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POLYCATION INHIBITION OF CHLOROPLAST PHOTOSYSTEM I*

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SUMMARY

A variety of large polycations are able to inhibit the Photosystem I activity of chloroplasts which have been exposed to a low salt environment. These swollen chloroplast membranes shrink in response to large polycations and the extent of volume contraction parallels the inhibitory response. Inhibition is prevented by low concentrations of salts and di- and trivalent ions are superior to monovalent ions in protecting the chloroplasts from polycation inhibition. The effect of various ions in preventing inhibition is roughly paralleled by the ability of these ions to induce shrinkage of the swollen chloroplasts. Photosynthetic lamellae which are not stacked in grana *in vivo* are protected from polycation inhibition by salts. Chloroplast membranes pretreated with polyanions are less sensitive to polycation inhibition. Light-driven Photosystem I activity causes insensitivity to polycation inhibition. Inhibition by histone or polylysine can be reversed by protease digestion of the membrane bound polycation. Polycation inhibition can be reversed by the detergent Triton X-100 under circumstances which suggest that the detergent creates a bypass of the polycation inhibited site.

INTRODUCTION

Polycations will completely inhibit the reduction of low potential oxidants by Photosystem I while only partially inhibiting reduction of high potential oxidants which may be reduced by both photosystems[1,2]. Spectroscopic evidence indicates that plastocyanin is the site of polycation inhibition[3]. A necessary precondition for inhibition is that salts be removed from the chloroplast environment before exposure to the polycation. Izawa and Good[4] have demonstrated that removal of salts causes reversable loss of the stacking of chloroplast grana membranes. Murakami and Packer[5] found that light could induce changes in the thickness of the photosynthetic membranes within the grana stacks. We find that both the ionic environment and light induced electron transport influence polycation inhibition. The photosynthetic lamellae of some plants or cell-types are not naturally arranged in grana stacks, but polycation inhibition and salt protection are observed with agranal chloroplasts

Abbreviations: TPCK-trypsin, L-(tosylamido-2-phenyl) ethyl chloromethyl ketone-treated trypsin to destroy contaminant chymotrypsin; TMPD, *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine dihydrochloride; MES, 2-(*N*-morpholino)ethanesulfonic acid.

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from several higher plant species. A variety of basic proteins can cause polycation inhibition and a variety of small molecules can prevent this inhibition. A systematic study of both inhibition and its prevention or reversal gives insights into the nature of the chloroplast membrane surface and the environment of the inhibitor susceptible catalyst in Photosystem I.

METHODS

Procedures for the isolation of spinach chloroplasts and for measurement of Photosystem I activity have been described earlier[2]. Chloroplast volumes were measured as described by Izawa and Good[6]. Bundle sheath chloroplasts from *Sorghum bicolor* were isolated by the procedure of Anderson *et al.*[7]. Corn seedlings were germinated and grown according to O'Neal *et al.*[8]. Chloroplasts were isolated from corn seedlings and from tobacco leaves by the method of Arntzen *et al.*[9]. Photosynthetic lamellae from blue-green algae were prepared by the method of Lee *et al.*[10].

Seeds of *Nicotiana*, John Williams Boardleaf and the Su/su variety containing agranal chloroplasts were the generous gift of Dr H. Menser of U.S.D.A., A.R.S., Beltsville, Md., U.S.A.[11]. Histones, polylysines, lysozyme Grade 1, horse heart cytochrome *c* Type III, ribonuclease, spermine, spermidine, lecithin, Triton X-100, DNA, bovine serum albumin fatty acid free, trypsin, trypsin inhibitor soybean Type I, glutathione and dithiothreitol were purchased from Sigma Chemical Co. L-(Tosylamido-2-phenyl) ethyl chloromethyl ketone-treated trypsin (TPCK-trypsin) was obtained from Worthington Biochemicals and tripalmitin from Calbiochem. Polygalacturonic acid, cysteine and cystine were purchased from Nutritional Biochemicals.

Occasionally, histone solutions which had been stored at 4 °C for several days would loose their inhibitory activity. Further storage gave precipitation of the protein. If these solutions were heated briefly at 100 °C, the histone redissolved and inhibitory activity was restored.

Other reagents were prepared or purchased as previously described[2]

RESULTS

Histone and synthetic polylysine polymers inhibit Photosystem I activity[2]. Fig. 1 shows that a variety of basic proteins inhibit Photosystem I activity when chloroplasts are exposed to these proteins in a salt-free medium. The complete inhibition caused by Type IIA histone can also be achieved with Type III or Type IV histone or with protamine. The basic proteins lysozyme, cytochrome *c* and ribonuclease are inhibitory but only to a limited extent. The polyamine spermine gives only slight inhibition and the smaller polyamines spermidine, lysyllysine and methyllysine do not inhibit. In other measurements, a polylysine polymer of 18 residues was found to inhibit completely while a polymer of 7 lysine residues gave a response like ribonuclease with a maximum inhibition of 30%.

The low salt environment necessary for polycation inhibition results in a swelling of the chloroplast membranes and a loss of the grana stack structure with concomitant increase in the packed chloroplast volume[4,6]. This swelling is reversed by exposure of the membranes to salts, sucrose or to polycations, but the grana

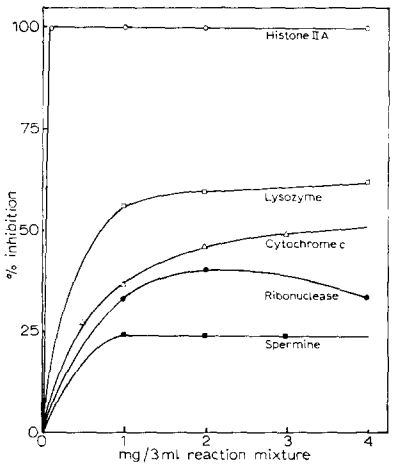


Fig. 1. Inhibition of Photosystem I by polycations. Changes in oxygen content of illuminated reaction mixtures were measured with a Yellow Springs Instrument Co. oxygen monitor equipped with a Clark-type oxygen electrode. The reaction chamber was illuminated with red light (Baird Atomic 680 nm narrow band pass filter) at an intensity of $6 \cdot 10^5$ ergs·cm^{-2}·s^{-1}. The chloroplasts were mixed with the polycation inhibitor prior to the addition of the other reaction components. Mixing the chloroplasts with the polycation before addition of other reaction mixture components is necessary to avoid protection against polycation inhibition by these other reagents. The reaction mixture contained the following in μmoles: sodium ascorbate, 50; TMPD, 0.2; methyl viologen, 0.4; Tris–MES buffer (pH 8.0), 150; and 3-(3,4-dichlorophenyl)-1,1-dimethylurea, 0.03 in a final volume of 3 ml. The reaction mixture also contained chloroplasts equivalent to 15 μg of chlorophyll and the other additions as noted. The chloroplasts had been washed and resuspended in 0.01 M Tris–MES buffer (pH 7.5) to achieve maximum sensitivity to polycation inhibition. The reaction mixtures were equilibrated in the dark with continuous stirring for 1.5 min prior to assay.

TABLE I

POLYCATION INDUCED SHRINKAGE OF SWOLLEN CHLOROPLASTS

The chloroplasts had been washed and resuspended in 0.01 M Tris–MES buffer (pH 7.5) to achieve maximum swelling and susceptibility to polycation effects. An aliquot of chloroplasts equivalent to 0.2 mg chlorophyll was mixed with salt, sucrose or 2 mg of the polycation to be tested in a final volume of 0.4 ml. Samples were then centrifuged in an hematocrit centrifuge for 20 min.

	Relative packed volume
Control	100
0.25 M KCl	20
0.2 M Sucrose	21
Histone	24
Lysozyme	45
Ribonuclease	56
Cytochrome *c*	56
Polylysine, mol. wt 3600	50
Polylysine, mol. wt 194000	25
Polylysine, mol. wt 400000	20

stacking is not reconstituted by sucrose alone[6]. The shrinkage effects are documented in Table I. It is apparent from these data that the extent of shrinkage caused by basic proteins roughly parallels the extent of inhibition by these polycations. While the three polylysines used in this study cause complete inhibition, their effectiveness on a molar basis is proportional to the polymer size. With the polylysines a relation of molecular size to the extent of membrane shrinkage is evident.

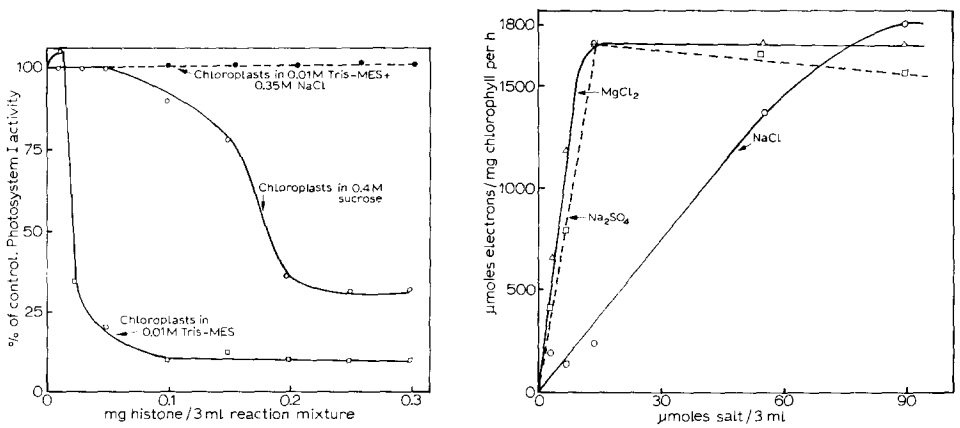


Fig. 2. Histone inhibition in the presence of sucrose or NaCl. The reaction conditions were identical to those described in Fig. 1. When the chloroplasts were mixed with the other reactants and sucrose to give a final concentration of 0.4 M prior to addition of histone, the data described by ○—○ was obtained. If the complete reaction mixture contained 0.35 M NaCl prior to addition of histone, the data described by ●—● were obtained.

Fig. 3. Salt protection against histone inhibition. The reaction conditions were identical to those reported in Fig. 1. The chloroplasts were first mixed with the specified concentration of salt, then 0.1 mg of histone Type IIA was added followed by all of the other reaction mixture components. ○—○, protective effect of NaCl; □---□, protective effect of Na_2SO_4; △—△, protective effect of $MgCl_2$.

The ability of sucrose to cause a reduction in packed chloroplast volume like that achieved with NaCl suggests that sucrose, like NaCl, might prevent polycation inhibition. This is not the case as shown by Fig. 2. Here one sees that chloroplasts which have been washed and resuspended in 0.01 M Tris–2-(*N*-morpholino)ethane sulfonic acid (Tris–MES) buffer (pH 7.5) are quite sensitive to histone inhibition. If the chloroplasts are resuspended and assayed in 0.4 M sucrose, they are still sensitive to histone inhibition although the maximum extent of inhibition is reduced to 65% and the concentration of histone needed to achieve this inhibition is doubled. If the chloroplasts are prepared and assayed in the presence of a high concentration of NaCl, histone does not inhibit.

Divalent ions are more effective than monovalent ions in protecting Photosystem I activity from polycation inhibition as can be seen in Fig. 3. Both Na_2SO_4 and $MgCl_2$ give complete protection against histone inhibition at a concentration of 5 mM while 6 times that amount of NaCl is needed to achieve the same protective effect. Other small polyvalent ions are quite effective in protecting Photosystem I from inhibition by large polycations. Relative effectiveness in preventing inhibition

is roughly paralleled by effectiveness in causing reduction in the volume of chloroplast membranes, as seen in Table II.

There are a variety of plant materials in which the photosynthetic membranes are not organized into grana stacks. Table III lists the results of experiments with several agranal preparations. Photosynthetic membranes from the blue-green alga *Anabaena variabilis* are not inhibited by polycations. Chloroplasts from young

TABLE II

SMALL ION EFFECTS ON MEMBRANE VOLUME AND SUSCEPTIBILITY TO POLYCATION INHIBITION

The chloroplast volume measurements were made as described in Table I with an extensive series of concentrations of each of the above compounds. In every case, maximum shrinkage was 60–80% reduction in the original volume. Photosystem I protection was measured as described in Fig. 3.

	Amount for 90% of maximum contraction		*Amount for 90% protection against histone inhibition of Photosystem I*	
	Molarity	*Ionic strength*	*Molarity*	*Ionic strength*
NaCl	0.2	0.2	0.067	0.067
Sodium acetate	0.15	0.15	0.033	0.033
Sodium succinate	0.05	0.15	0.003	0.009
Sodium citrate	0.012	0.072	0.0015	0.009
$MnCl_2$	0.024	0.072	0.003	0.009
Na_2HPO_4	0.024	0.072	0.003	0.009
Na_2SO_4	0.024	0.072	0.003	0.009
$K_3Fe(CN)_6$	0.005	0.030	0.0006	0.0036
Reduced glutathione	0.001	0.001	0.00045	0.00045
Oxidized glutathione	0.001	0.003	0.00045	0.00135

TABLE III

POLYCATION EFFECTS ON PHOTOSYNTHETIC LAMELLAE WHICH DO NOT FORM GRANA *IN VIVO*

Preparative procedures are described in Methods and the assay conditions are those detailed in Fig. 1. Histone inhibition was achieved with 0.1 mg histone Type IIA and salt protection with 200 μmoles NaCl added to the lamellae prior to histone addition.

Type of lamellae	*Photosystem I activity (μmoles O_2 uptake/mg chlorophyll per h)*		
	Control	*Plus histone*	*Plus NaCl, then histone*
Anabaena variabilis	5300	5300	5300
Corn seedlings 1 day after germination	1700	200	2100
Bundle sheath chloroplasts of *Sorghum bicolor*	800	0	1224
Su/su mutant of *Nicotiana tabacum*	5800	420	4100

corn shoot tips, from the bundle sheath cells of *Sorghum bicolor* and from the aurea variety of *Nicotiana tabacum* are all inhibited by histone and this inhibition is prevented by adding NaCl to these membranes prior to exposure to histone.

To probe the possibility that lipids on the chloroplast membrane surface might interact with the polycation inhibitor, an attempt was made to coat that surface or the lipid parts of it with exogenous lipid. Chloroplasts were first resuspended in a low salt medium to unstack the membranes, then were washed through an emulsion

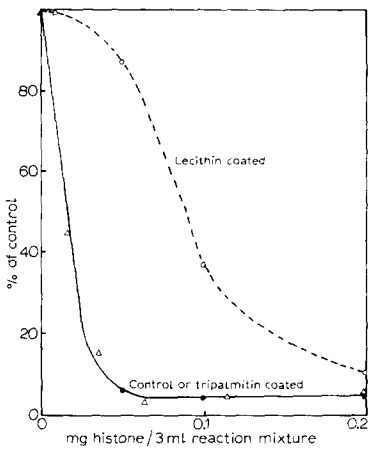


Fig. 4. The effect of lecithin or tripalmitin pretreatment on polycation inhibition of Photosystem I. The chloroplasts were washed and resuspended in 0.01 M Tris–MES buffer (pH 7.5), then a 1-ml sample of these chloroplasts containing 1 mg chlorophyll was added to 25 ml of emulsion containing 0.5 g of lecithin or tripalmitin. The emulsion had been prepared by sonicating the lipid in water until it was uniformly dispersed. The chloroplasts were centrifuged down from the emulsion by spinning at $10000 \times g$ for 10 min. The chloroplast pellet was then resuspended in 0.01 M Tris–MES buffer (pH 7.5). ○---○, lecithin-treated chloroplasts; △—△, tripalmitin-treated chloroplasts; ●—●, control chloroplasts.

of lecithin or tripalmitin. The chloroplast membranes were then resuspended in dilute Tris–MES buffer for assay. While no attempt was made to quantitate the amount of lipid bound to the chloroplast membranes by this treatment, it was clear from the copious white precipitates which appeared in the 80% acetone extracts used for chlorophyll measurements that much lipid had adhered to the membranes. As seen in Fig. 4, tripalmitin had no effect on histone inhibition while the phospholipid lecithin increased slightly the amount of histone needed to inhibit Photosystem I. Results similar to those obtained with lecithin were seen with chloroplast membranes which had been pretreated with DNA or polygalacturonate.

When chloroplast membranes are actively catalyzing the Photosystem I driven transfer of electrons from reduced *N,N,N',N'*-tetramethyl-*p*-phenylenediamine dihydrochloride (TMPD) to methyl viologen, they are resistant to inhibition by polycations. This is illustrated in Fig. 5A. If histone is injected into a reaction mixture actively reducing oxygen in the light, no diminution in the rate of Photosystem I activity is observed. If the chloroplasts are illuminated in the absence of one of the critical components needed for Photosystem I activity—TMPD, methyl viologen

or sodium ascorbate, and then exposed to histone prior to the addition of the missing component, activity is inhibited. This is illustrated in Fig. 5B where the absence of TMPD allows histone to inhibit in the light. Fig. 5C describes a control experiment in which TMPD is added to a reaction mixture after a preillumination period identical to that used in Fig. 5B, showing that histone, not preillumination with an incomplete reaction mixture, is the cause of inhibition.

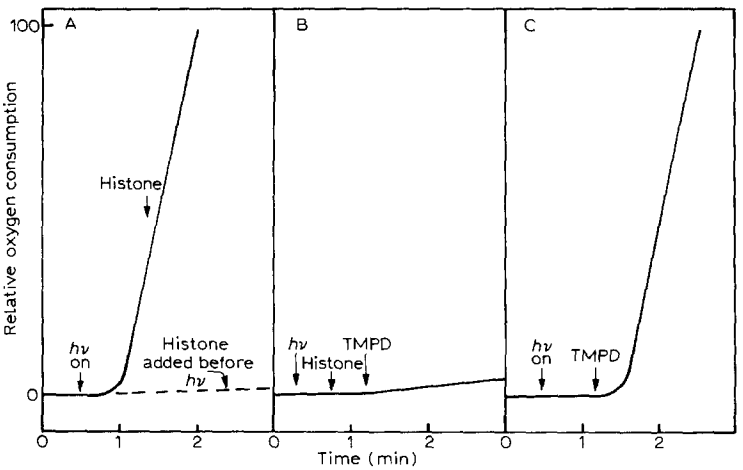


Fig. 5. Reaction conditions are the same as those described for Fig. 1 except that the concentration of sodium ascorbate was reduced to 10 μmoles. Histone (0.1 mg) or TMPD was injected into the reaction cell from a syringe through a Tygon capillary tube. The dashed line in A describes an experiment in which histone was injected just before turning the light on.

While a variety of conditions can prevent inhibition by polycations, reversal of inhibition by removal of the polycation has proved difficult. Washing the inhibited chloroplasts with various salts will not reverse inhibition of Photosystem I. However, polylysine is readily fragmented by trypsin and this enzyme does not destroy Photosystem I activity[12]. Table IV shows data indicating the reversal of polylysine inhibition by trypsin. Photosystem I activity is diminished by trypsin addition but this is due to trypsin acting as a polycation since the inhibitory effect is prevented by prior addition of salt. Polylysine causes inhibition and this can be partially reversed by exposure to trypsin. Other experiments indicated that the presence of salt during trypsin treatment interferes with reversal of inhibition. This seems likely to be the result of salt induced aggregation of the membrane interfering with trypsin accessibility to the polylysine. Trypsin reversal of polycation inhibition was prevented by treating the trypsin with heat or with soybean trypsin inhibitor. Similar experiments on the reversal of histone inhibition were successful only when the TPCK-trypsin was not used. Crystalline trypsin contains other proteases which assist in the dissolution of histone to non-inhibitory fragments. These proteases are destroyed in the TPCK-trypsin which is incapable of rendering histone inactive even when the histone is treated with TPCK-trypsin prior to mixing with chloroplasts.

A second kind of reversal of polycation inhibition of Photosystem I is accomplished with the detergent Triton X-100. Data in Table V show that the addition of

TABLE IV

REVERSAL OF POLYCATION INHIBITION BY TRYPSIN

Reaction conditions are same as those described for Fig. 1. Chloroplasts which had been washed and resuspended in 0.01 M Tris–MES buffer (pH 7.5) were mixed with polylysine, mol. wt 195000, at a ratio of 2 mg per mg chlorophyll. This mixture was divided and trypsin was added to half of it at a ratio of 1 mg trypsin per mg polylysine. All samples were stirred for 1 h then assayed. Controls without polylysine were treated in an identical fashion.

	Photosystem I activity (μmoles O_2 uptake/mg chlorophyll per h)
Control	1750
Control + polylysine	500
Control + polylysine + trypsin	1210
Control + trypsin	1250
Control + NaCl then trypsin	1600

TABLE V

REVERSAL OF POLYCATION INHIBITION BY A DETERGENT

Reaction conditions are the same as those described in Fig. 1. The histone addition was 0.1 mg and the plastocyanin was 2 nmoles.

	Photosystem I activity (μmoles O_2 uptake/mg chlorophyll per h)
Control	1480
Control + histone	180
Control + histone + 0.1% Triton X-100	760
Control, omit TMPD	0
Control, omit TMPD + plastocyanin	0
Control, omit TMPD + 0.1% Triton X-100	800
Control, omit TMPD + 0.1% Triton X-100 + plastocyanin	1600

Triton X-100 to inhibited chloroplast membranes restores Photosystem I activity. A possible explanation is that Triton X-100 exposes a part of the electron transport chain beyond the histone inhibited step to direct reduction by exogenous electron donors. This idea is supported by the data in Table V. Normally, Photosystem I activity is dependent on the presence of TMPD and exogenous plastocyanin will not substitute for it. In the presence of Triton X-100, activity is seen despite the omission of TMPD which is usually required to shuttle electrons in from ascorbate; this activity is stimulated by plastocyanin. Triton X-100 has changed the specificity of the preparation for electron donors.

DISCUSSION

Polycation inhibition of Photosystem I seems to depend on both the size and charge density of the polycation. While a chain of seven lysine residues gave only a partial inhibition even at very high concentrations, a polymer of eighteen residues gave complete inhibition at the same concentration as histones. The basic proteins ribonuclease, cytochrome *c*, trypsin and lysozyme all give partial inhibition. It is noteworthy that prior exposure to lysozyme does not protect the membrane from complete inhibition by subsequent addition of histone. Since DEAE-cellulose will inhibit Photosystem I activity when the exposure is at low salt concentration, it appears that there is no upper limit to the size of the polycations which can inhibit exposed chloroplast membranes.

Izawa and Good[4,6] found that a low-salt environment caused chloroplasts to lose their grana structure and greatly increase their volume. They found that several salts would cause contraction of the swollen membranes. In contrast to the data presented here, Izawa and Good found that sucrose gave a smaller extent of shrinkage than ionic salts. We have found that extent of shrinkage varies from 50 to 80% of the initial volume with swollen membranes from different batches of spinach and so we are hesitant to make precise comparisons with other data. Gross and Packer[13] have identified distinctive osmotic and divalent cation induced shrinkage in fragments of chloroplasts prepared by sonication and these two processes might well vary independently in different preparations. While histone and large polylysines give both complete inhibition and 70–80% shrinkage of chloroplast volume, lysozyme, ribonuclease and cytochrome *c* give only partial inhibition and partial shrinkage. The smaller polylysine, molecular weight 3600, is exceptional in that it can give complete inhibition but only moderate shrinkage in volume. Again both size and charge density of the polycations seem important in inducing contraction of the swollen membranes.

Salt protection against polycations might be due to a restacking of the grana membranes or simply to prior neutralization of charges to which the polycations must bind. Salts can cause restacking of parallel sheets of membrane and the reformation of grana-like structures (refs 6 and 14; Berg, S., Dodge, S., Krogmann, D. W. and Dilley, R. in preparation). Sucrose, while able to shrink the volume of the swollen membranes, does not prevent polycation inhibition and does not induce grana stacking. Further observations on the effects of sucrose, salts and polycations on grana stacking as monitored by electron microscopy will be reported separately. It is important to note that both divalent cations and divalent anions are much more effective than monovalent ions in preventing polycation inhibition. Prevention of inhibition is not simply cation competition for anionic sites on the membranes. Ohki *et al.*[14] have noted that either 5 mM $MgCl_2$ or 100 mM NaCl causes the appearance of groups of parallel membrane sheets in chloroplast preparations lacking grana as a result of exposure to salt-free medium. Since these concentrations correspond rather well with data presented here for salt protection against histone inhibition, it seems likely that salt protection is accompanied by considerable change in membrane conformation. However, Murakami and Packer[15] found that salt induced changes in chloroplast light scattering were cation dependent and that Na_2SO_4 gave responses like those with NaCl while $MgCl_2$ could induce a maximum response

at one fifth the concentration required of the monovalent cations. Thus, all salt effects are not the same. Table II shows an extended list of reagents which give parallel responses in the volume contraction assay and in preventing polycation inhibition. Ionic strength seems to be a critical determinant in these responses. The effects of oxidized and reduced glutathione are probably not due to disulfide–sulfhydryl interactions since dithiothreitol and mercaptoethanol did not prevent polycation inhibition and iodoacetate and *p*-chloromercuribenzoate were effective in preventing inhibition at the same concentration as sodium acetate.

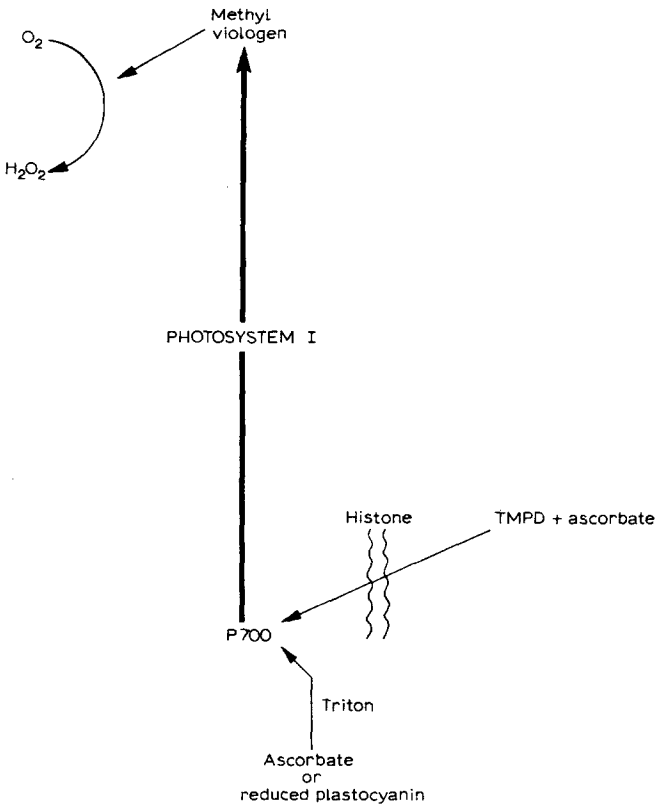
The experiments with agranal chloroplasts suggest that salt protection is achieved without reformation of grana stacks but this point will be clarified in our further studies with electron microscopy. The failure of histone to inhibit Photosystem I activity of blue-green algae may result from the plastocyanin having a positive net charge in these algae[16]. In any case, the inherent differences between agranal and granal chloroplasts do not result in marked differences in polycation susceptibility.

A coating of lecithin on the chloroplast membrane increases the concentration of polycation required for inhibition. Lecithin evidently provides additional anionic groups which compete for the inhibiting polycation but these groups do not prevent the polycation from attaching to the catalytic site which is inhibited. Cohen and Jagendorf[17] have described changes in chloroplasts induced by treatment with large polyanions. When we treated chloroplasts with sodium polygalacturonate or DNA using the same procedure as that used in the lecithin experiment, the same result of raising the histone concentration needed for inhibition was obtained. Thus hydrophilic and hydrophobic anions can bind to the membrane without blocking the polycation from its inhibition site. Tripalmitin, a neutral lipid, has no effect on polycation inhibition as would be expected from a mosaic membrane with distinct hydrophobic and hydrophilic regions.

Light induced changes in the size and shape of chloroplasts have been known for some time[18,19]. Murakami and Packer[15] have made detailed studies of light induced changes of the membrane within the chloroplast. They found that light flattens the membranes and decreases the spacing between thylakoids. Izawa and Good had found that light-driven electron transport produced a marked shrinking of chloroplast membranes which had been swollen in a low-salt environment. The light induced resistance to polycation inhibition is dependent on Photosystem I electron transport. While there are several effects of uncoupling agents on the light induced conformation changes of chloroplasts, we can only note that the uncouplers NH_4Cl and methylamine do not abolish light induced resistance to polycation inhibition. We have found that modification of carboxyl groups by carbodiimide activation and masking with glycine methyl ester prevents polycation inhibition[20]. This phenomenon will be dealt with more fully in a later report. Such results are consistent with the hypothesis that negative charges fixed on the membrane surface are involved in polycation binding and that such binding is essential for observing inhibition of electron transport. Protection against polycation inhibition by light induced electron transport might be due to a light induced alteration or protonation of carboxyl groups. Light induced protonation of negative charge groups has been indirectly demonstrated in previous work[21,22,5].

Selman and Bannister[12] established that trypsin destroys Photosystem II activity

without damaging Photosystem I. While large amounts of trypsin can inhibit the Photosystem I activity of chloroplasts in a low salt environment, this is due to the cationic property of the protein rather than its catalytic activity. Trypsin can cleave polylysine bound to the chloroplast membranes and partially reverse the polylysine inhibition. The presence of salts during trypsin treatment prevents reversal of inhibition perhaps by causing the membranes to aggregate and restack thus preventing the enzyme from reaching the polylysine bound to the membrane surface.



Scheme 1. Triton reversal of polycation inhibition.

Reversal of polycation inhibition by detergents is probably the result of exposure of P700 to direct reduction by added electron donors[23,24]. Scheme 1 illustrates our interpretation. Triton X-100 eliminates the need for TMPD as a catalytic mediator of electron flow between ascorbate and Photosystem I. After addition of Triton X-100 to the chloroplast membranes either ascorbate or reduced exogenous plastocyanin will donate electrons to Photosystem I. Since these activities are expressed in the presence of polycations, the newly exposed electron acceptor is believed to be beyond the polycation inhibition site. This kind of result is best obtained with Triton X-100 although similar if less dramatic results are seen with Tween-20. Neither digitonin nor sodium dodecyl sulfate would work in relieving polycation inhibition.

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