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BRIEF COMMUNICATION

Contribution of ΔpH and ΔE to photosynthesis of *Chlamydomonas reinhardtii*

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Abstract

The contribution of two components (ΔpH and ΔE) of the proton motive force to photosynthesis of *C. reinhardtii* was studied. Valinomycin, a photophosphorylation uncoupler, decreased significantly the fast phase (related mainly to the membrane electric potential) of millisecond delayed light emission (ms-DLE) of *C. reinhardtii*. Nigericin, another photophosphorylation uncoupler, decreased the slow phase (related mainly to the proton gradient) and partly also the fast phase of ms-DLE. Both valinomycin and nigericin decreased the net ATP content and photosynthetic rate of *C. reinhardtii*, but the inhibition by nigericin was stronger than that by valinomycin. Hence both components of the proton motive force contribute to photosynthesis and although the contribution of ΔpH is larger than that of ΔE, the latter is not negligible in photosynthesis of *C. reinhardtii*.

Additional key words: proton motive force; millisecond delayed light emission; nigericin; valinomycin.

Mitchell's chemiosmotic hypothesis suggests that under irradiation electron transport couples proton transport and generates proton motive force across membrane that drives ATP synthesis (Mitchell 1961). The proton motive force, the driving force for ATP synthesis of photophosphorylation (PSP), is composed of two components: the proton gradient, ΔpH, and the electric potential, ΔE. The photochemical events in thylakoids lead to the generation of the "assimilation power" (ATP and NADPH) that is required to drive carbon assimilation. The roles of ΔE in PSP and photosynthesis are still discussed. One opinion is that little or no ΔE is main-tained by thylakoids in steady state under irradiation (Avron 1987). On the other hand, the ΔE formed following a darkness-to-light transition is the major driving force for ATP synthesised during the first second or so after turning the light on. It is therefore the major energy source for ATP synthesis driven by a flash of radiation or a sequence of flashes (Vinkler *et al.* 1978, Witt 1979). ΔpH and ΔE are energetically equivalent driving forces for ATP formation (Hangarter and Good 1982). The role of ΔE in $\Delta\mu H^+$, besides ΔpH, is appreciable for driving ATP formation under steady state (Li and Du 1990). Li and Shen (1995) did not find any effect of ΔE on the leaf photosynthesis of higher plants *in vivo*. In this paper we incubated unicellular green algae (*Chlamydomonas* reinhardtii) with valinomycin or nigericin which uncouple photophosphorylation by eliminating ΔE or ΔpH across the thylakoid membrane. We measured their effects on the millisecond delayed light emission (ms-DLE, also called Delayed Luminescence) which was related closely to proton motive force (ΔE and ΔpH) in chloroplasts, net ATP content, and photosynthetic O_2 evolution, and studied the contribution of ΔpH and ΔE to photosynthesis.

The wild-type strain cc-125 of *C. reinhardtii* Dangeard was a gift of Dr. James Weifu Lee of Oak Ridge National Laboratory and Elizabeth Harris of *Chlamydomonas* Genetics Center, Duke University. The algae were cultured in the minimal medium at 25 °C under a light/darkness regime of 12/12 h, with an irradiance of about 60 μmol m^{-2} s^{-1}, and air bubbling. The basic reaction mixture contained 50 mM Tris-HCl (pH 7.0), 40 mM KCl, 10 mM $NaHCO_3$, and alga cells containing 20 μg cm^{-3} of chlorophyll. The cells were incubated with different concentrations of uncouplers for 1 h before various measurements. DLE measurement was accomplished by a Becqerell type phosphoroscope (Wei *et al.* 1998). Light flashes were generated by a 75 W 12 V movie-pro-

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jection incandescent lamp. The holes on the sector were so arranged that the measuring process may be divided into a series of 5.6 ms cycles for excitation and measurement, *i.e.*, 1 ms excitation followed by 4.6 ms darkness. The DLE between 2.8 and 3.8 ms after every flash was measured with an *EMI9558B* photomultiplier using a red glass filter. The signal transmitted through an amplifier was recorded continuously in a *SC-16* light beam oscillograph. Photosynthetic rate was determined by measuring oxygen evolution with Clark-type oxygen electrode under saturating irradiance of about 1 000 μmol m^{-2} s^{-1} from an incandescent lamp at 25 °C. ATP content was measured by the luciferin/luciferase luminescence assay according to Allnutt *et al.* (1991).

Previous work indicated that the fast phase of ms-DLE of chloroplasts (the first uprising 0.1 s of ms-DLE at beginning of flash irradiation) is mainly correlated with a rapid establishment of ΔE (Mayne and Clayton 1966, Wraight and Crofts 1971, Wei *et al.* 1998). Part of it is correlated to the amount of protons released from water oxidation (Xu and Shen 1984), but the slow phase (the part after the fast phase of ms-DLE, which reaches a steady level within a few seconds) is related mainly with ΔpH formed across the thylakoid membrane and is little affected by ΔE. Valinomycin and nigericin are common uncouplers for PSP. In the presence of K^+ in reaction mixture, valinomycin can equilibrate the potassium ion concentrations on both sides of the membrane and thereby diminish the ΔE formed across the thylakoid membrane. Nigericin is an ionophore, which stimulates the exchange of K^+ and H^+ between two sides of the thylakoid membrane that abolishes ΔpH, and thereby inhibits ATP formation.

In the presence of K^+ in reaction mixture, lower concentration of valinomycin or nigericin promoted the fast phase or slow phase of ms-DLE, but their higher concentrations lowered the ms-DLE (for part of results see Fig. 1). After the addition of 5 μM valinomycin, the fast phase of ms-DLE decreased dramatically, yet its slow phase changed very little. But after the addition of 5 μM nigericin, the slow phase of ms-DLE decreased obviously, but the fast phase went down partly. If valinomycin and nigericin were added to the reaction mixture together, both the fast and slow phase of ms-DLE of *C. reinhardtii* declined more than in the presence of valinomycin or nigericin alone. Hence the ms-DLE may be used for the *in vivo* research in *C. reinhardtii* similarly as it is used in studying the relation between the proton motive force and photosynthesis in higher plant leaves (Li and Shen 1995).

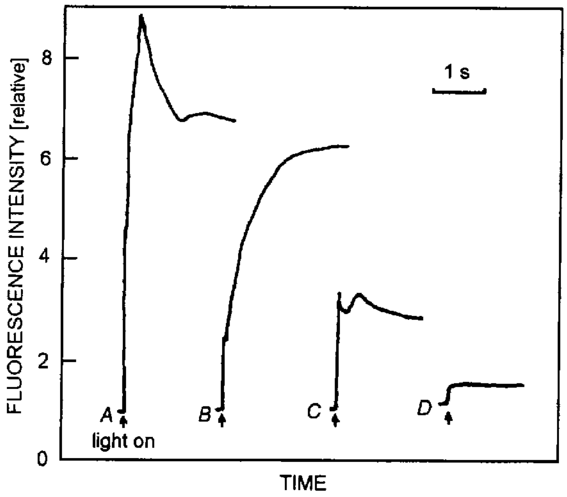

Fig. 1. The effect of uncouplers on the ms-DLE of *Chlamydomonas reinhardtii*. *A*: control; *B*: +5 μM valinomycin; *C*: +5 μM nigericin; *D*: +5 μM valinomycin + 5 μM nigericin.

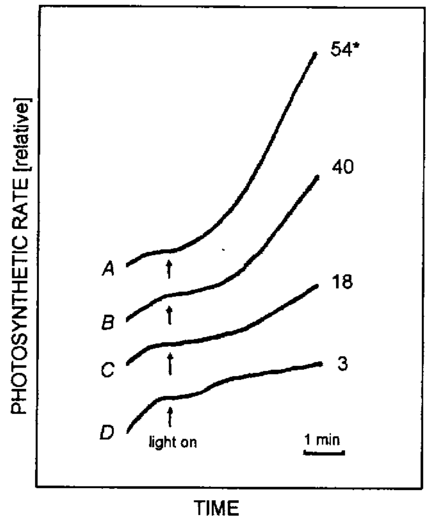

Fig. 2. The effect of uncouplers on photosynthetic rate of *Chlamydomonas reinhardtii*. *A*: control; *B*: +5 μM valinomycin; *C*: +5 μM nigericin; *D*: +5 μM valinomycin + 5 μM nigericin.

We tested also the effect of different concentrations of uncouplers on the net ATP synthesis of alga cells under irradiation. In the presence of K^+, lower concentration of valinomycin or nigericin promoted the net ATP content, but higher concentration of valinomycin or nigericin decreased the ATP content. In the presence of 5 μM valinomycin or 5 μM nigericin in the reaction mixture at 25 °C, the net ATP content (*minus* the dark control) of the alga cells decreased by about 30 and 70 % [61 and 21 mmol(ATP) kg^{-1}(Chl)], respectively, compared to the control [88 mmol(ATP) kg^{-1}(Chl)] after irradiation for 3 min. When valinomycin and nigericin were added together into the reaction mixture, the net ATP content was close to the dark control. This result indicates that valinomycin and nigericin affec-ted the chloroplast PSP activity of *C. reinhardtii in vivo*.

We also observed that in the presence of K^+, low concentration of valinomycin or nigericin could promote the photosynthesis rate. Higher concentration of valinomycin or nigericin inhibited the photosynthetic rate (part of the values is in Fig. 2). After adding separately 5 μM valinomycin or 5 μM nigericin into the reaction mixture, the photosynthetic rate of *C. reinhardtii* decreased by about 25 and 60 %, respectively. When valinomycin and nigericin were added together into the reaction mixture, the photosynthetic rate of *C. reinhardtii* came down to nearly zero.

The above results show that: (*a*) Diminishing either ΔpH or ΔE can lower the photosynthetic rate of *C. reinhardtii*, which indicates that both components (ΔpH and ΔE) of proton motive force contribute to photosynthesis of *C. reinhardtii in vivo*. The diminishing effect on ΔpH by nigerincin was stronger than the diminishing effect on ΔE by valinomycin. This demonstrates that ΔpH contributes more than ΔE to the photosynthesis of *C. reinhardtii*, but the contribution of ΔE cannot be neglected. This is different from the previous results (Li and Shen 1995) in which valinomycin + K^+ added through vacuum-infiltration had little effect on the photosynthesis of higher plant leaves. This suggested that ΔE constitutes only a small part of the steady state proton motive force formed across the thylakoid membrane *in vivo*. But Li and Du (1990) observed that ΔE was almost totally eliminated by gramicidin D and valinomycin, yet ΔpH was little affected, but photophosphorylation acti-vity of chloroplast was inhibited by ~30 % (gramicidin D) and ~50 % (valinomycin), respectively (Li and Du 1990). This shows that in steady state PSP of chloroplast, ΔE provides 30~50 % of the needed energy. The microenvironment of the chloroplast in *C. reinhardtii*, a unicellular green alga, must be different from that in the leaf of a higher plant or its chloroplast *in vitro*. Maybe this is the cause of different contribution of ΔE to the photosynthesis of *C. reinhardtii* and higher plants. (*b*) The observation that low concentration of nigericin could enhance PSP activity corroborated the results got in our laboratory that treatment of the chloroplasts with low concentration of nigericin increased ATP formation (Li and Ma 1990). This fact substantiates the view that photophosphorylation may be carried out with localised proton *in vivo*.

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