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REDOX OF Q REGULATED SLOW FLUORESCENCE INDUCTION OF ISOLATED CHLOROPLAST[1,2]

YUNG-SING LI

Institute of Botany, Academia Sinica, Nankang, Taipei, Taiwan, Republic of China

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Abstract

A cell type slow fluorescence time course (induction), showing peak-minimum-peak, intensity variations is found in isolated chloroplast. All phases of the induction are sensitive to gramicidin, ascorbate, or the concentration of methylviologen. The ways fluorescence responding to the agents mentioned indicate that there is a rate-limiting coupling site existing on the water-side of Q, (primary electron acceptor of Photosystem II), and reveal the dynamic nature of the electron transport activities,at the beginning of an illumination.

Introduction

Upon illumination, broken chloroplasts supplemented with Mg^{2+} or intact chloroplasts show a slow fluorescence falling of large magnitude after the well known instantaneous rise to a peak. For reasons of its Mg^{2+} dependency and its reversibility by uncoupler addition, the fluorescence falling has been attributed to Mg^{2+}—efflux from the thylakoids in response to light-induced proton-uptake (Krause, 1974; Barber and co-workers, 1974). Jennings *et al* (1976), based on the observations that uncouplers stimulate slow fluorescence falling, whereas subjection of chloroplasts to an acid-base transition is inhibitory, suggest instead that the falling is due to a decrease in the probability of the back transfer of energy from Photosystem II (PS II) reaction centers to the fluorescent "light harvesting" chlorophyll system.

More recently, Sokolove and Marsho (1977) resolve the fluorescence falling into two distinct components, an ionophore-reversible and an ionophore-resistant components. The ionopore-reversible falling is attributed to Mg^{2+}—efflux from the thylakoid driven by proton uptake, whereas the ionophore-resistant falling is attributed to photoinhibition.

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The present study reports yet another type of fluorescence induction which resembles that of algae. And evidences show that in all phases of the induction, the redox of Q may be a prime regulating force.

Material and Methods

Chloroplast were isolated from oats seedling according to Li (1975), except that the buffer system was different. Tris-HEPES buffer was prepared fresh each day of experiment, while HEPES-NaOH buffer was prepared from stock solutions, compositions are indicated in the legends of figures. Fluorescence was excited with green light (broad-band interference filter, 500–640 nm plus Corning glass CS 4–96, plus 8 cm 2% $CuSO \cdot 5H_2O$), the intensity was 30 nano-einstains/cm^2 sec at the position of the cuvette (but without a cuvette). Emitting light was observed 90 degree from the exciting beam at 685 (monochrometer set at 685 nm plus auxillary red filters).

Results and Discussions

Unlike the above mentioned reports on chloroplast slow fluorescence induction (fluorescence time course), the present study shows that after a falling from an initial peak value, fluorescence rises again (second rise, Figs. 1, 2, and 3). When will the second rise commence and how fast will it ascend depend on the the concentration of a Hill acceptor and on the availability of an exogeneous Photosystem II electron donor—ascorbate. The fact that the second rise commences earlier at 1×10^{-8}M methylviologen (MV, an auto-oxidizable compound), where electron transport may be limited for a lack of enough electron acceptors (Li, unpublished observation), than at 1×10^{-4}M indicates that this rise is possibly related to a change of the redox state of Q; the observation that ascorbate accelerates the rise (Figs. 1, 2 and 3) may concur with this postulation.

Fig. 1 shows that the initial falling can be prevented by gramicidin D at low MV concentration but not at high MV concentration (1×10^{-4}M), under the latter condition gramicidin curtails the initial peak and *accelerates the falling* dramatically, while the second rise is started earlier than in a control sample; ascorbate in the presense of gramicidin eliminates all the more dramatic fluorescence changes, leaving a very slow, monotonous fluorescence declining in light. The ascorbate-reversible gramicidin effect (with high MV concentrations) on fluorescence can be explained as follows: gramicidin may accelerate Q^- oxidation by way of its action on the well-known photophosphorylation coupling site located between the two Photosystems (Losada *et al.*, 1961), hence keeps the peak low and accelerates the initial falling ascorbate (with gramicidin) simply keeps Q^- concentration high and therefore prevents the

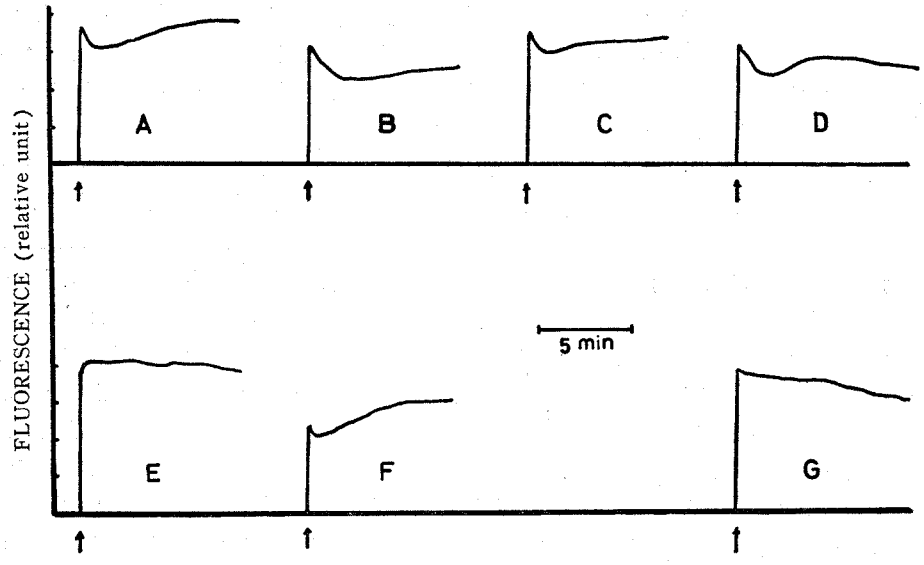


Fig. 1. Ascorbate and gramicidin effects on chlorophyll fluorescence, with high or low concentration of methylviologen. A and B, control samples, A with 1×10^{-5}M methylviologen (MV) added while B with 1×10^{-4}M; C and D, samples with ascorbate (1×10^{-2}M) added, same MV concentrations as A and B respectively; E and F, same as A and B respectively but in the presence of gramicidin (6×10^{-7}M); G, MV (1×10^{-4}M), both ascorbate and gramicidin were added with concentrations mentioned. Chloroplasts were isolated with $MgCl_2$ 5 mM; Tris (12 mM)-HEPES (21 mM), pH 7.5; sorbitol, 400 mM, and assayed in the same medium, chlorophyll 8.6 μ/ml. Upward arrow indicates light on.

falling. When MV is limiting, whether chloroplasts are in a coupled or an uncoupled state should make no difference on Q^- oxidation, if the above mentioned coupling site was the only limiting site. This expectation is only partially confirmed. Fig. 1 (gramicidin added to a sample with 1×10^{-8}M MV) shows that gramicidin acts not only on the wellknown coupling site but also somewhere else, for unexpectedly the falling is prevented altogether by the uncoupler.

The initial fluorescence falling in a control sample with limited amount of MV can sometimes be prevented also by ascorbate added alone (Fig. 2), which indicates that there may be a rate limiting site on the water side of Q in cnotrol samples and ascorbate may be able to bypass it, perhaps partially. This limiting site will be designated as Site II, while the well-known phosphorylation site as Site I. Site II seems to be uncoupler sensitive, for gramicidin added to a sample containing 1×10^{-8}M MV eliminates the fluorescence falling (Fig. 1), the gramicidin effect on Site I in this experiment is not manifested for reasons stated in last paragraph.

There have been two other fluorescence observations which also suggest the existence of an uncoupler sensitive rate limiting site on the water side of

Q, i.e. firstly, the Gimmler's observation (1973) that uncoupler accelerates fast fluorescence rising as predicted by Cheniae (1970) on the assumption that there is a photophosphorylation coupling site on the water side of Q, and secondly, the Li's observation (1973) that gramicidin D enhances fluorescence and photochemistry simultaneously. Around the same period when these reports appear, electron transport and phosphorylation experiments show that there may be a second photophosphorylation coupling site (Böhme and Trebst, 1969; Izawa *et al.*, 1973; Trebst and Reimer, 1973). Its precise location is not determined by these photochemistry experiments, and whether it is rate limiting is controversial (Izawa *et al.*, 1973; Heathcote and Hall, 1974). For reasons of its sensitivity to uncoupler and in the absence of any formidable evidences which indicate otherwise, rate limiting Site II will be identified with the newly found coupling site. By this identification, the mentioned coupling site is then assumed to be rate limiting and is located on the water side of Q.

How is the second rise related to the properties of Site I and Site II? Evidences show that the second rise of flluorescence may be the result of an increase of the rate constant of Site II, rather than a decrease of that of Site I, for in general, at about the same time when the second fluorescence rise occurs, photochemistry rate is also increased (Li, unpublished results, rate and fluorescence were measured separately), i.e. rate and fluorescence are non-complemental in this phase of induction; the increase of photochemistry indicating that electron flow from Q to MV is not retarded, i.e. Site I is not becoming more limitative in this phase of induction than the preceding one when fluorescence is at its minimum. Bannister and Rice (1968) have observed the same non-complementary in algae when the two parameters are measured simultaneously, and they have concluded that the result is due to activation process. (Phenomenologically, the fluorescence induction of algae and that of isolated chloroplast reported in this study are the same). However, the present study shows that as far as fluorescence is concerned Photosystem II reacion center is fully active, since in the presence of ascorbate fluorescence is high and there is no second rise, in fact some units may be inactiviated in light (later this report).

Generally, ascorbate alone can not prevent the initial fluorescence falling whether the MV concentration is low or high (Fig. 1). In some chloroplast preparations, however, ascorbate arrests the more dramatic fluorescence changes at low MV concentration (Fig. 2). What makes ascorbate being effective in preventing the fluorescence falling in one chloroplast preparation but not in another? The following observations may provide clues. In general, gramicidin is required for ascorbate to elimnate the initial fluorescence falling; but in an experiment where photochemistry measurement showing that

chloroplasts for unknown reasons are uncoupled, ascorbate eliminates the falling even at a MV concentration of 1×10^{-4}M and without gramicidin (data not shown). In other experiments I found long period of ascorbate incubation (15 minutes, for instanc) evicted the fall and the rise even at high MV concentrations and in the absence of gramicidin; the incubation time depended on the storage time of isolated chloroplasts and other known (temperature, for example) and unknown factors (Li, unpublished results). The long incubation requirement of the ascorbate effects on fluorescence indicates that there may be diffusion barriers between ascorbate in solution and the site of electron donation (assuming ascorbate incubation doesnot uncouple), this explanation is in accord with the concept that the physiological electron donor for Q is located on the inside of the thylakoid membrane, and in accord with the gramicidin requirement. It is not certain from the present study whether the gramicidin requirement is all due to diffusion effect. However, the fact that ascorbate supports both Site II and Sit I photophosphorylation (Böhme, Trebst and 1969; Ort and Izawa, 1974) indicates that ascorbate donates electrons via Site II. Ort and Izawa (1974) have found that the P/2e ratio of ascorbate supported electron transport is smaller than that of water supported electron transport, indicating that some electrons are bypassing Site II, or ascorbate incubation may induce partial uncoupling.

Incidentally, the fluorescence experiments show that ascorbate does donate electron to Q in normal, O_2 producing chloorplasts provided that a diffusion barrier can be overcome (see Allen and Hall, 1973; Ort and Izawa, 1974 for an

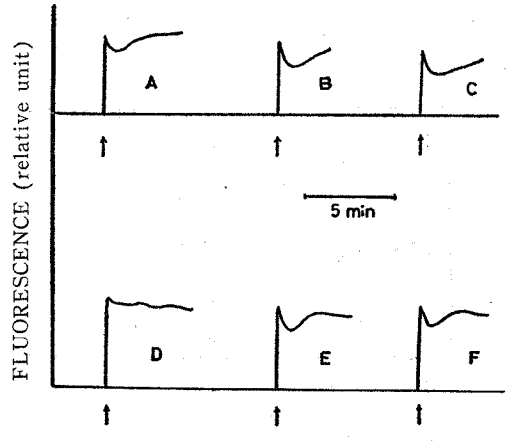


Fig. 2. Effects of methylviologen concentration on chlorophyll fluorescence with and without ascorbate. Upper row traces A, B and C, control samples with methylviologen concentration 1×10^{-8}M, 1×10^{-6}M and 1×10^{-4}M respectively; lower row traces D, E, and F, in the presence of added ascorbate (1×10^{-2}M) same methylviologen concentration as in A, B, and C, respectively. Experimental condition were the same as in Fig. 1 except 18 μg chlorophyll/ml.

argument against ascorbate being an electron donor to Q in normal chloroplat).

Fig 3. shows the effects of ascorbate concentrations on fluorescence in the presence of gramicidin. At low concentration, asorbate increases the fluorescence intensity in all the induction phases, however there is still a reminiscence of the more dramatic induction changes. At about 1.25 mM ascorbate, the dramatic changes are all gone, leaving a slow, monotonous fluorescence declining. Although higher ascorbate concentrations can slow down the remaining very slow, monotonous fluorescence declining in light, it can not stop it altogether at a concentration of 5 mM (10 mM in other experiments, data not shown). This remaining fluorescence falling in the presence of high ascorbate concentration may be caused by photoinhibition of the Photosystem II reaction center, whereas the ascorbate reversible fluorescence declining may be attributed to uncoupler induced water-splitting system inactivation (Renger, 1969; Homann, 1971).

The foregoing discussion, ignoring the light induced inhibitions of PS II reaction center and water-splitting system, and the diffusion barriers for

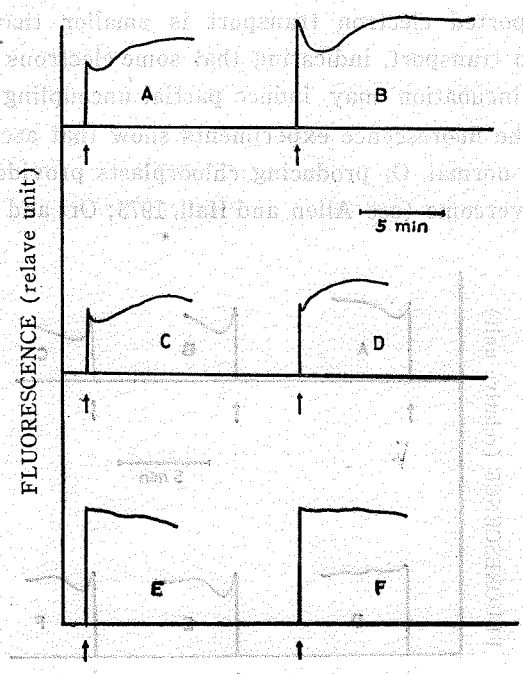
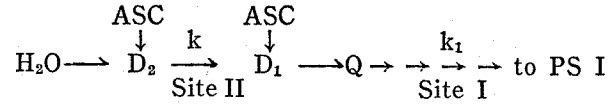


Fig. 3. Effects of ascorbate concentration on chlorophyll fluorescence in the presence of gramicidin. All samples contain 1×10^{-4}M methylviologen, A, no further addition; B, ascorbate (5×10^{-3}M); C, gramicidin (6×10^{-7}M); D, gramicidin plus ascorbate (1.5×10^{-4}M); E, gramicidin plus ascorbate (1.25×10^{-3}M); F, gramicidin plus ascorbate (5×10^{-3}M). Chloroplasts were isolated with HEPES-NaOH, 40 mM (pH 8); sorbitol 400 mM, and assayed in the same plus $MgCl_2$, 5 mM. Chlorophyll, 7.4 μg/ml. Buffer was prepared from HEPES-NaOH and Sorbitol stock solutions. Other conditions were the same as in Fig. 1.

accorbate, (ASC) can be summarized by the following simplified electron transport scheme,



where k and k_1 are two uncoupler-sensitive reaction constants (both may be composite) for reactions leading to Q reduction and Q^- oxidation respectively; D_1 and D_2 are primary and secondary electron donors respectively, and ascorbate can donate electron to both D_1 and D_2.

Because of the finding of limiting Site II, the non-complemental rate-fluorescence relationships, which could not be understood in terms of the Q-hypothesis of Duysens and Sweers (1963) if Q reduction were not sometimes to be limiting, can now be explained. New rate and energy conservation equations have been introduced in view of this newly found limiting site (Li, 1977), which explain all types of rate-fluorescence relationships in terms of Q-hypothesis of Duysens and Sweers. Admittedly, other processes which regulate the fluorescence quantum yield may operate during the induction phase, however, for the observations just reported, the conclusion is that the major fluorescence changes may be redox of Q-related.

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游離葉綠體中由Q的氧化還原態所控制的慢速螢光遞變

李 永 興

中央研究院 植物研究所

Q爲第二光系統的初級電子受子。一般而言，當Q處於氧化態時，光合單元的螢光强度低，當Q處於還原態時光合單元的螢光强度高。本文報告游離葉綠體螢光遞變以及利用加入的人工電子受子的濃度的不同，或加入解藕劑，或加入人工電子施者（維生素丙）或同時加入兩種以上上述作用物時對螢光遞變的影響。

照光後，葉綠體中的葉綠素螢光迅即昇至一峯值，其後即漸漸下降，歷時約一分鐘後再次慢慢上昇該種螢光强度的變化，統稱爲螢光遞變。在低電子濃度時，這種遞變現象可因解藕劑或維生素丙之加入而不復出現。維生素丙可代替水成爲第二光系統的一種電子施者。在高電子受子濃度時，解藕劑不但不能抑制遞變現象反而降低螢光峯值，加速其下降率，而螢光再上昇現象仍然存在。同樣，在高受子濃度時維生素丙也不能抑制遞變，但與解藕劑合用則屬有效。這些現象明示Q的還原與氧化率皆會受到某些限制，這些限制可用解藕劑解除之。