloroplast structure. *J. Biol. Chem.* **239**, 2061-2069.

(*12*) G. HIND and A. T. JAGENDORF. 1965. Light scattering changes associated with the production of a possible intermediate in photophosphorylation. *ibid.*, **240**, 3195-3201.

(*13*) G. HIND and A. T. JAGENDORF. 1965. Effect of uncouplers on the conformational and high energy states of chloroplasts. *ibid.*, **240**, 3202-3209.

(*14*) P. A. SIEGENTHALER. 1966. Phenylmercuric acetate and phosphate control of light-dependent shinkage and reactions in chloroplasts. *Physiol. Plantarum*, **19**, 437-447.

(*15*) L. PACKER. 1963. Control of chloroplast structure by adenosine triphosphate. In *Photosynthesis Mechanism in Green Plants. Natl. Acad. Sci.* and *Natl. Res. Council.*, pp. 587-598.

(*16*) A. T. JARGENDORF and J. NEUMANN. 1965. Effect of uncouplers on the light-induced *p*H rise with spinach chloroplasts. *J. Biol. Chem.*, **240**, 3210-3214.

(*17*) S. IZAWA and N. E. GOOD. 1966. Effect of salts and electron transport on the conformation of isolated chloroplasts. I. Light-scattering and volume changes. *Plant Physiol.*, **41**, 533-543.

(*18*) S. IZAWA and N. E. GOOD. 1966. Effect of salts and electron transport on the conformation of isolated chloroplasts. II. Electron microscopy. *ibid.*, **41**, 544-552.

(*19*) P. S. NOBEL. 1965. Light-induced size changes, charge, and ion translocation of spinach chloroplasts in vitro. *Doctral Dissertation*, University of California(Berkeley).

(*20*) L. PACKER and P. A. SIEGENTHALER. 1966. Control of chloroplast structure by light. *Int. Rev. Cytology*, **20**, 97-124.

(*21*) L. PACKER, D. W. DEAMER and A. R. CROFTS. 1967. Conformational changes in chloroplasts. *Brookhaven Symp. Biol.*, **19**, 281-302.

(*22*) R. M. LEECH. 1966. Comparative biochemistry and comparative morphology of chloroplast isolated by different methods. In *Biochemistry of Chloroplasts.* vol. **1**. edt. T. W. GOODWIN, Academic Press, pp. 65-74.

(*23*) S. MURAKAMI and P. S. NOBEL. 1967. Lipid and light-dependent swelling of isolated chloroplasts. *Plant & Cell Physiol.*, **8**, 657-671.

(*24*) P. S. NOBEL and L. PACKER. 1964. Energy-dependent ion uptake in spinach chloroplasts. *Biochim. Biophys. Acta*, **88**, 453-455.

(*25*) P. S. NOBEL, S. MURAKAMI and A. TAKAMIYA. 1966. Localization of light-induced strontium accumulation in spinach chloroplasts. *Plant & Cell Physiol.*, **7**, 236-275.

(*26*) P. S. NOBEL and S. MURAKAMI. 1967. Electron microscope evidence for the location and amount of ion accumulation by spinach chloroplasts. *J. Cell Biol.*, **32**, 209-211.

(*27*) A. R. CROFTS. 1968. Ammonium ion uptake by chloroplasts and the high-energy state. In *Regulatory Functions of Biological Membranes* (B. B. A. Library, vol. **11**) edt. J. JÄRNEFELT. Elsevier Pub. Co. pp. 247-263.

(*28*) P. S. NOBEL and L. PACKER. 1965. Light-dependent ion translocation in spinach chloroplasts. *Plant Physiol.*, **40**, 633-640.