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Zn^{2+} OR SILICOTUNGSTATE AFFECTS STEADY STATE CHLOROPHYLL A FLUORESCENCE OF CHLOROPLASTS SUSPENDED IN DISTILLED WATER

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Abstract

Neither silicotungstate nor $MgCl_2$ alone has any pronounced effect on fluorescence of chloroplasts suspended in distilled water. In combination, they lower fluorescence. Zn^{2+} lowers fluorescence, and its effect is suppressed by $MgCl_2$. Silicotungstate, in the absence of $MgCl_2$, enhances the fluorescence lowering effect of Zn^{2+}, whereas 3-(3, 4-dichlorophenyl)-1, 1-dimethylurea (DCMU) slows down the Zn^{2+} effects. These reagents may react with a hypothetic fluorescence regulating domain (FRD), adjacent to the reaction center of photosystem II, to affect the quenching properties of the center. Q-B-apoprotein may be a constituent of FRD.

Introduction

Silicotungstate practically abolishes variable fluorescence while either has no effect on electron transport (Li and Ueng, 1980), or greatly stimulates the rate of O_2 evolution (Zilinskas and Govindjee, 1975). Zn^{2+} also lowers fluorescence (Li, 1975a). Both Zn^{2+} and silicotungstate interfere with the actions of photosystem (PS) II inhibitors: Zn^{2+} reverses the inhibitory effect of O-phenanthroline on Q^- re-oxidation (Kautsky *et al.*, 1960; Bennoun and Li, 1973), whereas silicotungstate interferes with that of DCMU (Giaquinta *et al.*, 1974; Girault and Galmiche, 1974; Zilinskas and Govindjee, 1975; Li and Ueng, unpublished results). Q^- is the first stabilized reduced electron acceptor of PS II; in its oxidized form, Q is a fluorescence quencher (Duysens and Sweers, 1963).

Renger (1976) postulated that Q is covered on the outer side of the thylakoid membrane by a proteinaceous shield, the latter regulates, allosterically, the photosynthetic electron transfer on the reducing side of PS II; and DCMU binds reversibly to a special place of the protein shield, leading to an allosteric inhibition of the re-oxidation of Q^- (see also Malkin and Michaeli, 1972).

Since both DCMU and O-phenanthroline affect the same reaction of the photosynthetic electron transport, one may suggest that O-phenanthroline also binds to the protein shield. On the basis of the facts that both silicotungstate and Zn^{2+} interfere with the actions of PS II inhibitors, one may further postulate that silicotungstate and Zn^{2+} alter the physical state of the protein shield (recently designated as Q-B-apoprotein, Renger *et al.*, 1981), to affect fluorescence intensities. In this report the interactions between DCMU and Zn^{2+}, and between Zn^{2+} and silicotungstate effect on fluorescence are presented.

Materials and Methods

Chloroplasts were isolated from oat seedlings in a buffer system (pH 6.6) consisting of 2-(*N*-morpholino) ethane sulfonic acid, 15 mM; $MgCl_2$, 5 mM; sorbitol, 400 mM by a method described by Li (1975b). The chloroplasts were washed twice with the above mentioned buffer but without sorbitol, and resuspended in the wash buffer as a stock and kept on ice. Methods of oxygen evolution and fluorescence measurements have been described elsewhere (Li, 1975b, 1978, respectively).

A small aliquot of the chloroplast stock was diluted with double distilled water immediately before each measurement; but for experiments reported in Fig. 1, chloroplasts were diluted with the isolation buffer. The light intensity for fluorescence measurements was 100 kerg/cm^2·sec (green light, see Li, 1978 for details) in all experiments reported except that for Fig. 10 which was 170 kerg/cm^2·sec (green light). Concentrations of chloroplasts for fluorescence measurements were in the range between 3 to 4 μg chlorophyll/ml. Zn^{2+} was provided as zinc acetate, and Mg^{2+} was provided as $MgCl_2$. The stock solution of sodium dithionite was prepared immediately before each experiment and flushed continuously with N_2 gas. For O_2 measurements, incandescent light filtered with 12 cm 0.38% $CuSO_4 \cdot 5H_2O$ solution was used, its intensity, at the center of the empty cuvette, was 1.3×10^5 erg/cm^2·sec.

Results and Discussion

Zn^{2+} lowers the fluorescence yield of chloroplasts suspended in distilled water (Fig. 1). $MgCl_2$ slows down the fluorescence lowering effect of Zn^{2+} (Fig. 2). The Zn^{2+} effect is therefore not a divalent cation effect in general. At a concentration of 2.5 μM or below, silicotungstate alone does not affect fluorescence much in the present case; Zn^{2+} (Fig. 3) or Mg^{2+} (Fig. 4, $MgCl_2$ was used but $MgSO_4$ was also effective, result not shown) is required for silicotungstate to lower fluorescence. But in water (instead of buffer) washed chloroplasts, divalent cations are not required to observe the fluorescence

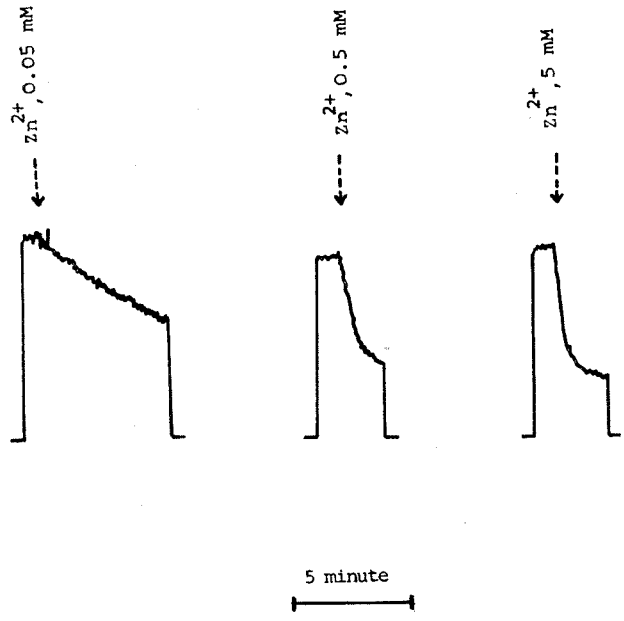

Fig. 1. Fluorescence lowering effect of zinc acetate—concentration dependency. Up-ward movement of recording trace represents an increase of the yield of fluorescence of chlorophyll a of chloroplasts.

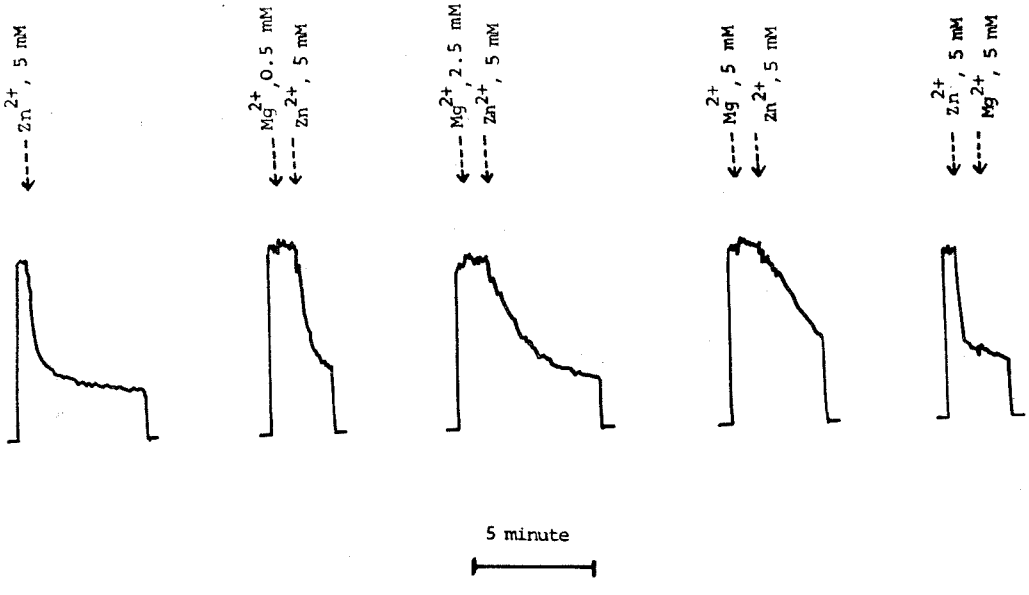

Fig. 2. Mg^{2+} retards the rate of fluorescence lowering induced by Zn^{2+}.

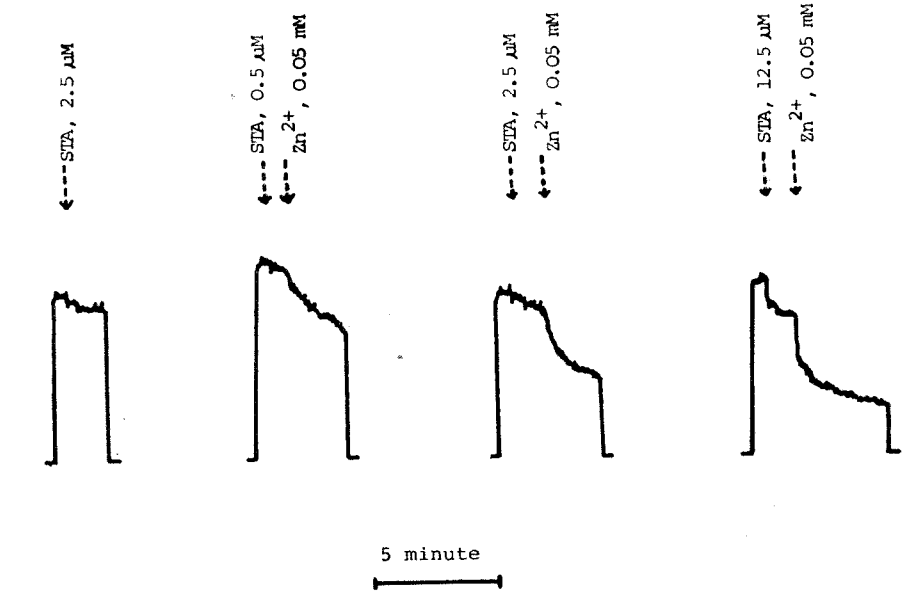

Fig. 3. Silicotungstate (STA) accelerates the rate of fluorescence lowering induced by Zn^{2+}.

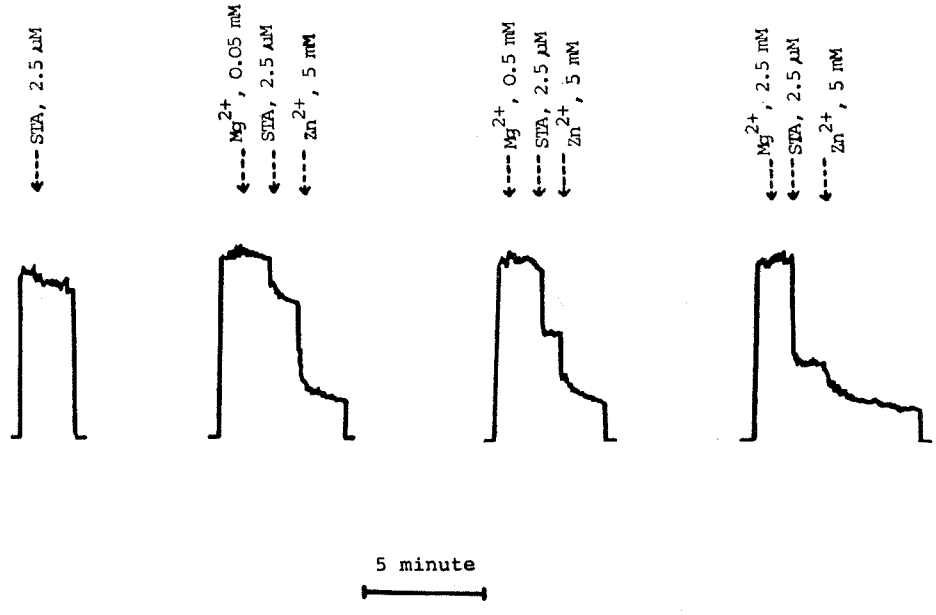

Fig. 4. Mg^{2+} modifies the fluorescence effect of silicotungstate and that of Zn^{2+}.

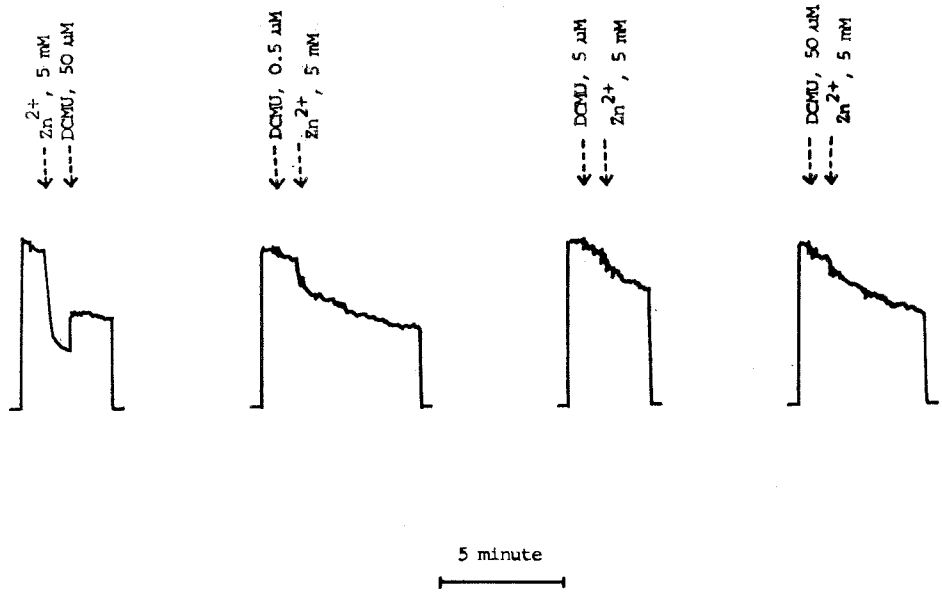

Fig. 5. DCMU modifies the effect of Zn^{2+} on fluorescence.

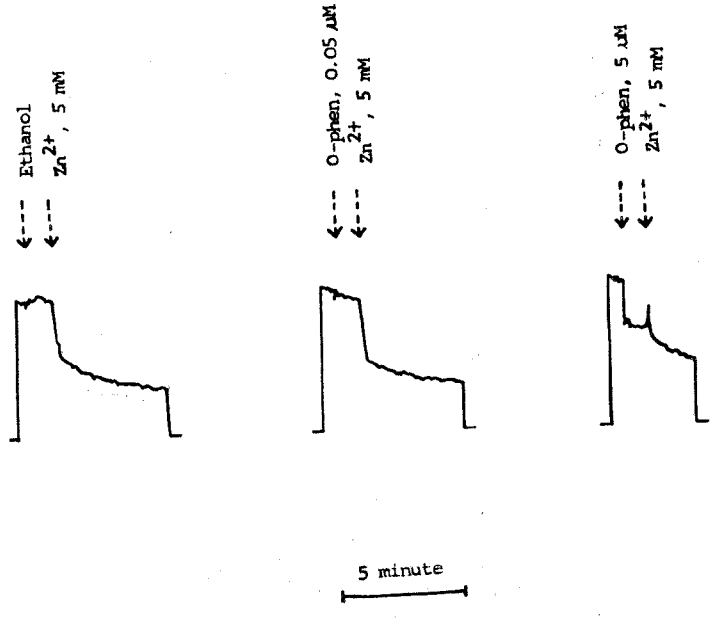

Fig. 6. Effects of O-phenanthroline on fluorescence.

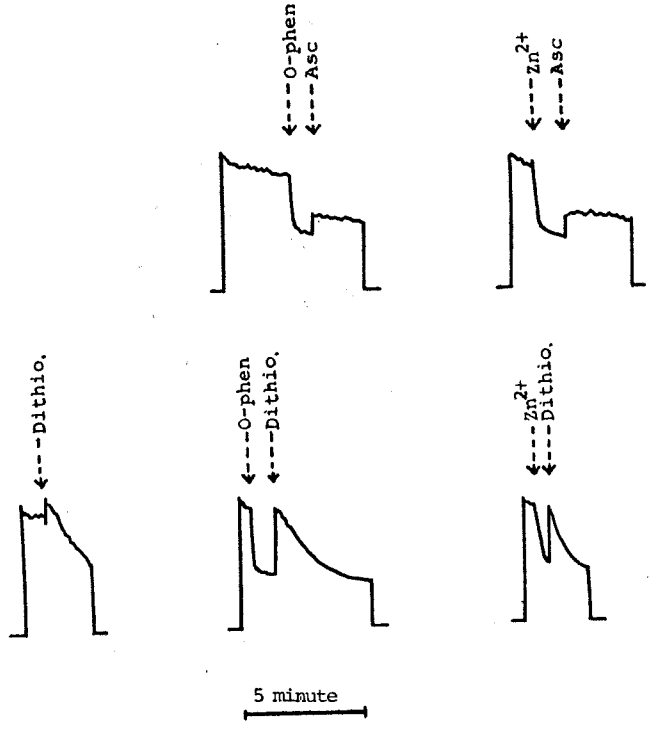

Fig. 7. Restoration of Zn^{2+} or O-phenanthroline lowered fluorescence yield by reducing agents.

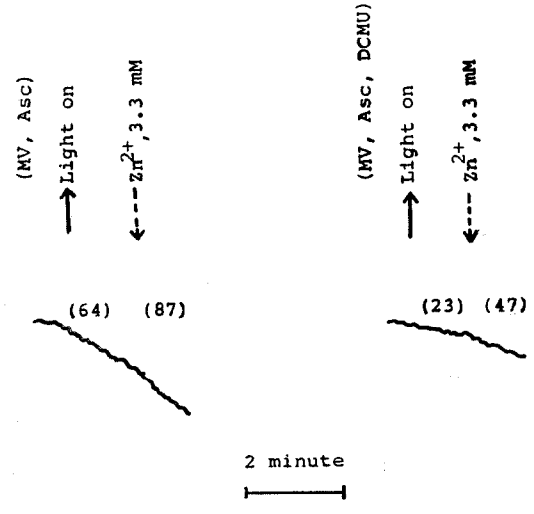

Fig. 8. Zn^{2+} releases, partially, the inhibitory effect of DCMU on ascorbate-methyl viologen mediated electron transport.
Chloroplasts, suspended in doubly distilled water, had a concentration equivalent to 12 μg chlorophyll/ml. Concentrations of reagents used were: Methyl viologen (MV), 67 μM; sodium ascorbate (Asc), 3.3 mM; DCMU, 33 μM; zinc acetate (Zn^{2+}), 3.3 mM.
Rates in μmoles O_2 uptake/mg chl•hr are shown in parentheses in the figure.

lowering effect (manuscript submitted elsewhere), it therefore is not clear whether divalent cations are required to neutralize the negative charges on the thylakoid membrane to facility the anionic silicotungstate in reaching the membranes, or not. (Our results showed that 2-(*N*-morpholino) ethane sulfonic acid, a buffer used in the present experiments, can affect the properties of thylakoid membrane profoundly, manuscript submitted elsewhere.)

DCMU retards the fluorescence lowering initiated by Zn^{2+} (Fig. 5). O-phenanthroline, at 5 μM, lowers fluorescence, and subsequent addition of Zn^{2+} causes a fast increase of fluorescence, followed immediately by a decrease of fluorescence intensity (Fig. 6), the transient fluorescence stimulation induced by Zn^{2+} can only be observed when fluorescence is suppressed by O-phenanthroline. The inhibitor effects may either be a pure redox effect or something else.

Under a high light intensity, ascorbate restores partially, and dithionite restores fully but only temporarily fluorescence lowered either by Zn^{2+} or by

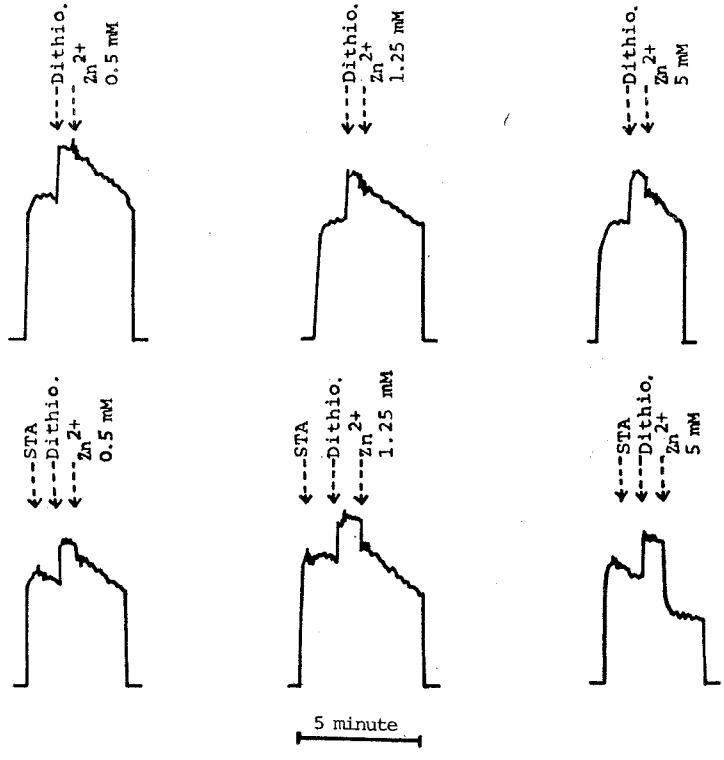

Fig. 9. Zn^{2+} lowers fluorescence of chloroplasts incubated with sodium dithionite at lower light intensity.
Concentrations of reagents used were: Sodium dithionite (dithio), added in solid form; silicotungstate (STA), 0.5 μM.

O-phenanthroline (Fig. 7), indicating that Zn^{2+} may inhibit electron transport on the donor side of PS II. Indeed, Zn^{2+} inhibits slightly electron transport using H_2O as a donor and methylviologen as an acceptor (result not shown), but, it stimulates electron transport using ascorbate as a PS II donor and methylviologen as an acceptor, Zn^{2+} also reverses partially the inhibitory effect of DCMU (Fig. 8). The reductant experiments suggest that results of Figs. 5 and 6 may be due to redox effects.

But, Zn^{2+} lowers fluorescence in the presence of both dithionite and silicotungstate (Fig. 9). Silicotungstate itself lowers fluorescence in the presence of both dithionite and $MgCl_2$ (Fig. 10). Fig. 10 also shows that phosphotungstate decreases fluorescence, and the level of the suppressed fluorescence intensity is lower in dithionite incubated chloroplasts than that in chloroplasts

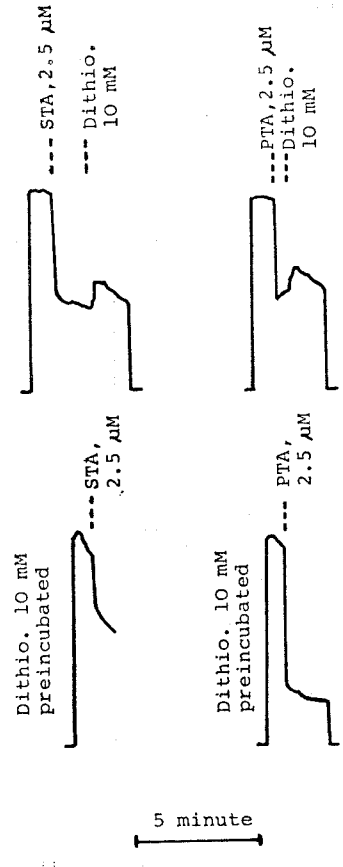

Fig. 10. Sodium dithionite assists phosphotungstate (PTA) to further suppress fluorescence.
Upper row: 10 mM sodium dithionite (dithio) was added in light.
Lower row: Chloroplasts were preincubated with 10 mM dithionite.

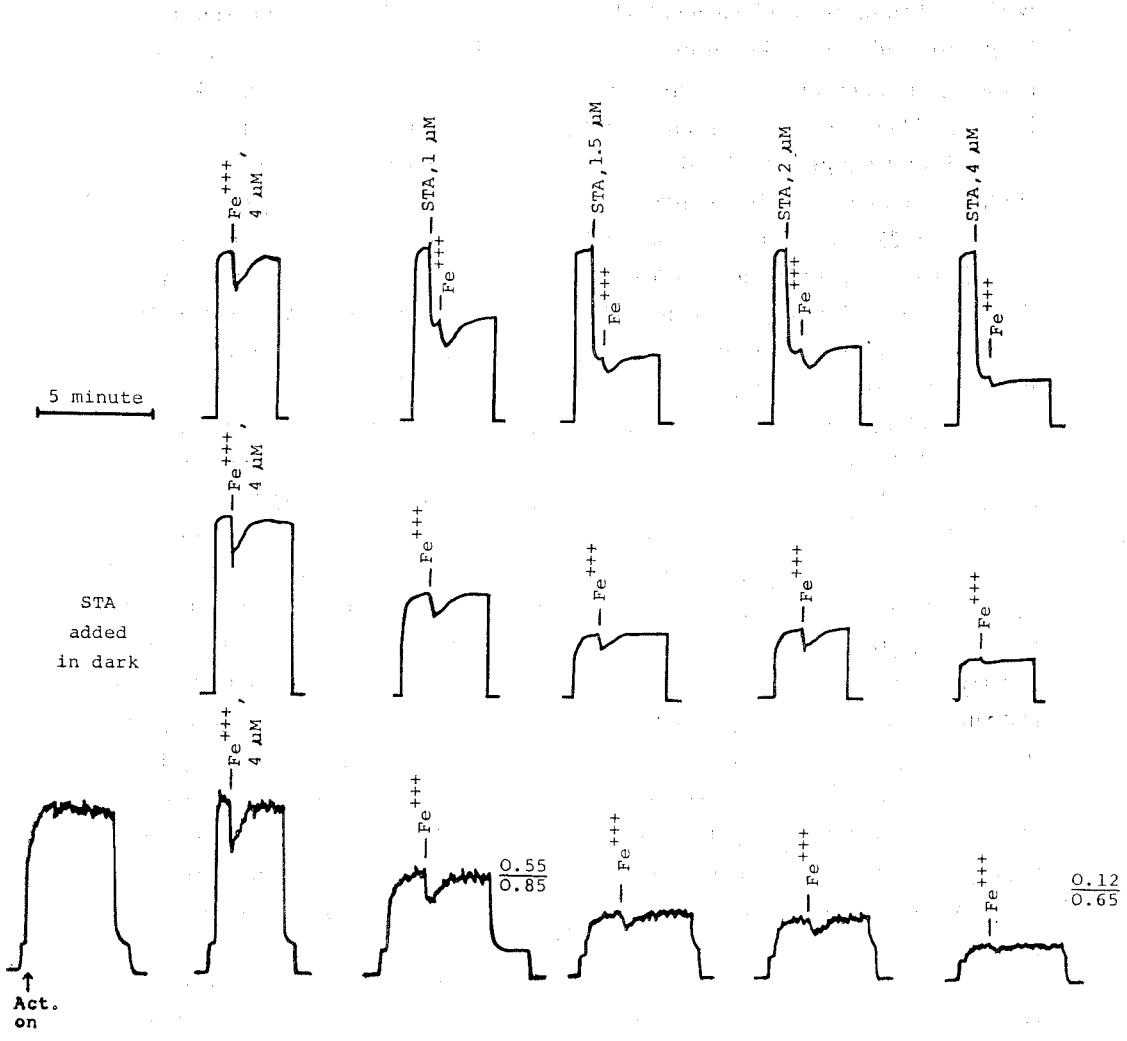

Fig. 11. Differential effects of silicotungstate on variable and on constant fluorescence. Recordings of the first (silicotungstate added in light) and second (silicotungstate added in dark) rows were fluorescence results obtained by a DC amplifier. Recordings of the third row (silicotungstate added in dark) were results of AC fluorescence measurements in which fluorescence were excited by a weak modulated light in the absence (constant fluorescence) or in the presence of a strong un-modulated actinic light (constant and viariable fluorescence). Act. on: Actinic light on. See Li *et al.* (1981) for a description of the AC fluorescence measurement. Both variable and constant fluorescence of a control sample are normalized to 1. The pair of number adjacent to a treated sample are normalized values of variable (upper) and constant (lower) fluorescence.

without dithionite pretreatment (the order of the dithionite and the phosphotungstate addition makes differences). Ascorbate is also effective (results not shown). Reductants may affect the physical properties of thylakoid membranes. These results of Figs. 9 and 10 reverse our conclusion based on Figs. 7 and 8, and suggest that inhibitors may affect the physical properties of the thylakoid membranes to modify the effect of Zn^{2+} on fluorescence.

Figure 11 shows that silicotungstate lowers preferentially variable fluorescence. At a concentration of 1 μM, silicotungstate quenches 45% of variable fluorescence, while 85% of constant fluorescence remains. At a concentration of 4 μM, silicotungstate quenches 88% and 35% of the variable and constant fluorescence respectively.

We set out the present investigation by predicting that Zn^{2+}, silicotungstate and PS II inhibitors may interact, and have found out that this is the case. We also postulated that silicotungstate reacts with Q-B-apoprotein to affect fluorescence. Whereas the results do not support the later hypothesis unequivocally, observations reported here do suggest some in depth studies to reveal their real significance. In fact, some of these studies have been taken, results suggest that there exists a fluorescence regulating domain (FRD), including Q-B-apoprotein as one of its constituents, which controls the fluorescence quantum yield (manuscripts submitted elsewhere). This domain may consist of one or several macromolecules. One of them, being a main regulator, may sense the redox changes of Q to undergo a conformational change to affect the quenching properties of the PS II reaction center (Li, 1977); the others may be peripheral molecules bearing sites which react with various physiological and non-physiological reagents to transmit information to the main regulator.

Silicotungstate may react with FRD so that either FRD does not sense the redox change of Q, or it does not undergo a conformational change to affect fluorescence to any large extent. The fluorescence lowering effect of reductant in phosphotungstate treated chloroplasts (Fig. 10), a hither-to unfound phenomenon, deserves special attention, it suggests that the physical state of a PS II unit is sensitive to redox changes of its environments, providing an experimental evidence to support the hypothesis of Li (1977). These observations also show that reductants can affect fluorescence in ways not related to the redox state of Q, one must therefore be cautious in interpreting reductant's effect on fluorescence.

Acknowledgements

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鋅離子及矽鎢酸鹽影響懸浮於蒸餾水中葉綠體的螢光

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鋅離子、鎂離子、矽鎢酸鹽及兩種第二光系統抑制劑能影響葉綠體螢光。上列某一試劑對葉綠體螢光效應，可被另一試劑所改變。該等試劑可能影響一個接近第二光合系統反應中心的"域"使該中心淬光螢光的能力改變。該"域"暫定名爲"螢光控制域"。