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Photoinactivation of chloroplasts already inhibited on the oxidizing side of Photosystem II

Steven M. Theg, Lisa J. Filar and Richard A. Dilley

Department of Biological Sciences, Purdue University, West Lafayette, IN 47907 (U.S.A.)

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We have investigated the irreversible inhibition produced in the photosynthetic electron-transport chain by illuminating Cl^--free or Tris-washed chloroplasts. Our data indicate that the site of this inhibition is on the oxidizing side of Photosystem II, either at the reaction center, P-680, or its immediate electron donor, Z; the possibility that the primary photoinduced lesion is at the second stable Photosystem II electron acceptor, Q_B, has been excluded. Comparison of our data with those in the literature lead us to postulate that the photoinactivation under study here is mechanistically the same as photoinhibition in living plants (Powles, S.B. (1984) Annu. Rev. Plant Physiol. 35, 15–44), the former occurring on an accelerated time scale.

Introduction

It has long been known that chloroplasts depleted of Cl^- develop a lesion in the electron-transport chain on the oxidizing side of PS II that can be reversed by subsequent Cl^- addition [1]. It has been further recognized that while in the Cl^--depleted state, chloroplasts are more susceptible to irreversible inhibition by heating [2], incubation with amines [2–5] and Mn^{2+} [6], and by illumination with relatively low light intensities [7–9], i.e., intensities necessary just to saturate the uncoupled Hill reaction.

Recently, Theg et al. [10] and Itoh et al. [11] demonstrated that reversible inhibition of PS II electron transport caused by Cl^- depletion is due to an inhibition of the S_2 to S_3 transition in the oxygen evolving complex. The former authors also noted that upon illumination of Cl^--deficient chloroplasts, a new irreversible lesion was apparently formed at a site much closer to P-680. Here, we present further data supporting our earlier postulate that photoinactivation of Cl^--depleted chloroplasts results in either destruction of the PS II reaction center or an inhibition of electron transport between the secondary electron donor to PS II, Z, and P-680. We also present evidence that the same lesion is found in Tris-washed chloroplasts after illumination, and discuss the similarities between the results of our experiments and those investigating photoinhibition of chloroplasts in vivo.

Abbreviations: Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DPC, 1,5-diphenylcarbazide; Hepps, 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Mops, 4-morpholinepropanesulfonic acid; P-680, Photosystem II reaction center; PS II, Photosystem II; Q_A, first stable Photosystem II electron acceptor; Q_B, second stable Photosystem II electron acceptor; SiMo, sodium 12-molybdosilicate ($Na_4SiMo_{12}O_{40}$); Taps, 3-{[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino}-1-propanesulfonic acid; Tricine *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; Z, physiological electron donor to the Photosystem II reaction center.

Materials and Methods

Chloroplasts were prepared in the absence of Cl^- from growth-chamber or market spinach by homogenizing leaves in a medium containing 150 mM Na_2SO_4/30 mM Na-Taps (pH 7.8)/3 mM $MgSO_4$/0.5 mM Na-EDTA. The pellet formed during centrifugation was washed twice and resuspended in 200 mM sucrose/5 mM Na-Hepes (pH 7.5)/2 mM $MgSO_4$/0.5 mg bovine serum albumin per ml. Tris-washed chloroplasts were prepared from pea seedlings as above, except: (1) a 20 min Tris-soaking step (0.8 M Tris-HCl at pH 8.4, 0°C, illumination from room lights) preceded the washes with resuspension media; (2) Cl^- salts (at the same ionic strengths) replaced the SO_4^{2-} salts in the grinding and resuspension media. Thylakoids were used either immediately or after storing less than 2 months at 77 K in the presence of 30% ethylene glycol. Measurements of oxygen evolution and chlorophyll fluorescence were carried out as in Ref. 10 in the assay media described in the figure legends.

For the experiment reported in Table I, the three protocols used were as follows: protocol A (no photoinactivating treatment): $t = 0$, chloroplasts added to O_2 electrode vessel containing assay medium in the presence or absence of Cl^-; $t = 2$ min, actinic light on. Protocol B (photoinactivation in the presence of DCMU): $t = 0$, chloroplasts added to vessel containing assay medium and 0.3 μM DCMU in the presence or absence of Cl^-; $t = 2$ min, 30 s illumination with photoinactivating light (heat-filtered white light at 28 mW/cm^2); $t = 2.5$ min, Cl^- added to Cl^--free samples. All samples were then diluted approximately 2-fold with ice-cold medium containing 200 mM sucrose/5 mM Na-Hepes (pH 7.5)/3 mM $MgCl_2$/20 mM NaCl/0.5 mg bovine serum albumin per ml. The samples were pelleted by bringing the centrifuge to $7700 \times g$ and then immediately turning it off and allowing it to coast down. The pellets were washed 3 times in the same buffer and pelleted as above, except that they were allowed to stand 5 min in the rotor before spinning to facilitate the partition of DCMU into the solution. The samples were resuspended in the same buffer, and then assayed for O_2 evolution in the original assay medium in the presence of Cl^-. The Chl recovery was monitored for each sample. Protocol C (photoinactivation in the absence of DCMU): $t = 0$, chloroplasts added to assay medium in the absence of DCMU and in the presence or absence of Cl^-; $t = 2$ min, plus or minus 30 s photoinactivating light; $t = 2.5$ min, Cl^- added to Cl^--free samples; $t = 4$ min, one-half of sample volume assayed for O_2 evolution; $t = 6$ min, remaining half assayed.

Trypsin treatment was performed by incubating chloroplasts with endogenous Cl^- present in a pH 7.1 medium containing 200 mM sucrose/2.5 mM Na-Mops/5 mM K_2SO_4/5 mM $MgSO_4$/50 μg Chl per ml/100 μg trypsin per ml. After 3 min, 67 μg trypsin inhibitor/ml (enough to inhibit 100 μg trypsin/ml) was added, followed by 50 mM Na-Hepps to raise the pH to 7.7, 1 mM $K_3FeCN_6^{3-}$, 5 μM DCMU and 2.6 μM nigericin. This protocol represented the best compromise between the low pH requirement for selective destruction of the DCMU-binding site by trypsin [12] and the high pH requirement for the release of Cl^- from the oxygen-evolving complex [13].

Light intensities were measured with a radiometer (United Detector Technologies, Inc., model 111c). 'White' light was measured either through a dilute $CuSO_4$ solution (Table I) or through a single Schott KG-2 infrared-blocking filter (all others). Values measured through the KG-2 filter are approx. 2.5-times higher than those measured through the $CuSO_4$ due to photosynthetically inactive infrared light that passed the filter.

All reagents were purchased from commercial vendors; trypsin (type III from bovine pancreas) and trypsin inhibitor (type II-s from soybean) were obtained from Sigma Chemical Company.

Results

Inability of artificial electron donors to donate to PS II after preillumination of Cl^--deficient chloroplasts

$P\text{-}680^+$ is known to be a quencher of variable fluorescence [14]. While normally reduced in less than 1 μs after a bright actinic flash, its lifetime can be extended to a few hundred μs in chloroplasts inhibited in the oxygen-evolving system. Thus a fluorescence measuring flash given to inhibited thylakoids at 80 μs after an actinic flash will reveal a high fluorescent state if $P\text{-}680^+$ is

immediately rereduced, or a low fluorescent state if the oxidizing side of PS II is unable to supply electrons for P-680$^+$ rereduction. Recently, we [10] and Itoh et al. [11] used this technique to demonstrate that two electron donors were still connected to P-680 in Cl^--depleted chloroplasts. We also noted [10] that after illuminating Cl^--free chloroplasts for 30 s to produce an irreversible inhibition, no electron donors appeared to remain associated with PS II. This suggested that either the PS II reaction center had been destroyed in the light-inactivated sample, or a block had developed between P-680 and the secondary electron donor to PS II, Z.

Yerkes and Babcock [15] carried out an extensive study of the mechanism of electron transfer from artificial donors to PS II in inhibited chloroplasts. They concluded that most artificial donors donate preferentially to Z rather than to P-680 directly. Therefore, if Cl^--depleted light-inactivated samples are inhibited between Z and P-680, then artificial donors should no longer restore electron transport in them. This postulate was tested in the experiments depicted in Figs. 1 and 2

Fig. 1 shows the effect of light inactivation of Cl^--free chloroplasts on the ability of 1,5-diphenylcarbazide (DPC) to support electron transfer to methyl viologen. Traces a and b show electron transport from H_2O to methyl viologen in chloroplasts that had been incubated for 2 min in the presence (trace a, 100% activity) and absence (trace b, 20% activity) of Cl^-. When Cl^- was added back after a 2 min dark Cl^--depletion regime, i.e., when the electron-transport activity was inhibited by 80%, the rate returned to that of the control (trace c). In this reversibly inhibited state, DPC was able to maintain a fairly high rate of electron transport (trace e). Trace d shows that after 30 s illumination with white light in the absence of Cl^- (the same light used to run the assay) the inhibition of electron transport was no longer reversed by Cl^- addition. Under these conditions DPC was able to donate electrons at a rate of less than one-half that observed in trace e (trace f). Trace g shows that if DPC was present during the preillumination period, its ability to donate electrons efficiently to PS II was largely preserved. These data suggest that the site of inhibition induced by illuminating

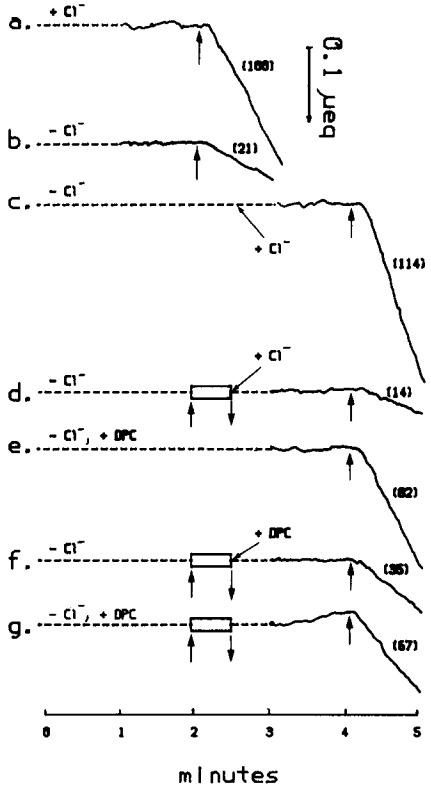

Fig. 1. The effect of preillumination on the ability of DPC to donate electrons to PS II in Cl^--depleted chloroplasts. Traces show oxygen uptake with methyl viologen as the terminal electron acceptor. The numbers in parentheses correspond to the percentage of control (100% = 425 μeq e^- per mg Chl per h). $t = 0$ is the time of chloroplast addition (50 μg Chl/ml) to the reaction vessel containing 25 mM Na-Hepes (pH 8.1)/5 mM K_2SO_4/5 mM $MgSO_4$/0.1 mM methyl viologen/1 μM nigericin, and, when present, 10 mM NaCl and 0.5 mM DPC. The upward arrows indicate light on; the downward arrows, light off. The diagonal arrows show the time of addition of the indicated compounds; compounds listed without arrows were present at $t = 0$. Open boxes show the time and duration of the inactivating preillumination. Heat-filtered white light at 0.125 W/cm^2 was used for both the photoinactivating and actinic illumination.

Cl^--depleted samples was toward the P-680 side of the preferred DPC donation site, and further, that oxidizing conditions between P-680 and the oxygen evolving system favored photoinactivation.

As in other treatments that inhibit electron flow on the oxidizing side of PS II, Cl^- depletion is known to result in a loss of the variable component of chlorophyll *a* fluorescence in the absence

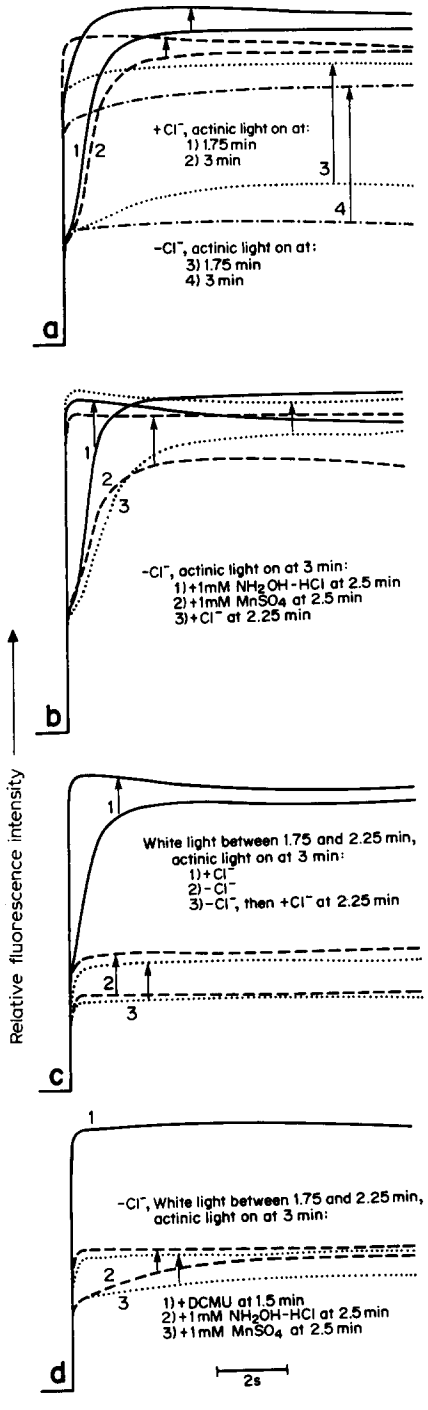

Fig. 2. The effect of preillumination on the ability of NH_2OH and $MnCl_2$ to donate electrons to PS II in Cl^--depleted chloroplasts. In all panels, curves 1 are drawn with solid lines, curves 2 with dashed lines, curves 3 with dotted lines and curves 4 with dot-dashed lines. The assay medium contained 5 μg Chl per ml/100 mM sucrose/25 mM Na-TAPS (pH 8.3)/5 mM K_2SO_4/5 mM $MgSO_4$/1 μM nigericin, and, when present, 10 mM NaCl, 4 μM DCMU, 1 mM NH_2OH and 1 mM $MnSO_4$. For each sample, the lower curve was recorded with DCMU absent, then DCMU was added and the curve pointed to by the vertical arrows was recorded. The intensity of the white photoinactivating illumination was 0.38 W/cm²; the blue actinic light intensity was 10 mW/cm².

of DCMU [16]. Electron donor activity of a compound can easily be recognized by its ability to restore the variable fluorescence in such chloroplasts [17]. Fig. 2 shows the behavior of Mn^{2+} and NH_2OH as electron donors in samples reversibly inhibited by Cl^- depletion in the dark, and irreversibly inactivated by illumination of Cl^--deficient thylakoids. In panel a are fluorescence rise curves of Cl^--sufficient (traces 1 and 2) and deficient (traces 3 and 4) chloroplasts. In all cases, the lower traces were recorded at the indicated times after chloroplast addition to the cuvette, then DCMU was added and the upper curves pointed to by the vertical arrows were recorded. The loss of the variable fluorescence by Cl^- depletion is evident. Panel b shows that the variable fluorescence could be recovered either by Cl^- readdition to the Cl^--deficient samples (trace 3), or by addition of the electron donors, Mn^{2+} (trace 2) or NH_2OH (trace 1). It can be seen in panel c that although 30 s preillumination did not significantly alter the fluorescence rise curve of the Cl^--sufficient samples (trace 1), it did block the recovery of fluorescence in the Cl^--depleted sample upon subsequent Cl^- addition (trace 3). Interestingly, the fluorescence curves recorded after DCMU additions also failed to rise significantly after light inactivation (traces 2 and 3). Panel d shows that the variable components of fluorescence in either the absence or presence of DCMU could not be restored by Mn^{2+} or NH_2OH after light-inactivation, indicating that, like DPC, these compounds were rendered ineffective as electron donors to PS II by this treatment.

Protection by DCMU of photoinactivation of Cl^--depleted chloroplasts

Trace 1 in panel d of Fig. 2 shows that DCMU, when present during the preillumination period, appeared to protect the Cl^--depleted samples from inactivation. This was tested further in the experiment reported in Table I, in which Cl^--free chlo-

TABLE I

THE EFFECT OF DCMU ON PHOTOINACTIVATION OF Cl^--FREE CHLOROPLASTS

The Cl^- depletion and assay medium contained 25 mM Na-Taps (pH 8.1)/5 mM K_2SO_4/5 mM $MgSO_4$/0.1 mM methyl viologen/2 μM nigericin and, when present, 20 mM NaCl and 0.3 μM DCMU; Chl was present at 50 μg/ml. Assays and treatments were performed at 18–22°C. Protocol A: chloroplasts assayed in the presence or absence of Cl^- without prior photoinactivation treatment. Protocol B: chloroplasts assayed in the presence of Cl^- after photoinactivation treatment with DCMU present and in the presence or absence of Cl^-. Samples were washed to remove the DCMU prior to the assays. Protocol C: chloroplasts assayed in the presence of Cl^- after photoinactivation treatment without DCMU and in the presence or absence of Cl^-. Details of the procedures are given in Materials and Methods. Corrected rate: rate normalized to 'protocol A, $+Cl^-$' sample to account for incomplete DCMU removal (protocol B) and photoinactivation unrelated to Cl^- depletion (protocol C). All rates are an average of two determinations and are expressed in μequiv. per mg Chl per h. The protection afforded by DCMU against photoinactivation can be seen by comparing the last lines of protocols B and C.

Sample	Cl^- present during assay	Actual rate	Corrected rate	Percentage of maximum
Protocol A; control, no photoinactivation:				
$+Cl^-$	+	736	736	100
$-Cl^-$	–	67	67	9
$+Cl^+$ + DCMU	+	59	59	8
Protocol B; photoinactivation in the presence of DCMU:				
$+Cl^-$ + DCMU – light	+	176	736	100
$-Cl^-$ + DCMU – light	+	186	778	106
$+Cl^-$ + DCMU + light	+	164	686	93
$-Cl^-$ + DCMU + light	+	84	351	48
Protocol C; photoinactivation in the absence of DCMU:				
$+Cl^-$ – DCMU + light	+	536	736	100
$-Cl^-$ – DCMU + light	+	81	111	15

roplasts were preilluminated in the presence or absence of DCMU and subsequently assayed for remaining Hill activity in Cl^--sufficient media after washing out the DCMU. It can be seen by comparing the last lines for protocols B and C that, relative to the respective controls, the DCMU-poisoned chloroplasts suffered significantly less Cl^--dependent photoinactivation (52% inhibition) than did the DCMU-free samples (85% inhibition).

Photoinactivation of Cl^--depleted chloroplasts does not involve Q_B

The pattern of inhibition produced by short (30 s) illumination periods in the absence of Cl^- is similar to that described for the phenomenon known as photoinhibition [18–20]. In photoinhibition, electron transport in plants or algae grown under relatively low light intensities is inactivated by prolonged intense illumination. The same damage can be mediated by lower light intensities if the plants are temperature, water or nutrient stressed [18]. Whereas most researchers of this topic concluded that the site of photoinhibition is on the oxidizing side of PS II close to the reaction center, Kyle et al. [21] postulated that photoinhibition is the result of destruction of the Q_B protein on the reducing side of PS II. The latter conclusion was based partly on an observed preservation of DCMU-insensitive electron transport from H_2O to SiMo in photoinhibited samples. In order to determine if our pattern of photoinactivation coincided with that seen by Kyle et al. [21], we examined electron transport from water to Q_A after illuminating our Cl^--deficient samples.

Initially we tried using the H_2O to SiMo reaction, but found that, in contrast to the results of Kelly and Izawa [2], we could not demonstrate a Cl^- requirement (data not shown). Rather than continuing with SiMo, we decided to check for the involvement of Q_B in our photoinactivation experiments by examining a different DCMU-insensitive electron transport reaction sequence, namely H_2O to Q_A to $Fe(CN)_6^{3-}$ in trypsin-treated thylakoids [12].

Table II shows two repetitions of an experiment which examined the effect of preilluminating

TABLE II

THE EFFECT OF PREILLUMINATION ON THE RECOVERY OF ELECTRON-TRANSPORT ACTIVITY UPON Cl^- ADDITION TO TRYPSIN-TREATED CHLOROPLASTS

All samples were incubated for two min at pH 7.0 with 100 μg trypsin/ml. At 2 min the following additions were made: 67 μg trypsin inhibitor/ml (sufficient to inhibit all trypsin present), 50 mM Hepps (bringing final pH to approx. 7.7), 5 μM DCMU, 1 mM $Fe(CN)_6^{3-}$, and in Expt. 1, 2 μM nigericin; Cl^- additions were 20 mM NaCl. See Materials and Methods for further details. All rates are an average of two determinations and are expressed in μeq e^- per mg Chl per h.

Time after Chl addition (min)				Rate *	% of $+Cl^-$ rate	% stimulation of $-Cl^-$ rate
0	3.75	4	6			
Experiment 1						
$+Cl^-$	–	actinic light	–	335	100	–
No Cl^-	–	actinic light	–	74	22	–
No Cl^-	–	$+Cl^-$	actinic light	140	42	+91
No Cl^-	15 s light *	$+Cl^-$	actinic light	90	27	+23
Experiment 2						
$+Cl^-$	–	actinic light	–	381	100	–
No Cl^-	–	actinic light	–	94	24	–
No Cl^-	–	$+Cl^-$	actinic light	187	49	+104
No Cl^-	15 s light *	$+Cl^-$	actinic light	73	19	−21

* Preillumination with saturating (for electron transport), heat-filtered white light.

Cl^--free chloroplasts on DCMU-insensitive electron transport from H_2O to $Fe(CN)_6^{3-}$ after a short incubation with trypsin. The trypsin incubation itself rendered much of the Cl^--dependent drop in electron flow irreversible even without illumination. However, in both experiments Cl^- addition still stimulated the rates approximately twofold in non-preilluminated samples. This stimulation by Cl^- was clearly suppressed after a 15 s preillumination period. These data indicate that the irreversible inhibition produced by illuminating Cl^--free chloroplasts was on the oxidizing side of PS II and did not involve the Q_B protein.

Photoinactivation of Tris-washed chloroplasts

The observation that the presence of DCMU or DPC during the preillumination period protected the Cl^--free chloroplasts from photoinactivation suggests that the damage was produced by the long-lived $P\text{-}680^+$ formed after the donor side of the electron-transport chain had been oxidized. If true, then a similar photoinactivation should occur upon illumination of any chloroplasts already inhibited on the oxidizing side of PS II. We tested this prediction using Tris-washed chloroplasts in the experiments shown in Fig. 3. It can be seen that, as in Cl^--depleted chloroplasts, preillumination of Tris-washed thylakoids blocked the recovery of the variable component of fluorescence upon addition of DCMU or of either Mn^{2+} or NH_2OH. Also shown in this figure is the protection, albeit incomplete, afforded against photoinactivation by DCMU (panel b, trace 4), and the observation that fluorescence still rose after preillumination when dithionite was added subsequently (trace b, panel 3).

Discussion

The data presented here corroborate the earlier observation that illumination of Cl^--depleted thylakoids results in a different type of inhibition than is observed in Cl^--free chloroplasts held in the dark. In the latter case, two electron donors appeared to remain associated with P-680 [10,11], electron transport could be restored by artificial donors to PS II [20], and fluorescence, while low initially, could be increased by DCMU [16]. In contrast, the data in Ref. 10 and presented here show that: (a) no electron donors were connected to PS II after illumination of Cl^--free chloroplasts; (b) fluorescence could not be recovered by DCMU addition; (c) nor could electron transport be restored by artificial electron donors. The latter

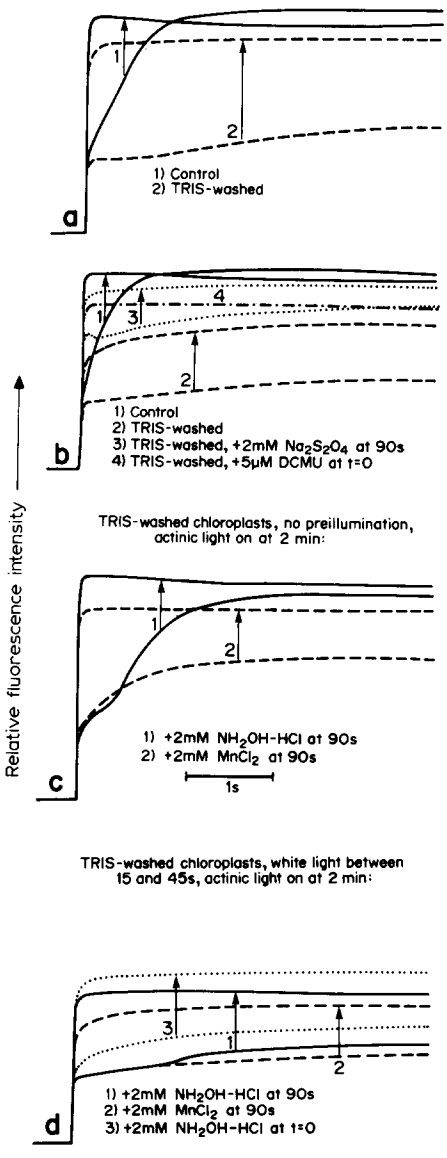

Fig. 3. The effect of preillumination on the ability of NH_2OH and $MnCl_2$ to donate electrons to PS II in Tris-washed chloroplasts. In all panels, curves 1 are drawn with solid lines, curves 2 with dashed lines, curves 3 with dotted lines and curves 4 with dot-dashened lines. The assay medium contained 10 μg Chl/ml, 200 mM sucrose, 25 mM Na-Tricine at pH 8.1, 10 mM KCl, 5 mM $MgCl_2$ and when present, 5 μM DCMU, 2 mM NH_2OH, 2 mM $MnCl_2$ and 2 mM $Na_2S_2O_4$. For each sample, the lower curve was recorded with DCMU absent, then DCMU was added and the curve pointed to by the vertical arrows were recorded. The intensity of the white photoinactivating illumination was 90 mW/cm^2; the blue actinic light intensity was 2.4 mW/cm^2.

two observations were also seen using Tris-washed chloroplasts.

Yamashita and Butler [23] described a photoinduced bleaching of chlorophyll and carotenoids in Tris-washed chloroplasts. In a single experiment we did not observe any appreciable pigment bleaching under our conditions (data not shown), probably because the illumination time was so short. Nonetheless, our fluorescence and artificial donor experiments are in agreement with theirs, and probably we have both examined the same phenomenon.

Our data suggest that illumination of Cl^--deficient or Tris-washed chloroplasts produced a lesion in the electron-transport chain somewhere between Z and Q_A. The experiments shown in Table II, in which electron transport from H_2O to Q_A was investigated after trypsin treatment, gave evidence that the site of light-induced inhibition was not at Q_B. Since P-680$^+$ is known to be a quencher of fluorescence, our data (Figs. 2 and 3) appear to be most easily explained by postulating the site of inhibition to between Z and P-680. Furthermore, the recovery of fluorescence upon dithionite addition and the persistence of a low rate of electron donation by DPC in photoinactivated thylakoids both suggest that the lesion is not at P-680 itself. Nevertheless, we cannot rule out the possibility that an increase in non-radiative energy dissipation may accompany destruction of reaction centers. This point might be decided through investigation of the EPR [24] and optical [25,26] signals for Z^+, P-680$^+$ and Q_A^-. In either case, the protection against light inactivation afforded by DCMU, DPC (and normal electron flow) indicates that the long-lived oxidant, P-680$^+$, produced in the absence of competent electron transport out of the water-splitting complex, is probably responsible for the inhibition.

With the exception of the H_2O to SiMo reaction [21] (and possible effects on the Q_B protein [21] which were not investigated) the type of inhibition we observed after illuminating Cl^--depleted or Tris-washed chloroplasts was indistinguishable from that observed with the better-characterized photoinhibition [18–20]. (The protective effect of DCMU in our experiments (Table I) may provide another exception [27–29]). However, the differences in experimental protocols

make direct comparisons difficult). In most cases of inhibition on the oxidizing side of PS II, i.e., that caused by Tris, NH_2OH, Cl^--depletion, ultraviolet irradiation, heating or alkaline pH, fluorescence yield can be increased to the normal F_{max} level by DCMU, and electron transport can be restored by artificial donors to Z^+. This is not true after preillumination of either Cl^--deficient, Tris-washed thylakoids nor of photoinhibited chloroplasts [18–20] (but see Ref. 21). The similarities between these two cases suggest that photoinactivation of Cl^--depleted or Tris-washed chloroplasts and photoinhibition are mechanistically the same phenomenon, the former appearing on an accelerated time-scale. Given that the photoinactivating damage appears to be mediated by P-680$^+$, one can postulate that the initial event in the process of photoinhibition in vivo is a reduction of water-splitting activity, followed secondarily by the development of a lesion very close to the reaction center as a result of reduced electron flow into P-680$^+$. This hypothesis is supported by the following observations: (1) plants become more susceptible to photoinhibition if they are heat-, cold- or water-stressed [18]; (2) the water-oxidizing system can be specifically damaged by heating [30] or chilling [31,32] leaves in the dark; (3) some potential PS II activity can be lost in weakly illuminated leaves undergoing severe water stress, although increased sensitivity towards photoinactivation generally occurs at somewhat higher leaf water potentials, i.e., potentials that may not be harmful to darkened leaves [33,34]. Furthermore, it has recently been suggested that the oxygen-evolving enzyme is specifically inhibited by dessication of leaves held in the dark (Canaani, O., personal communication).

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