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Short Communication

Dark Induction of the Non-Photochemical Quenching of Chlorophyll Fluorescence by Acetate in *Chlamydomonas reinhardtii*

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Addition of acetate to a suspension of *Chlamydomonas reinhardtii* cells in darkness induced transient and biphasic non-photochemical quenching of Chl fluorescence (qN) due to ΔpH-dependent down-regulation of PSII and the transition from state 1 to state 2. We propose that acetate-induced stimulation of the chlororespiratory electron flow triggers the regulation of PSII.

Key words: Acetate — *Chlamydomonas reinhardtii* — Chlorophyll fluorescence — Chlororespiration — P700 — State transition.

Respiratory electron transport in chloroplasts, known as chlororespiration (Bennoun 1982), has been demonstrated in algae (Maione and Gibbs 1986, Peltier et al. 1987, Wilhelm and Duval 1990, Ting and Owens 1993) and higher plants (Garab et al. 1989, Asada et al. 1992, 1993, Singh et al. 1993). One possible role for chlororespiration is the control of PSII activity by changing the flow rate of electrons and the redox level of the photosynthetic intersystem chain. The quantum yield of PSII is regulated by the pH gradient across the thylakoid membranes and the transition from state 1 to state 2, which are governed, respectively, by the rate of electron flow and redox state in the intersystem chain. Thus, it seems likely that PSII can be controlled by the chlororespiratory electron flow. The ΔpH-dependent down-regulation of PSII has been shown to be associated with the de-epoxidation of violaxanthin and the resultant lower rate of energy transfer from LHC II to PSII reaction center or with lower activity of electron donation at the donor side of PSII (for reviews, see Demmig-Adams 1990, Krause and Weis 1991, Schreiber et al. 1994). The transition from state 1 to state 2 is associated with a shift in the distribution of light energy in favor of PSI, at the expense of PSII. This process is triggered by reduction of either the plastoquinone pool or the Cyt *b/f* complex, which subsequently induces the phosphorylation of LHC II (for review, see Allen 1991).

The regulation of PSII activity, via a decrease in the potential photochemical yield of PSII, is inversely related to the non-photochemical quenching of Chl fluorescence (qN). Horton and Hague (1988) resolved total qN into qE (energy quenching; the ΔpH-dependent down-regulation of PSII), qT (the transition from state 1 to state 2) and qI (photoinhibition of PSII) in illuminated barley protoplasts. In the present study, we examined the effects on photosynthesis of acetate, a respiratory substrate that is commonly used in the heterotrophic culture of *Chlamydomonas reinhardtii*. Our analysis was based on the assumption that addition of acetate to the medium stimulates not only mitochondrial respiration but also chlororespiration. We show here that the addition of acetate to a suspension of cells induces the qE and qT types of quenching of Chl fluorescence. A role in the regulation of PSII is proposed for chlororespiratory electron input into the photosynthetic intersystem chain.

Chlamydomonas reinhardtii c-9 (IAM Culture Collection, Institute of Molecular and Cellular Biosciences, University of Tokyo) was cultured photoautotrophically under fluorescent lamps (5 W m^{-2}) in the medium of Orth et al. (1966) on a rotary shaker (100 rpm) at 27°C in an atmosphere of 2% CO_2 in air. Cell culture at the early stationary phase of growth was diluted with an equal volume of 40 mM MES/KOH (pH 6) and kept under dim light for at least 10 min for the relaxation of qE associated with the original culture conditions. Then the suspension of cells was used for measurements of Chl fluorescence and the redox state of P700. Changes in absorbance of $P700^+$ at 820 nm and Chl fluorescence were monitored with a PAM Chlorophyll Fluorometer (Walz, Effeltrich, Germany). The details of the conditions for measurement were described previously (Endo et al. 1995). The nomenclature of van Kooten and Snel (1990) was used for the parameters of Chl fluorescence.

Under weak actinic light, addition of 1 mM sodium

Abbreviations: *F*, variable Chl fluorescence; LHC II, light-harvesting complex of PSII; *Fo*, yield of instantaneous Chl fluorescence at open PSII centers; *Fm*, maximum yield of Chl fluorescence at closed PSII centers, induced by a pulse of saturating light; *Fm'*, maximum yield of Chl fluorescence at a given time during measurements; NDH, NAD(P)H dehydrogenase; P700, reaction center of PSI; qE, a part of qN associated with membrane energization; qI a part of qN induced by photoinhibition of PSII; qN; coefficient representing non-photochemical quenching of Chl fluorescence $(Fm-Fm')/(Fm-Fo)$; qT, a part of qN induced by the transition from state 1 to state 2.

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acetate to a suspension of *C. reinhardtii* induced a transient decrease in the maximum fluorescence (*Fm'*), as estimated by application of a saturating light pulse (Fig. 1) and an increase in the variable Chl fluorescence, *F*. The decrease in *Fm'* and the increase in *F* induced by acetate were more evident at pH 6 than pH 7 (data not shown), a phenomenon that might be explained the by low permeability of the plasma membranes to the dissociated form of acetate. Addition of 1 mM HCl did not induce transient quenching, an indication that pH change by the addition of acetate was not responsible for the quenching. The decrease in *Fm'* induced by acetate was biphasic, as was most evident at pH 6. The phase corresponding to a rapid lowering and recovery was completed within a few minutes after the addition of acetate, while the slow phase was completed within 20 min. Quenching of Chl fluorescence at the *Fm'* level can be represented by the non-photochemical quenching [qN, $(Fm-Fm')/(Fm-Fo)$]. The addition of an ionophore nigericin almost completely suppressed the rapid decrease in *Fm'*, but the slow phase was unaffected, indicating that the initial phase was attributable to qN which was dependent on the formation of ΔpH (qE). Considering the low intensity of the actinic light employed, we assumed that the slower and ionophore-insensitive decrease in *Fm'* was due to the transition from state 1 to state 2 (qT). To confirm this possibility, we examined the effects of the phosphatase inhibitor NaF, which suppresses the transition from state 2 to state 1 (Bennett 1980), on the recovery of decreased *Fm'* (Table 1). The decrease in *Fm'* recorded at 1 and 10 min after the addition of acetate represent those of the fast and slow phases, respectively. Addition of NaF did not significantly suppress either phase of *Fm'* lowering. However, in the presence of NaF, the recovery of *Fm'* after the slow-phase quenching (30 min after the addition of acetate) was suppressed, evidence that the slow phase of the quenching was due to the transition to state 2.

The effective photochemical yield of PSII can be evaluated as $(Fm'-F)/Fm'$ (Genty et al. 1989). The value of $(Fm'-F)/Fm'$ was 0.71 before the addition of acetate and it was reduced to a minimum of 0.37, twelve minutes after the addition of acetate, namely, during the slow quenching (Fig. 1). The photochemical evolution of oxygen was measured under the same conditions as those used to obtain the results shown in Fig. 1 with 2 mM bicarbonate as an electron acceptor under sub-saturating light (2 W m^{-2}). The rate of oxygen evolution also decreased from 32 (control) to 11 (10 min after the addition of acetate) μmol (mg Chl)$^{-1}$ h^{-1}. Under the same conditions, the uptake of ox-

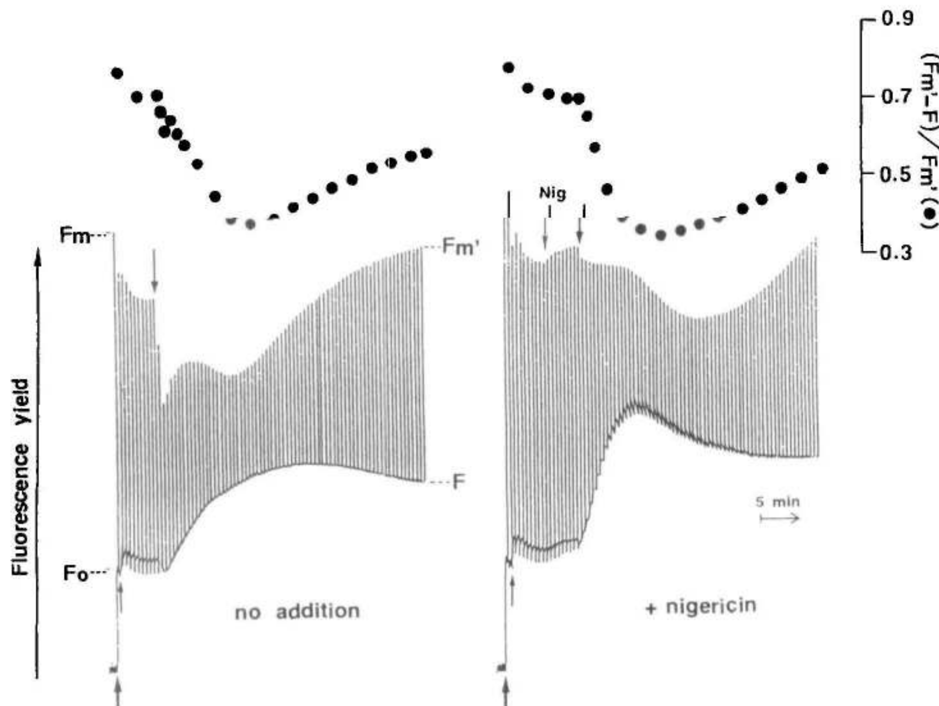

Fig. 1 The decrease in *Fm'* induced by acetate in *Chlamydomonas reinhardtii* cells. A cell culture (ca. 50 μg Chl ml^{-1}) was diluted with an equal volume of 40 mM MES/KOH (final pH, 6.0). A pulse of saturating white light (1 s, 350 W m^{-2}) was applied every 30 s for estimatation of *Fm'*. At the time indicated by the upward wide arrow, the measuring light was turned on, and at the time indicated by the upward slender arrow, the actinic light (3 W m^{-2}, 650 nm) was applied. At the time indicatd by the downward arrow, acetic acid (final conc., 1 mM) was added to the cell suspension. Where indicated, 1 μM nigericin (nig) was added.

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Table 1 Effects of NaF on the decrease in *Fm'* induced by acetate

Time after addition of acetate (min)	Decrease in *Fm'* (%)	
	Control	+NaF
1	35	29
10	24	17
30	0	11

Sodium acetate (0.5 mM) was added to an 8-day old suspension of cells (pH 6). Sodium fluoride (30 mM) was added 30 min before the addition of acetate. Values are expressed as percentages relative to *Fm'* just before the addition of acetate.

ygen in darkness was stimulated by acetate, increasing from 2.3 (control) to 9.9 μmol (mg Chl)$^{-1}$ h^{-1}. Thus, induction of qN was associated with a reduction in the photochemical yield of PSII.

In order to determine whether or not the decrease in *Fm'* by acetate was light-dependent, induction of qN after addition of acetate in darkness was examined (Fig. 2). To avoid excitation of PSII by repeated application of a saturating light pulse (Schreiber et al. 1995), a single, saturating pulse of light was applied at the indicated times for determination of *Fm'*. As was the case in the light, as shown in Fig. 1, nigericin-sensitive qN was induced first, being gradually replaced by nigericin-insensitive qN. This result indicates that the acetate-induced quenching was independent of the light conditions. In darkness, however, once quenching had been induced, it was maintained and the fluorescence yield did not returned to the higher level during the rest of the experiment, by contrast to the results in the light (Fig. 1). Excitation of PSI by actinic light oxidizes the electron carriers in the intersystem chain and contributes to the recovery of *Fm'*, since the reverse transition from state 2 to state 1 is triggered by the oxidation of the intersystem electron carriers.

The rate of the re-reduction of P700$^+$ after a short saturating flash of light in the presence of DCMU is an indicator of the redox level of the photosynthetic intersystem chain. The rate of the re-reduction of P700$^+$ was partly depressed by inhibition of the donation of electron from PSII by DCMU. Addition of acetate stimulated the re-reduction of P700$^+$ in the presence of DCMU (Fig. 3), suggesting the stimulated donation of electron from acetate to P700$^+$ via the plastoquinone pool.

Pyruvate, oxalacetate, glycolate and acetaldehyde at 2 mM also induced biphasic *Fm'* quenching in 20 mM MES at pH 6. The extent of the quenching induced by these compounds at 2 mM was roughly similar to that by acetate at 100 μM, showing that acetate was very effective in inducing the quencing of fluorescence at the *Fm'* level. By contrast, succinate, malate, ascorbate, glycerol, and ethanol did not induce quenching under the same conditions. It is noteworthy that NADPH-requiring oxalacetate was also able to induce a decrease in *Fm'*.

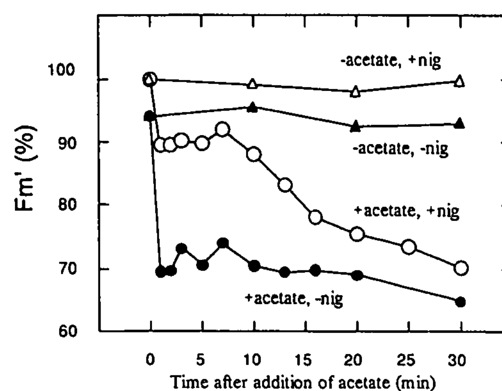

Fig. 2 Time courses of the nigericin-sensitive and nigericin-insensitive decreases in *Fm'* induced after the addition of acetic acid (1 mM) in darkness. A cell culture was diluted with an equal volume of 40 mM MES/KOH (pH 6). *Fm'*, estimated by application of a 1-s pulse of a saturating light (350 W m^{-2}), is expressed as a percentge of that in the dark-adapted cells at 27°C in the presence of 1 μM nigericin. Nigericin (nig) was added to the cell suspension 2 min before the measurement of *Fm'*.

Addition of acetate to cultures of *C. reinhardtii* in darkness or in low light induced the biphasic non-photochemical quenching (qN) of Chl fluorescence. According to Horton and Hague's nomenclature (1988), the rapid phase of qN, which is sensitive to uncouplers, is qE (energy quenching) and the slow phase, which is insensitive to uncouplers, is qT (the transition from state 1 to state 2). Both types of qN are induced by the enhanced donation of electrons to the photosynthetic intersystem chain. Therefore, the present results indicate that added acetate provides reducing equivalents not only in mitochondria but also

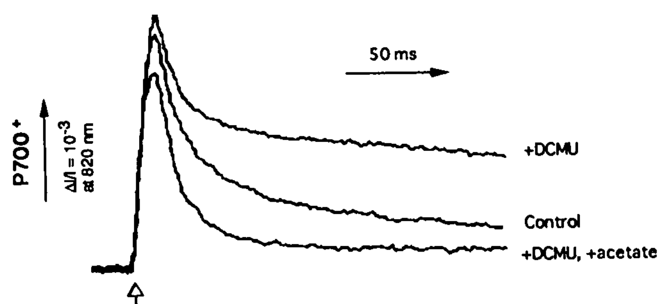

Fig. 3 Photooxidation of P700 by a 5-ms pulse of saturating light (1,500 W m^{-2}), and its re-reduction before and 5 min after the addition of acetic acid (1 mM). The cells were collected by centrifugation (3,000 × *g*, 5 min) and resuspended in 20 mM MES/KOH (pH 6) at 100 μg Chl ml^{-1}. DCMU (10 μM) was added to the suspension 2 min prior to the measurements, as indicated. The open arrow head indicates the triggering of a 5-ms flash.

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in chloroplasts, which stimulates the electron flow in the photosynthetic intersystem chain. For the production of reducing equivalents, acetate must be metabolized in the tricarboxylic acid cycle, which is localized exclusively in mitochondria. Therefore, inter-organelle transport to chloroplasts of reducing equivalents, through the oxalacetate-malate shuttle or phosphoglycerate-dihydroxyacetone phosphate shuttle, must occur. The transport of reducing equivalents between chloroplasts and mitochondria has been suggested in *C. reinhardtii* (Rebeille and Gans 1988, Lemaire et al. 1988, Gans and Rebeille 1990, Bulté et al. 1990, Bennoun 1994) and also in higher plants (Krömer et al. 1988, Krömer and Heldt 1991, Reddy et al. 1991, Vani et al. 1990, Saradadevi and Raghavendra 1992). The electron flow due to chlororespiration seems to be the only mechanism that explains this type of electron donation to the plastoquinone pool in darkness. Activated chlororespiration might result in the formation of a trans-thylakoid pH gradient, which induces qE, and in the reduction of the intersystem electron carriers, which triggers the transition from state 1 to state 2 and the concomitant induction of qT. The presence of chlororespiratory NAD(P)H dehydrogenase (NDH) in the thylakoid membranes has been demonstrated in cyanobacteria (Berger et al. 1991, Mi et al. 1992a, b, 1994, 1995) and in *C. reinhardtii* (Godde and Trebst 1980, Godde 1982, Ravenel et al. 1994).

In the physiological context, the suppression of PSII activity by acatete might function as a mechanism to protect cells against over-reduction of the intersystem chain, which could result in the production of active species of oxygen and consequent damage to cells. Addition of acetate in darkness reduces the electron carriers in the intersystem chain (Fig. 3). Therefore, even in low light, the electron input from water via PSII can over-reduce the intersystem carriers if this type of regulation of PSII is not operative in the heterotrophically grown cells. Thus, it is physiologically reasonable that PSII activity should be temporarily suppressed when acetate or some other source of carbon becomes available. A mutant of the cyanobacterium *Synechocystis* PCC 6803 that lacks respiratory NDH is locked in state 1 in darkness while the wild type is in state 2 as a result of the donation of electrons to the plastoquinone pool from cytosolic donors (Schreiber et al. 1995). This finding further supports our hypothesis that electron input to the plastoquinone pool through chlororespiration can regulate PSII in darkness.

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