# SLOW FLUORESCENCE QUENCHING OF TYPE A CHLOROPLASTS

## Relationship to electron-flow with $CO_2$ as acceptor

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## 1. Introduction

Krause [1,2] and Barber and his coworkers [3] have reported the occurrence of slow fluorescence quenching in dark-adapted, isolated, intact chloroplasts. This quenching is sensitive to uncouplers and is reversed by the divalent cation-specific ionophore A23187 [4]. It is lost upon osmotic shock, but restored by addition of $Mg^{2+}$ in the dark. Slow fluorescence quenching has therefore been attributed to $Mg^{2+}$-efflux from the thylakoids in response to light-induced proton-uptake.

If slow fluorescence quenching is driven by proton-uptake, the question then arises, what pathways of electron-transport are responsible for this proton-uptake in the functioning chloroplast? Barber and coworkers originally reported [5] that the rate of slow fluorescence quenching was dependent on the ability of the chloroplasts to fix $CO_2$. More recently, Telfer et al. [6] have suggested, on the basis of differential sensitivity of non-cyclic electron-flow and slow fluorescence quenching to DCMU, that cyclic electron-flow can occur in intact chloroplasts and can result in slow fluorescence quenching. We demonstrate here that net oxygen evolution linked to $CO_2$-fixation is not required for the observation of slow fluorescence quenching. Further, we present evidence that pseudocyclic electron-flow, with $O_2$ as terminal electron-acceptor, can support slow fluorecence quenching and in fact, does so during induction of net oxygen evolution and under conditions where the Calvin cycle is inhibited.

## 2. Methods

Intact (Type A [7]) chloroplasts were isolated from spinach as described previously [8], except that leaves were picked and illuminated with bright white light for 10 min immediately prior to use. Chloroplasts were more than 75% intact as determined by the ferricyanide reduction method [9,10] and fixed $CO_2$ at rates in excess of 100 mol/h/mg Chl when assayed polarographically in the presence of 10 mM bicarbonate [11] and catalase (85 units/ml) at pH 8.

Fluorescence and $O_2$-evolution were measured as reported earlier [12,13]. In both cases, broad-band blue actinic illumination (78 kerg/cm$^2$/s) was provided via a Shott KG-3, Corning 4-96 filter combination. Temperature was maintained at 20°C. Chloroplasts were diluted in assay medium [8] without $MgCl_2$ to a concentration of 7.5 μg chlorophyll/ml and 12.8 μg chlorophyll/ml for fluorescence and polarographic measurements respectively. In all experiments, the ionophore-reversible component of slow fluorescence quenching was monitored by the addition of $MgCl_2$ and the divalent cation-specific ionophore A23187 ([12] see also fig.1C).

## 3. Results and discussion

In fig.1, traces A–C, the time course of net $O_2$-evolution is superimposed on the trace representing slow fluorescence quenching for dark-adapted, intact spinach chloroplasts supplemented with $NaHCO_3$.



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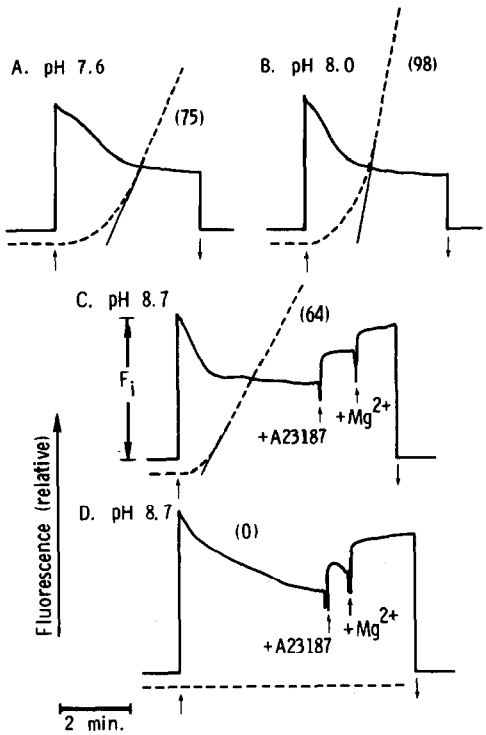


Fig. 1. Comparison of the kinetics of slow fluorescence quenching (solid traces) and net oxygen evolution (dotted lines) for Type A spinach chloroplasts. Trials A–C contained 10 mM $NaHCO_3$. Actinic light on (↑) and off (↓). Rates of $O_2$-evolution (in μmol/hr/mg Chl) observed in each trial are indicated in parentheses. The procedure for determination of ionophore-reversible quenching [12] is shown in C and D. After 4 min illumination, A23187 (1.25 μg/ml) and $MgCl_2$ (12.5 mM) are added. The resultant fluorescence increase, expressed as a percent of the original fluorescence level ($F_i$), is termed ionophore-reversible quenching.

Results obtained at several pH-values are presented. It can be seen that the length of the induction lag for $O_2$-evolution decreases with increasing pH, but that the rate of quenching increases such that the bulk of slow fluorescence quenching always occurs during the induction lag for $O_2$-evolution, prior to the attainment of maximal $CO_2$-fixation rates [14]. This observation led us to question the need for electron-flow to $CO_2$ during slow fluorescence quenching.

Figure 1D demonstrates that slow fluorescence quenching can, in fact, proceed in the absence of net $O_2$-evolution. The trace in fig.1D was obtained at pH 8.7 in the absence of added bicarbonate. Considerable ionophore-reversible quenching can be seen, yet under these conditions no net $O_2$-evolution could be detected.

The separability of these two events, slow fluorescence quenching and electron-flow to $CO_2$, can also be demonstrated with inhibitors of the Calvin cycle. As shown in fig.2, slow fluorescence quenching is essentially unaltered by DL-glyceraldehyde [15,16] and supraoptimal concentrations of inorganic phosphate [17,18]. The effects of these inhibitors on the net rate of $O_2$-evolution and on slow fluorescence quenching are summarized in table 1. DL-Glyceraldehyde, 10 mM, which completely eliminates $O_2$-evolution, reduced ionophore-reversible quenching by less than 12% in this experiment. Inorganic phosphate (2 mM) was a less stringent inhibitor of $O_2$-evolution (80% inhibition), but its effect on slow fluorescence quenching (again < 12% inhibition) was minor by comparison. DL-Glyceraldehyde addition to chloroplasts in the light had no perceptible effect

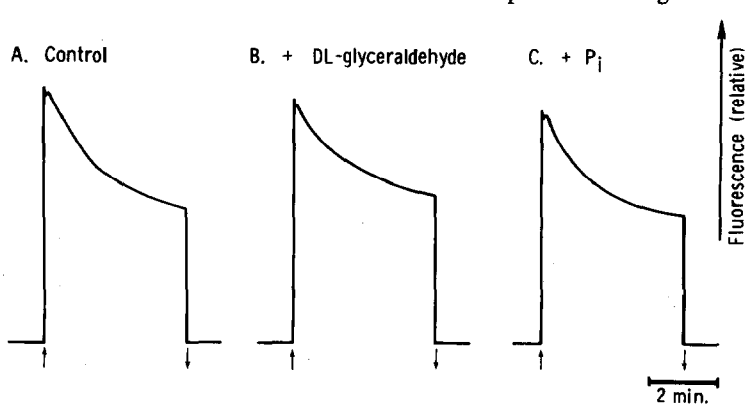


Fig.2. Effect of Calvin cycle inhibitors on slow fluorescence quenching measured in the presence of 10 mM $NaHCO_3$. (A) Control, (B) 10 mM DL-glyceraldehyde, (C) 2 mM $K_2HPO_4$. The pH was 8.3. Actinic light on (↑) and off (↓).

Table 1
Effect of inhibitors of $CO_2$ fixation on slow fluorescence quenching[a]

| Inhibitor | Rate of $O_2$ evolution ($\mu$mol/hr/mg Chl) | Total quenching (% $F_i$) | Ionophore-reversible quenching (% $F_i$) |
|---|---|---|---|
| None | 106 | 47.6 | 37.6 |
| 10 mM DL-glyceraldehyde | 0 | 39.7 | 33.3 |
| 2 mM Inorganic phosphate | 21.2 | 44.8 | 33.6 |

[a] Details as in fig.2

on the quenched fluorescence level, i.e., quenching was not reversed.

The occurrence of slow fluorescence quenching in the absence of net $O_2$-evolution can be explained in several ways. Quenching might result from the movement of a number of protons so small that the associated electron-flow is below the limits of polarographic detection. If, as suggested, the quenched state represents the high energy state, this seems unlikely. Alternatively, slow fluorescence quenching may result from electron-flow that is not detectable polarographically, i.e., cyclic or pseudocyclic electron-flow.

Pseudocyclic electron-flow has recently been demonstrated in whole-cells [19] and in isolated, intact chloroplasts under light-limiting conditions [20] by direct mass spectrographic measurement. The stimulatory effect of catalase on $CO_2$-fixation and on the associated net $O_2$-evolution by intact chloroplasts [20] is also consistent with the occurrence of pseudocyclic electron-flow.

Several lines of evidence suggest that pseudocyclic electron-transport can produce the slow fluorescence quenching seen in intact chloroplasts. First, as illustrated in fig.3, slow fluorescence quenching measured in the presence of DL-glyceraldehyde is severely inhibited by anaerobic conditions. Inhibition of ionophore-reversible quenching is particularly striking. We have recently shown [12] that there are two components of slow fluorescence quenching. One component, ionophore-reversible quenching, is correlated with light-induced proton-uptake. The second component, which is not reversed by ionophore A23187 and $Mg^{2+}$, is identified as photo-inhibition. It can be seen (fig.3B) that the latter constitutes the bulk of the quenching remaining under anaerobic conditions. Inhibition of quenching is relieved by restoration of aerobic conditions. Slow fluorescence quenching measured in the absence of DL-glyceraldehyde is also sensitive to anaerobic conditions, but to a lesser extent. These data are summarized in table 2.

The data clearly demonstrate the dependence of ionophore-reversible, slow fluorescence quenching on $O_2$ when $CO_2$-fixation is prevented. Lowered sensitivity to anaerobic conditions when the Calvin cycle is not inhibited may reflect the higher affinity of the electron-transport chain for $CO_2$ than for $O_2$ [21]. Quenching would then be due to electron-flow

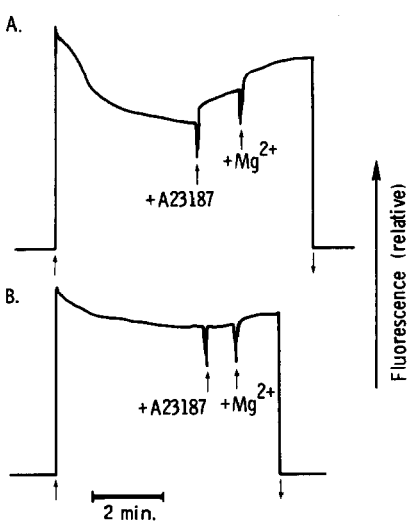


Fig.3. Effect of anaerobic conditions on slow fluorescence quenching in the presence of DL-glyceraldehyde. The reaction mixtures for A and B are identical: pH 8.0, 10 mM DL-glyceraldehyde, no added bicarbonate. The medium for B was equilibrated with $N_2$ by bubbling vigorously for 15 min immediately prior to chloroplast addition and assay. Actinic light on (↑) and off (↓).

Table 2
Effect of anaerobic conditions on slow fluorescence quenching

| Reaction conditions[a] | Total fluorescence quenching (% $F_i$) | Ionophore-reversible quenching (% $F_i$) | Percent inhibition of ionophore-reversible quenching |
|---|---|---|---|
| Aerobic | 54.1 | 32.5 | 0 |
| Anaerobic | 30.9 | 12.1 | 63 |
| Aerobic plus DL-glyceraldehyde | 44.9 | 28.7 | 12 |
| Anaerobic plus DL-glyceraldehyde | 22.2 | 6.2 | 81 |
| Anaerobic → aerobic[b] plus DL-glyceraldehyde | 37.7 | 26.9 | 17 |

[a] DL-Glyceraldehyde was 10 mM, when added. Anaerobic conditions were obtained by bubbling the medium vigorously with $N_2$ for 15 min prior to chloroplast addition. Other details as in fig.3.
[b] Following equilibration with $N_2$ the medium was bubbled for an additional 15 min with air

either to $CO_2$ or to the resultant endogenously produced $O_2$.

A second line of evidence implicating pseudocyclic electron-flow in slow fluorescence quenching depends on direct mass spectrographic measurements of pseudocyclic electron-flow. When dark-adapted, intact chloroplasts are illuminated, $O_2$-evolution of significant magnitude (15–30% maximum rate) begins immediately. This $O_2$-evolution is balanced initially by an equivalent $O_2$-uptake reaction [22] thus producing the familiar induction lag in net $O_2$-evolution [14]. The $O_2$-uptake reaction and the associated $O_2$-evolution (i.e., pseudocyclic electron-transport) are thus correlated in time with slow fluorescence quenching whereas non-cyclic electron-flow to $CO_2$ is not (see fig.1). Furthermore, this pseudocyclic electron-flow is insensitive to concentrations of DL-glyceraldehyde and inorganic phosphate that completely inhibit the Calvin cycle (22). Slow fluorescence quenching is similarly insensitive to these inhibitors (fig.2 and table 1).

## 4. Conclusions

The results presented suggest that electron-flow to $CO_2$, as monitored by net $O_2$-evolution, is not required for slow fluorescence quenching. Periods of most rapid quenching and most rapid net $O_2$-evolution are not correlated in time. Slow fluorescence quenching can be demonstrated under conditions where net $O_2$-evolution has been eliminated.

The data do not allow us to conclude that non-cyclic electron-flow to $CO_2$ cannot drive fluorescence quenching, simply that such electron-flow is not required. Our data suggest, however, that slow fluorescence quenching can be driven by pseudocyclic electron-transport, at least when electron-flow to $CO_2$ is limited, i.e., during induction, when $CO_2$ availability is low (high pH) and when the Calvin cycle is inhibited.

To the extent that slow fluorescence quenching represents generation of the high energy state, the results are consistent with the proposal [21,23] that pseudocyclic electron-flow contributes to meeting the ATP-demand of the Calvin cycle.

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