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STUDIES ON THE PHOTOOXIDATION OF MANGANESE BY ISOLATED CHLOROPLASTS

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SUMMARY

The photooxidation of manganese by isolated chloroplasts has been investigated in some detail in order to evaluate the possible relationship of this reaction to that of the normal Hill reaction. Comparable kinetics were observed for the effects of light intensity and temperature. The photooxidation of manganese is inhibited by typical poisons of photosystem II, *i.e.*, 3(3,4-dichlorophenyl)-1,1-dimethylurea and simazine, and by sodium cyanide. Removal of light petroleum-soluble components from chloroplasts decreases their capacity for manganese oxidation. This effect is partially reversed by addition of plastoquinone A. Several additional components of chloroplasts were examined for their effects upon manganese oxidation. Photosynthetic pyridine nucleotide reductase, purified ferredoxin, and a chloroplast diaphorase were ineffective at low concentration and inhibitory at high concentrations. FMN, at low concentrations, causes a marked stimulation of manganese oxidation. Both the normal and FMN-stimulated reaction is inhibited by the flavin-antagonized acriflavin. The requirement for flavin, the need for oxygen in the photooxidation of manganese, and the observed inhibition by cyanide, suggest that peroxide is a product of the reaction and furthermore that peroxidase, and perhaps also catalase, stimulates manganese oxidation through the removal of the newly formed peroxide. The photooxidation of Mn^{2+} to Mn^{3+} is dependent upon reactions of photosystem II but it was not possible to conclude definitely that the process is exclusively a photosystem II reaction.

INTRODUCTION

One of the least understood portions of the overall mechanism for photosynthesis is that of oxygen evolution. Many recent review articles have drawn attention to this fact rather emphatically[1,2]. A number of observations on the influence of manganese deficiency on photosynthesis and on various photochemical reactions of isolated chloroplasts indicate a specific role for this element in the oxygen-producing mechanism of photosynthesis[3–6]. This specific requirement and the rapidity with which deficiency effects can be restored[6] suggest that perhaps manganese ion functions directly. Because no photosynthetically active compound is known which specifically

Abbreviation: DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea.

contains manganese, *e.g.*, such as a manganoporphyrin[7], and further since no enzyme system of photosynthesis so far studied shows a specific requirement for manganese as a co-factor, it appears logical to suggest that the manganese ion itself undergoes a rapid photooxidation and reduction as part of the electron-transport system incident to photosystem II of photosynthesis. KENTEN AND MANN[8,9] noted some time ago that chloroplasts catalyze a photooxidation of $Mn^{2+} \rightarrow Mn^{3+}$. More recently SWENSON AND VERNON have demonstrated that manganipyrophosphate serves as a Hill oxidant and also supports oxygen evolution and non-cyclic photophosphorylation[10]. This latter observation adds another Hill reaction oxidant to an already long list. However, the findings of KENTEN AND MANN may be more directly related to the observed role of manganese in photosynthesis.

This investigation reports additional findings on the characteristics of the photooxidation of Mn^{2+} by spinach chloroplasts. The characteristics of the reaction parallel those of normal Hill reactions by showing similar sensitivity to DCMU, simazine, and by having identical light-saturation kinetics. The reaction requires oxygen, is stimulated by FMN, and is augmented by peroxidase. Unlike the Hill reaction, the photooxidation of Mn^{2+} is inhibited by cyanide and by acriflavin.

MATERIALS AND METHODS

Chloroplast preparation

Chloroplasts were prepared from leaves of spinach (*Spinacea oleracea*) in two ways. For experiments on manganese oxidation, chloroplasts were extracted according to the method of KENTEN AND MANN[9] but for comparative studies on photophosphorylation and manganese oxidation intact chloroplasts were prepared according to AVRON's procedure[11]. In the former method, deveined spinach leaves were ground in 0.05 M potassium phosphate buffer (pH 7.0) in a Waring blendor for 2 min. The homogenate was filtered through a double layer of cheesecloth to remove leaf debris and the filtrate was centrifuged at 1000 $\times$ *g* for 15 min. The pellet was discarded and the supernatant fluid spun at 3500 $\times$ *g* for 15 min. This supernatant fluid was discarded; the pellet was resuspended in 0.05 M phosphate buffer (pH 7.0) and filtered through glass-wool. The chloroplasts were either used immediately or frozen for later use. The entire extraction procedure was performed at approx. 4°. Lyophilized chloroplasts were prepared by resuspending the pellet from the final centrifugation in a small volume of ice-cold distilled water and immediately snap-freezing in a conventional lyophilization jar. The volume of material added was kept minimal in order that the vacuum drying was complete within 4 h.

Protein determinations were made using the Folin phenol reagent. Chlorophyll was determined by the modification of ARNON[12].

In all inhibitor studies, the inhibitor was added immediately before initiating the reaction. The concentrations as expressed represent final concentration in the reaction mixture unless otherwise indicated.

Instrumentation

All reactions which required light were run on a Gilson differential respirometer. Spectrophotometric determinations were conducted on a Zeiss PMQ II spectrophotometer to which a Photovolt Varicord Model 43 recorder was attached for time-course

studies. Centrifugation was conducted in either a clinical or a Sorvall Superspeed Model RC-2 refrigerated centrifuge.

Oxidized manganese assay

KENTEN AND MANN's assay for photooxidized manganese is based on the manometric measurement of N_2 evolved upon the addition of hydrazine to reaction mixtures containing Mn^{3+}. It was felt that a more sensitive and reproducible assay should be developed. The methods finally adopted correlated to some degree with techniques developed by KENTEN AND MANN. The complex between Mn^{3+} and pyrophosphate (manganipyrophosphate) has an absorption maximum at 258 nm. A standard curve at this wavelength was obtained with chemically oxidized manganese which was prepared by allowing MnO_2 and $MnSO_4$ to stand overnight in 0.1 M sodium pyrophosphate at pH 7.0. The residue was centrifuged out and the manganipyrophosphate poured off. This solution was standardized either by following the oxidation of ferrocyanide by Mn^{3+} at 460 nm or by determining the remaining ferrocyanide with the sensitive method of AVRON AND SHAVIT[13]. Although the two methods gave close agreement for manganese standardization, they were not adequate for estimation of Mn^{3+} formed by illuminated chloroplast mixtures.

The assay selected involved the disappearance of the 258-nm absorption of manganipyrophosphate upon reduction with hydrazine. Levels of Mn^{3+} as low as 0.05 μmole were readily detectable with this technique. The reaction mixture for the biological oxidation of manganese consisted of the following: 20 μmoles of $MnSO_4$, 100 μmoles sodium pyrophosphate, chloroplasts equivalent to 1 mg of chlorophyll, all in a volume of 5.0 ml of water adjusted to pH 7.0. The chloroplasts were always added in the dark because of the rapid initial photooxidation. Unless otherwise indicated the reaction conditions were as follows: temperature, 25°; light intensity, 1000 ft candles; time of illumination, 1 h; gas phase, air; light color, white. Experiments were terminated by turning off the lights and centrifuging the contents of each reaction flask for 10 min in a clinical centrifuge. The supernatant was placed in a cuvette and the absorbance determined prior to and following complete reduction of the oxidized manganese with hydrazine. Dark controls were always employed but little or no oxidation was noted.

Peroxidase assay

Peroxidase activity was assayed by following the oxidation of guaiacol. Reaction rates were determined by following the appearance of absorption at 470 nm, as produced by the tetraguaiacol[14]. Hydrogen peroxide solutions were standardized by titration with a standardized solution of potassium permanganate.

RESULTS AND DISCUSSION

The results of GERRETSEN[15] and of KENTEN AND MANN[8,9] have been used extensively to hypothesize about the mode of action of manganese in photosynthesis. Their work has, however, not been extended or verified to any great extent. Obvious extentions revolve about the following questions: Is the photooxidation of manganese by chloroplasts a phenomenon which is correlated with photosynthetic reactions known to require manganese or is it a non-specific photooxidation catalyzed by

chlorophyll? If photosynthetic reactions of system II are involved then this oxidation should possess properties similar to those of the Hill reaction. What is the oxidant in this reaction and where, in relation to the electron-transport sequence of photosynthesis, is manganese oxidized? And finally, what is the nature of the peroxidase stimulation in this reaction and is this stimulation indicative of an function *in vivo* for peroxidase? Our findings relevant to some of these questions are reported here.

Effect of temperature

The rate of manganese oxidation by spinach chloroplasts was investigated over the range of 7° to 30° and a response typical of an enzymatic controlled reaction was obtained. The optimum temperature was 25° and inactivation occurred at 30° and higher. Little or no activity was detected at 7°. These data are shown in Table I. Preliminary studies on the time course for this reaction indicated a linear response during the initial 30 min at 25°.

TABLE I

TEMPERATURE EFFECT ON MANGANESE OXIDATION

Reactions were run at 1000 ft candles for 30 min.

Temp.	*Absorbance at 258 mμ*	
	1	*2*
7°	0	0
10°	0.26	0.21
14°	0.52	0.49
21°	0.69	0.67
25°	0.91	0.83
30°	0.63	0.60

This response to temperature is very similar to that exhibited by the Hill reaction and it is apparent that thermolabile steps are involved in the photooxidation of manganese. Determination of the experimental activation energy for manganese photooxidation from the data contained in Table I provided a value nearly identical to that for the Hill reaction, *i.e.*, 10.8 kcal/mole (ref. 16).

Effect of light intensity

In the original report of KENTEN AND MANN[9] the effect of light intensity on the photooxidation of manganese was investigated over the range of 50 to 200 ft candles. Light saturation of the reaction was not attained and further their curves did not extrapolate to zero manganese oxidation in the dark. We have extended this aspect of the study to an upper range of 1000 ft candles and, further, attempted a comparison to the well documented response of the Hill reaction to light intensity[17]. Fig. 1 illustrates the light response for the photooxidation of manganese. The hyperbolic relationship between the rate of the reaction and light intensity indicates that this reaction is not strictly photochemical and, further, that such kinetics are identical to those of the Hill reaction. The experimental conditions incident to this type of experiment were not adequate for additional detailing of the absolute values of the

slope and intercept of such a plot which would allow approximation of values for the rate constant for the limiting dark reaction and for the limiting photochemical reaction respectively[17].

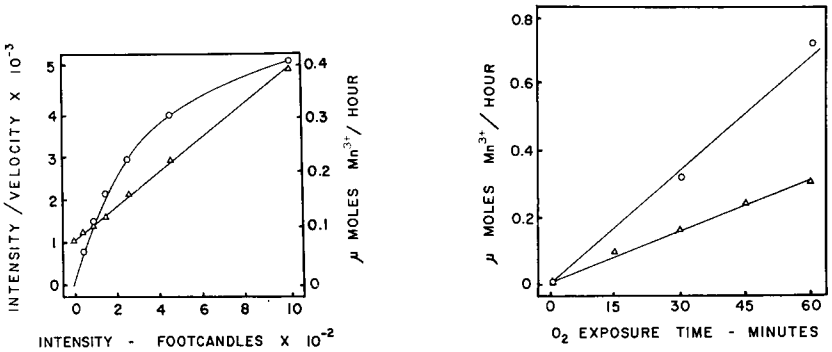

Fig. 1. The effect of light intensity on the rate of formation of oxidized manganese (○—○) and on the function light intensity/rate (△—△). See text for details of reaction conditions. See also ref. 17.

Fig. 2. The requirement for oxygen in the formation of Mn^{3+} by illuminated chloroplasts. All reaction vessels were initially gassed with nitrogen and were flushed with oxygen at the time intervals indicated. 0.04 μmole of FMN was included in the reaction mixture for the upper curve (○—○).

Requirement for oxygen

Little or no information is available as to the nature of the electron acceptor in the light-catalyzed oxidation of manganese. By comparing the rates of manganese oxidation in stirred and non-stirred reaction mixtures and by varying the depth of the reaction mixtures in open petri dishes it was demonstrated that the rates of manganese oxidation are higher in the stirred and thinner suspensions of chloroplasts[9]. In Fig. 2 the results indicating a definitive requirement for oxygen are presented. Clearly the amount of manganese oxidation is directly dependent upon the presence of oxygen. Several reactions of chloroplasts have been described in which oxygen can function as a terminal electron acceptor in chloroplast reactions. MEHLER[18] noted the utilization of molecular oxygen as a Hill oxidant and also NAKAMOTO *et al.*[19] observed an oxygen requirement for the FMN-mediated photophosphorylation. In the experiments summarized in Fig. 2 the addition of 0.04 μmole of FMN resulted in at least a 2-fold stimulation of the amount of manganese oxidized. Since the reduction of molecular oxygen through reduced flavins results in the formation of hydrogen peroxide, the production of hydrogen peroxide may account then for the stimulation of manganese oxidation by peroxidase[9].

FMN enhancement of manganese oxidation

The requirement of flavin in the photooxidation of manganese, as suggested from the data of Fig. 2, was examined over a broader concentration range. This stimulation by FMN is better illustrated in Fig. 3; saturation of this effect occurs at about 0.04 μmole. This concentration gave about a 7-fold stimulation under the conditions of this particular assay. The quantity required for stimulation emphasized the catalytic nature of the requirement. Also the rate of oxygen uptake accompanying

manganese oxidation is increased. The reaction between reduced flavin and oxygen may account for the utilization of molecular oxygen in manganese oxidation by chloroplasts not supplemented with flavin. A number of flavin-containing enzymes are known to occur in chloroplasts although there is little evidence for the existence of free flavin, particularly, in well-washed chloroplasts.

Further evidence for the participation of an endogenous flavin in the photo-oxidation of manganese was obtained by employing acriflavin as an inhibitor. This compound is a standard competitive inhibitor of flavin-catalyzed reactions. In Fig. 4 the absorbance at 258 nm is graphed as a function of acriflavin concentration (%, w/v). Since acriflavin is a mixture of isoalloxazine derivatives the concentration cannot be accurately expressed in molarity. However, the concentration required for 50 % inhibition is approx. $1 \cdot 10^{-3}$ M and agrees with the sensitivity seen with other flavin-catalyzed reactions.

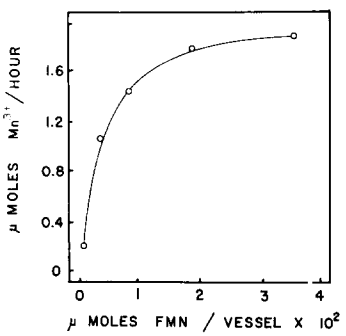

Fig. 3. The stimulation of manganese oxidation by FMN.

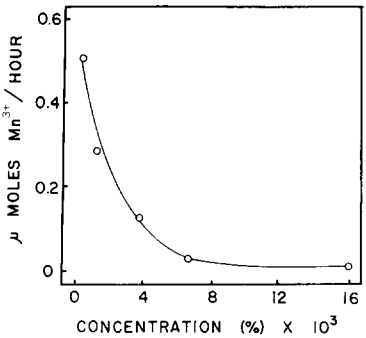

Fig. 4. Inhibition of manganese oxidation by acriflavin. See text for experimental details.

Inhibitor response

Three additional inhibitors were employed to extend the comparison of a manganese oxidation to oxygen evolution or Hill reaction. Of particular interest was to determine whether or not manganese oxidation required the participation of both photosystems I and II. Since DCMU and simazine are known preferentially to inhibit oxygen evolution, hence system II, the action of these two herbicides on manganese oxidation was tested. The results from these studies are presented in Fig. 5. The concentration required for 50 % inhibition (10^{-6} M) closely approximates the concentration required for a similar inhibition of the Hill reaction. Similar behavior is evident for simazine and it is apparent that both inhibitors are about equally specific. No attempt was made to reverse this type of inhibition by supplying an artificial hydrogen-donor system, *i.e.*, dichlorophenolindophenol–ascorbic acid, because of the reactivity of the oxidized manganese in such a system.

Because of the requirement for oxygen and the stimulatory effect of both flavin and peroxidase on manganese oxidation it appeared obvious that cyanide would be an effective inhibitor for this reaction. Peroxidase is extremely sensitive to cyanide while the Hill reaction is practically insensitive. Manganese photooxidation, as seen in Fig. 6, demonstrates intermediate sensitivity to cyanide. The concentration required to inhibit manganese oxidation is far greater than that required to inhibit

the assays for peroxidase *in vitro* but less than that required to inhibit the Hill reaction[20].

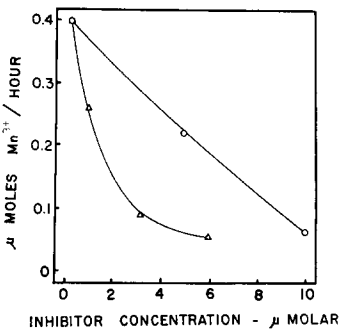

Fig. 5. Inhibition of manganese oxidation by simazine (O—O) and DCMU (△—△).

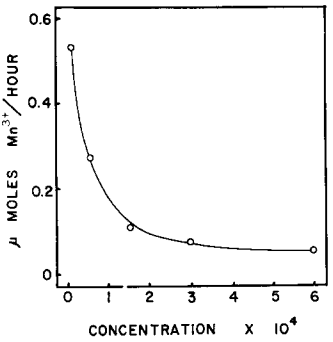

Fig. 6. Cyanide sensitivity of the chloroplast-catalyzed oxidation of manganese.

Requirement for lipid-soluble components of the chloroplast

From the preceding description of the effects of inhibitors of system II-type reactions of photosynthesis, it appears that the oxidation of manganese by isolated chloroplasts proceeds at least *via* system II. Further evidence for this interpretation was gained from studies on light petroleum extraction of lyophilized chloroplasts. Using the extraction method described by BISHOP approx. 75 % inhibition of both normal and FMN-enhanced manganese photooxidation (Table II) was observed. By adding back the extracted material partial restoration of activity was achieved; addition of purified plastoquinone A resulted in an equal recovery. For the experiments reported in Table II complete restoration of manganese oxidation capacity was not

TABLE II

LIPID-SOLUBLE COMPONENTS IN MANGANESE OXIDATION

FMN, when added, was 0.036 μmole per reaction mixture; plastoquinone A, 0.1 mg per mg chlorophyll.

	Absorbance at 258 mμ per mg chlorophyll	*Per cent of control activity*
Expt. 1		
Lyophilized chloroplasts	1.46	100
Extracted chloroplasts	0.32	22
Extracted chloroplasts *plus* extract	0.76	52
Lyophilized chloroplasts *plus* FMN	2.77	100
Extracted chloroplasts *plus* FMN	0.94	34
Extracted chloroplasts *plus* extract *plus* FMN	1.41	51
Expt. 2		
Lyophilized chloroplasts	2.89	100
Extracted chloroplasts	0.70	24
Extracted chloroplasts *plus* extract	1.22	42
Extracted chloroplasts *plus* plastoquinone A	1.47	51

obtained. This difficulty was due largely to the inability to prevent the rapid uptake of moisture which made it extremely difficult to extract from, or add back, the extract or plastoquinone to the chloroplasts. However, the technique was sufficient to illustrate a requirement for a fat-soluble component. Since pure plastoquinone A gave a better restoration than the crude extract, it would appear that plastoquinone A is the fat-soluble component required in the photooxidation of manganese. No attempt was made to determine if other plastoquinones gave similar or better restoration.

Requirement for co-factors

Our studies to this point indicate that an intact electron-transport system indigenous to photosystem II, including plastoquinone, is essential for photooxidation of manganese. Other known components of the photosynthetic electron-transport

TABLE III

CO-FACTORS IN MANGANESE OXIDATION (I)

Co-factor			*Per cent of control activity*
FMN (mμmoles)	*Photosynthetic pyridine nucleotide reductase (ml)*	*Diaphorase (ml)*	
0.0	0.0	0.0	100
	0.05		121
	0.1		140
	0.5		68
		0.1	114
		0.5	127
	0.1	0.1	113
5.0			223
5.0	0.05		218
5.0	0.1		159
5.0	0.1	0.1	129

system were studied to determine if any requirement was evident. Also, components known to stimulate the MEHLER reaction were investigated. Tables III and IV present the results of these experiments. Catalase and peroxidase were commercial preparations obtained from beef liver and horse radish, respectively. The photosynthetic pyridine nucleotide reductase and chloroplast diaphorase were partially purified from spinach and showed high activity in specific assays.

The stimulation by high concentrations of peroxidase agrees with the results of KENTEN AND MANN[9]. There appears to be a soluble factor which is partially removed in preparations and extensive washing of the chloroplasts. However, the amount of peroxidase removed by this treatment is exceedingly small compared to the amount of the purified enzyme necessary to restore the initial activity. Addition of catalase and ethanol, factors necessary for the MEHLER reaction, gave no stimulation. Catalase alone is slightly inhibitory at high concentrations. Even with the addition of FMN, a condition which would stimulate peroxide formation, no additive stimulation with catalase and ethanol was noted. We must assume that the level of activity of endo-

TABLE IV

CO-FACTORS IN MANGANESE OXIDATION (2)

Chloroplast preparation	*Additions*				*Per cent of control activity*
	Peroxidase (μg)	*Catalase (mg)*	*Ethanol (ml)*	*FMN (mμmoles)*	
Phosphate-extracted and unwashed	0.0	0.0	0.0	0.0	100 (control)
	50				174
Phosphate-extracted and washed in NaCl–EDTA					69
	10				85
	50				108
Phosphate-extracted and washed in phosphate					80
	10				80
	50				113
Phosphate-extracted and washed in phosphate	0.0	0.0	0.0	0.0	100 (control)
		0.2			91
		0.1			104
		0.2	0.2		102
				5.0	223
		0.1		5.0	135
		0.2	0.2	5.0	182
Sucrose-phosphate-extracted					104
				5.0	241

genous catalase was sufficiently high to result in the rapid removal of peroxide. Hence, the catalase level or the addition of a trapping agent would not further augment activity.

Chloroplast diaphorase and spinach photosynthetic pyridine nucleotide reductase were studied as potential co-factors for manganese oxidation because of their flavin content and because of their known role in electron-transport system. The stimulation expressed by both (Table III) was meager in comparison to that caused by FMN. The stimulation noted may be due to free flavin—or that freed from the protein complex during the reaction—from the two enzymes. Photosynthetic pyridine nucleotide reductase was inhibitory in high concentrations and both enzymes inhibited manganese oxidation with FMN present. No consistent additive effect of the two enzymes was observed.

The action of DCMU and KCN on the dark oxidation of manganese, which occurs during the reaction between peroxide and a phenol, as catalyzed by peroxidase was also studied. This reaction, as described by KENTEN AND MANN[8], occurs supposedly *via* an oxidation intermediate of the phenol. DCMU at $5 \cdot 10^{-6}$ M was ineffective while $2.5 \cdot 10^{-4}$ M KCN completely inhibited the oxidation of manganese when 25 μg of commercial peroxidase was employed. This concentration of KCN is only partially inhibitory to the chloroplast-catalyzed photooxidation (Fig. 6).

The nature and extent of the peroxidase stimulation was studied in further

detail by extracting chloroplasts several times with either phosphate buffer or NaCl–EDTA solution. These chloroplasts were then illuminated in the presence of varying concentrations of peroxidase and with the appropriate agents for manganese photooxidation. Thoroughly washed chloroplasts were still able to photooxidize manganese and added peroxidase caused at most only a 74 % increase in rate. No peroxidase activity was detected in the washed chloroplasts.

Studies on reactions of isolated chloroplasts have involved primarily observations on the photoreduction of a variety of oxidants with water serving as the primary electron donor. Since one of the fundamental processes on photosynthesis involves the photooxidation of water, studies on a similar type of chloroplast reaction, wherein alternate electron-donor systems might be utilized, would be of considerable theoretical interest in terms of the mechanism of photosystem II reactions. Other photooxidations catalyzed by chloroplasts and having DCMU sensitivity are the photooxidation of ascorbic acid, the photooxidation of certain hydroquinones, and the quinone-sensitized photooxidation of ascorbic acid[21]. These reactions are oxygen dependent and result in the formation of hydrogen peroxide in the presence of cyanide. In contrast to the cyanide sensitivity of manganese oxidation as reported here, the oxidation of *o*-hydroquinones and of ascorbic acid are not influenced by this poison. In these types of reactions it appears that the photooxidation occurs *via* photosystem II.

At least two interpretations for the mechanism of manganese oxidation by chloroplasts are possible. First, a normal Hill reaction may occur wherein endogenous or added flavin serves as the oxidant. Its reoxidation by oxygen would result in the formation of a peroxide and in the presence of endogenous peroxidase, or perhaps even a polyphenoloxidase, would be employed in oxidation of the manganous ion. Such a mechanism would be inhibited by any characteristic poison of the Hill reaction and also by KCN. Second, the mechanism might involve the removal of electrons from the manganous ion as part of the photochemistry of system II, *i.e.*, electrons are donated into the electron-transport chain at a position prior to the site of action of DCMU or simazine. This electron would be ultimately transferred to oxygen with the resulting formation of peroxide. Cyanide inhibition can be accounted for because of the obvious non-enzymatic reaction which would occur between the manganic ion and peroxide, *i.e.*, cyanide inhibition of catalase would result in the accumulation of peroxide which would in turn react with the oxidized manganese and the net effect would be as if no manganese oxidation had occurred. The latter mechanism implies a photochemical oxidation–reduction system in chloroplasts that results in an equilibrium between Mn^{2+} and Mn^{3+}. KENTEN AND MANN noted in their original finding that oxidized manganese was only slowly reduced in the dark. However, SWENSEN AND VERNON[10] have reported that manganipyrophosphate serves also as a Hill oxidant and furthermore supports oxygen evolution. These authors provided evidence which suggests that hydrogen peroxide was not an intermediate in their reaction. The fact that chloroplasts utilize an oxidized form of manganese as a Hill oxidant provides further evidence for the assumption that the amount of manganese oxidized at best represents only an equilibrium value between the two reactions. Under the conditions of our assay the equilibrium apparently favors oxidation during illumination. The accumulation of oxidized manganese may also be favored because of an inhibition of the Hill reaction and of photophosphorylation by two of the substrates for the re-

action, *i.e.*, manganous sulfate and pyrophosphate. This observation may also explain the low rates of manganese oxidation that were routinely obtained from either phosphate buffer-extracted chloroplast or those prepared by more conventional means.

Because the kinetics of the Hill reaction and manganese oxidation are so nearly identical in regards to temperature, light intensity, selective inhibitors and the presence or absence of plastoquinone it is tempting to speculate that the latter process is exclusively a photosystem II reaction. However, since the accepted method for distinguishing between reactions of photosystems I and II in chloroplasts, *i.e.*, the 2,6-dichlorophenolindophenol–ascorbate by-pass of the DCMU block, could not be employed under our experimental conditions it is not feasible to carry this line of speculation further. Nevertheless, we have found that digitonin-solubilized chloroplast fractions, which are known to catalyze certain photosystem I reactions, do not catalyze the photooxidation of manganese.

Additional objections to the hypothesis that manganese photooxidation is a system II reaction stem from the observed inhibitory action of cyanide and acriflavin and the requirement for flavin. These findings point to a mechanism involving reactions between a photochemically generated peroxide and either catalase or peroxidase which result in the photooxidation of manganese. This interpretation suggests that the mechanism of the chloroplast-catalyzed oxidation of manganese is not directly related to that of the known requirement for manganese in photosynthesis.

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