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PHOTOSTIMULATION OF NITROGEN FIXATION IN *ANABAENA CYLINDRICA*

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SUMMARY

Photostimulation of N_2 fixation in the blue-green alga *Anabaena cylindrica* was investigated using monochromatic light and by comparing action spectra of C_2H_2 reduction with that of photosynthetic O_2 evolution. Maximum nitrogenase activity per unit energy was measured at a wavelength which corresponds to the maximum light absorption of chlorophyll *a*. The action spectrum of C_2H_2 reduction indicates a primary involvement of Photosystem I in N_2 fixation by blue-green algae.

INTRODUCTION

In blue-green algae photosynthesis constitutes the only or the principal type of carbon assimilation, and therefore a close relationship between photosynthesis and N_2 fixation was envisaged for obvious reasons[1-3]. It was shown by Cox[4] that the main dependency of N_2 fixation upon light is for a supply of carbon to assimilate the fixed nitrogen. However, nitrogenase activity was stimulated by increasing light intensity also under conditions of carbon saturation, and when the products of the enzyme-catalyzed reaction, such as C_2H_4, were not metabolized by the alga[5]. This light stimulation of nitrogenase activity was unaffected in the presence of CMU (*p*-chlorophenyl-1,1-dimethylurea) concentration which inhibited CO_2 fixation over 90 %. From this it was assumed[5] that photostimulation of N_2 fixation in blue-green algae is probably through ATP generated in the photochemical reaction while reducing potential for N_2 fixation is furnished independent of the non-cyclic photo-electron transport.

The present study in which monochromatic light was used to resolve the complex effect of actinic light upon N_2 fixation was devised to test the above assumption and to see whether photostimulation of N_2 fixation can be assigned to a particular photochemical reaction.

METHODS

Anabaena cylindrica Lemm. was grown in the nitrogen-free Medium C1 of COBB AND MYERS[3] at 25° with fluorescent light (200 foot candles) and aerated with 0.5 % CO_2 in air.

Abbreviation, CMU, *p*-chlorophenyl-1,1-dimethylurea.

Heterocysts were isolated essentially as described by FAY AND WALSBY[6] except that vegetative cells were disrupted at maximum output of a Branson sonifier for 60 sec.

Absorption spectra were recorded with a Cary Model-14 spectrophotometer. Effects of scattering were minimized by the use of 3-mm translucent lucite diffusion plates immersed in the 10-mm cuvettes[7].

Action spectra were prepared using a Bausch and Lomb 500-mm grating monochromator with a grating blased for maximum efficiency at 500 nm. The light source, a Sylvania DWY lamp, was operated at constant voltage. The exit beam was protected by Corning cutoff filters 3391 (below 515 nm) or 3484 (above 515 nm). The beam was controlled by a circular neutral wedge and an electric shutter. The image of the exit slit, as focused on the reaction vessel, formed an illuminated area slightly larger than 1 cm^2 at a distance of 7 cm from the wedge. The optical system provided intensities in the range of 150–420 $\mu W/cm^2$. Light intensities were measured with a Cambridge thermopile exposing a diaphragm area of 1 cm^2.

Measurements of photosynthetic O_2 evolution were carried out using a Yellow Spring O_2 electrode with scale expanded and suppressed to allow recording of O_2 concentration at a sensitivity of about 0.02 μl O_2 per scale division. The electrode chamber of 1.5 ml capacity was water jacketed and thermostatically controlled, and the cell suspension magnetically stirred.

Nitrogenase activity was estimated by measuring the rate of C_2H_2 reduction[8]. Screw-cap serum bottles containing 1 ml of the algal suspension were filled with 10 % C_2H_2 in argon and incubated with magnetic stirring in light of selected wavelength. C_2H_4 production was assayed by gas chromatography using a Loenco Model 160 PMF gas chromatograph equipped with a 'Porapak R' column.

RESULTS

The absorption spectrum *in vivo* of the intact filaments of *A. cylindrica* shown in Fig. 1A demonstrates the preponderance of the principal pigments, chlorophyll *a* (absorption maximum at 675 nm) and phycocyanin (absorption maximum at 625 nm). From the absorption spectrum of isolated heterocysts (Fig. 1B) the absence or very low content of phycocyanin is apparent. This agrees with data of pigment analysis of isolated heterocysts[9]. The absorption peaks at 625–630 nm are attributed to secondary maxima of chlorophyll rather than to phycocyanin.

Fig. 2A shows the effect of light intensity at two selected wavelengths, 625 and 675 nm, on C_2H_2 reduction by intact filaments of *A. cylindrica*, in comparison with a similar effect on O_2 evolution seen in Fig. 2B. It demonstrates (1) a clear difference between the effect of light of 625-nm wavelength and of 675-nm wavelength on nitrogenase activity, and (2) that the rates of C_2H_2 reduction are linear with light intensity and extrapolate almost to zero.

Fig. 3A presents the action spectrum of C_2H_2 reduction by whole filaments of *A. cylindrica*. This indicates (1) that energy supplied at various wavelengths affects nitrogenase activity differently, and (2) that the maximum rate of C_2H_2 reduction occurred when this energy was supplied as light of 675-nm wavelength selectively absorbed by chlorophyll *a*. The action spectrum of C_2H_2 reduction markedly differs from the action spectrum of photosynthetic O_2 evolution shown in Fig. 3B. The

latter proceeded at highest rate in a spectral region which corresponds to the maximum absorption by phycocyanin.

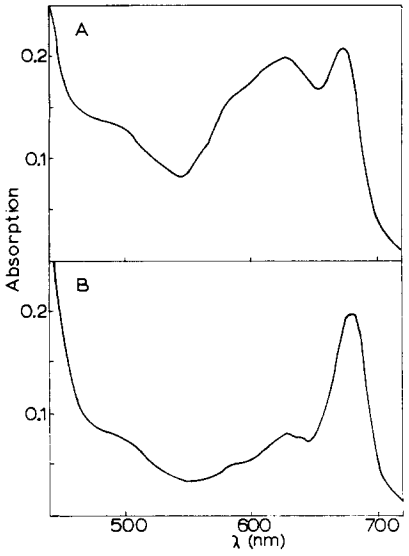

Fig. 1. Absorption spectra *in vivo* of intact filaments (A) and isolated heterocysts (B) of *A. cylindrica* obtained in a Cary spectrophotometer using a 3-mm lucite diffusion plate immersed in the 10-mm cuvette.

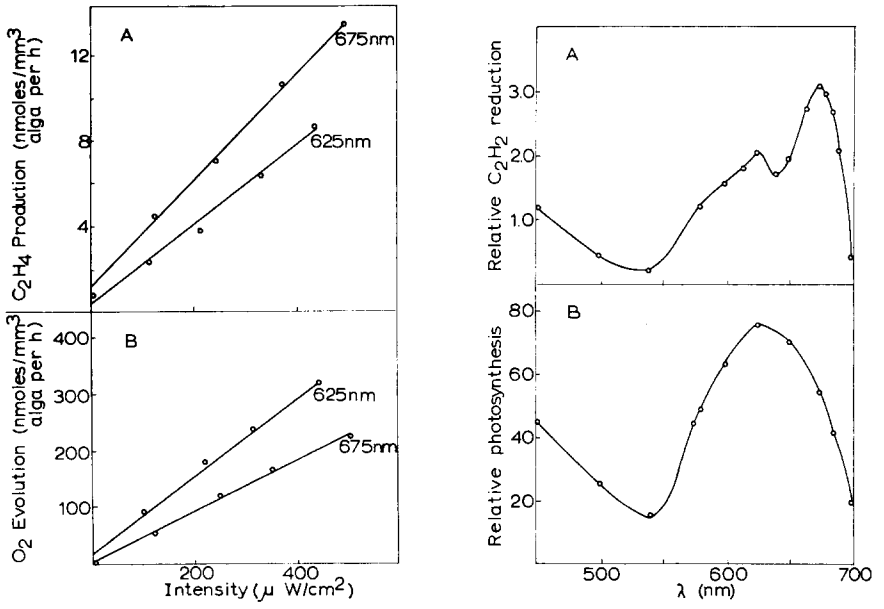

Fig. 2. A. Rate of C_2H_2 reduction *vs.* light intensity at 625 and 675 nm. B. Rate of net O_2 evolution *vs.* light intensity at 625 and 675 nm.

Fig. 3. Action spectra of C_2H_2 reduction (A) and O_2 evolution (B) by intact filaments of *A. cylindrica*. Action was calculated as relative response ((A), nmoles C_2H_4 produced per mm^3 alga per h; (B), nmoles O_2 evolved per mm^3 alga per h) per incident energy ((A) 500 $\mu W/cm^2$; (B) 100 $\mu W/cm^2$) at each wavelength.

DISCUSSION

The application of monochromatic light proved to be a useful approach in an attempt to resolve the nature of the effect of light on N_2 fixation in blue-green algae. The linear response of the rate of C_2H_2 reduction to light intensity is indicative of a direct photostimulation of nitrogenase activity. The action spectrum of C_2H_2 reduction clearly resembles action spectra of Photosystem I prepared with other algae[10], and supports the view of the primary involvement of this photosystem in N_2 fixation by blue-green algae[5].

The striking resemblance between the action spectrum of C_2H_2 reduction by intact filaments and the absorption spectrum of isolated heterocysts of *A. cylindrica in vivo* should also be mentioned. This is of special interest in view of the role of heterocysts in N_2 fixation by blue-green algae[11,12]. However, since vegetative cells in contrast with heterocysts possess both photosystems, Photosystem I and II, the resemblance may be coincidental. Hence this does not constitute direct evidence for nitrogenase activity in the heterocysts.

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