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SILICOMOLYBDATE AND SILICOTUNGSTATE MEDIATED DICHLOROPHENYLDIMETHYLUREA-INSENSITIVE PHOTOSYSTEM II REACTION: ELECTRON FLOW, CHLOROPHYLL *a* FLUORESCENCE AND DELAYED LIGHT EMISSION CHANGES

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SUMMARY

We have investigated the possible role of silicomolybdate and silicotungstate as acceptors of electrons in chloroplasts directly from Q, the primary electron acceptor of Photosystem II. Our data show:

1. Either of these compounds can accept electrons directly from Q in a 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU)-insensitive electron transport; however, the DCMU insensitivity is only short-lived, so initial rates must be used exclusively.

2. High concentrations of these silico compounds act as direct chemical quenchers of chlorophyll *a* fluorescence, but lower concentrations which also mediate O_2 evolution affect only the variable component of fluorescence in a manner suggestive of their electron-accepting capabilities.

3. Measurements of delayed light emission confirm the conclusions made from the fluorescence data. Also, they show the role of Q in delayed light emission as hydroxylamine data of other investigations have shown the role of Z, the electron donor of Photosystem II.

4. Silico compounds appear to be acting as electron acceptors and not as simple membrane modifiers allowing other acceptors to support a DCMU-insensitive electron transport.

INTRODUCTION

Girault and Galmiche [1] and Giaquinta et al. [2] have recently shown a 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU)-insentitive O_2 evolution from photosystem II in the presence of silicotungstate (SiTu) and ferricyanide (FeCy), and silicomolybdate (SiMo) and ferricyanide, respectively. Based on the fact that methylviologen, a Photosystem I acceptor of very low redox potential, did not support

Abbreviations: DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; SiTu, silicotungstate; SiMo, silicomolybdate; FeCy, ferricyanide.

a DCMU-insensitive O_2 evolution mediated by SiTu, the suggestion was made (1) that SiTu acts by altering membrane properties in such a manner as to allow ferricyanide (an acceptor of higher redox potential) to accept electrons between the Photosystem II reaction center and the DCMU site of inhibition, i.e. directly from Q, the primary electron acceptor of Photosystem II. Giaquinta et al. [2] supported similar conclusions with their data on the methylviologen-SiMo couple. Such a clear-cut Photosystem II partial reaction would greatly simplify many research problems in photosynthesis, and therefore, it is necessary to ascertain by other independent measurements that indeed one is measuring a DCMU-insensitive Photosystem II electron transport.

We have confirmed previous observations that O_2 evolution persists in the presence of DCMU with both FeCy and either SiMo or SiTu present. We studied more carefully the SiMo rather than the SiTu mediated O_2 evolution, as the rates of reactions were considerably higher in the former than in the latter case. We found that electron transport stops within 1 min after the addition of both DCMU and SiMo; monitoring, therefore, only initial rates after SiMo addition can be used for electron flow from H_2O to this chemical. Secondly, silico compounds are shown to be sufficient to sustain electron flow without the addition of FeCy; again, this reaction is DCMU-insensitive for only a limited time. SiMo appears to be acting as an electron acceptor itself.

If these silico compounds were indeed accepting electrons directly from Q, then one might predict that the variable chlorophyll *a* fluorescence yield (which reflects the level of reduced Q [3]) and delayed light emission (which reflects the back reaction between Q^- and Z^+, where Z is the electron donor to reaction center II [4]) in the presence or absence of DCMU would be severely depressed as Q would be kept in the oxidized state. The data reported in this communication, however, show that in addition to suppressing variable fluorescence, there is a remarkable decrease in "constant" fluorescence ("O" level), with concentrations of SiMo and SiTu greater than 25 μM. At concentrations lower than 25 μM of the silico acids where O_2 evolution is supported for only a brief time, the constant level of fluorescence is no longer depressed, yet the variable fluorescence decreases in proportion to the concentration of the silico-compounds; addition of DCMU in these cases only slightly raises the fluorescence maximum.

Measurements of delayed light emission of chloroplasts treated with concentrations of SiMo that only affect the variable fluorescence yield also give support to the fluorescence and oxygen data which show that these compounds are accepting electrons directly from Q.

MATERIALS AND METHODS

Chloroplast isolation from market spinach or romaine lettuce was as described previously [5], but with phosphate buffer of similar concentration and pH substituted for the Tris · HCl buffer; fresh, rather than frozen, chloroplasts were used in this study. Chlorophyll estimation was made according to the method of Mackinney [6].

O_2 evolution was measured with a Clark electrode, using a Yellow Springs oxygen monitor (model 53) and an Esterline Angus recorder (model E11015). The

time course of chlorophyll *a* fluorescence was measured according to the method of Munday and Govindjee [7] with a spectrofluorometer described elsewhere [8]. The method for measuring delayed light emission was esentially that of Jursinic and Govindjee [9].

Light-induced absorbance changes were measured at 540 nm using the split beam difference spectrophotometer of Sybesma and Fowler [10]. The measuring monochromator was set at 540 nm (half band width, 6.6 nm), and a 540-nm Farrand interference filter (half band width, 1.6 nm) was placed before the photomultiplier to eliminate the blue actinic light (Farrand interference filter 480, half band width, 5.5 nm).

Other details are given in the legends of the figures and tables.

RESULTS

SiMo and SiTu as electron acceptors: electron flow

As indicated in Fig. 1, curve D, SiMo can restore O_2 evolution to DCMU-inhibited electron flow to FeCy, as already shown by Giaquinta et al. [2]. However, these rates are initial rates, as after 45 s after addition of the SiMo, the rate of O_2 evolution begins to level off and is immeasurable at 1 min after addition. It is not a case of time-dependent photoinactivation of the chloroplasts as there is no change in

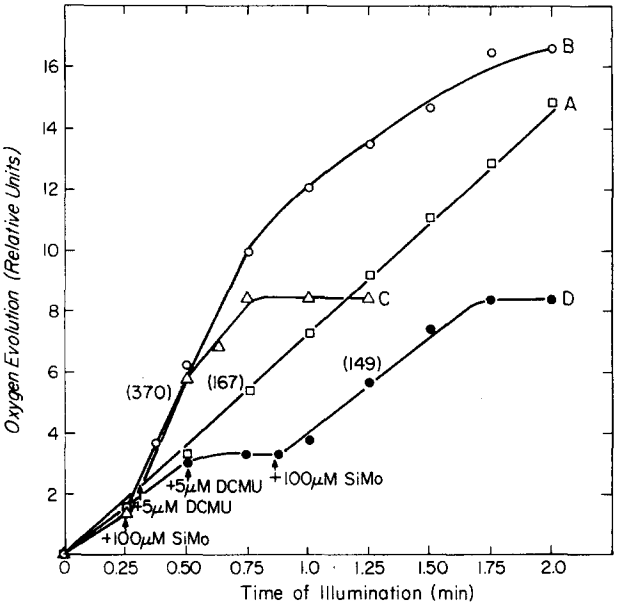

Fig. 1. FeCy-mediated O_2 evolution of spinach chloroplasts in the presence or absence of SiMo and DCMU. The reaction mixture contained in 1.7 ml: 100 mM KCl, 5 mM $MgCl_2$, 20 mM Tricine/KOH (pH 8.1), 0.5 mM FeCy and chloroplasts equivalent to 35 μg chlorophyll per ml. A. □ – □ FeCy as electron acceptor, no further additions. B. ○ – ○, 100 μM SiMo added at 0.25 s. C. △ – △, 100 μM SiMo added at 0.25 s and 5 μM DCMU added directly thereafter. D. ○ – ○, FeCy as electron acceptor with 5 μM DCMU added at 0.5 s, followed with SiMo at 0.85 s. Numbers in parentheses are initial rates of O_2 evolution expressed in μequiv/mg chlorophyll per h.

the rate of O_2 evolution supported by FeCy until after several minutes (curve A). Neither is it a case of limiting concentrations of SiMo, as when no DCMU is present, the rate is still measurable beyond 45 s for some time, although it does begin to slow down (curve B). Moreover, addition of a second aliquot of SiMo will almost completely restore the original rate to these chloroplasts, while similar additions to DCMU-treated chloroplasts will not restore O_2 evolution.

As can be seen in Fig. 1, curve D, rates of O_2 evolution as restored by SiMo to DCMU-treated chloroplasts are approximately the same as those rates with FeCy alone. Addition of SiMo to chloroplasts evolving O_2 with FeCy as electron acceptor results in an increased electron transport rate (curve B). As shown in curve C, if the SiMo addition is followed shortly thereafter with DCMU addition O_2 evolution is completely inhibited in approximately 30 s.

FeCy need not be present to see O_2 evolution with SiMo; in fact, as seen in Table I, the rate with SiMo alone (added in the light) is more than double that of the control rate with FeCy. It is interesting to note that the illumination of the chloroplasts in the acceptor-less reaction mixture prior to addition of the SiMo yields a much higher rate of electron transfer than if the compound is added to dark-adapted chloroplasts (Table I), while this is not the case for FeCy-mediated electron flow. Also, addition of DCMU to the chloroplasts in the dark followed by addition of SiMo in the light considerably decreases the initial rate of DCMU-insensitive O_2 evolution as compared to the rate when DCMU is added in the light after SiMo addition, or even when DCMU is added to illuminated chloroplasts prior to SiMo addition.

TABLE I

SILICOMOLYBDATE-MEDIATED OXYGEN EVOLUTION

Chl stands for chlorophyll in this and the following tables. The reaction mixture contained in 1.7 ml: 100 mM KCl, 5 mM $MgCl_2$, 20 mM Tricine/KOH (pH 8.1) and chloroplasts equivalent to 35 μg chlorophyll per ml. Where indicated, 0.5 mM FeCy, 100 μM SiMo and 5 μM DCMU are added. The rates represented are initial rates.

Chloroplasts	Electron acceptor	Treatment	O_2 evolution (μequiv/mg Chl per h)
Normal	FeCy added in dark	None	103
Normal	FeCy added in light	None	107
Normal	SiMo added in dark	None	82
Normal	SiMo added in light	None	250
Normal	SiMo added in light	DCMU added in dark (prior to SiMo)	98
Normal	SiMo added in light	DCMU added in light (prior to SiMo)	156
Normal	SiMo added in light	DCMU added in light (after SiMo)	250
None	SiMo added in light	None	0
Heat-treated 5 min, 50 °C	FeCy added in dark	None	0
Heat-treated 5 min, 50 °C	SiMo added in light	None	0

As has been adequately documented (see ref. 11), illumination of chloroplasts results in a reversible absorbance change at 540 mn, an indication of conformational and configurational changes in the membrane resulting from electron flow. Chloroplasts used in all experiments reported in this communication showed this light-induced reversible absorption change at 540 nm (Fig. 2). Under our experimental conditions, the chloroplasts had only endogenous electron acceptors; thus, the extent of the absorption change, reported here, was approximately ten times smaller than that observed when artificial electron acceptors are added [11]. We would like to suggest that SiMo alone because of its large size can perturb the membrane to some extent to make more accessible Q for direct reduction. However, if the chloroplasts are first exposed to light prior to SiMo addition, the light-induced change in the structural organization of the membrane might result in a repositioning of some of the electron transport components in the membrane such that Q is more exteriorly located on the membrane; in this manner Q can more efficiently donate its electrons to SiMo, which might explain the 3-fold increase in electron transport rates when SiMo is added to illuminated rather than dark-adapted chloroplasts. Addition of DCMU to dark-adapted chloroplasts results in the elimination of the light-induced absorbance change at 540 nm, as shown in Fig. 2, and a rate of O_2 evolution with SiMo added in the light (after dark DCMU addition) not greatly different from the rate seen with dark addition of the acceptor (Table I), which can be explained by the model described above. If DCMU and then the SiMo are added to illuminated chloroplasts, the rate of O_2 evolution is intermediate between the rate seen when SiMo is added to illuminated and dark-adapted chloroplasts, which might be understood in terms of the light-induced structural change of the chloroplast membrane being slowly relaxed with DCMU addition, resulting in a somewhat decreased availability of Q for SiMo. DCMU added to chloroplasts already evolving O_2 with SiMo does not alter the

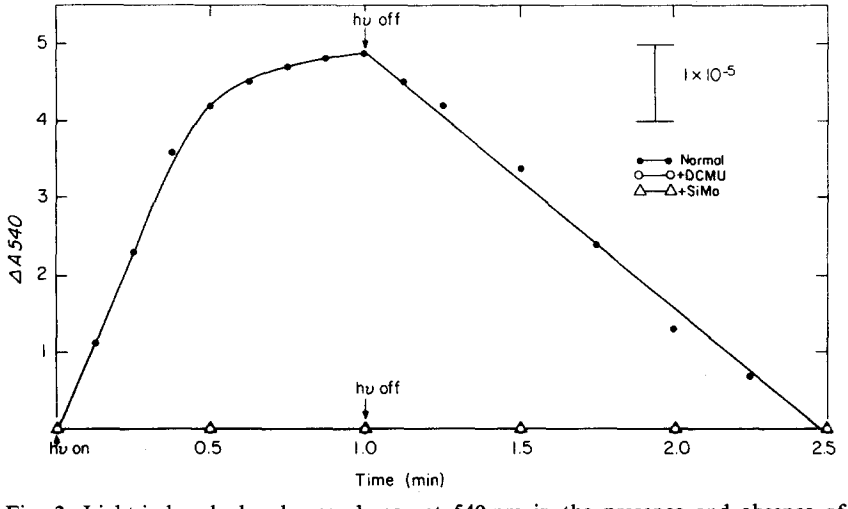

Fig. 2. Light-induced absorbance change at 540 nm in the presence and absence of DCMU or SiMo. Reaction mixture as in Table I with chloroplasts equivalent to 35 μg chlorophyll per ml contained in a 1-mm pathlength cuvette. ●—●, normal chloroplasts, no additions; ○—○, 5 μM DCMU; △—△, 100 μM SiMo. Additions made to fresh, dark-adapted samples. Instrumental conditions as in Materials and Methods.

initial rates, simply showing the initial insensitivity of SiMo reduction to DCMU.

Fig. 2 also shows that addition of SiMo to dark-adapted chloroplasts results in the elimination of the light-induced absorbance change at 540 nm, as is the case with DCMU. As we know that electron transport through Photosystem II is indeed occurring, we must postulate one of two possibilities: (1) that electron flow from Q to some component in the intersystem electron transport chain or in Photosystem I is necessary to support the structural changes that lead to 540-nm absorption changes, or (2) that the absence of phosphorylation, for reasons not yet clearly understood, in the SiMo system [2] stops the light-induced absorption changes.

SiMo and SiTu used alone are not catalyzing some light-induced, unprecedented type of O_2 evolution independent of photosynthesis, as illumination of the reaction mixture either in the absence of chloroplasts or in the presence of chloroplasts heated to 50 °C for 5 min, which have been shown to have an inhibited water oxidation reaction [12], does not produce any O_2, as shown in Table I.

Fig. 3 shows that as with FeCy and SiMo used in conjunction, DCMU will inhibit the Hill reaction supported by SiMo alone shortly after 30 s of DCMU addition. As in the case of FeCy and SiMo used with DCMU, the second addition of SiMo restores O_2 evolution only to those chloroplasts that have not seen DCMU (curve A versus curve B).

Data presented for SiMo essentially have been confirmed for the SiTu system (data not shown). Relatively low concentrations of silicotungstic acid (20 μM) can partially relieve the DCMU inhibition of O_2 evolution with FeCy as electron acceptor, and the O_2 evolution is DCMU-insensitive only for a limited time. SiTu can also

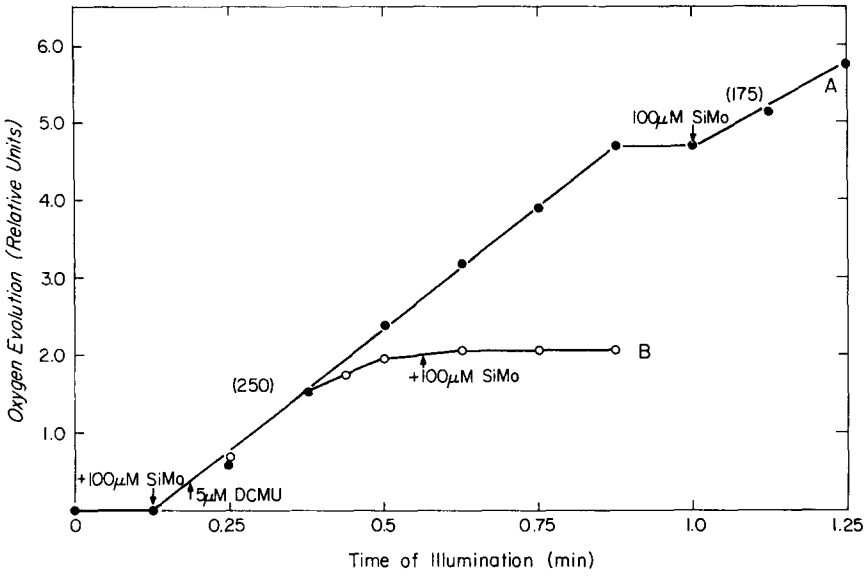

Fig. 3. SiMo-mediated O_2 evolution of spinach chloroplasts in the presence and absence of DCMU. Reaction mixture as described in the legend of Fig. 1 with FeCy omitted; 5 μM DCMU added to sample B only. Numbers in parentheses are initial rates of O_2 evolution expressed in μequiv/mg chlorophyll per h. The control rate of O_2 evolution in the water to FeCy reaction (minus DCMU and SiMo) was 103 μequiv/mg chlorophyll per h in this particular chloroplast preparation.

TABLE II

SiMo-MEDIATED O_2 EVOLUTION IN THE ABSENCE OF FERRICYANIDE

The conditions are as described in the legend of Table I. SiMo in concentrations given was added in the light. Only initial rates are reported. The control rate with electron flow to ferricyanide was 96 μequiv/mg chlorophyll per h.

SiMo concentration (μM)	O_2 evolution (μequiv/mg Chl per h)
0	0
5	102
10	124
25	209
50	246
100	240
200	231
300	192

act alone in supporting O_2 evolution, with rates, using 100 μM SiTu, approximately one-half of those with the same concentration of SiMo. Table II shows rates of O_2 evolution mediated by SiMo; very low concentrations of SiMo support O_2 evolution, although clearly the time in which electron transport is sustained is severely limited with the lower concentrations of the acceptor.

Chlorophyll a fluorescence

In the presence of DCMU, the fluorescence yield of broken chloroplasts rapidly rises to a maximum due to an accumulation of reduced Q and remains so throughout illumination [13]. Addition of FeCy to the DCMU-treated chloroplasts results in only a small decrease in the fluorescence yield due to the filter effect of the colored acceptor; this is expected as FeCy accepts electrons after the DCMU block (data not presented here). As first shown by Malkin and Kok [14], FeCy added to normal chloroplasts keeps Q in the oxidized state and the fluorescence level remains low (close to F_0), until the FeCy is reduced, after which the maximum fluorescence level (F_∞) is finally reached as Q becomes reduced. Only in the case where the electron acceptor is autooxidizable, does the fluorescence level remain low, as shown by Mohanty et al. [15] with methylviologen. As we had no reason to believe that SiMo is autooxidizable as it seems to become concentration limiting as it is reduced in mediating O_2 evolution, we expected a fluorescence transient similar to that seen for FeCy, but DCMU-insensitive.

We looked at the chlorophyll *a* fluorescence induction in the presence of the silico-compounds and DCMU to arrive at an independent measurement for the proposed electron acceptor role for the silico compounds (i.e. before the DCMU block and directly from Q). It was, however, surprising to find that SiMo or SiTu at 100 μM concentration (the same concentrations we routinely used to measure O_2 evolution) is a very potent quencher of chlorophyll *a* fluorescence in the chloroplasts; as expected, DCMU does not affect this quenching, as shown in Fig. 4(A). It is interesting to no tethat neither SiMo nor SiTu has any quenching effect on chlorophyll

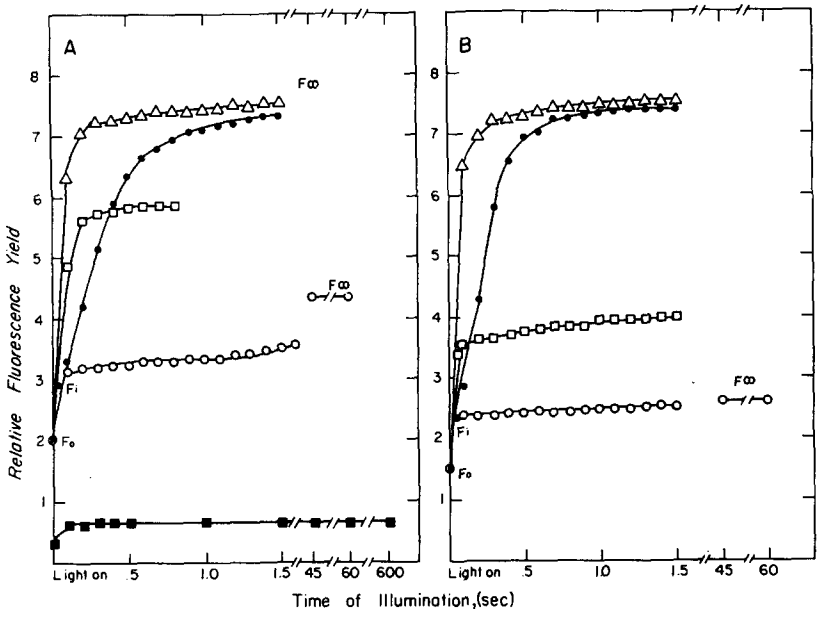

Fig. 4. Time course of chlorophyll *a* fluorescence yield in spinach chloroplasts with and without SiMo and DCMU. Fluorescence was measured at 685 nm (half-band width, 6.6 nm) (A) ●—●, normal chloroplasts without any addition; ○—○, 5 μM SiMo; ■—■, 100 μM SiMo (with and without 5 μM DCMU); △—△, normal chloroplasts with 5 μM DCMU; □—□, 5 μM SiMo and 5 μM DCMU. (B) ●—●, normal chloroplasts without any addition; ○—○, 25 μM SiMo; △—△, normal chloroplasts with 5 μM DCMU; □—□, 25 μM SiMo and 5 μM DCMU. Excitation, broad band blue light (C.S. 4-96 and C.S. 3-73), incident intensity, approx. 10^4 ergs · cm^{-2} · s^{-1}; concentration chlorophyll, 70 μg in 2 ml buffer, 20 mM Tricine/KOH (pH 8.1), containing 100 mM KCl and 5 mM $MgCl_2$; chloroplasts dark-adapted before each measurement.

a in solution, as indicated in Table III. The dramatic quenching phenomenon observable in the chloroplast appears to rely upon the chlorophyll as being bound in the membrane as some chlorophyll-protein complex. This is quite unlike the quenching properties of other well-studied chemicals, such as dinitrobenzene which effectively quenches chlorophyll fluorescence both in solution and in the chloroplast [16]. At lower concentrations of SiMo, as shown in Fig. 4, the constant fluorescence (F_0) and

TABLE III

EFFECT OF SILICOMOLYBDATE AND SILICOTUNGSTATE ON CHLOROPHYLL *a* FLUORESCENCE IN SOLUTION

Chlorophyll extracted from spinach chloroplasts with 80 % acetone and subsequently centrifuged to remove non-chlorophyllous materials; 70 μg chlorophyll in 2 ml of 80 % acetone used as sample. Instrumental conditions as in legend of Fig. 4.

Additions	Relative fluorescence yield at 685 nm
None	53.5
100 μM SiMo	55.0
100 μM SiTu	54.0

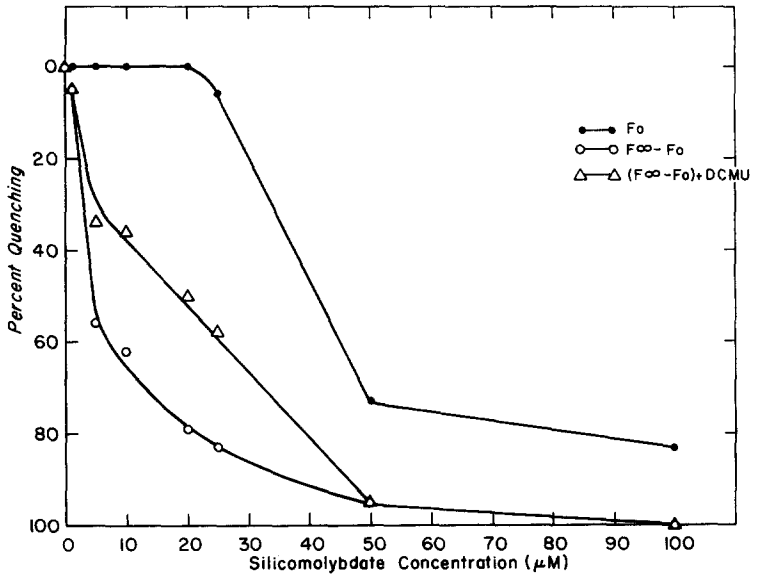

Fig. 5. Percent quenching of chlorophyll *a* fluorescence at F_0, and at F_∞-F_0 (in the presence and absence of 5 μM DCMU) as a function of SiMo concentration. To calculate percent quenching of the variable fluorescence yield in the presence of SiMo plus DCMU, the constant fluorescence was assumed to be the same in the presence of DCMU as in the SiMo-treated chloroplasts. Conditions as indicated in the legend of Fig. 4.

the rise in fluorescence corresponding to the photochemical reaction alone (F_0 to F_i) are not altered by the SiMo, while the rise to F_∞ is much slower, and the maximum level as seen in the control chloroplasts is not even met when DCMU is added. Effects on fluorescence kinetics are shown for both 25 and 5 μM SiMo additions to illustrate the different degrees of quenching of the variable fluorescence level. Percent variable fluorescence of the total fluorescence may vary in different chloroplast preparations, as is the case here; therefore, it is mandatory to use appropriate controls for each measurement. As shown in Fig. 5, a 1-μM concentration of SiMo or SiTu does not significantly affect the fluorescence transient, while concentrations above 5 μM and below 25 μM do not affect the fluorescence at the 0 level but do have a large quenching at the *P* (F_∞) level, and at these concentrations, DCMU only partially restores the maximum level of fluorescence. Concentrations of SiMo and SiTu above 25 μM greatly depress the 0 level of fluorescence. These observations parallel the quenching properties described for dinitrobenzene [16], although there are some differences noted here.

Lavorel and Joliot [17], using moderately quenching concentrations of dinitrobenzene, found that the sigmoidal 0 to *P* rise typical of normal chloroplasts changes to exponential with dinitrobenzene addition, and they have extended this data to support the connected units model for energy migration. Fig. 6 shows an oscilloscope trace of chlorophyll *a* fluorescence of normal and 25 μM silicomolybdate treated samples; the photographic film was purposely exposed twice to emphasize the fact that the two transients are virtually identical. Equally superimposable induction curves in the first 40 ms after onset of illumination were obtained with control chloro-

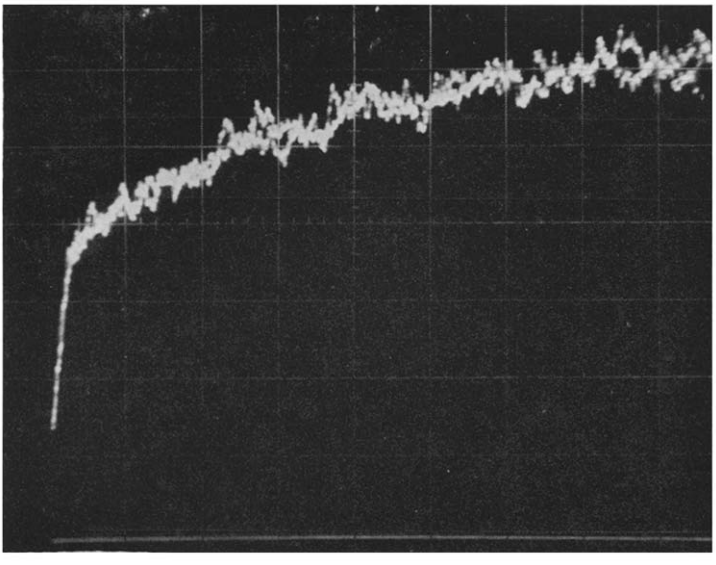

Fig. 6. Time course of chlorophyll *a* fluorescence yield in spinach chloroplasts; normal chloroplasts and chloroplasts plus 25 μM SiMo superimposed upon each other. Oscilloscope setting: 0.2 V vertical division; 5 ms horizontal division. Conditions as in Fig. 3.

plasts compared with the same chloroplasts treated with 5 or 10 μM SiMo (data not shown). The significant differences in the shapes of the two induction curves in the case of the dinitrobenzene-treated chloroplasts are lacking here. (Whether this data presents new possibilities for alternative models for energy migration is not yet clear to the authors and, thus, will not be discussed further.)

Etienne and Lavergne [16], working with dinitrobenzene, separated the action of the same quencher on the basis of concentration into a quencher of the photo-

TABLE IV

IRREVERSIBILITY OF THE QUENCHING EFFECTS OF THE 100-μM SILICO COMPOUNDS

Conditions as in legend of Fig. 3 with 100 μM additions of the silico compounds given to the control chloroplasts as indicated below. Washing was done by preincubating the chloroplasts, suspended to 35 μg chlorophyll per ml in the usual reaction mixture, with 100 μM SiMo or SiTu for 5 min in the dark at 0 °C, followed by centrifugation at $1000 \times g$ for 10 min, and resuspension of the chloroplasts in the same volume of buffer. The control was washed in the same manner without any additions.

Treatment	Additions	Relative fluorescence at F_0	Relative fluorescence at F_∞
I. Normal	None	33.5	97.5
	100 μM SiMo	3.5	6.5
	100 μM SiTu	4.0	7.5
II. Washed	None	34.0	94.0
	100 μM SiMo	3.0	6.5
	100 μM SiTu	4.5	8.0

TABLE V

EFFECT OF SALTS ON THE QUENCHING OF CHLOROPHYLL *a* FLUORESCENCE BY 100 μM SILICOMOLYBDATE

Conditions as in legend of Fig. 4. Additions and reaction mixtures as indicated.

Reaction mixture	Additions	Relative fluorescence at F_0	Relative fluorescence at F_∞
20 mm Tricine, 100 mM KCl, 5 mM $MgCl_2$ (usual reaction mixture)	None	18	72
	100 μM SiMo	3	6
2 mM Tricine	None	14.5	61.5
	100 μM SiMo	7	16
2 mM Tricine, 0.5 M $MgCl_2$	None	44	60
	100 μM SiMo	5	5
2 mM Tricine, 50 mM $MgCl_2$	None	16	88
	100 μM SiMo	10	19

chemical ($0 \rightarrow I$) and thermal ($I \rightarrow P$) phases of fluorescence. They found that the quenching was irreversible as far as the thermal rise was concerned but reversible with respect to the photochemical rise. We found that both phases were affected irreversibly with 100 μM SiMo or SiTu, as indicated in Table IV, by incubating the chloroplasts with the silico compounds for 5 min in the dark, followed by centrifugation and resuspension of the chloroplasts in the same volume of buffer.

Lastly, we looked briefly at the effect of cations on the degree of quenching by the SiMo. Papageorgiou and Argoudelis [18] reported that high concentrations of divalent cations increased the quenching capabilities of dinitrobenzene by enhancing the hydrophobicity of the membrane and thereby making chlorophyll more accessible to the quencher. This appears also to be the case with SiMo and SiTu, although lesser concentrations of divalent cations and significant amounts of monovalent cations can be more effective than the extremely high concentrations of $MgCl_2$ Papageorgiou and Argoudelis used in quenching chlorophyll *a* fluorescence both at the constant and variable levels, as indicated in Table V.

It is obvious that the apparent chemical quenching of these silico compounds at high concentrations makes it impossible to compare the effects of DCMU on leveling, within a short time, of the initial rates of O_2 evolution. From parallel O_2 measurements, if these compounds were not acting as direct chemical quenchers, one might expect fluorescence to rise when the original 100 μM SiMo becomes limiting, i.e. within 2 min after its addition. Indeed, these contradictions existing between the fluorescence and oxygen data simply point out that these compounds are not acting as simple acceptors such as FeCy but have the additional effect of directly quenching fluorescence.

Delayed light emission

Table VI shows the effect of SiMo on quenching the intensities of the slow component (100 ms after cessation of illumination) of delayed light emission from

TABLE VI

EFFECT OF SILICOMOLYBDATE ON DELAYED LIGHT EMISSION (DLE) AT 100 ms FOLLOWING CESSATION OF ILLUMINATION

1 ml of sample, containing 35 μg chlorophyll in reaction mixture as described in Table I, was illuminated for 15 s with blue light (Corning filter: C.S. 4-96). The intensity of the exciting light was approx. 10 kergs $\cdot$ cm^{-2} $\cdot$ s^{-1}. Delayed light emission was measured approx. 100 ms after the cessation of the illumination. Additions were made in the dark; DCMU, in 5 μM concentrations, was added to the chloroplasts in the dark after SiMo addition.

SiMo concentration (μM)	% quenching of DLE	% quenching of DLE with DCMU added
100	100	100
50	89	89
25	85	75
10	75	62
5	55	51
1	5	3

spinach chloroplasts. If SiMo does in fact accept electrons directly from Q, then the electron donor to Photosystem II, Z, can become oxidized and accumulate charges, but Q will be oxidized very quickly by the SiMo. The elimination or quenching of the slow component of delayed light with the addition of SiMo can be interpreted to be due to the inhibition of the back reaction between oxidized donor Z^+ and reduced acceptor Q^-, as has been the interpretation with hydroxylamine and DCMU-treated chloroplasts [19–21]. In fact, this experiment will provide the role of Q in delayed light emission as hydroxylamine data does for Z.

100 μM SiMo eliminates delayed light emission, and DCMU addition has no effect on the delayed light. As the concentration of SiMo is decreased, the percent quenching of delayed light emission is likewise decreased, and finally at 1 μM concentrations, the intensity of delayed light is not appreciably less than that of the control. DCMU added after SiMo appears to slightly increase the intensity of delayed light emission that has been quenched by concentrations of SiMo that have an effect only on the variable yield of fluorescence. The above results (in Table VI) thus provide an experimental suggestion for Q to be indeed playing a role in delayed light emission, as the hydroxylamine data [19–21] did for Z. Furthermore, the percentage quenching of delayed light emission by SiMo may reflect the average rate of SiMo reduction during illumination prior to delayed light measurement.

DISCUSSION

Three lines of evidence presented in this communication indicate that both SiMo and SiTu can act as direct electron acceptors from Photosystem II before the DCMU site of inhibition: (1) SiMo and SiTu mediated O_2 evolution in the presence of DCMU, but in the absence of additional electron acceptors, (2) the effects of the compounds on chlorophyll *a* fluorescence induction curves, and (3) the effects of the compounds on delayed light emission.

The first and most direct indication is that both SiMo and SiTu can accept electrons flowing from water in the absence of FeCy and that this electron transport

is DCMU-insensitive for a limited period of time. We have shown that if the SiMo- or SiTu-mediated O_2 evolution in the presence or absence of FeCy is to be used, one must necessarily be concerned with initial rates only, as the incipient insensitivity to DCMU is only short-lived. The leveling of O_2-evolving rates in the absence of DCMU at concentrations less than 50 μM appears to be due to limiting concentrations of the acceptor, as further additions of the silico compounds repeatedly restore the original rate. At higher concentrations of SiMo and SiTu, this leveling probably also reflects the interference of these chemicals in the transfer of excitation energy among the chlorophyll molecules (or alternately changes the rate constant of internal conversion) as witnessed by the dramatic quenching effects on "constant" fluorescence, although this explanation is perhaps oversimplified, as the quenching effect on fluorescence is manifested the moment the chloroplasts are illuminated, while the decrease in O_2 evolution is seen only after many seconds.

Fluorescence data also support the suggestion that these silico compounds accept electrons directly from Q. In these studies, one must look only at the chlorophyll *a* induction curves of samples to which only low concentrations of silico compounds have been added, as high concentrations ($\geqslant$ 50 μM) greatly suppress the constant (F_0) level of fluorescence, apparently acting as direct chemical quenchers of fluorescence. The slower kinetics of the variable rise in fluorescence and the proportional quenching of the maximum fluorescence level with increasing SiMo concentrations suggest that SiMo is behaving as an electron acceptor. DCMU somewhat decreases the quenching of F_∞, but not sufficiently to return the fluorescence level to that seen with DCMU-treated control chloroplasts. These data raise two questions if one is to compare these findings with those for O_2 evolution. First, we find that O_2 evolution with low concentrations of SiMo levels within a short time, suggesting as mentioned above, that all SiMo is reduced; it is not clearly understood why the fluorescence rise to F_∞ is not met after several minutes of illumination, as might be expected if one is to draw an analogy with the effect of other non-autoxidizable acceptors; eventually, when all the acceptor is reduced, F_∞ should be the same as that in the control sample. One very simple explanation might be that fluorescence is a much more sensitive measure of electron flow than is the use of the concentration electrode, and very low levels of O_2 evolution may be read as zero, while one can still see changes in fluorescence. Secondly, the fact that DCMU can somewhat decrease the quenching of fluorescence by SiMo may possibly show that although electrons preferentially flow to SiMo, as the compound becomes reduced, there may be additional electron flow diverted to the "A" pool, and addition of DCMU blocks this electron transport.

Delayed light is one additional, independent confirmation of the action of SiMo as electron acceptor from Q. Quenching of delayed light emission by SiMo (and SiTu, data not presented here) is a reflection of the fact that these silico compounds keep Q in the oxidized state so that there can be no back reaction between reduced Q and oxidized Z to produce delayed light. The illumination time of the chloroplasts in the presence of SiMo was 15 s, so in very low concentration ranges of SiMo, it is conceivable that the lessened quenching seen may be due to the fact that most of the acceptor is in this reduced state. DCMU again has an effect comparable to that seen in fluorescence, relieving some of the quenching of delayed light emission by SiMo at concentrations lower than 50 μM. It is interesting to note that

this effect is opposite to that seen in our control chloroplasts, where DCMU decreases the intensity of the slow component of delayed light.

The fact that SiMo or SiTu can support O_2 evolution in the absence of any (additional) electron acceptor suggests to us that the previously proposed mode of its action, i.e. altering membrane properties such that FeCy can accept electrons directly from Q, cannot be the situation, at least not in all cases. Both SiMo and SiTu are extremely large molecules, and therefore, probably non-penetrating. As shown, cations effectively increase the quenching of chlorophyll *a* fluorescence by these compounds, which suggests that an increased hydrophobicity of the membrane permits better accessibility to the chlorophyll. Preillumination of acceptor-less chloroplasts prior to addition of the SiMo results in a 3-fold greater rate of O_2 evolution as compared to rates when SiMo is added to dark-adapted chloroplasts, suggestive of a light-induced conformational change in the membrane that makes the silico compounds more accessible to Q.

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