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SILICOTUNGSTATE INDUCED DISCREPANCY IN THE RATES OF PHOTOSYNTHETIC ELECTRON TRANSPORT DETERMINED BY FLUORESCENCE DIP AND ABSORPTION METHODS

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Abstract

Silicotungstate, at low concentrations, prolonged the 2, 6-dichloroindophenol (DCIP) induced fluorescence dip, while stimulated the DCIP-Hill reaction (photosystem II inhibitor sensitive) measured by transmission changes. ($t_{1/2}$ of fluorescence dip, which is a function of Hill rate, is expected to be shortened when rate is enhanced). At a higher concentration (10 μM), silicotungstate abolished all variable fluorescence without affecting the initial transmission change. It is suggested that there are two types of photosystem II units: types A and B. Fluorescence is lowered while electron transport is stimulated in A units, whereas in B units, fluorescence is not affected but rate is retarded by silicotungstate at low concentration. At high concentration, silicotungstate abolishes fluorescence and decreases rates of both types of units. Or, these observations may suggest that the free plastoquinone molecules in the pool between the two photosystems are not on the main chain of electron transport. A third explanation is that silicotungstate slows down plastoquinone reaction, while stimulates DCIP Hill reaction, the latter in the present case accepts electron mainly from a site, which is sensitive to inhibitors, before plastoquinone in the pool. Ferrous ion chelating agent O-phenanthroline prevented the silicotungstate effects, suggesting the involvement of an iron atom in the quenching. A fluorescence control model, based on the O-phenanthroline-silicotungstate effect, is proposed.

Introduction

Chloroplasts convert light energy into chemical energy by two light reactions (photosytems I and II) operated in series. Photosystem (PS) II is responsible for the oxidation of H_2O, and the reduction of an electron carrier Q. Reduced Q, Q^-, supplies electrons to PS I which reduces NADP. There is a plastoquinone pool situated between the two photosystems.

Not all light quanta are converted into chemical energy, some re-emitted as fluorescence. At room-temperature, fluorescence, its intensity is a function

of Q, arises from PS II. Illuminated isolated chloroplasts, in the absence of Hill oxidants, emit high fluorescence. Introducing a small amount of oxidant to these chloroplasts causes an instant quenching of chlorophyll fluorescence, which re-rises following the exhaustion of the added oxidants and the subsequent reductions of plastoquinones in the pool then Q. The duration of the fluorescence dip or quenching can be used to estimate the rate of electron transport (Malkin and Kok, 1966; Li, 1973a, b; Li *et al.*, 1981).

Using this fluorescence dip technique and simultaneous absorption (direct) measurement of Hill rate, we have found and report here, two unusual effects of silicotungstate on chloroplast fluorescence and Hill rate: (i), low concentrations of silicotungstate prolongs the fluorescence dip while accelerates Hill rate; (ii), higher concentrations of silicotungstate abolishes completely the fluorescence re-rise without total inhibition of Hill rate.

The significance of these observations are discussed.

Materials and Methods

Chloroplasts were isolated from lettuce according to Li (1975), with the following modifications: isolation medium consisted of 15 mM MES (pH 6.6), 5 mM $MgCl_2$ and 400 mM sorbitol; a washing process was added with the same medium but without sorbitol, and chlorplasts were resuspended in the washing medium. The assaying medium was the same as the isolation medium plus 4 μM gramicidin.

The instrument for fluorescence observation had been described (Li, *et al.*, 1981), a second phototube was added to the system for simultaneous fluorescence and transmission measurements. An incandescent light was filtered by a broad interference filter (500–640 nm), a Corning filter (CS4–96) (the center of transmission of the combined filters was at 538 nm, half band width ca. 70 nm), and a solution of $CuSO_4$. The resulting beam served the following purposes: fluorescence excitation, actinic and transmission monitoring. Fluorescence and transmission were observed 90° and 180° respectively to the above beam. Transmission was monitored at 589 nm for 2,6-dichloroindophenol (DCIP) reduction. This wavelength was selected, because of the availability of interference filters.

Figure 1 plots the relationship between % transmission change and the concentration of DCIP: up to 50 μM, the two are linear. In the following experiments, the concentrations of DCIP were kept below 50 μM.

Because of the linear relationship between % transmission and the concentration of DCIP (Fig. 1), and becaus of the noise level, the rate of DCIP reducton was estimated by the half-time ($t_{1/2}$) of the transmission change.

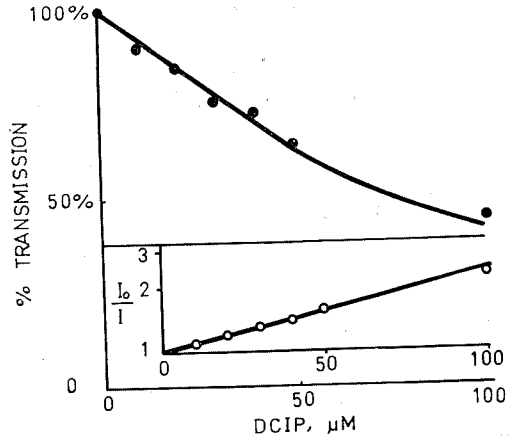

Fig. 1. Percentage transmission as a function of the concentration of DCIP. Main figure: transmission vs. concentration. Inset: I_0/I vs. concentration (semilog scale). Chloroplast concentration: 10 μg chlorophyll/ml. Light intensity: 14 kerg/cm^2 per sec.

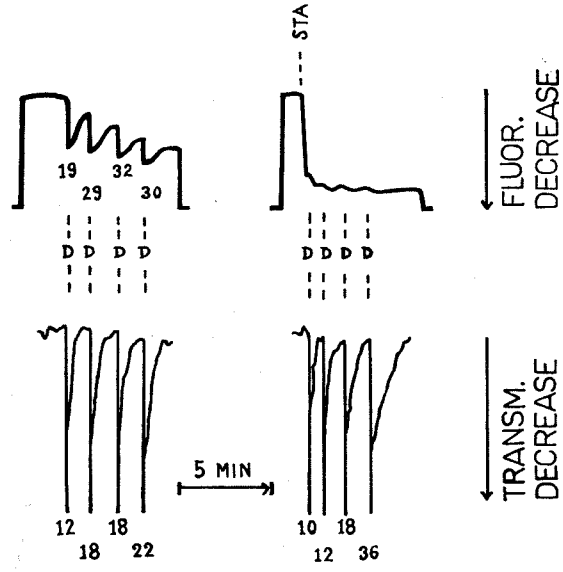

Fig. 2. Effects of repetitive DCIP additions, in the absence (left panels) or presence (right panels) of silicotungstate, on the durations of fluorescence dips and the rates of electron transport. Upper traces: fluorescence recordings. Lower traces: transmission measurements. The addition of DCIP (D, 20 μM) caused abrupt decreases in both fluorescence and transmission signals, which re-rise as DCIP were reduced progressively (recording traces moved upward). Silicotungstate (STA) caused a dramatic change in fluorescence but not in transmission signal. Numbers under the curves are half time in unit of second. See Fig. 1 for other experimental conditions. The small increase of transmission upon silicotungstate addition was due to a decrease of scattering light, the latter did not affect our results if the light guide used for the transmission measurement was in direct contact with the cuvette (not shown). STA concentration, 4 μM.

Results and Discussion

Repetitive additions of 20 μM DCIP, which oxidized Q instantly, induced fluorescence and transmission dips (Fig. 2). The half-times ($t_{1/2}$) of the first dips were shorter than those of the second ones, which in turn were shorter than those of the third ones, and so on so forth, indicating that rate of DCIP reduction went down with time. Silicotungstate seemed to slow down the deterioration of the electron transport activities (see also Li and Ueng, 1980), for its stimulating effect on the rate is more pronounced at the second than at the first DCIP addition (Fig. 2). But it became inhibitory at the end of the experiment, in agreement with Zilinskas and Govindjee (1975).

Well formed fluorescence dips could be obtained in silicotungstate treated chloroplasts, if the concentrations of silicotungstate were low. In these cases, silicotungstate prolonged the $t_{1/2}$ of fluorescence dip but shortened the transmission dip (Fig. 3). At a higher concentration, 10 μM, silicotungstate delayed the fluorescence-rise indefinitely without affecting the initial transmission change*.

A faster recording, performed at a lower light intensity, gives a more clear comparison in rates (Fig. 4). At this intensity, 1 or 2 μM

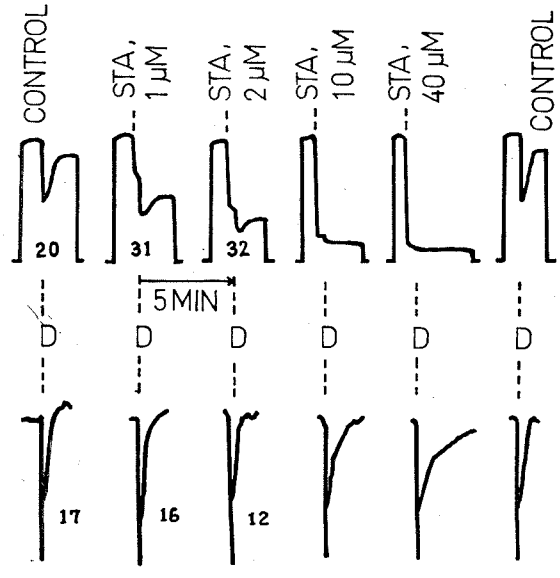

Fig. 3. Concentration effects of silicotungstate on fluorescence (upper traces) and the rate of DCIP reduction (lower traces). The addition of DCIP (20 μM) is marked by D. Numbers underneath the curves are $t_{1/2}$ in unit of second. See Fig. 1 for other experimental conditions.

* The initial rates of DCIP reduction, in the presence of 0-10 μM silicotungstate, were about the same (156 μ equivalent/hr. mg chl).

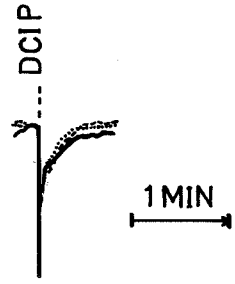

Fig. 4. Low concentrations of silicotungstate does not inhibit DCIP reduction. The concentration of DCIP was 10 μM, and the light intensity was 8.5 kerg/cm^2/sec. Solid curve, control; dashed curve, 1 μM silicotungstate added; dotted curve, 2 μM silicotungstate added. The $t_{1/2}$ ratios of the absorption changes of silicotungstate-added and control samples are less than 100%, while those of the durations of fluorescence dip are 153% for both 1 and 2 μM silicotungstate-added samples.

silicotungstate slow down the fluorescence re-rise by 53% of the rise-time in the absence of the agent (results not shown), while had only a little effect on the rate of transmission changes.

The fact that 1 μM silicotungstate prolonged** the $t_{1/2}$ of the fluorescence dip without slowing down the rate suggests that there may be two types of PS II units: types A and B. Silicotungstate makes type A units non-fluoresent while stimulates their ability to reduce DCIP. These units do not exhibit fluorescence change upon the exhaustion of DCIP. On the other hand, silicotungstate does not affect fluorescence of type B units, but inhibits their ability to reduced DCIP. There may be more A than B units, so that the net result of the silicotungstate effect is to stimulate the rate of Hill reaction.

The fluorescence dip technique measures mainly electron transport mediated by type B units, whereas the absorption method measures electron transport mediated by units of both types A and B in the presence of silicotungstate, this causes the discrepancy in rate determinations. We have no idea if A and B units are in fact α and β units of Melis and Homann (1975), respectively.

** For the following reasons, silicotungstate prolonged the fluorescence dip not because of it is an oxidant. Firstly, there was about 20 sec time lapse between silicotungstate addition and the introduction of DCIP, a time sufficient to reduce 10 nano-equivalent of oxidant under the condition of Figs. 2 and 3 (1 μM oxidant represents 1 nano-mole/ml of the oxidant). Secondly, a doubling of the silicotungstate concentration from 1 to 2 μM did not affect the fluorescence rise time (Fig. 3 and results not shown). Thirdly, 1 or 2 μM silicotungstate did not slow down the rate of DCIP reduction.

Earlier, we have suggested that silicotungstate may act on the Q-B-apoprotein to affect ϕ_f (Li and Ueng, 1982). Vermaas and Govindjee (1981) suggested that Q-B-aporotein may be different in α and β units. These lead to the inference that Q-B-apoproteins of types A and B units may also be different. Note also that ϕ_f of α units but not that of β units is sensitive to Mg^{2+} (Melis and Homann, 1978). There are evidence suggesting that Mg^{2+} reacts with Q-B-apoprotein (unpublished results).

The suggestion that in the presence of silicotungstate, type A units are non-fluorescent but photochemically active implies that ϕ_f changes and the redox of Q changes are two different events. A model is presented as follows to explain these experimental results.

There may exist a sensor which relays the redox change of Q to a protein, Q-B-apoprotein for instance, its conformation dictates the fluorescence yield of the PS II units (Li, 1977). Silicotungstate may interrupt the communication between either Q or the apoprotein and the sensor.

How Q-B-apoprotein affects the ϕ_f of a photosynthetic unit? Franck and Rosenberg (1964) suggested that iron of cytochrome may increase the probability of metastable state formation in chlorophyll molecules to quench fluorescence. This remains us the following possibility.

The low fluorescence state of a Q unit may be due to the existence of an iron atom near the chlorophyll of the reaction center. The iron in its functional position (Q unit) may facilitate primary charge separation between P680 and pheophytin (I), the primary electron donor and an intermediate electron carrier of PS II respectively. Upon Q reduction, the iron may move away from the center, resulting in a decrease in the rate of charge separation and an increase of fluorescence.

This suggestion is substantiated by the observation that O-phenanthroline, a ferrous ion chelating agent, prevents the silicotungstate effect (Fig. 5). The existence of a ferrous ion in the reaction center is demonstrated by the split EPR signal of reduced pheophytin (Klimov *et al.*, 1980; Rutherford *et al.*, 1981). [PS II inhibitor 3-(3,4-dichlorophenyl)-1, 1-dimethylurea had no effect.]

A schematic diagram which summarizes the above discussion is presented in Fig. 6. Q-B-apoprotein is imagined to have two hands, one carries Q while the other carries B. B is the acceptor of Q (Bouges, 1973; Velthuys and Amesz, 1974). These hands are responsible for the changes in the steric relationship between electron carriers P680, I, Q and B. Whereas the hand that carries Q may senses the latter's redox state, the movement of Q around is effectuated by a portion of the apoprotein marked by S which is exposed to exogeneous agents. The attack of S by silicotungstate may also expose Q to a hydrophilic environment.

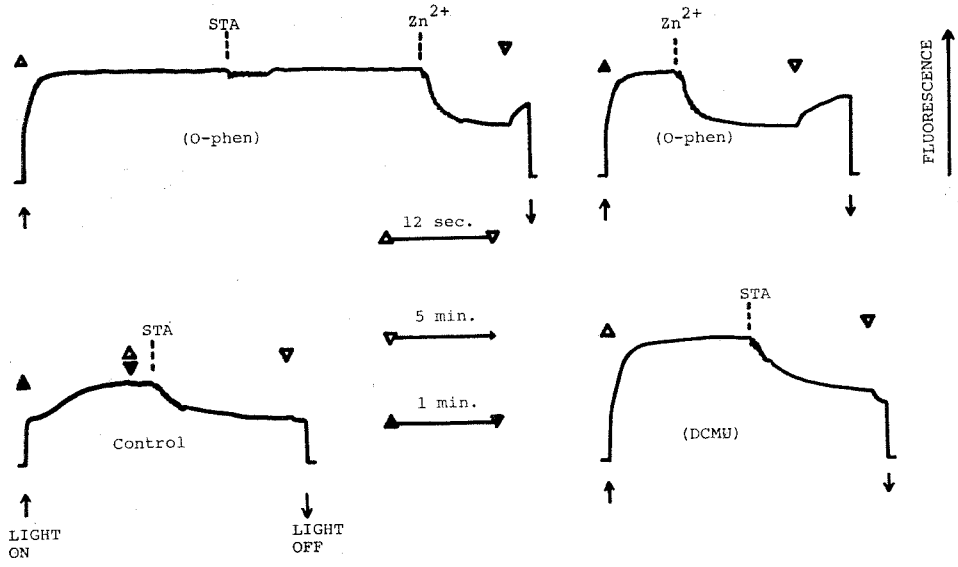

Fig. 5. O-phenanthroline prevents silicotungstate from lowering fluorescence. The different markers on top of each recording signal the change of recording speed. O-phenanthroline (60 μM) and 3-(3, 4-dichlorophenyl)-1, 1-dimethylurea (DCMU, 4 μM) when indicated in the figure were added in dark. Concentrations of Zn^{2+} (as Zn acetate) and silicotungstate were 4 mM and 4 μM respectively. Light intensity was 1.2 kerg/cm^2 per sec. Chloroplasts were washed twice with H_2O before use.

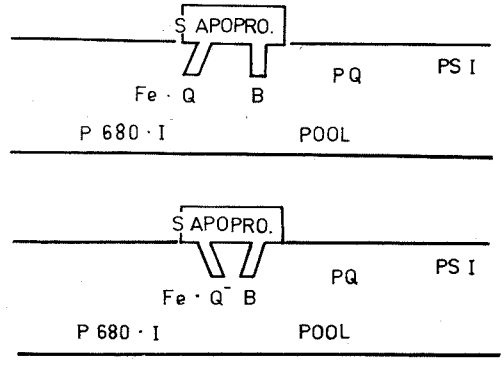

Fig. 6. Changing steric relationship, upon the reduction of Q, between the P680•I and Fe•Q couples. Upper figure: conformation of Q-B-apoprotein when Q is oxidized. Lower figure: conformetion of Q-B-apoprotein when Q is reduced (Q^-). Note, the steric relationship between P680 and Fe is also a function of the redox state of Q. See text for further explanation. Apopro: Q-B-apoprotein.

When Q is oxidized (upper panel, Fig. 6), Q and B are separated but Q and I are in touch. Fe is now near P680 to facilitate charge separation between I and P680. Upon Q reduction (lower panel, Fig. 6), Q^- and B moves towards each other. The iron goes with Q. The effeciency of the primary charge separation goes down a little and fluorescence rises. The degree of the exposure of the S portion in types A and B units may be different to account for the differential sensitivity of A and B units to silicotungstate. Incidentally, Malkin and Michaeli (1972) suggested that the steric relationship between Q and its acceptor changes as temperature lowers.

We must discuss another aspect of the silicotungstate effect. Under certain conditions, the silicotungstate suppressed fluorescence can be restored, partially, by dithionite (Li and Ueng, 1982). Dithionite may reduce the sensor or other part of the apoprotein directly and brings about a conformational change to affect the fluorescence yield. Alternatively, silicotungstate may raise the redox potential of Q so that it can not reduce the sensor.

In any case, dithionite can not restore all the fluorescence lowered by silicotungstate, the latter may also affect the process of the redox induced change of the apoprotein conformation so that PS II units can not attain their normal ϕ_f. There may be two types of conformational changes of Q-B-apoprotein: one allows Q^- and B to react, the other allows ϕ_f to change.

Let us come back to the discrepancy in rate-determination shown in Figs. 3 and 4. There are other interpretations besides the two-types-of-unit hypothesis.

Silicotungstate may accelerate electron transport from carriers before plastoquinone pool to DCIP, while slow down electron transport from Q to the pool. (The reduction of plastoquinone pool is responsible for the final fluorescence re-rise.) Assuming DCIP accepts electron at a site that is sensitive to PS II inhibitor, for electron transport in the presence of silicotungstate is sensitive to 3-(3, 4-dichlorophenyl)-1, 1-dimethylurea (Li *et al.*, 1982).

Alternatively, free plastoquinone in the pool, their reduction is slowed down by silicotungstate, are not on the main pathway of photosynthetic electron transport. According to this explanation, silitotungstate stimulates electron transport of the main chain but not the side steps of plastoquinone in the pool. Then, questions arise: what are the functional roles of these plastoquinones? Do these reduced plastoquinones diffuse to locations where PS I out-numbered PS II?

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在葉綠體中矽鎢酸鹽使兩種測定電子傳遞速率的方法產生矛盾的結果

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本文報告並解釋矽鎢酸鹽所引起的兩種異常現象。(1)當電子傳送率用兩種不同方法去測定時，矽鎢酸鹽使兩種方法產生不相同的結果。方法一顯示該鹽促進電子傳送，但方法二却顯示它抑制電子傳送。這兩種測定電子傳送的方法分別是吸收光譜法及葉綠素螢光法。(2)較高濃度的矽鎢酸鹽消除全部可變的葉綠素螢光，却未能抑制所有的電子傳送活動。