Bot. Bull. Academia Sinica **21**: 67-74 (1980)

DISSOCIATION OF CHANGES IN FLUORESCENCE OF CHLOROPHYLL A FROM CHANGES IN RATE OF ELECTRON TRANSPORT IN SILICOTUNGSTATE-TREATED CHLOROPLASTS(1,2)

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(Received November 29, 1979; Accepted December 31, 1979)

Abstract

Silicotungstate, at low concentrations, decreases fluorescence drastically without inhibiting electron transport, suggesting in conjunction with other evidences that the fluorescence yield of chlorophyll a of chloroplasts is regulated, by the physical state of the thylakoid membrane, rather than by the redox state of the primary electron acceptor.

Introduction

Silicomolybdate or silicotungstate renders photosynthetic oxygen evolution supported by ferricyanide insensitive to 3-(3,4-dichlorophenyl)-1,1-dimethylurea (Giaquinta *et al.*, 1974: Girault and Galmiche, 1974; Zilinskas and Govindjee, 1975), suggesting that these compounds alter membrane properties of thylakoid (Girault and Galmiche, 1974; Zilinskas and Govindjee, 1975).

These compounds also suppress chlorophyll a fluorescence yield of chloroplasts, which is attributed either to the electron accepting capabilities of these compounds or to direct chemical quenching by them, depending on the concentrations of the compounds added (Zilinskas and Govindjee, 1975). These explanations are put forward assuming, implicitly, that fluorescence and photochemistry compete.

Our observations, to be presented here, show that at the instance of silicotungstate addition there is a dramatic fluorescence change, while the rate of electron transport remains the same. These observations together with some other reports in the literatures may indicate that fluorescence does not

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(1) This work was financially supported by the National Science Council of the Republic of China.

(2) Paper No. 235 of the Scientific Journal Series, Institute of Botany, Academia Sinica.

compete with photochemistry. Fluorescence may be governed by the physical state of thylakoid membrane which is, presumably, responsive to the redox state of Q (Li, 1977, 1978a); silicotungstate either dissociates the physical state change from the redox change of Q, or alters the physical state change; either way, the variable fluorescence is diminished or abolished. These observations therefore substantiate, for a different reason, the proposition that silicotungstate modifies the property of thylakoid membrane (Girault and Galmiche, 1974; Zilinskas and Govindjee, 1975).

Materials and Methods

Chloroplasts were isolated from oat seedlings according to Li (1975), with the following modifications: the isolation medium consisted of MES (15 mM), pH 6.6; TES (15 mM), pH 6.6; $MgCl_2$ (5 mM); and Sorbitol 400 mM; a washing process was added with the same medium but without sorbitol, and chloroplasts were resuspended in the washing medium. The assaying medium was the same as the isolation medium. Methods of oxygen evolution and fluorescence measurements have been described (Li, 1975, 1978b respectively).

Results and Discussion

Silicotungstate lowers, dramatically, the intensity of chlorophyll a fluorescence of chloroplasts in the presence of ferricyanide under a higher light intensity but not under a lower light intensity (Fig. 1). Under the lower light intensity, ferricyanide is able to keep the variable fluorescence low. What if then when ferricyanide is absent? Fig. 2 shows that under the same lower light intensity, but in the absence of ferricyanide, silicotungstate is again effective in lowering the intensity of fluorescence. These experiments suggest, in agreement with Zilinskas and Govindjee (1975), that silicotungstate, at low concentrations, may affect only the variable fluorescence.

Silicotungstate may quench fluorescence in its capacity as an oxidant (suggested by Zilinskas and Govindjee, 1975), or it may quench fluorescence in other ways. If it quenches fluorescence because it accepts electron from Q, one should only see a transient fluorescence lowering when a small amount of an oxidant is added (see Li, 1973 for example); however, the fluorescence lowering effect of silicotungstate, in the absence of ferricyanide, sustains (result not shown).

At concentrations sufficient to repress fluorescence dramatically and instantly, silicotungstate has no appreciable effect on the rate of electron transport, i.e., the rates measured before and after silico compound addition are the same; but at higher concentrations, it inhibits rate (Fig. 3). For control samples, there is a slowing down of rate in light, which can be

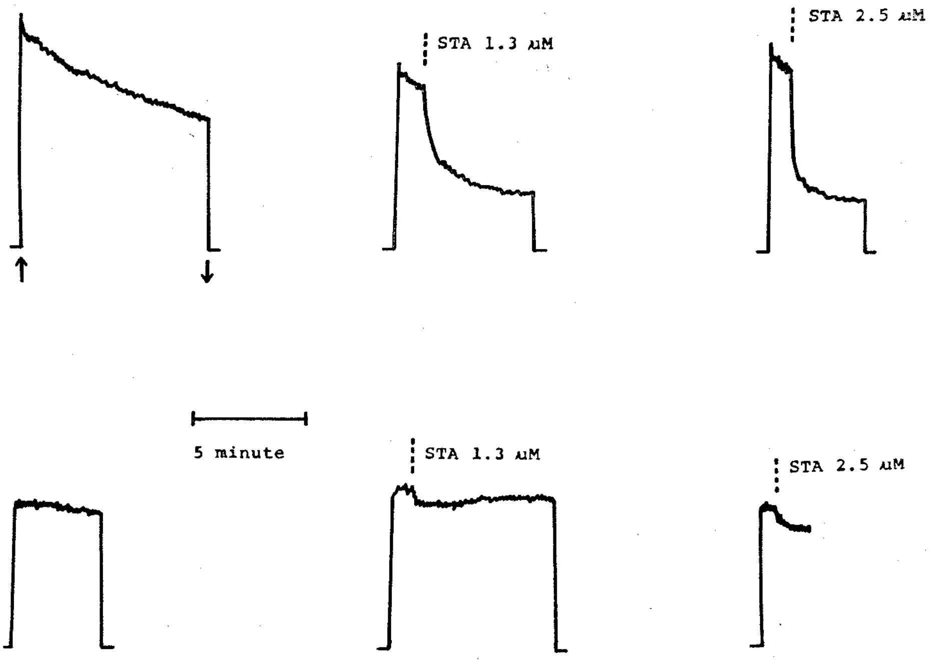

Fig. 1. Effects of silicotungstate (STA) on chlorophyll a fluorescence of chloroplasts (in the presence of ferricyanide) at two light intensities. Chloroplasts (10 µg chlorophyll/ml) were assayed in the isolation buffer. Concentration of ferricyanide was 7.5 mM. Fluorescence intensities are expressed in relative units, the units for the upper (exciting light intensity, 170 kerg/cm^2.sec) and lower (10 kerg/cm^2.sec) traces are not comparable. Upperward solid arrow, light on; downward solid arrow, light off. Dashed lines indicate when reagents were added.

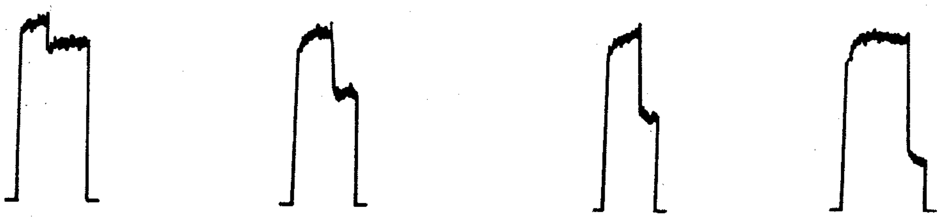

Fig. 2. Effects of silicotungstate on chlorophyll a fluorescence of chloroplasts (in the absence of ferricyanide). Addition of silicotungstate to a sample is evidenced by the abrupt dropping of the fluorescence intensity in each of the recording traces. The concentration of silicotungstate added are 0.075, 0.25, 2.5 and 12.5 µM respectively from left to right traces. Chloroplasts, 2 µg chlorophyll/ml. Light intensity was 10 kerg/cm^2.sec. For other conditions, see legend of Fig. 1.

prevented by the addition of silicotungstate. The rate slowing down can also be prevented by using a different buffer system (results not shown), and we have not determined which are the factors that is responsible for the rate-declining and its prevention in our buffer systems.

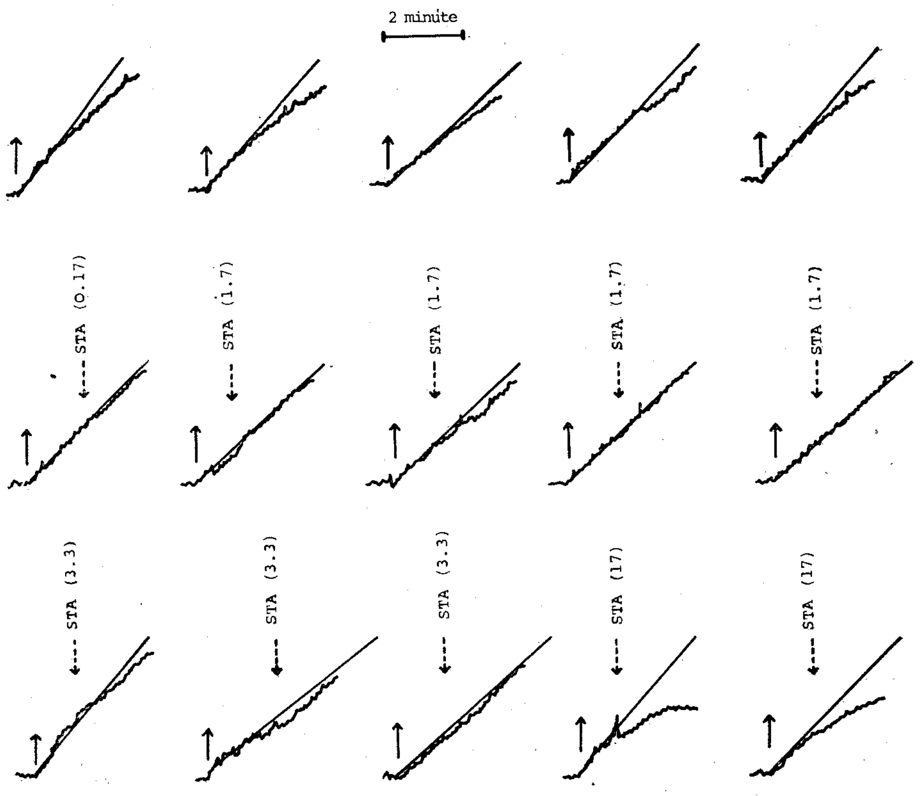

Fig. 3. Effects of silicotungstate (STA) on ferricyanide-supported O_2 evolution. Chloroplasts ($13\,\mu g$ chlorophyll/ml) were assayed in isolation buffer plus ferricyanide (5 mM) and gramicidin D ($0.33\,\mu M$). The mean initial rate of control experiments (upper row) $62\pm2\mu$moles O_2/mg chl. hr. Recording traces of experimental samples are shown in the middle and lower rows. The initial rate (before silicotungstate addition) of the middle row traces is $61\pm2\,\mu$moles O_2/mg chl. hr. That of the lower row is slightly lower than those of the upper and middle rows, owing to, possibly, chloroplasts ageing; the sequence of experiments were alternatively control assay, assay of low STA, and that of high STA. Light intensity was 130 kerg/cm^2. sec. Upward solid arrow means light on; dashed arrow marks the time when STA, with concentration in μM shown in parentheses, were added.

Even though the observations that a large fluorescence change goes without any change in electron transport indicate that photochemistry may not be affected detrimentally, our results are nevertheless not convincing, for they are from experiments performed in high light intensity. However, the findings of Zilinskas and Govindjee (1975) that high concentrations of silicomolybdate in fact stimulates rate of electron transport by about 150%, while lowers fluorescence below its constant level*, and that fluorescence remains low after the exhaustion of oxidant (a resupplement of oxidant revives electron transport) are more convincing results indicating that a suppress of variable fluorescence does not necessarily results in a lowering of photochemistry efficiency as predicted by the proposition that fluorescence and photochemistry compete directly. There are other instances of lowering variable fluorescence without or without proportionally affecting electron transport (Yamashita and Butler, 1968; Yamashita and Butler, 1969; Li and Ueng, unpublished results).

These observations are therefore difficult to understand in the framework of Q hypothesis of fluorescence (Duysens and Sweers, 1963) as such, the Q hypothesis implies that photochemistry and fluorescence compete directly. If we can assume that these two processes do not compete directly, rather fluorescence is responsive to a physical state change upon the reduction of Q (Li, 1977, 1978a), these experiments may be understood easily. Silicotungstate or silicomolybdate, added in light, may alter the conformation of thylakoid membrane in ways that the physical state changes upon Q reduction are altered or abolished so that fluorescence yield will not associate with the change in the redox of Q, without affecting photochemistry detrimentally.

Since Q is originally proposed to be a hypothetical molecule, it could as well be a hypothetical state.

Incidentally, several agents that affect the action of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), also lower fluorescence (Zilinskas and Govindjee, 1975; Renger *et al.*, 1976; Renger, 1976; Li and Ueng, unpublished results), pointing to the intimacy of the two phenomena. But the DCMU inhibition relieving effect and the fluorescence lowering effect of silicotungstate may have different concentration dependencies, suggesting that a difference exists between the two effects (Ueng and Li, results not shown). These DCMU experiments may provide clues to what are the machinery that controls the yield of fluorescence.

Here, it is relevant to recall a hypothesis put forword by Malkin and Michaeli (1972) that DCMU may induce either local or pronounced structure changes that affect the functioning of electron transport. And it is interesting to know whether the conformational changes suggested to be induced by

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* To explain the observation that high concentrations of silicomolybdate stimulates electron transport while represses fluorescence below the level of constant fluorescence, we assume that constant fluorescence represents the inevitable energy lose due to imperfect energy transfer, whereas variable fluorescence is controlled by the reaction center; silicomolybdate may perfect the energy transfer among bulk chlorophylls, at the same time making reaction centers permanent traps whether Q is reduced or oxidized.

silicotungstate, which relieve electron transport inhibition, and lower fluorescence, bear any relationship with the structure changes proposed by Malkin and Michaeli (1972).

Note Added in Proof

Studies of photosynthetic bacteria (Shuvalov and Klimov, 1976; Grondelle *et al.*, 1978; Godik and Borisov, 1979) suggest that part of the total emission designated as fluorescence may in fact be fast luminescence resulted from recombination reaction of P^+ and I^-, the oxidized reaction center bacteriochlorophyll and the reduced intermediate electron acceptor respectively.

If the same mechanism operates in Photosystem II of green plants one may explain the finding that silico compounds decrease fluorescence (including luminescence) without affecting electron transport detrimentally by assuming that silico compounds may hinder the recombination reaction, which may be in part responsible for the observation (Zilinskas and Govindjee, 1975) that silico compounds stimulate greatly electron transport while decreases drastically fluorescence. The hindrance hypothesis can not explain why fluorescence remains low after the cessation of measurable electron transport. Silico compounds may also affect, when the 'primary' acceptor is reduced, the rate of triplet formation of the biradical P^+I^-, and/or the rate constants of fluorescence, internal conversion of P^*, and energy transfer between it and bulk chlorophylls, whether P^* is created by recombination reaction or by photon absorption.

Since the bacteria experiments just mentioned were performed with primary acceptor prereduced, and since under certain conditions fluorescence is low when the primary acceptor is expected to be reduced, it remains to be seen whether there is a difference between the transformation from a low fluorescence (luminescence) state to a high fluorescence (luminescence) state and the reduction of the primary acceptor, whether there is, normally, a time delay between the two events.

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矽鎢酸鹽干擾葉綠體電子傳送率與葉綠素螢光強度的相關性

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在正常情形下，葉綠體電子傳送率與葉綠素螢光的强度成線性(或近線性)反相關；即當電子傳送率高時，螢光下降，而在電子傳送率低時，螢光上升。按 Duysens 及 Sweers，這是因爲第二光系統一電子載體，Q，在氧化狀態時（通常在高電子傳送率時）淬滅螢光。

本文報告低濃度的矽鎢酸鹽能使螢光大幅驟降，但不改變電子傳送率。該一發現表示Q的氧化還原或與螢光强度的相關並不是絕對的，或許不是直接的。