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# SILICOTUNGSTATE LOWERS DRAMATICALLY THE QUANTUM YIELD OF CHLOROPHYLL FLUORESCENCE IN SITU WITHOUT AFFECTING THE RATE OF ELECTRON TRANSPORT

YUNG-SING LI, SHIOW-HWEY UENG and BI-YU LIN

*Institute of Botany, Academia Sinica, Taipei, Taiwan (China)*

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**Electron-transport and fluorescence properties of chloroplasts in the presence of silicotungstate are studied by $O_2$-evolution and fluorescence-quenching techniques. Results show that silicotungstate lowers the fluorescence quantum yield of closed units, without affecting electron-transport activities of open units. Open and closed units are units with oxidized and reduced primary electron acceptor (Q), respectively.**

## Introduction

Silicotungstate, at low concentrations and under high light illumination, lowers dramatically fluorescence without affecting the rate of election transport [1]. At low light intensity and in the presence of a high concentration of ferricyanide, silicotungstate becomes ineffective in quenching fluorescence (upper panels of Fig. 1, results reproduced and rearranged from Ref. 1). At low light intensities, ferricyanide may keep most of the primary electron acceptor (Q) of Photosystem (PS) II, oxidized. These observations then suggest that silicotungstate at low concentrations suppresses, mainly, the fluorescence quantum yield ($\phi_f$) of $Q^-$ units. This is explained as follows.

In general, as the rate of electron transport increases, fluorescence falls, and vice versa; sometimes their relationships are complementarily linear [3,4]. This is because: (i) fluorescence is mainly emitted from PS II [5]; (ii) the rate is normally limited by reaction steps between the two photosystems [5] which limit the oxidation of the reduced primary electron acceptor, $Q^-$, of PS II; and (iii) Q is a fluorescence quencher [6].

A residual fluorescence exists even when all Q are oxidized which is defined as constant fluorescence. Upon Q reduction (units closed) the fluorescence increases; the increment is variable fluorescence [7]. Normally, constant fluorescence is 25–35% of total fluorescence [5,8]. If the higher value (35%) is taken, Fig. 1 shows that at the high light intensity used when electron transport is in effect, silicotungstate eliminates most of the variable fluorescence.

The intensity ($F$) of variable fluorescence is determined by three factors [5], namely, intensity ($\alpha_2 I$), yield ($\phi_f$) and concentration ($q^-$)

$$F = \alpha_2 I \phi_f q^- \quad (1)$$

where $\alpha_2 I$ is the number of quanta per unit time collected by PS II, $\phi_f$ has already been defined and $q^-$ is the normalized concentration of $Q^-$. The rate ($R$) of electron transport is also determined by three factors [5]:

$$R = \alpha_2 I \phi_{2c} q \quad (2)$$

Abbreviations: Tes, *N*-tris(hydroximethyl)methyl-2-aminoethanesulfonic acid; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCIP, 2,6-dichlorophenolindophenol; PS, photosystem; Chl, chlorophyll.

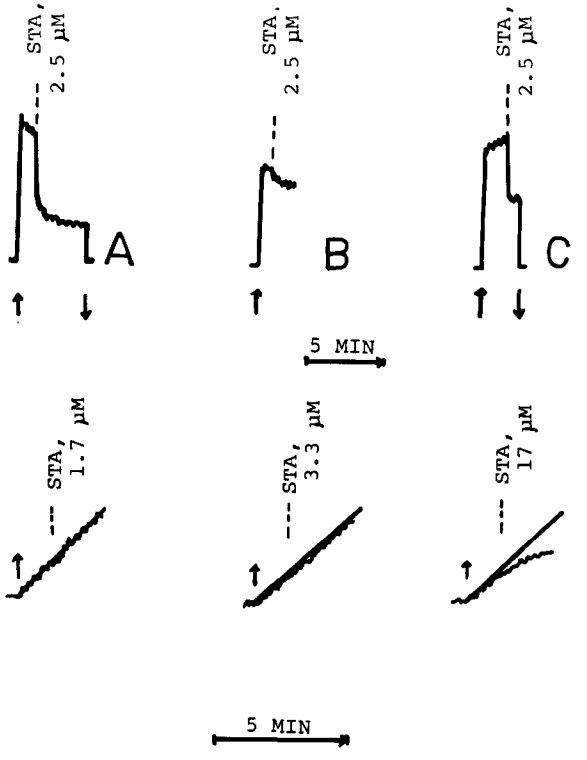


Fig. 1. Effects of silicotungstate on fluorescence (upper) and on $O_2$ evolution (lower). Upward arrows, light on; downward arrows, light off. Dashed lines mark the times when silicotungstate (STA) was added during illumination. Fluorescence traces: (A) Light intensity (green light [2]) 170 kerg/cm$^2$ per, in the presence of 7.5 mM ferricyanide. (B) Light intensity 10 kerg/cm$^2$ per s, in the presence of 7.5 mM ferricyanide. (C) Light intensity 10 kerg/cm$^2$ per s, in the absence of ferricyanide. The fluorescence scales are not the same for traces A–C. For $O_2$ measurements, the intensity was 130 kerg/cm$^2$ per s (incandescent light filtered by a solution of $CuSO_4$). The initial rate (slope, shown in the figure as a slanting line) of the left trace is about 61 μmol/mg Chl per h. The concentration of chloroplasts for $O_2$ measurement was 13 μg Chl/ml. Those for fluorescence experiments were 10 μg Chl/ml in the cases of traces A and B, 2 μg Chl/ml in the case of trace C.

where $\phi_{2c}$ is the photochemical quantum yield of PS II, and $q$ the normalized concentration of Q. The complementarily linear relationship between $R$ and $F$ arises from the fact that $q + q^- = 1$.

But we emphasize the fact that variable fluorescence arises from $Q^-$ units whereas photochemistry occurs in Q units. This permits an agent to change $F$ without affecting $R$, if it modifies only $\phi_f$. Silicotungstate may be such an agent. Under an extreme condition, silicotungstate eliminates all variable fluorescence while still permitting $O_2$ evolution to take place, albeit with a lower efficiency [9].

To establish firmly the $\phi_f$ hypothesis of the silicotungstate effect, we have examined the silicotungstate effect on the area of fluorescence dip ($F_{quen}$) induced by a tiny amount of oxidant. In response to this addition of oxidant, chloroplasts evolve a small amount ($A$) of $O_2$. Using the relationship $q + q^- = 1$ to combine Eqns. 1 and 2, we have:

$$F = \alpha_2 I \phi_f - \phi_f R / \phi_{2c} \tag{3}$$

in which

$$f_{quen} = -\phi_f R / \phi_{2c} \tag{4}$$

where $f_{quen}$ is the amount of fluorescence quenched at any instant, $f_{quen}$ gives Eqn. 5 on integration,

$$F_{quen}\ (\text{area of fluorescence dip}) = -(\phi_f / \phi_{2c}) A \tag{5}$$

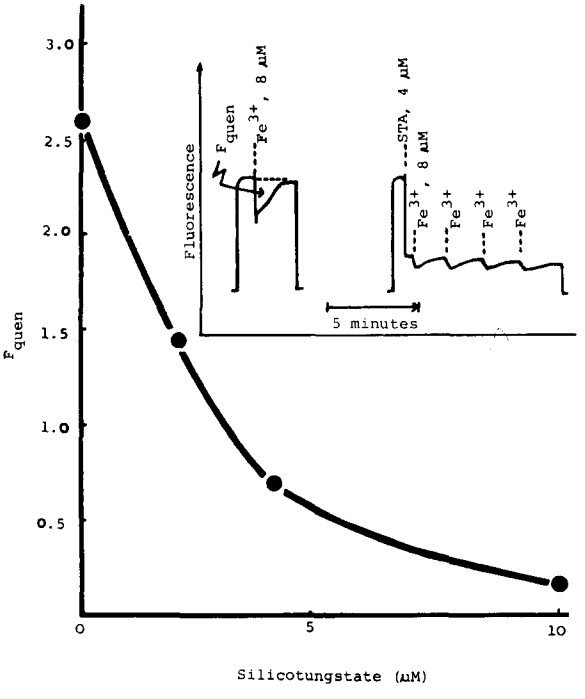


Fig. 2. The area of ferricyanide-induced fluorescence dip ($F_{quen}$, see inset, this figure) as a function of silicotungstate concentration. Light intensity was 14 kerg/cm$^2$ per s.

where $A = \int_0^t R\,dt$ is the total amount of $O_2$ evolution induced by a definite amount of Hill oxidant. There is no $\alpha_2 I$ term, which is not possible to determine experimentally in a quantitative and unequivocal way, in this equation. The rate $R$ is also absent. In other words, in the determination of the silicotungstate effect on $\phi_f$, changes in rates of dark reactions of electron transport and $\alpha_2 I$ are not relevant.

We report here that silicotungstate affects $F_{quen}$ without greatly altering $\phi_{2c}$ and $A$. This confirms our postulation that silicotungstate lowers fluorescence intensity by decreasing $\phi_f$ [1].

## Materials and Methods

Chloroplasts were isolated and assayed by methods described in Ref. 1, except Tes was omitted from both the isolation and assay media, and lettuce instead of oats was used as the source of chloroplasts (see Refs. 2 and 10, respectively, for methods of fluorescence and $O_2$ studies).

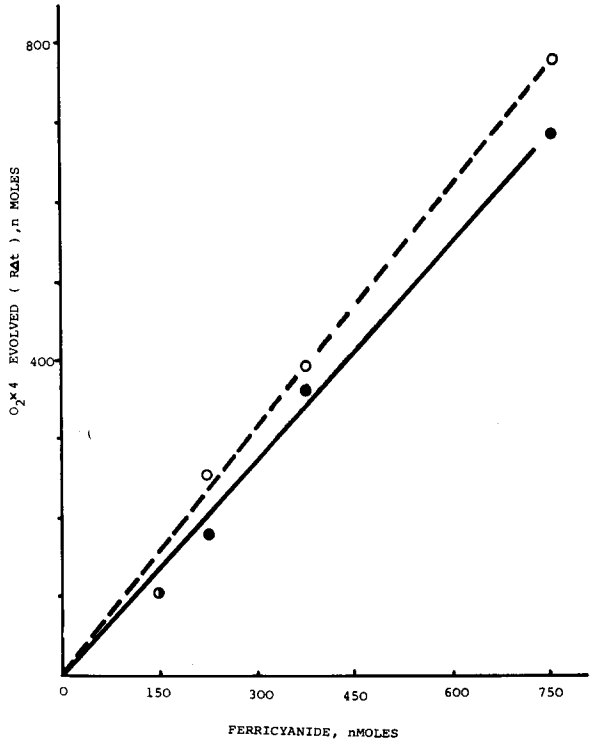


Fig. 3. Relationship between the amount of ferricyanide added and the amount of $O_2$ evolved in the absence (○) and presence of silicotungstate (●). Light intensity was near saturation. Gramicidin D (3.3 μM) was added to all samples. Silicotungstate when added had a concentration of 3.3 μM.

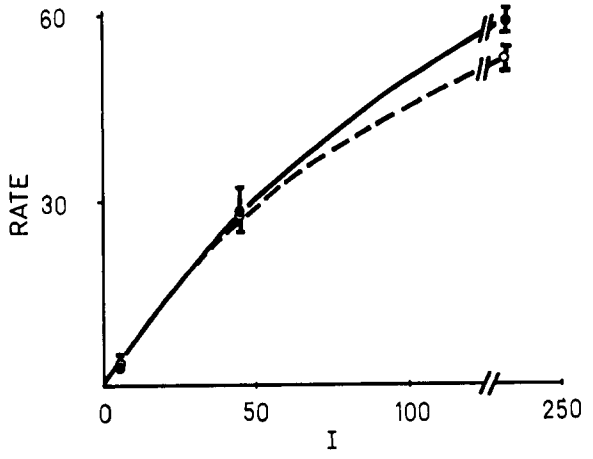


Fig. 4. Effects of silicotungstate on the rate (μmol $O_2$/mg Chl per h) of $O_2$ evolution as a function of light intensity ($I$, kerg/cm$^2$ per s). Concentrations of reagents: ferricyanide, 50 μM; gramicidin D, 3.3 μM; silicotungstate when added 3.3 μM. The dominant, not necessarily the initial, rates were plotted. The dominant rate, i.e., the rate responsible for the major part of $O_2$ evolved, was also the fastest rate observed in a particular sample. (○), Control, (●) silicotungstate added.

## Results and Discussion

Recording traces in the inset of Fig. 2 show that a small amount of oxidant induces a transient fluorescence dip. The area of the dip ($F_{quen}$) can be decreased by silicotungstate (Fig. 2). However, silicotungstate does not affect greatly the amount of $O_2$ evolved ($A$) (Fig. 3) nor does it stimulate $\phi_{2c}$, for it does not stimulate $O_2$ evolution at low light intensity (Fig. 4). This, according to Eqn. 5:

$$F_{quen} = -(\phi_f/\phi_{2c})A \qquad (5)$$

suggests that silicotungstate lowers $\phi_f$.

In order to avoid any detrimental effect of silicotungstate on electron transport, the con-

* Lately, our chloroplasts exhibit two unusual properties: (a) following the reduction of a small amount of oxidant, either DCIP or ferricyanide, the fluorescence remains far below its original level [9]; (b) electron transport is inhibited by low levels of silicotungstate (Fig. 6, and simultaneously measured fluorescence dips, results not shown).

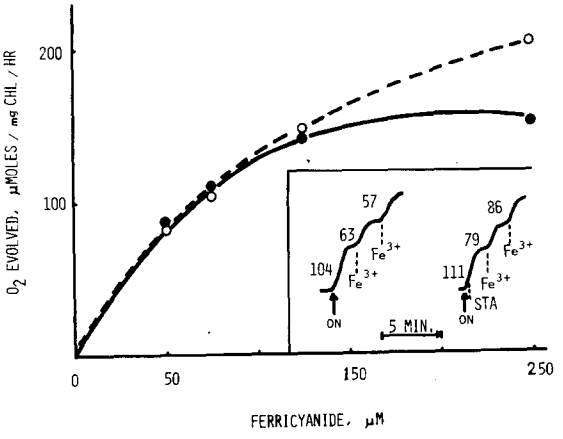


Fig. 5. Effects of silicotungstate on the rate of $O_2$ evolution as a function of the concentration of ferricyanide. (Inset) Traces of $O_2$ evolution. (Left) Control, (Right) silicotungstate added immediately following the onset of illumination. 75 μM ferricyanide was added in the dark, the second and third doses of ferricyanide (50 μM) were added in the light. Main figure: (O— — —O) control, (●———●) samples with silicotungstate (3.3 μM) added. All points are the rates of the first $O_2$ rises (inset). When the 50 μM points on the curves are compared with the rates following the second and third additions of ferricyanide (inset), one can see that in the control sample, the rates of the second and third rises are significant lower (see also Ref. 5); whereas in silicotungstate-treated chloroplasts the rates are not lowered. Light intensity was near saturating. Silicotungstate seems to counteract the well known detrimental effect of ferricyanide on electron transport (see Ref. 11), but at higher concentrations of ferricyanide, it aggravates the bad effect of the latter.

centration of ferricyanide has to be kept low (Fig. 5). The inset of this figure shows that upon exhaustion of the ferricyanide (75 μM) added, new additions of ferricyanide (50 μM) induce new bursts of $O_2$ evolution.

The PS II inhibitor DCMU inhibits the Hill reaction in the presence of silicotungstate * (Fig. 6). Yet DCMU fails to restore fluorescence suppressed by silicotungstate (Fig. 7). Only when the light intensity and the concentration of silicotungstate are both low does DCMU restore partially fluorescence. The lowest light intensity used in the experiments presented in Fig. 7 cannot keep all Q reduced in the control sample (Fig. 7, inset). DCMU may stimulate fluorescence of the sample supplemented with silicotungstate by converting some Q units to $Q^-$ units. It is not known if DCMU only stimulates fluorescence of units that are not yet affected by silicotungstate.

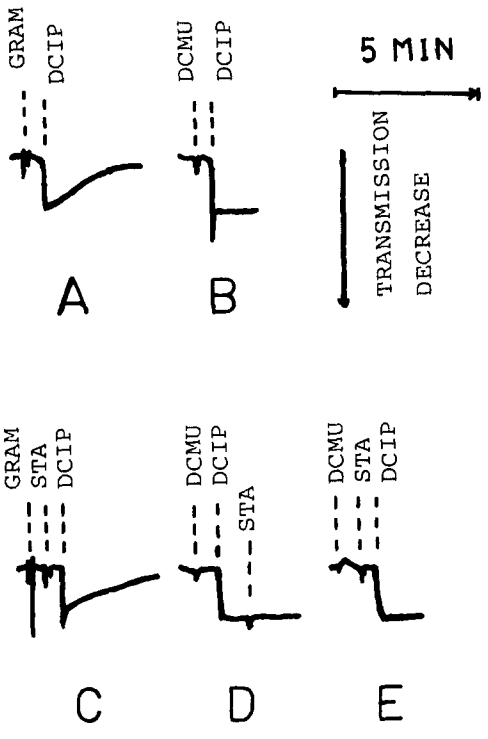


Fig. 6. DCMU inhibits the Hill reaction in the presence of silicotungstate (STA). Unless stated otherwise, reagents as indicated in the figure were added following the switching on of actinic light (see Ref. 9 for methods). The downward movement of a trace following the addition of DCIP was due to a decrease in light transmission. Traces: (A) control, (B) DCMU added to a control sample. (C) silicotungstate added to a control sample. (D) DCMU added followed by DCIP and silicotungstate additions in that order. (E) DCMU added but silicotungstate was introduced before DCIP. Concentrations of reagents when added: DCIP, 50 μM; silicotungstate, 1 μM; DCMU, 4 μM; gramicidin D (Gram), 4 μM. For samples B, D and E, gramicidin was added in the dark.

At a high light intensity, when all Q are reduced in a control sample, DCMU cannot stimulate fluorescence of a sample supplemented with silicotungstate in a sustained way (see trace D of Fig. 7). We ought not to neglect the information implied by the transient stimulation of fluorescence by DCMU at high light intensity. One possible way to explain this phenomenon is as follows. The transient stimulation signifies the failure of the inhibitor in bringing PS II units back into a normal fluorescence state by affecting the physical state of PS II reaction centers. It then indicates

that both the inhibitor and silicotungstate affect the same or related molecule (s), perhaps the Q B-apoprotein [12], to affect fluorescence.

We have therefore documented a case where a dramatic decrease in $\phi_f$ has no appreciable effect on the rate of electron transport. Although we do not know how $\phi_f$ is lowered by silicotungstate, we find it difficult to believe that at such low concentration, silicotungstate can keep most of Q oxidized to lower fluorescence dramatically and sustainedly [13]. In this connection it may be worthwhile to recall a suggestion made earlier [14] that the fluorescence increase upon Q reduction reflects a physical state rather than a redox state change of the reation center. Silicotungstate may alter the physical state change of the PS II reaction center, induced by the act of Q reduction, to affect $\phi_f$.

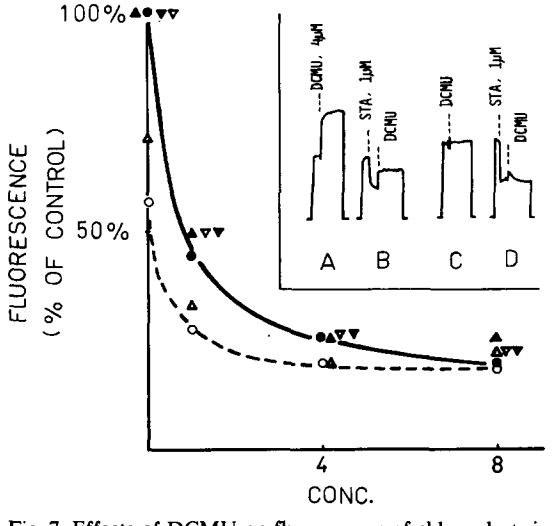


Fig. 7. Effects of DCMU on fluorescence of chloroplasts in the presence of silicotungstate as a function of the concentration of silicotungstate (in $\mu$M) at three different light intensities. (Open symbols) Fluorescence intensities in the absence of DCMU; (Solid symbols) in the presence of DCMU. When more than one symbol occupies the same points, only one of them is shown at the correct position, the rest have been moved along the $x$-axis. (○, ●) 1.6 kerg/cm$^2$ per s; (△, ▲) 3.6 kerg/cm$^2$ per s; (▽, ▼) 14 kerg/cm$^2$ per s. (Inset) A, B, 1.6 kerg/cm$^2$ per s; C, D, 14 kerg/cm$^2$ per s.

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