PHOTOSYSTEM I INHIBITION BY POLYCATIONS[1]

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Summary. Histones and other polycations inhibit the photosystem I reactions of isolated chloroplasts. Photosystem II activity is readily measured in preparations where histone has completely blocked photosystem I activity. Histone inhibition is prevented by preincubation of the chloroplasts in solutions containing salt.

A number of specific inhibitors of photosynthetic reactions are now known. Some of these inhibit selected sites in photosystem II (1,2) while others act to uncouple (3) or to block energy transfer (4,5) in photophosphorylation. However, no report has yet appeared on an inhibitor which specifically blocks electron flow through photosystem I. We wish to report here on such an inhibitor.

METHODS

Fresh spinach chloroplasts were prepared by grinding 100 gm of spinach leaves in a Waring blender with 150 ml of a solution containing 0.05M

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phosphate buffer, pH 7.4, 0.5M sucrose and 0.01M potassium chloride. The homogenate was passed through four layers of cheese cloth and one layer of Miracloth, then centrifuged at 600 x g for 10 minutes. The supernatant was centrifuged at 7,500 x g for 10 minutes to pellet the chloroplasts. This pellet was resuspended in 0.01M Trizma/MES buffer, pH 7.4 and pelleted at 7,500 x g for 10 minutes. The resulting pellet was resuspended in 0.01M Trizma/MES buffer, pH 7.4 for assays.

Trizma/MES buffer was prepared by balancing the desired concentration of these two buffers to obtain the correct pH without the addition of any other ions.

Chlorophyll concentration was determined by the method of Arnon (6).

DCPIP (2,6-dichlorophenolindophenol) photoreduction was measured spectrophotometrically at 600 nm. The standard reaction mixture contained 0.125 μmoles of DCPIP, 1.50 μmoles of Trizma/MES buffer, pH 7.0 and chloroplasts containing approximately 30 μgm of chlorophyll. Where indicated, 1.5 μmoles DPC (diphenylcarbazide) was added as an alternate electron donor (7). The optical density of the reaction was measured before and after illumination with 1 x 10^5 ergs/cm^2/sec of red light for 2 minutes. The volume of the reaction mixture was 3 ml. Reactions were measured at room temperature.

NADP photoreduction was measured in a fashion similar to that used for DCPIP photoreduction except 2.5 μmoles of NADP and a saturating amount of ferredoxin were added instead of DCPIP. When ascorbate/TMPD (tetramethylphenylenediamine) was used as the electron donor system, the reaction also contained 50 μmoles of sodium ascorbate, 0.2 μmoles of TMPD and 0.03 μmoles of DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea), and the pH of the Trizma/MES was adjusted to 8.0.

Oxygen electrode assays of methyl viologen reduction were measured as described previously (8). The standard reaction mixture contained in 3 ml: 150 μmoles Trizma/MES, pH 8.0, 0.03 μmoles DCMU, 50 μmoles sodium ascorbate,

0.5 μmoles TMPD, 0.4 μmoles methyl viologen and chloroplasts containing approximately 15 μgm of chlorophyll.

Ferredoxin was prepared by the method of Böger, et al. (9).

Ferricyanide photoreduction was measured spectrophotometrically at 420 nm. The basic reaction mixture contained 0.15 μmoles of ferricyanide, 150 μmoles of Trizma/MES buffer, pH 7.0 and chloroplasts containing approximately 30 μgm of chlorophyll in a 2 ml volume. The reaction mixture was illuminated at 25°C with red light of 1.2 x 10^5 ergs/cm^2/sec intensity. 0.2 ml of 20% trichloroacetic acid and 0.8 ml of water were added to the reaction mixture after illumination and the chloroplasts were pelleted in a clinical cnetrifuge. The optical density of the supernatant was then measured against a zero time blank.

Type II-A histone and polylysines were purchased from Sigma Chemical Company. All other chemicals were reagent grade.

RESULTS

Figure 1 shows the effect of histone on photosystem I activity as measured by the ascorbate/TMPD to NADP reaction. The reaction is completely inhibited when 0.075 mg of histone is present in the reaction mixture, representing a ratio of 50 chlorophyll molecules per histone, assuming the molecular weight of the histone is 2 x 10^4. The inhibition is only seen when reagents are added to the assay mixture in the correct order. When chloroplasts and histone are incubated together before the addition of other reagents to the reaction mixture, complete inhibition is observed. However, when the other reagents are added before the addition of chloroplasts, the reaction is only partially inhibited and the extent of inhibition varies widely from one assay to the next.

When 0.8 mmolar potassium chloride is present in the assay mixture with histone before the addition of chloroplasts, histone gives no inhibition of photosystem I. However, when histone and chloroplasts are preincubated without salt, then 0.8 mmolar potassium chloride is added, the reaction is

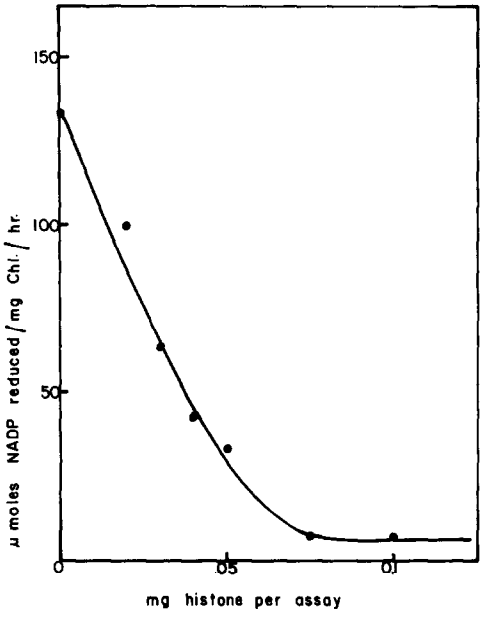

Figure 1

Effect of Histone on Photosystem I

Assays were carried out as described in the methods.

completely inhibited. Thus salt appears to protect the chloroplasts from inhibition rather than to reverse the inhibition. Numerous other salts could replace potassium chloride in affording protection against histone inhibition.

Table 1 shows the effect of histone on several photochemical reactions. The water to NADP reaction requires both photosystems and is completely inhibited by either DCMU or histone. The ascorbate/TMPD to methyl viologen reaction involves photosystem I only. It is insensitive to DCMU but completely blocked by histone. The reduction of either ferricyanide or DCPIP by electrons from water is completely inhibited by DCMU, demonstrating the dependence of these reactions on photosystem II. However, these reactions are only partially inhibited by histone, indicating that part of the ferricyanide and DCPIP reactions continue to function despite complete inhibition of photosystem I by histone. The photoreduction of DCPIP by diphenylcarbazide is also partially resistant to histone inhibition. Maximum

Table 1

Differential Sensitivity of Chloroplast

Electron Transport Reactions

Assay System	μmoles of acceptor reduced per mg chlorophyll per hour				
	Additions to standard reaction mixture				
	----	DCMU	Histone	Histone + DCMU	KCl Histone
H_2O→ NADP	240	0	3	0	240
TMPD→ methyl viologen	2400	2400	95	95	2640
H_2O→ Ferricyanide	382	0	208	0	413
H_2O→ DCPIP	244	2	106	0	285
DPC→ DCPIP	267	11	92	11	320

All assays were performed as described in the methods. 0.10 mg histone, 10.0 μmoles NH_4Cl, 0.25 mmoles KCl, 0.03 μmoles DCMU, and 1.5 μmoles of DPC were present where indicated.

inhibition of all reactions occurred at that histone concentration which completely inhibits photosystem I and no further inhibition occurred, even when a five fold excess of histone was added. In every case histone inhibition was prevented by adding KCl to the reaction mixture prior to histone. KCl alone had no effect on the reaction rates.

The complete inhibition of electron flow through photosystem I by histone is achieved at a ratio of about one histone molecule per 50 chlorophylls. Selective inhibition of photosystem I can also be achieved with polylysines and with other basic proteins, although with considerable variation in the molar ratio of chlorophyll to inhibitor. Dilley had identified polylysine as an uncoupler of photophosphorylation at a concentration similar to that concentration which we observed to inhibit

photosystem I activity (10). Further studies are in progress to better characterize the interaction of polycations with the electron transfer process.

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