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PHOTOSYSTEM I-INITIATED POSTILLUMINATION CO_2 BURST IN A CYANOBACTERIUM, *ANABAENA VARIABILIS*

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In *Anabaena variabilis*, a postillumination CO_2 burst originating from a pool of HCO_3^- is described here. This burst is insensitive to the electron-transport inhibitor, 3-(3,4-dichlorophenyl)-1,1-dimethylurea, but is abolished by carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone and *N,N'*-dicyclohexylcarbodiimide (inhibitors of photophosphorylation). The action spectrum for the burst shows that only Photosystem I is involved.

Cyanobacteria (blue-green algae) are photosynthetic prokaryotes which have existed for over billions of years. Unlike other prokaryotes, they have both PS I and PS II, drive electrons from water to CO_2 and thus evolve oxygen. Light harvesting in cyanobacteria is largely accomplished by Chl *a* and phycobilins. Chl *a* is preferentially associated with PS I while phycobilins are preferentially associated with PS II [1]. In order to characterize their photosynthetic properties, we measured the gas exchange of a cyanobacterium, *Anabaena variabilis*, using an open-gas analysis system and found a postillumination CO_2 burst defined as CO_2 evolution in the dark immediately following a light period. A similar type of CO_2 burst has recently been observed in a marine cyanobacterium, *Synechococcus* sp., by Badger and Andrews [2] using a mass spectrometer. This paper demonstrated that the CO_2 burst is initiated by excitation of PS I only and originates from a pool of HCO_3^- accumulated in the cells likely by cyclic photophosphorylation.

Cells of *A. variabilis*, strain M-2, from the algal collection of the Institute of Applied Microbiology (Tokyo University), were grown at 27°C in the medium described by Kratz and Myers [3] under aeration with 3% CO_2 in air (v/v). Continuous illumination was provided by fluorescent lamps (700 $\mu W/cm^2$). Cells were harvested by centrifugation at room temperature at 500 × *g*, resuspended in 30 ml of 40 mM Hepes-KOH buffer (pH 7.6) and then placed in a cylindrical reaction vessel (diameter, 3.8 cm). The gas exchange of the algae was measured at 27°C with an open-gas analysis system [4], which records the rate of gas exchange directly. Nitrogen gas containing 650 μl CO_2/l was led into the reaction vessel at a flow rate of 1.0 l/min. The exchanged gas was dried and then measured with an infrared CO_2 analyser (model ZAP; Fuji Electric Co., Tokyo) and a trace oxygen analyser (model 316; Teledyne Analytical Instrument Co., U.S.A.). Interference filters (bandwidth at half-height, 12–15 nm) were used to obtain

Abbreviations: Chl, chlorophyll; PS, photosystem; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCCD, *N,N'*-dicyclohexylcarbodiimide; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

various wavelengths of monochromatic light. The light source was a 650 W halogen lamp with a fan-cooled heat-absorbing filter. Absorption spectra were measured with a Shimadzu MPS-5000 multipurpose recording spectrophotometer, using cells 1.0 cm thick (path length) and 2.0 cm wide. Fractional absorption and absorbance of cell suspensions in the reaction vessel were calculated from the values of absorbance obtained using a 1 cm cell and the average length of the light path of the reaction vessel (3.0 cm).

When the light was switched on there was a decline in CO_2 followed by a transient rise and then gradual decline to a steady-state level (curve A in Fig. 1). Switching the light off resulted in a sharp increase in CO_2 to a level higher than the initial level followed by a decline to the initial level. This postillumination CO_2 burst was insensitive to DCMU, an inhibitor of PS II and noncyclic electron transport (curve B in Fig. 1; see also Table I). To describe the CO_2 burst quantitatively, we calculated the initial rate of the burst by assuming that the rate of CO_2 evolution followed first-order kinetics. The rate of CO_2 evolution into the medium ($-dC/dt$) is given by the equation:

$$-dC/dt = kC$$

where C is the concentration of a carbon source which gives rise to the CO_2 burst and k the rate constant for the conversion of the carbon source to CO_2. From this equation, we obtain:

$$\log(-dC/dt) = \log kC_0 - kt/2.303$$

where C_0 represents the concentration of the carbon source before the light is turned off and t is the time after the CO_2 level starts to increase in the dark (see Fig. 1). The change in CO_2 level in the

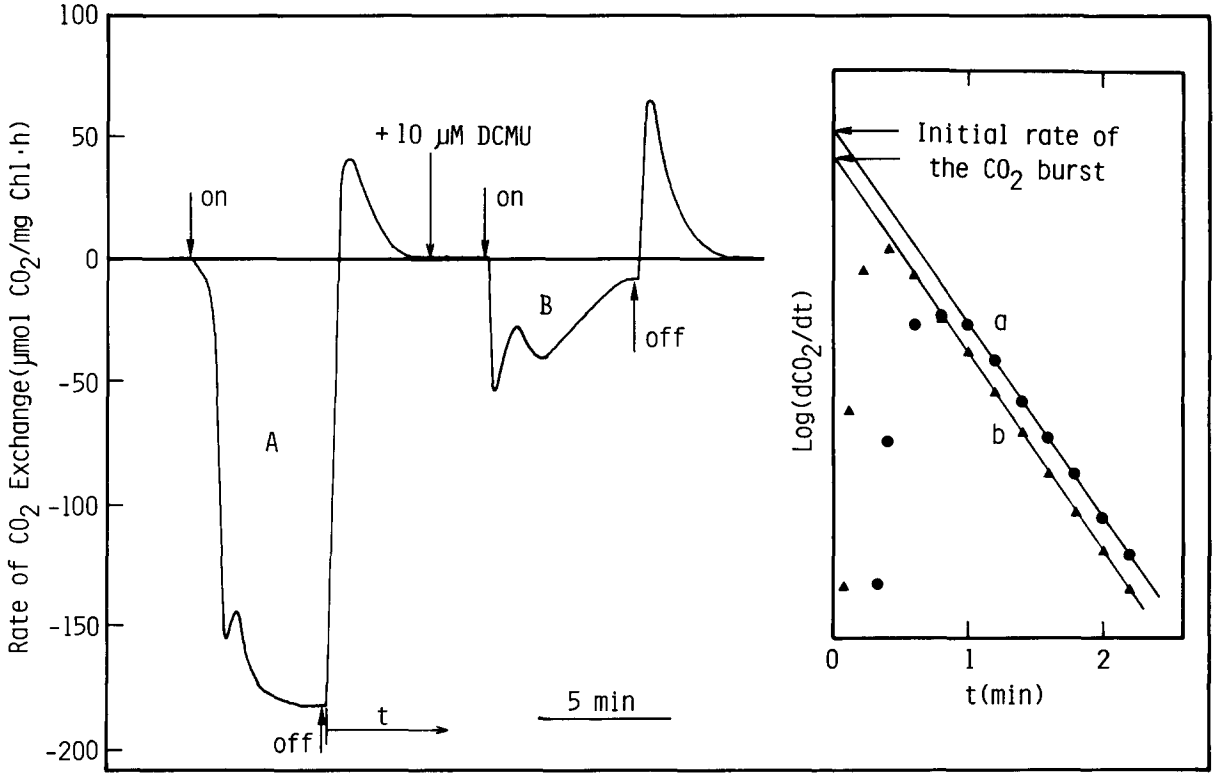

Fig. 1. Changes in CO_2 concentration in the gas phase after switching the white light (23 mW/cm^2) on and off. (A) Control, (B) with 10 μM DCMU. The cell suspension contained 9.6 μg Chl/ml. (Inset) Semilogarithmic plots of dCO_2/dt vs. t for the CO_2 burst of curves A (●, a) and B (▲, b). For details, see the text.

gaseous phase, dCO_2/dt, is equal to $-dC/dt$ and, therefore, a semilogarithmic plot of dCO_2/dt vs. t gives a straight line (see a and b in the inset of Fig. 1 for the CO_2 bursts of curves A and B, respectively). The intercept of each line with the ordinate gives the initial rate of the burst (kC_0) and the slope gives the value of $-k/2.303$. The C_0 and k values calculated from the CO_2 burst of curve A in Fig. 1 were 1.73 μmol/mg Chl and 0.026 s^{-1}, respectively, and the initial rate of the burst was 162 μmol/mg Chl per h. The validity of this extrapolation method was confirmed by measuring the kinetics of HCO_3^- transport in *A. variabilis* cells using the silicon oil centrifugation methd described by Kaplan et al. [5] (Ogawa, T. and Ogren, W.L., unpublished data). The concentration of ($HCO_3^- + CO_2$) in the cells in the light was 1.8 μmol/mg Chl (average of three experiments), which is in agreement with the C_0 value calculated by the extrapolation method. The concentration decreased without any lag after turning off the light. The decrease followed first-order kinetics with a rate constant of 0.020–0.033 s^{-1}, which is also in agreement with the rate constant calculated from the CO_2 burst. There was no change in the amount of photosynthetic products during the dark incubation after illumination. It is, therefore, evident that the extrapolation method is valid for the determination of C_0, k and the initial rate of the burst.

Table I summarizes the effects of inhibitors of photosynthesis on the CO_2 burst and O_2 evolution. None of these inhibitors affected the rate constant of the CO_2 burst (data not shown). Therefore, the percent of the control for the initial rate of the CO_2 burst is regarded also as that for the total amount of CO_2 evolved. Both the uncoupler of photophosphorylation, FCCP [6], and the inhibitor of ATP synthesis and proton transport, DCCD [6], had a strong inhibitory effect on the CO_2 burst. This suggests that ATP may be required to accumulate HCO_3^- involved in the CO_2 burst. The absence of inhibition by DCMU indicates that the HCO_3^- accumulation does not require noncyclic electron transport from PS II and that ATP is likely provided by cyclic photophosphorylation.

As a further test that the postillumination CO_2 burst is indeed driven by PS I, experiments were carried out to measure the action spectrum and

TABLE I

EFFECTS OF INHIBITORS OF PHOTOSYNTHESIS ON THE CO_2 BURST AND O_2 EVOLUTION

Results are expressed as μmol/mg Chl per h. The values in parentheses indicate the percent of the control. These values are valid for the total amount of CO_2 evolved in the burst. For details, see the text.

	CO_2 burst	O_2 evolution
Control	214 (100)	276 (100)
+ 1 μM DCMU	214 (100)	28 (10)
+ 10 μM DCMU	177 (83)	7 (2.5)
+ 3 μM FCCP	0 (0)	0 (0)
+ 0.2 mM DCCD	24 (11)	25 (9)

quantum yield for HCO_3^- accumulation (the accumulation of HCO_3^- which produces the CO_2 burst). The action spectrum for HCO_3^- accumulation (Fig. 2, upper panel), obtained by measuring the initial rates of the CO_2 burst following 5-min illumination with various wavelengths of monochromatic light, showed a peak around 684 nm (due to Chl *a* in PS I) while that for O_2 evolution showed a broad peak in the 600–700 nm region (due to phycocyanin and allophycocyanin in PS II); the latter is in agreement with the reported spectrum for oxygen evolution [1]. The quantum yield of O_2 evolution or HCO_3^- accumulation defined as the number of O_2 molecules evolved or CO_2 molecules evolved divided by the number of photons absorbed (number of incident photons × fractional absorption; see middle panel in Fig. 2) is shown in the bottom panel in Fig. 2. The quantum yield for the HCO_3^- accumulation was 0.3 at 700 nm which is much higher than the value (0.07 at 630 nm) obtained for O_2 evolution. In contrast to the drop at longer wavelengths in the quantum yield of O_2 evolution [7], a rise in that region was observed for the CO_2 burst. These results clearly demonstrate that the HCO_3^- accumulation is driven only by PS I.

The above quantum yield for the HCO_3^- accumulation was estimated assuming that the initial rate of the CO_2 burst in the dark is equal to rate of CO_2 evolution in the light and, consequently, to the rate of HCO_3^- accumulation. This assumption could lead to an overestimation of the quantum yield. The cytoplasmic pH reported for *Anacystis*

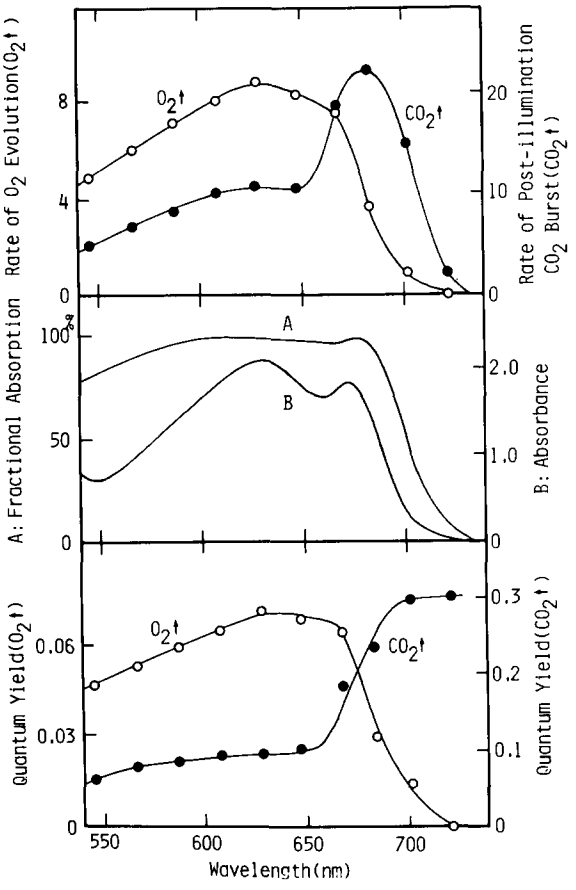

Fig. 2. Action spectra (upper panel) and quantum yields (bottom panel) for O_2 evolution (O_2 ↑) and HCO_3^- accumulation (CO_2 ↑). The rates in the upper panel are expressed in μmol O_2 or CO_2 evolved/mg Chl per h and are normalized to the rates at a quantum flux of 1 nE/cm^2 per s. The incident quantum fluxes of monochromatic light were 3.3–5.3 nE/cm^2 per s. The middle panel shows the absorption spectra of the cell suspension expressed as fractional absorption (A) or absorbance (B). The average length of the light path of the reaction vessel was 3.0 cm and the cell suspension in the reaction vessel contained 24.0 μg Chl/cm^2 (8.0 μg Chl/ml). The quantum yields for HCO_3^- accumulation in the bottom panel may be overestimated as discussed in the text.

nidulans is 7.5 in the light and 6.9 in the dark [8]. The rate constant for dehydration of HCO_3^- to CO_2 at 25°C calculated using the constants reported by Kern [9] is 0.015 s^{-1} at pH 6.9 which is close to the rate constant for the CO_2 burst observed above (a value of 0.0037 s^{-1} was obtained at pH 7.5). The much smaller rate constant at pH 7.5 suggests that the rate of CO_2 evolution may be lower in the light than in the dark. It is, however, not possible at present to measure the rate of CO_2 evolution in the light. Further studies are required to verify the quantum yields obtained in this study.

The postillumination CO_2 burst reported here was observed under anaerobic conditions where dark respiration and photorespiration were completely suppressed. Thus, it is not due to CO_2 evolution related to dark respiration or photorespiration. The activity of HCO_3^- transport has been demonstrated in various species of cyanobacteria [2,5,10]. It is unlikely that the HCO_3^- transport is driven by a change in the cytoplasmic pH. For example, if we accept the sorbitol-impermeable space of 0.39 ml/mg Chl reported by Kaplan et al. [5] and calculate the HCO_3^- concentration in the cells in equilibrium with 650 μl CO_2/l in the atmosphere using the equation of Henderson-Hasselbach and the constants reported by Umbreit [11], we obtain a concentration of 0.11 μmol/mg Chl at 25°C at pH 7.5; thus, a value much smaller than the HCO_3^- concentration accumulated in the cells in the light. Therefore, HCO_3^- transport for the CO_2 burst may require ATP, which may be produced by cyclic photophosphorylation.

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