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Short Communication

Effect of Monochromatic Light on Proton Efflux of the Blue-Green Alga *Anabaena variabilis*[1]

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ABSTRACT

Light-induced proton efflux of *Anabaena variabilis* was found to be biphasic, the second phase being inhibited by the ATPase inhibitor nitrofen (2,4-dichloro-1-[4-nitrophenoxy]benzene). The first, fast phase was triggered by monochromatic light of 707 nanometers, whereas the second, slower phase was not. With 707 nanometers, light, respiratory O_2 uptake was inhibited. Using light composed of two wavelengths (616 and 707 nanometers) a marked enhancement of both O_2 evolution as well as the second phase of proton efflux was observed. The first phase was not enhanced. Thus, phase II is driven by both photosystems. As concluded from the action spectrum phase I is markedly determined by photosystem-I activity. Altogether the data show that two different mechanisms of light-induced proton efflux exist on the cytoplasmic membrane of *Anabaena*, the slower one being dependent on ATP and linear photosynthetic electron flow.

Light-induced proton efflux of *Anabaena* has been described first by Scholes *et al.* (15), but no mechanism was suggested as being responsible for the light-induced acidification of the medium. It has been assumed that light-induced proton efflux of *Plectonema boryanum* may be due to a respiratory electron transport chain localized on the cytoplasmic membrane (2). In contrast, vanadate sensitivity of light-induced proton efflux (13) provided strong evidence for a unidirectional, proton-translocating ATP-hydrolase being active on the cytoplasmic membrane of *Anabaena* and *Nostoc*. In this communication an Emerson enhancement effect on proton efflux of *Anabaena* is reported, giving evidence for different mechanisms mediating the light-induced acidification.

MATERIALS AND METHODS

Anabaena variabilis Kütz. (ATCC 29413) was grown as described previously (12) with N_2 as nitrogen source. Filaments were washed and resuspended (30 μg Chl/ml) in a medium containing 3 mM glycylglycine/NaOH buffer (pH 6.3), 75 mM KCl, 75 mM NaCl, 5 mM $MgCl_2$ (pH 6.3). O_2 evolution and proton flux were measured according to Mitchell and Moyle (10), as described by Scherer *et al.* (14). The proton efflux actually mediated by phase II was calculated by numerically adding the kinetics of O_2 evolution (which is coupled with OH^--efflux, [5]) and proton efflux. Phase I was not corrected for phase II. Apparently the latter was not active 5 to 10 s after turning on the light, since no O_2 evolution was observed during this time interval. The sum of proton effluxes between 40 and 60 s after illumination is defined as phase II. A correction for phase I is not possible at the moment. For details and justification of this method see Hinrichs *et al.* (3).

Monochromatic light was produced by passing the light of a 100-W iodine lamp (Osram Halogen Bellaphot No. 64610) through interference filters (Balzers, Liechtenstein) determining the light intensity with either a wattmeter (YSI Kettering 65 A, Yellow Springs, OH) or a quantum sensor (LI-190 SB, LI-COR, Lincoln, NE). Saturating red light was provided by a RG-610 cut-off filter (Schott, Mainz, FRG).

RESULTS AND DISCUSSION

The kinetics of light-dependent proton efflux is shown in Figure 1, consisting of two different phases as indicated. The absolute rates of proton efflux are difficult to obtain, since proton efflux is accompanied by an OH^--efflux (5, 9). So, the rates given for phase II were calculated by adding the rate of proton efflux and OH^--efflux, the latter estimated from O_2 evolution (for details, see Hinrichs *et al.* [3]). Nitrofen inhibited phase II of proton efflux as well as photosynthetic O_2 evolution, but not phase I (Fig. 1). With the concentrations used, nitrofen has been shown to inhibit the F_0/F_1-ATPase (4, 7). We conclude, therefore,

Table I. *Influence of Light with Different Wavelengths on Proton Efflux and O_2 Exchange of A. variabilis*

Respiratory O_2 uptake in the dark was 10.6 μmol/mg Chl·h. Intensity of 616 nm light was 220 $\mu E/m^2 \cdot s$; with the quantum sensor available, the quantum flux of 707 nm light could not be determined. Therefore the 707 nm light was adjusted to yield the same light-induced phase-I proton efflux as was measured with 616 nm light.

Wavelength	Proton Efflux		O_2 Gas Exchange
	Phase I	Phase II	
nm	*μmol/mg Chl·h*		
616	22	3	−4[a]
707	25	5	−11
616 + 707	48	20	+11
Saturating red light (610)	94	47	+74

[a] O_2 uptake denoted by a (−) sign.

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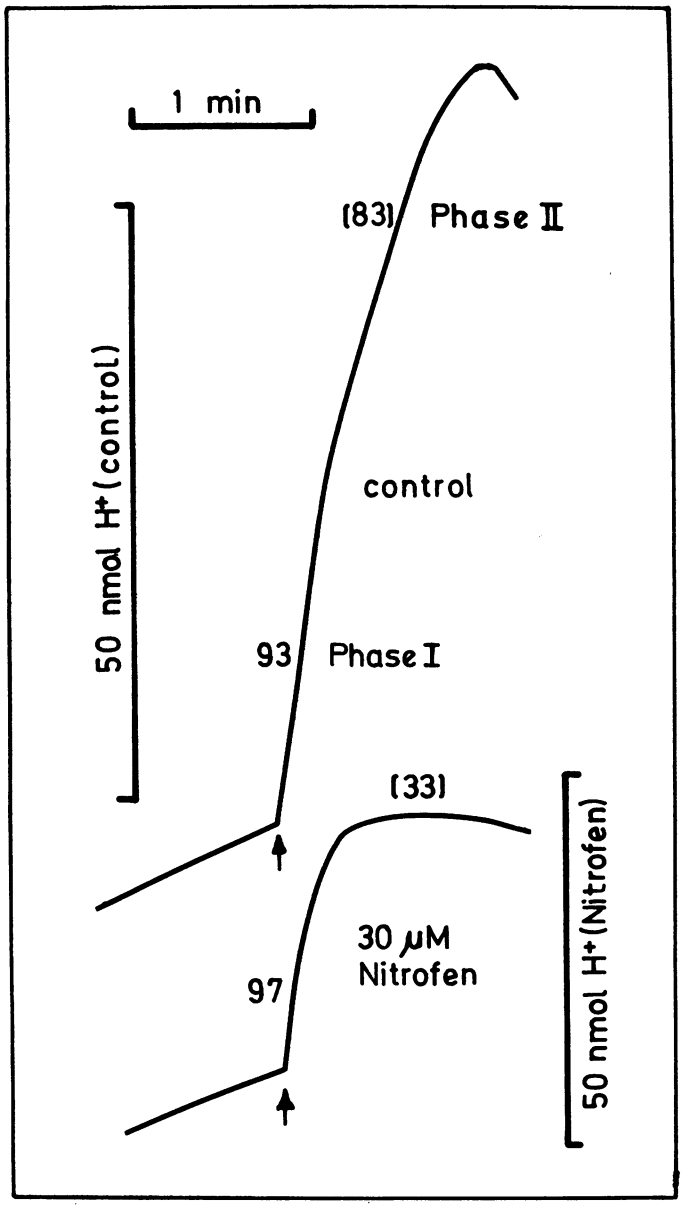

FIG. 1. Light-induced proton efflux of *A. variabilis*, influence of nitrofen. The figures represent the rate of proton efflux in μmol/mg Chl·h. Those in brackets are calculations of proton efflux including OH^--efflux. Arrows indicate when saturating red light (with wavelengths above 610 nm) was switched on. The rates of the two phases, and the extent of the difference between them, depend on the physiological condition of the filaments, which are not yet understood.

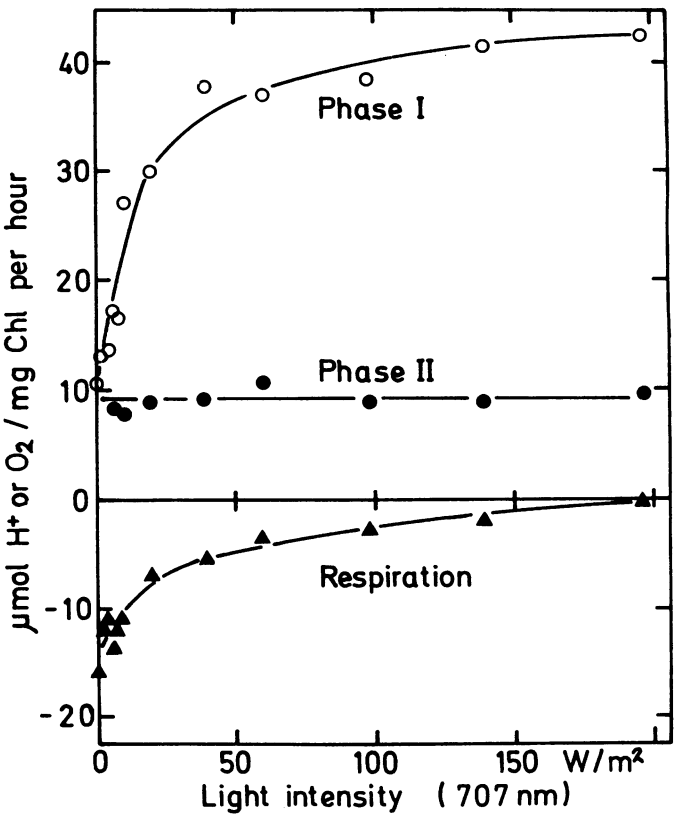

FIG. 2. Light dependence of proton efflux and O_2 exchange of *A. variabilis* using 707 nm light.

that phase II of the light-induced proton efflux is ATP-dependent, while phase I is not. We tried to demonstrate specific effects of DCCD[2] and diethystilbestrol, but found these inhibitors affecting unspecifically proton efflux of both phase I and phase II, together with O_2 evolution and respiration. The ATP dependence of phase II is in good accordance with the inhibitory effect of vanadate (13), indicative of an unidirectional, proton-translocating ATP-hydrolase being responsible for the phase-II efflux.

The effect of monochromatic light (707 nm) on proton efflux and O_2 exchange is shown in Figure 2. Quite low light intensities saturated phase I and inhibited O_2 uptake, but phase II was not detectable. It should be noted that the maximum rate of phase I with 707 nm light was about 40% of its rate in saturating red light (>610 nm). As shown previously, at 80 $\mu E/m^2 \cdot s$ phase I was active with 70% of the maximum reached with saturating red light while phase II and O_2 evolution were not detectable (Fig. 3 of Ref. [3]). The influence of light containing 616 and 707 nm wavelengths is shown in Table I (see legend for experimental details). Comparatively low light intensities were applied ensuring that all activities measured with light of either one or two wavelengths were at best in the half-saturated state. The intensities of the monochromatic lights, however, were adjusted to yield identical rates of phase-I proton efflux. With these light intensities used no phase-II proton efflux could be discriminated from acidification measurements in the dark (*cf.* Figs. 1 and 2). Obviously, the proton efflux of phase II as well as photosynthetic O_2 evolution exhibited an Emerson enhancement, indicative of phase II being dependent on both PSI and PSII, whereas phase-I proton efflux apparently is not dependent on the cooperation of the two photosystems.

The action spectrum shown in Figure 3 could not yield conclusive evidence as to whether phase I exclusively depends on PSI only. Apparently, PSI is quite effective since DCMU inhibition of proton efflux slightly shifted the maximum activity of phase I to longer wavelengths.

Light-induced proton efflux has been described for several eukaryotic algae (1, 17). For *Cyanidium* it has been reported to be driven by a PSI dependent phosphorylation (6). It was assumed for *Anacystis*, that the light-induced amino acid uptake depends on a PSI driven proton efflux (8). The DCMU-resistant, light-induced proton efflux of this species is completely inhibited by DCCD (11). It has been suggested that light-induced proton efflux of *Plectonema* is due to a respiratory electron transport localized on the cytoplasmic membrane oxidizing pyridine nucleotides produced in the light (2). At present the data available do not allow for a final general conclusion on regulation or on

[2] Abbreviation: DCCD, dicyclohexylcarbodiimide.

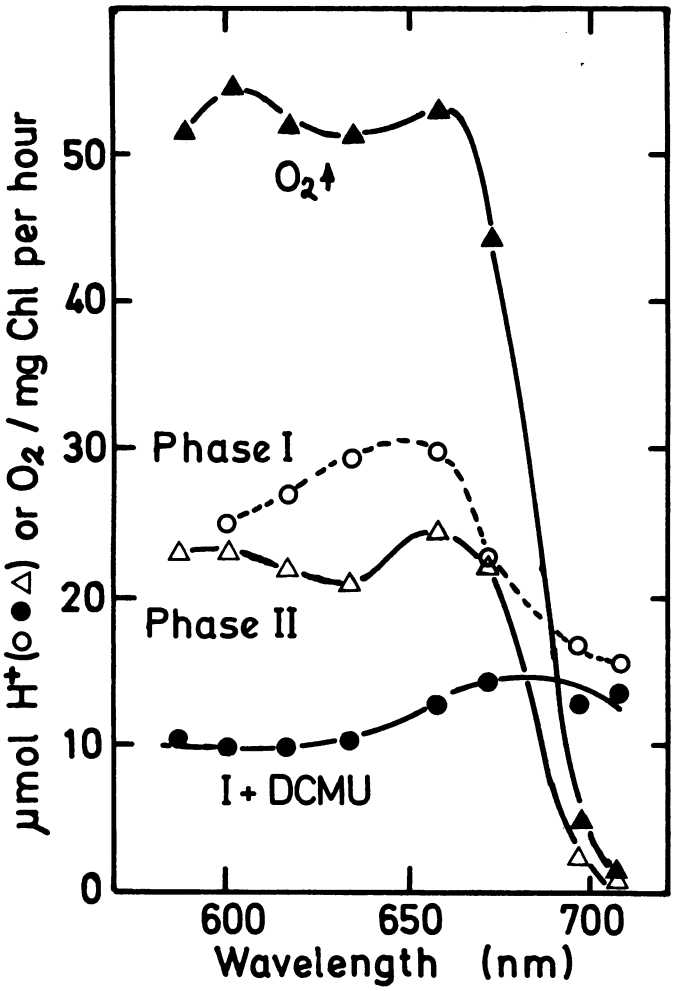

FIG. 3. Action spectrum of photosynthetic O_2 evolution and proton efflux of *A. variabilis*. Light intensity was 700 $\mu E/m^2 \cdot s$, Chl in the reaction chamber 30 $\mu g/ml \cdot$(●), Proton efflux with 10 μM DCMU present.

mechanism of phase-I proton efflux, but provide no evidence for a respiratory electron transport chain being localized on the cytoplasmic membrane of *A. variabilis* (*cf.* [3]).

Our experiments with monochromatic light indicate that two different processes of light-induced proton efflux occur on the cytoplasmic membrane of *A. variabilis*. Phase-II proton efflux is vanadate-sensitive, ATP-dependent, insensitive against 100 μM cyanide (3), and exhibits an Emerson enhancement. Conclusively, this efflux is produced by a unidirectional, proton-translocating ATPase, most likely similar to enzymes found on the plasmalemma of plant cells and fungi (*cf.* 16, 18). We have evidence (unpublished data) that this proton efflux may be important in osmoregulation and pH-adjustment of the cell. Additionally, the proton gradient produced may be useful for the uptake of substrates.

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